31ST ANNUAL SYMPOSIUM OF THE INTERNATIONAL CANNABINOID RESEARCH SOCIETY

JERUSALEM
ISRAEL

JUNE 21-24, 2021

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SYMPOSIUM OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

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ICRS2021 Day 1 - Live From Jerusalem
Monday, June 21st

07.00 EST 11.00 UTC 14.00 Local	Welcome to ICRS2021: Opening Remarks	
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Oral Session 1. Endocannabinoid Regulation and Signaling

Moderators: Cecilia Hillard, Ph.D. and Todd Stollenwerk

09.00 13.00 16.00	Veronika Kondev*, Yulong Li, Ao Dong and Sachin Patel	**UTILIZING A NOVEL ENDOCANNABINOID BIOSENSOR TO ELUCIDATE CIRCUIT-SPECIFIC ENDOCANNABINOID REGULATION OF VENTRAL HIPPOCAMPUS-BASOLATERAL AMYGDALA**	1
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09.45 13.45 16.45	Heather Bradshaw*, Clare Johnson, Gabriel H.D. de Abreu, Ken Mackie and Hui-Chen Lu	**CANNABINOID EXPOSURE DURING LACTATION SIGNIFICANTLY CHANGES THE LIPID CONTENT OF BREAST MILK INCLUDING THE ENDOCANNABINOIDS ANANDAMIDE AND 2-AG**	4
10.00 14.00 17.00	Ao Dong*, Kaikai He, Barna Dudok, Jordan S Farrell, Wuqiang Guan, Daniel J Liput, Henry L Puhl, Ruyi Cai, Jiali Duan, Eddy Albarran, Jun Ding, David M Lovinger, Bo Li, Ivan Soltesz and Yulong Li	**A FLUORESCENT SENSOR FOR SPATIOTEMPORALLY RESOLVED ENDOCANNABINOID DYNAMICS IN VITRO AND IN VIVO**	5
10.15 14.15 17.15	Bogna M Ignatowska-Jankowska*, Aysen Gurkan-Ozer, Alexander Kuck, Micah J Niphakis, Daisuke Ogasawara, Benjamin F Cravatt and Marylka Yoe Uusisaari	**DISTINCT KINEMATIC CHARACTERISTICS OF MAGL AND FAAH INHIBITION IN HIGH PRECISION 3D MOTION CAPTURE ANALYSIS OF FREELY BEHAVING MICE**	6
10.30 14.30 17.30	Francesca Palese*, Silvia Pontis, Natalia Realini, Massimiliano Lanfranco, Andrea Armirotti and Daniele Piomelli	**TARGETING NAAA COUNTERS DOPAMINE NEURON LOSS AND SYMPTOM PROGRESSION IN A MOUSE MODEL OF PARKINSON'S DISEASE**	7
10.45 – 11:30 14.45 – 15:30 17.45 – 18:30	**ICRS PRESIDENTIAL PLENARY LECTURER** A CANNABINOID LINK BETWEEN BRAIN BIOENERGETICS AND BEHAVIOR GIOVANNI MARSICANO, DVM, PH.D. Deputy Director, NeuroCentre Magendie U1215 INSERM and Université Bordeaux, France		

11.30 – 12.00 15.30 – 16.00 18.30 – 19.00	**EPM SPONSORED SESSION** **CANNABINOID ESTERS** EFFECT OF CANNABIDIOLIC ACID DERIVATIVE IN TREATING DIET-INDUCED OBESITY Elad Ben-Cnaan*, Anna Permyakova, Shahar Azar, Shira Hirsch, Liad Hinden and Joseph Tam METHYL ESTER CANNABINOIDS ARE POTENT ANTI-INFLAMMATORY COMPOUNDS Dan Peer*		
ORAL SESSION 2. CB2 PHARMACOLOGY AND SIGNALING **MODERATORS:** RAFAEL MALDONADO, M.D., PH.D. AND ELENA MARTÍN-GARCÍA, PH.D.			
12.00 16.00 19.00	Kishore Aravind Ravichandran, Nico Reusch, Bolanle Fatimat Olabiyi, Joanna Komorowska-Müller, Jan N. Hansen, Thomas Ulas, Marc Beyer, Andreas Zimmer and Anne-Caroline Schmöle*	**INTERACTION BETWEEN CB2 AND TOLL-LIKE RECEPTORS SHAPES MICROGLIAL ACTIVITY**	8
12.15 16.15 19.15	Samuel Ruiz de Martín Esteban*, M. Teresa Grande, Diego Herráez, Rocío Palenzuela, Irene Benito-Cuesta, Rosa M. Tolón, Ricardo Mostany, Cecilia J. Hillard and Julián Romero	**IN VIVO STUDIES OF THE ROLE OF CANNABINOID CB2 RECEPTORS IN MICROGLIAL ACTIVITY IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE BY MULTIPHOTON MICROSCOPY**	9
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12.45 16.45 19.45	Hongfeng Deng*, Clifton Leigh, Tama Evron, Ping Zhang, Zhuang Jin and Barbara White	**DEVELOPMENT OF NOVEL CB2 AGONISTS FOR TREATING INFLAMMATORY AND FIBROTIC DISEASES**	11

13.00 17.00 20.00	Idan Carmon*, Sai Meka, Jinan Elayyan, George Batshon, Eli Reich, Andras Bilkei-Gorzo, Andreas Zimmer, Raphael Mechoulam and Mona Dvir-Ginzberg	**HU308-MEDIATED CANNABINOID SIGNALING MITIGATES AGE AND TRAUMA-RELATED OSTEOARTHRITIS IN MICE**	12
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13.30 – 14.30 17.30 – 18.30 20.30 - 21.30	**ICRS LIFETIME ACHIEVEMENT AWARD** PRO-ADDICTION AND ANTI-ADDICTION EFFECTS OF CANNABINOIDS: QUELLE SURPRISE - DIFFERENT CANNABINOIDS HAVE DIFFERENT EFFECTS ELIOT GARDNER PH.D. Senior Investigator Molecular Targets and Medications Discovery Branch, Neuropsychopharmacology Section, NIDA Bethesda, MD USA		
ORAL SESSION 3. CANNABINOIDS AND REWARD/ADDICTION **MODERATORS:** ARON LICHTMAN, PH.D. AND KENNEDY GOLDSBOROUGH			
14.30 18.30 21.30	Elena Martin-Garcia*, Maria Cajiao, Eric Senabre and Rafael Maldonado	**NEUROBIOLOGICAL MECHANISMS UNDERLYING VULNERABILITY AND RESILIENCE TO CANNABIS ADDICTION**	13
14.45 18.45 21.45	Samantha M. Ayoub*, Reem Smoum, Mathew Farag, Harkirat Atwal, Cheryl L. Limbeer, Erin M. Rock, Stephen A. Collins, Fabiana Piscitelli, Fabio Arturo Iannotti, Vincenzo Di Marzo, Francesco Leri, Aron H. Lichtman, Raphael Mechoulam and Linda A. Parker	**THE EFFECT OF OLEOYL ALANINE ON OPIOID WITHDRAWAL BEHAVIOURS IN MALE SPRAGUE DAWLEY RATS**	14

15.00 19.00 22.00	Andrew J. Kesner*, Stephanie Ramos-Maciel, Yolanda Mateo, Alexa L. Gracias and David M. Lovinger	**MODELING SPONTANEOUS THC WITHDRAWAL SYMPTOMS IN MICE**	15
15.15 19.15 22.15	Alexis F. League*, Benjamin L. Gorman, Ian R. Jacobs, Justin L. Poklis, Micah J. Niphakis, Benjamin F. Cravatt, Aron H. Lichtman, Bogna M. Ignatowska-Jankowska and Sylvia Fitting	**INHIBITION OF MONOACYLGLYCEROL LIPASE ALTERS DENDRITIC BRANCHING COMPLEXITY AND REWARD-RELATED BEHAVIOR IN TAT TRANSGENIC MICE**	16
15.30 19.30 22.30	Catharine A. Mielnik*, Claudia Lutelmowski, Ali Salahpour, Heather Bradshaw and Ruth Ross	**MONOACYLGLYCEROL LIPASE (MAGL), BUT NOT FATTY AMIDE ACID HYDROLASE, INHIBITION EXACERBATES HYPERDOPAMINERGIC PHENOTYPES IN DOPAMINE TRANSPORTER-KNOCKOUT (DATKO) MICE**	17
15.45 – 17.00 19.45 – 21.00 22.45 – 24.00	NIDA CAREER WORKSHOP PRESENTATION BUILD UNIQUE EXPERTISE TO SUCCEED CAREER TRANSITION AND INDEPENDENCE Yu (Woody) Lin, PhD Roger Sorensen, PhD, MPH Sylvia Fitting, PhD		
15.45 – 17.00 19.45 – 21.00 22.45 – 24.00	CHAT ROOMS OPEN FOR DISCUSSION		

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Day 2
Tuesday, June 22nd

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07.00 – 09.00 **11.00 – 13.00** 14.00 – 16.00	**Poster Session 2**	P2

Oral Session 4. CB1 Pharmacology and Signaling

Moderators: George Kunos, M.D., Ph.D. and Malliga Iyer, Ph.D.

09.00 **13.00** 16.00	Ariel Rothner*, Liad Hinden, Majdoleen Ahmad, Alina Nemirovski, Ifat Abramovich, Bella Agranovich, Aviram Kogot-Levin, Eyal Gottlieb, Gil Leibowitz and Joseph Tam	**THE DELETERIOUS ROLE OF CANNABINOID-1 RECEPTOR IN THE PROGRESSION OF ACUTE TO CHRONIC KIDNEY DISEASE**	18
09.15 **13.15** 16.15	Mark B. Wiley* and Nicholas V. DiPatrizio	**CB1 RECEPTORS IN THE INTESTINAL EPITHELIUM ARE PROTECTIVE AGAINST GUT-BARRIER DYSFUNCTION IN DIET INDUCED OBESITY**	19
09.30 **13.30** 16.30	Anna Vázquez-Oliver*, Silvia Pérez-García, Nieves Pizarro, Lydia Garcia-Serrano, Gabriela Bordeanu, Pier-Vincenzo Piazza, Rafael De la Torre, Rafael Maldonado and Andrés Ozaita	**CANNABINOID TYPE-1 RECEPTOR INHIBITION AS A LONG-TERM PHARMACOLOGICAL APPROACH FOR MEMORY ENHANCEMENT IN A MOUSE MODEL OF DOWN SYNDROME**	20

09.45 13.45 16.45	Richard C. Kevin*, Elizabeth A. Cairns, Samuel D. Banister, Michelle Glass, Mark Connor and Iain S. McGregor	**PRO-CONVULSANT SYNTHETIC CANNABINOID RECEPTOR AGONISTS: PHARMACOLOGICAL AND TOXICOLOGICAL ASSESSMENT, AND IN VIVO EVALUATION OF FIRST-LINE EMERGENCY TREATMENTS**	21
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11.00 – 11.30 **15.00 – 15.30** 18.00 – 18.30	**WILLIAM A DEVANE YOUNG INVESTIGATOR AWARD SPEAKER** DRILLING DOWN ON "CANNABIS" IN THE HUMAN LABORATORY RYAN VANDREY, PH.D. Professor of Psychiatry and Behavioral Sciences Johns Hopkins Bayview Medical Center, Behavioral Biology Research Center, Baltimore, Maryland, USA		
ORAL SESSION 5. PAIN, LEARNING & MEMORY, NEUROPATHOLOGY **MODERATORS:** DAVID FINN., PH.D. AND STEPHANIE BOURKE			
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ORAL SESSION 6. CANNABIS HUMAN STUDIES - PART 1 **MODERATOR:** ZIVA COOPER., PH.D.			
14.15 **18.15** 21.15	Kevin F. Boehnke*, Joel J. Gagnier, Lynne Matallana and David A. Williams	**SUBSTITUTING CANNABIDIOL FOR OPIOIDS AND PAIN MEDICATIONS AMONG INDIVIDUALS WITH FIBROMYALGIA: A LARGE ONLINE SURVEY**	31
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Day 3
Wednesday, June 23rd

07.00 EST **11.00 UTC** 14.00 Local	Welcome to ICRS2021: Opening Remarks	
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Oral Session 7. Cannabidiol and Phytocannabinoids

Moderators: Alessia Ligresti, Ph.D. and Ali Mokhtar Mahmoud, Ph.D.

09.00 **13.00** 16.00	Lyndsey L. Anderson, Marika Heblinski, Nathan L. Absalom, Nicole A. Hawkins, Somayeh Mirlohi, Michael Bowen, Michael Udoh, Melissa J. Benson, Fan Zhang, Dilara Bahceci, Peter T. Doohan, Chris Bladen, Mark Connor, Mary Chebib, Iain S. McGregor, Jennifer A. Kearney and Jonathon C. Arnold*	**PHYTOCANNABINOID ACIDS DISPLAY ANTICONVULSANT ACTIVITY IN A MOUSE MODEL OF DRAVET SYNDROME**	37
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09.45 13.45 16.45	Sara Jane Ward*, William A. Kinney and Douglas E. Brenneman	**BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF CANNABIDIOL (CBD) AND THE CBD ANALOGUE KLS-13019 IN MOUSE MODELS OF PAIN AND REINFORCEMENT**	40
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10.45 14.45 17.45	Lucy J. Sloan*, Wei Liang, Max Duff, Sarah Shrader, Yongqing Liu and Zhao-Hui Song	**CANNABIDIOL INHIBITS ANGIOGENIC PROCESSES IN RETINAL MICROVASCULAR ENDOTHELIAL CELLS**	44
11.00 15.00 18.00	**BREAK**		
11.15 – 12.15 15.15 – 16.15 18.15 – 19.15	**ICRS PRESIDENTIAL PLENARY LECTURER** ENDOCANNABINOID SYSTEM: AN UP-AND-COMING SOURCE OF TARGETS FOR OSTEOARTHRITIS TREATMENT KATARZYNA STAROWICZ, PH.D. Institute of Pharmacology of the Polish Academy of Sciences Kraków, Poland		

Oral Session 8. Cannabinoid Drug Development & Delivery

Moderators: Ruth Ross, Ph.D. and Claudia Lutelmowski

12.15 16.15 19.15	Benoit Hornsperger, Jörg Benz, Michael Honer, Carsten Kroll, Bernd Kuhn, Fionn O'Hara, Andrea Martella, Catarina Raposo, Hans Richter, Manfred Schneider, Juliane Stephanus, Mario van der Stelt, Matthias Wittwer, Ludovic Collin and Uwe Grether*	**DISCOVERY OF REVERSIBLE HIGHLY POTENT AND SELECTIVE BICYCLOPIPERAZINE DERIVED MAGL INHIBITORS**	45
12.30 16.30 19.30	Shira Hirsch*, Meital Naim, Gershon Golomb and Joseph Tam	**ENCAPSULATION OF A CENTRALLY ACTING CANNABINOID-1 RECEPTOR (CB1R) BLOCKER IN PLGA-BASED NANOPARTICLES FOR THE TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE**	46
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13.30 17.30 20.30	Evangelos Dadiotis*, Vangelis Mitsis, Eleni Melliou and Prokopios Magiatis	**DIRECT QUANTITATION OF PHYTOCANNABINOIDS BY ONE DIMENSIONAL ^{1}H qNMR AND TWO DIMENSIONAL ^{1}H-^{1}H COSY qNMR IN COMPLEX NATURAL MIXTURES**	50
13.45 17.45 20.45	Antonius P. A. Janssen*, Johanna G. Bovenberg, Gerard J.P. van Westen and Mario van der Stelt	**COMPUTATIONAL DE NOVO DRUG DESIGN: AN APPLICATION OF A RECURRENT NEURAL NETWORK MODEL TRAINED THROUGH REINFORCEMENT LEARNING TO THE CASE OF CB2-RECEPTOR AGONISTS**	51
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ORAL SESSION 9. CANNABINOID INTERACTIONS WITH DRUGS OF ABUSE **MODERATORS:** MATTHEW HILL, PH.D. AND CATHERINE HUME, PH.D.			
15.15 19.15 22.15	Grzegorz Godlewski*, Luis Santos Molina, Resat Cinar, Jie Liu, Malliga Iyer and George Kunos	**PERIPHERAL HYBRID CB1 RECEPTOR/INOS ANTAGONIST MRI 1867 REDUCES ALCOHOL DRINKING BEHAVIOR AND SOME INFLAMMATORY MARKERS IN THE GI TRACT**	52

15.30 19.30 22.30	Yannick Fotio*, Francesca Palese, Pablo Guaman Tipan, Faizy Ahmed and Daniele Piomelli	**INHIBITION OF FATTY ACID AMIDE HYDROLASE IN THE CNS PREVENTS AND REVERSES MORPHINE TOLERANCE IN MALE AND FEMALE MICE**	53
15.45 19.45 22.45	Ewa Galaj*, Guo-Hua Bi, Allamar Moore, Eliot Gardner, Herbert Seltzman and Zheng-Xiong Xi	**THERAPEUTIC POTENTIAL OF PIMSR1, A NOVEL CB1 NEUTRAL ANTAGONIST, FOR COCAINE USE DISORDER: EVIDENCE FROM PRECLINICAL RESEARCH**	54
16.00 20.00 23.00	Grzegorz Godlewski*, Luis Santos Molina, Resat Cinar, Jie Liu, Malliga Iyer and George Kunos	**CB1 RECEPTORS ON SENSORY AFFERENTS OF THE GASTROINTESTINAL TRACT MAY CONTRIBUTE TO THE CONTROL OF VOLUNTARY ALCOHOL DRINKING IN MICE**	55
16.15 20.15 23.15	Julien C. Dodu*, Rebecca K. Moncayo, Aiden Jones, Joel E. Schlosburg, M. Imad Damaj, Dai Lu, Debra Kendall and Aron H. Lichtman	**THE CB1 POSITIVE ALLOSTERIC MODULATOR, ZCZ011, ATTENUATES NALOXONE-PRECIPITATED WITHDRAWAL SIGNS IN OXYCODONE-DEPENDENT MICE**	56
16.30 – 17.00 20.30 – 21.00 23.30 – 24.00	CHAT ROOMS OPEN FOR DISCUSSION		

* Presenting author

Day 4
Thursday, June 24th

07.00 EST **11.00 UTC** 14.00 Local	Welcome to ICRS2021: Opening Remarks	
07.00 – 09.00 **11.00 – 13.00** 14.00 – 16.00	**Poster Session 4**	P4

Oral Session 10. Cannabis Human Studies - Part 2

Moderators: David Meiri, Ph.D. and Iris Wyrobnik

09.00 **13.00** 16.00	Colleen Trevino, Aniko Szabo, Terri A. deRoon-Cassini and Cecilia J. Hillard*	**CIRCULATING CONCENTRATIONS OF THE ENDOCANNABINOID, 2-ARACHIDONOYLGLYCEROL, AT TIME OF TRAUMATIC INJURY ARE ASSOCIATED WITH CHRONIC PAIN**	57
09.15 **13.15** 16.15	Erica N. Peters*, Irina Mosesova, Laura MacNair, Ryan Vandrey, M. Hunter Land, Mark A. Ware, Cynthia Turcotte and Marcel O. Bonn-Miller	**MULTIPLE-DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF SPECTRUM YELLOW OIL IN HEALTHY PARTICIPANTS**	58
09.30 **13.30** 16.30	Jennifer Spohrs*, Laura Bindila, Michael Prost, Paul Plener, Georg Groen, Birgit Abler and Martin Ulrich	**FAAH GENETIC VARIATION MODULATES NEURAL CORRELATES OF EXTINCTION RECALL – AN FMRI STUDY**	59

09.45 13.45 16.45	Elyad Davidson*, Ester Goldberg, Salhab Kalil and Josh Schroeder	**EFFECT OF A CANNABIS EXTRACT ON ACUTE RADICULAR PAIN AND ON ANALGESICS REQUIREMENT: A DOUBLE-BLINDED, RANDOMIZED, 24 HOURS FOLLOW-UP STUDY**	60
10.00 14.00 17.00	Vered Hermush*, Liora Ore, Noa Stern, Nisim Mizrahi, Malki Fried, Marina Krivoshey, Ella Staghon, Violeta E. Lederman and Lihi Bar-Lev Schleider	**CANNABIDIOL OIL FOR BEHAVIORAL DISTURBANCES IN DEMENTIA, A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL TRIAL**	61
10.15 14.15 17.15	Xintian Lyu*, Silvia Illamola, Stephen Dahmer, Paloma Lehfeldt, Ilo Leppik and Angela Birnbaum	**DOSAGE AND FORMULATIONS RECEIVED BY PATIENTS WITHIN A US STATE CANNABIS PROGRAM**	62
10.30 14.30 17.30	**BREAK**		
10.45 – 11.45 14.45 – 15.45 17.45 – 18.45	ICRS PRESIDENTIAL PLENARY LECTURER CANNABIS AND NEURODEVELOPMENTAL VULNERABILITY: WHAT DO WE REALLY KNOW YASMIN HURD, PH.D. Departments of Psychiatry, Neuroscience and Pharmacology and Systems Therapeutics, Ward-Coleman Chair of Translational Neuroscience Director, Addiction Institute at Mount Sinai, NY, USA		
ORAL SESSION 11. NOVEL CB1 MODULATORS **MODERATORS:** STEVEN KINSEY, PH.D. AND OLIVIA VANEGAS			
11.45 15.45 18.45	Resat Cinar* Ziyi Liu, Malliga R. Iyer, Grzegorz Godlewski, Tony Jourdan, Jie Liu, Nathan J. Coffey, Charles N. Zawatsky, Henry L. Puhl, Jeih-San Liow, Robert B. Innis, Sergio A. Hassan, Yong-Sok Lee and George Kunos	**FUNCTIONAL SELECTIVITY OF A BIASED CANNABINOID-1 RECEPTOR (CB1R) ANTAGONIST FOR ANTI-DIABETIC EFFICACY WITHOUT CNS SIDE-EFFECTS**	63

12.00 16.00 19.00	Sumanta Garai*, Luciana M. Leo, Anna-Maria Szczesniak, Dow P. Hurst, Peter C. Schaffer, Ayat Zagzoog, Tallan Black, Roger G. Pertwee, Mary E. Abood, Melanie E. M. Kelly, Patricia H. Reggio, Robert B. Laprairie and Ganesh A. Thakur	**DISCOVERY OF G-PROTEIN BIASED CB1R ALLOSTERIC MODULATOR FOR THE TREATMENT OF GLAUCOMA WITH LONG-DURATION OF ACTION**	64
12.15 16.15 19.15	Asaad Gammal*, Anna Permyakova, Naama Adi-Hen, Julie Asfour, Amit Badihi, Taher Nassar, Simon Benita and Joseph Tam	**DUAL TARGETING OF CANNABINOID-1 RECEPTOR AND MODULATION OF PPARα ATTENUATES OBESITY-INDUCED FATTY LIVER DISEASE**	65
12.30 16.30 19.30	Malliga R. Iyer*, Resat Cinar, Charles Zawatsky, Casey Wood, Nathan J. Coffey, Kyu Ah Kim, Jasmina Abdalla, Alexis Katz and George Kunos	**STUDIES ON PERIPHERALLY RESTRICTED 3,4-DIARYLPYRAZOLINES AS POTENT ANTAGONISTS OF CANNABINOID-1 RECEPTOR (CB1R)**	66
12.45 16.45 19.45	Tama Evron, Suzie Ferreira, Hongfeng Deng, Andrew Kolodziej* and Barbara White	**THE DEVELOPMENT OF PERIPHERAL CB1 INVERSE AGONISTS WITH METABOLIC, ANTI-INFLAMMATORY, AND ANTI-FIBROTIC ACTIVITIES**	67
13.00 17.00 20.00	BREAK		

13.15 – 14.15 **17.15 – 18.15** 20.15 – 21.15	ICRS Presidential Plenary Lecturer EPIGENETICS: FROM PLURIPOTENT STEM CELLS TO ANCIENT DNA Eran Meshorer, Ph.D. Arthur Gutterman Chair in Stem Cell Research, Edmond and Lily Safra Center for Brain Sciences, Hebrew University, Jerusalem, Israel

Oral Session 12. THC

Moderators: Pascal Degrace, Ph.D. and Tony Jourdan, Ph.D.

14.15 **18.15** 21.15	Clare Diester*, James Gillespie, Hallie Lappin, Aron Lichtman, Dana Selley, Laura Sim-Selley and Steve Negus	**EFFECTS OF ENDOCANNABINOID CATABOLIC ENZYME INHIBITORS AND Δ⁹-TETRAHYDROCANNABINOL ON ACUTE PAIN-DEPRESSED NESTING IN MICE**	68
14.30 **18.30** 21.30	Jacqueline-Marie N. Ferland*, Randall J. Ellis, Gregory Rompala, Joseph A. Landry, James E. Callens, Annie Ly, Micah Frier, Teddy Uzamere, Graeme Betts, Mason M. Silveira, Catharine A. Winstanley and Yasmin L. Hurd	**ADOLESCENT THC HAS DOSE-DEPENDENT EFFECTS ON REWARD, STRESS REACTIVITY, AND DECISION MAKING IN ADULTHOOD VIA PERTURBATIONS IN ASTROCYTES**	69
14.45 **18.45** 21.45	Kwang-Mook Jung*, Hye-lim Lee, Erica Squire, Yannick Fotio, Francesca Palese, Alexa Torrens, Shiqi Su, Christina Renee Wong, Lin Lin, Jade Ramirez and Daniele Piomelli	**PERSISTENT EFFECTS OF FREQUENT ADOLESCENT THC EXPOSURE ON SOCIAL DEFEAT STRESS**	70

15.00 19.00 22.00	Marieka V. DeVuono*, Olivia La Caprara, Megan T. Sullivan, Alexandra Bath, Gavin N. Petrie, Erin M. Rock, Cheryl M. Limebeer, Matthew N. Hill and Linda A. Parker	**THE ROLE OF THE ENDOCANNABINOID SYSTEM AND STRESS IN CANNABINOID HYPEREMESIS SYNDROME**	71
15.15 19.15 22.15	Lin Lin*, Kwang-Mook Jung, Steve Mahler, Faizy Ahmed, Erica Squire, Shiqi Su, Alexa Torrens, Yannick Fotio, Jade Ramirez, Hyelim lee, Francesca Palese and Daniele Piomelli	**ADOLESCENT THC EXPOSURE PERMANENTLY REPROGRAMS ADIPOSE ENERGY METABOLISM**	72
15.30 19.30 22.30	Bryant Avalos* and Nicholas V. DiPatrizio	**CHRONIC EXPOSURE TO WHOLE CANNABIS EXTRACTS AMELIORATES METABOLIC DYSFUNCTION ASSOCIATED WITH DIET-INDUCED OBESITY IN MICE**	73
15.45 19.45 22.45	Hyelim Lee*, Erica Squire, Yannick Fotio, Francesca Palese, Alexa Torrens, Shiqi Su, Cristina Wong, Lin Lin, Jade Ramirez, Kwang-Mook Jung and Daniele Piomelli	**ADOLESCENT THC EXPOSURE PERSISTENTLY SUPPRESSES MICROGLIA FUNCTION IN MICE**	74
16.00 – 16.40 20.00 – 20.40 23.00 – 23.40	ICRS Founder's Lecturer AN OVERVIEW OF CANNABINOID RESEARCH OVER ALMOST 60 YEARS Raphael Mechoulam, Ph.D. Professor Emeritus Hebrew University of Jerusalem, Israel		
16.40 – 20.40 – 23.40 –	CHAT ROOMS OPEN FOR DISCUSSION		

* Presenting author

ICRS2021 POSTER SESSION TOPICS

P1 // Monday, June 21, 2021
07.00 – 09.00 EST // **11.00 – 13.00 UTC** // 14.00 – 16.00 LOCAL

A. Endocannabinoid Regulation and Signaling
B. CB2 Pharmacology and Signaling
C. Cannabinoids and Reward/Addiction
LB. Late-breaking research

P2 // Tuesday, June 22, 2021
07.00 – 09.00 EST // **11.00 – 13.00 UTC** // 14.00 – 16.00 LOCAL

D. CB1 Pharmacology and Signaling
E. Pain, Learning & Memory, Neuropathology
F. Cannabis Human Studies - Part 1

P3 // Wednesday, June 23, 2021
07.00 – 09.00 EST // **11.00 – 13.00 UTC** // 14.00 – 16.00 LOCAL

G. Cannabidiol and Phytocannabinoids
H. Cannabinoid Drug Development & Delivery
I. Cannabinoid Interactions with Drugs of Abuse

P4 // Thursday, June 24, 2021
07.00 – 09.00 EST // **11.00 – 13.00 UTC** // 14.00 – 16.00 LOCAL

J. Cannabis Human Studies - Part 2
K. Novel CB1 Modulators
L. THC

POSTER SESSION 1 – TOPICS A-C
DAY 1, MONDAY, JUNE 21ST

A. ENDOCANNABINOID REGULATION AND SIGNALING

ABSTRACT WITHDRAWN		P1 - 1
Uma Anand*, Barbara Pacchetti, Praveen Anand and Mikael H. Sodergren	**INVESTIGATING CANNABINOID ENTOURAGE EFFECTS IN CULTURED DRG NEURONS**	P1 - 2
Luara A. Batista*, Thiago S. Moreira and Ana C. Takakura	**INHIBITION OF ANANDAMIDE HYDROLYSIS AS A STRATEGY TO COUNTERACT RESPIRATORY ABNORMALITIES OBSERVED IN AN ANIMAL MODEL OF PARKINSON'S DISEASE**	P1 - 3
Hagar Bauminger*, Hiba Zaidan, Inna Gaisler-Salomon and Irit Akirav	**ANANDAMIDE HYDROLYSIS REVERSES THE PROLONGED BEHAVIORAL AND GENE EXPRESSION ALTERATIONS-INDUCED BY ADOLESCENCE NMDA RECEPTOR HYPOFUNCTION**	P1 - 4
Tallan Black*, Ayat Zagzoog, Andrew Roebuck, Quentin Greba, John G. Howland and Robert B. Laprairie	**CHARACTERIZATION AND TARGETING OF THE ENDOCANNABINOID SYSTEM IN TRAUMATIC BRAIN INJURY**	P1 - 5
Laura Boullon*, David P. Finn and Alvaro Llorente-Berzal	**SEX-DEPENDANT BRAIN REGIONAL ALTERATIONS IN THE ENDOGENOUS CANNABINOID SYSTEM IN A RAT MODEL OF PERIPHERAL NEUROPATHIC PAIN**	P1 - 6

Elizabeth A. Cairns*, Dilini Gunatilake, Adam Ametovski, Lewis J. Martin, Jonathan C. Arnold, Samuel D. Banister, Iain S. McGregor and Jessamy C. Tiffen	**THE EXPRESSION OF COMPONENTS OF THE ENDOCANNABINOID SYSTEM PREDICTS PATIENT SURVIVAL IN MELANOMA BUT CANNABINOIDS LACK SELECTIVITY AS POTENTIAL THERAPEUTICS**	P1 - 7
Ian de Bus*, Sandra van Krimpen, Han Zuilhof , Renger Witkamp, Bauke Albada and Michiel Balvers	**CHARACTERIZATION AND IMMUNE MODULATION OF COX-2-DERIVED DHEA METABOLITES IN LPS-STIMULATED MACROPHAGES**	P1 - 8
Ian de Bus*, Twan America, Norbert de Ruijter, Han Zuilhof, Renger Witkamp, Bauke Albada and Michiel Balvers	**CHEMICAL ENDOCANNABINOID-DERIVED PROBES SUGGEST A POTENTIAL REGULATING ROLE IN RAC1 SIGNALLING**	P1 - 9
Ryan. R Hall*, Damian Cohall and Andrew B. Tobin	**CROSSTALK BETWEEN MUSCARINIC RECEPTORS AND CANNABINOID RECEPTORS: IDENTIFYING POTENTIAL THERAPIES IN TEMPORAL LOBE EPILEPSY**	P1 - 10
Mary Hopkins*, Ana Bagüés, Yolanda López-Tofiño, Raquel Abalo and Alvaro Llorente-Berzal	**CHANGES IN THE ENDOCANNABINOID SYSTEM OF MALE RATS TREATED WITH A SINGLE SUB-NOXIOUS DOSE OF CISPLATIN**	P1 - 11
Clare T Johnson* and Heather B Bradshaw	**GPR55 ANTAGONIST ML193 AND AGONIST O-1602 HAVE CELL AND TIME-DEPENDENT EFFECTS ON LIPID REGULATION**	P1 - 12
Rupali Vyawahare, Ewa Wasilewski, Rex Siming Wang and Lakshmi P. Kotra*	**DIFFERENTIAL EFFECTS OF CANNABINOID RECEPTOR LIGANDS ON THE PRODUCTION OF CAMP IN MALE AND FEMALE CELLS OF NEURONAL ORIGIN**	P1 - 13

Gitit Kra*, Uzi Moallem, Radka Kočvarová, Alina Nemirovski, Joseph Tam, G. A. Contreras and Maya Zachut	**EFFECTS OF PERIPARTUM OMEGA-3 FATTY ACID SUPPLEMENTATION ON ENDOCANNABINOID TONE AND INFLAMMATION IN LIVER OF DAIRY COWS**	P1 - 14
Gitit Kra*, Uzi Moallem, Majdoleen Ahmad, Alina Nemirovski, Joseph Tam and Maya Zachut	**SEASONAL HEAT STRESS AFFECTS ENDOCANNABINOID GENE EXPRESSION IN ADIPOSE TISSUE OF POSTPARTUM DAIRY COWS**	P1 - 15
Claudia Lutelmowski*, Catharine Mielnik, Ali Salahpour, Amy J. Ramsey, Heather B. Bradshaw, Marija Milenkovic, Wendy Horsfall and Ruth Ross	**THE BEHAVIOURAL AND LIPIDOMIC EFFECTS OF INHIBITING ENDOCANNANINOID METABOLISM IN A HYPO-GLUTAMATERGIC MODEL OF PSYCHOSIS-LIKE PHENOTYPES**	P1 - 16
Sara Standoli, Cinzia Rapino, Camilla Di Meo and Mauro Maccarrone*	**EFFECT OF SPHINGOSINE-1-PHOSPHATE ON ENDOCANNABINOID SIGNALING**	P1 - 17
Alessandra Piccirilli, Roberto Coccurello, Sergio Oddi, Mariagrazia Perilli and Mauro Maccarrone*	**EFFECT OF ULTRAMICRONIZED PEA ON THE FAECAL MICROBIOME OF A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE**	P1 - 18
Alessandro Leuti, Marina Fava, Niccolò Pellegrino and Mauro Maccarrone*	**ENDOCANNABINOIDS AND PRO-RESOLVING MEDIATORS ACT SYNERGICALLY IN PRIMARY HUMAN MACROPHAGES**	P1 - 19
Chanté A. Muller*, Diane L. Lynch, Dow P. Hurst and Patricia H. Reggio	**UNIQUE ENTRY OF ANANDAMIDE AT TRPV1**	P1 - 20
Gavin Petrie*, Georgia Balsevich, Hiulan Yau, Tamas Fuzesi, David Rosenegger, Robert Aukema, Mario Van Der Stelt, Jaideep Bains and Matthew Hill	**TONIC ENDOCANNABINOID SIGNALLING GATES STRESS-LIKE STEREOTYPIC BEHAVIORS AND HPA AXIS ACTIVATION**	P1 - 21

Erin M. Rock*, Cheryl L. Limebeer, Reem Smoum, Raphael Mechoulam and Linda A. Parker	**EFFECT OF THE FATTY ACID AMIDES OLEOYL GLYCINE AND OLEOYL ALANINE ON LITHIUM CHLORIDE-INDUCED NAUSEA IN RATS AND VOMITING IN SHREWS**	P1 - 22
Syed Haneef Syed Askar*, Marina Santiago and Mark Connor	**COMPARISON OF THE PHARMACOLOGY OF THE HUMAN AND ZEBRAFISH CANNABINOID RECEPTORS**	P1 - 23
B. CB2 Pharmacology and Signaling		
Ana M. Martínez Relimpio*, M. Teresa Grande, Samuel Ruiz de Martín Esteban, M. Andrea Arnanz, Rosa M. Tolón, Cecilia J. Hillard and Julián Romero	**A COMPREHENSIVE ANALYSIS OF MICROGLIAL CANNABINOID CB2 RECEPTORS IN THE CONTEXT OF AMYLOID-INDUCED NEUROINFLAMMATION**	P1 - 24
Bolanle F. Olabiyi*, Eike Geissmar, Andreas Zimmer and Anne-Caroline Schmöle	**THE METABOLIC SIGNATURE OF CB2 IN LPS-INDUCED MICROGLIA STRESS RESPONSE**	P1 - 25
C. Cannabinoids and Reward/Addiction		
Maria T Rivera-Garcia* and Adrianne R Wilson-Poe	**EFFECTS OF CANNABIS VAPOR INHALATION IN THE ESTABLISHMENT AND EXPRESSION OF MORPHINE REWARD**	P1 - 26
Samah Shahen-Zoabi*, Reem Smoum, Tehila Beiser, Raphael Mechoulam and Rami Yaka	***N*-OLEYOLGLYCINE AND ITS DERIVATIVES ATTENUATE THE ACQUISITION AND EXPRESSION OF COCAINE INDUCED BEHAVIOURS**	P1 - 27
S. Olivia Vanegas*, Kristen R. Trexler and Steven G. Kinsey	**NOVELTY-INDUCED HYPOPHAGIA AS A MEASURE OF SPONTANEOUS CANNABINOID WITHDRAWAL**	P1 - 28

POSTER SESSION 2 – TOPICS D-F
DAY 2, TUESDAY, JUNE 22ST

D. CB1 PHARMACOLOGY AND SIGNALING

AyoOluwa O. Aderibigbe*, Pankaj Pandey and Robert J. Doerksen	**KNOWN MOLECULAR SWITCHES AND NEW GEOMETRIC DESCRIPTORS FOR THE CANNABINOID RECEPTOR 1 (CB1)**	P2 - 1
Courtney A. Bouchet*, Amy J Eshleman, Aaron Janowsky and Susan L. Ingram	**CANNABINOID 1 RECEPTOR FUNCTION IS REDUCED IN THE VENTROLATERAL PERIAQUEDUCTAL GRAY AFTER PERSISTENT INFLAMMATION**	P2 - 2
Sumner Burstein	**INFLAMMATION-RESOLVING ACTIONS OF AJULEMIC ACID**	P2 - 3
Nadia Di Franco*, Guillaume Drutel, Valerie Lacarriere, Valerie Lalanne, Nicole Etchamendy, Agnes Grel, Filippo Caraci, Giovanni Marsicano, Aline Marighetto, Piervincenzo Piazza, Monique Vallée and Jean-Michel Revest	**CB1 AS A POTENTIAL TARGET FOR COGNITIVE DEFICITS IN DOWN SYNDROME**	P2 - 4
Alessandra Gargano*, Eva Beins, Andreas Zimmer and Andras Bilkei-Gorzo	**LACK OF CANNABINOID RECEPTOR TYPE-1 LEADS TO ENHANCED AGE-RELATED NEURONAL LOSS IN THE LOCUS COERULEUS**	P2 - 5
Barbara L. F. Kaplan*, Brittany Szafran, Ashleigh Nicaise, Matthew K. Ross and James Nichols	**CB1 INACTIVATION INCREASES PRO-INFLAMMATORY CYTOKINE PRODUCTION IN RESPONSE TO LIPOPOLYSACCHARIDE**	P2 - 6

Hye Ji (Jay) Kim*, Teagan Holt, Udoka Ezeaka and Robert Laprairie	***IN VIVO* EVIDENCE FOR FUNCTIONAL INTERACTIONS BETWEEN CANNABINOID AND OREXIN RECEPTORS IN THE ADULT MOUSE BRAIN**	P2 - 7
Cristina Manenti*, Erica Zamberletti, Marina Gabaglio and Tiziana Rubino	**THE ENDOCANNABINOID SYSTEM IS A RELEVANT PLAYER IN ADOLESCENT MYELINATION**	P2 - 8
Madison N. Myers*, C. Javier Rendon, Maya Zachut, Joseph Tam and G. Andres Contreras	**CANNABINOIDS MODULATE ADIPOGENESIS AND LIPOGENESIS IN DAIRY COWS**	P2 - 9
Alexis Papariello*, David Taylor, Ken Soderstrom and Karen Litwa	**CB_1 ANTAGONISM INCREASES EXCITATORY SYNAPTOGENESIS IN A CORTICAL SPHEROID MODEL OF FETAL BRAIN DEVELOPMENT**	P2 - 10
Richard Slivicki*, Jiwon Yi, Victoria Brings, Phuong Huynh and Robert Gereau IV	**THE PERIPHERALLY RESTRICTED CANNABINOID CB-13 PRODUCES PERIPHERALLY-MEDIATED ANALGESIA BUT REPEATED DOSING ELICITS TOLERANCE AND SIGNS OF CENTRAL NERVOUS SYSTEM ACTIVITY**	P2 - 11
Michelle St. Pierre*, Sarah Daniels, Tatiana Sanchez and Zach Walsh	**VALIDATION OF THE NATURALISTIC CANNABIS ADMINISTRATION PROTOCOL (NCAP)**	P2 - 12
Carsten C. F. Walker* and Lorraine M. Sordillo	**ANANDAMIDE ENHANCES BARRIER INTEGRITY OF BOVINE VASCULAR ENDOTHELIAL CELLS DURING ENDOTOXIN CHALLENGE VIA CANNABINOID RECEPTOR-1 ACTIVATION**	P2 - 13
Courtney P. Wood*, Pedro A. Perez and Nicholas V. DiPatrizio	**ROLE OF CANNABINOID RECEPTOR SUBTYPE-1 IN THE INTESTINAL EPITHELIUM IN ANXIETY-LIKE BEHAVIORS**	P2 - 14

Alexander Young*, Shawn Adderley, Melanie Kelly and Eileen Denovan-Wright	**CANNABINOIDS MODULATE THE PRO- AND ANTI-INFLAMMATORY PROPERTIES OF POLARIZED M1/M2 MICROGLIA *IN VITRO***	P2 - 15
Charles Zawatsky*, Jasmina Abdalla, George Kunos and Resat Cinar	**CANNABINOIDS INDUCE LUNG INFLAMMATION VIA CB_1R ACTIVATION**	P2 - 16
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Konstantin Andrianov*, Inna Gaisler-Salomon and Irit Akirav	**DOUBLE EXPOSURE TO STRESS AS A RISK FACTOR FOR SEVERE PSYCHIATRIC DISORDERS: THE INVOLVEMENT OF ENDOCANNABINOID MARKERS**	P2 - 17
Geraint Berger*, Juan Zhou, Melanie Kelly and Lehmann Christian	**INTRAVESICAL BETA-CARYOPHYLLENE AMELIORATES LPS-INDUCED BLADDER INFLAMMATION AND PAIN IN A MURINE MODEL OF ACUTE CYSTITIS**	P2 - 18
Henry Blanton*, Melissa McHann, Kelsey Donckels, Isabel Castro, Kevin Pruitt and Josee Guindon	**ACEA (CB_1) AND CP55,940 (MIXED CB_1/CB_2) SEX-SPECIFIC ANTINOCICEPTIVE TOLERANCE IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY**	P2 - 19
Erik J. Fleischel* and Sara Jane Ward	**THE EFFECTS OF CANNABINOIDS IN THE PREVENTION OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN**	P2 - 20
Kennedy Goldsborough*, Rebecca Moncayo, Lauren Moncayo, Lesley D O' Brien, Kalpna Gupta, M Imad Damaj, Wally Smith, Joyce Lloyd and Aron Lichtman	**TARGETING MONOACYLGLYCEROL LIPASE TO REDUCE CHRONIC PAIN IN A HUMANIZED MOUSE MODEL OF SCD**	P2 - 21
Bryan W. Jenkins*, Shoshana Buckhalter, Melissa L. Perreault and Jibran Y. Khokhar	**IMPACT OF VAPOURIZED CANNABIS CONSTITUENTS ON OSCILLATORY ACTIVITY IN A NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA**	P2 - 22

Alex Mabou Tagne*, Yannick Fotio, Lin Lin, Erica Squire, Faizy Ahmed, Tarif Ibne Rashid, Elnaz Karimian Azari and Daniele Piomelli	**HEMP OIL AND PALMITOYLETHANOLAMIDE EXERT SYNERGISTIC ANTI-NOCICEPTIVE EFFECTS IN MOUSE MODELS OF ACUTE AND CHRONIC PAIN**	P2 - 23
Lesley D O'Brien*, Delaney Place, Kimberley Karin, Jane Roberts, David A Gewirtz, M Imad Damaj and Aron H Lichtman	**TARGETING THE ENDOCANNABINOID SYSTEM AS A STRATEGY TO ADDRESS AROMATASE INHIBITION-INDUCED SELECTIVE DISRUPTION OF LEARNING AND MEMORY IN MICE**	P2 - 24
Anna Portugalov*, Hiba Zaidan, Inna Gaisler-Salomon and Irit Akirav	**INCREASING ANANDAMIDE LEVELS RESTORE DEPRESSION-LIKE PHENOTYPE AND ALTERATIONS IN MICRO-RNAs IN RATS EXPOSED TO EARLY LIFE STRESS**	P2 - 25
Brenda Sbarski*, Eric Parise, Eric Nestler and Irit Akirav	**THE INVOLVEMENT OF B-CATENIN IN THE MEDIAL PFC IN PREVENTING STRESS-INDUCED EFFECTS IN A RAT MODEL FOR PTSD**	P2 - 26
Joshua A. Bilbrey, Yuma T. Ortiz, Lance R. McMahon and Jenny L. Wilkerson*	**EVALUATION OF THE TERPENES β-CARYOPHYLLENE, α-TERPINEOL, AND γ-TERPINENE IN THE MOUSE CHRONIC CONSTRICTION INJURY MODEL OF NEUROPATHIC PAIN: POSSIBLE CANNABINOID RECEPTOR INVOLVEMENT**	P2 - 27

F. Cannabis Human Studies - Part 1

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Ana Gabriela Hounie, Marco Agassiz Almeida Vasques and Wilson da Silva Lessa Júnior*	**CLINICAL EVOLUTION OF TREATMENT OF PROGRESSIVE SUPRANUCLEAR PALSY PATIENT WITH MEDICAL CANNABIS IN A PERIOD OF TWELVE MONTHS**	P2 - 30
M. Kathryn Dahlgren*, Kelly A. Sagar, Ashley M. Lambros, Rosemary T. Smith, Celine El-Abboud and Staci A. Gruber	**VETERANS DEMOSTRATE IMPROVED CLINICAL STATE AND HEALTH FOLLOWING SIX WEEKS OF TREATMENT WITH A HIGH-CANNABIDIOL PRODUCT**	P2 - 31
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UTILIZING A NOVEL ENDOCANNABINOID BIOSENSOR TO ELUCIDATE CIRCUIT-SPECIFIC ENDOCANNABINOID REGULATION OF VENTRAL HIPPOCAMPUS-BASOLATERAL AMYGDALA

*Veronika Kondev[1], Yulong Li[2], Ao Dong[2] and Sachin Patel[1,3]

[1]Vanderbilt Brain Institute; [2]Peking University School of Life Sciences; [3]Department of Psychiatry and Behavioral Sciences, Vanderbilt University School of Medicine

Introduction: Anxiety disorders and stress-related disorders are one of the most common mental illnesses in the world. However, current pharmacotherapies are only partially effective. Recent evidence demonstrates that the endocannabinoid system may present a novel functional target for future drug development. Here, we use a novel endocannabinoid biosensor to elucidate the circuit-specific and experience-dependent role endocannabinoids play in modulating stress responsivity, focusing on a ventral hippocampus (vHIPP) – basolateral amygdala (BLA) pathway known to regulate stress reactivity and fear learning.

Methods: Male, C57 mice underwent stereotaxic intracranial surgery. The novel endocannabinoid biosensor, AAV9-GRAB-eCB2.0, was delivered to the vHIPP and a fiber optic was implanted above the BLA to simultaneously excite and record changes in fluorescence. Mice were subject to a variety of behaviors, including restraint stress and exposure to the synthetic predator odor analog, 2-methyl-2-thiazoline (2MT). A separate cohort of mice underwent the same stereotaxic intracranial surgery but were also virally injected with AAV5-CaMKII-Gq-DREADD-mCherry (Addgene) or AAV5-Syn-ChrimsonR-tdT (Addgene), into the BLA before fiber optic implantation. A signal processor (RZ10X; Tucker-Davis Technologies) using light-emitting diodes at 465nm and 415nm modulated each laser's output. Data was analyzed with Matlab and presented as raw fluorescent traces of the 465nm wavelength and the 415nm wavelength (control channel). Data is also presented as a change in fluorescence $\Delta F/F$.

Results: The endocannabinoid biosensor was validated via i.p. injection of various compounds. The CB1 receptor (CB1R) agonist, CP55-940 (1mg/kg), increased fluorescence (i.e. increased GRAB-eCB2.0 activation), while the CB1R inverse agonist, Rimonabant (10mg/kg), decreased fluorescence in CP55,940-treated mice. Mice that were injected with Gq-DREADD in the BLA showed increased eCB sensor activation when injected with clozapine-N-oxide (CNO) (10mg/kg), which could be blocked by previously administering the DAGL inhibitor, DO34 (50mg/kg). Mice that were injected with Chrimson in the BLA showed frequency-dependent endocannabinoid synthesis/release and subsequent eCB sensor activation. The length of this GRAB-eCB2.0 activity was enhanced with the MAGL inhibitor, JZL184 (8mg/kg). Finally, restraint stress and 2MT exposure both induced robust GRAB-eCB2.0 activation at vHIPP-BLA synapses.

Conclusions: After rigorous validation of this novel endocannabinoid biosensor, it is concluded that different stressors result in endocannabinoid release in the BLA, and suggests CB1R activation at vHIPP terminals in the BLA.

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HUMAN EPITHELIAL STEM CELL SURVIVAL WITHIN THEIR NICHE REQUIRES "TONIC" CB1-SIGNALING – LESSONS FROM THE HAIR FOLLICLE

Tamás Bíró[1*], Koji Sugawara[2], Nóra Zákány[1], Stephan Tiede[3], Talveen Purba[4], Matthew Harries[4,5], Daisuke Tsuruta[2] and Ralf Paus[1,4,6]

[1]Monasterium Laboratory, Münster, Germany, [2]Department of Dermatology, Osaka City University, Japan, [3]Department of Biochemistry, University of Hamburg, Germany, [4]Centre for Dermatology Research, University of Manchester, UK, [5]The Dermatology Centre, Salford Royal NHS Foundation Trust, Salford, UK, [6]Dr Phillip Frost Department of Dermatology & Cutaneous Surgery, University of Miami, FL, USA

Introduction: The endocannabinoid system (ECS) regulates multiple aspects of human skin epithelial physiology, including inhibition/stimulation of epidermal and hair follicle (HF) keratinocyte proliferation/apoptosis, respectively. In addition, we have shown that activation of cannabinoid receptor-1 (CB1) coupled signaling, either by the endocannabinoid anandamide or by the phytocannabinoid tetrahydrocannabinol, markedly inhibited *ex vivo* human hair growth. Yet, how the ECS impacts on human adult epithelial stem cell (eSC) functions remains unknown. Therefore, by using the best pre-clinical human model systems offering a physiologically and clinically relevant opportunity for studying eSCs directly within the native human stem cell niche (i.e. the HF bulge), we explored the role of CB1 signaling in human eSC biology.

Methods: In human HF and skin organ cultures, as well as selected human eSC cell cultures, CB1 activity/expression were modulated by selective pharmacological agents or by targeted siRNA technology, and then various functional parameters were assessed by complementary immunolabeling methods. In addition, skin samples of CB1 knockout mice and of patients with lichen planopilaris (LPP) or frontal fibrosing alopecia (FFA), prototypic eSC pathologies of the HF bulge, were also assessed.

Results: Here, we show in organ-cultured human HFs that, unexpectedly, selective activation of CB1-mediated signaling by the selective CB1 agonist ACEA, via the MAPK (MEK/Erk 1/2) and Akt pathways significantly increases the number and proliferation of cytokeratin CK15+ or CK19+ human HF bulge eSCs *in situ,* and enhances CK15 promoter activity *in situ*. In striking contrast, CB1-stimulation promotes apoptosis in the differentiated progeny of these eSC (CK6+ HF keratinocytes). Instead, intrafollicular *CB1* gene-knockdown or CB1 antagonist treatment by AM251 or AM6545 significantly reduces human HF eSCs numbers and stimulates their apoptosis, while CB1 knockout mice exhibit a reduced bulge eSCs pool *in vivo.* Microarray analysis of laser capture-microdissected HF bulge cells from five patients with LPP or FFA showed a >2-fold significantly reduced expression of CB1 in the bulge epithelium of lesional LPP/FFA HFs compared to that of non-lesional HFs from the same patient. Consistent with these findings, CB1 protein expression *in situ* was also significantly reduced in lesional LPP HF bulge region, compared to those of healthy individuals This renders it plausible that insufficient CB1-mediated signaling may contribute to the depletion of bulge eSCs in scarring alopecia.

Conclusions: Taken together, these experiments identify novel, clinically important, and diametrically opposed effects of CB1 signaling in closely related epithelial cell populations within the same healthy human mini-organ, the scalp HF. Our data show that "tonic" CB1-mediated signaling is essential for the maintenance of adult human skin eSCs *in situ*, while growth of their more differentiated, but rapidly proliferating progeny (matrix keratinocytes) is strongly inhibited by CB1 activation. This novel concept is translationally important for the future therapeutic targeting of the ECS of human skin and introduces CB1 as new key signaling pathway in the regulation of adult human eSC physiology.

CHARACTERIZATION OF MOUSE BRAIN ENDOCANNABINOID DYNAMICS IN CIRCADIAN RYHTHMICITY AND SLEEP ONSET

Dorit Farfara[1*], Ben Korin[1], Hytham Hajjo[1], Hilla Azulay-Debby[1], Liron Sulimani[2,3], Gil M. Lewitus[4], Asya Rolls[1] and David Meiri[4]

1. Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel.
2. The Kleifeld Laboratory, Department of Biology, Technion-Israel Institute of Technology, Haifa, 3200003, Israel;
3. Cannasoul Analytics, Caesarea, Israel
4. The Laboratory of Cancer Biology and Cannabinoid Research, Department of Biology, Technion-Israel Institute of Technology, Haifa, 3200003, Israel.

Introduction: The endocannabinoid system (ECs) is involved in homeostatic physiological processes such as sensory perception, thermogenesis, energy balance, including mood and cognition, appetite, and sleep. Circadian rhythmicity is a key synchronizing mechanism that orchestrates these biological processes. Furthermore, initiation of sleep was shown to be involved in ECs modulation. The primary mediators of the ECs are the endocannabinoids that are metabolites derived from the long-chain fatty acids and are classified according to the lipid class they belong to. More than 20 different lipid classes have been suggested to interact with the ECs, making this group of extremely diverse metabolites. However, the dynamic of these lipids during the day and specifically during sleep initiation are elusive.

Methods: We characterized endocannabinoids and other cannabimimetic lipids in seven different areas from the mouse brain (brainstem, cerebellum, cortex, hippocampus, striatum thalamus, and hypothalamus), every six hours in a circadian rhythm (zeitgeber; ZT0, ZT6, ZT12, ZT18), and in initiation of sleep (ZT0, ZT1, ZT-sleep deprived; SD). We used a novel targeted cannabinoidomics (liquid chromatography high-resolution tandem mass spectrometry; LC/HRMS/MS) to create large data sets of ECs and cannabimimetic lipids level in the mouse brain. In addition, we analyzed changes in the receptors and metabolic enzymes of the ECs using qPCR.

Results: Out of seven screened endocannabinoid metabolites families, 2-MAGs, N-EAs, and FA families significantly changed during circadian rhythm in all seven analyzed brain regions. Levels of N-EAs at ZT12-18 were significantly elevated compared to ZT0-6, as opposed to 2-MAGs, which decreased at the same time points. FA, which showed the highest levels in all examined brain regions, changed significantly in circadian rhythms (ZT0-6 vs. ZT12-18), specifically in the striatum. During sleep initiation, the NAPE-PLD synthesis enzyme was increased in the cortex and hippocampus, resulting in elevated levels of N-EAs. Degradation enzymes, FAAH, and MAGL were increased in the cerebellum, resulting in decreased 2AG, 2DHG, 2EPG, and increased FAs. 2-SG, PA, and SA were increased during circadian rhythms in the cerebellum, striatum, thalamus, and hypothalamus.

Conclusions: Our results provide useful and important data on the EC system's brain pathways during circadian rhythms and sleep onset. Utilizing these data of endocannabinoid molecules in the brain may promote novel therapeutic strategies for sleep-related disorders.

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CANNABINOID EXPOSURE DURING LACTATION SIGNIFICANTLY CHANGES THE LIPID CONTENT OF BREAST MILK INCLUDING THE ENDOCANNABINOIDS ANANDAMIDE AND 2-AG

Heather Bradshaw*, Clare Johnson, Gabriel H.D. de Abreu, Ken Mackie and Hui-Chen Lu

Indiana University, Bloomington, IN USA

Introduction: With the growing legality and availability of Cannabis products more women are reporting using these products during pregnancy and lactation. Recently, we showed that mouse pups exposed to CBs during lacation from dams demonstrated significant differences in a variety of behavioral assays and that there were differential effects depending on the treatment regimen (e.g., THC or CBD alone or in combination). We wanted to test the hypothesis that significant level CBs are being delivered via breast milk and if there are any other measurable changes to the endogenous lipid content of the milk depending on treatment group.

Methods: Here, using a mouse model, we injected lactating dams daily with 3mg/kg of THC, CBD, THC:CBD (T+C), or vehicle from postnatal day zero (P0) through P10. On P10 pups were sacrifices 2 hours after the last injection and milk was collected from the stomachs of the pups. Lipidomic analysis was performed on the milk and >80 lipids were analyzed using methanolic extractions and HPLC/MS/MS analysis.

Results: Levels of THC and CBD were measured in all pups in dam CB-injected treatment groups. Levels of THC were significantly *lower* than levels of CBD from groups that were injected individually or together. However, levels of THC from the T+C group were significantly higher than those from THC alone. Likewise, levels of CBD were significantly higher in the T+C groups than CBD alone. Therefore, pups appeared to be exposed to significantly different levels of CBs depending on the treatment group. Levels of endogenous lipids were also significantly different among treatment groups. Levels of 2-AG and Anandamide were unchanged in the CBD group; however, both were significantly decreased in both the THC and T+C groups. Interestingly, levels of the linoleic acid derivatives, 2-linoleoyl glycerol (2-LG) were significantly lower in all treatment groups; however, LEA levels were significantly higher in the THC and T+C groups. Most all other NAEs were significantly decreased with all CB treatments; however, there is a mosaic of differences in many of the additional lipoamine NAE congeners, though the largest overall effects were measured in in the THC or T+C groups.

Conclusions: These data validate the hypothesis that CBs are present in breast milk and are ingested via lactation. We further this finding to demonstrate that significantly different levels of THC and CBD are available in milk depending on the CB exposure of the dam and that levels of CBD in milk are highest in each type of treatment. It is unknown what the specific roles are for milk-derived endocannabinoids and related lipids regarding pup development; however, here we demonstrate that the presence of CBs in the dam alter milk production of these lipids and may have significant effects on overall pup development and long-term health.

A FLUORESCENT SENSOR FOR SPATIOTEMPORALLY RESOLVED ENDOCANNABINOID DYNAMICS *IN VITRO* AND *IN VIVO*

Ao Dong[1*], Kaikai He[1], Barna Dudok[2], Jordan S Farrell[2], Wuqiang Guan[3], Daniel J Liput[4], Henry L Puhl[4], Ruyi Cai[1], Jiali Duan[1], Eddy Albarran[2], Jun Ding[2], David M Lovinger[4], Bo Li[3], Ivan Soltesz[2] and Yulong Li[1]

[1]Peking University School of Life Sciences, Beijing, China; [2]Stanford University School of Medicine, Palo Alto, CA, USA; [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; [4]National Institute on Alcohol Abuse and Alcoholism, Bethesda, USA;

Introduction: Endocannabinoids (eCBs) are retrograde lipid neuromodulators involved in many physiological important processes. However, the dynamics of eCBs in the brain remains largely unknown, mainly due to lack of tools for detection of eCB with good spatiotemporal resolution as well as with chemical specificity. Here, we developed a novel genetically-encoded eCB sensor ($GRAB_{eCB2.0}$, short for eCB2.0) based on the human CB1 receptor and a circular permutated GFP (cpGFP), and studied eCB dynamics both in vitro and in vivo.

Methods: eCB2.0 was engineered by inserting the circular permutated GFP into the 3rd intracellular loop of human CB1 receptor. eCB2.0 was characterized and applied in human cell lines, primary cultured neurons, acute brain slices and mice in vivo.

Results: eCB2.0 exhibited good cell membrane trafficking, second-resolution kinetics, high specificity for eCBs (both 2-AG and AEA), and a robust fluorescence response at physiological eCB concentrations. Using the eCB2.0 sensor, we monitored evoked changes in eCB dynamics in both cultured neurons and acute brain slices. Moreover, by expressing eCB2.0 in the mouse brain, we readily observed foot shock-elicited and running-triggered eCB transients in the basolateral amygdala and hippocampus, respectively. Lastly, we used eCB2.0 in a mouse seizure model and observed a spreading wave of eCB release that followed a Ca^{2+} wave through the hippocampus.

Conclusions: $GRAB_{eCB2.0}$ is a robust new probe for measuring the dynamics of eCB release under both physiological and pathological conditions. New improved sensors with 2-AG and AEA specificity are also being developed.

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DISTINCT KINEMATIC CHARACTERISTICS OF MAGL AND FAAH INHIBITION IN HIGH PRECISION 3D MOTION CAPTURE ANALYSIS OF FREELY BEHAVING MICE

Bogna M Ignatowska-Jankowska[1*], Aysen Gurkan-Ozer[1], Alexander Kuck[1], Micah J Niphakis[2], Daisuke Ogasawara [2], Benjamin F Cravatt[2] and Marylka Yoe Uusisaari[1]

[1]Neuronal Rhythms in Movement Unit, Okinawa Institute of Science and Technology, Japan
[2]Department of Chemical Physiology, Scripps Research Institute, La Jolla, CA 92037, USA

Introduction: One of well-known behavioral effects of cannabinoid CB_1 receptor activation is inhibition of locomotor activity. However, effects of endogenous cannabinoids on behavior have not been extensively studied. Here we aimed to determine whether enhancing endocannabinoid signaling produces effects similar to exogenous cannabinoid agonists using novel approach to study behavior.

Methods: To elevate signaling of endocannabinoids 2-Arachidonoylglycerol (2-AG) and Anandamide (AEA) we used selective inhibitors (MJN110 and PF3845, respectively) of enzymes responsible for their degradation: Monoacylglycerol lipase (MAGL) and Fatty Acid Amide Hydrolase (FAAH), respectively. High-speed, high-resolution marker-based 3D motion capture system (Qualisys) was used to track movement (3D trajectories and velocity of markers) during voluntary locomotor tasks: open field exploration and vertical climbing, in C57BL/6 male mice.

Results: The results revealed distinct behavioral phenotypes induced by different treatments. We have previously shown that low doses of synthetic cannabinoid agonist CP55,940 (0.03, 0.1, 0.3 mg/kg) produced significant bidirectional, task-dependent effects: a decrease of activity and widened stance in the open field, but increased activity in the vertical climbing task (n=10). Inhibition of endocannabinoid degradation with MJN110 (1.25, 2.5 mg/kg) and PF3845 (10, 30 mg/kg) produced distinct, opposite effects on locomotor behavior in both open field and climbing tasks (n=10-12). MJN110 (2.5 mg/kg) significantly increased locomotor activity in the open field and produced similar but less pronounced effect at a lower dose (1.25 mg/kg) and in the climbing task. PF3845 significantly reduced locomotor activity in the open field as well as climbing tasks at both doses. Moreover, MJN110 and PF3845 had distinct effects on gait parameters. PF3845 significantly decreased speed of ankle swing, shortened the distance and height of the swing. MJN110 increased swing distance but not height or speed of the swing. Stance width was unaffected by MJN110 and PF3845.

Conclusions: The results suggest that selective elevation of 2-AG and AEA signaling results in distinct, bidirectional effects on behavior that are different from exogenous cannabinoid agonists. Furthermore, the work highlights the strength of 3D motion capture as precise and sensitive tool to evaluate wide range of behaviors in rodents.

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TARGETING NAAA COUNTERS DOPAMINE NEURON LOSS AND SYMPTOM PROGRESSION IN A MOUSE MODEL OF PARKINSON'S DISEASE

Francesca Palese[1]*, Silvia Pontis[2], Natalia Realini[2], Massimiliano Lanfranco[2], Andrea Armirotti[2] and Daniele Piomelli[1]

[1] Departments of Anatomy and Neurobiology, Pharmacology and Biological Chemistry, University of California, Irvine, CA 92697-4625, USA
[2]Department of Drug Discovery and Development, Fondazione Istituto Italiano di Tecnologia, via Morego 30, 16163 Genoa, Italy

Introduction: *N*-acylethanolamine acid amidase (NAAA) is an endosomal-lysosomal cysteine hydrolase that stops the signaling actions of the peroxisome proliferator-activated receptor-α agonist, palmitoylethanolamide (PEA). In peripheral tissues, NAAA-regulated PEA signaling participates in host-defense homeostasis by preventing the inappropriate launch of inflammatory and nociceptive responses. The functions of this signaling system in the brain are still unclear, however.

Methods: Here we used the unilateral striatal administration of 6-hydroxidopamine (6-OHDA, 3.2 μg/μl) in wild-type C57Bl6 mice (WT) and genetically modified mice that express NAAA in a frame-shifted catalytically inactive form ($NAAA^{-/-}$ mice) to investigate NAAA role in neurodegeneration. 6-OHDA treated mice were also treated with a highly selective and brain penetrant NAAA inhibitor, ARN19702 (30 mg/kg i.p. twice a day for three weeks).

Results: Unilateral striatal injection of 6-OHDA in mice caused a rapid (48h post toxin administration) and persistent (up to 15 days post-injection) increase of NAAA immunoreactivity in dopaminergic neurons of the substantia nigra (SN) accompanied by a ≈30% decrease in nigral PEA content. Two weeks after 6-OHDA injection, when inflammation had spread to the dorsal striatum, NAAA levels were increased also in $Iba1^{+}$ cells that displayed the characteristic morphology of reactive microglia.
We next administered 6-OHDA to $NAAA^{-/-}$ mice, observing a (*i*) enhanced survival of nigral TH^{+} neurons, (*ii*) higher striatal levels of dopamine and dopamine metabolites, and (*iii*) greater density of striatal TH^{+} fibers, when compared with WT littermates. Moreover, $NAAA^{-/-}$ mice displayed (*iv*) attenuated motor responses to the dopaminergic agonist apomorphine, (*v*) prolonged latency to fall in the rotarod performance test, and (*vi*) lower mortality rate. Heterozygous $NAAA^{+/-}$ mice showed a protective phenotype but less pronounced than the one observed in homozygous NAAA deleted mice.
Moreover, chronic treatment with the highly selective and brain penetrant NAAA inhibitor, ARN19702 recapitulates the protective effect observed with the removal of the *Naaa* gene. Finally, to gain insights into possible mechanisms through which NAAA might influence neuronal survival, we ran a proteomic analysis on the SN of $NAAA^{-/-}$ and WT mice 48h after 6-OHDA administration and we observed that $NAAA^{-/-}$ mice upregulated proteins involved in the Krebs' cycle, oxidative phosphorylation and glycolysis. Interestingly, PGC1α and β, crucial regulators of these pathways, were downregulated in lesioned SN of WT mice, whereas in $NAAA^{-/-}$ mutants they were constitutively elevated and not affected by 6-OHDA administration.

Conclusions: We are showing that NAAA enables the toxin-induced death of nigrostriatal dopamine neurons through a mechanism that involves suppression of cellular bioenergetics pathways responsive to peroxisome proliferator-activated receptor-α coactivator-1 (PGC-1), a family of transcription coactivators implicated in the pathogenesis of Huntington's disease and non-familial PD. We further show that genetic or pharmacological interruption of NAAA activity restores expression of PGC-1-regulated genes and enhances dopamine neuron survival. Together these results reveal unexpected role for NAAA in the control of dopamine neuron survival and identify this enzyme as a druggable target for PD modification.

INTERACTION BETWEEN CB2 AND TOLL-LIKE RECEPTORS SHAPES MICROGLIAL ACTIVITY

Kishore Aravind Ravichandran[1], Nico Reusch[2], Bolanle Fatimat Olabiyi[1], Joanna Komorowska-Müller[1], Jan N. Hansen[3], Thomas Ulas[2], Marc Beyer[4,5], Andreas Zimmer[1] and Anne-Caroline Schmöle[1*]

[1]Institute for Molecular Psychiatry, University of Bonn, Bonn, Germany
[2]Genomics and Immunoregulation,
Life and Medical Sciences Institute (LIMES), Bonn, Germany
[3]Institute of Innate Immunity, University of Bonn, Bonn, Germany.
[4]Molecular Immunology in Neurodegeneration,
German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany
[5]PRECISE Platform for Single Cell Genomics and Epigenomics, German Center for Neurodegenerative Diseases (DZNE) and the University of Bonn, Bonn, Germany

Introduction: The CB2 receptor is predominately expressed on immune cells and thus tightly connected to the immune response. However, it is not clear how the CB2 receptor interacts with the innate immune response. We demonstrated before that CB2 deletion influences microglial activity as a consequence to TLR stimulation *in vitro,* but also in an *in vivo* mouse model of Alzheimer's disease (AD). Therefore, we aimed to investigate how CB2 deletion influences TLR-mediated microglial activation.

Methods: Primary microglia cultures from neonatal wild type (WT) and CB2 knockout ($CB2^{-/-}$) mice were stimulated with different TLR ligands, namely LPS/IFNγ (TLR4), PolyI:C (TLR3) and CpG (TLR9), sorted for $CD11b^{+}$ cells and proceeded for RNA sequencing. Microglial activation was analyzed with MotiQ in TLR-stimulated organotypic hippocampal slice cultures (OHSC) from WT and $CB2^{-/-}$ mice. Subsequently, the activation profile of TLR-stimulated bone marrow-derived macrophages (BM-MΦ) was characterized by secretion and cell surface expression of inflammatory markers. Protein levels and phosphorylation of p38 in primary microglia was quantified by Western Blot WES technology.

Results: We found that microglia from $CB2^{-/-}$ mice showed a dampened response to TLR 4 and 9 stimulation compared to WT microglia. Interestingly, stimulation of TLR9 led to a gene expression profile that was completely different in $CB2^{-/-}$ microglia when compared to WT microglia. Subsequently, we demonstrated that reduced TLR-mediated microglial activity in $CB2^{-/-}$ mice was not an artefact, but also present in $CB2^{-/-}$-OHSC. We revealed that CB2 deletion does not influence TLR-mediated activation of microglia only, but also at least partially influences the activation profile of monocytes as shown in BM-MΦ. Finally, we identified p38 MAPK signaling as potential mechanism that connects CB2 signaling to TLR-mediated microglial activation.

Conclusion: Our data demonstrate that the presence of the CB2 receptor is necessary to develop a full TLR-mediated microglial activation, which is mediated by p38 MAPK activity. This effect is very prominent in microglia, but also partially present in BM-macrophages, even though to a lower extent. Our data strongly support the crucial role of CB2 in microglia activation which can be now connected to an interference of the CB2 receptor with TLR signaling.

IN VIVO STUDIES OF THE ROLE OF CANNABINOID CB_2 RECEPTORS IN MICROGLIAL ACTIVITY IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE BY MULTIPHOTON MICROSCOPY

Samuel Ruiz de Martín Esteban[1*], M. Teresa Grande[1], Diego Herráez[1], Rocío Palenzuela[1], Irene Benito-Cuesta[1], Rosa M. Tolón[1], Ricardo Mostany[2], Cecilia J. Hillard[3] and Julián Romero[1]

[1]School of Pharmacy, Faculty of Experimental Sciences, Universidad Francisco de Vitoria, 28223, Madrid, Spain; [2]Department of Pharmacology, Tulane University School of Medicine, 70112, New Orleans, LA, USA; [3]Neuroscience Research Center, Medical College of Wisconsin, 53226, Milwaukee, WI, USA

Introduction: One of the main features of Alzheimer's disease (AD) is the presence of protein precipitates in the brain parenchyma. These pathological structures (named "neuritic plaques") are mainly composed of amyloid peptides and trigger profound alterations in the brain milieu and, specifically, an intense neuroinflammation. Currently, several hypotheses exist on the role of these plaques and it is not clear yet whether these plaques are a consequence of amyloid accumulation or a putative way to dampen the toxic effects of other forms of these peptides, such as oligomers. Among other processes, neuritic plaques trigger the activation of microglial cells, and as we have previously shown, the induction of the expression of cannabinoid CB_2 receptors. These receptors are selectively expressed by activated microglia and their role in the interplay between them and amyloid plaques is still unknown.

Methods: We here present data on the effects that the genetic deletion of cannabinoid CB_2 receptors provoke on the ability of microglial cells to phagocytose amyloid peptides and in the characteristics of neuritic plaques, *in vivo*. To that end, we have employed multiphoton microscopy and taken advantage of recently developed transgenic mouse models, such as 5xFAD/$CB_2^{EGFP;f/f}$, 5xFAD/$CB_2^{-/-}$ and, more recently, 5xFAD/$CB_2^{EGFP;f/f}$/Cx3cr1$^{tm2.1(cre/ERT2)Jung}$ mice.

Results: With these tools we have been able to perform a time-course analysis of the impact of CB_2 deletion on the ability of microglia to phagocytose amyloid peptides *in vivo* as well as to analyze the main features of neuritic plaques in the presence or absence of microglial CB_2. Our preliminary data indicate that the deletion of CB_2 receptors leads to a profound alteration in the phagocytic activity of microglial cells, with a significant decrease in amyloid uptake in CB_2-null mice. In addition, subtle differences were observed in neuritic plaque growth and structural features, including plaque volume, protein density, skewness and others.

Conclusions: Microglial cannabinoid CB_2 receptors modulate phagocytosis of amyloid peptides and exert mild effects on the interplay between these cells and neuritic plaques.

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TARGETED ACTIVATION, BUT NOT INHIBITION OF CANNABINOID RECEPTOR 2 (CB2R), IS A NOVEL APPROACH TO REDUCE RENAL FIBROSIS

Janos Paloczi[1*], Partha Mukhopadhyay[1], Cody Savage[1], Eszter Trojnár[1], Csaba Matyas[1], Yuri Persidsky[2], Uwe Grether[3] and Pal Pacher[1]

[1]Laboratory of Cardiovascular Physiology and Tissue Injury, NIH/NIAAA, Bethesda, MD, USA; [2]Temple Univ., PA, USA; [3]Roche Pharma Research & Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., 4070 Basel (Switzerland).

Chronic kidney disease (CKD) is among the leading causes of morbidity and mortality worldwide with a prevalence of 14% in the general population. Renal fibrosis is a histological hallmark of CKD and also believed to be a pathogenic intermediate for CKD progression.

Recently it has been suggested that CB2R is expressed in kidney tubular cells and it promotes kidney fibrosis. An anti-fibrotic effect of a newly developed putative inverse CB2R agonist XL-001 in a mouse model of kidney fibrosis induced by ureter occlusion was shown, promoting CB2R antagonists XL-001 for the treatment of CKD (Kidney Int 2018 Oct; and 2021 Febr Zhou et al.).

In this study we explored the functional role of CB2R in kidney fibrosis utilizing unilateral ureteral occlusion (UUO) induced kidney fibrosis model in mice. The putative inverse CB2R agonist XL-001 (20 mg/kg) was resynthesized and characterized and used alongside with established CB2R inverse agonist SR144528 (3 mg/kg) for in vivo experiments. Furthermore, 4 additional CB2R agonists AM1241, HU910, RO6839828 and RO6871304 were also investigated in vivo (3-10 mg/kg i.p.). All drug treatments continued for 14 days following UUO.

We found that there was negligible, if any, CB2R expression in normal kidneys. In kidneys subjected to unilateral ureteral occlusion (UUO) there was a marked (up to 20 folds) increase of CB2R mRNA expression, which paralleled by marked infiltration of $CD45^+$ leukocytes and fibrosis 2 weeks following the insult. The CB2R expression in diseased kidneys positively correlated with number of various infiltrating immune cells, but not with tubular markers.

We confirmed XL-001 to be a potent inverse agonist on human CB2R, but to a less extent on mouse receptors. Furthermore, its bioavailability in mice was extremely poor compared to all other CB2R ligands used. Surprisingly, we found that neither XL-001 (20 mg/kg) nor SR144528 (3 mg/kg) attenuated kidney inflammation and fibrosis, they rather had a tendency to increase both.

In contrast, all 4 CB2 agonists administration for 14 days following UUO attenuated kidney fibrosis (as shown by Masson's trichrome and Sirius Red staining or by αSMA western blotting) and decreased collagen-1, -3 and TGFβ expression (revealed by qRT-PCR) when compared to vehicle-treated controls. CB2R agonists also attenuated the increased recruitment of bone marrow-derived $CD45^+$ leukocytes decreasing renal inflammation in CKD.

Moreover, treatment with the CB2-R agonists HU910, RO6839828 or RO6871304 dramatically decreased renal inflammation and oxidative stress and cell death in mouse model of cisplatin-induced acute tubular injury.

In conclusion, the CB2R is not expressed in normal kidneys. During chronic kidney injury the CB2R expression is positively correlating with infiltrating immune cells, but not expressed in tubular cells. Targeted activation, but not inhibition of cannabinoid receptor 2 (CB2R), is a novel approach to reduce renal inflammation and fibrosis in CKD.

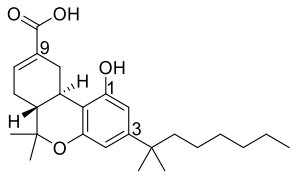
DEVELOPMENT OF NOVEL CB2 AGONISTS FOR TREATING INFLAMMATORY AND FIBROTIC DISEASES

Hongfeng Deng*, Clifton Leigh, Tama Evron, Ping Zhang, Zhuang Jin, and Barbara White

Corbus Pharmaceuticals, Norwood, MA 02062

Introduction: Cannabinoid type 2 receptor (CB2) is expressed on activated immune cells and fibroblasts and regulates multiple cellular activities to reduce inflammation and fibrosis. Selective CB2 activation offers a potential opportunity for treating inflammatory and fibrotic diseases, with greater selectivity for CB2 versus cannabinoid receptor type 1 (CB1) likely to reduce psychoactive adverse effects caused by CB1 activation in the central nervous system. The hypothesis of this work was that structural optimization would yield CB2 agonists with improved selectivity, yet retained or improved potency, compared to the parent compound.

Method: The structure of the parent compound is shown below. Guided by CB2 protein structure and structure-activity relationships, new analogs of the parent compound were designed with modifications at C9 position, the central phenol moiety, and C3 gem-dimethyl carbon chain portion. These analogs were tested for binding and functional activities. Promising compounds were further tested in animal models of inflammation and fibrosis.



Parent compound

Results: Significant improvement was achieved in CB2 binding affinity and selectivity against CB1. One new analog, compound EE*, showed CB2 affinity improvement from 150 nM to 7.7 nM, CB2 selectivity improvement from 2.8-fold to 38-fold in binding, from 46-fold to >943-fold in β-arrestin activity, and from 10-fold to 63-fold in cAMP activity. Compound EE reduced levels of the LPS-induced inflammatory cytokines MCP-1, TNFα, IL-1β, IL-6, and IL-23 produced by human PBMCs in a dose-dependent manner. These effects were inhibited by CB2, but not CB1, antagonist pretreatment. Compound EE reduced the transition of human primary fibroblasts to myofibroblasts, as measured by α smooth muscle actin levels in TGFβ-stimulated fibroblasts. Upon in vivo dosing, compound EE significantly reduced knee swelling in a rat monosodium urate-induced acute inflammation model and attenuated bleomycin-induced lung fibrosis in mice when administered in both preventative and therapeutic modes. Additionally, compound EE showed reasonable pharmacokinetic properties and no liabilities in preliminary safety assessments.

Conclusions: Information about CB2 receptor structure and the parent compound's structure-activity relationships led to the design of novel CB2 agonists with improved physicochemical properties and functional activities. Compound EE is a promising development candidate for treating inflammatory and fibrotic diseases.

* Full structure of compound EE cannot be disclosed to protect IP during early development.

HU308-MEDIATED CANNABINOID SIGNALING MITIGATES AGE AND TRAUMA-RELATED OSTEOARTHRITIS IN MICE

Idan Carmon[1,2]*, Sai Meka[1,2], Jinan Elayyan[2], George Batshon[2], Eli Reich[2], Andras Bilkei-Gorzo[3], Andreas Zimmer[3], Raphael Mechoulam[1] and Mona Dvir-Ginzberg[1,2]

[1]Multidisciplinary Center for Cannabinoid Research, Hebrew University of Jerusalem
[2]Institute of Dental Sciences, Hebrew University of Jerusalem
[3]Institute of Molecular Psychiatry, University of Bonn, Germany

Introduction: Osteoarthritis (OA) is characterized by progressive, irreversible erosion of articular cartilage that may be evoked during progressive age and after traumatic insult. This study aims to assess the attributes of HU308- a selective cannabinoid receptor type 2 (CB2) agonist, to serve as a potential Disease modified OA drug (DMOAD). We further attempted to elucidate if the mechanism bestowed by HU308 relies on Sirt1.

Methods: Wild type (wt) mice (female, 3 month-old, C57BL6/J) were subjected to post-traumatic OA induction using Destabilization of the Medial Meniscus (DMM) surgical procedure. The mice were Intra-articularly (IA) injected with HU308 (twice a week) from 4-8 weeks post-surgery. After sacrifice, the joints were assessed for local pain sensation and histopathologically. To further understand if HU308 relies on Sirt1 for preserving joint health, we systemically administered in aged wild type (wt; $Sirt1^{flox}$) mice and cartilage-specific Sirt1 KO mice, generated by inducible aggrecan-dependent Cre-recombinase driver, crossed with $Sirt1^{fl/fl}$ strain (i.e. ATC^{cre} $Sirt1^{fl/fl}$). Additionally, human chondrocytes were cultured with HU308 and monitored for anabolic and catabolic gene expression.

Results: Intra-articularly administered HU308 attenuated cartilage damage, osteophyte appearance and pain sensitivity following DMM procedure or in age-induced OA models. Interestingly, aged-mice bearing cartilage specific Sirt1 ablation (i.e. 16 months; ATC^{cre} $Sirt1^{fl/fl}$) exhibited similar OA severity following systemic IP administration of HU308 vs vehicle, indicating that HU308 might partially require Sirt1 to bestow its joint protective effects. Assessing human chondrocyte treated with HU308 (100nM), display a dose-dependent increase in aggrecan, COL2, PRG4, SOX9 and SIRT1 transcription levels**,** which was preceded by increased levels of pCREB.

Conclusions: Collectively, the results show that HU308 prevents joint damage in trauma and age-induced OA models, and increased thresholds for pain sensitivity. While the mechanism is fully to be determined, preliminary data indicate that HU308 exerts a pro-anabolic effect by enhancing pCREB and SIRT1 in chondrocytes, to maintaining joint health and integrity.

NEUROBIOLOGICAL MECHANISMS UNDERLYING VULNERABILITY AND RESILIENCE TO CANNABIS ADDICTION

Elena Martin-Garcia[1*], Maria Cajiao[1], Eric Senabre[1] and Rafael Maldonado[1,2]

[1]Laboratory of Neuropharmacology-Neurophar,
Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain. [2]Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain.

Introduction: A hallmark of addiction is the loss of inhibitory control, leading to compulsive behavior in addicted individuals. This disorder's complex multifactorial nature and the unknown neurobiological mechanistic correlation explain the lack of effective treatments. We aim to provide new inside into the neurobiological mechanisms underlying the vulnerability to develop addiction.

Methods: We used a mouse model of drug addiction using WIN 55,512-2 intravenous self-administration (0.0125 mg/kg/infusion) in C57Bl/6J, targeting the prelimbic medial prefrontal cortex (mPFC) to nucleus accumbens (NAc) pathway using chemogenetic approaches. We selectively expressed the inhibitory designer receptor exclusively activated by designer drug (hM4Di-DREADD) in mPFC projecting neurons by bilateral injections of a Cre-dependent AAV expressing hM4Di-DREADD into the prelimbic mPFC of mice and a retrograde AAV expressing Cre recombinase into the NAc core. Thus, hM4Di receptor expression only occurs in prelimbic neurons that directly project to the NAc core. For the activation of the hM4Di-DREADD, clozapine N-oxide was administered using Alzet osmotic minipumps implanted subcutaneously in the back of the mice that delivered a constant flow rate of 0.25 µl/h during 28 days.

Results: We found that the resilient or vulnerable phenotype of cannabis addiction can be obtained by altering the mPFC signaling. Thus, mice expressing hM4Di-DREADD showed a vulnerable phenotype. Hypoactivity of mPFC to NAc projecting neuron transmission by CNO-induced hM4Di-DREADD activation in prelimbic cortex promoted addictive-like behavior in C57Bl/6J mice.

Conclusions: Understanding the neurobiological mechanisms underlying resilience versus vulnerability to cannabis addiction is expected to pave ways for novel and efficient interventions to battle this mental disorder.

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THE EFFECT OF OLEOYL – ALANINE ON OPIOID WITHDRAWAL BEHAVIOURS IN MALE SPRAGUE DAWLEY RATS

Samantha M. Ayoub[1*], Reem Smoum[2], Mathew Farag[1], Harkirat Atwal[1], Cheryl L. Limbeer[1], Erin M. Rock[1], Stephen A. Collins[1], Fabiana Piscitelli[3], Fabio Arturo Iannotti[3], Vincenzo Di Marzo[3], Francesco Leri[1], Aron H. Lichtman[4], Raphael Mechoulam[2] and Linda A. Parker[1]

1. Department of Psychology and Neuroscience, University of Guelph, Canada. 2. Institute of Drug Research, Medical Faculty, Hebrew University of Jerusalem, Israel. 3. Institute of Biomolecular Chemistry, Consiglio Nazionale delle Richerche, Naples, Italy. 4. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, USA.

Introduction: Oleoyl glycine (OlGly) is an unexplored fatty acid amide that has been recently shown to alter acute opioid withdrawal in rats. More specifically, OlGly prevents acute naloxone-precipitated morphine withdrawal (NPMWD)-induced conditioned place aversion (CPA, Petrie et al., 2019) and largely reduces somatic NPMWD behaviours (Rock et al., 2019). However, endogenous OlGly is rapidly hydrolyzed by amidases in the body, which may limit its efficacy to treat opioid withdrawal in humans. Therefore, we designed experiments to evaluate the potential of a more stable analog of OlGly, Oleoyl alanine (OlAla; HU595), to interfere with acute and chronic opioid withdrawal behaviours in male Sprague Dawley rats.

Acute experiments: The effectiveness of OlAla (5 mg/kg, ip) was compared with OlGly (5 mg/kg, ip) to reduce acute NPMWD-induced CPA when administered 10 min or 60 min prior to naloxone. Both OlGly and OlAla prevented the NPMWD-induced CPA when given 10 min prior to naloxone, but only OlAla was effective across 60 min. OlAla's effects were found to be mediated by the CB1-receptor and PPAR*a*. OlAla also interfered with NPMWD- induced somatic withdrawal responses (abdominal contractions, mouth movements and lying on belly), including nausea-induced conditioned gaping. These experiments suggest OlAla reduces acute opioid withdrawal behaviours, and appears to be a more effective treatment option than OlGly.

Chronic experiments: Rats were trained to self-administer heroin for 3 hrs daily on a continuous schedule of reinforcement for 10 days. OlAla (5 mg/kg, ip) administration did not modify opioid self-administration on its own, which is consistent with previous work that has shown OlGly does not alter opioid reward (Donvito et al., 2019; Petrie et al., 2019); however, OlAla did interfere with naloxone-induced elevations of heroin self-administration. Different rats were treated chronically with escalating doses of morphine (10-80 mg/kg, ip every 12 hr) or saline for 14 days. Twenty-four hr after the final injection, they were injected with VEH 10 min prior to saline or naloxone and somatic withdrawal was measured. Immediately afterwards, rats were euthanized and their nucleus accumbens, amygdala, interoceptive insular cortex and prefrontal cortex was sent to the DiMarzo laboratory in Naples for assessment of endogenous OlGly and endocannabinoids. Both OlGly and 2-AG were found to be suppressed in the nucleus accumbens and amygdala in the morphine withdrawal rats. Then we evaluated the effect of OlAla and OlGly on spontaneous withdrawal from chronic exposure to heroin (7 mg/kg/day) or saline by osmotic minipumps for 12 days. Twenty-four hr following removal of the pumps, spontaneous somatic withdrawal was assessed for 30 min following an injection of VEH, OlAla (5 and 20 mg/kg, ip) or OlGly (5 and 20 mg/kg, ip). OlAla, but not OlGly, at 5 and 20 mg/kg suppressed somatic withdrawal behaviors of abdominal contractions, body stretching, mouthing movements and yawning.

Conclusion: OlAla may be a more effective treatment for opiate withdrawal than is OlGly.

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MODELING SPONTANEOUS THC WITHDRAWAL SYMPTOMS IN MICE

Andrew J. Kesner*, Stephanie Ramos-Maciel, Yolanda Mateo,
Alexa L. Gracias and David M. Lovinger

NIAAA, Rockville, MD. USA

Introduction: The diagnosis of cannabis withdrawal (DSM-V) has become less contentious at the clinical level given the accumulation of clinical studies reporting withdrawal symptoms upon cessation of cannabis use. Known consequences of withdrawal from chronic cannabis use in humans as outlined by DSM-V include irritability, nervousness, decreased appetite or weight loss, restlessness, depressed mood and hedonics, and uncomfortable somatic symptoms. These symptoms are thought to be driven by physiological changes that correspond with building tolerance to delta-9-tetrahydrocannabinol (THC) during prolonged cannabis use. THC is the major psychoactive ingredient in cannabis and in laboratory animal studies withdrawal symptoms are typically precipitated by treatment with a cannabinoid type 1 receptor antagonist or inverse agonist. However, this does not mimic the normal course of withdrawal in human cannabis users. In contrast, spontaneous THC withdrawal symptoms are thought to be difficult to observe in laboratory animal models, and as such, back-translational studies on the physiological mechanism of cannabis/THC withdrawal are lacking. We aimed to fill this knowledge gap by implementing mouse behavioral paradigms that target specific clinical withdrawal symptoms.

Methods: We modeled tolerance to chronic cannabis use using a THC treatment paradigm, consisting of 10 intraperitoneal injections of 10 mg/kg THC over 6 days, known to induce behavioral tolerance to THC. Behavioral measurements from pretreatment, and early and late THC abstinence epochs were obtained for all experiments. We performed between group (THC vs. Vehicle) and within subject comparisons of changes in behaviors driven by withdrawal from chronic THC, i.e. withdrawal symptoms.

Results: *Consumption and Locomotion.* During twenty four-hour home cage food and water intake and locomotion monitoring we observed potential sex differences during withdrawal for intake and locomotion. *Anhedonia.* In sucrose preference tests, mild anhedonia was observed in THC-treated males, but not females, as they consumed significantly less sucrose than the vehicle controls. *Reward seeking.* Mice performing an operant-cue discrimination task showed that both THC-treated males and females earned fewer rewards, suggesting reductions in motivation during THC abstinence. *Plasma corticosterone.* Changes in these behavioral indices do not appear to be mediated by stress per se, as circulating plasma corticosterone was only modestly increased in male but not female mice. *Neurochemistry.* The neurotransmitter dopamine plays a known role in manifestation of withdrawal symptoms, so we performed ex-vivo fast-scan cyclic voltammetry to measure dopamine levels within the striatum and observed alterations primarily during early withdrawal timepoints which corresponds to the strongest behavioral effects.

Conclusions: These studies describe several mouse behaviors with translatable relevance that are altered during spontaneous withdrawal from THC. We are beginning to uncover sex-differences in these behavioral changes that highlight importance of including sex as a biological variable at both the preclinical and clinical level when studying effects of THC administration and withdrawal. These data open the door for further pre-clinical research efforts to determine the neurobiological bases of, and potentially treat, primary withdrawal symptoms of cannabis use disorder.

INHIBITION OF MONOACYLGLYCEROL LIPASE ALTERS DENDRITIC BRANCHING COMPLEXITY AND REWARD-RELATED BEHAVIOR IN TAT TRANSGENIC MICE

Alexis F. League*[1], Benjamin L. Gorman[1], Ian R. Jacobs[1], Justin L. Poklis[2], Micah J. Niphakis[3], Benjamin F. Cravatt[3], Aron H. Lichtman[2], Bogna M. Ignatowska-Jankowska[4] and Sylvia Fitting[1]

[1]Department of Psychology & Neuroscience, University of North Carolina Chapel Hill, Chapel Hill, NC, USA; [2]Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, USA; [3]Department of Chemistry, Scripps Research Institute, La Jolla, CA, USA; [4]Okinawa Institute of Science & Technology, Neuronal Rhythms in Movement Unit, Okinawa, Japan

Introduction: Human immunodeficiency virus type 1 (HIV-1) -associated neurocognitive disorders are characterized by altered behavioral phenotypes, often involving inhibitory control deficits and motivation dysregulation. While combination antiretroviral therapy has increased the expected lifespan of individuals living with HIV-1, viral proteins such as transactivator of transcription (Tat) are poorly suppressed and drive neurocognitive deficits which persist beyond treatment. Previous work has demonstrated a neuroprotective role of targeted cannabinoid modulation of 2-arachidonoylglycerol (2-AG). Thus, aims of the present study were two-fold. First, we aimed to characterize differences in dendritic arborization complexity *in vitro* between frontal cortex neurons exposed to Tat and/or monoacylglycerol lipase inhibitor MJN110. Second, we aimed to investigate the therapeutic utility of MJN110 *in vivo* using a Tat transgenic mouse model. **Methods**: To assess morphological differences between treatments, mature primary frontal cortex neuron cultures were exposed to Tat and/or MJN110 for 24 hours, immunolabeled with MAP2 antibody, and dendritic arborization was assessed using Sholl analysis. To assess behavioral outcomes of Tat expression and MJN110 treatment, a novel assay developed to assess reward-related behavior was used to examine olfactory discriminative stimulus learning and cognitive flexibility. Subjects were trained to approach one of two odor cues, one of which predicted a salient reinforcer. Once subjects consistently interacted with only the reinforcer-predictive cue, the reward contingency was reversed such that the previous distractor odor was paired with the reinforcer. Following acquisition of the reversal task, brain tissue from subjects was dissected and snap-frozen. Ultrahigh performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was used to quantify brain region-specific protein expression differences by genotype and treatment. **Results**: Cultured Tat-exposed neurons treated with MJN110 displayed significant increases in branching complexity relative to vehicle-treated neurons exposed to Tat (p = .002; analyzed with Bonferroni-corrected two-way ANOVA). Tat(+) subjects acquired the shaping phase of the task significantly faster than Tat(-) controls (p = .011; analyzed with chi-square). In reversal training, no significant effect was noted for genotype. Interestingly, chronic MJN110 (1 mg/kg; 20 days subcutaneous injection) significantly increased the latency to reverse behavior in Tat(+) subjects (p = .043; analyzed with Welch's-corrected unpaired t-test), but not Tat(-) mice. UPLC-MS revealed significantly increased prefrontal and striatal 2-AG levels in both genotypes treated with MJN110 (p = .042 and < .0001, respectively; analyzed with Bonferroni-corrected two-way ANOVA). **Conclusions**: MJN110 shifted reward-related behavior and dendritic branching complexity in the presence of Tat to phenotypes resembling those of Tat(-) controls. Further experiments will clarify the striatum's role in the MJN110-driven effects observed, and ultimately seek to identify whether the obtained results implicate endocannabinoid activity in regulation of reward-related motivated behavior.

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MONOACYLGLYCEROL LIPASE (MAGL), BUT NOT FATTY AMIDE ACID HYDROLASE, INHIBITION EXACERBATES HYPERDOPAMINERGIC PHENOTYPES IN DOPAMINE TRANSPORTER-KNOCKOUT (DATKO) MICE.

Catharine A. Mielnik[1*], Claudia Lutelmowski[1], Ali Salahpour[1], Heather Bradshaw[2] and Ruth Ross[1]

[1]University of Toronto, Faculty of Medicine, Department of Pharmacology & Toxicology
[2]Indiana University Bloomington, College of Arts + Sciences, Psychological and Brain Sciences

Background: The endocannabinoid system is an important neurotransmitter system involved in many behaviours. It is also dysregulated in a number of psychiatric disorders, including psychosis, mania, and schizophrenia. Endogenous ligands of the eCBS include anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are inactivated by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) respectively. There is significant interest in targeting the inactivation of AEA and 2-AG for pharmacological intervention. Pharmaceutical companies have initiated clinical trials for FAAH and MAGL inhibitors to increase eCB levels at the synapse pharmacologically. Currently, FAAH inhibitors have advanced to phase II clinical trials for multiple indications, while only one MAGL inhibitor has successfully completed phase I trials. Both drugs show promising results for their respective indications, but it remains imperative to understand the effects of these drugs on dysregulated eCB systems; highly prevalent in neuropsychiatric disorders, along with alterations in dopamine signaling. We assessed the pre-clinical effects of FAAH and MAGL inhibition in a genetic model of hyperdopaminergia.

Methods: Adult DAT-knockout (DATKO) mice (male and female) were treated acutely with either a MAGL or FAAH inhibitor and tested on behavioural assays. Lipidomic analysis was completed (brain and plasma samples). Data were analyzed with three-way ANOVA (behaviour) and Student's t-test for lipidomics.

Results: DATKO mice present with subcortical hyperdopaminergia, manifesting in exploratory hyperactivity, mania-like behaviours and impaired sensorimotor gating. Following acute inhibition of MAGL, DATKO mice displayed exacerbated hyperlocomotive behaviour, without any amelioration of sensorimotor deficits. Lipidomic analysis highlighted baseline downregulation of key eCB molecules in subcortical forebrain structures of DATKO, further mirrored in plasma samples. Acute inhibition of FAAH in DATKO had no behavioural effects when compared to vehicle.

Conclusion: This data suggests that while inhibition of MAGL may have beneficial clinical effects for pain and Tourette's syndrome, it is imperative to understand baseline eCB tone and how states of hyperdopaminergia may diverge at the eCB level. Therefore, inhibition of MAGL may be contraindicated for certain neuropsychiatric disorders stemming from hyperdopaminergic states. Especially with MAGL inhibition, shown to enhance dopamine levels, it is essential to exercise caution with genetically vulnerable populations. The same contraindications may not be true for the inhibition of FAAH.

THE DELETERIOUS ROLE OF CANNABINOID-1 RECEPTOR IN THE PROGRESSION OF ACUTE TO CHRONIC KIDNEY DISEASE

Ariel Rothner[1*], Liad Hinden[1], Majdoleen Ahmad[1], Alina Nemirovski[1], Ifat Abramovich[2], Bella Agranovich[2], Aviram Kogot-Levin[3], Eyal Gottlieb[2], Gil Leibowitz[3] and Joseph Tam[1]

[1]Obesity and Metabolism Laboratory, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel; [2]The Ruth and Bruce Rappaport Faculty of Medicine, Technion- Israel Institute of Technology, Haifa, Israel; [3]Diabetes Unit and Endocrine Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Introduction: Acute kidney injury (AKI) is a risk factor for the development of chronic kidney disease (CKD), mainly attributed to a maladaptive repair following the acute episode. Whereas the overactivation and deleterious role of the endocannabinoid/CB_1R system in CKD is well established, its involvement in post-AKI disruptive healing is unknown.

Methods: We utilized a murine model of folic acid-induced AKI-to-CKD transition (240 mg/kg folic acid, single dose, i.p.) to evaluate the renal endocannabinoid 'tone', including the abundance of the main endocannabinoids, the expression of their metabolic enzymes and the canonical CB_1R. We further studied the effect of pharmacological inhibition of CB_1R on renal function, injury, inflammation, and fibrosis, in mice treated with the CB_1R inverse agonist, JD5037 (3 mg/kg/d, PO), for 14-days after folic-acid insult. Comparative metabolomics analysis was performed on the kidneys to explore the underlying mechanism by which CB_1R modulates AKI-to-CKD progression.

Results: The expression of renal CB_1R, and of the receptor's endogenous ligand, 2-arachidonoylglycerol (2-AG) and its biosynthetic enzyme, diacylglycerol lipase alpha (DAGLα) were all increased during the transition from AKI to CKD. Pharmacological CB_1R antagonism ameliorated the folic acid-induced kidney dysfunction and injury, evidenced by the decreased serum creatinine and urea nitrogen levels, reduced renal neutrophil gelatinase-associated lipocalin (NGAL) expression and attenuated tubular pathophysiology. Inflammatory and fibrogenic markers, known contributors to maladaptive renal repair, were significantly reduced following CB_1R inhibition. Kidney metabolomics profiling revealed that the beneficial effects of CB_1R blockade were mediated by altered renal glucose homeostasis and improved amino acid transport and metabolism.

Conclusions: Our results demonstrate that the endocannabinoid/CB_1R system plays a critical role in the transition from AKI-to-CKD. Inhibition of peripheral CB_1R attenuates the progression from AKI to CKD, and highlights novel molecular pathways associated with modulation of glucose and amino acid metabolism in the kidney. Taken together, these findings further support the development and clinical testing of peripheral CB_1R antagonists to limit AKI progression to CKD.

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GUT-BARRIER DYSFUNCTION ASSOCIATED WITH DIET INDUCED OBESITY IS EXACERBATED IN THE ABSENCE OF CB1R IN THE INTESTINAL EPITHELIUM

Mark B. Wiley* and Nicholas V. DiPatrizio

Division of Biomedical Sciences, University of California, Riverside, Riverside, CA, USA

Introduction: More than 70% of Americans are considered overweight or obese which leads to the development of several chronic comorbidities. One ailment associated with obesity is Inflammatory Bowel Disease (IBD) which has an idiopathic etiology and more than 3 million Americans diagnosed. The expansion of IBD development is observed exclusively in well-developed regions of the world where a western-style diet high in both fat and carbohydrates, derived from sucrose, has been adopted. Western-diet induced obesity leads to disruptions in the endocannabinoid (eCB) system throughout the intestinal tract and has been shown to contribute to gut-barrier function. Gut-barrier function is essential to providing protection from potentially pathogenic organisms ingested in a meal and its dysfunction directly contributes to IBD pathogenesis. Here, we tested the hypothesis that intestinal epithelial cannabinoid receptor subtype-1 (CB_1R) signaling provides protection from diet-induced gut-barrier dysfunction.

Methods: Male wild type (WT) or intestinal epithelial CB_1R knockout mice ($iCB_1R^{-/-}$) and controls were given *ad libitum* access to standard vivarium chow (SD) or a high fat, high sucrose western diet (WD) for 60 days. In a subset of experiments, $iCB_1R^{-/-}$ or control mice received daily IP injections of either THC or vehicle for 14 days at day 60 of exposure to the diets. Paracellular permeability was measured via FITC-conjugated dextran (4 kDa) with retro-orbital serum collection occurring at 4 hours post-gavage to provide an *in vivo* measurement of large intestinal gut-barrier function.

Results: Large intestine mucosal samples from WD-fed mice displayed significantly reduced levels of the eCB, 2-arachidonoyl-*sn*-glycerol (2-AG), when compared to SD-fed mice. Baseline tight-junction protein mRNA was unaffected when comparing $iCB_1R^{-/-}$ and control mice and no changes in large intestine gut-barrier function were observed at baseline. Mice fed WD for 60 days displayed an increase in intestinal paracellular permeability which was exacerbated in the absence of intestinal epithelial CB_1R. Furthermore, chronic THC treatment in WT mice for 14 days ameliorated the diet-induced disruption in gut-barrier function.

Conclusions: These data provide evidence that intestinal epithelial CB_1R signaling provides protection from diet-induced gut-barrier dysfunction. Furthermore, chronic THC administration rescues the increase in large intestinal paracellular permeability after 60 days on WD. Future experiments will include analysis of the mechanism in this response. Furthermore, chronic THC administration to $iCB_1R^{-/-}$ after 60 days on WD will be performed to identify if intestinal epithelial CB_1R signaling is required for the THC-induced rescue of gut-barrier function.

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CANNABINOID TYPE-1 RECEPTOR INHIBITION AS A LONG-TERM PHARMACOLOGICAL APPROACH FOR MEMORY ENHANCEMENT IN A MOUSE MODEL OF DOWN SYNDROME

Anna Vázquez-Oliver[1]*, Silvia Pérez-García[1], Nieves Pizarro[2,3], Lydia Garcia-Serrano[1], Gabriela Bordeanu[1,4], Pier-Vincenzo Piazza[5], Rafael De la Torre[1,2], Rafael Maldonado[1,2] and Andrés Ozaita[1,2]

[1.]University Pompeu Fabra, Barcelona, Spain.
[2.]IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain.
[3.]Autonomous University of Barcelona, Bellaterra, Spain.
[4.]Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom.
[5.]INSERM U1215, Neurocentre Magendie, Bordeaux, France.

Introduction: Down syndrome (DS), is the most significant genetic cause of intellectual disability for which there is no approved clinical treatment yet. By the age of 40 an increasing proportion of DS individuals present Alzheimer's disease (AD), with dementia incidence increasing among this population due to longer lifespan. The endocannabinoid system plays a crucial function in memory performance and has been proposed to be implicated in the pathophysiology of DS. Indeed, cannabinoid type-1 receptor (CB1R) function and expression were enhanced in the hippocampus of young-adult trisomic Ts65Dn mice. The sub-chronic administration of rimonabant, a CB1R antagonist/inverse agonist, restored the main neurological deficits in the mouse model, but whether a long-term sustained CB1R inhibition would be efficacious, or even prevent neurodegenerative processes in the aging trisomic model, has not been addressed so far.

Methodology: Male and female Ts65Dn trisomic and wildtype (WT) mice were treated with a low dose of rimonabant through the drinking water. A similar cohort of mice was exposed in parallel to a placebo solution as control/vehicle condition. Mice were included in the study at the age of weaning (postnatal day 21). Rimonabant dose was titrated in the same cohort of mice to the final concentration of 0.5 mg/kg/day at fourth months of age, to follow this dosage until mice were euthanized at ten months of age. All mice were behaviorally assessed for non-emotional long-term memory performance with the novel object-recognition memory paradigm, for anxiety-like behavior with the elevated-plus maze test and for locomotor activity with actimetry boxes. Rimonabant levels were quantified in brain homogenates and plasma samples by mass spectrometry analysis at the end of the treatment.

Results: Rimonabant oral treatment did not modify overall body weight or survival rate for the length of the experiment. During this period, mice were behaviorally assessed at different time points. After 3 months of treatment with rimonabant (aprox. 4 months of age), object-recognition memory performance of Ts65Dn trisomic mice was similar to that of wildtype controls and significantly better than Ts65Dn mice exposed to placebo. Instead, rimonabant treatment did not affect the anxiety-like behavior or the hyperlocomotor phenotype of Ts65Dn mice. When this cohort of mice was assessed at 10 months of age, a time point when Ts65Dn mice display a well-described neurodegenerative phenotype, trisomic mice treated with rimonabant showed a sustained improvement of object-recognition memory performance compared to placebo-treated trisomic mice.

Conclusions: These results reinforce the potential of CB1R chronic inhibition as a pharmacological approach relevant for the improvement of cognitive deficits associated with AD in the context of DS.

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PRO-CONVULSANT SYNTHETIC CANNABINOID RECEPTOR AGONISTS: PHARMACOLOGICAL AND TOXICOLOGICAL ASSESSMENT, AND *IN VIVO* EVALUATION OF FIRST-LINE EMERGENCY TREATMENTS

Richard C. Kevin[1*], Elizabeth A. Cairns[1], Samuel D. Banister[1], Michelle Glass[2], Mark Connor[3] and Iain S. McGregor[1]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, NSW, Australia
[2]Department of Pharmacology and Toxicology, Otago University, New Zealand
[3]Faculty of Medicine and Health Sciences, Macquarie University, NSW, Australia

Introduction: Synthetic cannabinoid receptor agonists (SCRAs) are the fastest growing class of novel psychoactive substance and are used and abused as intoxicants despite numerous associated health risks. In most cases, newly detected SCRAs are uncharacterised in terms of their pharmacology and toxicology. Seizures are a commonly reported adverse effect of SCRA use, but the mechanism by which this occurs is poorly understood. Moreover, current emergency treatments for SCRA-induced seizures remain untested in a controlled setting due to the lack of an appropriate animal model.

Methods: We routinely screen novel SCRAs using radioligand binding assays and fluorescence-based plate reader membrane potential assays, coupled with in vivo testing using radio-biotelemetry, to evaluate cannabimimetic activity and toxicology. During this screening, a subset of compounds produced substantial convulsant behaviours in mice. These behaviours were characterised and quantified using a modified Racine scale of seizure severity. The newly characterised convulsant response was then used as an animal model of the SCRA convulsant toxidrome. We tested whether several potential treatments, including SR141716, AM-630, diazepam, and cannabidiol (CBD), could block SCRA-induced convulsant behaviours.

Results: In vitro, many current-generation SCRAs were potent, cannabinoid 1 (CB1) receptor preferring agonists with low nanomolar EC50s and binding affinity. In mice, substantial dose-dependent hypothermic effects were observed for most - but interestingly, not all - SCRA compounds. These effects could be blocked by CB1 receptor antagonist SR141716, but not CB2 receptor antagonist AM-630, indicating the expected CB1 receptor mediated mechanism. Seizure-like behaviours, which occurred at doses as low as 0.3 mg/kg for multiple compounds, could similarly be blocked with SR141716 but not AM-630 or CBD, and were attenuated with high doses of diazepam.

Conclusions: These data show that, with some exceptions, the latest generation of SCRAs are highly potent CB1 receptor-preferring agonists, some of which produce convulsant effects in mice. These properties may underpin the toxicity associated with SCRA use in humans. In vivo pharmacological experiments support the use of first-line emergency administration of benzodiazepines for controlling SCRA-induced seizures.

CANNABINOID-1 RECEPTORS (CB1R) MODULATE WHITE ADIPOSE TISSUE LIPOLYSIS IN LEAN BUT NOT IN OBESE RODENT AND HUMAN

Chloé Buch*, Tania Muller, Julia Leemput, Patricia Passilly-Degrace, Pablo Ortega-Deballon, Jean-Paul Pais de Barros, Bruno Vergès, Tony Jourdan, Laurent Demizieux and Pascal Degrace

Team Pathophysiology of Dyslipidemia, INSERM-UB 1231, Dijon, France

Introduction: Endocannabinoid system (ECS) activity is positively correlated to fat mass and associated with metabolic risk in both mice and human. Accumulating evidence indicates that cannabinoid-1 receptors **(**CB1R) present in white adipose tissue (WAT) are involved in the regulation of fat storage, tissue remodeling and secretory functions but their role in lipid mobilization control is unclear.

Methods: Lean mice or rat WAT explants were treated with JLZ195 to inhibit endocannabinoid degrading enzyme activity and increase endocannabinoid tone while in other experiments, rat WAT explants were directly incubated with anandamide. Endocannabinoid tissue levels and production were determined by LC-MS/MS. Then, the consequences on lipolysis and regulatory pathways were evaluated measuring glycerol production, cAMP content and Akt activity. These parameters were also analyzed in obese mice fed a high fat diet for 16 weeks. In parallel, lipolytic activity was determined in abdominal visceral and subcutaneous adipose tissue collected from lean and obese subjects.

Results: JLZ195 led to an increase in anandamide tissue contents that was associated with a CB1R-dependent decrease in lipolysis in lean mice or rat WAT explants. Direct exposure of rat WAT explants with anandamide also inhibited glycerol production while mechanistic studies revealed the activation of Akt-signaling pathway. Interestingly, anandamide decreased lipolysis both in visceral and subcutaneous WAT collected on lean subjects suggesting that ECS may also reduce fat store mobilization in humans. In obese mice, WAT content and secretion rate of anandamide were higher than in control while glycerol production was reduced. However, CB1R blockade with Rimonabant did influence lipolysis neither *in vitro* in obese mice and human explants nor *in vivo* in obese mice.

Conclusions: Taken together, these data suggest that activation of ECS in WAT, by limiting fat mobilization, may participate in the progressive tissue remodeling which finally leads to organ dysfunction. The present findings also indicate that CB1R blockade is inefficient in regulating lipolysis in obese WAT and raise the possibility of an alteration of CB1R signaling in conditions of obesity.

THE OPPOSING ROLE OF CB1 RECEPTOR IN MODULATING RENAL PROXIMAL TUBULE MTORC1 SIGNALING

Liad Hinden[1*], Majdoleen Ahmad[1], Sharleen Hamad[1], Alina Nemirovski[1], Gergő Szanda[2], Sandra Glasmacher[3], Aviram Kogot-Levin[4], Bernard Thorens[5], Jürg Gertsch[3], Gil Leibowitz[4] and Joseph Tam[1]

[1]Obesity and Metabolism Laboratory, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel. [2]Department of Physiology Semmelweis University, Budapest, Hungary. [3]Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland. [4]Diabetes Unit and Endocrine Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel. [5]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.

Introduction: Diabetic chronic kidney disease (CKD), the renal manifestation of diabetes, contributes to increased morbidity and mortality of patients with diabetes. Activation of the cannabinoid-1 receptor (CB_1R) and the mammalian target of rapamycin complex 1 (mTORC1) in the renal proximal tubular cells (RPTCs) contributes to the development of diabetic CKD. However, whether these two signaling molecules interact with each other to regulate kidney function during health and disease has not been described yet.

Methods: By using a multidisciplinary approach that includes pharmacological and genetic manipulations of CB_1R and mTORC1, we assessed the effect of CB_1R activation/inhibition on mTORC1 activity under conditions of acute and chronic hyperglycemia or normoglycemia in RPTCs and mice.

Results: We show that hyperglycemia-induced endocannabinoid/CB_1R stimulation, mainly via the synthesis of 2-AG. This stimulation increased mTORC1 activity in a Gi and phosphoinositide 3-kinases (PI3K) dependent manners, enhancing the expression of sterol regulatory element-binding protein 1c (SREBP1c) and its nuclear translocation, which in turn, induced the transcription of the facilitative glucose transporter 2 (GLUT2), thus leading to the development diabetic CKD in mice. This effect was ameliorated by CB_1R nullification or GLUT2 ablation specifically in RPTCs. However in non-diabetic conditions, CB_1R maintained normal activation of mTORC1 by preventing the cellular excess of various amino acids via regulating their transporters, megalin and SLC6A19.

Conclusions: Our findings highlight two novel molecular mechanisms by which the activation of mTORC1 in RPTCs is tightly controlled by CB_1R, either by enhancing the reabsorption of glucose and inducing renal dysfunction in diabetes or by preventing amino acid uptake and maintaining normal kidney function in healthy conditions. Moreover, this work highlight the therapeutic potential of targeting peripheral CB_1Rs for the treatment of diabetic CKD, and on the other hand, avoiding its use in non-diabetic patients due to their possible effect in enhancing mTORC1 signaling.

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RENAL PROXIMAL TUBULE CELL CANNABINOID-1 RECEPTOR REGULATES BONE REMODELING AND MASS VIA A KIDNEY-TO-BONE AXIS

Saja Baraghithy[1*], Yael Soae[1], Dekel Assaf[1], Liad Hinden[1], Shiran Udi[1],

Adi Drori[1], Yankel Gabet[2] and Joseph Tam[1]

[1]Obesity and Metabolism Laboratory, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 9112001, Israel;
[2]Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

Introduction: The renal proximal tubule cells (RPTCs), well-known for maintaining glucose and mineral homeostasis, play a critical role in the regulation of kidney function and bone remodeling. Consequently, deterioration in RPTC function may therefore lead to the development of diabetic kidney disease (DKD) and osteoporosis. Previously, we have shown that the cannabinoid-1 receptor (CB_1R) modulates both kidney function as well as bone remodeling and mass. Here, we aim to assess whether RPTC CB_1R is the missing link between osteoporosis onset secondary to loss of kidney function in diabetes.

Methods: We employed genetic and pharmacological approaches in mice that target CB_1R in the RPTCs under normal and diabetic (Streptozotocin; 50 mg/kg/d for 5 days) conditions. Skeletal characterization was performed by utilizing combined μCT/histomorphometric analysis, measuring serum markers of bone turnover, and assessing bone strength by a 3-point bending test. Assessment of gene and protein expression levels *in vivo* and in two cell lines of RPTCs (HK-2 and LLCPK1) was performed by qPCR, western blot, and immunohistochemistry.

Results: Specific nullification of CB_1R in RPTCs preserved bone mass and remodeling under both normo- and hyper-glycemic conditions. Chronic daily blockade (for 16 weeks) of CB_1R (by SLV-319, 3 mg/kg, PO) prevented the development of diabetes-induced bone loss. These protective effects of negatively targeting CB_1R specifically in RPTCs were associated with its ability to modulate erythropoietin (EPO) synthesis in the kidney, a hormone known to affect bone mass and remodeling.

Conclusions: Our findings highlight a novel molecular mechanism by which CB_1R in RPTCs remotely regulates skeletal homeostasis via a kidney-to-bone axis that involves EPO. Antagonism of CB_1R in RPTCs has a therapeutic potential in treating diabetes-induced osteoporosis.

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THE ENDOCANNABINOID SYSTEM GATES FAST LEARNING

Charlotte Piette*, Sylvie Perez and Laurent Venance

Dynamics and Pathophysiology of Neuronal Networks Team, Center for Interdisciplinary Research in Biology, College de France, CNRS, INSERM, PSL University, Paris, France.

Introduction: Fast learning, designating the behavioral and neuronal mechanisms underlying the acquisition of a long-term memory after a unique and brief experience, is a crucial mechanism for developing efficient and adaptive responses. Despite its behavioral prevalence, the neural correlates of fast learning remain elusive (see for review: Piette et al., Front. Cell. Neurosci. 14 (2020) 575915). In this study, we aimed at elucidating how changes in cortico-striatal dynamics contribute to fast learning. Considering that a brief exposure to a stimulus involves only a small number of spiking events and based on our earlier *in vitro* work uncovering a new form of synaptic potentiation induced by a very low number of temporally coupled cortical and striatal spikes and dependent on the endocannabinoid system (Cui et al., J Physiol. 593 (2015) 2833-49; Cui et al., eLife 5 (2016) e13185; Cui et al., Science Rep. 8 (2018) 8139; Xu et al., Nat. Comm. 9 (2018) 4118; for review see: Piette et al., Front. Mol. Neurosci. 13 (2020) 132), we hypothesize that the endocannabinoid system could underlie the acquisition of fast learning in the striatum.

Methods: We first developed a fast learning task in which mice learn to avoid contact with an adhesive tape after a single exposure. We then used *in vivo* and *ex vivo* electrophysiological recordings in the striatum of behaving mice to probe cortico-striatal plasticity and the specific contribution of the endocannabinoid system. Finally, we test the performance of transgenic mouse strains in which endocannabinoid-dependent potentiation (eCB-LTP) is altered.

Results: We observed the emergence of a cortico-striatal potentiation 24h after the first exposure to the adhesive tape. *Ex vivo* occlusion plasticity experiments confirmed the implication of the endocannabinoid pathway. Furthermore, mice in which eCB-LTP was impaired by knocking-out cortical presynaptic CB1 or dopaminergic type-2 receptors (Xu et al., Nat. Comm. 9 (2018) 4118) exhibited degraded fast learning performance.

Conclusions: Cortico-striatal eCB-LTP could represent a molecular substrate for the fast learning of associations and goal-directed strategies. Therefore, manipulation of endocannabinoid levels in the striatum could allow to control the learning performance in one-shot tasks.

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ENDOCANNABINOID-MEDIATED MODULATION OF NEURON-GLIA CROSSTALK REVERSES NEUROPATHOLOGY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Rocío Palenzuela[1*], Gonzalo Ruiz-Pérez[1], Sharai Marqués[1], Noelia Aparicio[1], M. Teresa Grande[1], Irene Benito-Cuesta[1], Ana M. Martínez-Relimpio[1], Samuel Ruiz de Martín Esteban[1], María Posada[1], Rosa M. Tolón[1], Benjamin F. Cravatt[2], José A. Esteban[3] and Julián Romero[1]

[1]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, Pozuelo de Alarcón, 28223, Madrid, Spain; [2] The Skaggs Institute for Chemical Biology and Departments of Cell Biology and Chemistry, The Scripps Research Institute, La Jolla, California, 92037, USA; [3]Centro de Biología Molecular Severo Ochoa, CSIC-Universidad Autónoma de Madrid, 28049, Madrid, Spain.

Introduction: Alzheimer's disease (AD) is a devastating disorder that entails a complex pathophysiological scenario. The lack of effective treatments to prevent neurodegeneration highlights the need for further clarification of the underlying cellular and molecular mechanisms. Neuroinflammation, which crucially depends on neuron-glia interaction, seems to play a critical role in disease progression; nevertheless, its specific contribution to AD pathogenesis remains elusive. Several regulatory systems, such the endocannabinoid system (ECS), influence both neuroinflammation and neuron-glia crosstalk, thus positioning the ECS as a strong candidate to target to modify disease outcome. We have previously shown that the modulation of the ECS renders beneficial effects in a context of amyloid-triggered neuroinflammation. In the 5xFAD mouse model, the genetic inactivation of the enzyme that degrades anandamide (AEA), the fatty acid amide hydrolase (FAAH), was associated with a significant amelioration of the memory and synaptic plasticity deficits that characterize this model of AD.

Methods: To unveil the detailed mechanisms underlying the phenotypic rescue in 5xFAD/FAAH$^{-/-}$ animals, we determined dendritic spine density and morphology in CA1 pyramidal neurons from 5xFAD and 5xFAD/FAAH$^{-/-}$ mice. Furthermore, we carried out a search for molecular markers that correlate with the cognitive and functional improvement associated with FAAH deletion in 5xFAD animals.

Results: We now show that dendritic spine density in CA1 pyramidal neurons, which is notably decreased in 6-month-old 5xFAD animals, is restored when FAAH is genetically inactivated in this model. Importantly, we also reveal that while neuronal markers linked to synaptic function are not modified, the expression of microglial factors associated with phagocytic activity, such as TREM2, and of astrocytic factors involved in astroglia-microglia crosstalk, such as complement component C3, are specifically upregulated in 5xFAD/FAAH$^{-/-}$ mice.

Conclusion: In summary, our findings point to an intricate scenario in which the interaction between neurons, microglia and astroglia is crucial to prevent neuropathology and thus, restore neuronal functionality in the AD pathological context.

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MODULATION OF ENDOCANNABINOID SIGNALING IN THE HIPPOCAMPUS BY CHRONIC PAIN

Marta Bryk[1*], Jakub Mlost[1], Serena Boccella[2], Monica Iannotta[2], Rosmara Infantino[2], Sabatino Maione[2] and Katarzyna Starowicz[1]

[1]Maj Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland; [2] Department of Experimental Medicine, Division of Pharmacology, University of Campania "L. Vanvitelli", Naples, Italy

Introduction: Reflecting its role in several brain functions such as learning, memory and affective processing, the hippocampus is a very plastic brain structure. The science of pain research has for the most part ignored the involvement of the hippocampus in pain perception, most likely due to a lack of convincing nociceptive projections to the structure. However, Mutso et al. [1] were also able to show physiological, molecular, and neurogenesis abnormalities in the hippocampus of neuropathic injury rodents. Therefore, we aimed at expanding the idea by placing the brain learning and memory circuitry as central to an adequate understanding of chronic pain. The involvement of cannabinoid system (EC) is widely supported by recent studies on different models of chronic pain management [2]. The aim of our study was to examine hippocampal changes in animals suffering from chronic pain associated with osteoarthritis (OA) and the role of EC signaling in hippocampal functioning in rats with persistent pain.

Methods: In this study, male Wistar rats underwent intra-articular knee injection of NaCl (sham) or 1 mg of monoiodoacetate (MIA), to induce OA-like lesions. On day 28 post-MIA injection, following analysis were performed: mechanical pain threshold through Pressure Application Measurement (PAM) test, tactile allodynia assessed by von Frey test, long-term potentiation (LTP) in the dentate gyrus (DG) induced by theta-burst stimulations (TBS) of lateral entorhinal cortex (LEC) and extracellular levels of dopamine and serotonin in the DG collected through microdialysis in vivo, and analyzed by using HPLC method. Experimental groups were divided in Sham or MIA rats that received vehicle or URB597 (1 mg/Kg, i.p.) (selective inhibitor of FAAH enzyme) alone or in combination with the CB1 inverse agonist, AM251 (1 mg/Kg, i.p.).

Results: Behavioral tests, showed that systemic injection of URB 597 was able to reduce knee hyperalgesia in MIA rats, 28 days post-induction, at the peak of activity between 60-120 minutes post-drug. This effect was blocked by AM251 pre-treatment. Moreover, the same treatment significantly restored the altered LTP in DG-LEC connectivity, by increasing amplitude and slope of fEPSPs, in MIA rats. Finally, MIA animals showed lower dopamine and higher serotonin levels in hippocampal DG in comparison with naïve individuals. URB597 treatment significantly reversed altered dopamine and serotonin levels in hippocampal DG in MIA animals.

Conclusions: Current results indicate not only analgesic effect of URB597, but also its role in LTP formation in LEC-DG pathway and neurotransmitters extracellular levels in hippocampal DG. We observe robust behavioral, neurochemical, and synaptic changes in the hippocampus of animals with OA, therefore, targeting the reversal of these systematic changes in chronic pain could improve both patient quality of life and actual pain behavior.

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ENDOCANNABINOID-MEDIATED ENHANCEMENT OF HIPPOCAMPAL LONG-TERM POTENTIATION

Eric S. Levine* and Fouad Lemtiri-Chlieh

Department of Neuroscience, University of Connecticut School of Medicine, Farmington, CT, USA

Introduction: It is widely accepted that exogenous cannabinoids can impair short-term memory and cognition in humans and other animals. This is likely related to the inhibition of long-term potentiation (LTP), a form of synaptic plasticity, by the global and sustained activation of CB1 cannabinoid receptors in the presence of exogenous agonists. Conversely, the temporally and spatially-restricted release of endogenous cannabinoid ligands may enhance synaptic plasticity in a synapse-specific manner. The functional roles of endocannabinoids (eCBs) are complex because they can modulate synaptic transmission via suppression of GABA and glutamate release, with opposing effects on postsynaptic excitability.

Methods: All experiments were performed on postnatal day 15-30 CD-1 mice using protocols approved by the University of Connecticut Institutional Animal Care and Use Committee. Coronal slices containing the hippocampus were cut with a vibratome and transferred to a recording chamber continuously perfused with carboxygenated artificial cerebrospinal fluid at room temperature. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of the hippocampus in response to Schaffer collateral stimulation. LTP was induced by theta-burst stimulation (TBS) of the Schaffer collaterals or by exposure for 15 min to forskolin and rolipram in the absence of extracellular magnesium, which increases cAMP levels and enhances NMDA receptor activation.

Results: We examined the role of eCB signaling in LTP by recording fEPSPs in the CA1 stratum radiatum in hippocampal slices from juvenile mice. Significant LTP (~50% increase from baseline) was induced by either 1 or 3 trains of TBS. The magnitude of this potentiation was significantly reduced by preventing cannabinoid receptor activation with the selective CB1 receptor antagonist NESS-0327 (~25% increase from baseline). LTP was inhibited to a similar extent by preventing the synthesis of the endogenous ligand 2-AG using the DAG lipase inhibitor DO34. A comparable effect of CB1 blockade was also observed with pharmacologically-induced LTP.

These results suggest that activation of CB1 receptors by endogenous 2-AG normally enhances the magnitude of LTP, leading to the prediction that the predominant eCB effect under these conditions is to suppress inhibition. We therefore examined TBS-induced LTP while blocking inhibitory synapses with the GABA-A receptor blocker picrotoxin (PTX). The addition of PTX alone caused a slight, but not significant, increase in LTP. Consistent with our hypothesis, PTX completely prevented the effect of blocking CB1 receptors, indicating that intact GABAergic transmission is required for the eCB effect. The presence of PTX did not unmask any enhancing effect of CB1 receptor blockade, suggesting minimal eCB-mediated suppression of glutamate release under these conditions.

We have previously shown that TBS stimulation can cause a BDNF-dependent increase in eCB release at inhibitory synapses. Consistent with this, we found that blocking TrkB receptor activation with ANA-12 inhibited LTP to the same extent as blocking CB1 receptors. Interestingly, however, the effect of TrkB antagonism on LTP was still observed in the presence of PTX. This suggests that endogenous BDNF may enhance LTP by acting directly on glutamatergic synapses, in addition to triggering eCB release. Ongoing experiments are further exploring the role of BDNF-eCB interactions in the modulation of LTP.

Conclusions: These results indicate that 2-AG activation of CB1 receptors enhances TBS-induced and pharmacologically-induced LTP, and this effect is caused by the actions of 2-AG at inhibitory synapses, with minimal effects at excitatory synapses.

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ROLE OF HIPPOCAMPAL TRPV1 CHANNELS IN MEMORY: A TRPV1-CB1 BALANCE

Lia P. Iglesias[1*], Carlos Sorgi[2] and Fabricio A. Moreira[1]

Department of Pharmacology, Universidade Federal de Minas Gerais, Minas Gerais, Brazil.[1] Faculty of Pharmaceutical Sciences of Ribeirão Preto, Universidade de São Paulo, São Paulo Brazil.[2]

Introduction: Certain psychiatric disorders can be conceptualized as maladaptation in associative learning mechanism. Thus, pharmacological approaches that impair associative memories have been investigated as new treatments. The dorsal hippocampus (dHPC) is one of the key structures in processing contextual stimulus in associative learning. Moreover, hippocampal TRPV1 channels seem to modulate biological processes related to memory and learning, its role seems to be intensity-dependent and limited by age-dependent expression of the channel. TRPV1 channels are activated by AEA, but this compound has twenty-times more affinity for CB1. In this vein, we hypothesized that TRPV1, in balance with CB1 and AEA, may be a potential target in treating maladaptive memory underlying psychiatric disorders. The aim of this work was to evaluated the role of TRPV1 channels in dHPC dependent-memories (appetitive, aversive and neutral) in different conditions and phases as well as its relation with other pieces of the endocannabinoid system.

Methods: Nine or twenty weeks-old C57BL/6J mice were submitted to cannulation of dHPC and tested in different behavioural paradigms: cocaine-induced conditioned place preference (appetitive memory), novel object recognition test (neutral memory) and contextual fear conditioning (aversive memory, 3x 1s 500mA shock or 3x 2s 500mA shock). A TRPV1 blocker, SB36679 (1, 3 or 10nmol) was administered into the dHPC to evaluate the involvement of TRPV1 channels in these paradigms. In order to further investigate the role of CB1 and AEA involved in SB366791 effect, animals were pre-treated with a subeffective dose of AM251 (75pmol), a CB1 antagonist. In addition, hippocampal levels of AEA were quantified after different intensities of contextual fear conditioning using high performance liquid chromatography followed by Mass Spectrometry. Behavioural results were evaluated by t-student or ANOVA followed by Bonferroni post-hoc. The relation between freezing and AEA levels were evaluated by Pearson's. Values of $p<0.05$ was considered significant. Ethical approval, CEUA: 176/2020.

Results: TRPV1 blockers impaired retrieval of aversive memory without interfering with appetitive and neutral memories dependent on dHPC. In addition, TRPV1 blockers had no effect in other memory phases of aversive memory, as acquisition, consolidation or extinction. Curiously, in nine-weeks old mice TRPV1 blockers impaired retrieval when the animals were conditioned using more aversive (2s) but not low aversive (1s) procedures. In contrast, in twenty-weeks old mice, despite the low expression of the channel, SB366791 reduced freezing even in low aversive conditions. This effect of SB366791 in more aversive conditions was prevented by a subeffective dose of AM251, suggesting that blocking TRPV1 increases the availability of AEA to act trough CB1. In the same vein, AEA levels correlated with freezing, which may suggest that low aversive conditioning induces low AEA release which would not be enough to recruit TRPV1.

Conclusion: Hippocampal TRPV1 channels seem specifically involved in retrieval of aversive contextual memory. Our results suggest that the intensity-dependent recruitment of TRPV1 is due to the increased availability of AEA in more aversive procedures. In conclusion, TRPV1 seems a potential target to interfere high intense aversive memories underlying psychiatric disorders.

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HOW DANGEROUS ARE SYNTHETIC CANNABINOID RECEPTOR AGONISTS?

Pascal Vogiaridis and Steve Alexander*

School of Life Sciences, University of Nottingham Medical School,
Nottingham NG7 2UH ENGLAND

Introduction: Synthetic Cannabinoid Receptor Agonists (SCRAs), also known as Spice, Black Mamba, etc., are drugs intended to mimic the action of THC. They moved from the lab to the streets as a "legal, safe high", initially as an attempt to avoid detection by standard drug tests. The current usage in the US and UK appears to be most frequent amongst the incarcerated and homeless, with a perception of harm and even fatalities. The objective of this study was to conduct a systematic review of the current literature of SCRA-associated harms.

Methods: An initial search of PubMed was made in November 2020 resulting in an accumulation of 439 papers. Specific inclusion/exclusion criteria led to a focus on 50 studies, 26 of which allowed quantification of the relative frequency of multiple symptoms. The remainder explored individual symptom profiles in more detail.

Results: The most common clinical manifestations involved the central nervous or cardiovascular systems, appearing in 26/26 and 24/26 studies, respectively. Symptoms included cardiac arrhythmias, blood pressure abnormalities, an altered state of consciousness, agitation/aggression, anxiety/confusion, instances of nausea and vomiting, and in more severe cases, hallucinations/perceptual changes, seizures, acute kidney injury and respiratory failure.

Conclusions: The precise mechanisms by which these agents elicit these effects are unclear. SCRAs in general have higher potency and efficacy at CB_1 cannabinoid receptors compared to THC, and clearly lack the manifold other chemical components found in *Cannabis* preparations. Additionally, illicit SCRAs vary tremendously in composition and concentration. Whether these effects are CB_1 receptor-mediated or off-target will require additional investigation. Current treatments in emergency departments and admission units will need to continue to focus on the alleviation of severe symptoms until a greater mechanistic insight allows focus on more precise pathways.

SUBSTITUTING CANNABIDIOL FOR OPIOIDS AND PAIN MEDICATIONS AMONG INDIVIDUALS WITH FIBROMYALGIA: A LARGE ONLINE SURVEY

Kevin F. Boehnke[a*], Joel J. Gagnier[b], Lynne Matallana[c] and David A. Williams[a]

[a]Anesthesiology Department, University of Michigan Medical School, Ann Arbor, MI, USA
[b]Orthopaedic Surgery, University of Michigan Medical School, Ann Arbor, MI, USA
[c]National Fibromyalgia Association, Newport Beach, CA, USA

Introduction: People substitute cannabis for pain medications, but whether cannabidiol (CBD) is used similarly remains unknown. CBD-containing products can be categorized as CBD alone (isolate), hemp extract (containing <0.3% Δ-9-tetrahydrocannabinol [THC], other cannabinoids, and terpenes), or CBD-cannabis (containing >0.3% THC). We previously showed that over 30% of people with fibromyalgia report current CBD use, mainly due to inadequate relief from medications. In the current study, we conducted a secondary analysis to investigate medication substitution patterns among n = 878 individuals with fibromyalgia currently using CBD products.

Methods: We sub-grouped participants by most commonly used CBD product category (CBD isolate, hemp, CBD-cannabis, no preference) and whether they substituted CBD for medications. We investigated whether participants substituted CBD products for medications, substitution-driven medication changes (e.g., cessation of use), rationale for substituting, and changes in pain-related symptoms (e.g., sleep, anxiety). Analyses included ANOVA with post-hoc Tukey's tests and t-tests for continuous variables and Pearson's chi-square tests for categorical variables.

Results: The study population was 93.6% female, 91.5% Caucasian, and aged 55.5 years on average. The majority (n = 632, 72.0%) substituted CBD for pain medications, most commonly NSAIDs (59.0%), opioids (53.3%), gabapentanoids (35.0%), and benzodiazepines (23.1%). Most substituting participants reported decreasing or stopping use of these pain medications (70.4% - 93.6% depending on medication class). The most common reasons for substitution were fewer side effects (43.2% - 63.0%) and better symptom management (17.9% - 38.3%), with ranges reflecting differences by medication class. Participants using CBD-cannabis reported significantly more substitutions than any other group and larger improvements in health, pain, memory, and sleep than other CBD product subgroups (all p's < 0.05). Those who substituted reported significantly larger improvements in health, pain, anxiety, and sleep than those who did not (all p's < 0.05).

Conclusions: This is one of the first reports of substituting CBD products for pain medications among people with fibromyalgia. This widespread naturalistic substitution of CBD products for pain medications suggests the need for more rigorous study designs examining the therapeutic effects of different CBD products as well as stricter CBD-product regulatory standards to ensure accurate CBD product labeling and safety. Further study is also needed to better identify why substituting products with more THC were associated with greater benefit.

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ABSTRACT WITHDRAWN

GLOBAL ADVERSE EVENTS AMONG CANNABIS USERS IN 2020

Maja Kalaba, Erica Peters, Chanez N. Kebache and Mark A. Ware*

Canopy Growth Corporation, Montreal, QC, CAN

Introduction: An estimated 188 million individuals use cannabis worldwide. As of 2020 Canopy Growth Corporation (CGC) primary consumer markets include: Canada, US, Germany, UK and Australia. The objective of this analysis is to describe the adverse events (AEs) collected globally by CGC in 2020, stratified by product profile, in support of the development of safety guidelines for cannabis products.

Methods: Between January and December 2020, AEs in the CGC global pharmacovigilance database were collected from multiple solicited and unsolicited sources. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), and seriousness criteria were defined by the International Conference of Harmonization (ICH). Products consumed were classified by content of tetrahydrocannabinol (THC) and cannabidiol (CBD). Where an individual was consuming multiple products, the AE reported was counted for each suspected product. For this analysis, we excluded AEs that were coded as "lack of efficacy", "product issue" and "invalid cases". All AEs included in the analysis were verified by a healthcare professional. This analysis pertains to data extracted as of Jan. 29, 2021. Because the outcome of AEs initially reported in 2020 was not always available or reported by the date of data extraction, data on resolution status of AEs reported in 2020 were not analyzed.

Results: A total of 2238 individual case safety reports, and 5765 AEs were reported in 2020. Ninety-five percent (n=5480) of AEs were reported as non-serious. AEs were distributed across 22 System Organ Class (SOC) categories. Of the 285 (5%) serious AEs, no differences were observed across product profile: CBD-dominant (n= 87, 30.5%), Balanced (n=78, 27.4%), and THC-dominant (n=73, 25.6%).

Formulations included: oil (63%), softgel (24%), flower (9%), unknown (2%), beverage (<1%), cartridge (<1%), chocolate (<1%), cream (<1%), gummies (<1%).

MedDRA **SOC** Non-serious AEs n=5480[1]	**Total Adverse Events n (%)**	Product Profile: Balanced (THC:CBD) product n (%)	Product Profile: CBD-dominant product n (%)	Product Profile: THC-dominant product n (%)	Product Profile: Unknown n (%)
Nervous system disorders	**1172**	447 (38)	417 (36)	287 (24)	21 (2)
Gastrointestinal disorders	**1142**	343 (30)	544 (48)	233 (20)	22 (2)
Psychiatric disorders	**929**	369 (40)	287 (31)	246 (26)	27 (3)
General disorders and administration site conditions	**850**	264 (31)	382 (45)	193 (23)	11(1)
Respiratory thoracic and mediastinal disorders	**389**	91 (23)	147 (37)	146 (37)	5 (1)

[1]Due to abstract limit only the 5 most prevalent SOC categories are presented above.

Conclusions: The vast majority of AEs reported in 2020 with CGC products were non-serious; this prevalence is similar to the prevalence of non-serious AEs reported in a systematic review of prescription cannabinoid use. Differences were observed in the type of AE experienced by product profile, with Psychiatric disorders most common among THC-dominant and balanced products, and Gastrointestinal disorders more common among CBD-dominant products. The findings provide an important description of the safety profile of cannabis and demonstrate the need for global pharmacovigilance programs for licensed cannabis producers.

CANNABIDIOL EFFECTIVENESS ON SYMPTOM BURDEN: A RETROSPECTIVE OBSERVATIONAL STUDY ON CANADIAN PATIENTS

Lucile Rapin[1*], Cynthia El Hage[1], Maria-Fernanda Arboleda[1], Michael Dworkind[1,2] and Erin Prosk[1]

[1]Santé Cannabis, Montreal, QC, Canada;
[2]Department of Family Medicine, McGill University.

Introduction: Cannabidiol (CBD) is gaining attention for claims of its multi-symptom modality. In recent decades, scientific research has consolidated some evidence of CBD as a potential therapeutic to treat several medical conditions. With a beneficial safety profile compared to delta-9-tetrahydrocannabinol (THC) but often prohibitive price point, questions of the effectiveness of CBD in clinical practice remain. This retrospective study assessed CBD-rich treatments on overall symptom burden and the effect of transition from a CBD-rich to a CBD:THC combination in patients treated at a network of medical cannabis clinics in Quebec, Canada.

Methods: Adult patients, over 18 years of age initially treated exclusively with CBD-rich products and with available Edmonton Symptom Assessment System – revised version (ESAS-r) scores and product information at 3-month follow up (FUP3M) were included in this study. ESAS-r was used to assess the very common clinical symptom expression of pain, depression and anxiety as well as overall well-being at baseline (BL) and at FUP3M. Participants were grouped according to the number of clinically relevant symptoms (defined as a score of 4 and above at BL on the ESAS-r). Three-way mixed ANOVAs were used to assess ESAS-r scores differences between groups, product change (CBD to CBD:THC at FUP3M) and between visits. A p value of ≤ 0.05 was taken as statistically significant.

Results: A total of 279 patients were analysed, with 73% of patients having clinically relevant ESAS-r scores on at least 2 of the examined symptoms (Sx) (Table 1). Improvement at FUP3M was observed on all symptoms for the 4Sx group (Figure 1) and for pain, depression and anxiety symptoms in the 3Sx group. This clinical improvement was not present in groups with zero, one or two clinically relevant Sx. Groups 2Sx, 3Sx and 4Sx experienced significant improvement from baseline to FUP3M in pain ($p < 0.003$) and overall well-being ($p < 0.001$), whereas pain scores did not change between visits for group with zero or one Sx and wellbeing scores actually significantly increased for patients with no clinically relevant Sx. Anxiety and depression scores from patients in the 3Sx and 4Sx groups significantly improved between visits whereas groups with zero, 1 or 2 Sx did not experience changes in scores (both $p < 0.001$). There was no main effect of product as well as no significant interactions between visit and product or group and product.

Conclusion: Our results show that oral CBD-rich products may be effective to treat multiple symptom expression simultaneously. Precisely, this study indicates a more significant positive impact of initiation of CBD treatments in patients who present with a higher number of severe symptoms, regardless of the product used after the first follow-up visit. The examination of treatment plan details during the first follow-up constitutes a novel information to the existing literature and offers insight to prescription guidelines. This retrospective observational study demonstrates the importance of real-world evidence in medical cannabis research, especially in the current global environment where access to CBD treatments outpaces results from randomized clinical trials.

FOUR WEEKS OF TREATMENT WITH A PLANT-DERIVED, FULL-SPECTRUM HIGH-CANNABIDIOL PRODUCT FOR ANXIETY: RESULTS FROM AN OPEN-LABEL CLINICAL TRIAL

Staci A. Gruber[1*], Ashley M. Lambros[1,2], Rosemary T. Smith[1,2], Kelly A. Sagar[1,2,3] and M. Kathryn Dahlgren[1,2,3]

[1]Cognitive and Clinical Neuroimaging Core (CCNC), McLean Imaging Center, Belmont, MA
[2] Marijuana Investigations for Neuroscientific Discovery (MIND) Program, McLean Hospital Imaging Center, Belmont, MA
[3]Department of Psychiatry, Harvard Medical School, Boston, MA, USA

Introduction: Cannabidiol (CBD) is a non-intoxicating cannabinoid believed to have significant therapeutic potential. Acute administration studies have shown that CBD has anxiolytic properties and may mitigate negative physiological and cognitive effects associated with delta-9-tetrahydrocannabinol (THC). However, clinical trials are needed to assess the impact of high-CBD products on anxiety.

Methods: Fourteen patients exhibiting moderate to severe anxiety were enrolled in an open-label phase of the first clinical trial assessing a plant-derived, full-spectrum high-CBD sublingual product, specifically designed to address symptoms of anxiety. Patients administered 1mL of the custom-formulated product containing 10mg/ml CBD three times/day. In addition to assessing anxiety and related symptoms, patients completed a cognitive battery assessing executive function, verbal learning, and memory.

Results: No adverse events or feelings of intoxication were reported. After four weeks of treatment, patients exhibited significant reductions on measures of anxiety (Overall Anxiety Severity and Impairment Scale; Beck Anxiety Inventory), improved mood (Beck Depression Inventory), and better sleep (Pittsburgh Sleep Quality Index) (all $ps<.001$). Improvements on tasks of executive function were also generally noted (e.g., Stroop Interference time: $p<.001$). Although decreased performance was observed on a measure of verbal learning (Rey Auditory Verbal Learning Test [RAVLT] Trials 1-5 Correct: $p=.04$), memory remained stable relative to baseline (e.g., RAVLT Long Delay, $p=.40$).

Conclusions: Initial results are promising and suggest that a full-spectrum, high-CBD product may be efficacious for treating anxiety. A definitive assessment of the impact of this novel treatment on clinical symptoms and cognition will be ascertained in the upcoming double-blind, placebo-controlled phase.

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CANNABINOID HYPEREMESIS SYNDROME SURVEY AND GENOMIC ASSESSMENT

Ethan Russo[1*], Chris Spooner[2], Len May[2], Ryan Leslie[2] and Nishi Whiteley[1]

[1]CReDO Science, [2]EndoCannaHealth

Introduction: Cannabinoid hyperemesis syndrome (CHS) is a constellation of symptoms and signs consisting of intractable vomiting, abdominal pain and hot bathing behavior. CHS solely occurs in the context of heavy chronic use of cannabis or extracts containing high amounts of tetrahydrocannabinol (THC). Diagnostic criteria have been recently tabulated (Sorensen, *J Med Tox* 2017): history of regular cannabis use for over 1 year (74.8%), severe nausea and vomiting (100%), vomiting that recurs in a cyclic pattern over months (100%), resolution of symptoms after stopping cannabis (96.8%), compulsive hot baths/showers with symptom relief (92.3%), male predominance (72.9%), abdominal pain (85.1%), at least weekly cannabis use (97.4%), history of daily cannabis use (76.6%), and age less than 50 at time of evaluation (100%). The syndrome is increasingly identified, particularly in the USA. It is associated with frequent emergency visits for treatment and diagnosis, with high diagnostic expense ($30-90K) and general resistance to treatment with anti-emetics and analgesics. Considerable morbidity and even some fatalities have been reported. The definitive treatment is abstention from cannabis usage, but cutaneous application of capsaicin ointment (TRPV1 agonist/desensitizer) can provide symptomatic relief. The pathophysiology/biochemical basis of CHS may relate to biphasic dose responses to THC, a paradoxical shift of THC from partial agonist to antagonist of CB_1, or to changes in the TRPV1 receptor. The current study investigated mutations that could explain the pathophysiology.

Methods: After Western IRB approval, a screening questionnaire was posted online. Kits were sent to assess the DNA of patients fulfilling CHS criteria to assess single nucleotide polymorphisms (SNPs) or other mutations as compared to controls without this disorder.

Results: 585 people took the survey. Most were high frequency users of cannabis flower or concentrates (93%), using multiple grams/d of THC-predominant material. 15.6% carried diagnoses of cannabis dependency of addiction, and 56.6% experienced withdrawal symptoms. 87.7% of patients with diagnosis or symptoms indicative of CHS were improved after cannabis cessation, most suffering recurrence rapidly after resumption of use. 103 patients who carried formal CHS diagnosis and had consistent symptom profiles were invited to submit oral swabs for genomic testing, 40 patients returned kits for genomic analysis, 28 CHS patients and 12 controls. Findings included mutations in genes coding COMT ($p=0.0009$), TRPV1 ($p=0.021$), CYP2C9 ($p=0.0414$), DRD2 ($p=0.027$) and ABCA1 ($p=0.008$), providing several lines of evidence relating to CHS pathophysiology and clinical manifestations.

Conclusion: This is the largest patient cohort of CHS examined to date, and first to note associated mutations in genes affecting the ECS and neurotransmitter systems that may elucidate the pathophysiology of cannabinoid hyperemesis syndrome.

PHYTOCANNABINOID ACIDS DISPLAY ANTICONVULSANT ACTIVITY IN A MOUSE MODEL OF DRAVET SYNDROME

Lyndsey L. Anderson[1,2], Marika Heblinski[1,2], Nathan L. Absalom[3], Nicole A. Hawkins[4], Somayeh Mirlohi[5], Michael Bowen[1,6], Michael Udoh[1,2], Melissa J. Benson[1,6] Fan Zhang[3], Dilara Bahceci[1,2], Peter T. Doohan[1,2], Chris Bladen[5], Mark Connor[5], Mary Chebib[3], Iain S. McGregor[1,6], Jennifer A. Kearney[4] and Jonathon C. Arnold[1,2*]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Australia.
[2]Discipline of Pharmacology, The University of Sydney, Australia.
[3]School of Pharmacy, The University of Sydney, Australia.
[4]Department of Pharmacology, Northwestern University Feinberg School of Medicine, United States of America.
[5]Faculty of Medicine and Health Sciences, Macquarie University, Australia
[6]School of Psychology, Faculty of Science, The University of Sydney, Australia.

Introduction: Cannabis-based medicines hold great promise as a new class of drugs for the treatment of intractable childhood epilepsies. While the purified cannabidiol (CBD) formulation Epidiolex™ has been approved for the treatment of these epilepsies, many families of children with drug-resistant epilepsies continue to utilise medicinal cannabis formulations. Indeed, the CBD doses utilised in these cannabis formulations are generally much lower than those demonstrated to be effective in clinical trials opening the possibility that other cannabinoid components display anticonvulsant properties. We know that several phytocannabinoids exhibit anticonvulsant properties but there are >100 phytocannabinoids that could be probed for their anticonvulsant potential.

Methods: Conventional seizure models have assisted the discovery of anticonvulsant agents for drug-sensitive epilepsies, but they have failed to predict compounds effective in treating drug-resistant epilepsies such as Dravet syndrome. Dravet syndrome is a severe epileptic encephalopathy which typically presents with febrile seizures during the first year of life, with eventual progression to spontaneous, recurrent seizures. We screened 15 phytocannabinoids for their anticonvulsant properties in the *Scn1a*$^{+/-}$ mouse model of Dravet syndrome. We also assessed whether the phytocannabinoids affect receptors and channels implicated in the pathophysiology of Dravet syndrome.

Results: We discovered three novel phytocannabinoids with anticonvulsant properties against hyperthermia-induced seizures in *Scn1a*$^{+/-}$ mice: cannabigerolic acid (CBGA), cannabigerovarinic acid (CBGVA) and cannabidivarinic acid (CBDVA). CBGVA and CBDVA were as effective as CBD, whereas CBGA was effective at lower doses than CBD. Interestingly, the anticonvulsant activity of the compounds correlated with potency to antagonise human GPR55 receptors expressed in mammalian cells, where CBGA exhibited the lowest IC_{50}. We also found that CBGA antagonised the anticonvulsant drug targets TRPV1 and $Ca_v3.1$ receptors *in vitro*. While CBGA was a positive allosteric modulator of the $GABA_A$ receptor, prolonged exposure to this compound rapidly desensitised these receptors.

Conclusion: We have identified several phytocannabinoid acids that exhibit anticonvulsant properties in a mouse model of Dravet syndrome. CBGA's anticonvulsant activity may involve multiple drug targets.

THE EFFECTS OF CANNABIDIOL ON THE RECOVERY OF MICE AFTER TRAUMATIC BRAIN INJURY

Sigal Liraz-Zaltsman[1,2*], Chen Shemesh[2], Ruben Sachsse[3], Yael Friedman- Levi [1], David Last[4], Shirley Sharabi[4,] Yael Mardor[4], Raphael Mechoulam[1] and Esther Shohami[1]

[1]Institute for Drug research, The Hebrew University of Jerusalem, Jerusalem, Israel, [2]The Joseph Sagol Neuroscience Center, Sheba Medical Center, Israel, [3]Center for Drug Research, Department of Pharmacy,
Ludwig-Maximilians-Universität, Munich, Germany, [4]The Advanced Technology Center, Sheba Medical Center, Tel-Hashomer, Israel;

Introduction: Cannabidiol (CBD), one of the major nonpsychoactive cannabinoids produced by Cannabis sativa has a broad pharmacological profile, including anti-inflammatory and anti-oxidant properties. It was shown to exert neuroprotection in different brain pathologies (e.g. cerebral ischema, neurodegenerative diseases). In the present study we investigated the beneficial effects of CBD on functional recovery, pathological outcomes and BBB integrity after TBI, and the role of CB2 receptors in mediating these effects.

Methods: Male C57bl mice were subjected to closed head injury, using a well-established TBI model. One hour later they were randomly assigned to receive either 5mg/kg CBD i.p, alone, with 1mg/kg SR144528 (CB2R antagonist) or vehicle. Their neurobehavioral deficits was determined by Neurological Severity Score (NSS), a scale based on the ability to perform various tasks. Spatial learning and memory was tested using the Barnes maze (BM), short-term memory was assessed by the novel object recognition test (NORT) and spatial recognition memory by Y-maze test. MRI imaging was performed and its sequences included contrast-enhanced T1-weighted MRI and T2-weighted MRI. Treatment Response Assessment Maps (TRAMs), a novel method (developed at Sheba Hospital) was employed to calculate the integrity of the BBB. Mice were sacrificed at different time points, brain sections were stained with hematoxylin-eosin (H&E) to evaluate lesion area and ventricle size, and immunohistochemical analysis of neurofilament (NF) was performed in the dentate gyrus (DG) of the hippocampus to evaluate protection from axonal degeneration.

Results: Functional deficits (NSS) decreased significantly in the CBD-treated mice. They also learned significantly faster to locate the hidden platform in the BM (group main effect $p=0.001$ by repeated measures ANOVA). CBD-treated mice performed better than controls, with longer exploration time of the novel arm in the Y-maze ($p=0.051$), and displayed the largest difference in exploration time of the novel object in the NORT ($p=0.0001$). Moreover, significantly smaller lesion area (~70% decrease, $p=0.04$) and reduced ventricle size were found in CBD-treated mice. NF staining of the hippocampal DG cell layers were significantly upregulated at 3.5 months post injury in the CBD- vs vehicle-treated group. TRAM analysis 7 months after injury revealed enhanced contrast agent accumulation in the vehicle-treated, but not in CBD treated group indicating long-term protection of the disrupted BBB. Interestingly, a striking observation was that all CBD effects, including improvement of neurobehavioral and cognitive functions (NSS, BM, NORT, Y-maze) and the decrease in structural impairment (lesion area and BBB integrity) were abolished or decreased by the CB2 antagonist (SR144528), although no evidence for direct binding of CBD to the CB2R was previously shown.

Conclusions: The diverse pathology of TBI is well addressed by the multi-target CBD in the mouse CHI model with a significant improvement of motor and cognitive functions, survival of tissue, protection of axons from degeneration, and preservation of BBB integrity. Collectively, these results suggest CBD as a very promising candidate for future TBI therapy. The mechanism by which it affords its neuroprotection needs to be further addressed, with special emphasis on the involvement of the CB2R.

ABSTRACT WITHDRAWN

BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF CANNABIDIOL (CBD) AND THE CBD ANALOGUE KLS-13019 IN MOUSE MODELS OF PAIN AND REINFORCEMENT

Sara Jane Ward[1*], William A. Kinney[2] and Douglas E. Brenneman[2]

[1]Center for Substance Abuse Research, Lewis Katz School of Medicine, Temple University, Philadelphia PA 19140

[2]KannaLife Sciences Inc. Pennsylvania Biotechnology Center, 3805 Old Easton Rd, Doylestown, PA 18902

Background: Cannabidiol (CBD) is a non-euphorigenic component of *Cannabis sativa* which prevents the development of paclitaxel-induced mechanical sensitivity in a mouse model of chemotherapy-induced neuropathic pain (CIPN). We recently reported that the CBD structural analogue KLS-13019 shows efficacy in an *in vitro* model of CIPN. The goal of the present study was to characterize the behavioral pharmacological effects of KLS-13019 in comparison to CBD and morphine in mouse models of CIPN, nociceptive pain, and reinforcement.

Methods: Prevention or reversal of paclitaxel-induced mechanical sensitivity were assessed following i.p. or oral administration of CBD, KLS-13019, or morphine. Antinociceptive activity using acetic acid-induced stretching and hot plate, anti-reinforcing effects on palatable food or morphine self-administration, and binding to human opioid receptors were also determined.

Results: Like CBD, i.p. or oral KLS-13019 prevented the development of mechanical sensitivity associated with paclitaxel administration. In contrast CBD, KLS-13019 was also effective at reversing established mechanical sensitivity. KLS-13019 significantly attenuated acetic acid-induced stretching and produced modest effects on the hot plate. KLS-13019 was also devoid of activity at μ, δ, or κ opioid receptors. Lastly, KLS-13019, but not CBD, attenuated the reinforcing effects of palatable food or morphine.

Conclusion: KLS-13019 shares CBD's ability to prevent the development of CIPN, while KLS-13019 uniquely attenuated established CIPN. Because KLS-13019 binds to fewer biological targets, these findings can bring us closer to identifying molecular mechanisms shared by the two compounds as well as those unique to KLS-13019. Lastly, KLS-13019 may possess the ability to attenuate reinforced behavior, an effect not observed in the present study with CBD.

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CBGVA INHIBITS RECOMBINANT HUMAN T-TYPE CALCIUM CHANNELS

Michael Udoh[1*], Iain McGregor[1], Mark Connor[2] and Jonathon C. Arnold[1]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Sydney, Australia; [2]Dept Biomedical Sciences, Macquarie University, Sydney, Australia,

Introduction: T-type calcium channels (Ca_V3) are voltage-gated ion channels that allow inflow of calcium ions across the plasma membrane of both electrically excitable and non-excitable cells. They play essential regulatory roles in the conductance of neuronal action potential, and there is substantial evidence that the activity at neuronal T-type channels may contribute to pathological conditions such as epilepsy.

Tetrahydrocannabinolic acids (THCA), Tetrahydrocannabinol (THC) and Cannabidiol (CBD) have been shown to modulate human Ca_V3. THCA and THC are potent inhibitors of $Ca_V3.1$ peak current amplitude, but while THC causes significant hyperpolarisation shift in $Ca_V3.3$ steady-state inactivation, THCA is much less effective (Mirlohi *et al.*, 2020). There is also evidence that CBD, but not THC, interacts significantly with closed states of $Ca_V3.1$ channels (Ross, Napier and Connor, 2008). These interactions may contribute to the therapeutic benefits of THC and CBD.

However, there is no information on the activities of minor phytocannabinoids such as Cannabigerovarinic acid (CBGVA), Cannabigerovarin (CBGV), Cannabichromevarin (CBCV), Cannabichromevarinic (CBCVA) on T-type channels.

Methods: We examined the actions of CBCVA, Cannabigerorcin (CBGO), Cannabinolic acid (CBNA), Cannabichromenic acid (CBCA), CBGV, CBGVA, CBCV and CBCVA in HEK T-rex cells stably transfected with T-type $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ channels. We used FLIPR calcium 5 dye to measure changes in intracellular calcium in Flexstation 3 and characterised the concentration-response of compounds with ≥80% inhibition at 10 μM.

Results: NNC55940 (10 μM), a non-selective Ca_V3 inhibitor, blocked $Ca_V3.1$ (88 ± 6%), $Ca_V3.2$ (97 ± 0.5%) and $Ca_V3.3$ (100 ± 0.1%). We found that while CBGVA (10 μM) significantly inhibited calcium ion influx by 86 ± 8%; 91 ± 3% at $Ca_V3.1$ and $Ca_V3.2$ channels respectively, it weakly inhibited $Ca_V3.3$ by 35 ± 11%. CBGV (10 μM) weakly inhibited $Ca_V3.1$ by 47 ± 12%, but activities at $Ca_V3.2$ and $Ca_V3.3$ were considerably less. Interestingly we observed that CBCVA (10 μM) inhibited $Ca_V3.2$ channels by 62 ± 8%, while CBCV (10 μM) appeared to potentiate calcium influx by 37 ± 20%. We determined the inhibitory potency of CBGVA at CaV3.2 to be 1.8 μM which appeared to be less than its activity at Cav3.1.

Conclusion: We have identified that CBGVA shows significant inhibitory activity at human T-type Cav3.1 & Cav3.2 channel with similar efficacy to NNC55940. Our data also shows the effect of phytocannabinoids on T-type channels may be either potentiation or inhibition, and it further underscores the significance of investigating the bioactivity of each constituent of cannabis. Further studies will reveal the biophysical activities of CBGVA at these channels. CBGVA may be further investigated for its therapeutic potential in conditions such as epilepsy.

CANNABIDIOL ALTERS SOCIAL AND REPEPTITIVE BEHAVIORS IN A MODEL OF IDIOPATHIC AUTISM SPECTRUM DISORDERS

Sarah H. Shrader[1*], Nicholas Mellen[2], Gregory Barnes[2] and Zhao-Hui Song[1]

[1]Department of Pharmacology and Toxicology and [2]Department of Neurology, University of Louisville School of Medicine, Louisville, KY, USA

Introduction: The prevalence of Autism Spectrum Disorders (ASD) drastically risen over the last two decades from 1 in 150 children to 1 in 54 children in the U.S. according to the CDC. This heterogenous group of neurodevelopmental disorders is characterized by communication deficits, impaired social interactions and restricted interest plus repetitive behaviors. Autistic individuals also commonly present with a myriad of comorbidities, such as attention deficit hyperactivity disorder and anxiety. To date, a pharmacological intervention for the treatment of core autistic (and comorbid) symptoms has not been identified. While cannabidiol (CBD), the major nonpsychoactive constituent of *Cannabis sativa*, is suggested to have multiple therapeutic applications, the phytocannabinoid's effect(s) on idiopathic autism is unknown. We hypothesized that CBD will effectively attenuate the autism-like behaviors and autism-associated comorbid behaviors in BTBR $T^{+}Itpr3^{tf}$/J (BTBR) mice, an established mouse model of idiopathic ASD.

Methods: Mouse behavior assays were used to evaluate the effects of CBD on the behaviors of male BTBR mice, as compared to age-matched vehicle-treated male C57BL/6J (B6) mice. Weaned juvenile BTBR mice were administered by intraperitoneal injection either vehicle, 20 mg/kg CBD or 50 mg/kg CBD treatment daily beginning at postnatal day 21 for two weeks. Following injection on the final treatment day, mice were subject to the repetitive self-grooming assay to measure restricted interest/repetitive behavior, the open field assay to measure locomotor activity and anxiety-like behavior, and the 3 chamber social interaction assay to measure sociability. Data were analyzed by one-way ANOVA followed with Bonferroni's post-hoc tests. p-values of <0.05 were considered significant.

Results: Vehicle-treated BTBR mice spent significantly more time self-grooming compared to vehicle-treated B6 mice. This repetitive behavior was reduced in BTBR mice treated with 50 mg/kg CBD, but not 20 mg/kg CBD. In the open field assay, vehicle-treated BTBR mice demonstrated significantly higher levels of locomotion compared to their B6 counterparts, as measured by distance traveled. The locomotor activity was reduced in BTBR mice treated with both 20 mg/kg and 50 mg/kg CBD. In the three-chamber assay, vehicle-treated BTBR mice showed no social preference for the novel mouse compared to the novel object, unlike control B6 mice that spent more time near the novel mouse. The social deficit in BTBR mice was rescued with 20 mg/kg, but not 50 mg/kg, CBD treatment.

Conclusions: Our data indicate that CBD treatment can attenuate the excessive repetitive self-grooming behavior and alleviate the social deficit exhibited by BTBR mice. The effects of CBD on specific behaviors of BTBR mice are dose-dependent. Our results suggest that CBD may potentially be an effective drug to ameliorate repetitive, restricted behaviors and social deficits, two of three core symptoms observed in ASD patients.

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CANNABIDIOL TREATMENT IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS INHIBITS PRODUCTION OF THE PRO INFLAMMATORY CYTOKINE IL-1B

Nicholas Dopkins*, Kiesha Wilson, Kathryn Miranda,
Prakash Nagarkatti and Mitzi Nagarkatti

Dept. of Pathology, Microbiology and Immunology,
University of South Carolina School of Medicine, Columbia, SC

Introduction: Cannabidiol (CBD) is a phytocannabinoid that encompasses a major constituent of extracts isolated from cannabis plants. CBD has garnered wide-spread attention due to the non-toxic and affordable nature of its therapeutic application in neuroinflammatory disorders. Despite growing consumption of CBD as an alternative medicine, there is still little understanding on how CBD limits excessive inflammation. For this purpose, we aimed to further define how CBD limits excess neuroinflammation in a murine model of multiple sclerosis (MS) known as Experimental Autoimmune Encephalomyelitis (EAE).

Methods: Experimental Model: Chronic progressive EAE was induced by immunization of 6-week-old C57BL/6 mice with myelin oligodendrocyte glycoprotein segment 35-55. scRNA Sequencing: scRNA Seq was conducted using the 10X Genomics Chromium Controller Instrument and Chromium single cell 5' library & gel bead kit according to manufacturer instructions. Cell Ranger version 3.1.0 (10x Genomics) was used to process raw sequencing data. Seurat Suite version 3.0 was used to do downstream analysis of the Cell Ranger outputs. BMDM Cultures: Bone marrow derived macrophages (BMDMs) were generated from the bone marrow cells isolated from the femur of 6-week-old naïve female C57BL/6 mice. Cells after isolation were cultured in complete DMEM/F12 medium containing 10% FBS, 1% penicillin/streptomycin, 2mM L glutamine and 1U/ml of M-CSF for 7 days. After 7 days, the BMDMs were activated with lipopolysaccharide (LPS) at a concentration of 100ng/ml for 24 hours in media supplemented with 10μg/mL CBD.

Results: Oral administration of CBD alleviates EAE symptomology by suppressing macrophage mediated inflammation in the CNS. scRNA sequencing further demonstrates that CBD specifically limits IL-1β+CCR2+ macrophages in the CNS tissue of EAE mice. Amongst the myeloid cells, CBD treatment of BMDMs stimulated ex-vivo results in a significant reduction of IL-1β, indicating this is a result of a direct interaction with inflammatory macrophages.

Conclusions: It is concluded that CBD selectively inhibits encephalitis in a murine model of experimental MS by selectively inhibiting IL-1β+ macrophages from infiltrating the CNS and contributing to pathogenic encephalitis. This effect of IL-1β inhibition was shown to result from CBD directly inhibiting inflammatory macrophages, as demonstrated in vitro.

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CANNABIDIOL INHIBITS ANGIOGENIC PROCESSES IN RETINAL MICROVASCULAR ENDOTHELIAL CELLS

Lucy J. Sloan*, Wei Liang, Max Duff, Sarah Shrader, Yongqing Liu and Zhao-Hui Song

Department of Pharmacology and Toxicology, James Brown Cancer Center, Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, USA

Introduction: Angiogenesis is the process of forming new blood vessels from existing ones and is involved in physiological processes as well as pathological conditions. It is a multi-step process consisting of endothelial cell proliferation, degradation of the basement cellular membrane and extracellular matrix, endothelial cell migration and morphological differentiation, and the formation of the lumen and a tubular structure. Retinal microvasculature endothelial cell function is very important for the blood-retinal barrier and control of vascular permeability. Aberrant angiogenesis in the retina is involved in ocular pathological conditions such as neovascular age-related macular degeneration, uveitic neovascularization, and diabetic retinopathy. Previously, cannabidiol (CBD) has been shown to be both anti-angiogenic and pro-angiogenic, depending the types of vascular endothelial cells studied. In the present study, we investigated the effect of CBD on cellular processes and markers related to angiogenesis using mouse retinal microvascular endothelial cells (mRMVECs).

Methods: The cell migration assay (scratch assay) was used to study the effects of CBD on the migration of mRMVECs. In the tube formation assays, cells were grown in Matrigel and tubules formed in the presence and absence of CBD were compared. Western blot analyses were performed to examine the effects of CBD treatment on protein levels of slug, snail, Zeb1, vimentin, pSTAT, and STAT3. Quantitative RT-PCR was performed to investigate the effects of CBD on vascular endothelial growth factor (VEGF) mRNA levels. Data were analyzed with the NIH Image J software and plotted with GraphPad Prism software. Statistical analyses were performed using either T test or one-way ANOVA with Newman-Keuls post-tests and a level of significance at $p<0.05$.

Results: CBD (10 μM) slowed migration and inhibited tube formation of mRMVECs. Treatment of mRMVECs with CBD also downregulated angiogenic protein markers including slug, Zeb1, and vimentin in mRMVECs. Moreover, CBD inhibited phosphorylation STAT3 as shown by decreased ratio of pSTAT3/total STAT3. Lastly, CBD treatment decreased VEGF mRNA levels in mRMVECs.

Conclusions: Taken together, these results indicate that CBD is able to inhibit the angiogenic processes and cellular markers in mouse retinal microvascular endothelial cells. Therefore, this phytocannabinoid could be explored for its therapeutic potentials for retinal pathological conditions involving angiogenesis.

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DISCOVERY OF REVERSIBLE HIGHLY POTENT AND SELECTIVE BICYCLOPIPERAZINE DERIVED MAGL INHIBITORS

Benoit Hornsperger[1], Jörg Benz[1], Michael Honer[1], Carsten Kroll[1], Bernd Kuhn[1], Fionn O'Hara[1], Andrea Martella[2], Catarina Raposo[1], Hans Richter[1], Manfred Schneider[1], Juliane Stephanus[1],
Mario van der Stelt[2], Matthias Wittwer[1], Ludovic Collin[1] and Uwe Grether[1*]

[1] Roche Pharma Research & Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., 4070 Basel (Switzerland);
[2] Department of Molecular Physiology, Leiden Institute of Chemistry, Leiden University, 2333 CC, Leiden (The Netherlands).

Monoacylglycerol lipase, MAGL (MGLL) is the key enzyme regulating arachidonoyl signalling in the central nervous system. Pharmacological inhibition or genetic inactivation of MGGL significantly increases brain levels of the main endocannabinoid 2-arachidonoylglycerol (2-AG) while concomitantly reducing arachidonic acid (AA) and proinflammatory eicosanoids. The latter are upregulated in neuroinflammatory processes which occur as a consequence of disease or injury and result in the activation of resident immune cells (microglia and astrocytes). Therefore, inhibition of MAGL holds great therapeutic potential for treating neurodegeneration in diseases such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis (MS) or Amyotrophic Lateral Sclerosis (ALS) for which chronic neuroinflammatory processes are characteristic features. Several predominantly covalent MAGL inhibitors have proven to be efficacious in various preclinical models of neurodegenerative diseases (i.e. MS, ALS) and pain. However, chronic administration of such covalent compounds induce cannabimimetic side effects which might hamper their clinical developability.

Here we report the discovery of novel highly potent and selective MAGL inhibitors which are reversible. A DNA-encoded library screen provided an attractive bicyclopiperazine derived starting point. Structure guided optimization work focussing on ligand efficiency and overall absorption distribution metabolism and excretion properties resulted in ligands with excellent CNS exposure. Advanced molecules exhibit a significant increase in mouse brain 2-AG levels, a concomitant reduction of AA and prostaglandins without affecting brain anandamide levels. Acute LPS-induced neuroinflammation could be prevented as indicated by a lowering of brain TSPO levels. Furthermore, it was demonstrated that upon chronic administration (14 d) establishment of neuropathic pain was prevented without inducing tolerance and cannabimimetic side effects. Data on extensive activity-based proteome profiling (ABPP) which demonstrate the high selectivity and potency of our inhibitors across species will be reported as well. In summary, our data support considering MAGL inhibitors as promising immunomodulatory and neuroprotective therapeutics for chronic neurodegenerative disorders.

ENCAPSULATION OF A CENTRALLY ACTING CANNABINOID-1 RECEPTOR (CB1R) BLOCKER IN PLGA-BASED NANOPARTICLES FOR THE TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE

Shira Hirsch[1*], Meital Naim[2], Gershon Golomb[2] and Joseph Tam[1]

[1]Obesity and Metabolism Laboratory, [12]The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Introduction: The endocannabinoid system, comprising receptors in both the central nervous system and peripheral tissues, is well-known to be activated in individuals suffering from obesity and its related comorbidities, most notably cardiovascular disease, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD). This has prompted the development of drugs that block the cannabinoid-1 receptor (CB_1R) as potential therapeutics for these metabolic diseases. The clicical use of the first-in-class compound, rimonabant, which was found highly effective in treating all metabolic abnormalities associated with obesity, including NAFLD, was withdrawn due to neuropsychiatric side effects. Here, we describe a novel nanotechnology-based drug delivery system of rimonabant encapsulated in polymeric nanoparticles for effective peripheral targeting.

Methods: Rimonabant was encapsulated in biocompatible poly(lactic-co-glycolic acid) nanoparticles (PLGA NPs) using an emulsion solvent diffusion technique. The physicochemical properties of the NPs including yield, size, charge, morphology and stability were assessed. Brain penetration of rimonabant-encapsulated NPs, CNS-mediated behavioural activities, and their pharmacological efficacy in ameliorating obesity and its metabolic abnormalities were determined in comparison to free rimonabant treatment in mice. Additionally, the dynamic cellular uptake of fluorescently-labelled NPs and molecular mechanistic insights have been elucidated in primary hepatocytes cells by means of confocal microscopy and qPCR analyses.

Results: Rimonabant-encapsulated NPs exhibited an average diameter of 240 nm with a low size distribution (PDI<0.2), a neutral zeta potential of -2.0 mV, and both good entrapment efficiency (8% w/w) and stability as a lyophilized powder (12 month at -20°C). Following intraperitoneal injection of rimonabant NPs (1 mg/kg) in mice the drug was mainly distributed to the liver, spleen and lungs, and only low levels were found in the brain. In contrast to free rimonabant treatment, no CNS-mediated behavioral activities were detected in animals treated with rimonabant-NPs. Chronic 28-day intraperitoneal treatment with rimonabant-NPs, in diet-induced obese mice, resulted with no effect on body weight, food intake and fat mass. Nevertheless, improved hepatic steatosis and liver injury, measured by histology and biochemistry analyses, as well as enhanced insulin sensitivity were found in animals treated with rimonabant-encapsulated NPs. These *in vivo* effects were associated with enhanced cellular uptake of the formulation into hepatocytes, and with remarkably improved metabolic signature of these cells under lipotoxic conditions.

Conclusions: We successfully developed a method of encapsulating the centrally acting CB_1R blocker in PLGA NPs with desired physicochemical properties. This novel drug delivery system allows peripheral targeting of rimonabant to restore the metabolic advantages of blocking CB_1R in peripheral tissues, especially in the liver, without the negative CB_1R-mediated neuropsychiatric side effects.

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OPTIMIZATION OF TRUPATH BRET^2 ASSAY AND CHARACTERIZATION OF CB1 RECEPTOR LIGANDS FOR G PROTEIN SUBTYPE-SPECIFIC ACTIVATION

Thomas F. Gamage*, Daniel Barrus and Jenny L. Wiley

RTI International, Research Triangle Park, North Carolina, USA

Cannabinoid receptor signaling through its canonical pathway involves exchange of guanosine-diphosphate for guanosine-triphosphate (GTP) and dissociation the heterotrimeric G protein subunits, Gα from Gβγ. Quantification of receptor activation is typically measured either downstream of G protein activation (e.g. cAMP) or at the level of the G protein (e.g. [^{35}S]GTPγS). However, signaling is often measured in test systems where G protein subtype expression/stoichiometry may not recapitulate that of the specific cell type/tissue for which the experiment is meant to model.[1] Further, potency and efficacy are impacted by receptor reserve and the signal amplification inherent in downstream assays, and as such are not necessarily predictive owing to the range of cell types in which the receptor of interest is expressed. Due to its minimal signal amplification, measurement of G protein activation is a particularly useful in GPCR pharmacology. Recently, a suite of BRET^2 G protein biosensors (TRUPATH) for 14 Gα subtypes were made available[2] through Addgene which provide an inexpensive and high-throughput means for quantifying G protein subtype specific signaling/bias.

Adherent HEK293 cells (1-1.4×10^5 cells/cm^2) stably expressing the human CB_1 receptor were transfected with TRUPATH plasmids (250-500 ng per construct) in 60 mm dishes in Opti-MEM and either linear polyethylenemeine (25,000; Polysciences) or Transit2020 (Mirus Biosciences) at DNA:reagent ratios of 1:1, 1:3 or 1:6. Transfection efficiency was quantified by fluorescence intensity of GFP2. Coelenterazine 400a was added to the plate 10 min before reading. All luminescence measurements were done with a Clariostar (BMG Labtech) with emission filters of 515-30 for GFP2 and 410-80 for Rluc8-coelenterazine 400a, and data were calculated as net luminescence (treatment − control) of the GFP2/Rluc8 ratio. DNA:reagent ratios of 1:3 produced the largest GFP2 signal. Transit2020 resulted in marginally better signal to noise and reduced variability in CP55,940's concentration response curve for Gαi1. Optimization of plate read times was balanced with reduction in read variance and spiraling average resulted in lower variance at faster reads than center read. Compounds were added 5 min before reading, synchronized to the start of respective column read, and plates were read by column sequentially from top to bottom.

Cannabinoid receptor orthosteric ligands CP55,940, THC, anandamide, WIN55,212-2, as well as the positive allosteric modulator CAL010 activated Gαi1, Gαi2, Gαi3, GαoA, and GαoB with similar relative potencies and efficacies as observed in [^{35}S]GTPγS. Notably, ligand-dependent leftward shifts in potency and increases in efficacy were observed across subsequent reads suggesting TRUPATH could provide insight into differences in binding and activation kinetics.

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POTENTIAL PERIPHERALLY RESTRICTED CANNABINOID-1 RECEPTOR BLOCKERS BASED ON A BICYCLIC SCAFFOLD

Noam Freeman[1*], Ozer Greenfield[1], Meital Naim[1], Yael Soae[1], Amit Badihi[1], Joseph Tam[2], Taher Nassar[3] and Simon Benita[3]

[1]BioNanoSim (BNS), Hadassah Ein Kerem Campus, Minrav Building (JBP), Jerusalem, 9112101, Israel
[2]Obesity and Metabolism Laboratory; [3]*Laboratory* of Nano Delivery Systems, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel.

Introduction: Cannabinoid-1 receptor (CB_1R) antagonists have been shown to reduce body weight, increase energy expenditure, and improve the metabolic abnormalities associated with obesity both in preclinical models and clinically in humans. However, neuropsychiatric side effects (such as, depression, anxiety, and suicidal ideation) mediated by brain-penetrating compounds limited their therapeutic potential. Several lines of evidence suggest that activation of the peripheral endocannabinoid/CB_1R system contributes to the development of diet-induced obesity (DIO) and its metabolic consequences. This suggests that CB_1R blockade in the periphery could serve as a treatment to DIO, while sparing CNS-mediated adverse effects. Here, we describe the synthesis and evaluation of a series of peripherally restricted CB_1R blockers, based on a previously described bicyclic scaffold [1].

Methods: A library of 28 compounds (BNS801 through BNS828) was synthesized by chemically linking different moieties to the core structure of 5,6,7,8-tetrahydrooxepino[3,2-c]pyrazol-8-amine, predicted to have reduced CNS exposure. CB_1R binding, activity, and selectivity against CB_2R were assessed. In addition, the potential brain penetration of selected compounds following oral administration was evaluated using bi-directional permeability study across MDR1-MDCKII cells, with and without P-glycoprotein (P-gp) inhibitor. The efficacy of a selected lead compound in ameliorating metabolic complications of DIO, including glucose homeostasis, dyslipidemia, hepatic injury and steatosis, were determined in DIO mice.

Results: Some key compounds demonstrated promising CB_1R antagonism, having *Ki* values in the nanomolar range and low CB_2R affinity. We identified BNS808 and BNS822 as highly potent and selective CB_1R antagonists, exhibiting *Ki* values of 0.6 nM and 1.3 nM, respectively. In addition, BNS808 was found to have a relatively high efflux ratio and suspected to be a P-gp substrate in MDR1-MDCKII permeability assay, predicting its reduced CNS exposure. Finally, chronic oral administration of BNS808 (1 mg/kg/day, for 21 days) significantly reduced body weight, improved the metabolic profile, ameliorated hepatic steatosis, and did not affect ambulatory activity in DIO mice.

Conclusions: Our results demonstrate the synthesis and rapid *in vitro* evaluation of a new library of potentially peripheral CB_1R blockers. Two compounds were identified as highly potent and selective peripheral CB_1R blockers, with one of them, BNS808, demonstrating encouraging safety and efficacy results in the treatment of obesity and its metabolic abnormalities. This library could be further optimized to identify additional peripherally targeted CB_1R antagonists.

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FENCHONE DERIVATIVES: A NOVEL CLASS OF CB2 RECEPTOR SELECTIVE LIGANDS

Reem Smoum[1*], Christeene Haj[1], Shira Hirsch[1], Zhannah Yekhtin[2], Ruth Gallily[2], Joseph Tam[1] and Raphael Mechoulam[1]

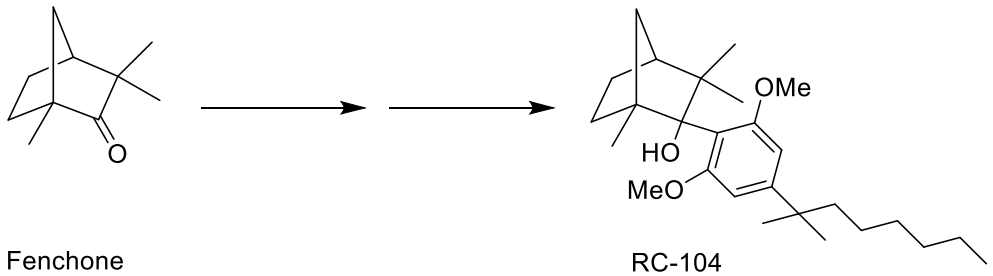
[1]Institute for Drug Research and [2]The Lautenberg Center for General and Tumor Immunology, Faculty of Medicine, The Hebrew University of Jerusalem

Introduction: Molecules that activate specifically the cannabinoid-2 receptor (CB2R) have a promising therapeutic potential as its activation reduces inflammation and exerts analgesic activity. Here we describe the synthesis, binding affinities, and pharmacological characterization of a novel series of fenchone-resorcinol derivatives acting as potent and selective CB2R ligands.

Methods: The fenchone-resorcinol derivatives were prepared by methylation of the resorcinols followed by lithiation and condensation with the fenchone. Some fenchone-resorcinol analogs were fluorinated using SelectFluor and demethylated using sodium thioethoxide. The structures of the products were determined by 1D and 2D NMR, GC-MS, LC-MS and X-ray. The binding affinities of the compounds at mCB1R and hCB2R were assessed in a competition displacement assays using [^{3}H]CP-55940 as a radioligand. The functional characterization of the compounds was determined using the [^{35}S]GTPγS binding assay. The anti-inflammatory and anti-nociceptive activities of fenchone-resorcinol derivatives were investigated using the mouse model of zymosan-induced inflammation.

Results: Twenty-four novel bicyclic monoterpenoid fenchone derivatives with different alkyl phenol or resorcinol groups were synthesized. The absolute configurations of four derivatives were determined by X-ray single crystal diffraction. The fenchone-resorcinol analogs exhibited potent binding and agonistic properties at the hCB2R with a very low affinity for the mCB1R. One of the analogs, RC-104, had a very high affinity (K_i = 3.5 nM) for the hCB2R with a 122-fold selectivity over the mCB1 receptor. In the [^{35}S]GTPγS binding assay, our lead compound was found to be a highly potent and efficacious hCB2R agonist (EC_{50} = 2.6 nM, $E_{(max)}$ = 89.6%). Two of the fenchone-resorcinol derivatives reduced zymosan-induced paw swelling and pain in mice.

Conclusions: In the present study, we report the design, synthesis, and biological evaluation of a series of a novel class of CB2R selective ligands. The fenchone-resorcinol analogs had high affinity and selectivity for the hCB2R. The design and development of new terpenoid-derived drugs with high affinity and selectivity for the hCB2R, may play a significant role in human disease treatment.



Fenchone RC-104

DIRECT QUANTITATION OF PHYTOCANNABINOIDS BY ONE DIMENSIONAL ^{1}H qNMR AND TWO DIMENSIONAL ^{1}H-^{1}H COSY qNMR IN COMPLEX NATURAL MIXTURES

Evangelos Dadiotis[1*], Vangelis Mitsis[2], Eleni Melliou[1] and Prokopios Magiatis[1]

[1] Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece
[2] Ekati Alchemy Lab SL, Barcelona, Spain

Introduction: The widespread use of phytocannabinoids or cannabis extracts as ingredients in numerous types of products, in combination with the legal restrictions of THC content, has created the need of development of new rapid analytical methods for their quantitation that ideally could be applied without separation and standards. Based on previously described qNMR studies we developed an extended ^{1}H qNMR and a novel 2D-COSY qNMR method for the rapid quantitation of ten major phytocannabinoids in cannabis plant extracts and cannabis-based products.

Method: All the experiments were performed with a DRX 400 MHz Bruker NMR, equipped with a 5 mm probe. For the quantitation with ^{1}H NMR, selected peaks between 3.2 – 8.2 ppm were used, syringaldehyde was used as an internal standard and the total analysis time was 3.5 minutes, without need of construction of calibration curves. The ^{1}H-NMR quantitation was based on the integration of the internal standard compared with the integration of selected non-overlapping peaks for each cannabinoid. The COSY qNMR method was developed with the use of tyrosol as internal standard, the total experiment time was 15 minutes and cross-peak correlations between 1.6-8.2 ppm were used. For the 2D qNMR method reference compounds were needed for the construction of calibration curves.

Results: The ^{1}H-qNMR method was successfully developed for the quantitation of cannabidiol (CBD), cannabidiolic acid (CBDA), cannabinol (CBN), cannabichromene (CBC), cannabichromenic acid (CBCA), cannabigerol (CBG), cannabigerolic acid (CBGA), Δ9-tetrahydrocannabinol (Δ9-THC), Δ9-tetrahydrocannabinolic acid (Δ9-THCA) and Δ8-tetrahydrocannabinol (Δ8-THC). The COSY qNMR method was applied for the quantitation of CBD, CBDA, CBN, CBG & CBGA and THC & THCA. The limits of quantitation for each compound were between 90-200 μg per analyzed sample. The two methods were applied for the analysis of hemp plants to find the appropriate time for their harvest and to determine the legally permitted THC & THCA content. The deviation between the two methods was less than 8%. The methods were also applied in olive oil products containing cannabis extracts.

Conclusion: qNMR is a powerful tool for the quantitative analysis of plant metabolites with short analysis time and without the need of a separation step and minimal solvent consumption. ^{1}H NMR method does not require the use of reference compounds and requires shorter time of analysis. However, complex extracts in ^{1}H-NMR may have a lot of signals and quantitation with this method is often hampered by peak overlap, with 2D NMR being a solution to this obstacle. The most important advantage of COSY NMR quantitation method was the determination of the legality of the plant based on its THC & THCA content, where in some samples the determination of THC & THCA content by ^{1}H qNMR was not feasible.

COMPUTATIONAL *DE NOVO* DRUG DESIGN: AN APPLICATION OF A RECURRENT NEURAL NETWORK MODEL TRAINED THROUGH REINFORCEMENT LEARNING TO THE CASE OF CB2-RECEPTOR AGONISTS

Antonius P. A. Janssen[1*], Johanna G. Bovenberg[1], Gerard .J.P. van Westen[2] and Mario van der Stelt[1]

[1]Department of Molecular Physiology Leiden University, Leiden, the Netherlands,
[2]Department of Computational Drug Discovery, Leiden University, Leiden, the Netherlands

Introduction: The cannabinoid 2 receptor (CB2R) is being investigated as a potential therapeutic target for various pathologies (e.g. pain, inflammatory bowel disease, multiple sclerosis and Alzheimer's disease). Selective CB2R agonists are required to avoid the psychoactive effects associated with the cannabinoid 1 receptor (CB1R) activation.

Methods: Here, we present the identification of novel CB2R-selective agonists via an implementation of DrugEx: a state-of-the-art computational *de novo* drug design algorithm, employing a deep recurrent neural network trained through reinforcement learning. Public data were leveraged to develop multiple CB2R computational models, including QSAR models for predicting CB2R-activity and selectivity for CB2R over CB1R.

Results: QSAR models were successfully trained for CB2R activity (RMSE: 0.80) and selectivity for CB2R (RMSE: 0.70). The DrugEx algorithm was successfully tailored to the task of generating CB2R-selective compounds. After hyperparameter tuning 10,000 compounds were sampled from the DrugEx molecular generator.

Conclusion: Seven clusters with at least ten druglike predicted CB2R-selective agonists were identified, totaling 305 unique agonists. Further studies are currently being carried out to increase novelty and drug-like properties of the generated hits and to experimentally validate the *in silico* generated compounds.

PERIPHERAL HYBRID CB_1 RECEPTOR/iNOS ANTAGONIST MRI 1867 REDUCES ALCOHOL DRINKING BEHAVIOR AND SOME INFLAMMATORY MARKERS IN THE GI TRACT.

Grzegorz Godlewski*, Luis Santos Molina, Resat Cinar, Jie Liu,
Malliga Iyer and George Kunos

Laboratory of Physiologic Studies,
National Institute on Alcohol Abuse and Alcoholism,
National Institutes of Health,
Bethesda, MD, USA

Chronic alcohol consumption is a major cause of morbidity and mortality worldwide. It produces a sustained systemic inflammatory state resulting from the disruption of intestinal barrier that leads to the development of alcoholic liver disease and neuroinflammation (Alcohol Res., 2017; 38: 163-171). The elevated levels of circulating endotoxins, proinflammatory cytokines and up-regulation of iNOS have been proposed as possible peripheral biomarkers of alcohol use disorder (AUD) (J Neuroimmune Pharmacol 2010; 5: 83–91).

We have recently shown that the brain non-penetrant CB1 receptor (CB1R) antagonist, JD5037, suppresses alcohol preference through gut-brain axis (Cell Metab 2019; 29: 1320-1333). Here we evaluate if peripherally active hybrid antagonist of CB1R / iNOS, *S*-MRI-1867 (JCI Insight, 2016; 1: e87336) offers a benefit in mitigating AUD.

Central administration of CB1R antagonists inhibited catalepsy and hypothermia in mice triggered by i.p. injection of WIN-55-212-2 but did not affect their voluntary alcohol drinking. In contrast, orally administered brain penetrant and non-penetrant CB1R antagonists (rimonabant and *S*-MRI-1867, respectively) were equally effective in reducing alcohol drinking in wild type mice but not in CB1R KO littermates. The inhibition of alcohol drinking by *S*-MRI-1867 was dose-dependent, as reflected by changes in blood alcohol and acetaldehyde levels, and independent of iNOS inhibition.

Alcohol drinking mice displayed elevated expression of iNOS and monocyte-macrophage marker F4/80 in the stomach, jejunum and liver, which were normalized by the treatment with *S*-MRI1867 (dual antagonist of CB1R / iNOS) and *R*-MRI-1867 (iNOS inhibitor).

We conclude that inhibition of drinking is mediated by CB1R outside the CNS and that simultaneous inhibition iNOS may limit alcohol-induced GI inflammation.

FATTY ACID AMIDE HYDROLASE INHIBITION IN THE CENTRAL NERVOUS SYSTEM PREVENTS AND REVERSES MORPHINE TOLERANCE IN MALE AND FEMALE MICE

Yannick Fotio[1*], Francesca Palese[1], Pablo Guaman Tipan[1], Faizy Ahmed[1,2] and Daniele Piomelli[1,2]

[1] Department of Anatomy and Neurobiology, School of Medicine, University of California, Irvine, USA.
[2] Center for the Study of Cannabis, University of California, Irvine, USA.

Introduction: Fatty acid amide hydrolase (FAAH) is an intracellular serine amidase that terminates the signaling activity of various lipid messengers involved in pain regulation, including agonists at cannabinoid receptors (e.g., anandamide) and peroxisome proliferator-activated receptor-α (PPAR-α) [e.g., palmitoylethanolamide (PEA)]. Here, we investigated whether pharmacological or genetic FAAH removal might prevent and/or reverse tolerance to the antinociceptive effects of morphine.

Methods: We induced tolerance in male and female mice by administering twice-daily morphine for 7 days while monitoring nociceptive thresholds (tail immersion test). The globally active FAAH inhibitor URB597 (1 and 3 mg/kg, intraperitoneal, i.p.) and the peripherally restricted FAAH inhibitor URB937 (3 mg/kg, i.p.) were administered daily 30 min prior to morphine, alone or in combination with the CB_1 cannabinoid receptor antagonist AM251 (3 mg/kg, i.p.), the CB_2 antagonist AM630 (3 mg/kg, i.p.) or the PPAR-α antagonist GW6471 (4 mg/kg, i.p.). Spinal levels of FAAH-regulated lipids were quantified by liquid-chromatography/tandem mass spectrometry. Gene transcription was assessed by RT-qPCR.

Results: URB597 prevented and reversed morphine tolerance in both male and female mice. The effect was mimicked by genetic FAAH deletion, but not by URB937. Antagonism of CB_2, CB_1 or PPAR-α either suppressed (CB_2) or attenuated (CB_1 and PPAR-α) the effects of URB597. Anandamide mobilization was enhanced in the spinal cord of morphine-tolerant mice. Transcription levels of the anandamide-producing enzyme *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD) and the PEA receptor PPAR-α were also elevated in spinal cord, whereas no changes were observed with CB_2, CB_1 or FAAH.

Conclusion: The results support a role for central FAAH-regulated lipid signaling in the modulation of opiate tolerance, and point to FAAH as a potential target for opiate-sparing medications.

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Conflict of interest: Daniele Piomelli is an inventor in patent applications owned by the University of California, which describe systemic and peripheral FAAH inhibitors. Other authors have no conflict of interest.

THERAPEUTIC POTENTIAL OF PIMSR1, A NOVEL CB1 NEUTRAL ANTAGONIST, FOR COCAINE USE DISORDER: EVIDENCE FROM PRECLINICAL RESEARCH

Ewa Galaj[1*], Guo-Hua Bi[1], Allamar Moore[1], Eliot Gardner[1], Herbert Seltzman[2] and Zheng-Xiong Xi[1]

[1] Molecular Targets and Medication Discovery Branch, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, USA; [2] Center for Drug Discovery, Research Triangle Institute, Research Triangle Park, NC, USA

Introduction: *Cocaine* is a highly *addictive* psychostimulant and its abuse often leads to the development of cocaine use disorder for which currently, there are no effective treatments. In the search for effective medications, CB1 receptors (CB1Rs) have become a major target of interest because of their abundant presence in the central nervous system and unique involvement in addiction. Unfortunately, traditional CB1 antagonists and inverse agonists have failed in clinical trials due to their adverse effects, necessitating different approaches in the development of effective therapeutics.

Methods and Results: Here, we evaluated therapeutic potential of PIMSR1, a neutral CB1R antagonist without an inverse agonist profile against cocaine abuse in a series of behavioral models. We found that PIMSR1 (3-30 mg/kg; ip) was effective in reducing cocaine self-administration maintained by low but not high doses of cocaine under a FR2 schedule of reinforcement, suggesting its ability to reduce cocaine reward and intake. In addition, PIMSR1 reduced lever responding for cocaine under a progressive-ratio schedule of reinforcement in rats, as indicative of its ability to reduce motivation to seek the drug. Importantly, systemic administration of PIMSR1 significantly and dose-dependently reduced cue-induced reinstatement of cocaine seeking, sugessing its preventative effects against relapse. In an optogenetic experiment PIMRS1 attenuated optical brain stimulation reward (BSR) maintained by stimulation of midbrain dopamine neurons in DAT-cre mice. These findings corroborate our previously presented data demonstrating PIMSR1's ability to reduce the reward-enhancing effects of cocaine in a rat electrical BSR paradigm.

Conclusions: Our findings suggest that PIMSR1 offers attractive prospects for pharmacotherapeutic exploration as it attenuates cocaine-driven behaviors without producing aversive or rewarding effects on its own.

CB1 RECEPTORS ON SENSORY AFFERENTS OF THE GASTROINTESTINAL TRACT MAY CONTRIBUTE TO THE CONTROL OF VOLUNTARY ALCOHOL DRINKING IN MICE

Grzegorz Godlewski*, Luis Santos Molina, Resat Cinar, Jie Liu, Malliga Iyer and George Kunos

Laboratory of Physiologic Studies,
National Institute on Alcohol Abuse and Alcoholism,
National Institutes of Health, Bethesda, MD, USA

The endocannabinoid system plays a key role in the regulation of alcohol craving. We have shown that the brain non-penetrant CB1 receptor (CB1R) antagonist, JD5037, suppresses alcohol preference through gut-brain axis. We proposed that endocannabinoids act via CB1R in ghrelin-producing cells to promote alcohol drinking by facilitating ghrelin acylation in a manner sensitive to the blockade by JD5037 (Cell Metab 2019; 29: 1320-1333).

Here we evaluate the possible contribution of CB1R located on sensory afferents of the gastrointestinal (GI) tract in the voluntary alcohol intake. We found that selective deletion of CB1R from ghrelin-producing cells only partially exhausted the inhibitory effect of JD5037 in alcohol drinking transgenic (Ghrl-Cre CB1$^{flox/flox}$) mice. The effect of JD5037 became occluded by the deletion of the CB1R gene from the nodose ganglion of Phox2b-Cre CB1$^{flox/flox}$ mice. Recent RNA sequencing of nodose ganglia indicate the existence of a non-homogenous clusters of neuronal, which differ with respect to genetic markers (Cell Rep., 2019; 27: 2508-2523). We found that the deletion of CB1R from the subpopulation Trpv1 positive neurons had only marginal effect on alcohol drinking (Trpv1-Cre CB1$^{flox/flox}$) mice, whereas the deletion of CB1R from advillin positive neurons (Avil-Cre/ERT2 CB1$^{flox/flox}$) mice abolishes the modulatory effect of JD5037 in alcohol drinking.

In conclusion, (i) CB1 receptor on sensory afferents of the GI tract may contribute to alcohol drinking behavior in mice. (ii) Further identification CB1 receptors on nodose ganglion nerve terminals projecting to the GI tract is ongoing.

THE CB1 POSITIVE ALLOSTERIC MODULATOR, ZCZ011, ATTENUATES NALOXONE-PRECIPITATED WITHDRAWAL SIGNS IN OXYCODONE-DEPENDENT MICE

Julien C. Dodu[1*], Rebecca K. Moncayo[1], Aiden Jones[1], Joel E. Schlosburg[1], M. Imad Damaj[1], Dai Lu[2], Debra Kendall[3] and Aron H. Lichtman[1]

Introduction: Opioid addiction remains a substantial public health problem that results in large numbers of morbidity and mortality. Current medications that treat opioid withdrawal are limited by their abuse liability and lack of efficacy in reducing all withdrawal symptoms. Although cannabinoid 1 (CB_1) receptor agonists ameliorate opioid withdrawal in both clinical and pre-clinical studies of opioid dependence, this strategy elicits cannabimimetic side effects. CB_1 positive allosteric modulators (PAMs) enhance CB_1 receptor signaling without cannabimimetic side effects in rodents. We hypothesize the CB_1 PAM, ZCZ011, attenuates naloxone-precipitated withdrawal signs in oxycodone-dependent mice.

Methods: Male and female ICR mice were made opioid dependent by two daily subcutaneous (s.c.) injections of 9-33 mg/kg oxycodone (or repeated saline) for eight days. On day 9, mice were administered 33 mg/kg oxycodone (or saline) followed by 1 mg/kg naloxone (s.c.) 2 h later and recorded for 30 min. Withdrawal signs measured were diarrhea, weight loss, jumps, paw flutters, and head shakes. A ZCZ011 dose-response experiment administered an acute intraperitoneal (i.p.) injection of 5-40 mg/kg ZCZ011 (or vehicle) 75 min prior to naloxone. To determine whether cannabinoid receptors mediate the anti-withdrawal effects of 40 mg/kg ZCZ011, complementary genetic (i.e., male and female CB_1 (+/+) and CB_1 (-/-) mice) and pharmacological (i.e., the CB_1 inverse agonist/antagonist, rimonabant, or the CB_2 antagonist, SR144528 were given the i.p. route of administration 85 min prior to naloxone) approaches were used. ZCZ011's effect on small intestinal GI transit was assessed using an oral gavage of charcoal solution prior to naloxone-precipitated withdrawal. Data were analyzed with Fisher's exact test, as well as one- and two-way ANOVA followed by Dunnett's and Tukey's post-hoc tests, respectively ($p < 0.05$ considered significant).

Results: Male and female ICR mice pre-treated with 40 mg/kg ZCZ011 significantly attenuated diarrhea, weight loss, paw flutters, and head shakes compared to vehicle-treated oxycodone-dependent mice. Importantly, ZCZ011 fully attenuated diarrhea and weight loss whereas it reduced paw flutters and head shakes by half. ZCZ011 did not affect jumping behavior. Pharmacological studies investigating cannabinoid receptor involvement revealed that CB_1, not CB_2, receptors mediate ZCZ011's attenuation of diarrhea, weight loss, and paw flutters. Likewise, ZCZ011's attenuation of naloxone-precipitated diarrhea, weight loss, and paw flutters was absent in CB_1 (-/-) mice, indicating CB_1 receptor involvement. Lastly, ZCZ011 attenuated naloxone-precipitated GI transit as evidenced by the leading edge of the charcoal in resected small intestines.

Conclusions: These studies indicate the CB_1 PAM, ZCZ011, reduces a subset of naloxone-precipitated withdrawal signs in oxycodone-dependent mice and may offer an alternative strategy to target CB_1 receptors to treat opioid dependence.

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CIRCULATING 2-ARACHIDONOYLGLYCEROL (2-AG) AT TIME OF TRAUMATIC INJURY IS ASSOCIATED WITH CHRONIC PAIN

Colleen Trevino, Aniko Szabo, Terri A. deRoon-Cassini and Cecilia J. Hillard*

Neuroscience Research Center, Medical College of Wisconsin, Milwaukee, WI 53226 USA

Introduction: Chronic pain is a non-productive and debilitating condition that occurs in 20-40% of individuals following a traumatic injury. The endocannabinoid signaling system (ECSS) and hypothalamic-pituitary-adrenal (HPA) axis are both stress-responsive, reciprocally related to each other, and can regulate pain. The purpose of this study was to develop a model of relationships among chronic pain, cortisol, and circulating endocannabinoids (eCBs) after traumatic injury.

Methods: One hundred forty-seven traumatically injured individuals completed a study in which clinical information and serum concentrations of the eCBs [2-AG and *N*-arachidonoylethanolamine (AEA)], and cortisol were measured both at the time of hospitalization and 6-9 months later. Demographic information and DNA were obtained at the time of hospitalization.

Results: The study sample was racially/ethnically diverse (50% Black and Latinx) and primarily male (69%); 34% percent endorsed a pain score of 4 or greater at the 6-9 month follow-up and were considered to have chronic pain. Those with chronic pain at follow-up were more likely to be injured by gunshot, have worse Injury Severity Scores (ISS), and greater pain at the time of injury. Those expressing two copies of G at rs324420 in the gene for FAAH were significantly less likely to develop chronic pain. Path analysis was used to model relationships among 2-AG, cortisol and reported pain, all measured at both time points, adjusting for sex and ISS. This modeling found that circulating 2-AG concentrations at the time of injury contributed to chronic pain in 3 ways: as a highly significant, independent positive predictor; as a mediator of the effect of ISS; and through a positive relationship with cortisol concentrations. Cortisol concentrations were significantly, negatively associated with contemporaneous pain in the pathway model.

Conclusions: These data indicate that 2-AG concentrations at the time of an injury, which are typically very high, are positively associated with the presence of chronic pain many months later and suggest that excessive activation of the ECSS contributes to the development of chronic pain and could be driven by the severity of the injury.

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MULTIPLE-DOSE STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF SPECTRUM YELLOW OIL IN HEALTHY PARTICIPANTS

Erica N. Peters[1*], Irina Mosesova[1], Laura MacNair[1], Ryan Vandrey[2], M. Hunter Land[1], Mark A. Ware[1], Cynthia Turcotte[1] and Marcel O. Bonn-Miller[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario, Canada
[2]Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Due to a lack of published pharmacokinetic (PK) and/or pharmacodynamic (PD) data, decision-making surrounding appropriate dosing of cannabis used for medical purposes is limited. This Phase 1, multiple-dose study evaluated the safety, tolerability, PK, and PD of Spectrum Yellow oil [20 mg/mL cannabidiol (CBD) / 0.9 mg/mL D^9-tetrahydrocannabinol (THC)], a cannabis-based product commercially available for medical use in Canada, Australia, the United Kingdom, and the Cayman Islands.

Methods: Participants (*N*=43) were randomized to one of five groups: 120 mg CBD and 5.4 mg THC daily, 240 mg CBD and 10.8 mg THC daily, 360 mg CBD and 16.2 mg THC daily, 480 mg CBD and 21.6 mg THC daily, or placebo. Participants were confined to a research facility and received study medication every 12 hours, approximately 60 minutes after a standardized meal for 6 days, plus a single dose in the morning of Day 7. Participants were discharged after a 32-hour post-dose blood draw on Day 8, and returned to the research facility on Days 9, 10, 11, and 13 for blood draws and study assessments. Treatment-emergent adverse events (TEAEs); plasma and urine concentrations of THC, CBD, and metabolites; and self-reported subjective effects were collected.

Results: Nearly all TEAEs (44/45) were of mild or moderate severity; none was serious. The most common TEAEs included dizziness, presyncope, insomnia, abdominal pain, and diarrhea [each reported by 3 participants (7.0%)]. The highest incidence of TEAEs (67%) was in the two higher-dose treatment groups. The highest number of TEAEs (17/45) occurred on the first treatment day. Steady-state plasma CBD concentrations were reached by Day 7. On Day 7, CBD exposure showed dose-proportionality ($AUC_{0\text{-}t}$ slope=1.03 [0.70, 1.36], C_{max} slope=0.92 [0.53, 1.31]). Most plasma THC concentrations were below the lower limit of quantification. Across Days 1 and 7, there were no consistent differences in subjective effects between placebo and active study medication.

Conclusions: Over a week of twice-daily dosing of Spectrum Yellow oil, daily doses of CBD up to 480 mg and of THC up to 21.6 mg were generally safe and became better tolerated after the first day of treatment. A prudent approach to improve tolerability with Spectrum Yellow oil might involve initial doses no higher than 240 mg total CBD and 10.8 mg total THC daily in divided doses, with titration upwards over time as needed based on tolerability. Because previous PK studies have examined cannabinoid formulations with a 1:1 or 2:1 ratio of THC to CBD, additional research is needed to further explore the impact of high levels of CBD concomitantly administered with low levels of THC, such as those studied here, on bioavailability.

This study was funded by Canopy Growth Corporation.

FAAH GENETIC VARIATION MODULATES NEURAL CORRELATES OF EXTINCTION RECALL – AN FMRI STUDY

Jennifer Spohrs[1*], Laura Bindila[3], Michael Prost[2], Paul Plener[1], Georg Groen[2], Birgit Abler[2] and Martin Ulrich[2]

[1]Dept. Child and Adolescent Psychiat., University Hospital Ulm, Ulm, Germany,
[2]Dept. Psychiat., Ulm University, Ulm, Germany,
[3]Facility of Endocannabinoids/Lipidomics, University Mainz, Mainz, Germany

Introduction: The past decades have shed tremendous insight into the mechanisms of the endocannabinoid system (ECS). Especially its putative role in the development and treatment of psychiatric, particularly anxiety-related disorders has been of great interest. Here, previous studies have revealed that in rodents and humans alike, elevated levels of the endocannabinoid (eCB) anandamide (AEA) are associated with lower levels of anxiety (Moreira et al., *Neuropharmac.* 2008), may act protectively against the anxiogenic impacts of stress (Morena et al., *Neurophychopharmac.* 2019; Hill et al., *Mol. Psychiatry* 2013), facilitate fear extinction (Moreira et al., 2008) and enhance extinction recall (Morena et al., 2008; Hill et al. 2013; Rabinak et al. *Curr. Pharm. Des.* 2014). Since AEA is degraded by fatty acid amide hydrolase (FAAH), and the single-nucleotide polymorphism (SNP) rs324420 in the FAAH-coding gene has been identified to modulate fear extinction learning and fear extinction recall (Mayo et al. *Biol. Psychiatry* 2019; Dincheva et al. *Nat. commun.* 2015), FAAH modulation has become a central topic of research.

Methods: To investigate differences in the neural underpinnings of extinction recall between the different FAAH-genotypes, we conducted a 'fear conditioning - extinction - extinction recall' fMRI study. Therefore, 51 healthy male subjects participated in a fear conditioning paradigm, in which geometric shapes were coupled with unpleasant thermal stimuli over the course of three days (day 1: fear conditioning, day 2: extinction, day 3: extinction recall). To elucidate the role of the FAAH-SNP, genotyping was performed via real-time qPCR using melting curve detection analysis. ECB levels were analysed using chromatography-mass spectrometry.

Results: Regarding the FAAH-polymorphism, baseline plasma levels of AEA were significantly ($t(49) = 2.8$, $p = 0.007$) higher in the 17 participants identified as carriers of the A allele ($[AEA]_{CA} = 0.49 \pm 0.16$ pmol/ml) compared to the 34 individuals that were CC homozygous ($[AEA]_{CC} = 0.38 \pm 0.13$ pmol/ml). Regarding neural correlates of fear conditioning and extinction, we were able to replicate the results of recent meta-analyses (Fullana et al., *Mol. Psychiatry*, 2015; Fullana et al., *Neurosci. Biobehav.*, 2018. Based on Fullana et al. (2018), to investigate extinction recall on day 3, we contrasted the signal of the un-extinguished CS minus the signal of the extinguished CS and observed a stronger neural signal in AC-heterozygotes in the following regions (Figure 2): the bilateral anterior insula, the inferior frontal lobe, anterior cingulate cortex extending into the superior frontal gyri, the right caudate nucleus and putamen, the inferior parietal lobe, the superior and middle temporal gyri (whole-brain analysis, voxel-level $p < 0.001$, cluster-level $p < 0.05$, FWE-corrected; Figure 2). Additionally, we found group differences between the AC- and CC-allele groups for post extinction recall anxiety, as assessed with the STAI-S ($t(49) = 2.27$, $p = 0.028$), indicating that AC-heterozygotes showed lower anxiety ratings. Furthermore, when looking at peripheral AEA-levels, they displayed significantly higher post-task AEA-levels on all three days (day 1: $t(49) = 3.01$, $p = 0.004$; day 2: $t(49) = 3.15$, $p = 0.002$; day 3: $t(49) = 2.67$, $p = 0.01$).

Conclusions: To put it in a nutshell, in our translational research set-up designed to investigate hypotheses from experimental animals in humans, we confirmed significant differences in the neural signature of extinction recall between FAAH-allele groups. Our data may facilitate the development of neurobiological models supporting suggestions to pre-treat patients with anxiety disorders with a FAAH inhibitor before interventions of cognitive behavioural therapy to promote consolidation of fear extinction learning.

EFFECT OF A CANNABIS EXTRACT ON ACUTE RADICULAR PAIN AND ON ANALGESICS REQUIREMENT: A DOUBLE-BLINDED, RANDOMIZED, 24 HOURS FOLLOW-UP STUDY

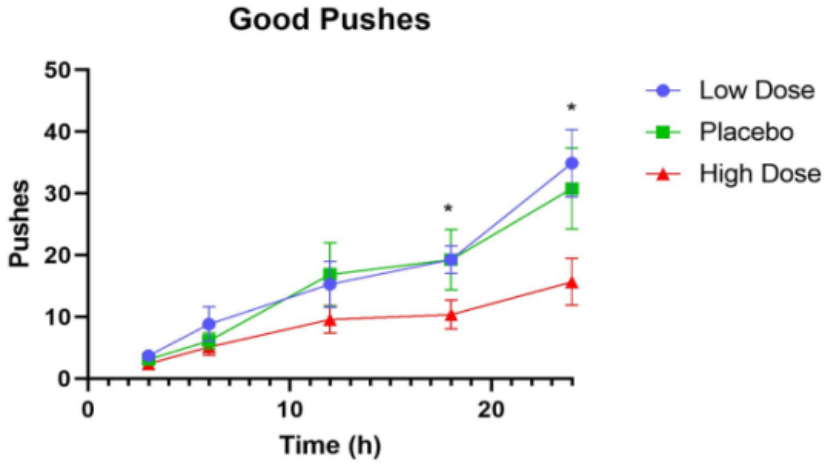
Elyad Davidson*, Ester Goldberg, Salhab Kalil and Josh Schroeder

Departments of Anesthesiology, CCM and Pain Relief, and Department of Orthopedics
Hadassah Hebrew University Hospital Jerusalem, Israel

Introduction: Recent surveys estimate that 10-20 % of adult populations have consumed cannabis in the past year. Medical cannabis consumers are a newer group of cannabis users. The most frequent indication for using medical cannabis is for managing chronic pain. There is only sparse data regarding the use of cannabinoids in acute pain conditions. Some have stated that cannabis has no role in management of acute pain while others have demonstrated primary evidence for opioid sparing and analgesic properties in post-operative or acute pain conditions. In this pilot study we wished to examine whether cannabis has an analgesic function in acute radicular pain in humans.

Methods: This was a double blinded, randomized, prospective study conducted on healthy adult patients naïve to cannabis, admitted to emergency room with recent onset, acute radicular pain symptoms. Radicular pain symptoms had dermatomal pattern corresponding to physical exam and a recent CT/MRI, demonstrating intervertebral bulging lumbar disk. Patients were randomly divided to one of three groups- high dose (THC-20 mg, CBD 20 mg), low dose cannabis (THC 10 mg, CBD 10 mg) and placebo administered sublingually. Patients were connected to a PCA (patient-controlled administration) morphine pump allowing administration of opioid for pain by self-administration. Patients were followed up for 24h with regard to pain, opioid consumption anxiety and other parameters.

Results: 36 patients were recruited 12 in each group. Patients receiving the higher dose of cannabis demonstrated a significant opioid sparing effect but no pain reduction.



Low Dose vs. High Dose: 18h- p=0.03, 24h- p=0.02

Conclusions: In this acute neuropathic clinical pain condition, we demonstrated significant opioid sparing effect in patient's naïve to cannabis who were administered one dose of sublingual high dose cannabis.

CANNABIDIOL OIL FOR BEHAVIORAL DISTURBANCES IN DEMENTIA, A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL TRIAL

Vered Hermush[1,2*], Liora Ore[3], Noa Stern[1,2], Nisim Mizrahi[1], Malki Fried[1], Marina Krivoshey[1], Ella Staghon[1], Violeta E. Lederman[4] and Lihi Bar-Lev Schleider[4,5]

[1]Geriatric Wing, Laniado Hospital, Netanya, Israel. [2]Technion School of Medicine, Haifa, Israel. [3]Department of Graduate Studies in Health Systems Management, The Max Stern Yezre'el Valley College. [4]Research Department, Tikun Olam -Cannbit Pharmaceuticals. [5]Clinical Research Center, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel

Background: Almost 90% of dementia patients suffer from some type of neurobehavioral symptom and there are no approved medications for these symptoms. The aim was to evaluate the safety and efficacy of CBD-A 30:1 compared with a placebo for the reduction of behavioral disturbances among dementia patients.

Methods: Participants were randomized 2:1 to receive either CBD-A 30:1 (30% CBD and 1% THC; n=42), or a placebo oil (n=22) three times a day for 16 weeks.

Results: There was a statistically significant difference in the proportion of subjects who had a Cohen-Mansfield Agitation Inventory score reduction of ≥ 4 points at week 16: 24 (57%) and 6 (27%) for treatment and control groups respectively (P=0.023). The ANOVA repeated measures analysis demonstrates significant better improvement in the CBD-A 30:1 group compare to the control group in week 14 and 16 (P=0.01). In the NPI-NH, results demonstrate significantly more than a 24% reduction in agitation/aggression (P=0.02). There was no difference in adverse event occurrences between the two groups.

Conclusion: CBD-A 30:1 is a safe and effective treatment option for patients suffering from behavioral disorders related to dementia and can significantly reduce agitation. Further research is required to evaluate the efficacy and side-effects of CBD in behavioral disturbances associatedwith various sub-types of dementia in larger scale.

DOSAGE AND FORMULATIONS RECEIVED BY PATIENTS WITHIN A US STATE CANNABIS PROGRAM

Xintian Lyu[1]*, Silvia Illamola[1], Stephen Dahmer[2,3], Paloma Lehfeldt[3], Ilo Leppik[1,4,5] and Angela Birnbaum[1,4]

[1]Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota; [2]Icahn School of Medicine at Mount Sinai; [3]Vireo Health; [4]Center for Clinical and Cognitive Neuropharmacology, College of Pharmacy, University of Minnesota; [5]MINCEP Epilepsy Care, University of Minnesota Physicians, Minneapolis, Minnesota

Introduction: Patients are exposed to varying medical cannabis (MC) products depending on state law. Minnesota has a well-defined and closely regulated state MC program requiring provider certification, pharmacist oversight, and third-party tested resulting in standardized extract products that provide consistency in cannabis products utilized by patients. Our objective was to best describe the formulations and maintenance doses distributed to patients across all reported conditions from one of two state approved manufacturers in Minnesota.

Methods: Four years (2016 - 2019) of dispensing data (n=14,316) from one MC Minnesota manufacturer reflecting ~50% of patients were analyzed. Data included age, type of product dispensed, amount, formulation, and route of administration. Exclusion criteria were: patients <6 weeks dispensation date, not regularly prescribed MC, and multiple certifying conditions. Data were stratified by certified medical condition and age (<18 years; ≥ 18 years). The formulation received was identified on the last visit or across all visits. Individuals were divided into two groups based on formulations: 1) capsules and/oral solutions only; 2) vaporizer only or along with other formulations. Maintenance daily dose (DD) (mg/day) of CBD and THC for each qualifying condition was calculated by using the last dose of their last stable dose. Stable dose was defined as individuals prescribed the same dose (± 10%) for more than 28 days. CBD and THC DD were expressed as median (range). DD between two formulation groups within each condition and between two age groups within each formulation group were compared using two sample t-test ($p<0.05$) via R software (V.4.0.3).

Results: Out of 4,600 patients with 13 unique certifying conditions, 3,977 (86%) were receiving one formulation at the last dispensing visit: cancer 87 (85%), intractable pain 2,936 (86%), autism 105 (94%), glaucoma 15 (79%), HIV/AIDS 24 (100%), Tourette syndrome (TS) 34 (92%), amyotrophic lateral sclerosis (ALS) 15 (88%), multiple sclerosis (MS) 245 (85%), inflammatory bowel disease (IBD) 143 (90%), terminal illness 8 (89%), obstructive sleep apnea (OSA) 158 (90.0%) Alzheimer's disease (AD) 3 (100%) and epilepsy 204 (93%). Considering all dispensing visits 1,384 (35%) patients received multiple formulations during overall treatment. CBD maintenance DD for formulation group one and two ranged from 3.5-142.5 mg/day and 4.8 - 99.0 mg/day, respectively. A significant difference of CBD maintenance DD between age groups was found only in formulation group one within TS ($p=0.002$). THC maintenance DD for formulation group one and two ranged from 7.5-64.1 mg/day and 20.8-130.2 mg/day, respectively. There were significant differences of THC maintenance DD between two formulation groups within cancer ($p=0.010$), intractable pain ($p<0.0001$), MS ($p=0.008$), IBD ($p=0.022$), and OSA ($p=0.012$).

Conclusions: Despite the availability of multiple products and formulations to patients, a majority of patients in a closely regulated MC state program received one formulation at maintenance. CBD and THC across formulations have complicated pharmacokinetics and changes in formulation may add additional variability in response within a patient. Tracking formulation changes may be useful in patients to account for variations in response with patients being treated for conditions such as epilepsy where fluctuations in pharmacokinetics should be avoided.

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FUNCTIONAL SELECTIVITY OF A BIASED CANNABINOID-1 RECEPTOR (CB_1R) ANTAGONIST FOR ANTI-DIABETIC EFFICACY WITHOUT CNS SIDE-EFFECTS

Resat Cinar[1*], Ziyi Liu[2], Malliga R. Iyer[3], Grzegorz Godlewski[2], Tony Jourdan[2], Jie Liu[2], Nathan J. Coffey[2], Charles N. Zawatsky[1], Henry L. Puhl[4], Jeih-San Liow[5], Robert B. Innis[5], Sergio A. Hassan[6], Yong-Sok Lee[6] and George Kunos[2]

[1]Section on Fibrotic Disorders, [2]Laboratory of Physiologic Studies, [3]Section on Medicinal Chemistry and [4]Section on Cellular Biophotonics, National Institute on Alcohol Abuse and Alcoholism, [5]Molecular Imaging Branch, National Institute of Mental Health, and [6]Bioinformatics and Computational Biosciences Branch, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 USA

Seven transmembrane receptors signal via heterotrimeric G proteins as well as G protein-independent, β-arrestin-dependent pathways. CB_1R antagonist rimonabant had shown promise as an anti-obesity agent that also improved metabolic complications, including fatty liver, insulin resistance and dyslipidemia but ultimately failed due to neuropsychiatric side effects. Dissociating therapeutic effects from unwanted side effects is a major challenge in drug development. In the case of CB_1R blockade, one way to achieve such separation is to limit the brain penetrance of the antagonist, which was exemplified by peripheral CB_1R antagonism. Another approach to selectively reduce side effects relies on biased signaling via identifying distinct functional coupling of CB_1R in CNS and periphery to retain therapeutic benefit. Here we present a peripheral CB_1R antagonist (MRI-1891) highly biased toward inhibiting CB_1R-induced β-arrestin-2 (βArr2) recruitment (IC_{50}: 21 pM) over G-protein activation (IC_{50}: 6 nM). In obese wild-type and βArr2-knockout (KO) mice, MRI-1891 treatment reduces food intake and body weight without eliciting anxiety even at a high dose causing partial brain CB_1R occupancy, as detected by CB_1R PET imaging. By contrast, the unbiased global CB_1R antagonist rimonabant elicits anxiety in both strains, indicating no βArr2 involvement. Interestingly, obesity-induced muscle insulin resistance is improved by MRI-1891 in wild-type but not in βArr2-KO mice, as revealed by hyperinsulinemic/euglycemic insulin clamps and 2-deoxyglucose uptake. In C2C12 myoblasts, CB_1R activation suppresses insulin-induced akt-2 phosphorylation, preventable by MRI-1891, βArr2 knockdown or overexpression of CB_1R-interacting protein. MRI-1891, but not rimonabant, interacts with non-polar residues on the N-terminal loop, including F108, and on transmembrane helix-1, including S123, a combination that facilitates βArr2 bias.

We conclude that CB_1R promotes muscle insulin resistance via βArr2 signaling, selectively mitigated by a biased CB_1R antagonist at reduced risk of CNS side effects.

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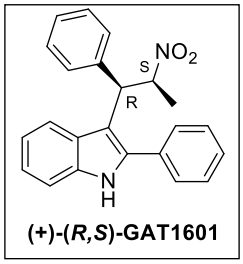
DISCOVERY OF G-PROTEIN BIASED CB1R ALLOSTERIC MODULATOR FOR THE TREATMENT OF GLAUCOMA WITH LONG-DURATION OF ACTION

Sumanta Garai,[†*] Luciana M. Leo,[¶] Anna-Maria Szczesniak, [Σ] Dow P. Hurst,[¥] Peter C. Schaffer,[†] Ayat Zagzoog,[Φ] Tallan Black,[Φ] Roger G. Pertwee,[§] Mary E. Abood,[¶] Melanie E. M. Kelly, [Σ] Patricia H. Reggio, [¥] Robert B. Laprairie[* Σ, Φ] and Ganesh A. Thakur[*,†]

[†] Department of Pharmaceutical Sciences, Bouvé College of Health Sciences, Northeastern University, Boston, Massachusetts; [¶] Center for Substance Abuse Research, Lewis Katz School of Medicine, Temple University, Philadelphia; [§] School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen; [Φ] College of Pharmacy and Nutrition, University of Saskatchewan, 104 Clinic Pl, Saskatoon; [¥] Center for Drug Discovery, University of North Carolina Greensboro, Greensboro; [Σ] Department of Pharmacology and Department of Ophthalmology and Visual Sciences, Dalhousie University, Halifax.

Introduction: The cannabinoid receptor, CB1(CB1R) is the most abundant G protein-coupled receptor in the CNS, where it mediates inhibition of neurotransmitter release at the presynaptic nerve terminal. The last few decades of research indicate that CB1R is an established therapeutic target for the treatment of several CNS-pathologies, including elevated intraocular pressure (IOP) a major risk factor for the development of glaucoma, a neurodegenerative disease that is one of the leading causes of irreversible worldwide blindness.[1] Although CB1R orthosteric agonists can decrease IOP in humans and animals, their general therapeutic use is essentially precluded by their short duration of action and the possibility of psychotropic side effects.[2] Importantly, a CB1R allosteric agonist-positive allosteric modulator (ago-PAM) does not exhibit these adverse properties[3], suggesting that CB1R ago-PAMs may constitute a potentially safer therapeutic approach for reducing IOP and obviating the risk of glaucoma-associated vision loss.

Methods & Results: 2-Phenyl indole based CB1R allosteric modulator, racemic **GAT211**[4,] and its enantiomers demonstrated preclinical IOP-lowering efficacy.[5] We applied the classical "magic methyl effect"[6] to improve the potency/efficacy of **GAT211**. Introducing a methyl group at α-position of nitro-group of GAT211 generated two diastereomers, the greater potency, and efficacy of *erythro*, (±)-**GAT562** *vs. threo*, (±)-**GAT582** constituting the first demonstration of diastereoselective CB1R-allosteric modulator interaction. The enantiomers of each diastereomer were separated and further pharmacological characterization showed (-)-(*S,R*)-**GAT1600** as a CB1R ago-PAM, whereas (+)-(*R,S*)-**GAT1601** was a CB1R allosteric agonist biased toward G protein- vs. β-arrestin1/2-dependent signaling. Computational studies further demonstrated that (-)-(*S,R*)-**GAT1600** docked into both a CB1R extracellular PAM and intracellular allosteric-agonist site(s), whereas (+)-(*R,S*)-**GAT1601** preferentially engaged only allosteric agonist site(s).[7]



(+)-(*R,S*)-GAT1601

Conclusion: (+)-(*R,S*)-**GAT1601** is, to the best of our knowledge, the first G-protein biased CB1R allosteric agonist to have been discovered. Our initial demonstration of the preclinical efficacy and unprecedented long duration (12h) of action of (+)-(*R,S*)-**GAT1601** as a biased allosteric agonist for lowering IOP and its inability to produce significant adverse cannabimimetic effects reveals its potential therapeutic value in treating glaucoma.

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DUAL TARGETING OF CANNABINOID-1 RECEPTOR AND MODULATION OF PPARα ATTENUATES OBESITY-INDUCED FATTY LIVER DISEASE

Asaad Gammal[1,2*], Anna Permyakova[1], Naama Adi-Hen[3], Julie Asfour[3], Amit Badihi[3], Taher Nassar[2], Simon Benita[2] and Joseph Tam[1]

[1]Obesity and Metabolism Laboratory;
[2]*Laboratory* of Nano Delivery Systems, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel,
[3]BioNanoSim (BNS), Hadassah Ein Kerem Campus, Minrav Building (JBP), Jerusalem, 9112101, Israel

Introduction: During the last few decades, there has been an epidemic increase in the worldwide prevalence of obesity and its metabolic complications. There is currently no available medication that simultaneously targets all of the metabolic consequences of obesity, justifying the search for novel approaches. Cannabinoid-1 receptor (CB_1R) antagonists reduce body weight and improve the associated hormonal/metabolic abnormalities in obese rodents and humans. However, their value as anti-obesity agents are limited by side effects mediated at CB_1R in the CNS. CB_1R and endocannabinoids are also present in peripheral tissues, and several lines of evidence suggest that activation of the peripheral endocannabinoid/CB_1R system contributes to diet-induced obesity (DIO) and its hormonal/metabolic consequences. Recently, we provided a strong evidence for a functional role of peroxisome proliferator-activated receptor-alpha (PPARα), an important modulator of hepatic lipid metabolism, in mediating the antisteatotic effect of peripherally restricted CB_1R blockade. Here, we describe the chemical modification of a currently available CB_1R blocking drug into a novel dual-targeted molecule that has the ability to block CB_1R in periphery and modulate PPARα activity in the liver.

Methods: A novel compound (BNS-110) was synthesized by chemically linking the core structure of SR141716A with Idebenone, a PPAR modulator molecule. BNS-110 was characterized by using LC-MS/MS, HPLC, and H-NMR analyses. CB_1R binding, activity, and selectivity against CB_2R as well as its brain penetration and behavioral profiles (Catalepsy, Ambulation, and Anxiety) were assessed. The efficacy of BNS-110 in ameliorating DIO and its metabolic complications, including glucose homeostasis, dyslipidemia, hepatic injury and steatosis, were determined in high-fat diet-induced obese mice chronically treated at 20 mg/kg/d, IP for 28 days. Hepatic PPARα expression and activity were assessed by qPCR, Western Blot, and functional assays.

Results: BNS-110 demonstrated a high affinity for human CB_1R (Ki = 46.7 nM) and a low selectivity against human CB_2R (Ki = 2890 nM). It reduced GTPγS binding, further suggesting inverse agonism properties. Following an acute *in vivo* administration of BNS-110, low levels of the compound were found in the brain. This was associated with negligible CNS-mediated behavioral effects, demonstrating the inability of BNS-110 to induce hyperactivity and anxiety or to antagonize CB_1R-mediated cataleptic behavior and hypomotility. Chronic treatment of DIO mice with BNS-110 significantly reduced body weight, food intake, and fat mass, reversed glucose intolerance and insulin resistance, reduced dyslipidemia, and improved kidney function. Additionally, BNS-110 was found very efficacious in reducing DIO-related hepatic injury and fatty liver by modulating the expression and activity of hepatic PPARα.

Conclusion: Collectively, our results highlight the therapeutic relevance of dual targeting the peripheral CB_1Rs (inhibition) and PPARα (stimulation) to treat obesity and its metabolic abnormalities, specifically fatty liver. Further preclinical studies are needed to promote these drugs into clinical evaluation in humans.

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STUDIES ON THE PERIPHERALLY RESTRICTED 3,4-DIARYLPYRAZOLINES AS POTENT ANTAGONISTS OF CANNABINOID-1 RECEPTOR (CB_1R)

Malliga R. Iyer,[1]* Resat Cinar,[2] Charles Zawatsky,[2] Casey Wood,[1] Nathan J. Coffey,[3] Kyu Ah Kim,[1] Jasmina Abdalla,[2] Alexis Katz[3] and George Kunos[3]

[1]Section on Medicinal Chemistry, [2]Section on Fibrotic Disorders, [3]Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 5625 Fishers Lane, Rockville, MD 20852, USA.

Introduction: Obesity and its metabolic complications, including cardiovascular disease and organ fibrosis, represent a global health problem with a need for new drug interventions. Among multiple signaling pathways implicated in such chronic conditions, an overactive endocannabinoid system (ECS) has emerged as a major pathogenic factor. The ECS exerts its effects mainly through its G-protein coupled receptors CB_1R and CB_2R that have been targeted for therapeutic gain in conditions related to the metabolic syndrome. The globally acting CB_1R antagonist rimonabant has been shown to inhibit food intake and mitigate insulin resistance and fibrosis, but neuropsychiatric side effects preclude its clinical use. To harness the therapeutic potential of CB_1R blockade, attention has turned to restrict drug action to the periphery. Here we report the synthesis and structure-activity relationships of novel dihydropyrazoline compounds designed to have limited brain penetrance and potent inhibitory activity at CB_1R. A series of racemic 3,4-diarylpyrazolines were synthesized and evaluated initially in CB_1R binding assays. The novel compounds had decreased lipophilicity and potent *in vitro* CB_1R antagonist activities. Tissue distribution studies of key compounds confirmed their limited brain penetrance. Compounds with potent CB_1R activity were then separated into component enantiomers and evaluated in a mouse model of the metabolic syndrome.

Methods: Design and synthesis of a novel series of 3,4-Diarylpyrazolines bearing various amidine pendants were carried out. The compounds were synthesized as racemates and evaluated in CB_1R/CB_2R binding and functional assays. The compounds were further evaluated for inverse agonism at the CB_1 receptor. Promising compounds were then separated by chiral HPLC to give pure enantiomers which were evaluated for peripheral restriction using tissue distribution studies.

Results: Several compounds showed nanomolar potencies on CB_1R and behaved as inverse agonists with acceptable selectivity over CB_2R. The compounds also showed low brain: plasma ratio (<3%) indicated their restricted central access.

Conclusion: A new series of peripherally restricted CB_1R agents with reduced lipophilicity were synthesized and pharmacologically evaluated. The novel compounds have therapeutic potential in obesity-related metabolic dysfunctions and will be optimized further for maximum efficacy.

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THE DEVELOPMENT OF PERIPHERAL CB1 INVERSE AGONISTS WITH METABOLIC, ANTI-INFLAMMATORY, AND ANTI-FIBROTIC ACTIVITIES

Tama Evron*, Suzie Ferreira, Hongfeng Deng, Andrew Kolodziej and Barbara White

Corbus Pharmaceuticals Inc., Norwood, MA, USA

Introduction: Inhibition of the cannabinoid receptor CB1 has been proposed as a promising therapeutic approach to treat metabolic diseases that are accompanied by inflammation and fibrosis, such as non-alcoholic steatohepatitis (NASH) and diabetic nephropathy. However, the clinical development of CB1 ligands has been limited due to their CNS on-target psychotropic effects. Corbus Pharmaceuticals is focused on developing peripherally restricted CB1 inverse agonists with metabolic, anti-inflammatory and anti-fibrotic activities.

Methods: Competitive binding, cAMP and βarrestin2 assays were used to evaluate receptor pharmacology. In vivo pharmacokinetic (PK) studies were performed in CD-1 mice upon oral administration of the compound. Brain/plasma ratio (B/P) was calculated based on drug exposure at C_{max} and area under the curve (AUC). Efficacy was evaluated in a diet-induced obesity (DIO) model in mice fed a high fat diet (HFD) for 14 weeks and then treated with test compound twice daily for 28 days. Body weight was recorded daily. Oral glucose tolerance test (OGTT) was performed on day 25. Effect of compounds on inflammation and fibrosis were tested in vitro, using LPS-stimulated human PBMC and TGFβ-stimulated human lung fibroblasts, respectively.

Results: Three novel CB1 inverse agonists with low brain exposure and no brain accumulation were identified. All three compounds displayed nanomolar affinity (Ki = 0.5 – 2.0 nM) to CB1, and > 1,000 – 15,000-fold selectivity for CB1 vs. CB2. The functional potencies of these compounds were confirmed in cAMP (IC50 = 1.0 – 5.0 nM) and βarrestin2 (IC50 = 0.4 – 5.0 nM) assays. Compounds C and D displayed low brain exposure with B/P_{AUC} of 0.06, whereas the exposure of compound E in the brain was negligible after single oral administration of 10 mg/kg to mice. At steady state, following 28 days of repeated oral twice daily administration, compound C and compound D maintained low B/P of < 0.05, which confers a significant margin between peripheral and central effects. In the DIO model, compound C at 5 and 10 mg/kg significantly reduced body weight, comparable to the effect of rimonabant, and improved glucose tolerance. At plasma exposures about 7-15-fold lower than those of compound C, both doses of compound D prevented the HFD-induced increase in body weight and 10 mg/kg improved glucose tolerance. Both compounds C and D dose-dependently reduced the LPS-induced production of pro-inflammatory cytokines such as IL-1β by human PBMC and reduced myofibroblast transformation of TGFβ-stimulated human primary fibroblasts. The biological activity of compound E is currently being evaluated.

Conclusions: Our data suggest that highly peripherally restricted CB1 inverse agonists are promising drug candidates for the treatment of metabolic diseases accompanied by inflammation and fibrosis.

Statement: To maintain IP during drug development, we are unable to disclose chemical structures at the time of presentation

EFFECTS OF ENDOCANNABINOID CATABOLIC ENZYME INHIBITORS AND Δ^9-TETRAHYDROCANNABINOL ON ACUTE PAIN-DEPRESSED NESTING IN MICE

Clare Diester[1]*, James Gillespie[1], Hallie Lappin[1], Aron Lichtman[1,2], Dana Selley[1], Laura Sim-Selley[1] and Steve Negus[1]

[1] Dept Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA
[2]School of Pharmacy, Virginia Commonwealth University, Richmond, VA

Introduction: Clinically relevant pain is often accompanied by behavioral depression and functional impairment, and preclinical assays of pain-depressed behavior can improve preclinical-to-clinical translation for candidate-analgesic testing. This study used an assay of pain-related depression of nesting behavior in mice to evaluate antinociceptive effectiveness of endocannabinoid catabolic enzyme inhibitors (eCB-CEIs) that ranged in selectivity for inhibition of monoacylglycerol lipase (MAGL) versus fatty acid amide hydrolase (FAAH).

Methods: Intraperitoneal dilute lactic acid (IP acid) served as an acute noxious stimulus in male and female ICR mice (N=6/sex/drug) to depress nesting behavior quantified as % Maximum Nestlet Consolidation (%MNC) during 90 min sessions. Antinociceptive effectiveness was defined by effectiveness of a test drug to alleviate IP acid-depressed nesting at doses below those that altered nesting in the absence of IP acid. eCB-CEIs were compared to a clinically effective cyclooxygenase inhibitor (ketoprofen) and to the direct cannabinoid receptor ($CB_{1/2}R$) agonist Δ^9-tetrahydrocannabinol (THC). Dose-effect curves were assessed for the following inhibitors in order of MAGL-to FAAH-selectivity: MJN110, JZL184, JZL195, SA57, URB-597, and PF3845. Time-course, antagonism, and repeated-dosing studies were conducted for MJN110. Following repeated-dosing studies, brain and spinal cord were collected 24 hours after the final treatment, and mixed-sex cohorts were analyzed for desensitization of CBR-stimulated G-protein activation using agonist-stimulated [^{35}S]GTPɣS binding in membranes from dissected CNS regions and [^{35}S]GTPɣS autoradiography in brain sections.

Results: Ketoprofen (0.1-10 mg/kg) produced robust antinociception (max %MNC=89), whereas THC (1-32 mg/kg) did not up to doses that decreased nesting in the absence of IP acid. Among the eCB-CEIs, MJN110 (0.1-3.2 mg/kg) produced the most effective antinociceptive profile (max %MNC=50) without altering control nesting. Antinociceptive effectiveness decreased as MAGL selectivity decreased, and PF3845 (1-32 mg/kg) failed to produce antinociception up to doses that decreased control nesting. Time course and antagonism studies for 1.0 mg/kg MJN110 showed a long duration of action (40min–6hr) mediated by CB_1R but not CB_2R and greater effectiveness in females. Chronic MJN110 (1.0 mg/kg/day x 7 days) showed sex-dependent antinociception and tolerance, with females showing antinociception on Day 1 but tolerance by Day 7, and males showing no significant antinociception across all 7 days. Results of G-protein activation studies in nucleus accumbens have shown reduced CP55,940-stimulated activity of MJN110 relative to vehicle-treated mice in both acid- and water-treated groups.

Conclusions: These results provide modest support for further consideration of MAGL-selective inhibitors, especially MJN110, as candidate analgesics, with future work needed to assess sex-dependent effects, tolerance, and utility in comparison to existing analgesics like ketoprofen.

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ADOLESCENT THC HAS DOSE-DEPENDENT EFFECTS ON REWARD, STRESS REACTIVITY, AND DECISION MAKING IN ADULTHOOD VIA PERTURBATIONS IN ASTROCYTES

Jacqueline-Marie N. Ferland*, Randall J. Ellis, Gregory Rompala, Joseph A. Landry, James E. Callens, Annie Ly, Micah Frier, Teddy Uzamere, Graeme Betts, Mason M. Silveira, Catharine A. Winstanley and Yasmin L. Hurd

Introduction: Cannabis is one of the most commonly used substances worldwide especially amongst teenagers. Despite the belief that cannabis is relatively harmless, exposure during adolescence is associated with increased risk of developing several psychopathologies in adulthood including addiction, depression, and cognitive deficits. In addition to the high levels of use amongst teenagers, the potency of delta-9-tetrahydrobcannabinol (THC), the main psychoactive constituent in cannabis, has increased more than fourfold compared to even twenty years ago. Determining the impact of adolescent THC exposure, especially high dose THC, on behaviors relevant to many psychopathologies observed clinically is essential to determine neural networks and molecular mechanisms underlying the development of psychiatric conditions.

Methods: To provide causal mechanistic insight into the protracted effects of adolescent THC exposure on behavior, we leveraged an animal model of recreational cannabis exposure to administer low (1.5 mg/kg) and high (5 mg/kg) dose THC to rats during their adolescent period and assessed effects of the drug on reward processing, anxiety, stress reactivity, and decision making in adulthood. RNA sequencing (RNAseq) was carried out on the basolateral amygdala, a region linked to reward processing, stress, and cognition to determine effects of THC on the transcriptome.

Results: Adolescent THC influenced behavior in a dose-dependent manner; while low dose THC influenced reward value and susceptibility to self-administer heroin, the high dose led to greater sensitivity to stressful conditions and re-exposure to THC later in life. RNAseq revealed that rats with prior history of high dose THC exposure had significant downregulation in genes specific to astrocytes, a subset of glia that maintain homeostasis of the synapse; these alterations were paired with upregulated genes of excitatory and inhibitory neurons. Furthermore, Gfap expression (an astrocyte marker) directly correlated with the cognitive deficits after adult re-exposure to THC.

Conclusions: The long-term effects of adolescent THC exposure are dose-dependent, and ex vivo data indicate that astrocytes, and the so-called "tripartite synapse," likely play a central role in THC-induced behavioral deficits after stress and drug re-exposure in adulthood.

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PERSISTENT EFFECTS OF FREQUENT ADOLESCENT THC EXPOSURE ON SOCIAL DEFEAT STRESS

Kwang-Mook Jung[1,2*], Hye-lim Lee[1,2], Erica Squire[1,2], Yannick Fotio[1], Francesca Palese[1], Alexa Torrens[1,2], Shiqi Su[1], Christina Renee Wong[1], Lin, Lin[1,2], Jade Ramirez[1,2] and Daniele Piomelli[1,2]

[1] Department of Anatomy and Neurobiology, School of Medicine, University of California, Irvine, USA.
[2] Center for the Study of Cannabis, University of California, Irvine, USA.

Introduction: Exposure to psychosocial stress has been linked to the development of depression. Bidirectional communication between the nervous and immune systems may contribute to the pathogenesis of this condition, which is characterized by elevated levels of stress mediators such as glucocorticoids and increased trafficking of proinflammatory immune cells. In mice, repeated social defeat (RSD) stress causes a robust depression-like phenotype marked by anxiety, anhedonia and social-avoidance, which is associated with an increased egress of monocytes from the bone marrow and recruitment of these cells to the brain. Migrating monocytes enhance inflammatory signaling through toll-like receptor (TLR)2/4-dependent microglial activation. This event contributes to neuropsychiatric pathology, as suggested by the finding that elimination of microglia during RSD or repopulation of RSD-affected microglia diminishes stress-related response. In unpublished work (see poster by Hye-lim Lee), we found that adolescent exposure to Δ^9-tetrahydrocannabinol (THC) persistently alters the microglial phenotype so that activation by damage-associated molecular pattern (DAMP) is significantly attenuated. The purpose of the present study was to determine whether adolescent exposure to THC may affect RSD stress in mice.

Methods: Mice were given THC (5 mg/kg) by intraperitoneal injection (i.p.) from postnatal day (PND) 30 to PND 43. When they reached adulthood (PND 70), they were subjected to daily RSD sessions under a standard protocol for 4 days. Social behavior was tested using the social interaction task, followed by molecular analysis of circulating cytokines, glucocorticoids and brain-wide gene expression. To assess the peripheral effects of adolescent THC exposure, mice received THC as described above, and at PND 70 they were treated with lipopolysaccharide (LPS, 0.33 mg/kg). 24 hours after LPS injection, their brain, blood and bone marrow were collected and immune cells (monocytes and lymphocytes) were quantified by flow cytometry.

Results: RSD caused severe social anxiety in control mice injected with vehicle during adolescence, as assessed by a significant reduction in the time spent by the mice in the social interaction zone and a significant increase in the time spent in the avoidance zone. In striking contrast, RSD had no such effect in mice that had been treated with THC in the adolescence. Molecular analyses revealed that RSD significantly elevated the plasma levels of corticosterone and IL-6, whereas no endocrine or inflammatory changes were observed in THC-exposed mice. The results indicate that adolescent THC exposure persistently abrogates social defeat-induced neuroinflammation and associated microglia-dependent deficits in social behavior. Next, we asked whether the persistent deficits caused by THC were associated with impairment in peripheral immune activation. We found that adolescent THC exposure persistently reduced the number of basal and LPS-induced monocytes and T lymphocytes in circulation. In the brain, the numbers of infiltrating macrophages and activated microglia were significantly reduced in THC-exposed mice compared to control animals.

Conclusion: The study shows that frequent exposure to THC in adolescence precludes RSD-induced neuroinflammation and associated microglia-dependent alterations in social behavior in adulthood. The results further suggest that these effects are associated with, and may be at least partially dependent on, a dampened peripheral innate immune response.

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THE ROLE OF THE ENDOCANNABINOID SYSTEM AND STRESS IN CANNABINOID HYPEREMESIS SYNDROME

Marieka V. DeVuono[1*], Olivia La Caprara[1], Megan T. Sullivan[1], Alexandra Bath[1], Gavin N. Petrie[2], Erin M. Rock[1], Cheryl M. Limebeer[1], Matthew N. Hill[2] and Linda A. Parker[1]

[1]Dept. of Psychology, University of Guelph, Guelph, ON, Canada
[2]Dept. of Cell Biology and Anatomy and Psychiatry, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada

Introduction: Cannabinoid Hyperemesis Syndrome (CHS) is characterized by nausea and vomiting due to high doses of Δ9-tetrahydrocannabinol (THC). It is thought that CHS is the result of endocannabinoid (eCB) system changes leading to hypothalamic-pituitary-adrenal (HPA) axis dysregulation. The eCBs are essential for regulation of the stress response, and HPA dysregulation is associated with similar nausea and vomiting disorders. Consistent with this hypothesis, we have shown that THC produces dose-dependent conditioned gaping, a rat model of nausea, and that high dose THC produces upregulation of the eCB degrading enzymes, monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), in the hypothalamus. To further establish the mechanism of CHS, the ability of several drugs to interfere with THC-induced conditioned gaping was tested in the taste reactivity test. The corticotrophin-releasing hormone (CRH) antagonist, Antalarmin, was used to determine if HPA activation is necessary for THC-induced nausea. Since eCBs inhibit the HPA axis, a MAGL inhibitor (MJN110) and FAAH inhibitor (URB597) were also tested. High doses of cannabinoids activate the stress response and Propranolol, a β-adrenergic antagonist, and WAY-100635, a 5-HT_{1A} antagonist, attenuate this effect and, therefore, may interfere with THC-induced nausea. In humans, CHS symptoms are not alleviated by typical anti-emetic drugs, such as Ondansetron, a 5-HT_3 antagonist; however, benzodiazepines are effective and commonly used. Therefore, Ondansetron and Chlordiazepoxide were administered as well.

Methods: Male rats received 3 daily conditioning trials where they were given one of the pre-treatments mentioned above or vehicle (VEH) before an intraoral infusion of saccharin paired with 10 mg/kg THC. The following day, rats underwent a drug-free test where they were only exposed to the infusion of saccharin, and gaping was measured. To confirm the involvement of the HPA axis, serum corticosterone (CORT) was analyzed from rats treated for 3 days with a nauseating (10 mg/kg) and non-nauseating dose (0.5 mg/kg) of THC.

Results: Pre-treatment with Antalarmin (10 and 20 mg/kg), MJN110 (10 mg/kg), URB597 (0.3 mg/kg), Propranolol (2.5 and 5 mg/kg),WAY-100635 (0.5 mg/kg) and Chlordiazepoxide (5 mg/kg) interfered with the establishment of THC-induced conditioned gaping. Similar to what is seen in humans, Ondansetron (0.1 and 0.01 mg/kg) did not interfere with THC-induced nausea. Rats treated with 10 mg/kg THC had significantly higher serum CORT than rats given VEH or 0.5 mg/kg THC.

Conclusions: Pre-treatments that interfere with stress interfere with THC-induced conditioned gaping, supporting the hypothesis that THC-induced nausea is a result of a dysregulated stress response due to eCB changes.

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ADOLESCENT THC EXPOSURE PERMANENTLY REPROGRAMS ADIPOSE ENERGY METABOLISM

Lin Lin[1*], Kwang-Mook Jung[1], Steve Mahler[2], Faizy Ahmed[1], Erica Squire[1], Shiqi Su[1], Alexa Torrens[1], Yannick Fotio[1], Jade Ramirez[1], Hyelim Lee[1], Francesca Palese[1] and Daniele Piomelli[1]

[1]Department of Anatomy and Neurobiology, University of California, Irvine, CA, USA
[2]Department of Neurobiology and Behavior, University of California Irvine, CA 92697

Introduction: Epidemiological studies show that frequent cannabis use is associated with lower body-mass index (BMI), improved fasting insulin and HDL-C levels and smaller waist circumference. This finding is paradoxical, because CB1 cannabinoid receptor activation by Δ^9-tetrahydrocannabinol (THC) or other agonists stimulates food intake and increases adipose lipogenesis in both experimental animals and humans. Since frequent cannabis use often starts during the teenage years, we evaluated the immediate and long-term effects of daily exposure to a moderate dose of THC (ado-THC) during adolescence on energy metabolism and adipose organ function.

Methods: Vehicle (Tween 80/saline) or THC (5 mg/kg) was administered to male and female C57BL/6j mice by intraperitoneal (IP) injection once daily on postnatal day (PND) 30-43. In some experiments, male mice also received AM251 (1 mg/kg) or AM6545 (3 mg/kg) once daily 60 min before THC. In other experiments, mice were placed on a high-fat diet (60 kcal% fat) on PND 57-130. We recorded meal parameters, body weight, motor activity, body composition (magnetic resonance spectroscopy), energy expenditure (metabolic chamber) and adipocyte size (hematoxylin-eosin). Levels of THC and endocannabinoids were quantified by LC-MS/MS. Fasting plasma was used for comprehensive blood panel analysis. Molecular analyses of epididymal white adipose tissue (WAT) were performed by RNA-sequencing (RNAseq) and quantitative PCR.

Results: Residual effects of ado-THC, assessed on PND44-49, included lower body-weight gain, increased energy expenditure and reduced WAT adipocyte area. There was no detectable effect on body length, food intake, motor activity, nutrient absorption or body composition. Enduring effects of ado-THC, assessed on PND70 (when THC was no longer detectable in the body), included lower fat mass, higher lean mass, and reduced adipocyte area in WAT. RNAseq data revealed a substantial increase in the transcription of thermogenesis-related genes in WAT. Global (AM251) or peripheral (AM6545) CB1 receptor blockade prevented these effects. Additionally, ado-THC mice were partly resistant to high-fat diet, assessed on PND57-130, as shown by reduced body-weight gain, increased energy expenditure, reduced fat mass and WAT adipocyte area, lower fasting glucose, and improved fasting cholesterol and triglyceride levels.

Conclusions: Frequent exposure to a moderate dose of THC during adolescence permanently reprograms WAT transcriptome and energy metabolism in mice. This striking effect may underpin the paradoxical negative association between cannabis use and metabolic syndrome in humans.

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CHRONIC EXPOSURE TO WHOLE CANNABIS EXTRACTS AMELIORATES METABOLIC DYSFUNCTION ASSOCIATED WITH DIET-INDUCED OBESITY IN MICE

Bryant Avalos* and Nicholas V. DiPatrizio

Division of Biomedical Sciences, School of Medicine, University of California, Riverside

Introduction: The peripheral endocannabinoid (eCB) system serves a critical role in regulating food intake and dietary preferences, and its activity becomes elevated in diet-induced obesity (DIO) and promotes overeating. Increases in appetite associated with elevated eCB signaling are similar to the effects found with acute consumption of *Cannabis sativa*. Paradoxically, however, retrospective studies suggest that long-term cannabis consumption is not only associated with a decreased body mass index (BMI) but also correlates with a lower prevalence of type-2 diabetes. Further research is necessary to understand how cannabinoid exposure affects the dynamics of eCB system function and its role in metabolic health and disease. In the current study, we aimed to investigate if chronic exposure to whole cannabis oil extracts or the primary intoxicating ingredient in cannabis, delta9 tetrahydrocannabinol (THC), affects metabolic dysfunction associated with DIO in mice.

Methods: Male C57BL/6Tac mice (8 weeks of age) were given ad-libitum access to a western-style diet (WD; 40% kcal from fat, 17% kcal from protein, 43% kcal from carbohydrates mainly from sucrose) for a total of 60 days. At day 30, animals were administered for the remainder of the study either pure THC (5 mg per kg) or whole cannabis oil extracts matched for THC content via our ultra-performance liquid chromatography/mass spectrometry methods (THC at 5 mg per kg). Body weights and food intakes were measured daily throughout drug exposure. An intraperitoneal glucose tolerance test (IP GTT) was performed at the end of the experiment.

Results: Chronic exposure to whole cannabis extracts and THC in DIO mice led to similar decreases in body weight and daily food intake when compared to control DIO mice treated with vehicle. Similarly, epididymal fat depots decreased in mass in both cannabis extract and THC-treated mice when compared to controls. Interestingly, the IP GTT revealed that dysregulated glucose clearance found in DIO mice was normalized in DIO mice treated with whole cannabis extracts but not in DIO mice treated with THC.

Conclusions: These results suggest that chronic cannabinoid exposure may possess pro-metabolic effects that include decreased body fat mass, reductions in the intake of a diet high in fat and sugar, and improvements in glucose homeostasis under conditions of DIO. Cannabis' therapeutic potential in the context of diabetes remains unclear; however, normalization of impaired glucose homeostasis in DIO mice treated with cannabis extracts, but not THC, suggests that THC alone is not responsible for this effect. The underlying mechanism(s) in these processes are currently under investigation.

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ADOLESCENT THC EXPOSURE PERSISTENTLY SUPPRESSES MICROGLIA FUNCTION IN MICE

Hye-lim Lee[1,2*], Erica Squire[1,2], Yannick Fotio[1], Francesca Palese[1], Alexa Torrens[1,2], Shiqi Su[1], Christina Renee Wong[1], Lin, Lin[1,2], Jade Ramirez[1,2], Kwang-Mook Jung[1,2] and Daniele Piomelli[1,2]

[1] Department of Anatomy and Neurobiology, School of Medicine, University of California, Irvine, USA.
[2] Center for the Study of Cannabis, University of California, Irvine, USA.

Introduction: Microglia are the major player of inflammation in the central nervous system (CNS). In particular, there is considerable evidence that chronic and uncontrolled activation of microglia may contribute to the pathogenesis of neurodegenerative diseases and other CNS disorders. Previous work found that administration of Δ^9-tetrahydrocannabinol (THC) during adolescence may disrupt normal developmental process, including synaptic pruning is mediated by microglia. However, it is not clear if the effect of THC on microglia is persistent and what are the long-term consequences. We hypothesized that adolescent THC exposure may cause both short- and long-term changes in the microglia, leading to dysregulated microglial activation that contributes to the persistent effect of THC.

Methods: Mice were injected with THC, 5 mg/kg by intraperitoneal injection (i.p.), from postnatal day (PND) 30 to PND 43. Then, they were left untreated until PND 49 or PND 70 when the brain was collected to assess the immediate or persistent effects of adolescent THC exposure. The brain was dissociated and microglia were isolated and purified through flow cytometry cell sorting. The microglial transcriptome was assessed by RNA sequencing (RNAseq). As an immune challenge, we injected 0.33 mg/kg of lipopolysaccharide (LPS), the bacterial endotoxin, by i.p., and the brain was collected 24 hours post injection.

Results: Bulk RNAseq analysis of microglia revealed that basal expression of certain endocannabinoid-related genes was altered by adolescent THC exposure both acutely and persistently, which was confirmed by real-time quantitative PCR (qPCR). Among them, adolescent THC significantly reduced expression of *Cnr1*, which encodes for CB_1 cannabinoid receptor, whereas it significantly increased *Faah*, which inactivates the endocannabinoid anandamide. The change in *Faah* transcription was observed both at PND 49 and PND 70, suggesting that the effect of THC on the brain endocannabinoid system may be persistent. The expression of FAAH protein was also increased by adolescent THC exposure, consistently with the gene expression data. In addition, we found that adolescent THC causes a marked down-regulation of inflammatory and immune response genes at both PND 49 and PND 70, suggesting that THC exposure may persistently dampen microglial activation. Immunohistochemistry experiments found that expression of the microglial marker IBA-1 was significantly reduced in prefrontal cortex and hippocampus of mice that had adolescent THC exposure compared to vehicle-treated control mice at both ages. Furthermore, expression of key immune response genes induced by LPS challenge was significantly impaired in adolescent THC mice at PND 70, as assessed by RNAseq and qPCR analyses. LPS-induced elevation of key cytokines in the brain was also significantly dampened in adolescent THC mice compared to controls.

Conclusion: Our microglial transcriptome analysis reveals that exposure to THC during adolescence significantly down-regulates inflammatory response genes, which is associated with alteration of the brain endocannabinoid system. Strikingly, these effects of adolescent THC exposure are maintained through adulthood and dampen the innate immune response of microglia to bacterial endotoxin.

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ABSTRACT WITHDRAWN

INVESTIGATING CANNABINOID ENTOURAGE EFFECTS IN CULTURED DRG NEURONS

Uma Anand[1], Barbara Pacchetti[2], Praveen Anand[3] and Mikael H. Sodergren[4]

[1]Surgery and Cancer, Imperial College London, UK
[2]EMMAC LIFE SCIENCES, UK
[3]Brain Sciences, Imperial College London, UK
[4]Surgery and Cancer, Imperial College London, UK

Background: The endocannabinoid 2-Arachidonyl glycerol (2-AG) exerts dose-related effects of antinociception, immobility, reduction of spontaneous activity, and hypothermia in mice (Mechoulam R et al 1995, Biochem Pharmacol, 50; 83-90). At sub-effective doses of 2-Arachidonyl glycerol (2-AG), its combination with the related, inactive 2-palmitoyl glycerol (2-PG) and 2-linoleoyl glycerol (2-LG), in the ratio 1:5:10 (A:P:L), induced significant behavioural effects, including analgesia in mice i.e. increased latency of response to heat pain. This potentiation of a biological effect by inactive compounds was described as the "entourage effect" by the Mechoulam group (Ben-Shabat S et al 1998, Eur J Pharm, 353; 23–31). We have investigated the effect of 2-AG, 2-PG and 2-LG, individually and in combination, on signaling via the heat and capsaicin receptor TRPV1, in a DRG culture model of neuronal hypersensitivity.

Method: Adult rat DRG neurons were cultured in modified BSF2 medium containing 2% fetal calf serum, NGF (100 ng/ml), and GDNF (50 ng/ml), in a humidified incubator at 37^{o}C. 48 hours later, the cultures were loaded with 2μM Fura2AM for calcium imaging, and treated with 2-AG, 2-PG (Tocris) and 2-LG (Cayman), singly or combined, or vehicle, for 5 minutes, followed by 1 μMol capsaicin, at 37^{o}C. The amplitude and latency of capsaicin responses were measured in individual neurons, and analyzed using the Mann Whitney test. Control values are derived from n=13, and others from n=3-7 experiments.

Results:
Controls: In vehicle-treated control experiments, 1 μMol capsaicin elicited immediate and sustained calcium influx in capsaicin sensitive neurons, with average latency of 1.27 ± 0.2 seconds and amplitude of 0.15 ± 0.01.
Effect of 2-AG alone: In dose-ranging studies, capsaicin responses after treatment with 1 or 50 μMol 2-AG were similar to controls; 100 μMol 2-AG did not significantly affect the amplitude, but markedly increased the latency of capsaicin responses to 229.27 ± 45 seconds (***P<0.001), which was unaffected by the presence of 10 μMol AM630 (CB2 antagonist) or 10 μMol Rimonabant (CB1 antagonist). Low concentrations of 2-AG elicited occasional transient calcium influx spikes in some neurons, and mild calcium influx of longer duration in a minority sub-set of neurons at 100 μMol 2-AG.
Effect of 2-PG alone: 2-PG alone did not affect capsaicin response latency at 10 nMol, 100 nMol, and 1 μMol, but this was increased at 5 μMol (320.5 ± 55 seconds, ***P<0.001), 10 μMol (394.11 ± 68 seconds, ***P<0.001), 100 μMol (299.6 ± 88 seconds **P<0.01) concentrations; capsaicin response latency was restored to control values after washout of medium. The increased latency was abolished in the presence of AM630 at 2-PG 10 μMol (to 0.77 ± 0.4 seconds), but not in the presence of Rimonabant (283.11 ± 3.2 seconds). No calcium influx was observed in response to 2-PG application, up to 100 μMol.
Effect of 2-LG alone: 2-LG increased capsaicin response latency at 12 μMol concentration (to 295.44 ± 32.3 seconds, ***P<0.001), which was unaffected by the presence of Rimonabant (352.1 ± 23.7), or AM630 (230.2 ± 93.4). No calcium influx was observed of 2-LG at 12 μMol, but intracellular calcium levels were increased in response to applications of 30 and 60 μMol 2-LG.
Effect of combined 2-AG, 2-PG and 2-LG: To mimic the reported entourage effect at a sub-effective dose of 2-AG (here 1 μMol), although 2-PG and 2-LG at the relevant dose ratios had some effects, we treated the cultures with 1 μMol 2AG + 5 μMol 2PG + 12 μMol 2LG. This combination significantly delayed capsaicin responses, to 281.5 ± 41.5 seconds (n=6, ***P<0.001), without affecting the amplitude; the response latency was restored after medium change. Studies at sub-effective doses of all three compounds are in progress.

Conclusion: 2-AG, 2-LG and 2-PG have differential effects on capsaicin mediated TRPV1 signaling, individually and in combination, potentially with diverse kinetic and receptor-mediated mechanisms that may be related to the integrity of the endocannabinoid molecules (see Starkus J et al 2019, Channels, 13; 172-191). Further studies are required at physiological doses, both in vitro and clinical trials.

INHIBITION OF ANANDAMIDE HYDROLYSIS AS A STRATEGY TO COUNTERACT RESPIRATORY ABNORMALITIES OBSERVED IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

Luara A. Batista[1], Thiago S. Moreira[2] and Ana C. Takakura[1]

[1]Department of Pharmacology, [2]Department of Physiology and Biophysics, Instituto de Ciências Biomédicas, Universidade de Sao Paulo, SP, Brazil.

Introduction: anandamide is an endocannabinoid with therapeutic potential to treat neurological and psychiatric disorders. Here, we tested the hypothesis that URB597, an anandamide hydrolysis inhibitor, counteracts the impaired response to hypercapnia observed in an animal model of Parkinson's Disease (PD).

Methods: male Wistar rats (300-360g; CEUA: 5513240518) received 6-OHDA (12 μg/2 μL) or vehicle into the dorsal striatum bilaterally and after 40 days respiratory parameters (respiratory frequency (f_R), tidal volume (V_T) and ventilation (V_E)) were recorded by whole-body plethysmography. For the recordings, animals received intraperitoneal injections of URB597 (0.3 mg/kg) or vehicle and were exposed to 15 min of normoxia (21% O_2, 79% N_2), 15 min of hypercapnia (7% CO_2, 79% N_2), and 15 min of recovery. Immunohistochemistry was also performed to evaluate expression of tyrosine-hydroxylase (TH) in the Substantia Nigra (SN) and Phox2b in the retrotrapezoid nucleus (RTN).

Results: Injections of 6-OHDA in the striatum led to a decrease in the total number of TH neurons in SN (6-OHDA + vehicle: 178±17 and 6-OHDA + URB597: 190±25 vs. vehicle + vehicle: 702±69, $p<0.05$) and Phox2b neurons in the RTN (6-OHDA + vehicle: 69±14, vs. vehicle + vehicle: 178±31 neurons, $p<0.05$). URB597 was able to decrease the number of Phox2b neurons in the RTN (Vehicle + URB597: 75±22, vs. vehicle + vehicle: 178±31 neurons, $p<0.05$). Animals treated with 6-OHDA and URB597 also presented a decrease in the number of Phox2b neurons in the RTN (6-OHDA + URB597: 59±20, vs. vehicle + vehicle: 178±31 neurons, $p<0.05$). Regarding ventilation, when data were analyzed as a time-course (10 sec interval) 6-OHDA animals presented higher variability for resting f_R when compared to vehicle animals (6-OHDA + vehicle: 13.41, vs. vehicle + vehicle: 8.96% Coefficient of Variation, $p<0.05$). Treatment with URB597 was able to increase this variability when compared to 6-OHDA animals (6-OHDA + URB597: 21.17, vs. 6-OHDA + vehicle: 11.29% Coefficient of Variation, $p<0.05$). Data were also analyzed as means during three time points (minutes 5, 10 and 15). We observed a reduction in f_R during hypercapnia in lesioned animals (6-OHDA + vehicle: 109±20, vs. vehicle + vehicle: 138±11 breaths/min, $p<0.05$). The means of V_T and V_E were not altered in lesioned animals. The attenuated tachypneic response observed in 6-OHDA-treated animals was not reversed by URB597 treatment (6-OHDA + URB597: 101±16 vs. 6-OHDA + Vehicle: 109±20 breaths/min, $p>0.05$). URB597 by itself also reduced f_R (93±8 vs. vehicle + vehicle: 138±11 breaths/min, $p<0.05$) and V_E during hypercapnia (830±95, vs. vehicle + vehicle 2663±310 mL/min/kg, $p<0.05$). Animals treated with URB597 that received 6-OHDA presented a reduction in f_R during hypercapnia (100±9 vs. vehicle + vehicle 138±11 breaths/min, $p<0.05$). There were no changes in V_E or V_T in animals with lesion of the SNpc that were treated with URB597.

Conclusion: we observed that the respiratory dysfunction observed in an animal model of PD may be of central origin. In addition, inhibition of anandamide hydrolysis by URB597 is not an effective treatment for the respiratory symptoms, which may have implications for the consideration of the endocannabinoid system as a target to develop treatments for PD.

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ANANDAMIDE HYDROLYSIS REVERSES THE PROLONGED BEHAVIORAL AND GENE EXPRESSION ALTERATIONS-INDUCED BY ADOLESCENCE NMDA RECEPTOR HYPOFUNCTION

Hagar Bauminger*, Hiba Zaidan, Inna Gaisler-Salomon and Irit Akirav

School of Psychological Sciences and the integrated Brain and Behavior Research Center, University of Haifa, Haifa 3498838 Israel

Introduction: NMDA receptor blockers are commonly used to mimic aspects of schizophrenia in rodents. Adolescence is a vulnerable time period for symptom development, characterized by dramatic changes in glutamate neurotransmission. The role of the endocannabinoid system (ECS) in the pathophysiology of schizophrenia is unclear. Here, we examined the efficacy of the anandamide hydrolysis inhibitor URB597 in restoring schizophrenia-like phenotypes induced by early-adolescence NMDA receptor blockade by MK-801.

Methods: The NMDA receptor antagonist dizocilpine (MK-801) was chronically administered to early-adolescent rats, followed by late-adolescence injection of URB597. In adulthood, rats were tested for behavioral abnormalities associated with schizophrenia. Then, brains were extracted for mRNA gene expression evaluation of glutamate, GABA, endocannabinoid and neuroinflammatory markers in the medial pre-frontal cortex (mPFC).

Results: Early-adolescence MK-801 impaired novelty recognition in the novel object recognition task, diminished social exploration in the social interaction test and altered mRNA expression of glutamate, GABA, cannabinoid receptors and neuroinflammatory markers in mPFC of adult rats. These behavioral and gene expression abnormalities were absent in rats that received late-adolescence URB597 treatment.

Conclusions: Early-adolescence MK-801 treatment induced long-term behavioral abnormalities in social behavior and novelty recognition which resemble the treatment-resistant negative symptoms and cognitive dysfunction in schizophrenia, and altered gene expression patterns in the PFC. The prevention of these abnormalities by late-adolescence URB597 point to the putative therapeutic capacity of endocannabinoid stimulation. Changes in neuroinflammatory markers parallelled the pattern of behavioral abnormalities and their reversal, suggesting that they may play a mechanistic role in the interaction between NMDA receptor blockade and the ECS.

CHARACTERIZATION AND TARGETING OF THE ENDOCANNABINOID SYSTEM IN TRAUMATIC BRAIN INJURY

Tallan Black[1], Ayat Zagzoog[1], Andrew Roebuck[2], Quentin Greba[2], John G. Howland[2] and Robert B. Laprairie[1]

[1]College of Pharmacy and Nutrition, and [2] Department of Anatomy, Pharmacology, and Physiology, College of Medicine, University of Saskatchewan, Saskatoon SK CAN.

Traumatic Brain Injuries (TBI) are considered one of the leading causes of death and disability worldwide. One emerging area of TBI research is the involvement of the endocannabinoid system (ECS) in response to brain injury. The ECS is modulated by exogenous cannabinoids such as Δ^9-tetrahydrocannabinol (THC) found in *Cannabis sativa*. THC is a partial agonist of both cannabinoid receptors CB1R and CB2R. CB1R activation contributes to analgesia and anxiolytic effects, whereas CB2R activation reduces inflammation. In the wake of *Cannabis* legalization in Canada, and as awareness surrounding post-traumatic complications increases, the need for research examining this intricate relationship between exercise, the ECS, and TBI is apparent. To date there has been a lack of research into the effects of TBI and exercise on the ECS, pre-clinical or otherwise. We hypothesized that treatment of rats subjected to TBI with THC post-injury will restore behavioral and molecular profiles of injured rats.

Methods: Eighteen Sprague-Dawley rats of both sexes aged 8-12 weeks at the time of TBI were randomly assigned to: 1) Sham-TBI + Vehicle; 2) Sham-TBI + 1 mg/kg THC *i.p.*; 3) 300 g weight drop TBI + Vehicle; or 4) 300 g weight drop TBI + 1 mg/kg THC *i.p.* Behavioral and physiological changes associated with CB1R activation were assessed using the tetrad battery: catalepsy, body temperature, open-field, and antinociception. Y-maze was used to assess spatial learning and memory, and rotarod assessed higher level motor function following injury. Changes to relative CB1R density in the cortex were analyzed using western blot, and a cytokine and chemokine analysis assess concentrations 8 days following injury. All experiments were performed with the approval of the University Animal Care Committee. Statistical analyses were 2x2 repeated measured ANOVA, $p < 0.05$ (Prism v. 8.0).

Results: There were no specific effect of 1 mg/kg of THC or TBI in the tetrad battery or Y-maze across all treatment. TBI robustly reduced male rotarod performance in both VEH and THC treated groups, and THC treatment decreased performance in Sham-TBI rats in comparison to vehicle controls. Despite exhibiting no change in rotarod performance, females who received a TBI and THC exhibited significantly lower relative CB1R density when compared to the Sham-TBI+THC group. The cytokine analysis revealed that TBI was a main effect for male Interleukin-4 (IL-4), THC significantly decreased levels of Interleukin-6 (IL-6) in the male Sham-TBI group, and THC treatment was a main effect for female CXCL5/LPS-induced chemokine (LIX).

Conclusions: Males appeared to be more susceptible to locomotor effects of both THC and TBI and may experience prolonged injury recovery and drug effect indicated by the cytokine analysis. Comparatively, females demonstrated an injury resistant behavioural phenotype to TBI, but experienced pronounced subtractive modulation of their endocannabinoid system from the combination of injury and drug treatment. These results support existing, but limited, sex differences in both TBI and endocannabinoid modulation, but indicate the need for further sex specific investigation of other phytocannabinoids, dosing regimens, behaviours, and injury magnitudes.

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SEX-DEPENDANT BRAIN REGIONAL ALTERATIONS IN THE ENDOGENOUS CANNABINOID SYSTEM IN A RAT MODEL OF PERIPHERAL NEUROPATHIC PAIN

Laura Boullon*, David P. Finn and Alvaro Llorente-Berzal

Pharmacology and Therapeutics/National University of Ireland, Galway

Introduction: Despite higher prevalence of chronic pain in women compared to men, sexual dimorphism in neuropathic pain is poorly understood. This study characterized sex differences in the development of pain-, depression- and anxiety-related behaviours, together with cognitive deficits, after peripheral neuropathy. Furthermore, we investigated alterations in the endogenous cannabinoid system (ECS) in brain regions involved in the descending modulation of nociception.

Methods: Sham or Spared Nerve Injury (SNI) surgery was performed in adult male and female Sprague-Dawley rats (n=12 per group). Von Frey and Acetone Drop tests (post-surgery days (PSD) 7, 14, 21, 63, 84, 99) investigated mechanical and cold allodynia, respectively. Depression-related behaviours, were analysed by the sucrose preference (PSD 54 and 75) and Forced Swim (PSD 92) tests, respectively. Elevated Plus Maze (PSD 59) and Light-Dark Box (PSD 70) tests determined anxiety-related behaviours. The development of recognition, social and spatial memory deficits were examined using Novel Object Recognition (PSD43), Social Interaction (PSD 75) and T-maze (PSD 86 and 87) tests. Post-mortem analysis (PSD100) of endocannabinoids [anandamide (AEA) and 2-arachidonoylglycerol (2-AG)] and N-acylethanolamines [oleoylethanolamide (OEA) and palmitoylethanolamide (PEA)] levels were determined in discrete brain regions of the descending pain pathway: prefrontal cortex (PFC), periaqueductal grey (PAG) and rostral ventromedial medulla (RVM), using high pressure liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). Non-parametric data were analysed using Kruskal-Wallis followed by Mann-Whitney U post hoc test with Bonferroni-Holm corrections. Parametric data were analysed using 2-way ANOVA followed by Student-Newman-Keuls post hoc test. $P<0.05$ was considered statistically significant.

Results: SNI surgery induced the development of mechanical and cold allodynia in both sexes, effects maintained during the full duration of the experiment. Interestingly, females exhibited lower paw withdrawal thresholds in the von Frey and acetone drop tests than their male counterparts. No between-group differences were observed in the sucrose preference test, however significantly higher immobility during the forced swim test was detected in female-SNI rats compared to sham counterparts. Reduction in the time spent in the open arms during the elevated plus maze test, a parameter indicative of anxiety-related behaviour, was exhibited in male-SNI rats compared to their sham counterparts. No effect of SNI was observed in the light-dark box test. SNI had no effect on recognition memory in either sex, however, alterations were observed in social-related behaviours and spatial memory of male-SNI but not female-SNI rats, compared to sham counterparts. Levels of 2-AG and PEA were significantly higher in PFC of male-SNI rats, compared to their sham counterparts; an effect not observed in females. PAG and RVM levels of endocannabinoids and N-acylethanolamines were unaltered due to SNI in either sex.

Conclusions: These results provide evidence for sex-dependant changes in pain- depression-, anxiety- and cognition-related behaviour and in PFC 2-AG and PEA levels following peripheral nerve injury. Further studies are required to determine whether the sex-dependent ECS alterations observed are causally linked to the behavioural sex dimorphism.

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THE EXPRESSION OF COMPONENTS OF THE ENDOCANNABINOID SYSTEM PREDICTS PATIENT SURVIVAL IN MELANOMA BUT CANNABINOIDS LACK SELECTIVITY AS POTENTIAL THERAPEUTICS

Elizabeth A. Cairns[1], Dilini Gunatilake[2], Adam Ametovski[1], Lewis J. Martin[1], Jonathan C. Arnold[1], Samuel D. Banister[1], Iain S. McGregor[1] and Jessamy C. Tiffen[2]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, NSW, Australia
[2]Melanoma Epigenetics Laboratory, The Centenary Institute, NSW, Australia

Introduction: The incidence and mortality rate of melanoma in Australia is the highest in the world, and is the fourth most common type of cancer in Australia. Even with recent advances in therapies, many patients develop treatment resistance, and metastatic melanoma still suffers a very poor survival rate. Novel therapies are desperately needed. Previous studies suggest that some cannabinoids can be cytotoxic to some melanoma cancer cells *in vitro*; however, these claims have typically involved effects in a very small number of melanoma cell lines. Here we sought to investigate the potential cytotoxic effects of cannabinoids and endocannabinoid system (ECS)-modulating drugs in a representative pool of multiple patient-derived melanoma cells.

Methods: RNA expression and survival data were acquired from the Skin Cutaneous Melanoma cohort of The Cancer Genome Atlas and compared data from non-tumor skin tissue (GTEx). Nine patient-derived melanoma lines (A06, C013, C027, C044, C055, C058, C074, C089, D22M1) collected at the Queensland Institute of Medical Research, Berghofer Medical Research Institute, and 2 non-cancerous cell lines (human dermal fibroblasts [HFF], and human epidermal melanocytes [HEMn-MP]) were used to evaluate effects of cannabinoids and/or ECS modulating drugs on cell proliferation. Cell growth was evaluated using CellTiter-Glo reagent after exposure to 0.3-300 μM of compound or vehicle for 72 hours.

Results: Patient survival data from the Cancer Genome Atlas suggested that levels of expression of CNR2, GPR18, GPR55, TRPV1, FAAH2 and NAAA genes were positively correlated with patient survival outcomes, while expression of DAGLB was negatively correlated. *In vitro* data showed that THC, CBD, CBG, CBC, equimolar THC+CBD, PEA, NAGly, ML-186 (a GPR55 agonist), and Orlistat and KT-109 (non-selective DAGLB inhibitors) were neither potent nor selective inhibitors of melanoma cell growth (mean IC50 range in melanoma cells 23.3 - >300 uM). At 10 μM, CBD, CBG, CBC, and THC+CBD actually promoted growth in melanoma cells (mean growth 147.8-187.5% relative to vehicle treatment, $p < 0.05$).

Conclusions: While expression of various components of the ECS do appear to be associated with long-term patient outcome, none of the diverse range of cannabinoids and ECS-modulating drugs tested here were potent or selective inhibitors of melanoma cell growth. Future studies might investigate the potential growth promotion effects of cannabinoids on melanoma cells, particularly within an *in vivo* context.

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CHARACTERIZATION AND IMMUNE MODULATION OF COX-2-DERIVED DHEA METABOLITES IN LPS-STIMULATED MACROPHAGES

Ian de Bus[1,2], Sandra van Krimpen[2], Han Zuilhof [1], Renger Witkamp[2], Bauke Albada[1,*] and Michiel Balvers[2,*]

[1] Laboratory of Organic Chemistry,
[2] Nutritional Biology Group Wageningen University & Research, Wageningen, The Netherlands

Introduction. The endocannabinoid docosahexaenoyl ethanolamide (DHEA) has potential immune modulating and anti-inflammatory properties. Just like other poly-unsaturated fatty acids (PUFAs) and PUFA derived endocannabinoids, DHEA is susceptible to enzymatic (and non-enzymatic) oxidation leading to potentially interesting, and largely unknown metabolites. Two recently discovered cyclooxygenase 2 (COX-2)-derived DHEA hydroxy metabolites, 13-HDHEA and 16-HDHEA, were analysed for their immune modulating effect in LPS-stimulated RAW264.7 murine macrophages.

Methods. RAW264.7 cells were pre-incubated with 2.5 μM or 5.0 μM 13-HDHEA and 16-HDHEA for 30 min. before stimulation with 1.0 μg/mL for 24h. Effect on NO production was determined using Griess assay, andIL-6, IL-1β, and IL-1Ra production were determined using ELISA assays. Effects on whole genome mRNA expression was performed by mRNA transcriptome analysis using Affymetrix Mouse Gene 2.1 ST arrays followed by functional and upstream regulator analysis in Ingenuity Pathway Analysis (IPA©) from Qiagen. Finally, effects of 13- and 16-HDHEA on prostaglandins and endocannabinoids were determined using a targeted UPLC-MS/MS method..

Results. Both 13-HDHEA and 16-HDHEA inhibited IL-1β and IL-1Ra, but not NO and IL-6 production. Transcriptomic analysis showed inhibition of pro-inflammatory regulating genes (InhbA, Ifit1) and suggested potential inhibition of pro-inflammatory regulating pathways including toll-like receptor 4 (TLR4), MyD88, and interferon regulatory factor 3 (IRF3), whereas anti-inflammatory genes (SerpinB2) and potential regulators IL-10, sirtuin 1 (Sirt-1), fluticasone propionate were induced. IPA analysis suggested a ROS-inducing and antiangiogenic effect for 13-HDHEA. 13-HDHEA and 16-HDHEA did not affect prostaglandin formation. Compared to DHEA the anti-inflammatory effects of 13-HDHEA and 16-HDHEA were small but distinct, indicating that there are differences in anti-inflammatory effects and mechanisms between DHEA and 13-HDHEA and 16-HDHEA.

Conclusion.. Both 13- and 16-HDHEA had a modest but distinct immune modulating effect in LPS-stimulated RAW264.7 macrophages. This implies that 13-HDHEA and 16-HDHEA have specific anti-inflammatory effects when compared to DHEA, but also suggests that COX-2 metabolism acts as a regulatory mechanism to limit the anti-inflammatory properties of DHEA. Future research is warranted to better understand DHEA metabolism and its implications in inflammatory models.

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CHEMICAL ENDOCANNABINOID-DERIVED PROBES SUGGEST A POTENTIAL REGULATING ROLE IN RAC1 SIGNALLING

Ian de Bus[1,2], Twan America[3], Norbert de Ruijter[4], Han Zuilhof[1], Renger Witkamp[2], Bauke Albada[1,*] and Michiel Balvers[2,*]

[1]Laboratory of Organic Chemistry, [2]Nutritional Biology Group, Division of Human Nutrition, [3]Plant Research International, Business Unit Bioscience, [4]Laboratory of Cell Biology, Wageningen University & Research

Introduction. Poly-unsaturated fatty acid (PUFA)-derived endocannabinoids like arachidonoyl ethanolamide (AEA) and docosahexaenoyl ethanolamide (DHEA) possess regulatory roles in various biological processes. Although previous studies have revealed some of their molecular targets, much of their mechanisms of action remains unknown. Chemical bi-functional probes containing both an UV-dependent diazirine and a terminal alkyne, can be used to selectively target and identify novel protein targets. Therefore, we synthesized bi-functional endocannabinoid probes to selectively identify new protein interaction targets of DHEA and AEA in RAW264.7 macrophages.

Methods. Organic synthesis was used to produce two DHEA probes, one AEA probe, and one control probe without any reported biological activity. IL-6 and PGE_2 ELISA were used to confirm that the probes mimic the (anti-)inflammatory properties of the natural compounds in LPS-stimulated RAW264.7 macrophages. 4h incubation of the probes in 8h LPS-stimulated RAW264.7 macrophages followed by copper(I) mediated azide-alkyne click reaction with a fluorophore or biotin was used to visualize the probes and selectively purify their proteomic targets. Characterization of binding targets was performed by affinity-based purification with magnetic Pierce beads followed by on bead tryptic digestion and LC-MS/MS proteomics. Bio-informatic analysis of the proteomic data was performed using Uniprot and Ingenuity Pathway Analysis (IPA).

Results. Confocal fluorescence microscopy showed that the endocannabinoid probes were effectively taken up by the macrophages, resulting in ER and liposomal localization. For DHEA 62 protein targets, and for AEA 114 protein targets were characterized. Interestingly, 38 of these proteins were shared between DHEA and AEA, amongst which the enzyme Ptgs2, and the regulatory protein Rac1. Interestingly, both DHEA and AEA probes also targeted many Rac1 interactome related proteins. Confocal microscopy using immunofluorescence with Rac1 and Ptgs2 antibodies proved colocalization of the proteins with the endocannabinoid probes. Bio-informatic analysis with IPA also suggested a role in ROS production for the endocannabinoids. In RAW264.7 macrophages no significant effects on ROS production were observed, but this model is probably not well-suited for ROS production assays.

Conclusion. Proteomic analysis with endocannabinoid-derived synthetic bi-functional probes suggested a novel regulatory target in endocannabinoid signalling, Rac1. Proteomic analysis showed that Rac1 as well as many Rac1 interactome proteins were selectively targeted by both DHEA and AEA probes. Microscopy experiments further supported the presence of specific Rac1-endocannabinoid sites in vitro. The biological implications of the Rac1-endocannabinoid interaction need future investigations.

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CROSSTALK BETWEEN MUSCARINIC RECEPTORS AND CANNABINOID RECEPTORS: IDENTIFYING POTENTIAL THERAPIES IN TEMPORAL LOBE EPILEPSY

Ryan. R Hall[1], Damian Cohall[1] and Andrew B. Tobin[2]

1. Faculty of Medical Sciences, University of the West Indies
Cave Hill Campus, Barbados
2. Institute of Molecular, Cell and System Biology, University of Glasgow, Scotland

Introduction: Epilepsy is a neurological condition categorised by recurrent seizures. Approximately 65 million people globally have epilepsy with 80% of cases living in developing countries. Forty percent of epileptics experience pharmaco-resistance and there is a need for new effective therapies. Studies have shown that the administration of delta-9-Tetrahydocannabinoid (THC) and Cannabinol (CBD) can reduce seizure activity and severity in epileptic animal models (Ham et al., 1975; Wallace et al., 2003). Also, CBD has shown to significantly reduce epileptic activity in children who suffered from Dravet Syndrome and Lennox-Gestaut Syndrome (Jones et al., 2012)(Devinsky et al., 2014). Temporal lobe epilepsy (TLE) is most common form of epilepsy with focal seizures and can be induced experimentally by the muscarinic agonist, pilocarpine. This study investigated the potential cross-talk between cannabinoid and muscarinic systems by focusing on the human M1 acetylcholine muscarinic receptor (hM1R) expressed in an in vitro transfected cell system together with Cannabinoid Receptor 1 (CB1R) and Cannabinoid Receptor 2 (CB2R).

Methods: Chinese Hamster Ovarian (CHO) cells expressing the hM1R were cultured and transiently transfected with cDNA encoding for CB1R or CB2R. Transfection efficiency was verified by Western Blotting. IP1 and pERK assays were performed with the muscarinic receptor activated with acetylcholine (ACh) at varying concentrations with cells expressing CB1R and CB2R with the EC_{20} dose of arachidonyl-2-chloroethylamide (ACEA) and HU-308 respectively. pERK and IP1 levels were measured in incubation cell lysates with fluorescence resonance energy transfer (FRET). Signals were expressed as the FRET ratio F = (fluorescence665nm/ fluorescence590nm) and normalized to the maximal response to ACh.

Results: Co-expression of CB1R with the hM1R reduced the hM1R pERK and IP1 response by 33% and 6% respectively compared to the response observed in cells expressing only the hM1R. Interestingly, co-stimulation of the CB1R with an EC20 dose of ACEA restored some of the hM1R signalling. Under these conditions the hM1R pERK and IP1 response was only reduced by 8% and 1% respectively. In similar experiments, co-expression of CB2R with the hM1R reduced the hM1R pERK and IP1 response by 21% and 7% respectively compared to the response observed in cells expressing only the hM1R. Here co-stimulation with CB2R with an EC20 dose of HU308 and acetylcholine made only a modest impact on the effect of co-expression of the two receptors. Under these conditions the hM1R pERK and IP1 reduced by 23% and 7% compared to the hM1R response in cells only expressing the hM1R.

Conclusion: The co-expression of either CB1R or CB2R with the hM1R in this in vitro recombinant system reduces the activation of the muscarinic receptor. The addition of ACEA, a CB1 full agonist to cells expressing the CB1R receptor and the hM1R receptor resulted in a partial reversal of the inhibition of the activation of the hM1R muscarinic receptor.

CHANGES IN THE ENDOCANNABINOID SYSTEM OF MALE RATS TREATED WITH A SINGLE SUB-NOXIOUS DOSE OF CISPLATIN

Mary Hopkins[1], Ana Bagüés[2], Yolanda López-Tofiño[2], Raquel Abalo[2] and Alvaro Llorente-Berzal[1]

[1]. Pharmacology and Therapeutics, School of Medicine, Galway Neuroscience Centre and Centre for Pain Research, NCBES, National University of Ireland Galway, Galway, Ireland.
[2]. Dpto Ciencias Básicas de la Salud, Fac Ciencias de la Salud, y Unidad Asociada al Instituto de Química Medica (IQM) del CSIC, Universidad Rey Juan Carlos, Alcorcón, Spain.

Introduction: The anticancer drug cisplatin is known to cause serious side effects, such as gastrointestinal disorders and peripheral neuropathy. Exogenous cannabinoids exert positive effects in chemotherapy-treated patients reducing nausea and vomiting and stimulating appetite, but they can also have analgesic effects. In fact, activation of cannabinoid receptors can reduce or prevent neuropathic-related symptomatology in rodents. In this study, we investigated the maladaptive changes to the endocannabinoid system (ECS) occurring at the brain and dorsal root ganglia (DRG) after a single and sub-noxious dose of cisplatin.

Methods: Male Wistar rats were injected i.p. with saline or cisplatin (5mg/kg). Immediately after, barium contrast was gavaged and serial X-rays were taken 0-24 h afterwards. Food intake and body weight were recorded 1, 2 and 7 days after treatment and locomotor activity and pain-related behaviours (von Frey and Hargreaves' tests) were measured on day 4. One week after treatment, animals were sacrificed, their brains dissected, and L4 and L5 DRGs extracted. All tissues were flash frozen and stored at -80ºC for further use. Rt-PCR was carried out to assess the gene expression of cannabinoid-related receptors (CB_1R and CB_2R) and catabolic enzymes (FAAH, MAGL), as well as that of the 3 subtypes of peroxisome proliferator-activated receptors (PPARα, PPARβ/δ and PPARγ). LC-MS/MS was used to assess levels of endocannabinoids and related N-acylethanolamines.

Results. Cisplatin 5 mg/kg failed to alter locomotor activity and pain-related behaviours, but induced a significant reduction of food intake, body weight and gastric emptying. Cisplatin-treated animals showed a significant increase of CB_2R, MAGL and PPARα mRNA expression in the prefrontal cortex, whilst in the amygdala a significant reduction of CB_1R and MAGL mRNA levels was observed. In addition, cisplatin significantly increased CB_1 receptor mRNA expression in L4 DRG, with a similar trend in L5 DRG. No significant alterations in the levels of endocannabinoids and the related N-acylethanolamines were found.

Conclusions. A single 5 mg/kg dose of cisplatin induced a significant effect on gastrointestinal motility but no changes in locomotor activity nor pain-related behaviours. Interestingly, alterations in the genic expression of proteins related to the endocannabinoid system were found in regions related to pain and emotional processing. Longer and/or increased dosing studies may reveal further alterations in the ECS.

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GPR55 ANTAGONIST ML193 AND AGONIST O-1602 HAVE CELL AND TIME-DEPENDENT EFFECTS ON LIPID REGULATION

Clare T Johnson* and Heather B Bradshaw

Indiana University, Bloomington IN 47405 USA

Introduction: G-protein-coupled receptor 55 (GPR55) is located throughout the central nervous system and interacts with both synthetic and phytocannabinoids. The complete pathway of cellular signaling by GPR55 has not been fully elucidated and is likely cell-type dependent. Likewise, understanding the cell-specific influence of GPR55 agonism and antagonism on lipid regulation will greatly enhance our understanding of these varied signaling systems. The present study addresses this gap in knowledge by determining the lipidomic profile of C6 glioma, BV2 microglial, and N18 neuronal cell lines after treatment with either the GPR55 agonist O-1602 or the GPR55 antagonist ML193.

Methods: Cells were incubated with 10μM of either ML193 or O-1602 for 2 or 24 hours, followed by fast methanolic lipid extraction. Lipids were then partially purified over C-18 solid phase extraction columns and analyzed using HPLC/MS/MS screening for 2-acyl glycerols, lipoamines including Anandamide free fatty acids, and prostaglandins.

Results: Results showed a variety of cell- and time-dependent effects. BV2 cells treated with ML193 showed significantly *lower* levels of prostaglandin E_2 (PGE_2) compared to vehicle after both 2 and 24 hours, and cells treated with O-1602 showed a time-dependent effect wherein PGE_2 levels were significantly *higher* after 2 hours and significantly *lower* after 24. BV2 cells also exhibited significantly *lower* levels of 2-arachidonoyl-sn-glycerol (2-AG) and arachidonic acid compared to vehicle after 24 hours of ML193 treatment, whereas O-1602 treatment for 24 hours resulted in significantly *higher* levels of 2-AG but no difference in levels of arachidonic acid. N18 cells showed significantly lower levels of 2-linoleoyl-sn-glycerol and 2-oleoyl-sn-gylcerol, but not 2-AG, after 2 hours with both ML193 and O-1602 treatment. Interestingly, the C6 glioma cell line was largely unaffected by 24-hour treatment with either compound, with the only significant differences being a decrease in N-oleoyl tyrosine after ML193 treatment, and an increase in N-palmitoyl taurine and decrease in oleic acid following O-1602 treatment.

Conclusions: These findings illustrate cell and time-specific differences in how these GPR55-related compounds drive changes in the cellular lipidome and therefore, intra and inter-cellular communication.

DIFFERENTIAL EFFECTS OF CANNABINOID RECEPTOR LIGANDS ON THE PRODUCTION OF CAMP IN MALE AND FEMALE CELLS OF NEURONAL ORIGIN

Rupali Vyawahare,[1] Ewa Wasilewski,[1] Rex Siming Wang,[1,2] and Lakshmi P. Kotra[1,2,3*]

[1] Centre for Molecular Design and Preformulations, and Krembil Research Institute, University Health Network, Toronto, Ontario, Canada, M5G 1L7
[2] Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Ontario, Canada, M5S 3M2
[3] Multi-Organ Transplant Program, Toronto General Hospital, Toronto, Ontario, Canada, M5G 1L7

Introduction: Sex is a factor in neuronal cell responses and these responses are also influenced by sex hormones. Understanding the effects of cannabinoids in male and female neuronal cells and the influence of sex hormones is an important step to relate to the physiological effects of cannabinoids.

Methods: Male and female neuroblastoma cells (LAN-5 and SH-SY5Y, respectively) were employed under differentiation conditions, in the presence and absence of physiologically relevant concentrations of 17β-estradiol (E2) and testosterone (T). Four popular cannabinoids (Δ^9-THC, CBD, Δ^9-THCA and CBDA) and a synthetic molecule (CP-55940) were evaluated as ligands to cannabinoid receptors, and adenylate cyclase (AC) activity was measured as it relates to cAMP production.

Results: Male and female (LAN-5 and SH-SY5Y) cells produced opposing functional responses to the CB_1R and CB_2R agonist, CP-55940, in the presence of RA, implying that the functional response of these receptors differs in male and female neurons. Overall, a trend of potentiating and opposing effects, was observed in both male and female cells in the presence of steroid hormones E2 and T.

Conclusions: There are important differences in the functional response of cannabinoid receptors to popular phytocannabinoids in male and female neuronal cells. This difference is further augmented in the presence of E2 and T. These studies imply that sex and sex hormones should be considered in studies involving cannabinoids for meaningful and clinically significant outcomes.

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EFFECTS OF PERIPARTUM OMEGA-3 FATTY ACID SUPPLEMENTATION ON ENDOCANNABINOID TONE AND INFLAMMATION IN LIVER OF DAIRY COWS

Gitit Kra*[1,2], Uzi Moallem[1], Radka Kočvarová[3], Alina Nemirovski[3], Joseph Tam[3], G. A. Contreras[4] and Maya Zachut[1]

[1]Department of Ruminant science, Institute of Animal Sciences, ARO Volcani Center, Israel; [2]Faculty of Agriculture, the Hebrew University in Jerusalem, Israel; [3]Obesity and Metabolism Laboratory, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel; [4]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, USA

Introduction: Dietary supplementation of omega-3 (n-3) fatty acids (FA) may affect the endocannabinoid system (ECS) by reducing the availability of arachidonic acid (AA; C20:4n-6), and elicit anti-inflammatory effects. The role of the ECS in dairy cows is recently emerging, but information on the ECS in bovine liver is lacking. We aimed to examine the effects of two sources of n-3 FA, encapsulated flaxseed oil (FLX) or fish oil (FO) on hepatic ECS 'tone', and inflammatory and metabolic markers in blood of peripartum dairy cows.

Methods: Late pregnant cows were fed from 21 d before expected calving a basal diet supplemented with encapsulated fat: i) CTL–saturated fat (*n*=14); ii) FLX (*n*=14); or iii) FO (*n*=14). Plasma metabolites [glucose, beta-hydroxybutyrate (BHBA)], inflammatory markers [tumor necrotizing factor alpha (TNF-α), interlukin-6 (IL-6), prostaglandin E metabolite (PGEM) and G-2-alpha metabolite (PGFM)], and FA profile were examined. Liver biopsies were obtained at 10 d postpartum (n = 5 per treatment) for testing the expression of ECS components and inflammatory genes (real-time PCR) and proteins (immunoblots). Endocannabinoids (eCBs) in liver and plasma at time of biopsy were examined by LC-MS/MS. Continuous measures were analyzed by PROC MIXED; genes, proteins, and eCBs were analyzed by GLM procedure of SAS.

Results: The n-6/n-3 ratio in plasma was lowest in FLX, with intermediate value in FO, and highest in CTL. Milk production was similar among groups, and feed intake during the first 21 days of lactation was lower in FLX than in CTL. Prepartum, plasma BHBA concentrations were lower in FLX, and TNF-α concentrations tended to be lower in FO compared to CTL. Postpartum, concentrations of IL-6 tended to be lower in FO compared to CTL. The FLX cows had lower PGEM concentration and tended to have lower PGFM in plasma during the first week in lactation compared to CTL. In liver, the relative gene expressions of the cannabinoid receptors *CNR1* and *CNR2* as well as the pro-inflammatory gene *IL-6* were lower in FLX than in other groups. Protein abundances of TNF-α, IL-10 and CB1 in liver were lower in FO than in CTL, while the abundance of NFKB was higher in FLX than in other groups. In liver, levels of the eCB 2-AG were 1.7-fold higher in FLX than in other groups, while in plasma AEA levels were lower in FLX and in FO compared to CTL, and AA was lower in FLX than in CTL.

Conclusions: Peripartum supplementation of n-3 FA seems to lower the ECS 'tone' in bovine liver, with some differential effects of FLX and FO on liver ECS receptors, eCB levels, systemic inflammatory markers and prostaglandin metabolites. We conclude that the ECS is involved in bovine liver metabolism and inflammation, and dietary n-3 can attenuate ECS 'tone' in liver of peripartum dairy cows.

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SEASONAL HEAT STRESS AFFECTS ENDOCANNABINOID GENE EXPRESSION IN ADIPOSE TISSUE OF POSTPARTUM DAIRY COWS

Gitit Kra*[1,2], Uzi Moallem[1], Majdoleen Ahmad[3], Alina Nemirovski[3], Joseph Tam[3] and Maya Zachut[1]

[1]Department of Ruminant science, Institute of Animal Sciences, ARO Volcani Center, Israel;
[2]Faculty of Agriculture, the Hebrew University in Jerusalem, Israel;
[3]Obesity and Metabolism Laboratory, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Introduction: High-yielding dairy cows are extremely sensitive to a hot environment due to their increased metabolic rate. Heat load has an added stress effect on the metabolic load in postpartum (PP) dairy cows. The role of the endocannabinoid system (ECS) in dairy cows is recently emerging, but information on the effects of heat stress on the ECS in bovine adipose tissue (AT) is lacking. We aimed to examine the effects of heat stress during summer compared to winter on AT gene and protein expression of the ECS components, levels of endocannabinoids (eCBs) as well as metabolic and inflammatory markers in blood of PP dairy cows.

Methods: Eighteen high-yielding, 255 ± 5 d pregnant, dry multiparous Israeli-Holstein dairy cows that were randomly available at the Volcani dairy farm during the summer (n = 9) or winter season (n = 9) participated in this study. Blood samples were collected twice weekly from 3 weeks before expected calving until 3 weeks PP, and subcutaneous AT biopsies were taken at 7 d after calving. The levels of eCBs were determined in AT and plasma at day of AT biopsy. Plasma concentrations of non-esterified fatty acids (NEFA), insulin, and TNF-α were examined. In AT, the expression of ECS components (real-time PCR) and proteins (immunoblots) were examined. Continuous measures were analyzed by PROC MIXED; genes and proteins were analyzed by GLM procedure of SAS. Unpaired two-tailed Student's t-test was used to determine differences in eCBs between groups.

Results: Dry matter intake and the average calculated energy balance were lower in summer than in winter cows PP. Plasma insulin concentrations were lower in summer than in winter while plasma NEFA concentrations were higher in summer than in winter. Plasma TNF-α concentrations were 3.4-folds higher in summer than in winter cows. In AT, the relative mRNA expression levels of *CNR1* and *CNR2* were 46% and 48% lower in summer than in winter, respectively. The expression of *MGLL* in AT was 46% lower in summer than in winter, and the relative expression levels of *FAAH, NAPEPLD* and *PPAR-α* were not different between seasons. The expression of the transient receptor potential cation channel subfamily V member 1 (*TRPV1*) was 58% lower in summer than in winter AT. The changes measured in the mRNA expression were not translated to differences in the protein abundances of CB1, CB2, FAAH and MGLL in AT nor in the average levels of the eCBs AEA, 2-AG, OEA, PEA and AA in plasma or AT in summer vs. winter cows.

Conclusions: The findings at the mRNA level suggest that the ECS activity in AT may be affected by seasonal heat stress in PP dairy cows, however as we could not find changes in protein abundance or in eCB levels, more research should be conducted to elucidate the role of the ECS in heat stressed dairy cows.

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THE BEHAVIOURAL AND LIPIDOMIC EFFECTS OF INHIBITING ENDOCANNANINOID METABOLISM IN A HYPO-GLUTAMATERGIC MODEL OF PSYCHOSIS-LIKE PHENOTYPES

Claudia Lutelmowski*[1], Catharine Mielnik[1], Ali Salahpour[1], Amy J. Ramsey[1], Heather B. Bradshaw[2], Marija Milenkovic[1], Wendy Horsfall[1] and Ruth Ross[1]

[1]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, CA.
[2]Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA.

Introduction: While the dysregulation of the endocannabinoid system (ECS) in the pathophysiology of schizophrenia is supported by multiple lines of evidence, the exact role of the ECS on behaviours of psychosis remains unclear. In a hypo-glutamatergic model of psychosis-like phenotypes, the elevation of endocannabinoid (EC) tone has mixed effects on the models phenotypic hyperlocomotion and cognitive impairment. Increasing anandamide (AEA), by inhibition of the enzyme fatty-acid amide hydrolase (FAAH), both ameliorated and impaired a cognitive deficit depending on the dose, without affecting locomotion. Meanwhile, increasing 2-arachydonyl glycerol (2-AG) by inhibiting the enzyme monoacylglycerol (MAGL), potentiated hyperlocomotive behaviour and the cognitive deficit (Kruk-Slomka et al., 2019). In this study, we continue to investigate how elevations in endocannabinoid (EC) tone affect psychosis-like phenotypes in the GluN1 knockdown (GluN1KD) mouse, a genetic model of N-methyl-D-aspartate (NMDA-R) hypofunction (Mohn et al., 1999).

Methods: Male and female, adult (PD >70) GluN1KD and littermate wild-type mice were subjected to a 120m pre-treatment with either MJN-110 (5 mg/kg i.p.), PF-3845 (10 mg/kg i.p.) or vehicle (1:1:18 - 95%EtOH: Tween80: saline). The cannabimimetic triad was evaluated by measuring catalepsy, rectal temperature, and locomotion immediately following treatment. On a subsequent testing day, the antipsychotic potential of the compounds was assessed by measuring the acoustic startle response (ASR), and pre-pulse inhibition (PPI) under the same treatment regimen. Finally, high-performance liquid chromatography-mass spectrometry (HPLC-MS) was used to identify baseline EC levels in GluN1KD plasma, striatal and prefrontal cortex samples.

Results: HPLC-MS revealed that key EC lipids and substrates are altered in the GluN1KD mice in comparison to their wild-types. Treatment with the inhibitors of 2-AG and AEA metabolism, MJN110 and PF3845 respectively, resulted in changes to locomotion and temperature, with the model showing greater responses to EC manipulation compared to wild-type animals. Meanwhile, little to no changes in cataleptic behaviour, PPI or ASR were observed.

Conclusion: The lipidomic and behaviour data suggest that NMDA-R hypofunction may be accompanied with alterations in baseline endocannabinoid tone. These differences may make model more sensitive to acute perturbations in EC signalling as was seen by changes in some psychosis-related behaviours in GluN1KD mice but not in wild-types. This study supports the need to characterize how components of the ECS correspond to psychosis-related behaviours and the considerations for the development of therapeutics that target EC enzymatic activity.

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EFFECT OF SPHINGOSINE-1-PHOSPHATE ON ENDOCANNABINOID SIGNALING

Sara Standoli[1], Cinzia Rapino[2], Camilla Di Meo[1] and Mauro Maccarrone*[3,4]

[1]University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Enviroment, 64100 Teramo, Italy;
[2] University of Teramo, Faculty of Veterinary Medicine, 64100 Teramo, Italy;
[3]University of L'Aquila, Department of Biotechnological and Applied Clinical and Sciences, 67100 L'Aquila, Italy;
[4]European Center for Brain Research, Santa Lucia Foundation IRCCS, 00143 Rome, Italy.

Introduction: Sphingosine-1-phosphate (S1P) and endocannabinoid (eCB) systems regulate the same intracellular signaling pathways involved in biological processes such as cell growth, proliferation and migration through G-protein coupled receptors (GPCRs). In particular, S1P binds to and activates five different GPCRs (S1PR1-5), whereas eCBs [especially *N*-arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG)] trigger type 1 and 2 (CB_1 and CB_2) cannabinoid receptors and GPR55, in addition to the transient receptor potential cation channel subfamily V member 1 (TRPV1) [Maccarrone M et al., *Nature Rev. Neurosci*. 15 (2014) 786-801]. S1P receptors show 20% sequence identity with CB_1 and CB_2 (Sanchez T et al., *J Cell Biochem*. 92 (2004) 913-922). Of note, interactions between S1P and eCBs have been documented in rat coronary artery (CA) reactivity [Mair KM et al., *Br J Pharmacol*. 161 (2010**)** 176-192].

Methods: Here, the molecular effects of S1P (used at 1 μM for 24h) on distinct elements of the eCB system have been investigated in murine myoblast C2C12 cells, by using *quantitative Real Time-Polymerase Chain Reaction* (qRT-PCR) and *Western blot* analyses. Moreover, the modulation of the mitochondrial membrane potential upon cell treatment with S1P and/or eCBs (used at 1 μM for 15', 30', 1h and 24h) and antagonists (used at 100 nM) of eCB-binding receptors was assessed by using the JC-1 fluorescent dye. Statistical analysis was performed by one-way ANOVA test followed by Bonferroni *post hoc* test ($p < 0.05$).

Results: *qRT-PCR* analysis showed that S1P led to a significant increase in GPR55 and TRPV1 mRNA expression, and *Western blot* confirmed the increase in TRPV1 with a decrease in CB_2 protein expression. Instead, gene and protein expression of any other element of the eCB system was affected. A significant depolarization of the mitochondrial membrane potential was found upon S1P treatment at 30', an effect that was significantly reverted by AEA and 2-AG through CB_1, CB_2, GPR55 and (in the case of 2-AG) TRPV1. A preliminary docking analysis suggested that CB_1 antagonism by S1P could be due to binding to the *N*-terminal allosteric pocket of the receptor.

Conclusions: In conclusion, S1P modulates the expression of some eCB-binding receptors at gene and protein level, and thus it may interfere with mitochondrial membrane potential regulation.

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EFFECT OF ULTRAMICRONIZED PEA ON THE FAECAL MICROBIOME OF A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

Alessandra Piccirilli[1], Roberto Coccurello[2,3], Sergio Oddi[3,4], Mariagrazia Perilli[1] and Mauro Maccarrone*[1,3]

[1]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, 67100 L'Aquila, Italy
[2]Institute for Complex Systems, National Council of Research (CNR), 00185 Rome, Italy
[3]European Center for Brain Research/Santa Lucia Foundation IRCCS, 00143 Rome, Italy
[4]Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy

Introduction: Over the past decade, the impact of the symbiotic microbiota on numerous host functions has been increasingly recognized, and has been linked to altered endocannabinoid signaling [Chevalier G et al., *Nat Commun*. (2020)11:6363]. In particular, in neurodegenerative pathologies like Alzheimer's disease (AD) microbiota alterations may impact on inflammation, a key process that is reduced by the endocannabinoid-like compound palmitoylethanolamide (PEA) [Leuti A et al., *Adv Drug Deliv Rev.* (2020) 159:133-169]. Here, we took advantage of a well-established AD-like animal model (Tg2576 mice) [Maccarrone M et al., *Pharmacol Res*. (2018) 130:366-373], that we treated with PEA to interrogate the possible effect on faecal microbiome.

Methods: Faecal pellets (100 mg per sample) were collected from Tg2567 mice and their wild-type (WT) counterparts that were administered for 3 months with ultramicronized PEA (um-PEA) via a delivery system consisting in drug pellets (80 mg/pellet) subcutaneous implantation. Both WT and Tg2576 control groups were treated via the same route and delivery system with non um-PEA-containing placebo pellets. Total genomic DNA was extracted by using MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (ThermoFisher Scientific, Monza, Italy). The Swift Normalase Amplicon Panel (SNAP) for 16S v2 was used to target all V1-V9 variable rRNA regions. Sequence analysis was performed by using Illumina MiSeq platform, and taxonomic classification was carried out using the Illumina 16S Metagenomics workflow.

Results: The composition of the gut microbiome was interrogated in WT, WT+PEA, Tg2576 and Tg2576+PEA mice, using 6 replicas per group. The relative abundance of different genera was found to be quite similar among the 24 fecal samples at the genus level. Indeed, the dominant bacterial phyla were represented by Bacteroidetes (ranging from 50% to 58%) and Firmicutes (ranging from 7% to 11%), followed by Proteobacteria that were represented only by the *Helicobacter* genus (4% in all groups). Among Firmicutes, *Clostridium* genus was present in all samples, with 11.42%, 7.42%, 9.82% and 9.84% in WT, WT/PEA, Tg2676 and Tg2676/PEA respectively, whereas *Acetatifactor* and *Eisenbergella* genus were always below 4%. The most represented genera of Bacteroidetes phyla were *Prevotella*, *Barnesiella*, *Coprobacter*, *Bacteroides*, *Odoribacter* and *Alistipes*, that accounted for > 50% of all microbioma. Interestingly, only *Prevotella* genus markedly increased in Tg2576/PEA mice compared to Tg2576 and WT animals (20.66% *vs* 14.19% and 12.71%, respectively).

Conclusions: Our preliminary data point to *Prevotella* as an interesting player in the microbiota-endocannabinoid signaling crosstalk, and its impact on neurodegenerative/neuroinflammatory diseases like AD. Notably, *Prevotella* genus was found largely reduced in AD-like APP/PS1 transgenic mice [Shen L et al., *J Alzheimers Dis.* (2017) 56:385-390], and our data provide the first evidence that chronic PEA treatment can contribute to rebalance gut bacterial populations. Further investigations will be necessary to understand gut dysbiosis in AD, and the role of the major endocannabinoid players both in pathogenesis and possible therapy of the disease.

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ENDOCANNABINOIDS AND PRO-RESOLVING MEDIATORS ACT SYNERGICALLY IN PRIMARY HUMAN MACROPHAGES

Alessandro Leuti[1,2], Marina Fava[2,3], Niccolò Pellegrino[1] and Mauro Maccarrone[2,4]

[1] *Department of Medicine, Campus Bio-Medico University of Rome, 00128 Rome, Italy*
[2] European Center for Brain Research/IRCCS Santa Lucia Foundation, 00143 Rome, Italy
[3]Faculty of Biosciences and Technology for Food Agriculture and Environment, University of Teramo, 64100 Teramo, Italy.
[4] Department of Biotechnological and Applied Clinical and Sciences, University of L'Aquila, 67100 L'Aquila, Italy

Introduction: Specialized pro-resolving mediators (SPMs) are a novel class of lipids that include D- and E-series resolvins, lipoxins, protectins and maresins. These lipids orchestrate resolution of inflammation, which avoids collateral damage coming from exaggerated immune responses. During resolution, macrophages represent a pivotal target of SPMs. Indeed, the latter compounds drive the differentiation of pro-resolving macrophagic phenotypes (i.e., M2-like), while blunting pro-inflammatory properties of the classically-activated ones (i.e., M1-like), thus promoting efferocytosis (i.e., removal and phagocytosis of dead [apoptotic] cells) and tissue debris. Endocannabinoids (eCBs) have long been postulated to participate in immune modulation by both exerting pro-inflammatory effects and acting as anti-inflammatory/pro-resolving-like compounds; however, their role in macrophage-dependent resolution, or their synergy with SPM-driven processes, has never been interrogated. Here, we investigated the effect of eCB treatment on inflammation/resolution profile of M1- and M2-like macrophages, as well as the effect of simultaneous administration of SPMs and eCBs on resolution-related immune properties of macrophages.

Methods: Freshy isolated primary monocytes were polarized into classical M1-like or alternatively-activated M2-like macrophages, in the continuous presence or absence of each of the two major eCBs (2-AG and AEA). Expression of the main SPM-related receptors – i.e., GPR32, GPR18, formyl peptide receptor 2 (FPR2), chemerin receptor 23 (ChemR23) – and enzymes – i.e., 5/12/15-lipoxygenase (5/1/15-LOX) – as well as functional readouts like efferocytosis, were assessed by means of qRT-PCR and polychromatic flow cytometry.

Results: M1-like and M2-like macrophages displayed different pro-resolving phenotypic profiles, and chronic treatment with 2-AG or AEA changed the pro-resolving profile of both M1- and M2-like phenotypes. In particular, AEA exerted a significant downregulation of GPR32 (the RvD1 receptor) in M1-like macrophages and an up-regulation of ChemR23 (the RvE1 receptor) in M2-like macrophages; instead, 2-AG elicited only a significant down-regulation of GPR32 in M2-like macrophages. Furthermore M2-like cells, differentiated in the presence of AEA and RvE1, showed enhanced efferocytosis.

Conclusions: Overall, eCBs and SPMs were able to act synergically to modulate the inflammatory and pro-resolving features of human primary macrophages. This evidence strongly suggests that, during resolution of inflammation, both lipid families might act in concert to modulate the activity of these cells, thus impacting on the inflammatory response.

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UNIQUE ENTRY OF ANANDAMIDE AT TRPV1

Chanté A. Muller*[1], Diane L. Lynch[1], Dow P. Hurst[1] and Patricia H. Reggio[1]

[1]Department of Chemistry and Biochemistry,
University of North Carolina at Greensboro, Greensboro, NC 27402.

Whether caused by inflammation or dysfunctional nerves, chronic pain affects nearly 10% of the world's population. Since there are few treatments that are effective while being non-invasive and non-addictive, new targets are being explored. Found in the peripheral nervous system, the transient receptor potential subfamily vanilloid type 1 (TRPV1) ion channel can be activated by a plethora of exogenous and endogenous stimuli including the spicy compound found in chili peppers, capsaicin, as well as temperatures above 43^{o}C and acidic conditions. In recent years, it has been discovered that TRP channels, including TRPV1, act as ionotropic cannabinoid receptors. The endocannabinoid anandamide has been shown to have a similar binding affinity to TRPV1 as capsaicin and can rapidly desensitize the channel producing an analgesic effect.

Previously, we constructed models of the open and closed structures of TRPV1 from the published cryo-EM structures (PDB: 5IRX, 5IRZ). Prime (Schrodinger, Inc. Version 11.8.012) was used to complete unresolved regions of extracellular loops and ankyrin repeat domains were incorporated in the model using the available crystal structure (PDB: 2PNN). These models have been equilibrated (500 ns) in a fully hydrated POPC bilayer for use in molecular dynamics simulations (AMBER 18).

Currently, our molecular dynamics simulations are focused on understanding ligand entry and activation of TRPV1. Our simulations have suggested that anandamide enters TRPV1 via a unique route, through a tunnel between helices S1-S4 rather than via the vanilloid binding pocket, where capsaicin is said to bind. Simulations microseconds in length have been performed with anandamide and TRPV1 and the results of these simulations will be presented.

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TONIC ENDOCANNABINOID SIGNALLING GATES STRESS-LIKE STEREOTYPIC BEHAVIORS AND HPA AXIS ACTIVATION

Gavin Petrie*[1,2], Georgia Balsevich[1,2], Hiulan Yau[1,2], Tamas Fuzesi[1,2], David Rosenegger[1,2] Robert Aukema[1,2], Mario Van Der Stelt[3], Jaideep Bains[1,2] and Matthew Hill[1,2]

[1]Hotchkiss Brain Institute,
[2]University of Calgary, Calgary, AB, Canada,
[3]Department of Molecular Physiology

Introduction: Endocannabinoid (eCB) signalling is known to gate many aspects of the stress response including the hypothalamic-pituitary-adrenal (HPA) axis. A nexus of the HPA axis is a cluster of corticotropin releasing hormone (CRH) producing neurons in the paraventricular nucleus of the hypothalamus (PVN). In both resting conditions, and in response to stress, disruption of eCB signalling can increase drive on the HPA axis, although the circuits through which this occur have yet to be identified. It has recently been demonstrated that optogenetic activation of CRH neurons exclusively produces a characteristic behavioural sequelae consistent with what is seen following exposure to stress, with noted increases in self-directed behaviors such as grooming. As such, using an array of cellular, endocrine and behavioral readouts associated to activation of CRH neurons in the PVN, we examined how tonic eCB signaling exerts inhibitory control over the generation of a stress response.

Methods: Our endpoint measurements of a stress response included the examination of self-directed homecage behaviors (i.e., grooming), activation of CRH neurons in the PVN and corticosterone (CORT) release were measured using respective immunohistochemical and ELISA approaches. Optogenetic procedures were performed by infecting the PVN of CRH-cre transgenic animals with cre-dependent Arch3.0 or control eGFP in order to photo-inhibit these neurons while recording their behaviors under the influence of AM251.

Results: The CB1 antagonist/inverse agonist AM251, neutral antagonist NESS243, and NAPE PLD inhibitor LEI401 all uniformly increased home-cage self-directed behaviors, c-fos activation in the PVN and circulating CORT, recapitulating the effects of stress. Increases in self-directed behaviors and CORT were also seen after direct administration of AM251 into the PVN, indicating that there is a local eCB tone in the PVN that is gating activation of CRH neurons. Importantly, optogenetic inhibition of PVN CRH neurons ameliorated the self-directed behaviors following CB1 blockade in a stress-free environment indicating that activation of this cluster of neurons is requisite for the generation of a stress response following disruption of eCB signaling.

Conclusions: These data suggest that tonic AEA signaling at CB1 receptors, possibly in the PVN proper, act to constrain activation of CRH neurons in the PVN and restrict activation of a stress response; disruption of this tonic signal results in the generation of a stress response. This work will help us to understand how eCB signalling regulates components of the HPA as well as behaviors associated with psychiatric conditions, such as depression and autism.

EFFECT OF THE FATTY ACID AMIDES OLEOYL GLYCINE AND OLEOYL ALANINE ON LITHIUM CHLORIDE-INDUCED NAUSEA IN RATS AND VOMITING IN SHREWS

Erin M. Rock*[1], Cheryl L. Limebeer[1], Reem Smoum[2],
Raphael Mechoulam[2] and Linda A. Parker[1]

[1]Department of Psychology and Collaborative Neuroscience Program,
University of Guelph, Guelph, ON, Canada;
[2] Medicinal Chemistry Laboratory, The Institute for Drug Research, School of Pharmacy,
Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.

Introduction: The fatty acid amide, oleoyl glycine (OlGly) has been shown to interfere with nausea-like somatic withdrawal reactions elicited by acute naloxone-precipitated morphine withdrawal (MWD) in rats (Rock et al., Psychopharmacology. 237 (2020) 375-384; Ayoub et al., Psychopharmacology. 237 (2020) 2753-2765). Furthermore, OlGly interfered with nausea-induced conditioned gaping reactions in rats, elicited by lithium chloride (LiCl)- and MWD, and LiCl-induced vomiting in *Suncus murinus* (house musk shrews). Recently, a more stable methylated version, oleoyl alanine (OlAla), has demonstrated sustained efficacy in preventing acute naloxone-precipitated MWD conditioned place aversion and interfered with MWD-induced conditioned gaping reactions in rats (Ayoub et al., Psychopharmacology. 237 (2020) 2753-2765). These fatty acid amides may therefore be effective new compounds for reducing LiCl-induced nausea and vomiting in rats and shrews, respectively.

Methods: Here we evaluated the potential of OlGly and OlAla to interfere with LiCl-induced conditioned gaping reactions (a rat model of nausea) when administered 20 or 70 min prior to LiCl. We also evaluated the ability of OlGly and OlAla to interfere with LiCl-induced vomiting in shrews when administered 20 or 70 min prior to LiCl.

Results: At doses of 5 and 20 mg/kg, ip, OlGly interfered with LiCl-induced conditioned gaping in rats when administered 20 min prior to LiCl, but not when administered 70 min prior to LiCl. At doses of 5 and 20 mg/kg, ip, OlAla interfered with LiCl-induced conditioned gaping in rats when administered either 20 or 70 min prior to LiCl, suggesting that the preventative effect of oleoyl alanine persists over 70 min, unlike that of oleoyl glycine. In shrews, both OlGly and OlAla at doses of 1 and 5 mg/kg ip reduced LiCl-induced vomiting when administered 20 min prior to LiCl, with 1 mg/kg OlAla trending towards greater anti-emetic effectiveness than 1 mg/kg OlGly. When administered 70 min prior to LiCl, both OlGly and OlAla (5 mg/kg, ip) similarly reduced vomiting in shrews.

Conclusions: These results suggest that oleoyl glycine and oleoyl alanine may be effective anti-nausea and anti-emetic treatments. Oleoyl alanine may be a more stable and effective compound.

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COMPARISON OF THE PHARMACOLOGY OF THE HUMAN AND ZEBRAFISH CANNABINOID RECEPTORS

Syed Haneef Syed Askar*, Marina Santiago and Mark Connor

Department of Biomedical Sciences, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, Australia

Introduction: Cannabinoid receptors (CBs) are involved in many physiological functions and are responsible for the psychoactive effects of *Cannabis* in humans. Zebrafish (*Danio rerio*) are widely used as a vertebrate model organism. Cannabinoid actions in models of addiction, anxiety and development have been studied in zebrafish. How zebrafish CBRs (zfCBRs) respond to drugs is unknown.

Methods: This study aims to understand the potential differences in signalling between human and zebrafish cannabinoid receptors. Responses of zfCBRs were examined after expressing zfCBRs in the mouse AtT20 cell line. Zebrafish have 3 cannabinoid receptors, zfCB1 (70% homology to human) and a paralogue pair of zfCB2, zfCB2A and zfCB2B (98% similarity to each other, 46% to human CB2). HA-tagged CB receptors activate K channels in AtT20 cells, and the receptor-induced hyperpolarisation of the cells was measured by a membrane potential assay in a FlexStation 3 plate reader.

Results: The reference synthetic cannabinoid CP 55,940 hyperpolarized hCB1 and zfCB1 cells with similar potency (hCB1 pEC_{50} 7.0 ± 0.1; zfCB1 pEC_{50} 7.2 ± 0.1), as did THC (hCB1 pEC_{50} 6.3 ± 0.1; zfCB1 pEC_{50} 6.2 ± 0.1). On the other hand, WIN 55,212-2 hyperpolarized zfCB1 cells more potently than hCB1 cells, with pEC_{50} of 7.9 ± 0.1 and 6.5 ± 0.1, respectively. AEA (pEC_{50} 6.8 ± 0.1) and 2-AG (pEC_{50} 7.1 ± 0.1) were substantially more potent at zfCB1 than hCB1 (pEC_{50} 5.7 ± 0.1, and 6.2 ± 0.1 respectively). CP 55,940 was less potent at zfCB2A (pEC_{50} 5.9 ± 0.1) than hCB2 (pEC_{50} 7.4 ± 0.1), while AEA and 2-AG had similar potency in zfCB2A (pEC_{50} 5.6 ± 0.1, 6.2 ± 0.1 respectively) and hCB2 (pEC_{50} 5.4 ± 0.1, 6.0 ± 0.1 respectively). No ligands produced a significant response in the cells expressing zfCB2B, however, an HA-tagged protein of appropriate molecular weight was detected in these cells. This suggests that zfCB2B is either not functional or not appropriately trafficked to the membrane of AtT20 cells.

Conclusions: This study reveals the pharmacological signalling profile of hCBRs and zfCBRs are not the same, with differences in the rank order of potency of synthetic and endogenous ligands. Our work highlights the need to understand the drug responses of zebrafish receptors when using drugs *in vivo*.

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A COMPREHENSIVE ANALYSIS OF MICROGLIAL CANNABINOID CB_2 RECEPTORS IN THE CONTEXT OF AMYLOID-INDUCED NEUROINFLAMMATION

Ana M. Martínez Relimpio*[1], M. Teresa Grande[1], Samuel Ruiz de Martín Esteban[1], M. Andrea Arnanz[1], Rosa M. Tolón[1], Cecilia J. Hillard[2] and Julián Romero[1]

[1]School of Pharmacy, Faculty of Experimental Sciences, Universidad Francisco de Vitoria, 28223, Madrid, Spain; [2]Neuroscience Research Center, Medical College of Wisconsin, 53226, Milwaukee, WI, USA

Introduction: The selective expression of cannabinoid CB_2 receptors in neuritic plaque-associated microglia suggests that these receptors may play a role in the multiple functions that these cells play in amyloid pathology and, specifically, in neuroinflammation. We have focused on microglial cells due to i) their prominent role in amyloid-triggered neuroinflammation, and ii) to the selective expression of CB_2 receptors in this cell type in the context of Alzheimer's disease.

Methods: To assess this topic, we have developed several animal models of amyloidosis in which the expression of CB_2 receptors may be prevented in a cell- and time-dependent manner (5xFAD/$CB_2^{EGFP;f/f}$, 5xFAD/$CB_2^{-/-}$ and 5xFAD/$CB_2^{EGFP;f/f}$/Cx3cr1$^{tm2.1(cre/ERT2)Jung}$ mice) and employed a novel approach by specifically isolating microglial cells from the brains of 6-month old mice. By using this strategy, we have been able to make a detailed characterization of the role of CB_2 receptors in microglial cells in the context of amyloid pathology and their influence on the expression of several markers of neuroinflammation (including IL1b, TNFa, IL6) as well as in the activity of different signaling cascades (cAMP, pCREB, pERK, NFkB).

Results: Our preliminary data suggest that the deletion of CB_2 receptors leads to a proinflammatory phenotype and to an impairment of some relevant functions, such as amyloid phagocytosis. In addition, we analyzed the consequences of CB_2 activation by the specific agonist HU-308 and found significant differences according to the concentration of the agonist used and as a function of the signaling cascade considered.

Conclusions: We speculate that cannabinoid CB_2 receptors exert a complex modulation of the activity of microglial cells in the context of amyloid-induced damage.

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THE METABOLIC SIGNATURE OF CB2 IN LPS-INDUCED MICROGLIA STRESS RESPONSE

Bolanle F. Olabiyi*[1], Eike Geissmar[2], Andreas Zimmer[1] and Anne-Caroline Schmöle[1]

[1]Institute of Molecular Psychiatry, Medical Faculty, University of Bonn, Germany
[2]Institute for Innate Immunity, Medical Faculty, University of Bonn, Germany

Introduction: The cannabinoid receptor type 2 (CB2) has long been explored in the area of neuroinflammation. It is known that the stimulation of macrophages with LPS changes their cellular metabolism in such a way that the downstream inflammatory phenotypes are altered (Nonnenmacher and Hiller, 2018; Lauterbach et al., 2019). However, whether the CB2 has a distinct metabolic remodelling activity in microglia upon inflammatory stimuli is not known. In this study, we investigated how CB2 deletion influences microglia metabolic rewiring in response to TLR4 stimulation.

Methods: Neonatal primary microglia obtained from wildtype (WT) and constitutive CB2 knock-out ($CB2^{-/-}$) mice were stimulated with TLR4 ligand (lipopolysaccharide, LPS) at 10 ng/mL or 100 ng/mL for 16 hours. The metabolic parameters: Oxygen Consumption rate (OCR) and Extracellular acidification rate (ECAR) were analysed using the Seahorse XF analyser. RNA sequencing of the microglia also characterized the metabolic gene expression profile.

Results: Our OCR result indicates that LPS stimulation causes a significant decrease in maximal respiration upon uncoupling of mitochondria with FCCP in WT microglia whereas $CB2^{-/-}$ displayed a higher respiratory capacity. We also observed that the mitoprotective phenotype in $CB2^{-/-}$ seems to be independent of glycolytic pathway as there was no significant difference in the ECAR between WT and $CB2^{-/-}$ microglia. Nevertheless, our RNA sequencing data set showed a downregulated glycolytic genes in CB2ko microglia upon LPS stimulation compared to the WT.

Conclusions: Taken together, our results suggest that CB2 plays a role in TLR4-mediated metabolic impairment. However, future experiments are needed to ascertain this. Next, we plan to perform mitochondrial staining for reactive oxygen species in the microglia using mitoSox and also to investigate single metabolites of glycolytic pathway and TCA cycle to provide a deeper mechanistic approach towards understanding how CB2 signaling interferes with microglia metabolism.

EFFECTS OF CANNABIS VAPOR INHALATION IN THE ESTABLISHMENT AND EXPRESSION OF MORPHINE REWARD

Maria T Rivera-Garcia* and Adrianne R Wilson-Poe

Introduction: Cannabidiol (CBD) has been used for its therapeutic effects in multiple medical conditions including pediatric epilepsy. Recently, it has also been proposed as relapse prevention tool in addiction. In humans, CBD reduces heroin-related craving, anxiety and seeking behavior and, morphine withdrawal symptoms. In preclinical models, CBD inhibits cue-induced heroin seeking behavior and potentiates the extinction of cocaine and amphetamine reinforcement. Smoking and vaping are the most common routes of administration in humans. However, because preclinical models rarely use routes of administration that mimic human cannabis use, the translational value of CBD therapeutic effects is limited. We wanted to evaluate the effects of high CBD cannabis vapor inhalation on the rewarding properties of morphine.

Methods: In Long Evans female rats (6-7 weeks old) we induced morphine (5 mg/kg/day or saline for 5 days) conditioned place preference (CPP). After the conditioning phase animals were pre-exposed to cannabis extract vapor (69% CBD of dry weight) or vehicle (Propylene Glycol and Vegetable Glycerin-PGVG) for 30 minutes and side preference was recorded. The extinction of morphine reinforcing effects was evaluated 7 and 14 days after the last morphine administration. On day 15, animals were acutely exposed to cannabis or control vapor PG/VG and immediately after drug reinstatement was induced using 1 mg/kg of morphine. To evaluate the effects of CBD in the development of morphine rewarding properties, independent groups of animals were exposed to either cannabis vapor or PG/VG before each conditioning session for 5 days. The next day, animals' preference for the drug-paired chamber of the CPP apparatus was evaluated without vapor inhalation or morphine injection.

Results: As expected, rats administered with morphine (5 mg/kg) showed a significant preference for the drug-paired chamber compared to saline-treated animals. Rats pre-exposed to cannabis vapor did not show preference for the morphine-paired chamber either in the post-conditioning test or after the reinstatement injection, while animals acutely pre-exposed to PG/VG showed the typical morphine response, spending more time in the drug-paired side. On the other hand, animals exposed to cannabis vapor during the development of morphine conditioning showed a significant increase in morphine-paired side preference in the post-conditioning test but not in the reinstatement session. Unexpectedly, PG/VG-treated rats did not show the morphine-induced side preference in any of the testing sessions after conditioning.

Conclusions: In line with previous clinical and preclinical reports, our results show that inhalation of high CBD cannabis plant extract prevents the expression of morphine rewarding properties. Although we did not see effects in the establishment of morphine rewarding effects, CBD vapor consistently prevented morphine reinstatement in our experimental conditions. Future studies are needed to better understand PG/VG effects in the mechanisms of reward and/or other reinforcing drugs and paradigms.

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N-OLEYOLGLYCINE AND ITS DERIVATIVES ATTENUATE THE ACQUISITION AND EXPRESSION OF COCAINE INDUCED BEHAVIOURS

Samah Shahen-Zoabi*, Reem Smoum, Tehila Beiser, Raphael Mechoulam and Rami Yaka

Institute for Drug Research (IDR), School of Pharmacy, Faculty of Medicine,
The Hebrew University of Jerusalem, Jerusalem, 91120, Israel

Introduction: Drug addiction is a chronically relapsing disorder involving complex interactions among brain circuits, genetics, environment and an individual's life experiences. Drug addicts are engaged in behaviours that become compulsive and often continue despite harmful consequences. The endocannabinoid system plays key modulatory role during synaptic plasticity and homeostatic processes in the brain and has been found to play an important role in the neurobiological processes underlying drug addiction. Impaired endocannabinoid signaling contributes to dysregulated synaptic plasticity, increased stress responsivity, negative emotional states and craving that propel addiction. Therefore, we hypothesized that the endocannabinoid system is involved in rebalancing the reward system following drug exposure, as a general neuroprotective mechanism.

Methods: C57BL/6 mice were used in this study, the behavioural paradigms included: psychomotor sensitization and conditioned place preference, and Liquid chromatography–mass spectrometry (LC-MS) analysis was used for quantitative profiling of endocannabinoids in mouse brain.

Results: We first measured the levels of endocannabinoids in different brain areas of the reward system following chronic cocaine treatment. We found that following chronic repeated daily treatment of cocaine (20 mg/kg), the levels of oleoyl glycine was significantly elevated in the nucleus accumbens. We next tested whether exogenous administration of oleoyl glycine will attenuate cocaine-induced behaviors, for this purpose, we use two behavioral paradigms; psychomotor sensitization that measures drug induced neuroadaptations and conditioned place preference which measure drug reward. We found that administration of oleoyl glycine (60mg/kg) during exposure to cocaine attenuated the expression of cocaine-conditioned reward and did not affect the development of sensitization. Administration of oleoyl glycine (60mg/kg) during withdrawal attenuated the expression of cocaine sensitization and did not affect cocaine-conditioned reward. In order to enhance the stability of oleoyl glycine and its duration of action, several novel methylated derivatives of oleoyl glycine were synthesized, of the molecules tested: methyl oleoyl glycine: HU-595 and dimethyl oleoyl glycine: HU-596. We found that administration of HU-595 (60mg/kg) or HU-596 (60mg/kg) during cocaine conditioning significantly decrease the expression of cocaine-conditioned reward. However, when given during withdrawal from repeated exposure it did not affect the expression of sensitization.

Conclusions: In summary, our findings suggest that endocannabinoid system is involved in the common neurobiological mechanisms underlying the development and expression of drug addiction, boosting the endocannabinoid system exogenously has beneficial effects against cocaine-induced behaviours.

NOVELTY-INDUCED HYPOPHAGIA AS A MEASURE OF SPONTANEOUS CANNABINOID WITHDRAWAL

S. Olivia Vanegas*[1,2], Kristen R. Trexler[3] and Steven G. Kinsey[1,3]

University of Connecticut [1]School of Nursing
[2]Department of Psychological Sciences, Storrs, CT, USA
[3]West Virginia University Department of Psychology, Morgantown, WV, USA

Cannabinoids that activate CB_1 can produce withdrawal symptoms following repeated administration. Gastric discomfort is a commonly reported symptom of cannabis withdrawal. Rodent models generally describe somatic signs of behavior, and cannabinoid withdrawal also affects behavior in common screens for anxiolytic and antidepressant drugs. The goal of the present study was to evaluate novelty-induced hypophagia, i.e., depressed feeding caused by environmental change, as a method to measure cannabinoid withdrawal. Adult male and female C57BL/6J mice were trained to drink a sweetened condensed milk mixture and were treated twice daily for 5 days with either delta-9-tetrahydrocannabinol (Δ^9-THC; 10 or 50 mg/kg, s.c.) or vehicle (s.c.). We have previously reported that somatic signs of spontaneous cannabinoid withdrawal peak at 36 h post abstinence, so milk consumption was measured at 12 and 36 h after the final Δ^9-THC injection. Mice developed tolerance to Δ^9-THC-depressed milk drinking after 4 days, but milk consumption did not differ between groups on test day, indicating that spontaneous Δ^9-THC withdrawal has no effect on sweetened milk drinking. Given that effects of spontaneous withdrawal from partial agonists are typically of smaller magnitude than precipitated withdrawal from full agonists, we treated mice with either the full CB_1 agonist JWH-018 (1 mg/kg, s.c.) or vehicle (s.c.). Mice developed tolerance to JWH-018-depressed milk drinking after 4 days. Withdrawal was precipitated on the 6th day using the CB_1-selective inverse agonist rimonabant (3 mg/kg, i.p.). Rimonabant, *per se*, depressed milk consumption regardless of cannabinoid treatment, making it difficult to determine a possible effect of cannabinoid withdrawal. Thus, follow-up studies using lower doses of rimonabant are ongoing.

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KNOWN MOLECULAR SWITCHES AND NEW GEOMETRIC DESCRIPTORS FOR THE CANNABINOID RECEPTOR 1 (CB1)

AyoOluwa O. Aderibigbe*, Pankaj Pandey and Robert J. Doerksen

Division of Medicinal Chemistry, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Introduction: Molecular switches are conserved changes in the spatial arrangement of certain amino acid residues that are unique to different functional states of a receptor. The canonical ionic lock and rotameric toggle switch are two molecular switches that have been widely used to study receptor conformations at unique functional states and/or their transition between different states of the CB1 receptor. However, these molecular switches are not sufficient to distinguish between the conformations of the experimental 3D structures of CB1 in the three functional states that have now been described.

Methods: Short classical molecular dynamics (MD) simulations were performed on seven experimental 3D structures of CB1. A generalized linear classification model with lasso penalty was applied to a dataset generated by extracting Cα–Cα distances from MD trajectories. In addition, χ1 and χ2 torsion angles of CB1 residues were assessed to identify a new set of geometric descriptors to better distinguish among the active-, intermediate- and inactive- states of CB1.

Results: Statistical analysis of the dataset revealed 11 Cα–Cα distances adequate to classify CB1 receptor conformations into the three functional states with no misclassification error. Four of the distances highlight increased space within the transmembrane bundle at the intracellular space, which is unique to active-state CB1 structures. Five Cα–Cα distances describe the increased orthosteric binding pocket volume that is unique to the inactive state, while the last two Cα–Cα distances are unique to the intermediate state. An assessment of the χ1 torsion angles also revealed a need for a modification to the definition of the rotameric toggle switch.

Conclusions: This work identifies 11 Cα–Cα distances to distinguish among conformations of the experimental 3D structures of CB1 based on their functional states. While the methodology may be applied to identify geometric descriptors to detect subtle but conserved conformational changes in different functional states of other G protein-coupled receptors, the distances identified in this study would not be directly applicable to other G protein-coupled receptors. This work provides a way to understand intramolecular signal transduction in the intermediate-state structure.

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CANNABINOID 1 RECEPTOR FUNCTION IS REDUCED IN THE VENTROLATERAL PERIAQUEDUCTAL GRAY AFTER PERSISTENT INFLAMMATION

Courtney A. Bouchet*[1], Amy J Eshleman[2], Aaron Janowsky[1,3] and Susan L. Ingram[1]

1. Neurological Surgery Department, Oregon Health & Science University
2. VA Portland Health Care System
3. Oregon Health & Science University

Introduction: Cannabinoid receptors (CBRs) are sensitive to environmental perturbations. We have previously observed that presynaptic cannabinoid 1 receptor (CB1R) inhibition of GABA release and CB1R protein levels are significantly reduced after persistent inflammation in the rostral ventromedial medulla (RVM). This CB1R downregulation is accompanied by increased cannabinoid 2 receptor (CB2R) function. The goal of this study is to determine the effect of persistent inflammation on CBRs within the ventrolateral region of the periaqueductal gray (vlPAG), a region upstream of the RVM.

Methods: CB1R-mediated inhibition of GABA release was assessed with whole cell patch clamp electrophysiology measuring spontaneous miniature inhibitory postsynaptic currents (mIPSC) and electrically evoked inhibitory postsynaptic currents (eIPSCs). CBR [^{3}H]CP-55,940 binding was measured in microdissected vlPAG tissue.

Results: `In brain slices from naïve rats, the CBR agonist WIN55,212-2 (3 μM) suppressed presynaptic GABA release and this effect was reversed by the CB1R-specific antagonist rimonabant (3 μM). After persistent inflammation (5-7 days) induced by subcutaneous injection of Complete Freund's Adjuvent (CFA) into the hindpaw, the suppression of GABA release induced by WIN55,212-2 was significantly reduced. This effect was selective for CB1R, as persistent inflammation did not impact mu-opioid receptor inhibition of GABA release. To determine if CFA-induced neural activity acutely elevates endocannabinoids that desensitize CB1Rs, we tested CB1R function 24h after CFA injection. WIN55,212-2 inhibition was consistent with that of naïve animals, indicating that the mechanism underlying the loss of CB1R function takes longer than 24h and is likely not due to the increased spontaneous neural activity immediately following CFA injection. Preliminary radioligand binding saturation curves in vlPAG tissue indicated that 5-7d CFA treatment did not alter CBR density or kinetics. Since CP-55,940 is a nonselective CBR agonist, the lack of changes in CBR binding could be due to increased CB2R expression; however, electrophysiological experiments indicate no increase in CB2R function in the vlPAG after persistent inflammation.

Conclusions: These data indicate that persistent inflammation reduces CB1R function in the vlPAG through a mechanism distinct from the immediate increases in neural activity induced by CFA injection. Data collected from radioligand binding saturation curves indicate that the CB1R is not downregulated in the vlPAG, potentially indicating that the receptor is desensitized or internalized. Future experiments are focused on the underlying mechanisms resulting in decreased CB1R function in presynaptic terminals following persistent inflammation.

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INFLAMMATION-RESOLVING ACTIONS OF AJULEMIC ACID

Sumner Burstein

Department of Biochemistry & Molecular Pharmacology
University of Massachusetts Medical School, 364 Plantation St.Worcester, MA 01605

Ajulemic acid (lenabasum) is a synthetic analog of the phytocannabinoid THC with which it has a significant structural overlap. Unlike THC it has minimal CNS activity, however, it does retain other actions of potential therapeutic value generally at much lower doses than THC. Currently, it is undergoing human clinical trials for four indications; scleroderma (SSC), cystic fibrosis (CF), dermatomyocitis (DM) and lupus (SLE). The DM trial is nearing the end of Phase 3 raising the possibility of its availability to patients in the foreseeable future. It has shown a remarkable safety profile thus far with just under 1000 subjects having received treatment some for as long as 2 years. Most importantly, there are no reports of CB1 receptor mediated (cannabimimetic) actions. This is in contradiction to animal model studies claiming the existence of such responses following dosing with ajulemic acid. One possible explanation relates to the question of drug purity. Many preparations of ajulemic acid contain measureable amounts of CB1 agonists resulting from inadequate purification during synthesis. When so-called “ultrapure” material was tested, it had no cannabimimetic activity in the tetrad assay at therapeutic doses. Thus, the long sought after goal of separation of therapeutic activity from certain CNS actions may now have been reached.

CB1 AS A POTENTIAL TARGET FOR COGNITIVE DEFICITS IN DOWN SYNDROME

N. Di Franco*[1,2]; G.Drutel[1,2] ; V. Lacarrière[1,2] ; V. Lalanne[1,2] ; N. Etchamendy[1,2] ; A.Grel[1,2] ; F. Caraci[3] ; G. Marsicano[1,2] ; A. Marighetto[1,2] ; PV. Piazza[4]; M. Vallée*[1,2] and JM. Revest*[1,2]

[1]Inserm U1215, Neurocentre Magendie and [2]University of Bordeaux, Bordeaux, France; [3]University of Catania, Catania, Italy; [4] Aelis Farma, Bordeaux, France; *equal contribution

Introduction: Down syndrome (DS), originated by the full or partial triplication of human chromosome 21, is one of the most common genetic cause of intellectual disability, which triggers several pathological phenotypes. Among them, executive functions and working memory (WM) are particularly deficient. WM can be defined as the ability to temporally store a small amount of information and to organize them for the execution of cognitive tasks (Baddeley et al. 2003), which mainly involves the dorsal hippocampus (dHPC) (Lee et al. 2003). Currently, there is no therapy for the rescue of neurocognitive impairment in DS. Endocannabinoid system (ECS) seems to be involved in cognitive deficits of DS animal models. The endocannabinoid 2-AG was overexpressed in the brain of Ts65Dn mouse model of DS (Lysenko et al. 2014) and the administration of an antagonist of the cannabinoid receptor type 1 (CB1R), rimonabant, rescued memory impairment in Ts65Dn mice (Navarro-Romero et al. 2019). The current work aims to assess the molecular basis of DS, exploring the ECS involvement in DS cognitive deficit and to propose a therapeutic approach.

Methods: We performed a battery of behavioral tests to explore different phenotypes in Ts65Dn mice and we focused on the WM impairment. For translational purposes in humans, we used a radial maze model to assess WM, which has been developed in preclinical and clinical research (Etchamendy et al. 2012; Al Abed et al. 2016). Using this model, similar WM deficits were found in Ts65Dn mice and DS patients. Then, we analyzed the expression of genes involved in ECS trough qPCR, fluorescent in situ hybridization (FISH) and proteins with western blot (WB).

Results: Both CB1R gene and protein resulted overexpressed in the dHPC. FISH showed that CB1 mRNA was significantly overexpressed in the stratum lacunosum-moleculare (SLM) and radiatum (SR) whereas it was downexpressed in the stratum pyramidale (SP) of dCA1. Coherently with the literature, CB1 is highly expressed in inhibitory GABAergic interneurons of SLM and SR, and in excitatory glutamatergic neurons of the SP. Thus, our findings suggest a CB1 overexpression in GABAergic interneurons and parallel downexpression in glutamatergic neurons. Since functional neuroanatomy of memory is highly dependent on those cell-types and GABA/Glu imbalance is crucial for cognitive dysfunctions in Ts65Dn (Contestabile et al. 2017; Gomez de Salazar et al. 2018). This hypothesis, need to be confirmed with a double FISH, colocalizing CB1-GAD (GABA interneurons marker) and CB1-VGLUT (glutamatergic interneurons marker).

Conclusion: Our data suggest that CB1 alteration can be crucial for WM impairment in Ts65Dn, demonstrating the critical role of CB1 as potential target for DS. As a result, decreasing CB1 activity would be a suitable therapeutic approach for cognitive deficits in DS. Interestingly, in collaboration with the biotech *Aelis Farma*, our group developed new synthetic analogs of endogenous steroid pregnenolone, acting as negative allosteric modulators of CB1. In contrast to full CB1R antagonists such as rimonabant (Mitchel et al. 2007), these compounds present excellent ADMET profile and no side effects (Piazza et al.; WO/2012/160006; WO/2014/083068; WO/2019/162328A1). Preclinical experiments with one of these compounds in Ts65DN mice are currently ongoing.

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LACK OF CANNABINOID RECEPTOR TYPE-1 LEADS TO ENHANCED AGE-RELATED NEURONAL LOSS IN THE LOCUS COERULEUS

Alessandra Gargano*, Eva Beins, Andreas Zimmer and Andras Bilkei-Gorzo

Institute of Molecular Psychiatry, Medical Faculty, University of Bonn, Germany

Introduction: The endocannabinoid system (ECS) is an important neuromodulatory system that plays a crucial role in a large variety of homeostatic processes essential for maintaining the integrity of brain function. Our laboratory and others have previously shown that cannabinoid receptor type-1 (CB1r) activity is neuroprotective and a modulator of brain ageing; a genetic disruption of CB1r signaling accelerates brain ageing, whereas the pharmacological stimulation of CB1r activity has the opposite effect. In this study, we have investigated how the components of the ECS affects noradrenergic neurons in the locus coeruleus (LC), which are vulnerable to age-related changes; their numbers are reduced in patients with neurodegenerative diseases and probably also in healthy aged individuals.

Methods: The number of LC neurons was determined using stereological counting at first in old (22 months) and young (3 months) wild-type animals to confirm a real ageing effect in our mouse model. Next, wild-type animals from six different age groups (from 2 to 24 months) were used in order to investigate the timeline of LC age-related neurodegeneration. Thereafter, the number of LC neurons was compared between old (18 months) and young (3 months) wild-type and cannabinoid 1 receptor knockout ($Cnr1^{-/-}$) mice. In addition, markers for noradrenergic innervation, inflammation, and also DAGLα protein expression have been analyzed by immunohistochemistry.

Results: Our data show that in wild-type mice there is a neuronal loss in LC comparable with the findings in humans, and that the number of noradrenergic neurons starts declining in middle aged animals (8 months). Moreover, our results reveal that old $Cnr1^{-/-}$ mice have less noradrenergic neurons compared to their age-matched wild-type controls. This result was also confirmed by the analysis of the density of noradrenergic terminals which proved that $Cnr1^{-/-}$ mice had less compared to the wild-type controls. Additionally, we assessed pro-inflammatory glial activity in the LC. Although the density of microglia in $Cnr1^{-/-}$ mice was enhanced, they did not show enhanced inflammatory profile. Interestingly, the analysis of DAGLα expression in LC during ageing revealed an accumulation of the enzyme in noradrenergic cells during ageing, starting at 9 months of age and becoming prominent as early as 11 months.

Conclusions: All together our data suggest an involvement of the entire endocannabinoid system in the aging process of brain areas sensitive to age-related changes such as LC. Furthermore, we hypothesize that CB1r activity is necessary for the protection of noradrenergic neurons, even though its anti-inflammatory effect probably only plays a minor role in it. Further in vivo studies will investigate how these alterations in neuronal number influence the noradrenergic system and the ageing process.

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CB1 INACTIVATION INCREASES PRO-INFLAMMATORY CYTOKINE PRODUCTION IN RESPONSE TO LIPOPOLYSACCHARIDE

Barbara L. F. Kaplan*, Brittany Szafran, Ashleigh Nicaise,
Matthew K. Ross and James Nichols

Center for Environmental Health Sciences, Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA

Introduction: While the role of CB_2 in immune homeostasis is well-established, questions still remain about the role of CB_1. Previously we published that clinical disease was exacerbated in experimental autoimmune encephalomyelitis (EAE) in *Cnr1*$^{-/-}$ mice as compared to wild type littermates. We also noted in these previously published data that splenocytes obtained from *Cnr1*$^{-/-}$ EAE mice produced more interferon (IFN)-γ as compared to wild type when restimulated ex vivo with the same peptide used to induce disease. Interestingly, an examination of T cells stimulated in vitro revealed the opposite results with wild type T cells making less IFN-γ than *Cnr1*$^{-/-}$ T cells in response to anti-CD3/CD28 stimulation. Together these data suggested that a non-T cell was responsible for producing the enhanced IFN-γ in *Cnr1*$^{-/-}$ mice. We hypothesized that innate cells in the splenocyte population were responsible for the enhanced IFN-γ in *Cnr1*$^{-/-}$ mice.

Methods: Splenocytes from male and female *Cnr1*$^{-/-}$ and wild type littermates were stimulated in vitro with lipopolysaccharide (LPS) at 0.1 and 1 μg/ml for up to 3 days. Separate studies using SR141716A-treated splenocytes were also conducted in vitro in response to LPS.

Results: ELISA analyses revealed that *Cnr1*$^{-/-}$ mice produced more IFN-γ than wild type littermates in cells from both males and females, with statistical significance in 1 μg/ml LPS-treated cells at day 3 in males. IL-6 also showed enhanced production from *Cnr1*$^{-/-}$ mice, again with statistical significance in male mice at days 1-2. A similar pattern for TNF-α was also observed with statistically significant differences in 1 μg/ml LPS-treated cells at day 1 in females. Enhanced trends in IL-1β from *Cnr1*$^{-/-}$ mice were also seen, albeit none were significant. Separate studies using SR141716A-treated splenocytes revealed enhanced IFN-γ production in LPS-stimulated cells as compared to vehicle-treated cells.

Conclusions: These data confirmed our hypothesis that CB_1 plays a role in controlling cytokine production, which likely came from innate cells. Future studies will evaluate mechanisms by which CB_1 alters immune cell signaling.

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IN VIVO EVIDENCE FOR FUNCTIONAL INTERACTIONS BETWEEN CANNABINOID AND OREXIN RECEPTORS IN THE ADULT MOUSE BRAIN

Hye Ji (Jay) Kim*[1], Teagan Holt[1], Udoka Ezeaka[2] and Robert Laprairie[1, 3]

College of Pharmacy and Nutrition, University of Saskatchewan[1], College of Arts and Science, University of Saskatchewan[2], Department of Pharmacology, College of Medicine, Dalhousie University[3]

Introduction: The endocannabinoid and orexin systems serve key roles in many of the same biological functions, such as sleep, appetite, and reward. The type 1 cannabinoid receptor (CB1R) and both types of the orexin receptor, OX1R and OX2R, co-localize in brain regions such as the hypothalamus, cingulate cortex, hippocampus, and periaqueductal grey. Accumulating evidence suggests that these receptors are not only expressed in the same regions and cells, but heterodimerize on the cell surface to evoke unique downstream effects.

Methods: Male and female C57BL/67 mice were intraperitoneally injected with the cannabinoid receptor agonist CP55,940 and the OX2R antagonist MIN-202. Following the injections, mice underwent tetrad testing consisting of catalepsy, body temperature, thermal nociception, and locomotion, respectively. All mice were then perfused, and their brain tissue collected for immunohistochemical analysis.

Results: When the CB1R agonist CP55,940 was administered alone, both sexes showed endocannabinoid-dependent effects across the tetrad, as expected. Co-administration of a constant dose of CP55,940 and increasing doses of the OX2R antagonist MIN-202 reduced the potency of CP55,940 to produce hypothermia and anti-nociception, with no effect on catalepsy or locomotion. Male mice demonstrated a higher sensitivity to MIN-202-mediated antagonism than females. These data demonstrate a functional *in vivo* interaction between the cannabinoid receptors and OX2R whereby inhibition of OX2R blocked CB1R-dependent activity in an outcome-specific manner that may be tied to the relative expression of both receptors in different brain regions, such as the hypothalamus (e.g. hypothermia) and spinal cord (e.g. nociception). Furthermore, immunohistochemistry will provide data on which brain regions have the highest density of receptor co-localization and potential interaction.

Conclusion: This study demonstrates a functional interaction between cannabinoids and orexins, which may be important in understanding how both systems regulate important homeostatic functions such as sleep, appetite, reward, and emotionality. The inclusion of both males and females also highlights important sex differences in these system interactions. Ongoing studies are presently assessing interactions with OX1R, molecular and immunohistological interactions between these receptors, and the consequences of agonist co-administration *in vivo*.

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THE ENDOCANNABINOID SYSTEM IS A RELEVANT PLAYER IN ADOLESCENT MYELINATION

Cristina Manenti*[1], Erica Zamberletti,[1] Marina Gabaglio[1] and Tiziana Rubino[1]

1 Dept. of Biotechnology and Life Sciences (DBSV),
Univ. of Insubria, Busto Arsizio (VA), Italy

Introduction: Although a lot of scientific evidence suggests that exposure to Cannabis during adolescence increases the risk of mental diseases later in life, this illicit drug remains the most commonly used among adolescents. Adolescence is a very sensitive period, characterized by changes in morphology such as a process of grey matter loss and an increase in white matter volume (myelination). In recent years, some studies have shown the relevance of the endocannabinoid system (ECS) in synaptic maturation during adolescence but its involvement in adolescent myelination remains completely unknown. The gain in axonal myelination, that serves to enhance communication efficiency, has previously been associated with the development of language and memory skills in adolescence. Interestingly, recent studies have shown that individual variability in myelin growth trajectories, investigated during the transition from adolescence to adulthood, can be also linked to the expression of impulsivity traits. Specifically it has been reported that impulsivity is associated with a reduction in myelin growth in the lateral and medial prefrontal cortex. On these grounds, our first aim was to study the role of the ECS in adolescent myelination. Moreover, since our preliminary data demonstrated that the ECS plays a relevant role in this process, our second aim was to investigate, through the wire-Beam Bridge Test, if any correlation exists between impulsivity and the development of white matter after ECS functionality blockade in adolescent rats.

Methods: In order to thoroughly analyze each step of brain maturation, the adolescent developmental window (from PND 28 to 52) was divided in five sub-periods of five days each. Sprague-Dawley female rats of each sub-groups were injected daily, for five days with AM251 (0.5 mg/kg, i.p.) a selective CB1 receptor antagonist. Five hours after the last injection, PFCs were removed, immediately frozen in liquid nitrogen and stored at -80 °C until processing. Then, through western blot analysis, we studied the impact of this modulation on two markers of myelination (MBP and MOG).To reach our second aim, Sprague-Dawley female rats received daily intraperitoneal injections of AM251 (0.5 mg/kg), starting from PND 28 to 45.Three days after the last injection, rat impulsivity was tested in the Wire-Beam Bridge Test. Four hours after the test, animals were sacrificed and the PFCs were prepared for electron microscopy.

Results: In control animals, MBP and MOG levels mainly increased during the period investigated and in parallel impulsivity decreased with the myelination enhancement. In animals administered with AM251, except for the last sub-period investigated, MBP and MOG levels were always lower and CB1 receptor blockade reduced the time that rats needed to cross the bridge, thereby leading to an increase of impulsivity. The appearance of myelin layer in the rat PFC studied by electron microscope confirmed that AM251 administration from PND 28 to 45 prevented the myelination enhancement in adolescent female rats.

Conclusions: Our preliminary data suggest that the endocannabinoid tone is fundamental for the occurrence of adolescent myelination process. The ECS blockade during this specific developmental window seems to prevent the increase of myelin and, in parallel, lead to a higher expression of impulsivity.

CANNABINOIDS MODULATE ADIPOGENESIS AND LIPOGENESIS IN DAIRY COWS

Madison N. Myers[1*], C. Javier Rendon[1], Maya Zachut[2], Joseph Tam[3] and G. Andres Contreras[1]

[1]Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA. [2]Department of Ruminant Science, Institute of Animal Sciences, Volcani Center, Rishon LeZion, Israel. [3]Obesity and Metabolism Laboratory, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.

Introduction: The periparturient period in dairy cows is characterized by increased lipolysis and reduced adipogenesis and lipogenesis in adipose tissues (AT). As lactation progresses, the intensity of these processes is reverted. However, if AT lipolysis dysregulation is protracted, it may lead to high disease susceptibility. In monogastrics, cannabinoids (CBs) enhance the adipogenic and lipogenic capacity of adipocytes via activation of cannabinoid receptor 1 (CB1). However, the effects of CBs on dairy cow AT are unknown.

Methods: Subcutaneous AT (SCAT) was collected from multiparous, non-lactating, non-gestating Holstein cows (N=8). Stromal vascular fraction was obtained via collagenase digestion. Pre-adipocytes were isolated following outgrowth of plastic adherent cells, plated, and induced to differentiate upon confluency using standard differentiation medium for 14 days. During the first 2 days of differentiation, adipocytes were exposed to the synthetic CB arachidonyl-2'-chloroethylamide (**ACEA**) at 0 (CON), 1 (**ACEA1**), 5 (**ACEA5**), or 10 (**ACEA10**) μM concentrations. ACEA was included with or without the CB1 antagonist rimonabant (**RIM**, 0.1 μM) for 48 h. Cell viability was assessed using Calcein AM according to the manufacturer's guidelines. Adipogenesis was evaluated using long-term live-cell imaging IncuCyte® S3 system and the IncuCyte ZOOM™ software. Neutral lipids were stained with Bodipy 493/503. We calculated adipogenesis efficiency as the ratio of cells with at least one lipid droplet over the total number of cells per well. At the last day of culture, lipogenesis was evaluated by TAG accumulation (Adipored®) and is reported in RFU/ng DNA.

Results: ACEA at any of the doses and RIM did not affect viability of cells during culture. ACEA10 (54.5±4.3%), but not ACEA5 (50.05±4.3%) and ACEA1 (50.89±4.3%) increased (P<0.05) adipogenesis efficiency compared to CON (44.23±4.3%). Across all ACEA concentrations, the presence of RIM (46.25±4.1%) reduced adipogenesis compared to those cells unexposed to the CB1 inhibitor (53.59±4.1%, P<0.05). Similar to adipogenesis, increased lipid accumulation (i.e., lipogenesis) in ACEA10 (3115.43±278) compared to CON (1849.45±278); and the presence of RIM reduced lipid content in ACEA10+RIM (2681.19±278) but not in CON+RIM (1840.40±278).

Conclusions: Our findings demonstrate that, in dairy cows, adipogenesis and lipogenesis are enhanced upon CB1 activation by CBs. Further testing will determine the implications of feeding CB precursors, such as omega-6 fatty acids, on the augmented production of endogenous CBs and the promotion of adipogenesis and lipogenesis in dairy cows.

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CB_1 ANTAGONISM INCREASES EXCITATORY SYNAPTOGENESIS IN A CORTICAL SPHEROID MODEL OF FETAL BRAIN DEVELOPMENT

Alexis Papariello*[1], David Taylor[1], Ken Soderstrom[1] and Karen Litwa[2]

[1]Brody School of Medicine at East Carolina University,
Department of Pharmacology and Toxicology, Greenville, NC 27834
[2] Brody School of Medicine at East Carolina University,
Department of Anatomy and Cell Biology, Greenville, NC 27834

Introduction: The endocannabinoid system (ECS) plays a complex role in the development of neural circuitry during fetal brain development. The cannabinoid receptor type 1 (CB_1) controls synaptic strength at both excitatory and inhibitory synapses and thus contributes to the balance of excitatory and inhibitory signaling. Imbalances in the ratio of excitatory to inhibitory synapses have been implicated in various neuropsychiatric disorders associated with dysregulated central nervous system development including autism spectrum disorder, epilepsy, and schizophrenia. We thus sought to evaluate the effects of acute CB_1 antagonism on synaptogenesis in a cortical spheroid model of the mid-gestational fetal brain.

Methods: The role of CB_1 in human brain development has been difficult to study but advances in induced pluripotent stem cell technology have allowed us to model the fetal brain environment. Cortical spheroids resemble the cortex of the dorsal telencephalon during mid-fetal gestation and possesses functional synapses, spontaneous activity, an astrocyte population, and pseudo-laminar organization. We first characterized ECS components CB_1, MAGL, and DAGLα in this model using STORM microscopy and confocal imaging. Next, we assessed the effect of CB_1 selective antagonist SR141615A on excitatory and inhibitory synapses using confocal image analysis to visualize synapses and micro-electrode array recording to measure synaptic output.

Results: We first observed that CB_1 localized to the presynaptic compartment of both excitatory and inhibitory synapses. Additionally, we found presynaptic DAGLα and postsynaptic MAGL at excitatory synapses. Next, using SR141716A, we observed an increase in excitatory, and to a lesser extent, inhibitory synaptogenesis. Further, CB_1 antagonism increased the variability of spontaneous activity within developing neural networks, as measured by micro electrode array. Consistent with the increased synaptogenesis, we also observed a decrease in synaptic RhoA-GTPase activation after SR141716A treatment.

Conclusion: We have established that cortical spheroids express ECS components and are thus a useful model for exploring endocannabinoid mediation of childhood neuropsychiatric disease. Additionally, CB_1 can be antagonized to create a phenotype which displays disrupted excitatory/inhibitory balance through increased excitatory synaptogenesis and increased variability of neural activity. Our results further confirm the role of the ECS in synaptic pathology and we propose the utilization of CB_1 as a targetable receptor for therapeutics in neurodevelopmental disorders.

THE PERIPHERALLY RESTRICTED CANNABINOID CB-13 PRODUCES PERIPHERALLY-MEDIATED ANALGESIA BUT REPEATED DOSING ELICITS TOLERANCE AND SIGNS OF CENTRAL NERVOUS SYSTEM ACTIVITY

Richard A. Slivicki[1], Jiwon Yi[1,2], Victoria E. Brings[1], Phuong Nhu Huynh[1] and Robert W. Gereau IV[1,3,4]

[1]Washington University Pain Center and Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO.
[2]Neuroscience Graduate Program, Division of Biology & Biomedical Sciences, Washington University School of Medicine, St. Louis, MO.
[3]Department of Neuroscience, Washington University, St. Louis, MO
[4]Department of Biomedical Engineering, Washington University, St. Louis, MO

Introduction: Peripherally restricted cannabinoid type 1 receptor (CB_1R) agonists have been developed with the hope of providing analgesia without unwanted effects, including psychoactivity and tolerance, associated with CB_1R activation in the central nervous system. The present study sought to evaluate the impact of long-term administration of a peripherally restricted cannabinoid agonist, CB-13, on reducing inflammatory nociception and producing CB_1R dependence in mice in vivo and evaluate cellular mechanisms underlying CB-13-induced antinociception in vitro using cultured mouse dorsal root ganglion (DRG) neurons.

Methods: A within-subjects dose-response of CB-13 in reducing mechanical allodynia induced by CFA was generated and the maximally efficacious dose (3 mg/kg i.p.) was evaluated for efficacy in reducing CFA-induced thermal allodynia. The peripherally restricted CB_1 antagonist AM6545 was used to evaluate the specificity of CB-13's anti-allodynic effects. Tolerance and CB_1R dependence was evaluated following repeated dosing To evaluate for other cardinal signs of central CB_1R activation, catalepsy, tail-flick antinociception and changes in body temperature were measured in naïve mice using the same doing paradigm as stated above. The effect of CB-13 on prostaglandin E2 (PGE_2)-induced TRPV1 sensitization and neuronal hyperexcitability was measured in lumbar DRG cultures isolated from naïve mice.

Results: CB-13 reduced inflammation-induced mechanical allodynia in a peripheral CB1R-dependent manner and relieved inflammatory thermal hyperalgesia. Phenotypes associated with central CB1R activation occurred only at a dose of CB-13 approximately 10-fold its ED_{50} for reducing allodynia. In cultured mouse DRG neurons, CB-13 reduced TRPV1 sensitization and neuronal hyperexcitability induced by the inflammatory mediator (PGE_2)-providing potential mechanistic explanations for the analgesic actions of peripheral CB_1R activation. Strikingly, repeated dosing with CB-13 resulted in both analgesic tolerance and CB_1R dependence, even at a dose that did not produce central CB_1R-mediated phenotypes on acute dosing.

Conclusions: These results suggest increased CNS exposure with repeated CB-13 dosing, leading to unwanted engagement of central CB_1Rs.Thus, caution is warranted regarding the use of CB-13 a therapy. Nonetheless, the clear analgesic effect of peripheral CB_1R activation suggest that the approach of developing peripherally restricted cannabinoids as novel analgesics should be pursued.

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VALIDATION OF THE NATURALISTIC CANNABIS ADMINISTRATION PROTOCOL (NCAP)

Michelle St. Pierre*[1], Sarah Daniels[1], Tatiana Sanchez[1] and Zach Walsh[1]

Department of Psychology, University of British Columbia, Kelowna, BC, Canada[1]

Introduction: To date, experimental research examining the effects of cannabis administration on human behaviour has been conducted primarily in laboratory settings. The highly controlled lab environment enables researchers to isolate drug effects from other contextual and individual variables and protects participants from the stigma and potential legal consequences of documented ingestion of a controlled substance. However, lab-based approaches may compromise ecological validity, which is a particular concern given the sensitivity of cannabis effects to contextual factors such as set and setting which may differ substantially between laboratory and typical self-administration contexts. Moreover, the category of cannabis contains diverse chemovars with distinct cannabinoid profiles and concentrations, meaning that a given chemovar administered in a laboratory may vary considerably with regard to its similarity to the chemovar typically used by a given research participant thereby further compromising ecological validity. Laboratory administration of cannabinoids also comes at a substantial cost to feasibility and accessibility, as obtaining licences from federal agencies to access, store and administer cannabis continues to be barrier in many jurisdictions. These limitations of conventional cannabis administration paradigms are particularly salient in light of the profound expansions of access to legal cannabis for adults across North America – the landscape of cannabis use and access has changed dramatically over the past decade while human research paradigms have remained largely static. This apparent disconnect mandates the development of new research paradigms for studying the acute effects of cannabis administration. To address this research need we have developed the Naturalistic Cannabis Administration Protocol (NCAP). The NCAP is a low-barrier, replicable, and an ecologically valid methodology to study acute cannabis effects in which participants self-administer their typical cannabinoid preparation in a familiar environment and undergo experimental manipulations and behavioral assessment via video conference. The present research reports on the feasibility of and acceptability of a pilot study of the NCAP.

Methods: Two appointments were counterbalanced such that participants were randomly assigned to either use cannabis at their first appointment or at their second appointment which was scheduled one month later. Appointments were scheduled at a time of day when the participant *"usually got high"* and included five cognitive tasks in domains including delayed verbal recall memory, divergent and convergent thinking, processing speed, working memory and verbal fluency. Following each experimental session, participants were interviewed about the protocol and a subjective performance evaluation.

Results: A university sample (N = 30; 57% female; M_{age} = 22.1, SD = 2.6) of cannabis users (i.e., use at least 3X/week) participated in this study. Preliminary results support the feasibility and acceptability of the NCAP in a university sample. Within-subjects data examining the effect of cannabis administration on cognition in a naturalistic setting will be presented.

Conclusions: The NCAP provides researchers with a low barrier and ecologically valid paradigm to research the effects of cannabis use on human behaviour. Previous studies on the psychological effects of cannabis use should be replicated with the NCAP to determine generalizability to this novel approach.

ANANDAMIDE ENHANCES BARRIER INTEGRITY OF BOVINE VASCULAR ENDOTHELIAL CELLS DURING ENDOTOXIN CHALLENGE VIA CANNABINOID RECEPTOR-1 ACTIVATION

Carsten C. F. Walker* and Lorraine M. Sordillo

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing 48824, United States

Introduction: Dysfunctional inflammation associated with coliform mastitis is a major contributor to the severity and potential lethality of systemic infections. Targeted breakdown of the vascular endothelium is a part of mastitis pathogenesis. The ability of the endocannabinoid (EC) system to modulate inflammation was shown in several non-bovine species. The EC system is comprised of the cannabinoid receptor 1 and 2 (CB1/CB2, respectively) and their ligands, fatty acid ethanol amides and glycerols. The EC arachidonoylethanolamide (AEA) improves network formation and proliferation in human and rodent endothelial cell models via activation of several receptors, including CB1. Increased plasma AEA during systemic coliform mastitis *in vivo* and elevated CB1 and 2 expression in cultured bovine aortic endothelial cells (BAEC) challenged with endotoxin (LPS) support the bovine EC system involvement in inflammation. Prior experiments using the electric cell-substrate impedance sensing (ECIS) technology showed a dose dependent increase in barrier resistance of BAEC challenged with LPS.

Methods: Rimonabant, a CB1 inverse agonist, was used to elucidate the involvement of CB1 in the increased barrier resistance observed in BAEC treated with AEA during LPS challenge. Confluent primary BAEC cells in ECIS arrays were treated with 25 ng/mL of LPS for 6-8 hours before addition of AEA/rimonabant.

Results: Rimonabant doses of 0.1, 0.5, and 1 μM decreased barrier resistance within the first 2 hours of treatment, with a loss of effect at 4 hours.

Conclusions: Timing and effect of rimonabant treatment indicate that AEA related increase in barrier resistance is CB1 mediated. However, AEA and rimonabant were shown to activate non-CB receptors involved in network formation and proliferation and further *in vitro* modeling is necessary to fully elucidate the mechanism of increased BAEC barrier resistance in response to AEA. Identifying regulatory points of AEA enhanced endothelial cell barrier resistance may lead to new therapeutic targets to optimize inflammatory responses during acute disease events, reducing dairy cow mortality via reduced incidence and severity of systemic infections.

ROLE OF CANNABINOID RECEPTOR SUBTYPE-1 IN THE INTESTINAL EPITHELIUM IN ANXIETY-LIKE BEHAVIORS

Courtney P. Wood*, Pedro A. Perez and Nicholas V. DiPatrizio

Division of Biomedical Sciences, School of Medicine, University of California, Riverside, Riverside, CA, USA

Introduction: The endocannabinoid system (ECS) participates in the behavioral expression of anxiety. For example, mice constitutively lacking the cannabinoid receptor subtype-1 (CB_1R) spend significantly less time exploring the open arms of an elevated plus maze when compared to wild-type mice, a behavior that is indicative of increased anxiety. Moreover, mice treated with a CB_1R inverse agonist behave similarly, while mice receiving cannabinoid agonists such as delta-9-THC and WIN55,212-2 exhibit an anxiolytic phenotype in the elevated plus maze. The ECS is expressed ubiquitously throughout the body, including in the intestinal epithelium where it controls gut-brain signaling important for food intake. In the current study, we aimed to determine if CB_1Rs located in the small-intestinal epithelium exert control over anxiety-like behaviors in the elevated plus maze test, and whether this behavior is sex-dependent.

Methods: For these studies, we utilized our novel conditional intestinal epithelium-specific CB_1R-null mice ($IntCB_1^{-/-}$, $Cnr1^{tm1.1\ mrl}$/vil-creERT2) and controls with normal expression of CB_1Rs in the intestinal epithelium to identify a role for intestinal CB_1Rs in anxiety-like behaviors. Male and female mice (6-8 weeks old) were treated with tamoxifen (40 mg/kg, I.P.) for 5 consecutive days to ablate CB_1R in the intestinal epithelium. Mice were allowed a 10-day recovery period, after which they were acclimated to the testing room for 3-4 hours. Mice were allowed to freely explore the elevated plus maze apparatus for a 5-minute period. Data was collected using Noldus EthoVisionXT software. RT-qPCR was performed to confirm knockdown of intestinal CB_1Rs, as previously described in Avalos et al., *Nutrients*, 2020.
Results: Male $IntCB_1^{-/-}$ mice spent significantly more time than controls exploring the open arms of the maze. Male $IntCB_1^{-/-}$ mice also participated in a head dipping behavior more frequently than controls. There were no significant differences in mean velocity, total distance moved, and cumulative duration of movement between genotypes. In contrast to males, female $IntCB_1^{-/-}$ mice did not exhibit any significantly different behaviors than control animals on the EPM. Female mice, however, explored the open arms more than the males and also spent more time performing the head dipping behavior. There were no differences in overall locomotion between genotypes or sex.

Conclusions: Collectively these results suggest that genetic deletion of CB_1Rs in the intestinal epithelium is associated with an anxiolytic phenotype in a sex-dependent manner, with a robust phenotype found for male mice. Further studies will investigate the mechanism(s) by which intestinal CB_1Rs control anxiety-like behaviors. We are actively evaluating other physiological readouts associated with sex-dependent anxiety-like behaviors, such as circulating cortisol levels and neuronal activity in brain regions associated with anxiety. Furthermore, we are testing the hypothesis that deletion of CB_1Rs in the intestinal epithelium contributes to this phenotype via gut-brain signaling mediated by the afferent vagus nerve.

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CANNABINOIDS MODULATE THE PRO- AND ANTI-INFLAMMATORY PROPERTIES OF POLARIZED M1/M2 MICROGLIA *IN VITRO*

Alexander P. Young*[1], Shawn J. Adderley[1], Melanie E.M. Kelly[1] and Eileen M. Denovan-Wright[1]

[1]Department of Pharmacology, Dalhousie University, Halifax, NS, Canada

Introduction: Microglia can take on an activated M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotype during states of neuroinflammation[1]. The M1 phenotype mediates host defense but sustained activation can damage neurons[2]. M1 microglia normally transition to the M2 phenotype to halt the inflammation[3]. However, this transition fails in many neurological disorders[3]. Microglia express cannabinoid type 1 (CB_1) and type 2 (CB_2) receptors, and cannabinoid signaling is linked to the transcription of some inflammatory factors which indicates therapeutic potential to limit neuroinflammation[4]. With this work, we aim to determine if cannabinoid receptors in M1 and M2 microglia represent a viable target to manipulate microglial phenotype.

Methods: Cultured SIM-A9 microglia were stimulated to acquire an M1 or M2 phenotype in the presence or absence of cannabinoid treatments. A CB_1 receptor agonist (ACEA; >1400-fold selective), CB_2 receptor agonist (HU-308; >440-fold selective), and nonselective agonist (CP 55,940) were used to target the cannabinoid receptors. Enzyme-linked immunosorbent assays were used to measure cytokine release, and reverse transcription quantitative PCR was used to measure the mRNA abundance of M1 and M2 markers. Impermeant dyes were used to mark dead ST*Hdh*$^{Q7/Q7}$ neurons treated with conditioned media from microglia. Statistical inference was performed using parametric or nonparametric tests as appropriate.

Results: CB_1 and CB_2 receptor-selective and nonselective cannabinoids dampened the inflammatory activity of cultured SIM-A9 microglia in a dose-dependent manner. ACEA reduced NO release to ~53% relative to M1 microglia that did not receive cannabinoids. HU-308 and CP 55,940 reduced NO release to ~72% and ~81%, respectively. ACEA and HU-308 also reduced the mRNA abundance of inducible NOS, TNFα, IL-1β, and IL-6 in M1 microglia relative to cells that received the pro-inflammatory stimuli without cannabinoids. Cannabinoids also upregulated anti-inflammatory markers in M2 microglia. Conditioned media from M1 microglia induced cell death in ~85% of ST*Hdh*$^{Q7/Q7}$ neurons compared to ~48% of neurons treated with media from resting microglia. Media was less neurotoxic when M1 microglia were treated with cannabinoids first as neuron death was reduced to ~65% by ACEA, ~58% by HU-308, and ~71% by CP 55,940.

Conclusions: Selective and non-selective cannabinoid treatments dampen the inflammatory response of M1 microglia and enhance the anti-inflammatory response of M2 microglia. This modulation of phenotype is indicative of the neuroprotective potential of drugs that interact with microglial cannabinoid receptors. Further work will expand on the signaling mechanisms that underlie the effects of cannabinoids on the inflammatory activity of microglia.

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CANNABINOIDS INDUCE LUNG INFLAMMATION VIA CB_1R ACTIVATION

Charles N. Zawatsky*[1], Jasmina Abdalla[2], George Kunos[2] and Resat Cinar[1]

[1]Section on Fibrotic Disorders, [2]Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, NIH, Rockville, MD, 20852, USA.

The use of synthetic cannabinoids (SC) has persisted despite their schedule-1 classification under the Synthetic Drug Abuse Prevention Act of 2012. Analysis of the National Poison Data System indicates that hospitalizations caused by SC use increased significantly between 2010 and 2015. Among those hospitalized, some patients exhibited respiratory failure, pulmonary infiltrates, alveolar damage or hemorrhage and histopathologic features of organizing pneumonia. SCs are often 30 to 100-fold more potent than Δ9-tetrahydrocannabinol (THC), the major psychoactive ingredient of cannabis, in activating cannabinoid receptor 1 (CB_1R). The mechanism by which SCs damage pulmonary tissue has yet to be elucidated- a crucial first step in optimizing treatment for patients presenting with SC-related respiratory distress. CB_1R is expressed in multiple cell types in the lung, with its overactivity linked to lung injury, alveolar inflammation, and fibrosis.

We explored the acute effects of the synthetic cannabinoid CP55,940 on CB_1R activation and inflammation in the lungs of C57BL6/J mice. CP55,940 (22.6 μg/kg [565 ng / 150 μl]) was delivered via oropharyngeal (OP) aspiration to mice weighing 25-27 g. The lung tissue was collected 4, 24 and 48 hours after CP55,940 delivery, and assessed via mass spectrometry and RT-qPCR to quantify markers of endocannabinoid system-related and inflammatory activity. Oropharyngeal CP55,940 administration significantly increased *Cnr1* and *Cnr2* expression as early as 4 hours, then normalized by 48 hours post drug delivery. The expression of a number of other genes was similarly upregulated, including those of inflammatory cytokines TNFα, IL1β and IL6; chemokines such as chemokine (C-X-C motif) ligand 1, chemokine (C-C motif) ligand 2, chemokine (C-C motif) ligand 3, and chemokine (C-X-C motif) ligand 10; as well as the pro-inflammatory transcription factor NACHT, LRR and PYD domains-containing protein 3 (*Nlrp3*) inflammasome, within a similar time course. Blockade of CB_1R by rimonabant (10mg/kg) completely prevented the cannabinoid-induced elevation of CB_1R gene expression along with inflammatory cytokines, chemokines, and pro-inflammatory transcription factors. CP55,940 exposure also caused significant, CB_1R-dependant increases in pro-inflammatory, cell-signaling oxidized phosphatidylcholines such as palmitoyl-oxo-valeryl-phosphatidylcholine (POVPC), prevented by rimonabant.

These findings demonstrate that pulmonary exposure to potent cannabinoids induces lung inflammation via CB_1R activation. This may justify the treatment of SC-induced lung disease with a CB_1R antagonist, a possibility to be investigated through intervention-focused studies. Although the CB_1R antagonist rimonabant is no longer available due to neuropsychiatric side effects, orally bioavailable, peripherally restricted CB_1R antagonists which are currently under clinical development may offer an alternative. Cytokines such as TNFα, IL1β, and IL-6 upregulated by CB1R activation are involved in the COVID-19 cytokine storm specifically, and acute respiratory distress syndrome (ARDS) broadly. Oxidized phosphatidylcholines such as POVPC are also involved in ARDS. Accordingly, future studies should explore the treatment efficacy of CB_1R antagonists in COVID-19 and ARDS models.

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DOUBLE EXPOSURE TO STRESS AS A RISK FACTOR FOR SEVERE PSYCHIATRIC DISORDERS: THE INVOLVEMENT OF ENDOCANNABINOID MARKERS

Konstantin Andrianov*, Inna Gaisler-Salomon and Irit Akirav

School of Psychological Sciences and the Integrated Brain and Behavior Research Center, University of Haifa

Background: The interplay between experiences during critical developmental periods and later adult life is crucial in shaping individual variability in stress coping strategies. We evaluated the impact of early adolescent and adult stress exposure on behavioral abnormalities across cognitive and emotional domains. We further asked whether these abnormalities are affected by pharmacological treatment with an endocannabinoid enhancer and are correlated with alterations in endocannabinoid markers in the medial prefrontal cortex (mPFC).

Method: Rats were exposed to daily footshock sessions (adolescence stress) at postnatal days (P)31-40. At P46-56 rats were injected with vehicle or treated with URB597, which increases anandamide levels**.** At P78-80, rats were exposed to one hour restraint stress (adulthood stress) followed by a battery of behavioral tests.

Results: Behavioral profiling revealed that only a subset of the rats that were exposed to adolescence or adulthood stress developed long-lasting behavioral emotional and cognitive symptoms. Moreover, the proportion of affected rats was enhanced by exposure to both stressors. Some of these changes were associated with alterations in cannabinoid type 2 receptors in the mPFC. Treatment with URB597 after adolescence stress did not attenuate behavioral.

Conclusion: Taken together, our findings suggest that the emotional and cognitive phenotype of adult males is shaped by both early and later life experiences in a non-additive way.

INTRAVESICAL BETA-CARYOPHYLLENE AMELIORATES LPS-INDUCED BLADDER INFLAMMATION AND PAIN IN A MURINE MODEL OF ACUTE CYSTITIS

[1]Geraint Berger, [2] Juan Zhou, [1,2,3] Melanie Kelly and [1,2] Christian Lehmann

[1]Department of Pharmacology, Dalhousie University, Halifax, NS, Canada
[2]Department of Anesthesia, Pain Management and Perioperative Medicine, Dalhousie University, Halifax, NS, Canada
[3]Department of Ophthalmology and Visual Sciences, Dalhousie University, Halifax, NS, Canada

Introduction

Interstitial cystitis (IC) is a chronic pain disorder of the urinary bladder causing symptoms of pelvic pain, nocturia, and dysuria with no curative treatment. Medical management is therefore directed toward pain and symptom management, however, many patients remain dissatisfied with the management of their condition. Experimental evidence has demonstrated the involvement of cannabinoid receptors in bladder inflammation and function. Assessing the anti-inflammatory and analgesic effects derived from local cannabinoid receptor 2 (CB_2R) activation in the urinary bladder is the overarching aim of this study.

Methods

Lipopolysaccharide was instilled into the urinary bladder to induce bladder inflammation. Intravital microscopy (IVM) and Luminex immunoassays were used to assess the anti-inflammatory effects of Beta-Caryophyllene (BCP) by quantifying leukocyte recruitment in the bladder microcirculation, and the levels of pro-inflammatory cytokines in the bladder tissue, respectively. Von Frey Aesthesiometry, and non-stimulus evoked animal behavior analysis were also used to assess the potential analgesic effects conferred by local BCP treatment. Data were analyzed by one-way, and two-way repeated measure's ANOVA, with statistical significance considered at $p<0.05$.

Results

IVM and Luminex data showed that local CB_2R-targeted treatment reduced leukocyte adhesion in the bladder microcirculation and inhibited the production of numerous proinflammatory mediators in the bladder, respectively. CB_2R deficient animals were also shown to exhibit an exacerbated inflammatory phenotype. Intravesical instillation of BCP significantly restored the suprapubic withdrawal threshold to baseline levels, suggesting an analgesic effect consistent with CB_2R activation.

Conclusion

This study shows that local treatment with a phyto-derived sesquiterpene (BCP) in the urinary bladder produces anti-inflammatory and analgesic effects, consistent with the well-established and reported effects of CB_2R activation. Pharmacological activation of the CB_2R could underscore a novel treatment strategy for reducing IC-induced pain and inflammation.

ACEA (CB_1) AND CP55,940 (MIXED CB_1/CB_2) SEX-SPECIFIC ANTINOCICEPTIVE TOLERANCE IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Henry Blanton*[1], Melissa McHann[1], Kelsey Donckels[1], Seth Brauman[1], Isabel Castro[2], Kevin Pruitt[2] and Josée Guindon[1]

[1]Department of Pharmacology and Neuroscience,
[2]Department of Immunology and Molecular Microbiology,
Texas Tech University Health Sciences Center, Lubbock, TX, 79430.

Introduction: Recent studies have found sex-differences in the response to cannabinoid compounds. Our group has recently demonstrated sex differences in chronic pain models. Chemotherapy-induced pain sensitivity (CIPS) is a clinical challenge for cancer patients. The development of novel targeted therapies with long term efficacy in alleviating CIPS is an ongoing focus of preclinical research. The endocannabinoid system has shown promise as a target for alleviation of CIPS in both preclinical and clinical studies. However, a complete understanding of the mechanisms underlying tolerance to cannabinoid compounds remains elusive as does the contribution of sex hormones to this process.

Methods: The objective of our current work is to assess in wild-type female and male mice whether ACEA (CB_1 receptor agonist) and CP55,940 (mixed CB_1/CB_2 receptor agonist) demonstrate sex-specific antinociceptive tolerance in a cisplatin (5 mg/kg/week) CIPS model, and assess the contribution of sex-hormones in mediating changes in expression of cannabinoid receptors and inflammatory factors. Data were analyzed using analysis of variance (ANOVA) for repeated measures or one-way ANOVA as appropriate followed by Bonferroni post hoc tests using SPSS statistical software (version 21.0).

Results: Our study found no sex differences in cisplatin-induced mechanical (digital electro von Frey) and cold (acetone 0.1 ml) allodynia in wild-type male and female mice receiving vehicle. Antinociceptive tolerance to ACEA (0.5 mg/kg) developed after 5 days of chronic administration for females and after 9 days for males for both mechanical and cold allodynia, respectively. CP55,940 (0.3 mg/kg i.p.) antinociceptive tolerance developed after 8 days for females and 11 days for males for mechanical allodynia and after 10 days for females and 15 days for males for cold allodynia. Chronic administration of ACEA and CP55,940 in females resulted in disturbance of the estrous cycle resulting in a sustained metestrus phase quantified for 27 consecutive days. Moreover, ACEA and CP55,940 chronic administration relative to vehicle treated groups correlated with a decrease in estradiol plasma levels in females and a decrease in testosterone plasma levels in males. In females, ACEA and CP 55,940 resulted in an increased ovarian expression of mRNA for the pro-inflammatory cytokines IL-6 and IL-1β. In males, ACEA increased CB_1, but decreased CB_2 and IL-6 mRNA levels in the testes. CP55,940 decreased aromatase mRNA levels in the testes.

Conclusions: These results illustrate the complexity of changes induced by sex-hormones and the influence of these hormones on cannabinoid receptors and inflammatory markers following chronic administration of cannabinoid compounds. Future studies are needed to better understand the role of sex-hormones in the development of chemotherapy-induced pain sensitivity (CIPS), response to cannabinoids, and the impact of hormonal changes on inflammatory and pain markers in different tissues.

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THE EFFECTS OF CANNABINOIDS IN THE PREVENTION OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

Erik J. Fleischel* and Sara Jane Ward

Center for Substance Abuse Research, Temple University, Philadelphia, PA, USA

Introduction: Treatment with conventional chemotherapeutic agents such as paclitaxel results in chemotherapy-induced neuropathic pain (CINP) in approximately 1/3 of patients. In some cases, these symptoms may be so excruciating that chemotherapeutic treatment must cease until pain is reduced. There is no drug that can safely and effectively attenuate symptoms of CINP. Humans have used Cannabis for several millennia, and while clinical and preclinical data is currently limited, an overwhelming body of anecdotal evidence suggests that it may be a promising candidate for pain relief. The psychoactive properties of Δ^9-Tetrahydrocannabinol (THC), a component of Cannabis, has created concern for the safety of its use as a pain-reliever. However, Cannabis has been found to contain several other molecules that are believed to reduce painful symptoms while having no psychoactive effects. Among these molecules are β-Caryophyllene (BCP) and cannabidiol (CBD). These compounds have shown promising results in other preclinical models of pain and their effectiveness was tested in our model of CINP.

Methods: Mimicking chemotherapy regimens, C57Bl/6 mice were treated with paclitaxel (8.0 $mg \bullet kg^{-1}$, i.p., days 1, 3, 5, and 7). To determine the efficacy of cannabinoid treatment, mice were given BCP, CBD, or a combination of BCP and CBD (p.o., days 1, 3, 5, and 7) 15 minutes prior to paclitaxel injection. Mechanical allodynia was assessed on days 0 and 14 using Von Frey Filaments to evaluate the development of peripheral neuropathy. These data were transformed into a percent maximum possible effect (%MPE) for each treatment group. On days 14 and 15, mice were euthanized and the L1-L4 regions of the spinal cord were collected and then stained for Iba-1 and DAPI. The number of doubly-stained cells in the dorsal horn of the spinal cord were counted and their features were measured using ImageJ. Allodynia data was analyzed by one-way ANOVA followed by Dunnett post-hoc tests ($p < 0.05$ was considered significant).

Results: Paclitaxel treatment consistently caused strong mechanical sensitivity throughout the course of the experiment. Several doses of the cannabinoids tested, either alone or in combination, prevented the onset of mechanical sensitivity. Microglia of paclitaxel-treated animals were larger than those of control groups and were irregularly shaped, while microglia of the cannabinoid-treated and vehicle groups were smaller and had a morphology characteristic of homeostatic microglia.

Conclusions: Our results suggest that BCP, CBD, and a combination of BCP and CBD prevent the onset of chemotherapy-induced neuropathic pain in a dose-dependent manner. These treatments may affect the central sensitization of pain through glial cell dependent mechanisms. Future experiments will be conducted to elucidate these molecular mechanisms and explore a wider range of doses and cannabinoids.

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TARGETING MONOACYLGLYCEROL LIPASE TO REDUCE CHRONIC PAIN IN A HUMANIZED MOUSE MODEL OF SCD

Kennedy Goldsborough*[1], Rebecca Moncayo[1], Lauren Moncayo[1], Lesley O'Brien[1], Kalpna Gupta[2], M. Imad Damaj[1], Wally Smith[3], Joyce Lloyd[4] and Aron Lichtman[1]

[1] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA
[2] Division of Hematology/Oncology, University of California - Irvine, Irvine, CA
[3] Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA
[4] Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA

Introduction: Sickle Cell Disease (SCD) is a major cause of morbidity and mortality worldwide, and affects more than 100,000 people in the US. Acute, vaso-occlusive pain crises are the hallmark of the disease and the primary cause of hospitalization. Recurrent, acute pain episodes compound and result in chronic pain for many patients, though the mechanisms of this transition are poorly understood. Although opioids remain the standard of care to treat SCD chronic pain, their myriad adverse side effects (e.g., constipation, respiratory depression, abuse liability, dependence) as well as the fact that SCD chronic pain requires prolonged opioid treatment that results in tolerance, severely limit their therapeutic utility. Thus, a pressing need exists to identify effective non-opioid analgesic strategies to reduce SCD chronic pain. Humanized mouse models of SCD, such as the Berkeley (BERK) model, provide a useful tool to investigate disease pathophysiology and evaluate novel therapeutic strategies. Inhibitors of the major degradative enzyme of 2-arachidonoylglycerol, monoacylglycerol lipase (MAGL), reduce nociceptive behavior in neuropathic and inflammatory preclinical models of pain through cannabinoid receptor-dependent and -independent mechanisms. MAGL inhibitors have yet to be tested in BERK mice and we hypothesize that they will ameliorate pain-stimulated and pain-depressed behavior. Here we evaluate a series of pain-stimulated and pain-depressed behaviors in BERK mice.

Methods: Male and female (2-6 months of age) BERK mice were used as subjects for these experiments. Pain-stimulated behaviors were assessed using the Von Frey and Hot Plate tests, while pain-depressed behaviors were assessed using the Grip Strength and Nesting tests. Anxiety-like behaviors were assessed using the Light-Dark (L/D) Box test. Cannabimimetic effects of MJN-110 were evaluated in the tetrad (antinociception, hypothermia, catalepsy, locomotion) assay. MJN-110 (1 and 5 mg/kg, i.p.) was given 30 minutes (for acute time-course) or two hours (for repeated daily injections) prior to testing. Data were analyzed as Student's t-test, One- and Two-way ANOVAs followed by Tukey or Sidak when appropriate ($p < 0.05$ considered significant).

Results: BERK mice displayed profound mechanical allodynia and thermal hyperalgesia, but not diminished grip strength at 2-3 months of age. BERK mice also demonstrated a reduction in time spent in the illuminated chamber of the L/D Box and diminished nesting behavior. MJN-110 (5 mg/kg) significantly reduced mechanical allodynia at 1 and 2 hours post-injection. Importantly, BERK mice given seven days of daily injections of MJN-110 (5 mg/kg) displayed sustained antinociception that did not undergo tolerance. MJN-110 produced partial effects in the tetrad assay, suggesting minimal cannabimimetic effects.

Conclusion: These initial findings validate that BERK mice show hypersensitive responses to mechanical and heat stimuli. A novel finding is that BERK mice display profound deficits in nesting behavior, suggesting functional behavioral deficits, as well as anxiogenic-like behavior as assessed in the L/D box. The data suggest that MAGL inhibition represents a viable strategy to reduce chronic pain behaviors in the BERK mouse model. Ongoing studies are examining whether MJN110 ameliorates functional alterations of BERK mice in nesting and the L/D box.

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IMPACT OF VAPOURIZED CANNABIS CONSTITUENTS ON OSCILLATORY ACTIVITY IN A NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA

Bryan W. Jenkins*, Shoshana Buckhalter, Melissa L. Perreault and Jibran Y. Khokhar

Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada

Background: Cannabis use is highly prevalent in patients with schizophrenia and worsens the clinical course of the disorder. To understand the causal impacts of cannabis with differing constituent proportions on schizophrenia-related disruptions, we investigated the impact of cannabis vapour containing either delta-9-tetrahydrocannabinol (THC) or both THC and cannabidiol (CBD) on corticolimbic oscillations in the neonatal ventral hippocampal lesion (NVHL) rat model of schizophrenia and controls.

Methods: Male Sprague Dawley rats underwent NVHL or sham-lesion surgeries on post-natal day 7. In adulthood, electrodes were implanted targeting the cingulate cortex (Cg), the prefrontal cortex (PFC), the dorsal hippocampus (dHIP), and the nucleus accumbens (NAc). Recordings of local field potentials (LFPs) were captured before and after rats were exposed to two strains of vapourized cannabis (~10% THC = "THC-only"; ~10% THC:CBD = "THC+CBD") in a cross-over design with a two-week wash-out period between exposures.

Results: Compared to controls, NVHL rats had reduced baseline gamma power in the Cg, dHIP, and NAc, and reduced high-gamma coherence between the dHIP-Cg. THC-only vapour broadly suppressed oscillatory power and coherence. THC+CBD vapour appeared to ameliorate the THC-induced impacts on power and coherence in both sham and NVHL rats. For NVHL rats, THC-only vapour also normalized the baseline dHIP-Cg high-gamma coherence deficits. NHVL rats also demonstrated a 20ms delay in dHIP theta to high-gamma phase-coupling, which was ameliorated by both exposures in the PFC and NAc.

Conclusion: Cannabis vapour without CBD suppressed oscillatory activity in NVHL and sham rats, while vapour with balanced THC:CBD ameliorated some of the THC-induced effects.

HEMP OIL AND PALMITOYLETHANOLAMIDE EXERT SYNERGISTIC ANTI-NOCICEPTIVE EFFECTS IN MOUSE MODELS OF ACUTE AND CHRONIC PAIN

Alex Mabou Tagne*[1], Yannick Fotio[1], Lin Lin[1], Erica Squire[1], Faizy Ahmed[1], Tarif Ibne Rashid[1], Elnaz Karimian Azari[2] and Daniele Piomelli[1]

[1]Department of Anatomy and Neurobiology, University of California, Irvine, CA, USA
[2]Metagenics, Aliso Viejo, CA, USA

Introduction: The use of products derived from hemp as self-medication for pain and other medical conditions is gaining in popularity, but evidence for their effectiveness remains very limited. In the present study, we set out to assess the efficacy of a full-spectrum hemp oil extract (HOE), alone or in combination with the anti-inflammatory and analgesic agent palmitoylethanolamide (PEA), in mouse models of acute and chronic pain.

Methods: We conducted the formalin and chronic constriction injury (CCI) tests in male CD-1 mice. HOE alone or in combination with PEA was given by the oral route. Formalin-induced acute nociceptive behaviors were recorded by a video camera for 60 min and quantified by researchers blinded to treatments. Mechanical allodynia and heat hyperalgesia were assessed using a dynamic plantar aesthesiometer and Hargreaves apparatus, respectively. Paw edema was monitored using a digital caliper. Transcription of interleukin (IL)-1β, IL-6, IL-10 and TNF-α was measured in spinal cord tissue by RT qPCR. CBD, CBDA, PEA, and the endocannabinoids AEA and 2-AG were extracted from plasma and lumbar spinal cords and their levels were quantified by LC/MS-MS.

Results: HOE alone reduced formalin-induced nocifensive behavior (phase II) by up to 27% ($P < 0.05$ vs. vehicle). Likewise, mechanical allodynia and heat hyperalgesia were attenuated in the injected paws by up to 27% ($P < 0.01$) and 41% ($P < 0.001$), respectively. HOE reduced paw edema by 63% at 50 mg-kg^{-1} ($P < 0.01$) but had no such effect at 10 and 100 mg-kg^{-1}. HOE (100 mg-kg^{-1}) had no effect on CCI-induced heat hyperalgesia or mechanical allodynia in the operated limbs, whereas a 7-day treatment with the extract decreased heat hyperalgesia by 33% ($P < 0.01$). On the other hand, the combination of sub-effective doses of HOE and PEA reduced phase II by 32% ($P < 0.01$). Likewise, formalin-evoked mechanical allodynia and heat hyperalgesia were attenuated by 49% ($P < 0.0001$) and 54% ($P < 0.0001$), respectively. CCI-induced mechanical allodynia and heat hyperalgesia were alleviated in the operated limbs by as much as 85% ($P < 0.0001$) and 118% ($P < 0.0001$), respectively. There was no effect on formalin-evoked paw edema. IL-6 and IL-10 mRNAs increased at least twofold in lumbar spinal cord tissue after CCI surgery, an effect that was attenuated to the same extent by HOE alone or by the HOE/PEA combination. Co-administration of HOE enhances and prolongs systemic exposure to PEA. By contrast, HOE alone or in combination with PEA did not affect plasma or spinal cord levels of AEA and 2-AG.

Conclusions: HOE exerts modest antinociceptive effects when administered alone, whereas the extract synergizes with PEA to produce a substantial greater-than-additive alleviation of pain-related behaviors. This synergism may result from the ability of HOE to prolong the lifetime of PEA in circulation.

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TARGETING THE ENDOCANNABINOID SYSTEM AS A STRATEGY TO ADDRESS AROMATASE INHIBITION-INDUCED SELECTIVE DISRUPTION OF LEARNING AND MEMORY IN MICE

Lesley D O'Brien*[1], Delaney Place[1], Kimberly Karin[1], David A Gewirtz[1], M Imad Damaj[1] and Aron H Lichtman[1]

[1]Pharmacology and Toxicology Department, Virginia Commonwealth University

Introduction: Breast cancer is the most common cancer in women, with two thirds of cases being estrogen dependent. Inhibition of aromatase, which leads to a reduction of estradiol production in breast and other tissues is used as a second line treatment to prevent breast cancer recurrence. Because aromatase inhibition (AI) is effective in reducing breast cancer recurrence, patients diagnosed with estrogen dependent breast cancer may be prescribed AIs for years or decades. However, serious side effects of AI include learning and memory deficits. In this study, we developed a mouse model of AI-induced memory deficits using single trial learning and memory tasks. Given the importance of the endocannabinoid ligands 2-arachidonoylglycerol (2-AG) and anandamide (AEA) in the regulation of learning and memory, experiments are underway to test whether inhibition of their respective degradative enzymes (monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH)) will prevent AI-induced learning and memory deficit.

Methods: Female ovariectomized (OVX) mice were given the AI Letrozole (0.5 mg/kg, p.o.) once daily for a week, and then administered this treatment on alternating days for the remainder of the experiment. In the first experiment, mice were evaluated in the object location (OL) task following a single acute dose of Letrozole. In the second experiment, subjects were administered Letrozole for two and four weeks and were evaluated in both the OL and object recognition (OR) tasks. A separate cohort of OVX mice were given the same Letrozole regimen for two weeks, followed by a two week washout period in their home cages, and tested in OL and OR tasks before and after washout. Hippocampal and prefrontal cortex tissue was collected and flash frozen in liquid nitrogen from mice receiving letrozole for 4 weeks for analysis by LC-MS-MS.

Results: Single acute dose of Letrozole had no impact on OL performance, whereas two or four weeks weeks of Letrozole administration significantly impaired OL, but not OR, performance. The Letrozole-induced impairment of OL performance spontaneously resolved after two weeks of drug washout.

Conclusions: The present data suggest that while AI has no immediate acute effects on hippocampal-dependent learning and memory, it selectively impairs performance when given repeatedly in a task specific manner, which spontaneously resolves upon cessation of treatment. Ongoing studies are evaluating whether Letrozole alters endocannabinoid tone (i.e., 2-AG, AEA and related lipids in hippocampus and prefrontal cortex), as well as if the MAGL inhibitor MJN110 will prevent AI-induced hippocampal-dependent learning and memory task deficits.

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INCREASING ANANDAMIDE LEVELS RESTORE DEPRESSION-LIKE PHENOTYPE AND ALTERATIONS IN MICRO-RNAs IN RATS EXPOSED TO EARLY LIFE STRESS

Anna Portugalov*, Hiba Zaidan, Inna Gaisler-Salomon and Irit Akirav

School of Psychological Sciences and the Integrated Brain and Behavior Research Center, University of Haifa, Haifa 3498838, Israel

Introduction: Early life adverse experiences significantly increase predisposition to psychopathologies, including depression. Here we compared the effect of treatment with the fatty acid amide hydrolase (FAAH) inhibitor, URB597 that increases anandamide levels and the selective serotonin reuptake inhibitor (SSRI), paroxetine, on depressive-like behavior and the expression of microRNAs (miRNAs) in the prefrontal cortex (PFC) of rats exposed to early life stress (ELS).

Methods: Male and female rats were exposed to the "neglectful mother" paradigm from postnatal day (P)7 to P14. During P45 to P60 (late-adolescence) URB597 (0.4 mg/kg) or paroxetine (5mg/kg), were administered i.p. for 2 weeks. On P90 (adulthood) rats were tested for depression-like behavior and microRNA's expression.

Results: Males and females demonstrated at adulthood depression-like behavior, such as decreased social behavior and increased immobility in the forced swim test. Chronic treatment with URB597 during post-adolescence, but not with SSRI, restored these behaviors. In the medial PFC, ELS males demonstrated a decrease in miR-16 and ELS females demonstrated a decrease in miR-135a. Importantly, URB597, that restored depression-like behavior in both sexes, also normalized PFC miR-16 and miR-135a expression. The ELS-mediated decrease in the expression of miR-16 was correlated with reduced social behavior in males.

Conclusions: Our findings show for the first time that enhancing anandamide signaling can prevent ELS-induced decrease in PFC miRNAs and associated depression-like phenotype in both sexes. This may advance our knowledge on pathways dysfunctional in depression in cortical areas and suggest a mechanism for the beneficial effects of enhancing the endocannabinoid system.

THE INVOLVEMENT OF B-CATENIN IN THE MEDIAL PFC IN PREVENTING STRESS-INDUCED EFFECTS IN A RAT MODEL FOR PTSD

Sbarski B*[1], Parise E[2], Nestler E[2], Akirav I[1]

[1] School of Psychological Sciences, University of Haifa, Haifa 3498838, Israel
[2] Nash Family Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA.

Introduction: Emerging evidence suggests that increasing anandamide levels may ameliorate anxiety and depression, although the underlying mechanisms are unknown. We aimed to determine whether the fatty acid amide hydrolase (FAAH) inhibitor URB597 prevents the effects of shock and reminders on alterations in corticotrophin releasing factor (CRF) and CRF type 1 receptor (CRFr1) via a mechanism involving the Wnt/β-catenin pathway in the medial PFC (mPFC).

Methods: Rats were exposed to shock and reminders and injected with URB597 (0.3 mg/kg, i.p.). CRF, CRFr1 and β-catenin protein levels were examined 3 weeks after the shock. In a following experiment, rats were taken to stereotaxic surgery in which β-catenin was down-regulated in the mPFC by viral-mediated gene transfer.

Results: Exposure to shock and reminders induced a depression- and anxiety-like phenotype, upregulation of CRF and CRFr1 and downregulation of β-catenin protein levels in the mPFC. URB597 restored the behavioral phenotype and the alterations in CRF, CRFr1 and β-catenin. Importantly, URB597 did not restore the behavioral phenotype or the alterations In CRF and CRFr1 levels following downregulation of mPFC β-catenin in rats exposed to shock and reminders.

Conclusions: The findings further support a potential therapeutic role for FAAH inhibition in stress-induced depression and anxiety and suggest that the effects of URB597 on behavior are associated with β-catenin function in the PFC.

EVALUATION OF THE TERPENES β-CARYOPHYLLENE, α-TERPINEOL, AND γ-TERPINENE IN THE MOUSE CHRONIC CONSTRICTION INJURY MODEL OF NEUROPATHIC PAIN: POSSIBLE CANNABINOID RECEPTOR INVOLVEMENT

Joshua A. Bilbrey, Yuma T. Ortiz, Lance R. McMahon and Jenny L. Wilkerson*

Department of Pharmacodynamics, College of Pharmacy
University of Florida, Gainesville, FL, USA

Introduction: Pain is one of the most common reasons to seek medical attention and chronic pain is a worldwide epidemic. Anecdotal reports suggest cannabis may be an effective analgesic. As cannabis contains the terpenes α-terpineol, β-caryophyllene, and γ-terpinene we hypothesized these terpenes would produce analgesia in a mouse model of neuropathic pain.

Methods: We used the chronic constriction injury of the sciatic nerve mouse model, which produces mechanical allodynia, assessed via the von Frey assay, as well as thermal hyperalgesia assessed via the hotplate assay. Compounds were further assessed in tests of locomotor activity, hypothermia, and acute antinociception.

Results: Each terpene produced dose-related reversal of mechanical allodynia and thermal hyperalgesia. Thermal hyperalgesia displayed higher sensitivity to the effects of each terpene than mechanical allodynia and the rank order potency of the terpenes was α-terpineol > β-caryophyllene > γ-terpinene. To examine the involvement of cannabinoid receptors, further tests were conducted in mice lacking either functional cannabinoid type 1 receptors (CB_1R (-/-)) or cannabinoid type 2 receptors (CB_2R (-/-)). Compared to wild type mice, CB_1R (-/-) mice treated with α-terpineol displayed a 2.91-fold decrease in potency to reverse mechanical allodynia; in CB_2R (-/-) mice, the potency of α-terpineol was decreased 11.73-fold. The potency of β-caryophyllene to reverse mechanical allodynia decreased 1.80-fold in CB_2R (-/-) mice. Each terpene produced a subset of effects in tests of locomotor activity, hypothermia, and acute antinociception.

Conclusions: These findings suggest α-terpineol, β-caryophyllene, and γ-terpinene may have differential cannabinoid receptor activity and a pharmacological profile that may yield new efficacious analgesics.

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ABSTRACT WITHDRAWN

INVESTIGATING THE RELATIONSHIP BETWEEN AFFECTIVE STATE, STRESS, SOMATOSENSORY SENSITIVITY AND CIRCULATING ENDOCANNABINOIDS IN HEALTHY HUMAN PARTICIPANTS: A PILOT STUDY

*Stephanie Bourke[1,3,4], *Therese O'Connor[4,5], Nikita N. Burke[1,3,4], Mary Hopkins[1,3,4], Massieh Moayedi[6], Brian E. McGuire [2,3,4] and David P. Finn[1,3,4].

[1]Pharmacology and Therapeutics, School of Medicine, [2]School of Psychology, [3]Galway Neuroscience Centre and [4]Centre for Pain Research, National University of Ireland Galway, [5]Sligo University Hospital, [6]Centre for Multimodal Sensorimotor and Pain Research, Faculty of Dentistry, University of Toronto. *Joint first authors.

Introduction: The endocannabinoid system is involved in a multitude of physiological processes including pain processing, affective state, and the stress response. Stress and affective state have a complex, modulatory influence on pain (Corcoran *et al*. 2015, *International Review of Neurobiology*, 125, 203-255). The aim of this study was to investigate the relationship between affective state, acute psychological stress, somatosensory sensitivity (using quantitative sensory testing [QST]), and circulating endocannabinoid levels, in healthy human volunteers.

Methods: Fourteen healthy volunteers were recruited and consented to procedures approved by the NUI Galway Research Ethics Committee (7 female, 7 male; mean age ± SEM: 25.7 ± 2.6 years). Blood samples, heart rate (HR), blood pressure (BP) and state-anxiety scores were obtained at 3 time points throughout the study: baseline, post-stress and post-QST. Following baseline measurements, participants completed State-Trait Anxiety Inventory and Patient Health Questionnaire 9 (PHQ-9). Participants were then exposed to acute psychological stress using the Montreal Imaging Stress Task (MIST), followed by QST comprising a standardized battery of tests to determine thermal (cool, warm) detection and pain (cold, heat) thresholds, heat pain tolerance and conditioned pain modulation (CPM). In a second session, at least two weeks later, the same participants underwent an identical protocol, except for the use of a non-stressful control version of the MIST. Quantification of plasma 2-arachidonoylglycerol (2-AG), anandamide (AEA), *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), was carried out by LC-MS/MS. Parametric or non-parametric statistical analyses were performed as required, $P<0.05$ was considered significant.

Results: State anxiety scores were significantly higher following exposure to the MIST, compared to its control. Cold detection threshold (CDT) was significantly increased (i.e. greater sensitivity) following exposure to the MIST. CPM was significantly lower post-MIST compared to post-control. There were no significant differences between 2-AG, AEA, PEA and OEA concentrations at baseline and those post-MIST (or post-control) or post-QST. However, AEA, PEA and OEA concentrations correlated positively with cold pain threshold (CPT) following exposure to the MIST as well as following QST. AEA and OEA were positively correlated with mean arterial pressure (MAP) following exposure to the MIST. PHQ-9 was negatively correlated with CDT and positively correlated with warm detection threshold (WDT) post-MIST, but not post-control. Trait anxiety was negatively correlated with CDT post-MIST, but not post-Control.

Conclusions: Exposure to the MIST, compared to control, resulted in higher state anxiety scores and increased cool sensitivity, but not cold pain thresholds. Following exposure to the MIST, participants had less efficient CPM, indicating an increased sensitivity to pain during CPM. Correlations reveal a relationship between negative affect, stress, circulating endocannabinoids/*N*-acylethanolamines and somatosensory sensitivity.

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CLINICAL EVOLUTION OF TREATMENT OF PROGRESSIVE SUPRANUCLEAR PALSY PATIENT WITH MEDICAL CANNABIS IN A PERIOD OF TWELVE MONTHS

Ana Gabriela Hounie, Marco Agassiz Almeida Vasques and Wilson da Silva Lessa Júnior*

Private Office, São Paulo, Brazil (Hounie), Armed Force (Agassiz),
Federal University of Roraima (Lessa Jr)

Introduction: Progressive supranuclear palsy (PSP) is a severe, debilitating, and often fatal disease that has no effective treatment to date. Its clinical and pathological features resemble other neurodegenerative disorders, such as Alz-heimer's (AD) and Parkinson's (PD) diseases. The de-velopment of new therapies for neurodegenerative disorders has been the subject of intense research in recent years, with several experimental evidence suggesting the possible efficacy of cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC) [1]. We herein report the case of improvement in motor and language functions in a patient with PSP after the administration of medical cannabis.

Methods: This article is a case report of a 71-year-old woman presented to the medical office with a history of 6 years of progressive gait disturbance, parkinsonism, and a severe language disturbance. She had lost the capacity to produce intelligible speech except for moans and unintelligible noises (dysarthria) and displayed ophthalmoplegia. She was also unable to walk and had rigidity of limbs.Serum levels of folic acid, total calcium, creatinine, iron, ferritin, magnesium, phosphorus, and vitamin B12 were within normal ranges.A full extract of cannabis oil with the approximate ratios of the main cannabinoids of 0.5% CBD, 2% THC, 10% CBDA (cannabidiolic acid), and 7% THCA (tetrahydrocannabinolic acid) (approximately 11 mg/mL of CBD + CBDA and 9 mg/mL of THC + THCA) was prescribed, beginning with 2 sublingual drops at night and doubling the dose on a weekly basis. The oil was acquired by the patient's family from ABRACE (a non-profit organization founded by relatives of children with refractory epilepsy that benefited from cannabis oil and was conferred the right to grow, extract, and commercialize cannabis products in Brazil). There were no side effects attributable to the oil. After a 3-week period, an improvement in dysarthria was perceived. The patient started to articulate words, although still in an unintelligible manner, attempting to communicate. One week later, she improved word articulation and presented a slight return of gaze function. After 5 weeks of cannabis oil administration, her oral output was further improved, and the family and clinical staff were able to understand the words and the meaning of her speech. She also showed improvement in hand tremor and regained the ability to move her limbs. Finally, the patient was also able to stand on her feet and walk with assistance.

Results: The case presented herein is probably the first report of PSP improvement in scientific literature. PSP is now considered a spectrum of motor and behavioral syndromes associated with a specific four-repeat (4R) tau neuropathology at autopsy.

Conclusions: This new treatment might represent some hope to patients suffering from this fatal disease. Research of medical cannabis is still in its beginnings but should be encouraged, especially in refractory patients or those for whom there is no specific treatment.

VETERANS DEMOSTRATE IMPROVED CLINICAL STATE AND HEALTH FOLLOWING SIX WEEKS OF TREATMENT WITH A HIGH-CANNABIDIOL PRODUCT

M. Kathryn Dahlgren[1,2,3], Kelly A. Sagar[1,2,3], Ashley M. Lambros[1,2], Rosemary T. Smith[1,2], Celine El-Abboud[1,2] and Staci A. Gruber[1,2,3]

[1]Cognitive and Clinical Neuroimaging Core (CCNC), McLean Imaging Center, Belmont, MA
[2] Marijuana Investigations for Neuroscientific Discovery (MIND) Program, McLean Hospital Imaging Center, Belmont, MA
[3]Department of Psychiatry, Harvard Medical School, Boston, MA, USA

Introduction: Cannabis use remains popular among veterans, who often consider cannabis to be lower-risk or safer than other substances. Further veterans frequently report medical cannabis use to treat service-related conditions including PTSD, anxiety, sleep disturbances, pain, and depression. There is significant evidence that several cannabinoids have therapeutic properties; in particular, cannabidiol (CBD) has been associated with anti-inflammatory and anxiolytic effects. However, in order to evaluate the clinical efficacy of widely available cannabis products, clinical trials and studies designed to evaluate the specific impact of individual products are necessary.

Methods: As part of an ongoing, observational, longitudinal study, veterans interested in medical cannabis treatment are enrolled in a quasi-clinical trial where a hemp-derived, full-spectrum, high-CBD sublingual product is provided at no cost from the product manufacturer (Charlotte's Web, Original 50 mg/ml CBD) as part of a veterans support program. Patients complete a baseline study visit prior to initiating use of the high-CBD product. Once patients receive the study product, they complete clinical assessments every two weeks for six weeks of treatment. Patients are not required to be cannabis naïve at baseline, but in the current analyses, a majority of patients (64.71%) were cannabis naïve. All cannabis use including CW product use is tracked in drug diaries to calculate individual product use and overall cannabis exposure.

Results: Thus far, seventeen patients have completed six-weeks of treatment with significant improvements noted on several measures of clinical state and general health relative to baseline. Specifically patients demonstrate significantly reduced PTSD symptomatology (PTSD Checklist; Life Events Checklist; both *ps*=.002) and anxiety (Beck Anxiety Inventory; State Trait Anxiety Inventory; all *ps*≤.026), improved sleep (Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Fear of Sleep Inventory, Insomnia Severity Index; all *ps*≤.022), lower pain (Visual Analog Scale, Pain Distress Scale, Pain Disability Index; all *ps*≤.044), and improved depression and mood (Beck Depression Inventory, Profile of Mood States Total Mood Disturbance; all *ps*≤.007). Patients also report significant improvements in their general health on all subscales of the RAND Short Form Medical Outcomes Survey (all *ps*≤.049) and on almost all subscales of the World Health Organization Quality of Life Scale (all significant *ps*≤.037).

Conclusions: These preliminary data suggest that the addition of a high-CBD product to their daily regimen may be beneficial to veterans looking for relief for a broad variety of clinical symptoms. Larger sample sizes will allow for comparison between veterans already using cannabis at baseline versus those who were not; currently the data indicates clinical improvements in both groups over the course of treatment. Additionally, more standardized clinical trials will help determine the efficacy of this specific product as well as other cannabis products with varied cannabinoid profiles.

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CANNFLAVINS – FROM PLANT TO PATIENT: A SCOPING REVIEW

Simon Erridge*[1], Nagina Mangal[1], Barbara Pacchetti[2] and Mikael H Sodergren[1,2]

1. Department of Surgery and Cancer, Imperial College London, UK
2. Emmac Life Sciences, London, UK

Introduction: Cannflavins are a group of prenylflavinoids derived from *Cannabis sativa L*. plant. Cannflavin A (CFL-A), B (CFL-B) and C (CFL-C) have been heralded for their anti-inflammatory properties in pre-clinical evaluations. This scoping review aims to synthesise the evidence base on cannflavins to provide an overview of the current research landscape to inform research strategies to aid clinical translation.

Methods: A scoping review was conducted of EMBASE, MEDLINE, Pubmed, CENTRAL and Google Scholar databases up to 26th February 2020. All studies describing original research on cannflavins and their isomers were included for review.

Results: 25 full text articles were included. CFL-A and CFL-B demonstrated potent anti-inflammatory activity via inhibition of 12-o-tetradecanoylphorbol 13-acetate induced PGE2 release (CFL-A half maximal inhibitory concentration (IC50): 0.7μM; CFL-B IC50: 0.7μM) and microsomal prostaglandin E synthase-1 (CFL-A IC50: 1.8μM; CFL-B IC50: 3.7μM). Outcomes were also described in preclinical models of anti-oxidation (CFL-A), anti-parasitic activity (CFL-A, CFL-C), neuroprotection (CFL-A) and cancer (Isocannflavin B, a CFL-B isomer). In-silico screening identified that CFL-A has binding affinity with viral proteins that warrant further investigation.

Conclusions: Cannflavins demonstrate a number of promising therapeutic properties, most notably as an anti-inflammatory agent. Low yields of extraction however have previously limited research to small pre-clinical investigations. Identification of cannflavin-rich chemovars, novel extraction techniques and recent identification of a biosynthetic pathway will hopefully allow research to be scaled appropriately. In order to fully evaluate the therapeutic properties of cannflavins focused research now needs to be embedded within institutions with a track-record of clinical translation.

THE EFFECT OF CANNABIS-BASED MEDICINE IN THE TREATMENT OF CACHEXIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

Samuel Hammond[1], Simon Erridge*[1], Nagina Mangal[1], Barbara Pacchetti[2] and Mikael H Sodergren[1,2]

1. Department of Surgery and Cancer, Imperial College London, London, UK
2. Emmac Life Sciences, London, UK

Introduction: Cachexia is a hugely prevalent condition associated with underlying chronic disease. Wasting of skeletal muscle and adipose tissue loss in cachectic patients is associated with higher rates of disability, reduced quality of life and worse prognosis. There is large unmet need to develop strategies to treat cachexia as there are currently no standard guidelines in the management of cachexia. Modulation of the endocannabinoid system, via exogenous cannabinoids, has demonstrated potential in modulating appetite, reducing catabolism and has shown anti-inflammatory properties. Since no single pharmacological agent is currently recommended for use in cachexia, the potential of cannabinoids as an appetite stimulant warrants further research and assessment of current evidence. This review aims to evaluate the evidence for the efficacy of cannabis-based medicinal products, against placebo and other active treatments, in anorexia-cachexia syndrome in improving appetite, weight and quality of life.

Methods: A literature search of the Medline, EMBASE, CENTRAL and the Web of Science Core Collection, for papers published up to February 2020, was conducted. All randomised controlled trials comparing the use of cannabis-based medicine versus placebo/active treatments for patients with cachexia were screened. The quality of evidence in included studies was assessed using the GRADE framework and any risk of bias was judged using the Cochrane risk of bias tool.

Results: A total of 5 studies, encompassing 934 participants, were found to be eligible. The pooled group effect size for change in appetite was -1.79 (95% CI: -3.77 to 0.19) favouring the control group (p=0.08). Additionally, no significant difference for weight change or change in quality of life for cannabinoids versus placebo/other treatment was observed. The quality of evidence for all 5 studies was assessed to be low.

Conclusion: There is a lack of high-quality evidence to recommend the use of cannabinoids in the treatment of cachexia. Given the limited available pharmacological options for cachexia and the potential for cannabinoids to modulate appetite and alter the immune system, further research is needed before clinical recommendations on the pharmacological management of cachexia can be made.

CANNABIS USE AND PERCEPTIONS AMONG COLLEGE STUDENTS IN CALIFORNIA

Selina Espinoza*, Claudia Rocha, Melissa-Ann Lagunas,
Courtney Crouse and Jennifer L. Lovell

Department of Psychology, California State University, Monterey Bay, Seaside, CA, USA

Introduction: More than half of the United States has legal medicinal and/or recreational access to adult-use cannabis. A group affected by legalization is college students over the age of 21. The purpose of the study was to explore cannabis use and perceptions among college students living in California. The current project used a concurrent mixed-methods online survey design and was approved by the University Institutional Review Board.

Method: Of the 203 participants, the majority were female (61.1%, $n = 124$) and identified as Latinx/Hispanic (46.8%, $n = 95$). The anonymous self-report survey included demographic questions and scales measuring cannabis use disorder (The Cannabis Use Disorders Identification Test-Revised; Adamso et al., 2010), cannabis consumption (Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory; Cuttler & Spradlin, 2017), and well-being (The Multicultural Quality of Life Index; Mezzich et al., 2011). Participants answered open-ended questions about the positive, negative, and spiritual impact/s of cannabis on their life and health, and how the COVID-19 pandemic impacted their cannabis use.

Results: A paired-sample t-test was used to determine the percent relief college students experienced when using cannabis versus non-cannabis treatments or medications. College students significantly found more relief using cannabis as a treatment ($M = 5.23$, $SD = 3.30$) compared to non-cannabis treatments or medications ($M = 3.53$, $SD = 2.94$), $t(104) = 4.36$, $p < .001$ (two-tailed), $d = .15$. There was no significant correlation between average symptoms of Cannabis Use Disorder and Multicultural Quality of Life, $r(112) = -0.02$, $p = .84$. Researchers used inductive content analysis for qualitative data. Analysis revealed the most frequent emergent themes for therapeutic benefits of cannabis included *relaxation/stress reduction* (55%, $n = 81$) and *anxiety symptom/s reduction* (14%, $n = 21$). In contrast, the most common theme for negative impacts of cannabis use also included *anxiety/mental health issues* (25%, $n = 36$). Most participants reported no spiritual benefits (63%, $n = 83$); however, some participants discussed cannabis use improving their *self love* (14%, $n = 19$) and *self awareness* (12%, $n = 16$). When asked about the impact of COVID-19 on their use, most participants reported increased cannabis use (28%, $n = 42$), while some reported decreased cannabis use (13%, $n = 20$).

Conclusions: Results will be discussed in the context of how college students' lived experience with cannabis and scale scores could inform university professionals and the community about the therapeutic use of cannabis and how to support prevention efforts when warranted.

CANNABIDIOL USE MAY BE ASSOCIATED WITH IMPROVEMENTS IN ARTHRITIS SYMPTOMATOLOGY: AN EXPLORATORY CROSS-SECTIONAL ANALYSIS

Nicholas Frane*, Erik Stapleton, Cesar Iturriaga, Maximillian Ganz, Rupa Vijayan, Vijay Rasquinha and Robert Duarte

Zucker School of Medicine at Hofstra/Northwell, Department of Orthopaedic Surgery, Huntington, NY, USA

Introduction: The purpose of our study was to evaluate evaluating the patient-perceived effect that CBD use has on pain and use of other oral medications in patients with arthritis. We hypothesized that patients who used CBD would report improvements in pain with no difference between groups when compared by type of arthritis.

Methods: A novel anonymous questionnaire was created to evaluate patients' perceived efficacy of CBD use for arthritis pain. Patients were recruited through online methods including social media accounts and newsletters (The Arthritis Foundation and Savvy Cooperative). 709 patients initially participated in the survey. Participants were excluded if they didn't have arthritis (N=75), had not tried CBD (N=133), or had incomplete surveys (N=73).

Responses to use and pain questions were stratified by subtypes of arthritis. Pearson's chi-squared and Fisher's exact tests were used in the analysis of categorical variables. Pain and pain reduction responses were evaluated with non-parametric Kruskal Wallis Tests. Individual comparisons were evaluated with Mann-Whitney U tests. A two-tailed p-value less than 0.05 was considered statistically significant.

Results: The influence of CBD was mostly positive in effect on pain intensity (37.9% much better, 45.1% little better), physical function (28.7% much better, 37.4% little better), and sleep quality (37.6% much better and 28.5% little better). Subgroup analysis by diagnosis type (osteoarthritis, rheumatoid, or other autoimmune arthritis) found differences in among groups for physical function (P=0.013), favoring the osteoarthritis group.

The cohort reported a 44% reduction in pain after CBD use (2.58-point reduction). The osteoarthritis group had greater percentage reduction (P=0.020) and point reduction (P<0.001) in pain compared to rheumatoid arthritis and other autoimmune arthritis.

The majority of respondents reported a reduction or cessation of other medications after CBD use (N=259, 60.5%): reductions in Anti-inflammatories (N=129, 31.1%), Acetaminophen (N=78, 18.2%), Opioids (N=36, 8.6%) and discontinuation of Anti-inflammatories (N=76, 17.8%), Acetaminophen (N=76, 17.8%), and opioids (N=81, 18.9%).

Conclusions: In our convenience sample, participants reported high rates of symptomatic relief. Furthermore, patients using CBD reported reduction and discontinuation of opioids, Tylenol, and anti-inflammatories. The present study suggests that there may be therapeutic benefit to CBD use in patients with arthritis.

EVALUATION OF THE DANISH PILOT PROGRAM OF MEDICAL CANNABIS

Carsten Hjorthøj*, Christine Merrild Posselt and Merete Nordentoft

Copenhagen Research Center for Mental Health – CORE, Hellerup, Denmark 2900.

Introduction: Cannabinoids are increasingly legalized and requested worldwide. Evidence regarding their efficacy and safety, however, is limited. Denmark introduced a pilot program of medical cannabis in January 2018. Objective: We aimed to evaluate the efficacy, side-effects, and non-specific effects of cannabinoids for patients with different indications, compared to controls.

Methods: Register-based, propensity-score-matched cohort study. We included all people who redeemed at least one prescription for cannabinoids since January 2018 (cases) matched exactly on prescribed indication and propensity-score matched on a range of potential confounders to people who redeemed non-cannabinoid prescriptions for the same indications. Use of cannabinoids was divided into use of pure / nearly pure THC, combined THC+CBD, and pure / nearly pure CBD. A range of outcomes such as use of pain medication, other types of medication, use of primary and secondary healthcare, work ability, mortality, etc. were investigated.

Results: We included 4,597 cases and 4,597 matched controls. Among pain patients, users of THC used more opioids during follow-up than controls. Among patients with neuropathic pain, however, users of either CBD, THC, or combined CBD+THC used less gabapentin than controls. Users of all three classes of cannabinoids were also hospitalized fewer days than controls among patients with neuropathic pain but not among patients with other or unspecified pain disorders. Cannabinoids were generally safe and even displayed some non-specific positive effects among patients with neuropathic pain. Among patients with multiple sclerosis, users of pure CBD had a range of better outcomes than controls, but this was not observed for users of mixed CBD+THC, including Sativex. We did not identify any positive effects for patients with cancer-related indications. Very few people had redeemed prescriptions for cannabinoids for anxiety, as this was not part of the Danish pilot program, but there were strong indications of positive effects, although it was difficult to identify which classes of cannabinoids were most effective due to the limited sample size. No patients redeemed prescriptions for cannabinoids for sleep disorders, but across other disorders, use of cannabinoids was associated with an increased use of conventional sleep medication. The side effect profile appeared mild.

Conclusion: Although not a randomized study, we used propensity score matching to identify indications for which cannabinoids might be useful. In particular, we identified patients with neuropathic pain, but not necessarily other pain disorders, as people who might benefit from cannabinoids. Patients with multiple sclerosis and patients with anxiety may also benefit. Based on a large, naturalistic yet well-controlled sample of users of cannabinoids, we thus have highly important findings regarding the usefulness of cannabinoids for treating a range of different disorders.

THE CHEMOTYPE LANDSCAPE OF COMMERCIAL *CANNABIS* IN THE UNITED STATES

Christiana J. Smith, Daniela Vergara, Brian C. Keegan and Nick Jikomes*

Center for Research and Education Addressing Cannabis and Health, University of Colorado-Boulder; Department of Science & Innovation, Leafly Holdings, Inc.

Introduction: A majority of adult US citizens now have state-legal access to *Cannabis*. These products contain a multitude of chemical compounds but the chemotaxonomic diversity of commercial *Cannabis* is incompletely understood. Commercial *Cannabis* is also heavily marketed based on distinct effects purportedly linked to common industry categories and a large number of allegedly unique "strains." In this study, we performed the largest chemotaxonomic analysis of *Cannabis* to date and quantified the extent to which industry labels reliably map to distinct chemical phenotypes.

Methods: We mined a large dataset compiled by the technology company, Leafly. This dataset comprised nearly 100,000 samples of *Cannabis* tested for cannabinoid and terpene content measured by labs in six US states. It also included common industry labels associated with samples, plus measures of consumer popularity from website data provided by Leafly. Using various computational techniques, we performed a thorough chemotaxonomic analysis of the chemical data and identified the extent to which industry labels and consumer popularity metrics correlated with the underlying chemical composition of legal *Cannabis* products.

Results: Using a dataset more than an order of magnitude larger than any previous study, we replicated previous findings including segmentation of *Cannabis* into distinct chemotypes defined by THC:CBD ratios and identification of robust correlations among various terpenes. We extended past work by identifying at least three chemotypes of THC-dominant *Cannabis* with distinct terpene and cannabinoid profiles that consistently show up across the US. We show that, with certain exceptions, industry categories (Indica, Hybrid, Sativa) generally do not map to these distinctive profiles. We also show that "strain names" attached to samples display a broad range of consistency across *Cannabis* producers, with some more variable than others.

Conclusions: Multiple, distinct chemotypes of commercial *Cannabis* are reliably found across US states, each defined by specific cannabinoid and terpene ratios. Although quantifiably distinct chemotypes of commercial *Cannabis* are common across commercial markets, the industry labels used to segment products do not reliably map to these chemotypes. The chemotypes identified here may serve as a guide for human clinical research aimed at investigating whether distinct cannabinoid and terpene ratios provide distinguishable effects. These results may also have implications for the regulation of product marketing in the legal *Cannabis* industry.

LONELINESS AND CIRCULATING ENDOCANNABINOID CONCENTRATIONS IN OLDER BEREAVED INDIVIDUALS

Minhi N. Kang*, Luisa Bohorquez Montoya, Timothy McAuliffe, Garrett Sauber, Stacy A. Claesges, Cecilia J. Hillard and Joseph S. Goveas

Departments of Psychiatry, and Pharmacology and Toxicology, and the Neuroscience Research Center; Medical College of Wisconsin, WI, USA

Introduction: Loneliness, one of the most significant consequences of bereavement in the elderly, increases the risk of developing mental illness in acutely grieving individuals. We recently found that loneliness at baseline in acutely grieving elderly was associated with worsening depressive symptoms over time. Our previous work also found grievers to have elevated circulating concentrations of endocannabinoids (eCBs), relative to nonbereaved individuals. Here, we examined the relationship between loneliness and eCBs in acutely grieving older adults.

Methods: We studied 74 older adults (acute grief with high loneliness: n=24; acute grief with low loneliness: n=30; healthy controls [HC]: n=20). Grief participants were within 13-months following the death of a loved one. Loneliness was measured using the UCLA loneliness scale; depressive symptoms using the 17-item Hamilton Depression Rating Scale (HAM-D); grief symptom severity using the Inventory of Complicated Grief (ICG) scale. Circulating eCB concentrations (*N*-arachidonoylethanolamine [AEA] and 2-arachidonoylglycerol [2-AG] were quantified in serum samples using isotope dilution, liquid chromatography-mass spectrometry. Analysis of covariance (ANCOVA) was used to compare circulating eCB concentrations among the 3 groups, after adjusting for age and gender. Linear regression models were used to examine the relationships of loneliness with eCBs, and univariate general linear models were used to test for the main and interactive effects of loneliness and HAM-D, and loneliness and ICG, on eCB concentrations.

Results: ANCOVA that examined serum AEA concentration differences between the three groups was significant (F=3.104; **p=0.02; Figure**); post hoc comparisons revealed that relative to nonbereaved HCs, grievers who endorsed high levels of loneliness had higher serum AEA concentrations (**p=0.007**). Similar 2-AG concentrations were observed between the groups (p>0.05). Loneliness was positively associated with AEA in grievers, after controlling for age, gender, and days since the loss (r=0.26; **p=0.05**), but after including HAM-D and ICG in the model, findings were no longer significant (r=0.02; p=0.32). HAM-D was noted to have a main effect on AEA (F=6.918; **p=0.01**), but the main effect of loneliness or loneliness-depressive symptom interaction on AEA concentrations were not significant.

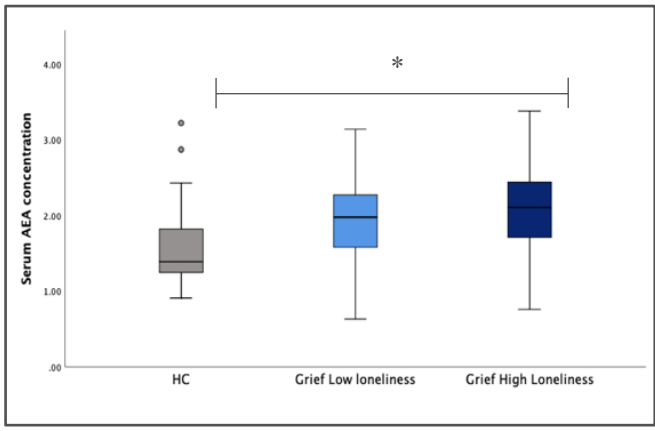

Figure. Serum AEA concentration differences between healthy control (HC), acute grief with low loneliness, and acute grief with high loneliness groups. Statistically significant difference between groups is indicated by * (p<0.01).

Conclusion: Grieving older adults who endorse intense loneliness were found to have higher serum AEA concentrations than nonbereaved healthy counterparts, but this relationship appears to be driven by baseline depression severity. Future longitudinal investigations should elucidate the role of circulating eCB concentrations in explaining the relationship of loneliness with depressive and grief symptom changes over time, and in the development of mental health complications such as major depressive disorder and/or prolonged grief disorder, in older bereaved individuals.

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OBSERVED IMPACT OF LONG-TERM CONSUMPTION OF ORAL CANNABIDIOL ON LIVER FUNCTION IN HEALTHY ADULTS

Robert Kaufmann*, Keith Aqua, Jeff Lombardo and Martin Lee

Springfield IL, Wellington FL, Buffalo NY, Los Angeles CA

Introduction: Previous studies have suggested cannabidiol (CBD) can cause elevations in liver function tests (LFT), specifically ALT, in humans. However, in these studies, most of the subjects were taking medications that have been known to cause LFT elevations, and the amount of CBD being consumed was considerably higher than ingested by most recreational CBD users. This study was undertaken to determine if the prevalence of elevated LFTs in a generally healthy adult population already taking CBD would be higher than that of the normal population and, if true, were there complicating factors identified in those subjects with elevated LFT.

Methods: Adults 18-75 years of age across the United States, known to be taking CBD orally for a minimum of 30 days, were recruited from consumers of 12 individual CBD product companies to participate in this decentralized, observational study. An app based, 21CFR Part 11 decentralized clinical study platform (ValidCare Study) was used to securely automate consent inclusion/exclusion criteria. Individuals selected were sent their standard CBD regimen (full-spectrum, broad spectrum, or CBD-isolate) from the company of their choice. Demographic information, medical history, reasons for taking, dosage, and current medications were collected via the app, along with daily journaling information on dosage, adverse effects, and efficacy. At the end of 30 days of journaling, LFT were obtained. Quantitative data were analyzed using the Wilcoxon rank-sum or signed-rank tests and qualitative data by the chi-square or binomial tests using an exact p-value calculation.

Results: 28,121 individuals were invited to participate in this study, 1475 were enrolled, and 839 (Female: 65.3%, Male: 34.7%) completed the study. The percent of individuals taking full spectrum hemp oil was 55.7%, CBD-isolate, 40.5% and broad spectrum 3.8%. The mean ± SD (median) daily dose of CBD was 53.9 $\pm$ 47.6 (38.4), overall, with full spectrum (47.5 $\pm$ 52.2 mg [31.1]) being significantly less than CBD-isolate (62.4 $\pm$ 40.9 mg [51.0]), $p<0.00001$ and broad spectrum (57.1 $\pm$ 25.2 mg [46.8]), $p= 0.0001$. The prevalences of elevated individual LFT's were: ALT 9.06%, AST 4.05%, ALP 2.03%, bilirubin 1.67%, with ALT and AST significantly higher than the general population value of 2.5% ($p\leq0.00001$ and 0.005, respectively). Of 76 individuals with elevated ALT, 65 were <2x ULN, 8 were between 1x and 2x ULN, and 3 individuals were >3x ULN. Only 1 individual had a mild elevated total bilirubin level (1.3). This individual's ALT was <2xULN and all other LFT were normal. 6 of the 8 individuals with ALT >1x and <2x ULN and all 3 with ALT >3x ULN had elevated AST. All individuals with ALT >3x ULN were on medications known to cause elevated LFT, 5 of the 8 with ALT >2x ULN group and 33 of 65 with ALT >1x ULN group were on similar medications. There was no significant difference in prevalence of elevated LFTs between companies nor was there any difference between CBD types.

Conclusions: This data shows that use of self-medication of CBD in a population of individuals is safe and although, it may be associated with increased LFT, it does not cause liver failure. Individuals taking CBD have a higher incidence of elevated liver enzymes than the general population, however, observed elevation by CBD use alone was not clinically significant (i.e., ALT>2x) and liver failure was not present in any individual. Any elevation of LFTs were most commonly associated with complicating factors of drug use and medical conditions known to potentially elevate LFTs.

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TOLERABILITY AND EFFICACY OF CANNABIDIOL ON MOTOR SYMPTOMS IN PARKINSON DISEASE: INTERIM REPORT ON TOLERABILITY

Maureen Leehey*, Ying Liu, Stefan Sillau, Sarah Fischer, Jost Klawitter, Cristina Sempio, Michelle Fullard, Trevor Hawkins, Lauren Seeberger, Emil Diguilio, David Vu, Sarah Baker, Tristan Seawalt, Grace Chin and Jacquelyn Bainbridge

Departments of Neurology, Clinical Pharmacy, Anesthesiology, and Regulatory Compliance, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Introduction: Cannabis use is frequent in PD, despite limited data regarding its effects. This study aimed to determine the tolerability of relatively high cannabidiol (CBD) and low 9-Δ-tetrahydrocannabinol (THC) in Parkinson disease (PD).

Methods: This randomized, double-blind, controlled, parallel study investigates a CBD cannabis extract (from National Institute of Drug Abuse) oral sesame oil solution with 100 mg/ml CBD and 3.33 mg/ml THC. Study drug started at 1.25 mg/kg/day, and increased to 2.5 mg/kg/day CBD (taken twice daily) for 10-14 days.

Results: Between 09/2018 and 04/2020, 51 persons enrolled, and 41 (66% men) took study drug: mean age 69.2 (SD 7.4), H&Y 2.6 (0.6). Mean final dose was 2.3 (0.4) mg/kg/day, i.e., 189 (55) mg CBD and 6.3 (1.8) mg THC, and participants were on study drug for 17.3 (7.0) days.

Unblinded study staff assigned participant data to two groups according to treatment randomization, which were matched in age, sex and H&Y. Group A (n=22), reported AEs 206 times, and 19 (86%) reported an AE: dizziness (56%); fatigue, feeling of relaxation, headache, decreased concentration, (36%); feeling abnormal, feeling drunk, nausea, somnolence (27%); confusion, (23%); dry mouth, fall, increased appetite (18%); agitation, disorientation, increased concentration, thinking abnormal, weakness (14%). Group B (n=19) reported AEs 93 times, and 17 (89%) reported an AE: headache (37%); somnolence (28%); feeling of relaxation (21%); cold, cough, diarrhea, fatigue, insomnia, nausea, (16%); anorexia, decreased concentration, depression, dizziness, elevated mood feeling drunk (11%). Most AEs were mild; in Group A they occurred more at 1.25 mg/kg/day, in Group B at 2.5 mg/kg/day. Two serious AEs occurred in Group A, none in Group B. No significant changes in physical exam, safety labs and EKG occurred. One participant withdrew due to intolerance.

Conclusion: Interim data suggests relatively high CBD/low THC (likely Group A) is tolerated in PD, with many mild AEs.

IMPLEMENTING CLINICAL TRIALS WITH CANNABIS PRODUCTS IN THE UNITED STATES: CHALLENGES AND LESSONS LEARNED

Heike Newman[1], Jacqueline L. Bainbridge[2] and Emily M. Lindley*[3]

University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
1. Office of Regulatory Compliance
2. School of Pharmacy, Department of Clinical Pharmacy
3. School of Medicine, Department of Orthopedics

Introduction: There is growing evidence for the efficacy of cannabis for the treatment of various health conditions. As such, there is a concomitant increase in the number of new investigators entering the realm of cannabis-based research. Implementing clinical trials of investigational cannabis products is a complex process, particularly for researchers at institutions without prior cannabis research experience. In addition to the arduous regulatory approvals required, there are various legal and infrastructural considerations that can be challenging to navigate. The goal of this presentation is to describe how we initiated a clinical trial of vaporized cannabis at the University of Colorado (PI Lindley) and to provide suggestions for avoiding obstacles when developing new cannabis research programs.

Methods: The timeline and obstacles faced for each of the regulatory approvals to begin the clinical trial was reviewed: FDA, local IRB, DEA, and NIDA. Further, the legal and infrastructural issues that were faced at the University level were summarized. The costs of infrastructure renovations were calculated.

Results: Overall, there was a delay of approximately 30 months from the time of funding award notice to implementation of the clinical trial. The study required human subjects to administer vaporized cannabis on the University campus, and thus renovations were needed to existing research exam rooms to add a high efficiency exhaust system. Further, the drug storage room in the Clinical and Translational Research Center needed renovations and additional equipment to meet DEA Schedule I security requirements. The total cost for these infrastructural renovations was approximately $55,000. These renovations, and University legal considerations took 18 months to complete. There were also delays in initiating the study due to DEA and NIDA approval processes (9 months) that can be avoided in the future. IRB and IND approvals were timelier in nature. Additional detailed descriptions of challenges and tips for researchers will be presented.

Conclusions: Researchers that will be administering cannabis products to human subjects should consult their University administration and legal counsel early in the proposal planning process to determine where the research can be conducted on campus, who can handle/dispense products, what renovations might be required, etc. Researchers should also know their local state regulations regarding pharmacy management of Schedule I products. While clinical research with cannabis products is possible, there are many regulatory and local state/University requirements that can be challenging, particularly for a new cannabis researcher.

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PLACEBO EFFECT SIZES IN US-REGISTERED CLINICAL TRIALS OF CANNABIS AND CANNABINOID-BASED INVESTIGATIONAL DRUGS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Mallory Loflin*[1], Karisa Jaime[2], Emily Wilhite[1,2], James Sottile[2], Jillian Carter[1], Anisha Choi[1], Shreya Sunkara[1] and Hannah Denton[1]

[1]University of California, San Diego School of Medicine, San Diego, CA
[2]US Department of Veterans Affairs, VA San Diego Healthcare System, San Diego, CA

Background: Estimating placebo response is a critical component of sample size calculation for randomized controlled trials (RCTs). However, placebo effect sizes tend to increase over time within drug classes (Montgomery, 1997; Robinson & Rickels, 2000). For example, the placebo effect size has more than doubled since the 1980s within antidepressant clinical trials (Walsh et al., 2002). This effect may be due to knowledge of the drug, reliance on subjective endpoints, and clinical target (affect), which are known predictors of placebo inflation. While there are limited cannabis/cannabinoid-based drugs prescribed in the US, "cannabis" is largely viewed as an effective treatment option for a range of conditions (including psychiatric) among the US general population. Cannabis RCTs might be at risk for inflated placebo response given this popular perception of medical cannabis' efficacy and current clinical trial targets. The aims of the current study were to estimate the effect size of placebo across all US-registered cannabis and cannabinoid RCTs and determine whether the placebo response for cannabinoids has increased over time.

Methods: Searches were conducted in November 2019 and February 2021 for the registration records and published results of all completed, double blind, placebo-controlled RCTs testing "cannabis", "cannabinoid(s)", "THC," or "CBD" registered on clinicaltrials.gov, the registration site required for all US-based trials. All registered studies that tested a cannabis or cannabinoid medication against placebo with an efficacy primary outcome were included. Standardized within- and between-subject effect sizes were calculated for placebo and treatment effects and examined against time and study size.

Results: Seventy-six RCTs registered between 2001 and 2019 met all search criteria and were included in effect size analysis. An additional seventeen trials completed in 2020 were identified that had not yet reported results. Of the initial 76 studies, 47% reported results on clinicaltrials.gov following study completion and 55% published findings in academic journals. Among studies registering results, within-subject effect sizes for placebo ranged between $d = .21$ and $d = 3.84$ (Mean $d = 1.01$, 95%CI = -.81 to 2.83; weighted $d = 1.12$). Year (midpoint) that the study was conducted was positively associated with size of placebo response ($F = 7.65$, $p = .01$, $b = .18$, 95%CI = .05 to .32).

Conclusions: There is substantial variability in placebo effect sizes in US-registered cannabis clinical trials, and average placebo effect sizes have modestly increased over time. The high percentage of studies failing to report findings suggest that effect size ranges reported here likely underestimate true placebo response. Funnel plots support this interpretation. These findings have important implications for researchers choosing sample sizes for future cannabis clinical trials, as placebo response is generally large and appears to be increasing.

TOBACCO CO-USE INFLUENCES TREATMENT OUTCOMES IN A PILOT TRIAL OF VARENICLINE FOR CANNABIS USE DISORDER

Erin Martin*[1], Nathaniel Baker[2], Brett Froeliger[3] and Aimee McRae-Clark[1,4,5]

1. Department of Neuroscience, Medical University of South Carolina, Charleston, SC
2. Department of Public Health Sciences, Medical University of South Carolina, Charleston SC
3. Department of Psychological Sciences, University of Missouri, Columbia, MO
4. Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC
5. Ralph H. Johnson VA Medical Center, Charleston, SC

Introduction Most people that use cannabis also use tobacco ("co-use"). Co-use is associated with increased severity of both nicotine and cannabis dependence, as well as worse outcomes when engaged in treatment. Thus, pharmacotherapeutic interventions that specifically target co-use may be of particular interest over the treatment of either substance alone. Varenicline, already an effective treatment for nicotine dependence, presents an attractive candidate medication.

Methods Participants (n=72) were randomized to receive either varenicline (goal dose 1 mg BID; n=13 smokers, 22 nonsmokers) or a placebo (n=19 smokers, 18 nonsmokers) for 6 weeks. Primary study outcomes were collected at baseline and weekly thereafter, and included both objective (urinary THC-COOH, cotinine) and subjective (negative affect, cannabis craving) measures. Daily cannabis and cigarette use data were obtained using Timeline Follow-Back. A response inhibition task was conducted at baseline and at end of treatment.

Results Cigarettes smoked per day was positively correlated with cannabis use sessions per day at baseline (rho=0.36, p=.04), but no differences were observed between smokers and nonsmokers in baseline measures of cannabis craving (48 (2.8) vs. 44.3 (2.5)) or negative affect (1.53 (0.31) vs. 1.30 (0.27)). During treatment, smokers receiving varenicline reported fewer cannabis use days on average than those receiving placebo (42% (11.3) vs. 76.4% (8.5)) and nonsmokers in either condition (Varenicline: 52.9% (10) vs. Placebo: 53.6% (11.7)), and cigarettes smoked per day was positively associated with cannabis use days (p=.001). Smokers receiving varenicline also reported a greater reduction from baseline in cannabis craving at end of treatment compared to the placebo (-28 (4.8) vs. -18.8 (4.8)) and compared to nonsmokers in either condition (Varenicline: -18.6 (4.4) vs. Placebo: -24.3 (5.2)). In contrast, nonsmokers receiving varenicline reported a greater reduction in negative affect from baseline compared to placebo (-1.04 (0.5) vs. -0.53 (0.61)) as well as smokers regardless of randomization (Varenicline: -0.66 (0.75) vs. Placebo: -0.50 (0.55)). Cigarette smokers performed worse overall on the response inhibition task (p=.07), and this was rescued by varenicline treatment. Urine results have not yet been analyzed.

Conclusion Preliminary results suggest varenicline may have differential effects in the treatment of cannabis use disorder as a function of tobacco use status.

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CANNABIDIOL EFFECTS ON ANANDAMIDE LEVELS IN INDIVIDUALS WITH COCAINE USE DISORDER; EXPLORATORY RESULTS FROM A RANDOMIZED CONTROLLED TRIAL

Violaine Mongeau-Pérusse*[1,2], Élie Rizkallah[1,2], Florence Morissette[1,2], Suzanne Brissette[2,3], Julie Bruneau[2,3], Simon Dubreucq[1,2], Guillaume Gazil[4] and Didier Jutras-Aswad[1,2]

[1]Department of Psychiatry and Addictology, University of Montréal, Canada, [2] Research Centre, Centre hospitalier de l'Université de Montréal, Canada, [3] Department of Family and Emergency Medicine, Université de Montréal, Canada [4]Unité de recherche clinique appliquée (URCA), Research Center, Centre hospitalier univerisaire Sainte-Justine.

Introduction: Administration of cannabidiol (CBD) has been shown to increase anandamide (AEA) signalling, possibly mediating some of its therapeutic properties. Whether it exerts such effect on AEA in individuals with cocaine use disorder (CUD) has not been studied. In this exploratory analysis of a clinical trial assessing CBD efficacy in decreasing cocaine craving and relapse, we aimed to evaluate CBD's effects on AEA plasma levels in individuals with CUD.

Methods: We conducted a phase II, double-blind, randomized, parallel groups, placebo-controlled trial of CBD as a treatment for CUD at the Centre hospitalier de l'Université de Montréal. After a 10-day inpatient detoxification period (phase I), participants were weekly followed for 12 weeks (phase II). We randomized 78 individuals with CUD into two study arms, receiving either a daily oral dose of 800 mg CBD (N=40) or placebo (N=38) starting on day 2, for a total of 92 days. We measured AEA levels at baseline, day 8 (phase I) and week 4 (phase II). We used a generalized estimating equation model to assess CBD's effects on AEA plasma levels.

R**esults:** We observed similar mean AEA levels in both treatment groups (p =0.357). At day 8, the mean AEA levels (standard deviation [SD]) were 0.26 (0.07) ng/ml in the CBD group and 0.29 (0.08) ng/ml in the placebo group. At week 4, they were 0.27 (0.09) ng/ml for participants treated with CBD and 0.30 (0.09) ng/ml for participants treated with placebo.

Conclusions: To our knowledge, this is the first study assessing AEA levels in individuals with CUD treated with CBD. Contrary to our hypothesis, CBD was not superior to placebo in increasing AEA levels in individuals with CUD.

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CANNABIS & YOUTH: ACUTE EFFECTS OF THC ON ADOLESCENTS

Conor H. Murray*, Sissi Huang and Harriet de Wit

Department of Psychiatry & Behavioral Neuroscience,
University of Chicago, Chicago, IL, USA

Introduction: Increased accessibility to cannabis and its primary psychoactive constituent THC has raised public health concerns surrounding risks associated with acute THC intoxication. One potential source of risk is age, specifically during the period of adolescence. Preclinical studies indicate that adolescent rats are more sensitive to the acute effects of THC than adults (Cha et al., 2006; Quinn et al., 2008; Wiley et al., 2007), apparently due to greater cannabinoid receptor expression in the brain. Specifically, cannabinoid receptors (CB1R) are more prevalent in prefrontal cortex (PFC) during adolescence than any other period of life (Gee et al., 2016; Meyer et al., 2018). This trajectory of expression is paralleled by CB1R function, with greater CB1 receptor-mediated signaling during adolescence, decreasing during adulthood (Heng et al., 2011). In humans, CB1R expression peaks at ages 15-17 and declines to base levels near age 35 (Choi et al., 2012; Long et al., 2012). These findings raise the possibility that human adolescents are more sensitive to effects of THC effects, which could lead to undesirable side effects such as cannabis-induced psychosis and higher risk for abuse.

Methods: Healthy volunteers aged 18-20 (adolescent; N=8) or 30-40 (adult; N=6) participated in 3 study sessions in which they received 0, 7.5, or 15 mg Marinol (dronabinol; delta-9-THC), at one-week intervals. To minimize the influence of tolerance, only participants who reported fewer than 20 total lifetime THC uses, and no use in the last 30 days were accepted. Subjective, behavioral, and physiological measures were obtained at regular intervals, including surveys (Profile of Mood States, Drug Effects Questionnaire, Addiction Research Center Inventory, 5-Dimensions of Altered States of Consciousness), cognitive tasks (time reproduction, Simple Reaction Time, Stop Task), and heart rate and blood pressure.

Results: The two groups did not statistically differ on demographic measures. Compared to adults, adolescents reported greater effects on the ARCI Marijuana scale after 7.5 mg THC, and exhibited greater increases in heart rate after both 7.5 and 15 mg THC. Adolescents also exhibited greater reductions in time reproduction than adults, and greater impairment on the Simple Reaction Time and Stop tasks. Finally, adolescents were also more sensitive than adults across several subscales of the 5-Dimensions of Altered States of Consciousness, including Disembodiment, Changed Meaning in Percepts, and Insightfulness.

Conclusion: These preliminary findings suggest that adolescents are more sensitive to the acute effects of THC relative to adults, on subjective, physiological and behavioral measures. It remains to be determined whether these differences in sensitivity to THC are associated with either increased risk for adverse effects of cannabis, or greater susceptibility to develop misuse or dependence.

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CANNABIS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE – CURRENT EVIDENCE AND PROSPECT FOR THE FUTURE

Timna Naftali

Gastroenterology, Head of IBD service, Meir Hospital, Kfar Saba, Israel

Introduction: Inflammatory bowel diseases (IBD) pose a significant personal and socioeconomic burden on patients due to their effects on quality of life, daily functioning and use of healthcare system. The overall response to treatment is limited to 40-60%. Therefore, it is not surprising that many patients seek alternative treatments such as the use of cannabis. Indeed, epidemiological data indicate that as many as 15% of patients with IBD use cannabis. However, data about the clinical therapeutic efficacy of cannabis is limited.

Methods: This presentation will aim to update the current evidence of cannabis use for IBD.

Results: In an observational prospective open label study on 30 patients with Crohn's disease (CD) a significant clinical improvement was observed with an average decrease in Harvey Bradshaw index from 14 ± 6.7 to 7 ± 4.7 ($P < 0.001$). The improvement was sustained over an average period of 2 years (rang 0.3- 9 years). In an 8 week double-blind placebo-controlled study of 21 patients with CD, we found a significant improvement in Crohn's disease activity index (CDAI) in the cannabis active group compared to the placebo group (152 ± 109 vs. 306 ± 143, $P < 0.05$). In a study on 20 patients with CD who were treated with cannabidiol vs. placebo over 8 weeks, no significant improvement in CDAI was observed. Another study using CBD enriched oil in CD patients showed a significant reduction of CDAI from 282 to 166 in the cannabis group ($p=0.004$).

In ulcerative colitis (UC), a 10 week study failed to show significant difference in remission rate in patients who were treated with cannabidiol (n=29) vs. placebo (n=31). In another study on 32 UC patients disease activity was reduced in the cannabis group (10.9 to 5), but not in the placebo group (11 to 8, $p=0.006$).

Conclusion: the current data is limited due to the small number of prospective placebo-controlled studies and the lack of assessment of cannabis effect on objective disease parameters including mucosal inflammation and inflammatory markers. Thus, the key question of whether the reported beneficial clinical effect of cannabis in patients with IBD relates to relief of symptoms, *or* to the anti-inflammatory effects of cannabis remains unanswered.

EFFECTS OF MENSTRUAL CYCLE PHASE AND CIRCULATING ESTRADIOL ON RESPONSE TO ORAL DELTA-9-TETRAHYDROCANNABINOL

Elisa Pabon* and Harriet de Wit

Department of Psychiatry & Behavioral Neuroscience, University of Chicago, Chicago, IL, USA

Introduction: Cannabis use is on the rise, especially among young women. There is evidence that women are particularly susceptible to adverse, stress-related responses to the drug, such as tachycardia and anxiety. Yet, little is known about sources of risk for adverse responses in women, especially risks related to the menstrual cycle. Preclinical evidence suggests that circulating estrogen levels are associated with adverse responses to cannabinoids. To determine the relationship between estrogen and response to cannabinoids in humans we tested the effects of oral Δ^9-tetrahydrocannabinol (THC) vs placebo in healthy female occasional cannabis users at two hormonally distinct phases of the menstrual cycle.

Methods: Forty women received oral THC (7.5 mg, 15 mg) during either the early (EF group) or late follicular (LF group) phase of the cycle. Women were randomly assigned to the two groups, and the drug was administered in a double-blind and counterbalanced design with at least 1 week between sessions. The primary outcome measures were subjective ratings of mood and drug effects and cardiovascular responses. Blood serum estradiol levels were measured at the start each session to confirm phase.

Results: As expected, estrogen levels were higher in the LF group. The cardiovascular effects of THC (increased heart rate and decreased high frequency heart rate variability) were similar in the EF and LF phase. Contrary to our hypothesis, the subjective effects of THC were greater, and occurred earlier, during the EF phase, on measures of feeling a drug effect and anxiety.

Conclusions: The findings suggest that cycle phase affects subjective, but not cardiovascular, responses to THC. It remains to be determined why the subjective effects of the drug were more pronounced during the EF, when estrogen levels are low. With additional subjects, we will examine relationships between estrogen and subjective responses within the phases, and in relation to estrogen levels. Studies of this kind will help to understand the risks of cannabis use, to maximize medical potential and minimize public health risks. Future studies identifying individual differences in response to THC will allow for more preventive action against cannabis-induced anxiety, paranoia, and psychosis.

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THE CBD CRAZE: MAKING A CASE FOR OBSERVATIONAL CLINICAL DATA TO ASSESS MEDICAL CANNABIS TREATMENT EFFECTIVENESS

Erin Prosk*[1], Rihab Gamaoun[1,2], Cynthia El Hage[1], Maria-Fernanda Arboleda[1] and Antonio Vigano[1,2]

[1]Department of Research, Santé Cannabis, Montreal, QC, Canada.
[2]Department of Oncology, McGill University.

Introduction: Medical cannabis, or cannabinoid-based products continue to gain global popularity driving the evolution of regulatory access frameworks in more than 30 countries to date. Many countries opt for an initial program of CBD-only products, likely due to its safety profile and despite limited clinical evidence. Regardless, global market estimates in the tens of billions CBD industry indicate significant consumer demand and pressure from patients and caregivers continues to increase. Validation of the therapeutic expectations of CBD products has fallen behind, and requires research commitment and availability of CBD and THC-based products in controlled regulatory frameworks.

Methods: Patients initiated on medical cannabis treatment at Santé Cannabis, a Quebec-based clinic network, between July 2017 and July 2019 were assessed utilizing a standardized medical questionnaire and validated assessment tools, including the revised Edmonton Symptom Assessment System (ESAS-r), Brief Pain Inventory-short form (BPI-sf) and EuroQol Quality of Life measure (EQ-5D) at baseline (BL), 3-month (FUP1), and 6-month (FUP2) follow-up, to assess treatment effectiveness. A comparison of patient groups who were treated with only CBD-rich (CBD group), and CBD-rich + Adjuvant THC (CBD+THC group) was completed for both descriptive and quantitative results. Effectiveness of treatment between Baseline, 3-month and 6-month follow-up appointments was assessed using One-way between-groups analyses of variance (ANOVA) for Mean score comparison.

Results: Both CBD group (n = 715) and CBD+THC group (n = 380) showed statistically significant ($p < 0.05$) improvement between Baseline and FUP1 for: 1) Pain severity (BPI-sf) of CBD =-0.64 vs CBD+THC=-0.69, 2) Pain interference (BPI-sf) CBD=-1.00 vs CBD+THC=-1.08, 3) Pain (ESAS-r) CBD =-0.80 vs CBD+THC=-0.90), 4) Tiredness (ESAS-r) CBD=-0.71 vs CBD+THC=-0.88), 5) Wellbeing (ESAS-r) CBD=-1.03 vs CBD+THC=-0.70), and for 6) Quality of life, Overall health (EQ5D) CBD=-3.88 vs CBD+THC=-7.89). However, when comparing effectiveness between the CBD and CBD+THC groups, the CBD+Adjuvant THC treatment group showed a more important improvement for almost all assessed elements (Pain severity and interference, all measures on the ESAS-r when comparison with the results for the CBD group) except for wellbeing (ESAS-r) that is both statistically and clinically significant in the CBD group. Very small differences were found between FUP1 and FUP2 mean scores, and differences were not statistically significant, suggesting that improvement achieved at FUP1 was generally maintained at FUP2.

Conclusion: Control or continued prohibition of cannabinoid-based or specifically THC-based products within CBD-only medical cannabis frameworks limits research opportunities and likely falls short of the needs of patients and healthcare professionals in today's social and cultural climate. With a 20-year history of legal medical cannabis access ~400,000 registered patients and a multitude of THC and CBD-based products under its federal access program, Canada serves as a regulatory model and an opportunity for real-word data collection. Findings from one clinical organization indicate a modest improvement in symptom management with CBD-rich treatments and the necessity of both CBD and THC-based medical cannabis treatments to improve treatment effectiveness, however additional data and a deeper investigation is required to further validate these findings and to address control for potential biases, including potential misclassification biases and selection biases.

SURVEY OF PATIENTS EMPLOYING CANNABIGEROL-PREDOMINANT PREPARATIONS WITH CLINICAL RESPONSES AND ADVERSE EVENTS

Ethan Russo[1*], Carrie Cuttler[2], Michelle Sexton[3], Amanda Stueber[2] and Nishi Whiteley[1]

[1]CReDO Science, [2]Washington State University, [3]University of California, San Diego

Introduction: Cannabigerol (CBG), and its precursor cannabigerolic acid before decarboxylation, is sometimes labeled the "mother of all cannabinoids," but normally is found in herbal cannabis only in trace amounts, as the plant most often acts as a high-throughput producer of tetrahydrocannabinol and cannabidiol. This situation changed with selective conventional mendelian breeding resulting in a plant in which 100% of the cannabinoid content consisted of CBG (de Meijer, 2005). Despite this development and its very interesting and potentially useful pharmacology, CBG has remained a rare commodity until quite recently, when CBG-predominant plants and extracts began to appear, especially in the US Northwest.

Methods: The study was determined exempt from the need for IRB. Participants were recruited via various listservs related to cannabis and cannabinoid research as well as social media. They completed an online survey assessing CBG use patterns, conditions treated with CBG, perceived efficacy, associated adverse events and withdrawal symptoms.

Results: A total of 106 adults (55 male, 44 female, 7 other/missing) who reported using CBG-predominant cannabis and residing in the US completed the survey. 27.4% reported a preference for pure CBG, 24.5% preferred material containing between 50-99% CBG, and the remainder reported a preference for <50% CBG. On average, respondents had used CBG for 9.18 months. Average quantity of use was 3.5 grams of flower, 1 gram of concentrates, and 29.25 mg of edibles per week. 60.4% reported obtaining CBG online, 21.7% from dispensaries. Over 50% reported using CBG at least once per day. Oral administration predominated (69.8%) with the remainder smoking (32.1%), vaping (46.2%) or employing topically (13.2%). 54.7% reported using CBG for medical purposes, 6.6% for recreational purposes, and 37.7% for both. The most common conditions people reported using CBG to treat were: anxiety (55.7%), chronic pain (41.5%), insomnia (33%), depression (33%), and migraine (18.9%). Efficacy was highly rated, with the majority reporting their conditions were very much improved or much improved by CBG. Further, 73.8% claimed superiority of CBG over conventional medicines for chronic pain, 74.3% for insomnia, 60% for migraine, 77.1% for depression, and 76.8% for anxiety. 46.2% of CBG users reported no side effects, with 19.8% noting dry mouth, 17.9% sleepiness, 13.2% increased appetite, and 10.4% dry eyes. 98% reported no withdrawal symptoms. 46.8% of respondents noted a preference for CBG-dominant cannabis over other types. 42.9% of the 7 respondents endorsing prescription opioid use noted opioid sparing.

Conclusion: This is the largest patient survey of cannabigerol use to date, and first to document anecdotal efficacy of CBG-predominant cannabis, particularly in anxiety, insomnia, and chronic pain. Most respondents claimed greater efficacy of CBG over conventional pharmacotherapy, and with a very benign adverse event profile. This study suggests the prospect that CBG-predominant cannabis-based medicines can be utilized safely in randomized controlled trials.

REPORTED SIDE EFFECTS AND IMPRESSION OF CHANGE FOLLOWING THREE MONTHS OF MEDICAL CANNABIS TREATMENT

Kelly A. Sagar*[1,2,3], M. Kathryn Dahlgren[1,2,3], Rosemary T. Smith[1,2], Ashley M. Lambros[1,2], Celine El-Abboud[1,2] and Staci A. Gruber[1,2,3]

[1]Cognitive and Clinical Neuroimaging Core,
McLean Imaging Center, McLean Hospital, Belmont, MA
[2] Marijuana Investigations for Neuroscientific Discovery (MIND) Program,
McLean Imaging Center, McLean Hospital, Belmont, MA
[3]Department of Psychiatry, Harvard Medical School, Boston, MA

Introduction: Legalization of medical cannabis continues to spread across the US, with increasing numbers of individuals using cannabis to treat a variety of medical symptoms. While studies have recently begun to examine the impact of medical cannabis (MC) treatment, a myriad of questions remain unanswered, including potential side effects related to MC use as well as patients' overall perception of change after initiating MC treatment.

Methods: As part of an ongoing longitudinal study, 33 patients were assessed prior to initiation of MC use and returned for follow-up visits after initiation of MC (i.e., using cannabis to treat a specific medical condition/symptoms). The current analyses examined data from patients' first follow-up visit, which occurred after 3 months of regular MC treatment. During this visit, patients completed a Side Effects Questionnaire (SEQ) in which they denied or endorsed 42 potential side effects related to MC use; all endorsed side effects were then rated for severity using a 3-point scale (mild, moderate, or severe). Side effects were categorized into the following domains: cardiovascular/respiratory, anxiety, mood/psychiatric, sleep, appetite/nausea/weight, physiologic, energy, cognitive, libido, and alcohol use. Patients also completed the Patient's Global Impression of Change (PGIC) scale, which asks patients to "describe the change (if any) in activity limitations, symptoms, emotions, and overall quality of life" using a 7-point scale ranging from "no change or condition has gotten worse" to "great deal better and a considerable improvement that has made all the difference".

Results: Few MC patients (<25%) reported side effects across cardiovascular/respiratory, anxiety, mood/psychiatric, libido, and alcohol use domains. More highly endorsed side effects included those in the following domains: sleep (39.4%), appetite/nausea/weight (45.5%), physiologic (63.6%), energy (57.6%), and cognitive (45.5%). Specifically, the most commonly reported individual side effects were: dry mouth (54.6%), cognitive cloudiness (33.3%), and sleepiness/fatigue (33.3%). Importantly, some of the "side effects" reported, such as increased sleepiness, are often related to the same issues patients are using MC to treat (i.e. poor sleep). Additionally, rank data on the severity of side effects appear to be skewed toward "mild" ratings. On the PGIC, patients generally reported positive changes (median response: 5 = moderately better, and a slight but noticeable change).

Conclusions: Taken together, results suggest that in the current sample, most patients' experience with MC treatment was positive overall. However, future analyses will provide additional information on which side effects are viewed negatively, and which may actually be indicative of positive change. In addition, future analyses will also examine how MC use variables, including frequency of use and exposure to specific cannabinoids influence patients' outcomes.

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EFFICACY AND SAFETY OF MEDICAL CANNABIS IN THE PEDIATRIC POPULATION – SYSTEMATIC REVIEW AND META-ANALYSIS

Nir Treves*[1], Noa Mor[1], Matitiahu Berkovitch[2], Orit E. Stolar[3] and Ilan Matok[1]

[1]Division of Clinical Pharmacy, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
[2]Clinical Pharmacology Unit, Shamir Medical Center (Assaf Harofeh), Zerifin, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel
[3]The Autism Center, Alut, Shamir Medical Center (Assaf Harofeh), Zerifin

Introduction: Despite the increased use of medical cannabis (MC), the efficacy and safety aspects for indications such as epilepsy and chemotherapy-induced nausea and vomiting (CINV) among children remain uncertain.

Methods: The meta-analysis screening included published and unpublished studies through May-2020 in MEDLINE, EMBASE, and clinicaltrials.gov databases. Selection criteria included studies evaluating efficacy and safety outcomes of MC vs. control among children (18 years old). All potentially eligible studies were independently assessed by two reviewers and considered regardless of study design. I^2 was calculated for heterogeneity, and meta-analyses, network meta-analysis, and trial sequential analysis were performed. We assessed the decrease in seizures in children with Dravet syndrome, serious adverse events, decreased appetite, and mental state changes. When heterogeneity was observed, we performed subgroup analysis and conducted a network meta-analysis (NMA) to estimate if a dose response is present.

Results: Out of 9,133 results, seven studies were included, all of which are RCTs with 486 patients. CBD was associated with a 50% reduction in seizures rate (RR=1.69, 95%CI[1.20 to 2.36]) in patients with Dravet syndrome, and the caregiver global impression of change statistically significantly improved (Median Estimated difference=(-1), 95%CI[-1.39 to -0.60]). A dose-response association between 10mg/kg/d and 20mg/kg/d CBD and decreased appetite was found as well (RR=1.23, 95%CI[0.61 to 2.47], RR=2.40 95%CI[1.39 to 4.15], respectively). MC was associated with mental state changes (RR=2.58, 95%CI[1.62 to 4.12]).

Conclusions: CBD was associated with clinical improvement in Dravet syndrome, although more evidence is warranted in other epilepsy syndromes and other indications. In addition, CBD in high dosage was associated with a decreased appetite. CBD products and other MC products were associated with changes in mental state as well.

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CANCER APPETITE RECOVERY STUDY (CARES): STUDY PROTOCOL FOR A DOSE-ASCENDING, MULTICENTER, RANDOMIZED CONTROLLED PHASE 1/2 TRIAL OF ART27.13 IN PATIENTS WITH CANCER ANOREXIA AND WEIGHT LOSS

Barry Laird[1], Andrew Yates*[2] and Steve D Reich[2]

[1] Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, EH4 2XU, UK And St Columba's Hospice, 15 Boswall Rd, Edinburgh, EH5 3RW, UK
[2]Artelo Biosciences, 888 Prospect Street, Suite 210, La Jolla, CA 92037, USA

Background: The cannabinoid 1 (CB_1) receptor regulates appetite and body weight, however, unwanted central side effects of either agonists (in wasting disorders) or antagonists (in obesity and diabetes) have limited their therapeutic utility. The next generation of these potential medicines have been peripherally restricted to mitigate these issues. ART27.13 (Artelo Biosciences) is a development stage CB_1/CB_2 receptor agonist with reduced brain penetration. In otherwise healthy subjects who participated in a Phase 1 pain study it was observed that low doses of ART27.13 rapidly increased body weight of more than 3% that was not explained by fluid retention and without serious or persistent side effects. Anorexia affects over 60% of late-stage cancer patients for which there is no pharmacologic intervention recognised as the standard of care. ART27.13 may prove to increase appetite, lean body mass, and weight in people with cancer anorexia cachexia syndrome (CACS).

Methods/design: CAReS is a two stage randomised, double-blind, placebo-controlled clinical trial to investigate the utility of **ART27.13 in cancer anorexia and weight loss**. Stage 1 of the study will determine the optimal dose of **ART27.13** to be used in the second stage efficacy study. CAReS will recruit adult patients of all cancers (except brain cancer or brain metastases) on no or stable anti-cancer therapy who have documented weight loss of >5% body weight in the prior 6 months from enrolment.

In stage 1, up to 24 cancer patients will be randomly assigned to receive orally administered dose-ascending ART27.13 for 12 weeks. Safety, dose-limiting toxicity, efficacy (lean body mass, weight gain and change in anorexia), quality of Life (QoL), pharmacokinetics, Karnofsky Performance Status (KPS), and exploratory mechanism of action endpoints will be investigated in order to select the dose of **ART27.13** to be used in Phase 2.

In stage 2, up to 25 cancer patients will receive the recommended ART27.13 dose, or placebo, for 12 weeks randomized 4:1 (drug to placebo). The primary endpoint of the phase 2 trial is to determine point estimates of activity of ART27.13 in terms of weight gain, lean body mass, KPS, and improvement of anorexia. The secondary endpoints will continue to assess safety, QoL measures, and exploratory endpoints will assess the anti-inflammatory and hormonal effects, physical activity, abuse potential, and any opioid sparing effects of ART27.13.

Discussion: Anorexia and the resulting weight loss in cancer patients can compromise health, weakening the immune system, causing discomfort and dehydration, ultimately reducing the patient's prognosis and quality of life. **ART27.13 represents a novel therapeutic strategy to stimulate appetite and weight gain known to arise from CB_1 receptor activation that could significantly benefit patients with CACS.** For the broader community, the data that will arise from this study will help elucidate the potential of modulating the peripheral cannabinoid system in the control of appetite and weight and inform the development strategies for trials targeting this underserved patient group.

Trial registration: EudraCT NUMBER:2020-000464-27

Research Ethics Committee reference: 20/NE/0198.

CANNABINOIDS/CANNABIS AND IMMUNITY – LESSONS FROM PRE-CLINICAL STUDIES

Osnat Almogi-Hazan* and Reuven Or

Cancer Immunotherapy Research Center,
Hadassah Hebrew University Medical Center, Jerusalem, Israel

Introduction: A variety of pathological conditions involve dysregulation of the immune system. For example, in autoimmune diseases, Graft versus Host Disease (GVHD) and secondary complications post infectious diseases, increased activity of the immune system causes inflammation and tissue damage. Unfortunately, in many cases the currently available therapies are unable to restore normal immune function, therefore there is a need for new approaches to balance immunity.

Cannabinoids, the biologically active constituents of cannabis (Marijuana), have important immunological effects. They possess a wide range of anti-inflammatory properties by inducing the production of anti-inflammatory cytokines and affecting the differentiation and function of both innate and adaptive immune cells.

Methods & Results: Our study compares the effects of THC, CBD and cannabis extracts on murine models of immune-associated diseases. In an animal model for GvHD, pathologic activation of the adaptive immune system was tested. Our results demonstrated, that although the *in-vitro* lymphocyte activation was more influenced by pure cannabinoids, cannabis extracts exhibited superior reduction of inflammation. In addition, we used a model of inflammatory bowel disease (IBD) to examine the pathologic innate immune response. In this model, as well we identified the significant activity of the cannabis extracts, compared to the pure cannabinoids.

Conclusions: Our results demonstrate that the effects of cannabis extracts and pure cannabinoids are different. The extract's unique effect could be a result of either, a synergistic function of THC/CBD with other components or from independent anti-inflammatory properties of other molecules in the plant.

Better understanding of the reciprocal relationship between cannabinoids and immunity is essential to design therapeutical strategies that will allow cannabinoids/cannabis incorporation into the clinic.

ASSESSMENT OF CANNABIDIOL AND Δ^9-TETRAHYDROCANNABINOL IN MOUSE MODELS OF MEDULLOBLASTOMA AND EPENDYMOMA

Clara Andradas*[1,2], Jacob Byrne[1], Mani Kuchibhotla[1], Mathew Ancliffe[1,3], Anya C. Jones[4], Brooke Carline[1], Hilary Hii[1], Alexandra Truong[1,2], Lisa C. D. Storer[5], Timothy A. Ritzmann[5], Richard G. Grundy[5], Nicholas G. Gottardo[1,6] and Raelene Endersby[1,2]

[1]Brain Tumour Research Program, Telethon Kids Institute, Nedlands, WA, Australia 6009.
[2]Centre for Child Health Research, University of Western Australia, Nedlands, WA, Australia 6009.
[3]Murdoch University, Murdoch, Western Australia, Australia 6150.
[4]Telethon Kids Cancer Centre, Telethon Kids Institute, Nedlands, WA, Australia 6009.
[5]Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, United Kingdom NG7 2RD.
[6]Department of Paediatric and Adolescent Oncology/Haematology, Perth Children's Hospital, Nedlands, WA, Australia, 6009.

Introduction: Medulloblastoma (MB) and ependymoma (EPN) are two aggressive pediatric brain cancers that affect young children. Despite multimodal treatment protocols that include surgery, radiotherapy and chemotherapy, survival rates for patients with high-risk disease have failed to improve significantly for several decades and recurrences are common. This combined with the devastating impact of current treatments, have led to an urgent need to identify more effective therapeutic strategies for these children. A large body of evidence has demonstrated that cannabinoids, the active compounds of the plant *Cannabis sativa*, exert anti-tumour actions in different cancer types. Specifically, it has been shown that cannabinoids can improve the effect of chemotherapy and radiotherapy in glioblastoma models. Despite the promising results in adult cancers, there is no existing data in paediatric brain tumour models. In this context, we aimed to investigate if the cannabinoids Δ9-tetrahydrocannabinoil (THC) and cannabidiol (CBD) have anti-tumour efficacy in MB and EPN.

Methods: Three patient derived group 3 MB cell lines (D425, D283 and PER547) and two EPN cell lines (IC-1425EPN and DKFZ-EP1NS) were used. Alamar blue was used to measure cell survival in the viability assays and drug interaction assays. Then, computational algorithms were used to determine if the anti-proliferative effect of the combination treatments was enhanced over single drug treatments. The ED50 and ED80 were used to study autophagy and apoptosis signalling pathways at different timepoints after treatment. CB1 and CB2 expression was analysed from two publicly available datasets downloaded from the Gene Expression Omnibus (GEO): GSE64415 and GSE85217. To study the effect of THC and CBD *in vivo*, orthotopic implant models of MB and EPN were used.

Results: Here we show that MB and EPN express cannabinoid receptors, CB1 and CB2. Furthermore, *in vitro* THC and CBD treatment inhibited MB and EPN cell proliferation and induced cell death in part mediated by the induction of autophagy. Additionally, the combination of THC and CBD with each other or with conventional chemotherapy synergistically reduced MB and EPN cell viability. However, these results did not translate effectively *in vivo* and failed to improve animal survival.

Conclusion: This study emphasizes the importance of preclinical models in validating therapeutic agent efficacy prior to clinical trials, ensuring that enrolled patients are afforded the most promising therapies available.

PHARMACOLOGICAL CHARACTERIZATION OF PHYTOCANNABINOIDS AT HUMAN CANNABINOID RECEPTORS

Daniel G. Barrus*, Thomas F. Gamage and Jenny L. Wiley

Discovery Sciences, RTI International, Research Triangle Park, NC, USA

Introduction: Since the United States changed the classification of hemp (i.e., *Cannabis* containing less than 0.3% Δ^9-tetrahydrocannabinol [Δ^9-THC]) from a Schedule I controlled substance to an agricultural commodity in 2018, public interest in non-Δ^9-THC □hytocannabinoids has increased. These compounds have been widely touted for their putative medicinal and/or psychoactive effects, despite little research into their pharmacological activity. We evaluated the in vitro cannabinoid pharmacology of 13 phytocannabinoids: Δ^9-THC, tetrahydrocannabinolic acid (THCA), tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA), cannabichromene (CBC), cannabichromenic acid (CBCA), cannabichromevarin (CBCV), cannabichromevarinic acid (CBCVA), cannabinol (CBN), cannabicyclol (CBL), cannabivarin (CBV), cannabicylolic acid (CBLA), and cannabigerivarinic acid (CBGVA).

Methods: Receptor binding affinities were determined using [^{3}H]CP55,940 competitive binding and receptor function was assessed using agonist-stimulated [^{35}S]GTPγS binding. hCB_1 and hCB_2 HEK293 P2 membranes and multiple concentrations of test compounds were incubated in assay buffer at 30°C for 90 min with 1 nM [^{3}H]CP55,940 or pre-equilibrated for 30 min in assay buffer with 30 μM GDP followed by addition of 0.1 nM [^{35}S]GTPγS and subsequent 1 h incubation.

Results: Of the tetrahydrocannabinols (Δ^9-THC, THCA, THCV, and THCVA), Δ^9-THC and THCV had moderately high affinities for hCB_1 and hCB_2. While Δ^9-THC exhibited partial agonism at both receptors with moderate potency, THCV had no efficacy at either. THCA and THCVA had low binding affinities to hCB_1 and hCB_2, with THCA moderately stimulating hCB_1 and THCVA producing slight stimulation of hCB_2, both with low potency. Of the cannabichromenes (CBC, CBCA, CBCV, and CBCVA), only CBC and CBCV had measurable binding affinities to both hCB_1 and hCB_2, with both compounds stimulating these receptors with low potency. CBCA had low, but measurable, affinity and efficacy at hCB_2. Of the other □hytocannabinoids tested (CBN, CBL, CBV, CBLA, CBGVA), all but CBGVA had measurable binding affinity to hCB_1 and hCB_2, though only CBN stimulated both receptors with moderate potency, while CBV and CBL stimulated only hCB_2.

Conclusions: In general, the "varin" forms of the □hytocannabinoids tested, which have shortened C-3 tails, had comparable affinities, potencies, and efficacies to their parent (C-5) compounds at both receptors. However, THCV lacked efficacy at both receptors and CBV only retained CBN's potency and efficacy at hCB_2—further research is needed to determine if either compound acts as a neutral antagonist, though Pertwee et al. (Br. J. Pharmacol. 150 [2007] 586-594) similarly found that THCV acts as a CB_1 neutral antagonist rather than as an inverse agonist. The acid forms of the □hytocannabinoids all had greatly diminished affinities, potencies, and efficacies at both receptors compared to their decarboxylated forms, except for THCA, which had similar efficacy to Δ^9-THC at hCB_1 but diminished potency. Future studies should investigate the pharmacological interactions between these compounds and how the present findings translate to in vivo effects.

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ATTENUATION OF OXIDATIVE STRESS AND INFLAMMATORY RESPONSE BY CHRONIC CANNABIDIOL ADMINISTRATION IS ASSOCIATED WITH IMPROVED N-6/N-3 PUFA RATIO IN THE RED SKELETAL MUSCLE IN A RAT MODEL OF HIGH FAT DIET-INDUCED OBESITY

Patrycja Bielawiec*, Ewa Harasim-Symbor, Klaudia Sztolsztener, Karolina Konstantynowicz-Nowicka and Adrian Chabowski

Department of Physiology, Medical University of Bialystok, Bialystok, Poland

Introduction: During the progression of obesity, the excessive amounts of lipids are deposited in non-adipose tissues such as skeletal muscle, which results in profoundly disrupted metabolic processes. Recent data indicate a growing interest upon using cannabidiol (CBD) as an agent with beneficial effects in the treatment of obesity. Therefore, our study aimed to investigate the influence of two-week CBD administration on the n-6:n-3 polyunsaturated fatty acids (PUFAs) ratio in different lipid fractions, oxidative stress parameters and inflammatory pathway in the red gastrocnemius muscle in a rat model of high fat diet-induced obesity.

Methods: All experiments were performed on Wistar rats fed high fat diet (HFD) or standard rodent diet for 7 weeks, and subsequently injected with CBD (10 mg/kg once daily for the last 2 weeks of a diet regime). Lipid content was assessed using gas-liquid chromatography (GLC), while colorimetric and immunoenzymatic methods were used to determined oxidative stress parameters. The total expression of proteins of an inflammatory pathway was measured by Western blotting.

Results: Our results revealed that, a HFD influences the fatty acids (Fas) composition in different lipid fractions, especially n-6 PUFA, in the skeletal muscles, as well as contributes to the development of oxidative stress and local inflammation. Importantly, we showed that CBD significantly improved the n-6:n-3 PUFA ratio, and shifted the equilibrium towards anti-inflammatory n-3 PUFAs (in the DAG, TAG and PL fractions). Additionally, CBD prevented lipid peroxidation products generation and attenuated inflammatory response.

Conclusions: In summary, our data provide new insight into the mechanism of CBD action at the cellular level in skeletal muscle and indicate that CBD presents potential therapeutic properties with respect to the treatment of obesity and related disturbances.

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GLIOBLASTOMA MULTIFORME TREATMENT BY COMBINATION OF PHYTOCANNABINOIDS AND COMMON BRAIN CANCER DRUGS

Mor Cohen Harel*, Dikla Vardi and David Meiri

Faculty of Biology, Technion – Israel Institute of Technology, Haifa, Israel

Introduction: Glioblastoma multiforme is a grade IV brain tumor that expresses high intra-tumor heterogeneity and presents a great challenge for treatment. Chemotherapies are insufficient due to fast and aggressive growth, resistance and recurrence by the cancer's stem cells. Therefore, finding treatments that can decrease the chemoresistance is essential. Studies have shown that two □hytocannabinoids from the *Cannabis* plant, Δ^9-tetrahydrocannabinol (Δ^9-THC) and Cannabidiol (CBD), improve glioblastoma cell response to radiation and chemotherapy, and moreover, directly affect tumor growth and cell viability. In the present study, we investigated the ability of multiple *Cannabis* extracts to exert a cytotoxic effect on various glioblastoma cell lines, in relation to their phytocannabinoid content, and to the cells susceptibility to Bortezomib (BTZ). BTZ is a proteasome inhibitor, a common treatment for Multiple Myeloma, currently being studied for the treatment of glioblastoma. It was suggested to have increased potency in cells expressing Transient Receptor Potential cation channel, subfamily V2 (TRPV2) and in combination with CBD, a familiar agonist of TRPV2.

Methods: Death rates of six glioblastoma cell lines were evaluated after 24h treatments of multiple *Cannabis* extracts (4 μg/ml), using Hoechst & PI staining, and clustered according to the level of sensitivity to *Cannabis* treatments. Apoptosis was evaluated by Annexin-PI staining and by mitochondrial membrane potential disruption (MMP) that was examined with tetramethylrhodamine ethyl ester (TMRE) staining using flow cytometry. The basal level of the cell lines' cannabinoid receptors gene expression was detected by qPCR analysis. Cells that expressed higher levels of TRPV2 were selected to assess the combination of *Cannabis* treatments with BTZ on cell death.

Results: The cell lines showed variability in sensitivity to different *Cannabis* extracts, both in cell death rates and by apoptotic markers. A combination of 12.5 ng/ml BTZ with low concentrations of a high-CBD extract (2 μg/ml) was able to induce cell death after 48h in cell lines that were not sensitive to *Cannabis* treatment, nor to BTZ alone. Susceptible cell lines were responsive already after 24h to combinations of the high-CBD extract and lower doses of BTZ (3, 6 ng/ml), and expressed high sensitivity (80% cell death) after 48 hours. Interestingly, when pure CBD in the same amount as in the extract was combined with BTZ there was no effect.

Conclusions: As the extracts were more effective than pure CBD, our results suggest that other *Cannabis* metabolites in addition to CBD are involved in the induction of cell death. The combination of these *Cannabis* compounds and BTZ suggest a novel way to overcome the resistance of Glioblastoma multiforme to treatment. The role of TRPV2 in the mechanism of induced cell death by a high-CBD *Cannabis* extract and BTZ combination remains to be further elucidated.

CANNABIDIOL (CBD) TREATMENT IMPROVES SPATIAL MEMORY IN 14-MONTH-OLD FEMALE TAU58/2 TRANSGENIC MICE

Fabian Kreilaus[a], Madilyn Coles*[a], Magdalena Przybyla[b], Lars Ittner[b], and Tim Karl[a,c]

[a]School of Medicine, Western Sydney University, Australia
[b]Dementia Research Centre, Faculty of Medicine and Health Sciences, Macquarie University, Australia
[c]Neuroscience Research Australia (NeuRA), Australia

Introduction: Frontotemporal dementia (FTD) and Alzheimer's disease (AD) share the pathological hallmark of intracellular neurofibrillary tangles, which result from the hyperphosphorylation of microtubule associated protein tau. The *P301S* mutation in human tau carried by TAU58/2 transgenic mice results in brain pathology and behavioural deficits relevant to FTD and AD. The phytocannabinoid cannabidiol (CBD) exhibits properties beneficial for multiple pathological processes evident in dementia.

Methods: 14-month-old female TAU58/2 transgenic and wild type-like (WT) littermates were treated with 100 mg/kg CBD or vehicle i.p. starting three weeks prior to conducting behavioural paradigms relevant to FTD and AD and continuing throughout testing.

Results: TAU58/2 females exhibited impaired motor function, reduced bodyweight and less anxiety behaviour compared to WT. A moderate reduction in sociability and an impaired spatial reference memory of vehicle-treated transgenic mice were restored by chronic CBD treatment. Chronic CBD also reduced anxiety-like behaviours and decreased contextual fear-associated *freezing* in all mice.

Conclusions: Chronic remedial CBD treatment ameliorated several disease-relevant phenotypes in 14-month-old TAU58/2 transgenic mice, suggesting potential for the treatment of tauopathy-related behavioural impairments including cognitive deficits.

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MEDIUM DOSE CHRONIC CANNABIDIOL TREATMENT REVERSES OBJECT RECOGNITION MEMORY DEFICITS OF *APP$_{SWE}$/PS1ΔE9* TRANSGENIC FEMALE MICE

Madilyn Coles*[a], Georgia Watt[a], Fabian Kreilaus[a] and Tim Karl[a,b]

[a]School of Medicine, Western Sydney University, Campbelltown, NSW 2560, Australia
[b]Neuroscience Research Australia (NeuRA), Randwick, NSW 2031, Australia

Introduction: Alzheimer's disease (AD) is a debilitating neurodegenerative disease that causes behavioural and cognitive impairments and the familial form of the disease is modelled by the *APP$_{Swe}$/PS1ΔE9* (*APPxPS1*) transgenic mouse model. The phytocannabinoid cannabidiol (CBD) has anti-inflammatory, anti-oxidant and neuroprotective properties and *in vitro* and limited *in vivo* evidence suggests that CBD possesses therapeutic-like properties for the treatment of AD. Cannabinoids are known to have dose-dependent effects and the therapeutic potential of medium dose CBD for *APPxPS1* mice has not been assessed in great detail yet. We aimed to evaluate the potential of chronic treatment with 5 mg/kg bodyweight CBD to reverse the behavioural deficits of adult *APPxPS1* mice.

Methods: 12-month-old *APPxPS1* transgenic female mice and their wild type-like littermates were treated (post-onset of AD-like symptoms) via daily intraperitoneal injection with 5 mg/kg bodyweight CBD (or vehicle). Daily treatment commenced three weeks prior to and continued during the assessment of behavioural domains including anxiety, exploration and locomotion, motor functions, cognition, and sensorimotor gating. Two-way ANOVA, mixed ANOVA, one-way and three-way RM ANOVA, and one sample t-test statistical analysis techniques were used, and significant differences were determined when $p < .05$.

Results: *APPxPS1* mice exhibited a hyper-locomotive and anxiogenic-like phenotype in the light dark test and had wild type-like motor abilities in the accelerod and pole test paradigms. The spatial learning abilities of *APPxPS1* mice in the cheeseboard paradigm were not impaired, although AD transgenic mice took generally longer to complete the cheeseboard training (due to a lower locomotion speed). All mice displayed intact spatial memory (although this was delayed in AD transgenic mice) and retrieval memory, but *APPxPS1* mice showed reduced levels of perseverance in the cheeseboard probe trial. Importantly, vehicle-treated *APPxPS1* mice were □hytocannabin by object recognition deficits in the novel object recognition test and delayed spatial learning in the cheeseboard paradigm, both of which were reversed by CBD treatment. Finally, impairments in sensorimotor gating of *APPxPS1* mice in the prepulse inhibition test were not affected by CBD.

Conclusions: Chronic administration of medium dose CBD appears to have therapeutic value for the treatment of particular behavioural impairments present in AD patients, including recognition impairments. Future research should consider the molecular mechanisms behind CBD's beneficial properties for AD transgenic mice.

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ESTABLISHMENT OF A HUMAN WHOLE BLOOD NLRP3 INFLAMMASOME ACTIVATION ASSAY FOR EVALUATING NOVEL INHIBITORS: ASSESSMENT OF CANNABIDIOL

Luke Davison* and Jack Prenderville

Transpharmation Ireland Ltd., Dublin, Ireland.

Introduction: Inflammation is the process by which the immune system responds to pathogen and damage-associated stimuli, and one of the main biological processes through which this is achieved is activation of the inflammasome. Inflammasomes are multi-protein complexes that regulate the production of the pro-inflammatory cytokines IL-1β and IL-18 in response to a wide range of stimuli. One of the best studied of these inflammasomes is the nucleotide-binding and oligomerization domain and leucine-rich repeat-containing pyrin domain containing 3 (NLRP3) inflammasome, which is classically activated by a two-step process involving sequential inflammatory stimuli. Cannabidiol (CBD) is a phytocannabinoid with a number of reported therapeutic benefits including anti-inflammatory, antioxidant and immunomodulatory activities (Atalay et al., Antioxidants. 2020; 9(1):21). Recently, it has been reported that CBD is capable of inhibiting the NLRP3 inflammasome in human THP-1 monocytes through modulation of the P2X7 receptor (Chang et al., Journal of Natural Products 2020 83 (6), 2025-2029). This suggests that CBD can inhibit inflammasome activation in human immune cells. Here, we established a human whole blood model of inflammasome activation and investigated the NLRP3-inhibitory potential of CBD.

Methods: Human whole blood was obtained from healthy donors (n=6) and stimulated *ex vivo* for 4 hours with 2ng/ml of the TLR4 agonist lipopolysaccharide (LPS; endotoxin). Whole blood was treated with increasing concentrations of CBD (1nM, 10nM, 50nM, 100nM, 1μM, 10 μM) 3 hours after LPS treatment for 30 minutes, followed by stimulation with ATP at 5mM for 30 minutes. The specific NLRP3 inhibitor MCC950 (5μM) was used as a positive control for inflammasome inhibition. Analysis of the cytokines IL-1β, TNF-α, IL-6 and IL-10 in the whole blood supernatant was performed using a MesoScale Diagnostics assay.

Results: A significant increase in the expression of IL-1β, TNF-α and IL-6 was observed following LPS stimulation alone ($P<0.0001$). IL-1β was further increased by ATP treatment ($P<0.01$). MCC950 was found to significantly reduce IL-1β release following LPS and ATP treatment ($P<0.0001$). There was no significant effect of CBD treatment at any concentration for any of the cytokines measured. Data was analysed using One-Way ANOVA and Fisher's LSD post-hoc test, data presented as mean ± SEM.

Conclusions: Here, we have established a robust model of NLRP3 inflammasome activation in human whole blood and have identified MCC950 as a specific NLRP3 inhibitor that potently hytocanna IL-1β production. Using this model, we investigated the use of CBD as a novel NLRP3 inflammasome inhibitor. It can be concluded that CBD had no effect on IL-1β production at the time points and concentrations selected. Future experiments will focus on increasing both the concentration of CBD and treatment time, as well as carrying out a similar experiment on human peripheral blood mononuclear cells to identify the potential impact that plasma protein binding in the whole blood milieu may have on the efficacy of CBD in this model.

OPPOSING EFFECTS OF CANNABIS EXTRACTS ON CD4 T REGULATORY CELLS DIFFERENTIATION AND FUNCTION

Yara Eid Mutlak*, Hila Novak-Kotzer, Dor Samet, Anat Gelfand and David Meiri

The Laboratory of Cancer Biology and Cannabinoid Research, Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel

Introduction: Regulatory T cells (Treg) are a subset of CD4 T cells, that play a critical role in maintenance of immunological tolerance to self-antigens and inhibit autoimmune responses and their impaired suppressive function. These cells are found in patients with multiple sclerosis (MS) but not in healthy individuals. Cannabis is nowadays used for relieving symptoms of many different medical conditions including neuropathic pain and spasticity induced by MS. Cannabinoids can modulate immune reactions in the periphery as well as in the brain, influence T cell subset balance and cytokine expression and play a role in the balance between neuroinflammation and neurodegeneration. In this work we tested whether Cannabis may up-regulate Treg functions and directly inhibit functions of effector T cells; both outcomes may lead to effective inhibition of MS.

Methods: MS animal model - experimental autoimmune encephalomyelitis (EAE), induced by myelin oligodendrocyte glycoprotein (MOG35-55) immunization of C57BL/6 mice, to explore the effect of cannabis extracts derived from the same high-CBD cultivar before and after the decarboxylation. In addition, we used in vitro approach of induced Treg (iTregs) suppression assays and biochemical analysis to characterize the intracellular molecular mechanisms of Cannabis-mediated regulation of T cell function.

Results: In our work we found two Cannabis extracts that showed opposing effects on T regulatory cells viability and forkhead box p3 (Foxp3) expression in-vitro. Treatment of Tregs with one extract promotes the suppressive function of regulatory T cells through up-regulation of FoxP3 expression. While treatment with the other extract reduces Foxp3 expressions in T regulatory cells. This effect was diminished by blocking the CB2 receptor. The administration of the first Cannabis extract in vivo downregulated the progression of EAE and resulted in better clinical scores. In contrast, the administration of the second extract in vivo exacerbated EAE progression.

Conclusion: The Cannabis extract that exhibited better suppressive abilities of T regulatory cells, contributed to EAE amelioration. In contrast, the Cannabis extract that impaired T regulatory function by reducing FoxP3 expression caused aggravation of the disease. Thus, the effect on T regulatory cells function appears to be instrumental in regulating the balance between proinflammatory and regulatory T-cell function. This study provides an important input into our understanding of molecular mechanisms of specific molecules from the Cannabis extracts which may act as enhancers of T regulatory cells activity, and thus may have therapeutic potential in MS and other autoimmune conditions.

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COMPARISON OF SYNTHETIC AND PLANT-ISOLATED (-)-CANNABIDIOL ON BINDING PROFILE AND ACTIVITY AT CANNABINOID RECEPTORS

Joerg Thilo Fischer*[1], Javier Fernández-Ruiz[2], Brahim Gargouri[4], Eduardo Muñoz[3], Matthias Winkler[1] and Bernd L. Fiebich[4]

[1] Symrise AG, Holzminden, Germany
[2] Instituto Universitario de Investigatión en Neuroquímica, CIBERNED and IRYCIS, Departamento de Bioquímica y Molecular, Facultad de Medicina, Universidad Complutense, Madrid, Spain
[3] Maimonides Biomedical Research Institute of Córdoba, Spain; Department of Cellular Biology, Physiology and Immunology, University of Córdoba, Spain; University Hospital Reina Sofía, Córdoba, Spain
[4] VivaCell Biotechnology GmbH, Freiburg, Germany

Introduction: Cannabidiol (CBD) is one of the major non-psychotropic phytocannabinoids with a broad spectrum of proven or suspected efficacy against for instance several epilepsy forms, schizophrenia, neuroinflammation or vomiting and nausea. The main physiological targets of CBD in human body are cannabinoid-receptors (CB1 and CB2), whereby some studies claim different binding profiles and intrinsic activities (signaling) at these receptors, depending on the origin of the CBD, either isolated from plant extracts or chemically synthesized. Naturally, CBD is present in the (-)-enantiomer form. The present study compares binding capacities as well as signaling of natural and chemical derived (-)-CBD on both receptor-types.

Methods: Synthetic (-)-CBD was produced according to the method developed at and patented by Symrise AG, Holzminden, Germany (WO2015032519). Plant-derived (-)-CBD was purchased at Freyherr (Ljubljana, Slovenia). Affinity of samples to cannabinoid receptors were determined via competition studies on commercially available membranes prepared from CB1- and CB2-receptor-stably transfected HEK-293 cells (RBHCB1M400UA and RBXCB2M400UA; Perkin-Elmer Life and Analytical Sciences, Boston) following procedures previously published by Gómez-Cañaz et al.. Intrinsic activity was investigated in HEK-293 cells transfected with CB1- and CB2-receptors in a cAMP luciferase assay. Statistical analysis was performed by one-way ANOVA, followed by post-hoc tests ($p<0.05$ was considered statistically significant).

Results: With Ki values in the micromolar range (>3 μM), both compounds show only low affinity on CB1- ($Ki_{(synthetic)}$: 8960 ± 371 nM vs. $Ki_{(plant\text{-}derived)}$: 6970 ± 735 nM) and CB2-receptors ($Ki_{(synthetic)}$: 4310 ± 1292 nM vs. $Ki_{(plant\text{-}derived)}$: 3370 ± 394 nM), whereas no significant differences are observed between synthetic and plant-derived (-)-CBD on both receptors. In regard of intrinsic activity at these receptors, experiments in transfected cells demonstrated that both, plant-isolated and synthetic (-)-CBD behaved as antagonists for CB1-receptors. At CB2-receptors, both compounds showed agonistic activity at only high concentrations (25 μM). No differences are observed between both varieties of (-)-CBD.

Conclusions: (-)-CBD of synthetic origin has the same binding affinity to CB1- and CB2-receptors and reveals the same receptor signaling activity than (-)-CBD extracted from cannabis plant. This supports that (-)-CBD can be used for the same fields of use, irrespectively of its origin.

Acknowledgements: This work was funded by Symrise AG, Holzminden, Germany.

References: Gómez-Cañaz, M., Morales, P., García-Toscano, L., Navarrete, C., Muñoz, E., Jagerovic, N., et al. (2016). Biological characterization of PM226, a chromenoisoxazole, as a selective CB2 receptor agonist with neuroprotective profile. *Pharmacological Research* 110, 205-215.

CANNABINOIDS ATTENUATE THE SEVERITY OF COLITIS IN A MURINE MODEL AND MODULATE CXCR4 EXPRESSION

Anat Gelfand*, Gil M Lewitus, Elazar Besser and David Meiri

Faculty of Biology, Technion - Israel Institute of Technology, Haifa, Israel

Introduction: Chemokines are small cytokines that govern leukocyte migration. They attract cells that express their cognate ligands and thus play key roles in inflammation and tissue injury. Among various chemokines, the expression of C-X-C Motif Chemokine Receptor 4 (CXCR4) and its cognate ligand have been shown to increase in the progression of several inflammatory conditions, including inflammatory bowel diseases (IBDs). Some studies demonstrated that cannabinoids, the active metabolites of the Cannabis plant, exhibit immunosuppressive properties that include modulation of immune cell migration. In the present study, we examined whether cannabinoids regulate the inflammatory response, in part, by modulating chemokine-mediated leukocyte infiltration into inflamed tissue of the gastrointestinal tract in colitis.

Methods: Activated splenocytes from immunized mice were cultured *ex-vivo* in the presence of either whole-plant Cannabis extracts, specific cannabinoids or vehicle control for 3 hours before being subjected to a flow cytometric analysis. The percentage of CXCR4 positive cells and its expression level (MFI) were evaluated on four designated cell populations: CD45+ leukocytes, CD11b+ monocytes and CD3+ or CD4+ T lymphocytes. Additionally, *in-vivo* effects of the cannabinoids in the acute inflammatory state were examined using Dextran Sulfate Sodium (DSS)-induced colitis model in mice. C57BL/6 female mice received 2.5% (w/v) DSS in drinking water for seven days. Treatment was administrated intraperitonially from day one to seven every other day. Severity of colitis was evaluated by disease activity index, body weight and colon length.

Results: Activation related CXCR4 upregulation was attenuated by several cannabis extracts on CD45+ leukocytes, CD11b+ monocytes, CD3+ T lymphocytes and CD4+ T helper cells (60%,50%,45%,45% respectively) as indicated by lower percentage of CXCR4+ cells and total CXCR4 cell surface expression (mean fluorescence expression, MFI). We identified two cannabinoids that only when combined had the same effect as the whole extract. Notably, we tested different ratios of the two cannabinoids and the original ratio as in the plant was the most effective in reducing CXCR4 expression. These cannabinoids had protective effects also in DSS-induced acute colitis *in-vivo*. Administration of a whole-Cannabis extract or the two specific cannabinoids significantly ameliorated the severity of the disease, as indicated by reduced percentage of body weight loss, lower Disease Activity Index and less colon shortening at day 7. Interestingly, each cannabinoid alone had no effect on disease progression.

Conclusions: Cannabinoids exerts therapeutic effects on experimental colitis by reducing colonic inflammation *in-vivo* and downregulate inflammation-related CXCR4 upregulation on immune cells *in-vitro*. These results suggest that modulation of this chemokine axis may have potential as a therapeutic target for the treatment of IBD.

CANNABINOIDS AND CANNABIS EXTRACTS AFFECT METABOLIC SYNDROME PARAMETERS INCLUDING MICROBIOME IN MICE FED HIGH FAT-CHOLESTEROL DIET

Jonathan Gorelick*, Tal Assa-Glazer, Noa Sela, Abraham Nyska,
Nirit Bernstein and Zecharia Madar

Eastern Regional R&D Center, Kiryat Arba, Israel
The Robert H Smith Faculty of Agriculture, Food and Environment,
The Hebrew University of Jerusalem, Rehovot, Israel
Department of Plant Pathology and Weed Research,
Volcani Center, Rishon LeZion, Israel
Sackler School of Medicine, Tel Aviv University, Timrat, Israel
Institute of Soil, Water and Environmental Sciences, Volcani Center, Rishon LeZion, Israel

Introduction: Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic syndrome, which often includes obesity, diabetes, and dyslipidemia. Several studies **in mice and humans** have implicated the involvement of the gut microbiome in NAFLD. While cannabis may potentially be beneficial for treating metabolic disorders such as NAFLD, the effects of cannabis on liver diseases and gut microbiota profile have yet to be addressed. In this study we evaluated the therapeutic effects of cannabis strains with different cannabinoid profiles as well as individual cannabinoids on NAFLD progression.

Methods: NAFLD was induced by feeding mice a high fat cholesterol diet (HFCD) for 6 weeks. During this period cannabis extracts or individual cannabinoids were administrated orally at a concentration of 5 mg/kg every 3 days. Profile of lipids, liver enzymes, glucose tolerance and gene expression related to carbohydrate lipids metabolism and liver inflammation were analyzed. The effect of cannabis strains or cannabinoids on microbiota composition in the gut was evaluated.

Results: A CBD-rich extract produced an increase in inflammatory related gene expression and a less diverse microbiota profile, associated with increased fasting glucose levels in HFCD fed mice. In contrast, mice receiving a THC-rich extract exhibited moderate weight gain, improved glucose response curves and a decrease in liver enzymes. Surprising HFCD fed mice given CBD alone exhibited improved glucose response curves, while THC alone did not affect glucose response. Similar effects on the microbiota composition were observed.

Conclusions: The results of this study indicate that while administration of cannabis containing elevated levels of THC may help ameliorate symptoms of NAFLD, THC alone may not be as effective. Interestingly, while CBD alone may be beneficial at alleviating at least some of the symptoms of NAFLD, a CBD-rich cannabis extract may cause a pro-inflammatory effect in the liver, linked with an unfavorable change in the microbiota profile.

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EFFECTS OF PHYTOCANNABINOIDS ON MICRORNA EXPRESSION IN TWO MODELS OF GENERALISED EPILEPSY

Mona Heiland*[1,2], Ngoc Thanh Nguyen[1,2], Gary P. Brennan[1,2], Thomas D. M. Hill[1,2], Gareth Morris[1,2] and David C. Henshall[1,2]

[1]Department of Physiology and Medical Physics,
Royal College of Ireland (RCSI), Dublin 2, Ireland
[2]FutureNeuro SFI Research Centre, Royal College of Ireland (RCSI),
Dublin 2, Ireland

Introduction: Epilepsy is one of the most common neurological diseases characterised by spontaneous recurrent seizures, affecting ~65 million people worldwide. There remains an urgent need for new therapies for drug-resistant epilepsy, as ~30% of affected people do not respond to current pharmacological treatments. Recently, the non-psychotomimetic phytocannabinoid cannabidiol (CBD) has been approved for the treatment of rare childhood epilepsies including Dravet syndrome (DS). However, the mechanisms by which CBD exert its anti-seizure effects are not fully elucidated. MicroRNAs (miRNA) are small non-coding RNA molecules which regulate protein expression. Their expression is extensively altered in epilepsy and targeting certain miRNAs can prevent seizures.

We hypothesised that modulation of miRNAs is a potential mechanism of action for the therapeutic effects of CBD. To test this, we measured changes to the levels of miRNAs in the hippocampus of mice after dosing with CBD using a regime known to produce anti-seizure effects.

Methods: We first identified a dose of CBD that produced acute anti-seizure effects in the pentylenetetrazol (PTZ) mouse model, a common first screening model for anti-seizure effects. Then, adult male C57BL/6 mice were treated for five days twice daily with CBD (200 mg/kg/day; i.p.) or vehicle control. Mice were then euthanised and the hippocampus processed for small RNA sequencing. A selection of differentially expressed miRNAs was validated using individual Taqman miRNA assays and levels of the miRNAs were measured using hippocampus from a mouse model of Dravet syndrome (Scn1a+/-). Data were analysed for normal distribution using D'Agostino and Pearson omnibus normality test. For the statistical analysis, a one-way ANOVA with post-hoc Dunnett's multiple comparison test or unpaired t-test was performed and data was considered significant at $p \leq 0.05$.

Results: A dose of 200mg/kg/day caused a significant reduction in seizure severity in the PTZ model. Using this dose, CBD treatment produced changes to 66 miRNAs in the mouse hippocampus ($p < 0.05/0.01$; non-FDR corrected). This included 20 up-regulated and 46 down-regulated miRNAs, including lower levels of miR-335. Analysis of the hippocampus from the DS mouse revealed levels of the same miRNA were increased.

Conclusions: Taken together, these data demonstrate that CBD treatment at a dose which produces anti-seizure effects results in selected changes to miRNAs in the brain of mice that may have relevance for its anti-seizure mechanism.

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CANNABIDIOL REGULATES MYOCARDIAL LIPID METABOLISM IN A RAT MODEL OF OBESITY

Ewa Harasim-Symbor*, Patrycja Bielawiec and Karolina Konstantynowicz-Nowicka

Department of Physiology, Medical University of Bialystok, Bialystok, 15-222, Poland

Introduction: Increased dietary fatty acid consumption leads to overweight, insulin resistance and type 2 diabetes mellitus along with lipids accumulation in such tissues as cardiac muscle or liver. Enhanced deposition of lipids in cardiomyocytes in obesity can cause hypertrophy of the left ventricle, contractility impairment as well as systolic and/or diastolic heart failure. Pivotal factor responsible for accumulation of lipids in different tissues (including cardiac muscle) is intensified intracellular transport of long chain fatty acids (LCFA), which primarily depends on fatty acid protein transporters expression (FAT/CD36 – fatty acid translocase, FABPpm – plasma membrane associated fatty acid binding protein, FATP1,4,6 – fatty acid transport protein). By regulating the expression of the above transporters the intensity of LCFA uptake as well as lipid content in the heart can be modulated. Therefore, we investigated whether cannabidiol (CBD) controls myocardial LCFA uptake along with the level of selected lipid fractions.

Methods: In an animal model of obesity male Wistar rats were fed a high-fat diet (HFD; kcal – 60% fat, 20% carbohydrate and 20% protein) for 7 weeks, while control rats were receiving standard rodent chow (kcal – 12.4% fat, 57.1% carbohydrate and 30.5% protein). Rats from the HFD groups and parallel control groups were administered CBD injections or its solvent (10 mg/kg per day, intraperitoneal) for last 14 days of the experiment. Cardiac muscle and plasma samples were collected. Free fatty acid (FFA), diacylglycerol (DAG) and triacylglycerol (TAG) contents as well as protein transporters expression were determined using gas liquid chromatography and Western blotting technique, respectively. Data were analyzed by one-way ANOVA followed by appropriate post-hoc test ($P<0.05$ considered significant).

Results: Chronic CBD administration caused substantial reduction of intramyocardial DAG and TAG fractions with parallel increase of FFA content in HFD group compared to HFD group alone. Importantly, the above changes probably resulted from decreased myocardial total expression of FAT/CD36, FABPpm and FATP1,4,6 in obese rats subjected to CBD injections compared to respective control group. Moreover, CBD apart from diminishing myocardial lipid content (DAG and TAG) also markedly elevated plasma concentrations of both FFA and TAG fractions in rats fed HFD compared to HFD alone.

Conclusions: In the light of the above data, it is likely that CBD downregulates LCFA uptake and thereby can be considered as a therapeutic agent protecting the heart against metabolic disturbances and contractile dysfunction caused by lipotoxicity.

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PHYTOCANNABINOID CONCENTRATIONS IN HEMP DERIVED PRODUCTS: LC-MS/MS ASSAY VALIDATION AND QUANTIFICATION OUTCOMES

Erin Johnson*¶, Shanna Babalonis§ and Michael Kilgore¶

Departments of Pharmacology and Nutritional Science¶ and Behavioral Science§,
University of Kentucky College of Medicine, Lexington, KY, USA

Introduction: The rapid development of new products in the hemp/CBD industry has outpaced the ability of regulatory bodies to establish standards and protocols to ensure the safety of consumers, including the potential presence of Δ^9-tetrahydrocannabinol (Δ^9-THC). The goal of the present study was to develop and validate methods for the identification and quantitative determination of specific phytocannabinoid compounds in complex mixtures widely available for consumption.

Methods: A liquid extraction method was developed to isolate phytocannabinoids from hemp-derived sublingual oil and hemp protein powder. The extracts were then analyzed by LC-MS/MS using a targeted method on a Thermo TSQ Vantage system with ionization in both positive and negative modes. Separation was carried out using a Phenomenex Kinetex® C8 column and 12-minute gradient program.

This method was validated for quantitative determination of cannabidiol (CBD) and Δ^9-THC. The method validation included experiments to determine selectivity, recovery, matrix effects, accuracy and precision. The validated method was then used to conduct product content analysis on commercially available sublingual oils and hemp protein powders (products acquired from the internet as well as brick and mortar stores).

Results: These method validation studies demonstrate robust and reproducible approaches to determining concentrations of cannabidiol and Δ^9-tetrahydrocannabinol. Linearity was demonstrated by an R^2 of at least 0.98 across three batches prepared and analyzed on three different days. Accuracy and precision were determined by intra-batch and inter-batch relative error within ± 15% and coefficient of variation within ± 15%. Recovery was 89% and 85% for cannabidiol and Δ^9-tetrahydrocannabinol, respectively. Minor ion suppression, less than 7%, was observed.

Quantitative analysis of 28 hemp-derived sublingual oil products indicated a wide range of cannabinoid concentrations: CBD range = 3 mg/mL to 109 mg/mL; Δ^9-THC range = not detected to 1393 µg/mL. Quantitative analysis of 24 hemp protein powders also indicated a wide-range of estimated concentrations: CBD range = not detected to 32195 ng/g; Δ^9-THC range = not detected to 1439 ng/g.

Conclusion: These studies validate LC-MS/MS methods for the detection of cannabinoids in both hemp-derived sublingual oils and hemp protein powders and provide quantification of cannabidiol and Δ^9-tetrahydrocannabinol concentrations in these matrices.

PHYTOCANNABINOIDS FOR TREATING NON-ALCOHOLIC FATTY LIVER DISEASE, DYSLIPIDEMIA, AND TYPE 2 DIABETES

Radka Kočvarová*, Shahar Azar and Joseph Tam

Obesity and Metabolism Laboratory, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Introduction: Obesity, a chronic progressive disease that is now reaching epidemic proportions globally, has been described as a catalyst for cardiovascular disease (CVD), type 2 diabetes (T2D), and non-alcoholic fatty liver disease (NAFLD). Cannabis, via its non-psychoactive phytocannabinoids, Δ^9-tetrahydrocannabivarin (THCV) and cannabidiol (CBD), has been shown to reduce weight gain, to inhibit the development of alcohol-induced hepatosteatosis, to improve insulin sensitivity and to decrease hepatic triglyceride accumulation in murine models of obesity. These data indicate that phytocannabinoids are able to potentially elicit positive metabolic effects under fatty conditions; however, whether CBD and/or cannabigerol (CBG) have the potential to treat obesity and its metabolic abnormalities has never reported yet.

Methods: Human hepatoma HepG2 cells exposed to lipotoxic conditions were used as an *in vitro* model to test whether CBD and/or CBG reduce lipid accumulation in hepatocytes. Furthermore, gene analysis was performed to measure the expression levels of several key molecules, enzymes and transcription factors associated with *de novo* lipogenesis and fatty acid oxidation. Subsequently, the *in vivo* therapeutic potential of CBD (10 mg/kg/day, IP) and/or CBG (25 mg/kg/day, IP) on hepatic steatosis, glucose and insulin homeostasis, dyslipidemia, and body weight gain was evaluated in high-fat diet (HFD)-induced obese mice by using biochemistry and histological analyses as well as by utilizing *in vivo* settings (ipGTT, ipIST, and body composition).

Results: *In vitro,* CBD alone (20 μM) demonstrated a significant reduction in lipid accumulation in hepatocytes, whereas CBG was ineffective at any concentration tested. The combination of CBD with CBG (20+20 μM, 20+50 μM, respectively) seemed to have a synergistic effect. Moreover, these combinations resulted in a positive change in the expression profile of many genes and transcription factors involved in lipogenesis (downregulation) and mitochondrial fatty acid β-oxidation (upregulation). *In vivo*, the HFD-induced hepatic steatosis, hyperglycemia, and glucose intolerance were significantly attenuated by CBD, CBG, and CBD+CBG. Normalization of HOMA-IR, a marker for β-cell function and insulin resistance, was only found in the HFD-fed mice treated chronically with CBG. Significant improvements in hypercholesterolemia and hypertriglyceridemia were noted in all the treated groups. All the above-mentioned findings were not related to changes in body weight. Yet, a significant reduction in fat mass and increased lean mass were found in the CBG-treated mice.

Conclusion: These data indicate that phytocannabinoids, specifically CBD and/or CBG, are potentially able to elicit positive metabolic effects under lipotoxic conditions. Further efforts to achieve a better understanding of the molecular mechanisms involved are crucial to the development and clinical testing of these phytocannabinoids in humans.

Acknowledgment: This study was funded by a CiiTECH Labs research grant to J.T.

EFFECT OF PHYTOCANNABINOIDS ON ANDROGEN DEPRIVATION THERAPY IN A HIGH-FAT DIET EXACERBATED PROSTATE CANCER

Magdalena Kostrzewa*[1], Ali Mokhtar Mahmoud[1], Marianna Cerasuolo[2], Roberto Ronca[4], Sébastien Lacroix[3], Cristoforo Silvestri[3], Vincenzo Di Marzo[1,3] and Alessia Ligresti[1]

[1]Institute of Biomolecular Chemistry, National Research Council of Italy, Italy, [2] University of Portsmouth, School of Mathematics and Physics, Hampshire, UK, [3]Canada Excellence Research Chair, Université Laval, Québec City, Canada, [4]Department of Molecular and Translational Medicine, University of Brescia, Italy

Prostate cancer (PCa) is the second most frequent cause of tumor-associated mortality worldwide. Tumor growth depends on androgens, therefore, the mainstay treatment, especially for advanced tumor, is based on androgen deprivation therapy (ADT). Many patients receiving ADT report accumulation of abdominal fat and metabolic syndrome development as side effects. Nevertheless, androgen blockade is a frequently used therapy also in over-weight and obese patients. Obesity is associated with elevated incidence of aggressive PCa and a higher frequency of complications following ADT (Derweesh et al. BJU Int 2007). Diets with high fat and sugar content promote prostate tumor carcinogenesis and contribute to negative prognosis, however, the underlying mechanisms are still poorly understood. The *gastrointestinal microbiome* plays a major role in the pathogenesis of several diseases and acts as a regulator of androgen metabolism potentially contributing to PCa development blockade and influencing the pharmacological outcome of ADT. Numerous studies consider phytocannabinoids promising anti-cancer agents and controllers of food intake and lipid metabolism.

Here, we investigated the effect of CBD and CBG (1:1, 37.5+37.5 mg/kg, i.p. twice per week) alone and combined with the anti-androgen enzalutamide (3 mg/kg, in drinking water, the consumption of which was not altered by any other treatment), on tumor progression and gut microbiota in a transgenic adenocarcinoma mouse prostate model (TRAMP) fed with high fat diet (HFD) and regular diet (RD). Mice were exposed to HFD after weaning at 4 weeks of age. Treatments were administered from 12 weeks of age, when RD-fed TRAMP mice developed multistage autochthonous prostate tumors. Animals were sacrificed at 25 weeks of age, and histopathological analysis was performed on dissected genitourinary tissues. 16S rRNA gene sequencing was performed on fecal samples to profile the diversity of the gut microbiome.

HFD exacerbated PCa development and induced gut microbiota dysbiosis increasing the Firmicutes to Bacteroidetes ratio ($p=0.0079$). Administration of anti-androgen ($p=0.0092$) or phytocannabinoids ($p=0.0016$) alone significantly inhibited tumour development only in RD-fed mice. Combined treatments instead were effective in mice fed with both diet regimens. As for gut microbiota in HFD-fed mice, enzalutamide, alone and in combination with phytocannabinoids, restored the lowered levels of phylum Bacteroidetes ($p \leq 0{,}0001$). Comparing single enzalutamide treatment with its treatment combined with CBD+CBG, we found that this latter treatment was associated with increased abundance of bacterial families such as *Anaerosporobacter* ($p<0.0001$), *Roseburia* ($p<0.01$) , *Lactobacillus* ($p<0.01$) and reduced abundance of *Turicibacter* ($p<0.0001$), *Phocea* ($p<0.05$) and *Bilophila* ($p<0.05$).

The study suggests that 3 mg/kg of the anti-androgen enzalutamide can modify gut microbiota composition in HFD-fed mice, but it becomes able to inhibit HFD-exacerbated tumour growth in these animals only if combined with phytocannabinoids. These findings indicate a novel, more beneficial with respect to monotherapy, therapeutic approach for PCa progression, when worsened by other comorbidities like diet-induced overweight and obesity.

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TOXICITY AND LIFESPAN EFFECTS OF CANNBIGEROL IN A PRECLINICAL *C. ELEGANS* MODEL

M. Hunter Land[1], Marton L. Toth[2], Laura MacNair[1], Siva A. Vanapalli[2,3], Marcel O. Bonn-Miller[1], Erica N. Peters[1] and Timothy W. Lefever*[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario, Canada.
[2]NemaLife Inc. Lubbock, Texas, USA.
[3]Texas Tech University, Lubbock, Texas, USA.

Introduction: Cannabigerol (CBG) is among the most prevalent commercially available cannabinoids derived from Cannabis*;* however, very little is known about its physiological effects. *Caenorhabditis elegans* (*C. elegans)* are often used in early preclinical studies to investigate life-long toxicity, effects on lifespan, and health related outcomes. Due to their short lifespan of approximately 2-3 weeks, highly conserved molecular and cellular mechanisms, and adopted use in toxicity and life extension studies, the *C. elegans* model was selected to examine acute and long-term effects of CBG exposure.

Methods: Acute toxicity was determined by treating day 1 adults with a wide range of CBG concentrations (0.075 µM, 0.75 µM, 7.5 µM, 75 µM, 375 µM, 750 µM, and 3750 µM) and assessing mortality and motility compared to control. Acute thermotolerance was examined by treating adult animals with CBG (0.075 µM to 3.75 mM) and exposing them to 37 ^{o}C for 4 hours, then scoring for the number of alive animals treated with CBG compared to controls. Long-term toxicity was assessed by exposing day 1 adults to 7.5 µM, 75 µM, 375 µM of CBG continuously until all animals perished, noting changes in lifespan duration and motility compared to controls.

Results: No acute or long-term toxicity of CBG was identified. C. elegans treated with CBG demonstrated significantly increased resistance to heat-induced molecular stress (thermotolerance), up to 127.7% at 75 µM compared to untreated controls. Extension in mean lifespan compared to controls was observed for 7.5, 75, and 375 µM CBG with 75 µM CBG, significantly extending mean lifespan by 19.1% ($p < 0.0001$). In addition, 75 µM CBG treatment increased the percentage of highly active animals by 183% on day 15 compared to controls.

Conclusion: Acute and lifelong exposure to CBG in the C. elegans model did not demonstrate long-term toxicity. Additional preclinical investigations should be conducted in other invertebrate and vertebrate models to verify the lack of short and long-term effects of CBG..

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ABSTRACT WITHDRAWN

THE EFFECT OF *CANNABIS* FLOWER FERTILIZATION ON THE ACCUMULATION OF SECONDARY METABOLITES

Carni Lipson Feder*[1], Anna Shapira[1], Ohad Guberman[1], Oded Cohen[2], Moshe Flaishman[2] and David Meiri[1]

[1]The Laboratory of Cancer Biology and Cannabinoid Research, Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel; [2]Agricultural Research Organization (ARO), Volcani Center, Institute of Plant Sciences, Rishon LeZion, Israel

Introduction: In the last couple of decades growing evidence has been showing the therapeutic abilities of *Cannabis* plants. These capabilities have been attributed to the specialized secondary metabolites stored in the female flowers. Among these metabolites there are two central groups: phytocannabinoids, which are exclusive to *Cannabis* and include more than 100 different molecules; and terpenoids, mostly volatile components that are found in many different plants, are responsible for the fragrance and taste of the plants, and may have defensive roles. The accumulation of the metabolites in the female plant flowers is highly versatile and influenced by a largely unknown regulation system, attributed to genetic, developmental and environmental factors. In this study, we examined whether fertilization of the female plant by different male plants influences the metabolite accumulation.

Methods: Two cultivars of female *Cannabis* plants, Δ^9-Tetrahydrocannabinol (THC) - or Cannabidiol (CBD)-rich, were fertilized by three different male plants, THC- or CBD-rich and a male-induced female plant. After a flowering stage of 6 to 8 weeks, inflorescences were picked and dried, followed by extraction of the metabolites. The concentration of over 100 accumulated phytocannabinoids was quantified by High-Performance Liquid Chromatography/Ultraviolet detection (HPLC/UV) and Liquid Chromatography-Mass Spectrometry (LC-MS). Additionally, over 100 Terpenoids were assessed by Gas chromatography-Mass Spectrometry (GC-MS).

Results: Fertilization caused a significant decrease in total phytocannabinoid concentration, 75% and 60% decrease on average in the THC-rich cultivar and the CBD-rich cultivar, respectively. Specific phytocannabinoids, such as Tetrahydrocannabinolic acid (THCA), Cannabidiolic acid (CBDA) and Cannabigerolic acid (CBGA) were reduced by over 80%. Total terpenoid concentration did not demonstrate a specific trend. However, distinguishing between monoterpenoids (10-carbon backbone) and sesquiterpenoids (15-carbon) revealed a significant decrease in monoterpenoids concentration and a mixed trend in sesquiterpenoids content. Monoterpenoids were significantly decreased by fertilization, in both female types, in a similar manner to that of the phytocannabinoids, 73% on average in the THC-rich cultivar and a 57% on average in the CBD-rich cultivar.

Conclusions: This study shows that fertilization of *Cannabis* results in a significant reduction in phytocannabinoids and monoterpenoids concentration. This suggests that both metabolite groups are commonly regulated. In support of this, some studies showed that monoterpenoids and phytocannabinoids (and not sesquiterpenoids) share a common biosynthesis precursor, and that they have common accumulation pattern in flowering plant. Further work is needed to clarify whether they are also downregulated by a common regulation system following fertilization.

UNDERSTATING THE MOLECULAR MECHANISM(S) OF CBD ANALGESIC PROPERTIES

Yossef Ma'atuf* and Avi Priel

Institute of Drug Research, Pharmacology section, Pharmacy School, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Introduction: To date, the most common clinical use of phytocannabinoids (pCBs) is for analgesic purposes, treating the most debilitating chronic pain disorders, such as neuropathic and cancer pains. However, little is known about their molecular mechanism(s) inducing pain relief. While two cannabinoid specific receptors (CB1 and CB2) have been recognized and extensively studied, their pharmacological or genetic blockage did not abolish the analgesic effects of cannabinoids, indicating that other, yet to be identified, receptors/channels mediate the cannabinoid-induced analgesia. Previous studies indicate that both phyto- and endocannabinoids interact with several types of voltage-gated ion channels, including sodium channels (Nav). Nav channels represent an important class of drug targets for pain and many other pathology conditions. Of particular interest to pain-related drug discovery are the Nav subtypes primarily expressed in sensory neurons, such as Nav1.8. Nav1.8 has been reported to be preferentially expressed in small-diameter unmyelinated sensory afferents specialized for detecting noxious stimuli. However, the role of the pain pathway NaV channels in the phytocannabinoids-induced analgesia is not known. Moreover, whether Nav1.8 is modulated by pCB is yet to be elucidated.

Methods: To initially define whether pain pathways Nav channels are modulated by pCB, we characterized the pharmacological effect of the major phytocannabinoids, CBD and THC, on nociceptors from trigeminal ganglion using both voltage- and current-clamp recordings. To specifically define the mechanism of pCBs on pain pathway-related Nav channels, we expressed human Nav channels in HEK and ND7/23 cells and characterized the biophysical properties of the pCBs current modulation.

Results: We found that both CBD and THC inhibits the generation of action potentials in TTX-resistant neurons, with CBD being more potent than THC. Moreover, CBD and THC significantly inhibit the Na+ currents in both TTX-resistant and TTX-sensitive neurons. The rate of inhibition was much higher in the TTX-resistant neurons compared to the TTX-sensitive. Accordingly, to examine the role of specific Nav channel subtypes, we used voltage-clamp recordings of transiently transfected HEK293T and ND7/23 cells to characterize CBD and THC's effects on Nav1.7 and Nav1.8 channels activity. Our results show that Nav1.8 has higher sensitivity to CBD in comparison to Nav1.7.

Conclusions: We found that TTX-resistant neurons have a higher sensitivity to CBD than TTX-sensitive trigeminal neurons. Moreover, we found that CBD dramatically inhibits Nav1.8 in clinically relevant concentrations.

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CBD SELECTIVELY TARGETS THE METABOLIC REPROGRAMMING OF HORMONE REFRACTORY PROSTATE CANCER CELLS

Ali Mokhtar Mahmoud*[1], Viviana Marolda[1], Magdalena Kostrzewa[1], Vincenzo Di Marzo[1,2], Roberto Ronca[3] and Alessia Ligresti[1]

[1]Institute of Biomolecular Chemistry, National Research Council of Italy, Italy
[2]Canada Excellence Research Chair, Université Laval, Québec City, Canada
[3]University of Brescia, Department of Molecular and Translational Medicine, Italy

Metabolic reprogramming is a hallmark of cancer used for the dominant growth and survival of cancer cells. Tumour evolves the metabolic reprogramming by driving the activation of some transcription factors and oncogenic signaling. Prostate cancer (PCa) relies on this mechanism only on its high-grade stage, the hormone refractory prostate cancer (HRPC). We previously reported that CBD is able to inhibit non-HRPC PCa growth via a mechanism partially mediated by TRPM8 (De Petrocellis et al., Br. J. Pharmacol. 2013). With its complex pharmacology and being able to interact with other receptors (Morales et al., Prog Chem Org Nat Prod, 2017), in different tumor types, CBD showed on different cancer types, anti-tumor properties mediated by different mechanisms. CBD may bind also VDAC1 (Rimmerman et al., Cell Death Dis. 2013), the mitochondrial outer membrane channel, suggesting that mitochondria-targeting might be responsible of the CBD-mediated anti-cancer effects. Mitochondria are prominent organelles governing the main metabolic pathways as well as oncogenic and cell death signals. This oncometabolic interaction is not fully understood yet and offers a potential new scope for promising interventions against metabolic reprogramming in cancer. Recently, we set-up and characterized *in vivo* and *in vitro* models of HRPC (Cerasuolo et al., Cancer Research 2020). These models have been generated by exposing a transgenic adenocarcinoma mouse prostate model (TRAMP) mouse and TRAMP-C2 cells to the standard anti-androgen chemotherapy drug enzalutamide (MDV3100). Combined treatment with purified (>99%) botanical CBD and CBG (1:1, 37.5+37.5mg/kg, i.p. twice per week) significantly reduced tumour relapse ($p=0.0052$) in TRAMP animals under the hormone refractory status. Similar results were observed *in vitro* where phytocannabinoid treatment (CBD and CBG, 1-10-30 μM) induced a significantly greater pro-apoptotic effect ($p<0.01$) in HRPC rather than parental non-HRPC cells. Untreated HRPC cells showed altered mitochondrial bioenergetics (reduced oxidative phosphorylation and increased glycolysis capacity) when compared with non-HRPC cells. Differently from CBG, whose effect was partially reverted by CB1 and TRPV1 antagonists, CBD inhibited HRPC cell viability in a cannabinoid receptor- and TRP channel- independent fashion (preliminary data). Moreover, in HRPC cells the exposure to CBD (3 μM for 24h) increased glycolytic rate and inhibited oxidative phosphorylation in HRPC cells, but not in non-HRPC cells. GBG exposure did not change these parameters in either cell type. Furthermore, preliminary data indicate that 3 and 6 μM CBD treatment increased mitochondrial reactive oxygen species (mtROS) production, decreased mitochondrial ATP production rate, and significantly decreases ($p<0.05$) mitochondrial membrane potential and mass in HRPC cells. High-resolution respirometry studies in permeabilized HRPC cells demonstrated that exposure to CBD (6 μM for 6h) reduces mitochondrial oxygen consumption, indicating the capacity of CBD to inhibit the mitochondria. Finally, again in HRPC cells, initial experiments ($n=2$) indicate that CBD (3-6 μM for 6h) modulated mitochondrial morphology and dynamics. Western blotting (Fis1 and MFN1 protein level) showed that CBD increased cellular capacity for mitochondrial fission in agreement with super-resolution confocal microscopy analysis. Furthermore, CBD induced significant modulation of specific oncogenic related signalling pathways in these HRPC cells (increased mRNA expression of HIF-1a, BNIP3, PTEN, AMPK/ULK-1, protein levels of PTEN and P53, and decreased protein levels of Akt).

Although these findings need to be confirmed, this study indicates novel metabolic targets in preclinical models of HRPC, and demonstrates how CBD, by targeting mitochondria, may modulate the oncometabolic regulation of these cells. These findings indicate that certain phytocannabinoids may offer an exciting potential therapeutic approach in the metabolic reprogramming of HRPC.

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THERAPEUTIC EFFECTS OF CANNABIDIOL IN COMBINATION WITH CHEMOTHERAPY IN PANCREATIC DUCTAL ADENOCARCINOMA

Nagina Mangal[1,2], Patrick Lawrence[2], Chanthirika Ragulan[2], Krisha Desai[2], Barbara Pacchetti[3], Vikash Reebye[1], Anguraj Sadanandam[2]* and Mikael H Sodergren[1]*

[1] Department of Surgery and Cancer, Imperial College London
[2] Systems and Precision Cancer Medicine Team, Division of Molecular Pathology, Institute of Cancer Research
[3] EMMAC Life Sciences, London, UK
*joint senior authors

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is highly complex and aggressive in nature. Those diagnosed with this lethal malignancy have a median 5 year-survival rate of 8%. Standard systemic therapy up until now has been limited to the use of nucleoside analogues such as gemcitabine or combinations drugs. There is an urgent need for novel therapeutics to improve treatment efficacy and clinical outcome. Recently, cannabidiol (CBD) has emerged as a potential agent with oncological effects. The aim of this study was to investigate whether CBD has potentiating effects with gemcitabine and nab-paclitaxel *in vivo*.

Methods: A mouse cell line derived from genetically engineered mouse model with mutations in *KRAS*, *INK4A* and *TP53* genes was injected (0.5×10^6 cells) subcutaneously in the right flank of 6-8 weeks old FVB/N mice after acclimatization. Post randomization, mice (N=10 per group) were treated with 100 mg/kg Cannabidiol alone (Adven150, EMMAC Life Sciences, London, UK) or in combination with 10 mg/kg Gemcitabine and 10 mg/kg Nab-paclitaxel; appropriate vehicle controls were maintained. Following end point (tumor burden or ulceration), animals were culled, tumors were harvested with one part prepared for flow cytometry staining and the remaining embedded in paraffin for further molecular analysis. Unpaired t-test with Welch's correction was used to compare vehicle and treated groups. A p-value of $p<0.05$ was considered statistically significant.

Results: CBD monotherapy did not show any significant effects on tumour burden or survival in our mouse model. However, a combination of CBD with gemcitabine and nab-paclitaxel, led to a significant reduction in tumour burden by 62.5% ($p=0.0006$) when compared to vehicle group along with a moderate survival benefit. Our flow cytometry data indicated a non-significant increase in $FOXP3^+$ (%$CD4^+$) T regulatory cell population and a decrease in $CD8^+$ T cells in tumours treated with CBD alone, suggesting an immune-suppressive tumour microenvironment. The decrease in tumour burden in the triple combination treated tumours was supported by a non-significant increase in $CD3^+$ T cells and PD-1^+ B cells and a decrease in $CD8^+$ T cells potentially leading to a pro-inflammatory anti-tumour activity.

Conclusions: Our study indicates that CBD in combination with gemcitabine and nab-paclitaxel offers therapeutic benefit in our *in vivo* model of PDAC. Further work will elucidate CBD mediated downstream biological pathways in sensitising the tumour to chemotherapy and immunotherapy. These findings will help to determine the mode of action, treatment dosing and the synergy as an adjunct for treating pancreatic cancer.

SUBCHRONIC TREATMENT WITH CANNABIDIOL REVERSED COMPULSIVE-LIKE AND ANXIOGENIC-LIKE EFFECT PROMOTED BY THE CONSUMPTION OF HIGH-REFINED CARBOHYDRATE DIET

Anna Paula Marçal*, Nícia Soares, Laila Asth and Daniele Aguiar

Pharmacology Department, Federal University of Minas Gerais, Brazil.

Introduction: Obesity and its comorbidities are a significant public health problem worldwide. The increase in ultra-processed food consumption, with low satiety capacity and high energy imbalance, is closely associated with its expansion. One main characteristic of obesity is the chronic low-intensity inflammatory state in white adipose tissue, which is a factor associated with the urgency of obesity and its comorbidities, such as type 2 diabetes and metabolic syndrome. Moreover, peripheral inflammation is also a characteristic of anxiety and compulsion disorders. In this sense, cannabidiol (CBD), the primary nonpsychoactive phytocannabinoid present in the Cannabis sativa plant, emerges as a potential therapeutic drug for treating psychiatric diseases. This study verified whether acute or subchronic treatment with CBD would attenuate the anxiety-like and compulsive-like behaviors observed after chronic consumption of a high-refined carbohydrate-rich (HC) diet in mice.

Methods: Balc/C mice received a control or HC diet for 12 weeks. The HC diet consisted of 40% condensed milk, 40% standard diet, 12% refined sugar, and 8% water. After the diet period, the animals were divided into vehicle and CBD (30mg/Kg) groups. The drugs were administered intraperitoneally in acute treatment, one injection thirty minutes before the behavioral tests, or sub-chronic treatment seven daily injections in the last week of diet treatment. The behavior tests employed were the Marble Burying test (MB) and Novel Suppressing Feeding test (NSF). Data were analyzed by two-way ANOVA followed by Bonferroni post-hoc tests ($p<0.05$ considered significant).

Results: The HC diet's chronic consumption induced an anxiogenic-like behavior in the NSF test ($F_{(1,43)}=4.37$; $p<0.05$ one-way ANOVA) and a compulsive-like behavior in the MB test ($F_{(1,25)}=4.86$; $p<0.05$ one-way ANOVA). The sub-chronic treatment with CBD (30 mg/kg) but not acute attenuated anxiogenic-like behavior ($F_{(1,24)} =5.86$; $p<0.05$ two-way ANOVA followed by Bonferroni) and compulsive-like behavior ($F_{(1,24)} =5.858$; $p<0.05$ two-way ANOVA followed by Bonferroni).

Conclusion: Our data reinforced the deleterious effects induced by chronic consumption of the HC diet in the compulsive and anxious behaviors and the use of CBD as a potential drug treatment. The mechanisms that account for the CBD effects in our model remains to be clarified.

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ANTITUMOR EFFECT OF THREE CANNABINOIDS ON LEUKEMIA THROUGH INHIBITION OF *NOTCH1* PATHWAY

Elazar Besser, Gil Moshe Lewitus, Hila Novak-Kotzer, Shiri Procaccia, Paula Berman, Inbar Shreiber-Livne, Yishai Ofran and David Meiri*

The Laboratory of Cancer Biology and Cannabinoid Research,
Faculty of Biology, Technion- Israel Institute of Technology, Haifa, Israel

Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic cancer, emerging from malignant T-cell progenitors. In humans, more than 50% of T-ALL cases exhibit auto-activation of Notch1 signaling, which results in oncogenic transformation and impaired apoptosis, thereby enhancing the malignant phenotype. In recent years, great strides were made in our understanding of the vast potential of phytocannabinoids, the active compounds of the Cannabis plant, in governing proliferation and apoptosis in cancer cells. As Cannabis is also a well-known modulator of the immune system, in this study we examined whether the antitumor effect of Cannabis can be harnessed to induce T-ALL elimination. We found a particular strain and identified the specific phytocannabinoids responsible for this effect, as well as the molecular mechanism by which these phytocannabinoids mediate their antitumor effects in this type of cancer.

Methods: Several T-ALL cell lines with different genetic mutations were challenged using numerous Cannabis extracts with varying phytocannabinoid profiles, and their viability was assessed. Both *in-vitro* and *in-vivo* experiments indicated that Notch1 mutated T-ALL cells are highly sensitive to a specific Cannabis chemovar. The effect of this Cannabis extract on the Notch1 signaling pathway was evaluated using western blot, quantitative real-time PCR, CRISPR gene deletion, luciferase reporter assays and RNA-seq. Finally, utilizing advanced biochemical and mass-spectrometry approaches, we succeeded in identifying and isolating three specific phytocannabinoids that in concert achieve the effect of the whole extract, and their ability to eliminate malignant T-ALL cells was shown by a series of *in-vitro* and *in-vivo* experiments.

Results: Here, we have identified a specific Cannabis extract that induces elimination of T-ALL cells by preventing Notch1 maturation. In turn, this leads to inactivation of Notch1 signaling and the induction of apoptosis. Three specific phytocannabinoids were isolated and purified from the Cannabis extract, together they had an effect as the whole extract in reducing cell viability, increasing apoptosis, reducing Notch1 intracellular domain (NICD) formation, and reducing tumor burden *in-vivo*. We identified the endocannabinoid receptors involved in the inhibition process and revealed the Notch1 upstream and downstream targets that take part in the mechanism of action.

Conclusions: Our results indicate that three specific phytocannabinoids have the capacity to strongly impair Notch1 signaling. These findings may pave the way for the establishment of a new pharmacotherapy for the treatment of T-ALL and additional Notch1-dependent cancers.

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TRANSFORMATION OF PHYTOCANNABINOIDS BY HEAT – ISOMERIZATION, DEHYDRATION, AND DERIVATIZATION OF CANNABINOIDS PRESENT IN *CANNABIS SATIVA* SMOKE

Christina Miyabe Shields

Real Isolates LLC, Boston, MA, USA

Introduction: Inhaling cannabinoids by smoking *Cannabis sativa* biomass, primarily flower, is the most common method of ingestion. Differences in the therapeutic effects of smoking cannabis versus ingesting cannabis edibles has been reported. These differences have been attributed to first-pass metabolism before distribution and variations in pharmacokinetics, but transformation of phytocannabinoids into active isomers and derivatives that are present in cannabis smoke may contribute to this phenomenon. This study investigates the transformation of phytocannabinoids by applied heat in environments that simulate the process of smoking.

Methods: Cannabidiol (CBD) isolate (>98% pure), high CBD hemp distillate, and hemp *Cannabis sativa* flower were placed in a small cylindrical glass chamber. The chamber was heated to temperatures between 200-500˚C and the smoke was passed through a selection media by vacuum flow. Compounds of interest were eluted from the filter and eluants were collected for analysis.

Results: Heating CBD isolate, high CBD hemp distillate, and hemp *Cannabis sativa* flower all resulted in dramatic changes in the total cannabinoid composition at temperatures relevant to smoking cannabis. One of the compounds of interest that was not present prior to the heating process was isolated and evaluated. The compound was identified as cannabinodiol (CBND), a dehydration product of CBD. CBND and other derivatives were not present in samples extracted using hexane or cryo-ethanol.

Discussion: Edible cannabis products are created from extractions that focus on preserving the primary phytocannabinoids present in the plant; the total cannabinoid composition present in cannabis smoke is substantially different. There are many variables that may contribute to the final cannabinoid composition in smoke such as temperature, pressure, heat source, gas composition, moisture content, ambient humidity, variations in pH, etc.

Conclusions: When smoking cannabis at high temperature, transformation of phytocannabinoids is significant. While the total activity of these cannabinoid compositions containing pyrolytic cannabinoids, or pyrocannabinoids, at endogenous targets is unclear, it is likely that their combined interactions contribute to the difference in effects between smoking cannabis and edible cannabis. A better understanding of these total cannabinoid compositions, especially the interaction of pyrocannabinoids, will aid in the understanding of the therapeutic potential of cannabis and the development of therapeutic cannabis products.

NETWORK-BASED ANALYSIS OF THERAPEUTIC TARGETS FOR CANNABIDIOL IN NEUROPATHIC COMPONENT OF OSTEOARTHRITIS

Jakub Mlost* and Katarzyna Starowicz

Department of Neurochemistry, Institute of Pharmacology,
Polish Academy of Sciences, Cracow, Poland

Introduction: Osteoarthritis (OA) is a chronic joint disease resulting in chronic pain development, which involves both nociceptive and neuropathic component. Unfortunately, the current understanding of OA pathophysiological mechanisms has not led to the development of disease-modifying drugs that are able to stop or slow down disease progression. Increasing attention is being paid to non-invasive strategies that may prevent further cartilage destruction and the role of the endocannabinoid system in the aforementioned treatment. Cannabidiol (CBD) has gained widespread acknowledgement and marketing success as safe and non-psychoactive cannabinoid. Much research has been conducted regarding CBD's mechanism of action, which revealed more than 60 molecular targets at the moment (summarized in [1]). The aim of the present study was to analyse the publicly available data in order to assess therapeutic potential of CBD in OA.

Methods: Molecular targets of CBD were established through literature search. Genes associated with osteoarthritis and neuropathic pain were obtained through Open Targets Platform. Venn analysis was performed to find common targets between CBD, neuropathic pain and OA. Subsequent analysis of protein-protein interactions and functional enrichment were performed with STRING and KEGG databases.

Results: Literature search allowed us to find 65 molecular targets of CBD, whereas Open Targets Platform provided 970 and 2473 genes associated with neuropathic pain and OA, respectively. Venn analysis revealed 22 common targets for CBD, OA and neuropathic pain. STRING database was able to cluster the targets as GABA receptors, TRP channels, GPCR receptors and enzymes involved in free fatty acid metabolism. Functional enrichments analysis revealed significant involvement of the detected targets in synaptic transmission, regulation of membrane potential, ion transport, inflammation and pain. Edge connectivity analysis revealed PPARγ and TRPA1 as the two most connected nodes with high affinity towards CBD.

Conclusions: Neuropathic pain management is considered as one of the most challenging issues in chronic pain treatment. Our analysis was based on publicly available data and allowed us to evaluate number of potential CBD targets involved in the neuropathic component of OA. These results imply CBD as potential adjuvant for existing OA treatment that could combat the neuropathic component of OA pain and PPARγ as the possible molecular target. Further experimental validation of our results is essential but if successful, it may prove our bioinformatic approach as the meaningful tool for prediction of drug-efficacy or the mechanism of action in the disease of interest.

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CANNABIDIOL AS A DISCRIMINATIVE STIMULUS: AN EXPLORATORY INVESTIGATION

Mohammed Mustafa*, Joel Schlosburg and Aron H. Lichtman

Department of Pharmacology & Toxicology,
Virginia Commonwealth University, Richmond, VA

The approval by the Food and Drug Administration of epidiolex (cannabidiol; CBD) for the treatment of severe seizure disorders has put this phytocannabinoid in the forefront of biomedical research. Other potential therapeutic applications of CBD supported by preclinical research, human studies, and anecdotal reports include pain, inflammation, and anxiety. The proven and purported therapeutic effects of CBD may be mediated by a diversity of pharmacological targets (e.g., 5-HT1a, GPR55, PPARγ) as well as within the endocannabinoid system (FAAH inhibition, CB_1 allosteric modulation). Traditionally referred to as a non-psychoactive compound, users report subjective effects such as feeling "different" or "relaxed" (Spindle et al. 2020). Although CBD does not substitute for delta-9-tetrahydrocannabinol or other CB1 receptor agonists in the drug discrimination paradigm, an operant assay used to evaluate subjective effects of CNS active drugs, no published reports of which we are aware have examined whether CBD produces a subjective stimulus of its own. Here, we test whether mice can learn to discriminate CBD from vehicle. An initial experiment in food-restricted C57BL6/J male mice trained to nose-poke on a fixed-ratio of 10 (FR10) for food reinforcement found that CBD (30-100 mg/kg) did not produce rate suppression. Subsequently, a training dose of 100 mg/kg CBD was selected in which male C57BL6/J mice are being trained to discriminate this drug from vehicle. Correct responses (FR10) on treatment-associated apparatus' result in food reinforcement. An ongoing experiment shows that mice select the injection appropriate apparatus with an average of 80% or greater correct responding. However, the first food reinforcement (FFR) accuracy of each mouse is ~50%, suggesting that the drug's subjective effects are not driving the operant responses. Therefore, on day 75 of training, we increased the dose of CBD to 200 mg/kg, with the aim to increase the FFR accuracy. This presentation will provide an update on whether CBD produces a reliable discriminative stimulus determined by passing training criteria (correct FFR, ≥ 80% treatment-associated responses) on 9 of 10 consecutive sessions. An outcome in which subjects learn to discriminate CBD from vehicle would provide a powerful in vivo approach to study both the pharmacology and underlying mechanisms of action of this drug.

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DISTINCT CANNABINOIDS ALTER HUMAN TH17 CELLS DIFFERENTIATION AND FUNCTION

Hila Novak-Kotzer*, Ronen Rosenblum, Yara Eid Mutlak and David Meiri

The Laboratory of Cancer Biology and Cannabinoid Research, Faculty of Biology, Technion- Israel Institute of Technology, Haifa, Israel

Introduction: Pro-inflammatory IL-17-producing T cells termed Th17 are actively involved in the pathogenesis of several autoimmune pathologies. Cannabinoids and endocannabinoids have been shown to exert potent anti-inflammatory activities in various experimental models of inflammation diseases, yet the mechanisms involved are still to be understood. The main psychoactive cannabinoid, Δ-9-tetrahydrocannabinol (THC), and the main nonpsychoactive cannabinoid, cannabidiol (CBD) were shown to down regulate the production and secretion of IL-17 but not to affect the levels of the proinflammatory cytokines TNFα and IFNγ.

Methods: By using a combination of variety of molecular techniques and cell-based functional assays we investigated the effect of different high CBD Cannabis extracts and specific cannabinoids on controlling of human Th17 and other T cell subsets activation and differentiation.

Results: Our work shows that the presence of specific cannabinoids markedly alters the ability of CD4 T cells to differentiate into Th17 cells. This results in a reduction of Th17 cells frequencies as well as its function. The extract showed no effect on cells viability. This work provides previously unidentified insights on the molecular mechanism of Cannabis-mediated modulation of the immune response in human CD4 T cells.

Conclusions: The study provides an important input to our understanding of molecular mechanisms of Cannabis treatment in humans and may aid for the design of safer and more effective Cannabis-based therapy of autoimmune diseases and other T cell mediated immune responses.

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THE EFFECTS OF CBD AND TMP COADMINISTRATION IN CANCER

Ryan F Maguire[1], Timothy J England[1] and Saoirse E O'Sullivan*[2]

[1]School of Health and Medicine, University of Nottingham, UK
[2]Artelo Biosciences, 888 Prospect Street, Suite 210, La Jolla, CA 92037, USA

Introduction: Cannabidiol **(**CBD) and tetramethylpyrazine (TMP) both display anti-cancer properties in *vitro* and *in vivo*. However, both suffer from poor bioavailability when administered orally. Co-crystallisation of CBD and TMP (ART12.11, Artelo Biosciences) could confer the new crystal form with a more desirable pharmacokinetic profile, with the added potential for positive pharmacodynamic interactions. To that end, in this initial study, the effects of CBD and TMP coadministration were investigated in cellular models.

Methods: Experiments were performed in proliferating and confluent CaCo2 (colorectal), SKOV-3 (ovarian) and DU145 (prostate) cancer cells (n=8). The metabolism of resazurin to resorufin was measured as a marker of cell viability. Scratch assays were performed on 24h serum starved (1%) SKOV-3 and DU145 cells at 70% confluence as an assay of cell migration. Percentage area closure at 24 and 48h was calculated as 100*value/baseline. Data was analysed using an unpaired t-test for IC_{50} values or one-way ANOVA with multiple comparison for all other data. Data is presented as mean ± standard error of the mean (SEM).

Results:

1. In proliferating CaCo2, DU145 and SKOV-3 cells, CBD caused a concentration-dependent decrease in resazurin metabolism (48h; IC_{50} = 18.5µM ±1.2, 9.5µM ±0.2 and 11µM ±0.4 respectively), indicating reduced cancer cell proliferation. TMP also reduced the metabolism of resazurin in all cell types. CBD in combination with TMP increased the potency of CBD, reducing the IC_{50} by -6.µM ±1.3 ($p<0.001$) in DU145 and by -9µM ±1.1 ($p<0.001$) in SKOV-3 cells.
2. In confluent cancer cells, CBD reduced resazurin metabolism (144h, repeated treatments, 10-20 µM). This cytotoxic effect of CBD was not affected by TMP, except at 15 µM, where the cytotoxic effects of CBD were enhanced in DU145 by 5% ±1.7 ($p<0.01$) and in SKOV-3 cells by 27% ±8.1 ($p<0.01$).
3. In scratch assays, CBD at 10µM and 20µM (48h) caused a reduction in the rate of wound closure in DU145 cells and SKOV-3 cells, indicating reduced migratory effects of the cancer cells. The presence of TMP had no effect on the anti-migratory effect of CBD at 10µM, but caused a small but significant reduction in the effect of 20µM CBD, by 23% ±2.6 ($p<0.05$) in DU145 cells and by 28% ±3.67($p<0.0001$) in SKOV-3 cells. CaCo2 cells did not possess enough migratory capacity to carry out this assay.

C**onclusion:** In proliferating and confluent cancer cells, the combination TMP and CBD increases the anti-cancer effects of CBD, particularly in ovarian cancer cells. However, CBD's anti-migratory effects were slightly attenuated by TMP at higher doses. Future work is required to test the CBD:TMP co-crystal *in vivo* in cancer models to establish whether further potential pharmacodynamic and pharmacokinetic interactions occur.

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IN VITRO CYTOTOXICITY OF CANNABINOIDS IN COMBINATION WITH COMPONENTS OF CHOP REGIMEN AGAINST NON-HODGKIN LYMPHOMA

Saba Omer*, Satyanarayana Pondugula, Muralikrishnan Dhanasekaran, Mansour Mahmoud, Brad Matz and Dawn Boothe

Department of Biomedical Sciences, Auburn University, Al, USA

Introduction: Cannabinoids are known for their palliative effects against chemotherapy-induced side effects (e.g. pain, nausea, vomiting, and loss of appetite) and some of the cannabinoids are currently FDA approved for the treatment of chemotherapy-induced side effects in cancer treatment. However, in the last two decades, various in vitro and in vivo studies have also demonstrated their effect against tumor growth and progression in many models of human cancers including non-Hodgkin lymphoma (NHL) and mantle cell lymphoma. Previously, we demonstrated the cytotoxic effect of endogenous and exogenous cannabinoids on human and canine B cell and T cell type NHL cell lines. The purpose of this study was to demonstrate the cytotoxic effect of cannabinoids in combination with the components of CHOP, a traditional NHL chemotherapy regimen. (cyclophosphamide, doxorubicin, vincristine, prednisone, lomustine). We hypothesized that the synergistic combination of cannabinoids and components of CHOP chemotherapy drugs might be more effective against cancer cells compared to their alone treatments.

Methodology: For this study, three cannabinoids were studied, one from each of the three major categories of cannabinoids (endocannabinoid AEA, Phytocannabinoid CBD, and synthetic cannabinoid WIN-55 212 22). Each cannabinoid was selected based on potency as determined in our previous experiments. For the combination, we have used five NHL chemotherapy drugs. We used canine malignant B type NHL cell line 1771, cells were cultured in RPMI. The cytotoxicity of each drug alone and combinations was analyzed by colorimetric MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide) cell proliferation assay and Combination index (CI) analyses based on the Chou-Talalay method was used to study the combinational effect.

Results: Our results demonstrate that the cytotoxic effects of all traditional NHL chemotherapy drugs are synergistically enhanced (interaction with CI <1) by each of the three cannabinoids when added to 1771 canine malignant B type NHL cells.

Conclusion: This work provides proof-of-concept for using cannabinoids and traditional NHL drugs in combination to reduce the dose, and therefore the toxicity, of chemotherapeutic drugs and possibly increasing the survival benefit in lymphoma clinical translation studies.

Acknowledgments: We are grateful to Dr. Steven Suter, North Carolina State University for sharing canine lymphoma cell lines.

CANNABIGEROL AND Δ^9-TETRAHYDROCANNABINOL FROM *CANNABIS SATIVA* INTERACT WITH ADDITIONAL PHYTOCANNABINOIDS FOR CYTOTOXIC AND CELL MIGRATION INHIBITORY ACTIVITY ON HUMAN GLIOBLASTOMA CELL LINES *IN-VITRO*

Hadar Peeri*[1,2], Nurit Shalev[2], Ajjampura C Vinayaka[2], Dvora Namdar[2], Seegehalli M Anil[2], Eduard Belausov[2] and Hinanit Koltai[2]

[1]The Mina and Everard Goodman Faculty of Life Sciences,
Bar-Ilan University, Ramat Gan, Israel
[2]Institute of Plant Science, Agriculture Research Organization,
Volcani Center, Rishon LeZion, Israel

Introduction: Glioblastoma multiforme (GBM) is one of the most lethal cancers. Despite aggressive multidisciplinary treatment, the median survival rate for diagnosed patients is 15 months and have shown no significance improvement in the last three decades. Therefore, new therapeutic strategies are urgently needed to improve the prognosis and quality of life of GBM patients. *Cannabis sativa* is used for the treatment of different medical conditions. Around 150 phytocannabinoids were identified in *C. sativa*. The phytocannabinoids Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) trigger GBM cell death. However, the combinations of cannabis molecules that may have optimal anti-GBM activity are unknown. This study aimed to identify active compounds from cannabis extract and to assess respective cytotoxic and anti-migration activity against GBM cells.

Methods: The cytotoxic activity of *C. sativa* extract and fractions was determined by XTT assay on A172 and U87 cell lines. Chemical content was determined by high-performance liquid chromatography (HPLC) and Gas chromatography mass spectrometry (GC/MS). Apoptosis and cell cycle were determined by Fluorescence-activated cell sorting (FACS) and F-actin structures by confocal micropscopy. Cell migration and invasion were determined by scratch and transwell assays, respectively. 3D structures were printed in extracellular matrix. Gene expression was determined by quantitative PCR. Data were analyzed by Tukey–Kramer test or Student's T-Test ($P \leq 0.05$) using the JMP statistical package and considered significant when $P \leq 0.05$.

Results: Fractions of extract from a high-THC cannabis strain had significant cytotoxic activity against GBM cell lines. Standard mix (SM) of the active fractions F4 and F5 induced apoptosis and expression of endoplasmic reticulum (ER)-stress associated-genes. Addition of CB2 receptor Inverse agonist led to a significant reduction in F4 and F5 cytotoxic effect and ER-stress genes expression. F4 and F5 inhibited cell migration and invasion, altered cell cytoskeleton and inhibited colony formation in both 2D and 3D models.

Conclusions: Combinations of cannabis compounds identified here convey both anti-proliferative and anti-migratory effect on GBM cell lines. Activity of F4 and F5 was higher than that of the crude extract, and their SMs were more cytotoxic than the corresponding fractions. Moreover, the fractions were highly effective in inhibiting cell motility and cell invasion. The formulations of active cannabis compounds may lead to new cannabis-based therapies and should be further examined for efficacy on GBM in pre-clinical studies and clinical trials.

CLASSIFICATION OF MEDICAL CANNABIS STRAINS FOR TREATMENT OF PAIN AND CONVULTIONS BY ZEBRAFISH MODEL

Paula Adriana Pitashny*[1,2], Ram Harari[2], Yoni Bar On[1,2] and Karen Jackson[1,2]

[1]Tel Hai College, Nutrition and Food Sciences Departments, K. Shmona, Israel
[2]Migal Research Institute, Human Nutrition and Health, K. Shmona, Israel

Introduction: The zebrafish (*Danio rerio*) larvae had become a popular organism in neuroscience research for modeling brain diseases. The larvae small size contributes to an efficient, rapid, and cheap method to screen large numbers of chemicals trough behavioral assays and is considered the only suitable vertebrate model for high throughput drug or medicinal plants screening. Medicinal Cannabis (MC) become a complementary therapy for several health problems such as anxiety, pain, post-traumatic stress disorder, sleep disorders, convulsions, tremors in Parkinson's disease and autism. Since the zebrafish larvae present the endocannabinoid system (ECS) in its whole showing the same neurotransmitters and receptors as mammals, this biological model is an appropriate tool for the detection and classification of different MC strains for different conditions. In the present work, we studied the effect of different MC strains with different concentrations of cannabinoids on pain and convulsions.

Methods: Pain assay-Six days-postfertilization zebrafish larvae in a 96-well plate were exposed to increasing doses of MC extracts of different strains or analgesic drug (Dipyrone-96 mM). The larvae were then exposed to a painful stimulus (high temperature), which caused an increase in motor activity. Convulsion assay- Six days-postfertilization zebrafish larvae in a 96-well plate were exposed to increasing doses (0-10μM/ml) of MC extracts of different strains or anticonvulsant drug valproic acid (VPA-5mM). The larvae were then exposed to the proconvulsant pentylenetetrazol (PTZ-15mM), which caused an increase in swimming distance. The change in motor activity and swimming distance in both assays were measured using a Danio vision larva tracking system, analyzed by EthoVision XT video tracking software and expressed as a motion index.

Results: Specific cannabis strains lowered the motor activity to control levels.

Conclusions: In conclusion, the zebrafish model could be an appropriate tool to identify effective MC strains for the treatment of pain and could be used for screening of different strains and further for clarifying the MC mechanism of action related to the analgesia.

COMBINED ISOLATED CANNABINOIDS AS A POTENTIAL TOOL FOR WOMEN'S HEALTH INDICATIONS

Prutchi Sagiv Sari*[1,2] and Maor Yehoshua[2]

[1]Gynica; [2]Lumir Lab

Introduction: There is an abundance of anecdotal evidence suggesting that medical cannabis has immense therapeutic potential in women's health. Ancient references found in Mesopotamia and China mention the use of Cannabis for menstrual pain, bloating, abnormal bleeding, menopausal symptoms and urinary tract infections.

Currently, much light has been shed on the mechanism of action on how the endocannabinoid system (ES) affects specific mechanisms that are critical to disease establishment and maintenance, especially the ES modulation of pain and inflammation.

Gynica is set to searching for new ways to address these conditions and is focusing currently in several indications with high unmet need such as dyspareunia (painful sex), dysmenorrhea (painful menstruation) and endometriosis. Importantly, these indications do not have currently many viable treatment options available.

Gynica is developing optimal cannabinoid combinations and proprietary delivery systems that will address the unmet needs in women's health diseases. Our development team focused on in vitro techniques aiming at evaluating the role of phytocannabinoids in different combinations and concentrations.

Methods: We evaluated the anti-inflammatory effects of different cannabinoid isolates (including CBD, THC, CBG, CBC and their carboxylated forms), terpenes (including beta myrcene and beta caryophyllene) and flavonoids as well as several specific combinations in monocyte/macrophage cell lines (murine RAW 264.7 and human THP-1) and endometriosis cell lines (Z11 and Z12). Parameters including cell viability, nitric oxide (NO), COX1 and COX2 and interleukin (IL)-6, are hallmarks of these diseases and were evaluated. In addition, migration and invasiveness of endometrial cells into an agarose spot.

Results: Main results include:

1. Synergistic effect of THC and THCA in reducing NO, COX1 and COX2 expression;
2. Significant anti-inflammatory effects of the flavonoid apigenin;
3. Significant effects of the terpenes and flavonoids in reducing NO;
4. We demonstrate the entourage effect between major and minor cannabinoids as compared to major cannabinoids alone;
5. Detection of synergistic effect between cannabinoids and terpenes in reducing all inflammatory parameters evaluated;
6. Two particular combinations of the plant compounds above result in a superior efficacy in all models evaluated.

Conclusions: Particular combinations of cannabinoids and their respective carboxylated forms, as well as terpenes and/or flavonoids, are highly effective as anti-inflammatory and pain reducing agents. Main results show: reducing interleukin-6 (IL-6) secretion, reducing cyclooxygenase (COX) expression, and reducing nitric oxide (NO) production, which represent a potential tool to specifically treat gynecological conditions.

BEHAVIORAL AND HISTOLOGICAL EFFECTS OF VAPORIZED FULL-SPECTRUM CBD EXTRACT

Rizelle Mae C. Rose*, Maria Rivera-Garcia and Adrianne R. Wilson-Poe

Legacy Research Institute, Portland, OR, USA

Introduction: One of the most common ways of cannabinoid delivery used by the population is through vaping (inhaling cannabis oil vapor). The majority of preclinical studies about vaping uses propylene glycol (PG), vegetable glycerol (VG), or a combination of both (PGVG) as their control, despite being linked to increased lung inflammation after chronic exposure. The use of full-spectrum CBD extract (hemp oil which contains CBD and other cannabinoids and compounds) in vaping products is widely popular, but studies of its effects in animals or humans is lacking. In the current study, we aim to compare the behavioral and histological effects of hemp oil and PGVG, focusing the effects on female as CBD is widely used for pain relief for its anti-inflammatory properties, and women are disproportionately affected by chronic pain. We explored the effects of these proposed vehicles through the tetrad test, a morphine dose-response test, and compared lung histology after chronic exposure to vapor.

Methods: Female Long Evans rats were divided into 4 treatment groups (saline injection with PGVG vapor, saline injection with hemp oil vapor, morphine injection (5mg/kg) with PGVG vapor, and morphine injection with hemp oil vapor) and had 30-minute twice daily vapor exposure for 2 days. The tetrad test was conducted at baseline, after treatment 1, and after treatment 4. On Day 3, the rats underwent a morphine dose-response test on thermal antinociception. Another cohort of female Long Evans rats were divided into 2 treatment groups (PGVG vapor and hemp oil vapor) and had 30-minute twice daily vapor exposure for 20 days. Body temperature was monitored daily, and lung tissue was collected for histology after the last exposure. Data were analyzed by two-way ANOVA.

Results: As expected, thermal antinociception was observed in morphine, but not saline-treated animals, but there was no significance between the PGVG exposed groups to the hemp exposed groups. Repeated morphine administration produced tolerance to locomotor sedation effects of morphine in both PGVG and hemp exposed animals. Cold and mechanical allodynia tests showed inconclusive results, which was not entirely unexpected given that these behavioral assessments are better suited to chronic pain models. Hypothermia was not observed in either PGVG or hemp vapor groups. Despite trace amounts of THC in the full-spectrum hemp vapor, catalepsy was not observed in any of the groups. There was no overall significant difference between PGVG vapor and hemp oil vapor; that is, full-spectrum hemp oil did not produce significant tetrad or morphine interaction results. Lung histology showed similar vascular/alveolar surface ratio for both chronic PGVG and hemp oil vapor exposure, which presented no pathology.

Conclusion: Because hemp vapor produced comparable behavioral effects to PGVG, and did not produce histopathology, it suggests that preclinical studies of inhaled vaporized cannabis could use this substance as more clinically relevant control excipient. These results will facilitate future studies of pain-relieving effects of THC, by allowing us to examine the so-called “entourage effect” or potentiated effects of THC in combination with CBD and other molecules found in full-spectrum cannabis oil.

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PHYTOL, NOT PROPYLENE GLYCOL, CAUSES SEVERE PULMONARY INJURY AFTER INHALATION DOSING IN SPRAGUE-DAWLEY RATS

Daniela Schwotzer*[2], Tim Lefever[1], Jake McDonald[2], Kristen Trexler[1], Marcel Bonn-Miller[1] and Mark Ware[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario, Canada.
[2]Lovelace Biomedical, Albuquerque, New Mexico, USA.

Introduction: The use of vaping pens for inhalation of cannabinoid derived products is rising and has become a popular alternative to smoking combustible products. For efficient product delivery, compounds such as Phytol or Propylene Glycol (PG) are sometimes added to vaping pens as thinning agents. This study aimed at comparing Phytol and Propylene Glycol with respect to potential toxicity and safe use in vaping products.

Methods: This study was designed as a standard 14-day rat nose-only inhalation study aimed at investigating potential adverse effects of Phytol and PG. Male and female Sprague Dawley rats were exposed to 5 mg/L of Phytol or PG for up to 6 hours over up to 14 days and monitored for clinical signs and changes in body weight. Gross necropsy and histopathology of respiratory tissue was performed to assess potential adverse effects. Dose levels were regulated by exposure duration and selected to cover a range from 30 minutes to 6 hours, in line with OECD recommendations for repeated dose inhalation studies (OECD, 2009a, OECD 2009b). As PG appears safe for human consumption (Cotta et al. 2017, Stratton et al. 2018), the highest feasible doses were selected. For proper comparison of the two compounds similar aerosol concentration and exposure durations were targeted for Phytol, given the limited research on Phytol inhalation.

Results: Phytol exposed animals expressed severe clinical signs, body weight loss, and mortality after one or two exposure days, leading to termination of all dose groups for this compound. Lung weights were increased, and respiratory tissue was severely affected, demonstrating dose-responsive tissue degeneration, necrosis, edema, haemorrhage, and inflammation. A low observed adverse effect level was determined for Phytol at ≤ 109.0 / 10.9 mg/kg/day presented/deposited dose, indicating that its use as an excipient in vaping products is not recommend; a safe exposure range was not established. Propylene Glycol exposed animals did not show any adverse reactions after 14 days of high dose exposure and was considered safe with a no observed adverse effect level at a 1151.7/115.2 mg/kg/day presented/deposited dose in rats.

Conclusion: This study demonstrated that, in high dose rodent studies, PG causes minimal biological response while Phytol shows severe pulmonary toxicity. Since the same aerosol characteristics used for PG were targeted for Phytol, the effects observed in this study correspond to higher than expected human doses. It is also unclear in how well rodent inhalation effects translate to human exposure. However, the severity of effects after single dose exposure raises concern for the safe use of Phytol in humans.

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THE ANTI-TUMOR EFFECTS OF CANNABIS EXTRACTS ON FAP SYNDROME AND COLORECTAL CANCER

Lior Spektor*, Liat Rahamim-Ben Navi, Meshi Sadot and David Meiri

Technion – Israel Institute of Technology, Israel

Introduction: Familial adenomatous polyposis (FAP) syndrome, a genetic disorder caused by a germline mutation in the adenomatous polyposis coli (APC) gene, is characterized by numerous adenomatous polyps affecting individuals from a young age. Unless detected and managed at early stages, FAP patients will certainly develop colorectal cancer (CRC). CRC is one of the leading causes of cancer-related death; therefore, developing treatments that prevent polyp proliferation and induce their regression is needed. Interestingly, according to hospitals' observational cases, Cannabis may reduce the number of polyps in FAP patients. Consequently, phytocannabinoids, the active metabolites of the Cannabis plant, present such potential new therapy. Therefore, we set out to examine the anti-tumor effects of different Cannabis extracts in different FAP models.

Methods: The anti-tumor effects of various Cannabis extracts were assessed *in-vitro* on a human cell line (LS1034 cells), *in-vivo* on transgenic mice with a mutation in the APC gene ($APC^{Min/+}$ mice), and *ex-vivo* on intestinal organoids derived from $APC^{Min/+}$ mice; using cell viability, biochemical, and immunofluorescence tools.

Results: LS1034 cells treated with high CBD extracts were significantly less viable than untreated cells or cells treated with high THC extracts, and this effect was mediated mainly via the CB2 receptor. Furthermore, high CBD extracts induced cell death on LS1034, but not on normal human colon (CCD841 cell line). We have also established an *in-vivo* mouse model of FAP syndrome and used it to derive $APC^{Min/+}$ organoids (from $APC^{Min/+}$ mice). The anti-tumor effects of the high CBD extracts were also observed *ex-vivo*, as treated $APC^{Min/+}$ organoids displayed elevation in the protein level of cleaved caspase-3 and a decrease in the proliferative marker Ki-67, alongside a reduction in organoids size, compared to untreated $APC^{Min/+}$ organoids or organoids treated with high THC extracts. In addition, high CBD (but not high THC) extracts inhibited the Wnt/β-catenin signaling pathway in both LS1034 cells and $APC^{Min/+}$ organoids, as was observed by the significant reduction in the protein levels of active β-catenin, HES1, and C-Myc.

Conclusion: Based on the *in-vitro* and *ex-vivo* results, our findings suggest that Cannabis-based treatment has the potential to serve as a preventive therapy for colorectal polyps development. The $APC^{Min/+}$ mice will be used to assess the anti-tumor effects of different high CBD extracts in various doses.

CANNABIDIOL EFFICACY TO IMPROVE VOCAL RECOVERY IS ASSOCIATED WITH ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY

Mark A. Tripson*, Katherine N. Johnson and Ken Soderstrom

Department of Pharmacology and Toxicology, The Brody School of Medicine at East Carolina University, Greenville, NC, USA

Introduction: The non-euphorigenic phytocannabinoid cannabidiol (CBD) has been used successfully to treat childhood epilepsy, a condition associated with delayed development that includes vocal communication. Translational to human speech, zebra finch song is a complex behavior learned during a sensitive period of vocal development. Like language, quality of adult zebra finch song is maintained through continuous sensorimotor refinement involving multiple circuits controlling vocal learning and production. Song syntax and phonology slowly degrade following deafening (that interferes with sensorimotor maintenance) and are rapidly, but transiently disrupted following partial lesions of a vocal motor cortical region called HVC (proper name). We found previously that CBD both reduces the magnitude of lesion-related song pattern disruption, and speeds vocal recovery. Because in other systems CBD has anti-inflammatory and antioxidative effects, we suspected that similar processes may be important to vocal recovery. We therefore tested the hypothesis that expression of inflammatory, and anti-inflammatory mediators, as well as oxidative stress were altered following lesions in a manner opposed by CBD treatments.

Methods: Adult songbirds were given once daily injections in a volume of 50 µl IM to pectoralis. Six daily treatments were given prior to surgical procedures, followed by two daily postoperative treatments. Microlesion surgery targeted HVC unilaterally for partial ablation (about 6% of its volume). Treatments included vehicle or 10 mg/kg/day CBD (>99% pure CBD provided by GW Research Ltd, Cambridge, UK). Brain tissue dissection of two cortical vocal motor (HVC & RA) and two cortical-striatal learning regions (lMAN & Area X) were performed using a sterile micro punch and followed with Trizol RNA extraction and oligo-dT-primed cDNA synthesis. Expression of inflammatory (IL-1β, IL-6, TNFα), and anti-inflammatory mediators (IL-10), as well as oxidative stress (SOD2) were measured using qRT-PCR. Data were analyzed by two-way ANOVA followed by Sidak's post-hoc tests ($p<0.05$ considered significant).

Results: Microlesion surgeries induced expression of inflammatory mediators in lesioned hemispheres without contralateral effects. CBD significantly reduced lesion-related gene expression within the vocal motor regions. CBD also increased expression of anti-inflammatory IL-10 selectively within vocal motor regions. Additionally, the mitochondrial stress marker SOD2, was distinctly elevated following microlesions within lMAN, HVC and RA, but not Area X, and this was also significantly reduced by CBD treatments.

Conclusions: Using a songbird pre-clinical animal model we have found that CBD-improved vocal recovery involves reduced expression of inflammatory cytokines, markers of mitochondrial stress and increased expression of anti-inflammatory IL-10. These effects were found within distinct regions of circuits controlling vocal learning and production. These results will guide investigation and determination of circuit- and region-specific neuroplastic changes responsible.

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TREATMENT OF SEB-INDUCED ARDS WITH CBD AMELIORATES FATAL INFLAMMATORY RESPONSE

Kiesha Wilson*, Muthanna Sultan, Alkeiver Cannon,
Prakash S. Nagarkatti and Mitzi Nagarkatti

Department of Pathology, Microbiology and Immunology,
University of South Carolina School of Medicine, Columbia, SC, USA

Background: The novel SARS-CoV-2 virus known to cause the COVID-19 outbreak has claimed the lives of over 240,000 Americans and 1,270,000 people worldwide, with cases consistently rising daily. Thus, understanding the mechanisms behind this disease are vital at this time. With the absence of appropriate research infrastructure to handle the virus and a refractoriness of rodents to this disease, Staphylococcus enterotoxin B (SEB)-induced Acute Respiratory Distress Syndrome (ARDS) model mimics the cytokine storm and fatality presented in patients with severe COVID-19.

Methods: When C3H/HeJ mice were exposed to a dual dose of SEB, their survival dropped to 0% in ~5 days. In this study, we administered cannabidiol (CBD) intraperitoneally for 3 days pre- and post-SEB dosing and found that the survival rate increased to 100% indefinitely.

Results: Initial evaluation of scRNASeq data from lungs comparing naïve to SEB-induced ARDS mice illustrated an increase in neutrophils, inflammatory macrophages, and a loss in lung epithelial cells in the latter group. When evaluating the effect of CBD treatment on SEB-induced ARDS, we were able to demonstrate that CBD reduced the macrophage population. To characterize the mechanism by which CBD treatment ameliorated the inflammatory response, we found that CBD treated mice had significant reduction in TNF-α and IL-1β. The expression of these cytokines is directly associated with the presence and activation of inflammatory macrophages and neutrophils presented in ARDS. MicroRNA microarray differential expression analysis showed a significant increase in the expression of mmu-miR-124-3p, mmu-miR-21a-5p, and mmu-miR-140-5p with CBD treatment. Ingenuity Pathway Analysis (IPA) indicated that mmu-miR-21a-5p targets IL-1β, and mmu-miR-140-5p targets TNFα, while mmu-miR-124-3p targets both IL-1β and TNFα. The miRs were also implicated in pathways associated with respiratory disease and inflammation.

Conclusions: This finding offers insights for the development of preventive and therapeutic strategies in the treatment of ARDS, including that induced in COVID-19.

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THE EFFECT OF CANNABIS ON MYELOID-DERIVED SUPPRESSOR CELLS IN MURINE MELANOMA

Iris Wyrobnik*, Hila Novak-Kotzer and Dedi Meiri

Biology Faculty, Technion - Israel Institute of Technology, Haifa, Israel

Introduction: During solid tumor progression, the tumor microenvironment evolves into a highly immunosuppressive milieu that enables continuous growth and metastatic spreading. Key players in establishing this immunosuppressive environment are the immature myeloid cells called Myeloid-derived suppressor cells (MDSC), who are categorized in mice into monocytic-MDSC (MO-MDSC) and polymorphonucler-MDSC (PMN-MDSC). MDSC are recruited, polarized and activated through tumor secreted cytokines and chemokines, such as colony-stimulating factor 1 (CSF1), MCP-1 and MIP-2. One promising strategy in targeting MDSC is the depletion of such tumor secreted cytokines to achieve a reduction in MDSC recruitment and activation. A few studies focused on the effect of single cannabinoids on MDSC, such as (-)-Δ^9-trans-tetrahydrocannabinol (Δ^9-THC) or cannabidiol (CBD), however, the effect of cannabinoids and whole cannabis extracts on the MDSC population in a tumor model has not yet been studied.

Methods: To investigate MDSC frequency *in-vivo*, murine melanoma cells (B16F10) were injected subcutaneously into wildtype C57BL/6 mice. Cannabis extract or solvent control were injected intraperitoneally and MDSC frequency was measured in tumors using flow cytometry. Cytokine and chemokines in cell supernatants were measured using a cytokine array kit and ELISA. Cytokines and chemokines concentrations in tumors were detected by ELISA. Cannabinoid receptors expression in murine and human skin cancer cell line (A375) were detected using Real Time-PCR.

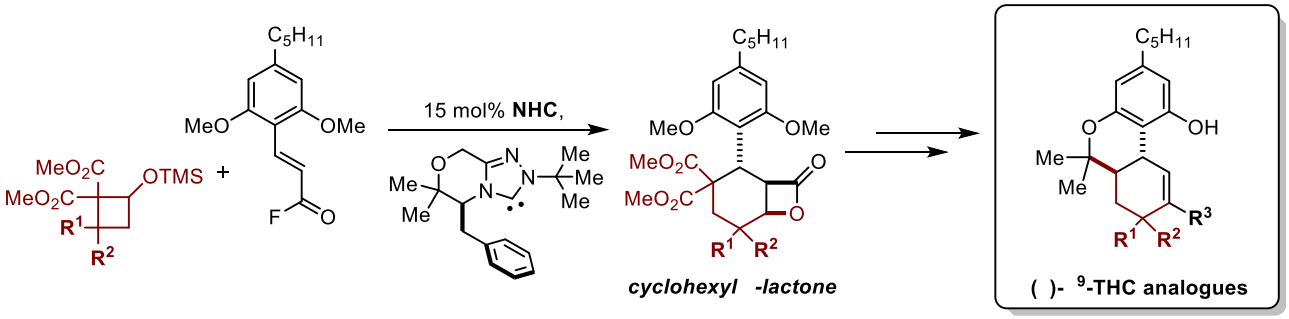
Results: CAN treated mice showed a reduction of MO-MDSC percentages and an increase of PMN-MDSC percentages in tumors compared to control treated mice. Additionally, we identified the reduction of the several cytokines and chemokines *in-vitro*. Validation of the myeloid-related cytokines and chemokines in tumors and in cell supernatants showed the specific reduction of CSF1. Moreover, using the fractionation method previously developed in our lab, we identified a specific single cannabinoid out of the cannabis extract that was shown to most efficiently reduce CSF1 secretion by B16F10 and A375 cells *in-vitro*. Further analysis of the relevant cannabinoid receptor showed high expression of TRPV2 in B16F10 and A375 cell lines, suggesting its involvement in the reduced CSF1 secretion.

Conclusions: Our results show that a specific single cannabinoid reduces the secretion of CSF1 by melanoma cells via TRPV2 activation. This reduction results in a decrease in MO-MDSC cells frequency and an increase in PMN-MDSC cells frequency in the tumors. This shift within the MDSC subpopulations might reduce the immune suppressive tumor microenvironment leading to decreased tumor progression and reduced metastatic spreading.

ENANTIOSELECTIVE SYNTHESIS OF (−)-Δ^9-THC AND NOVEL C8-SUBSTITUTED (−)-NORMETHYL-Δ^9-THC ANALOGUES

Adam Ametovski*,[1,2] Simon Cromwell,[1] Hayley Wilson,[1] Charlotte Fletcher,[2] Samuel Banister,[2] David Finlay,[3] Michelle Glass[3] and David W. Lupton[1]

[1] Department of Chemistry, Monash University, Clayton, Victoria 3800, Australia
[2]Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, The University of Sydney, Camperdown, NSW 2050, Australia
[3]Department of Pharmacology and Toxicology, The University of Otago, Dunedin 9016, New Zealand



Introduction: From a medicinal chemistry perspective, significant attention has been directed towards exploring Δ^9-tetrahydrocannabinol (Δ^9-THC) analogues substituted at the C5' aromatic position, and to a lesser extent, the C9 methyl group. Conversely, little is known about the structure-activity relationship of analogues bearing C8 substitution, highlighting the need to employ new synthetic methods to access such analogues in order to explore their pharmacology.

Methods: An enantioselective synthesis of (−)-Δ^9-THC, (−)-normethyl-Δ^9-THC and series of novel C8-substituted analogues was realised by utilising a new NHC-catalysed (4+2) annulation of donor-acceptor cyclobutanes and α,β-unsaturated acyl fluorides. All compounds were evaluated for binding and functional activity at human CB_1 and CB_2 receptors using radioligand competition and fluorescence-based plate reader membrane potential assays.

Results: The (4+2) annulation provided a heavily functionalised cyclohexyl β-lactone in good yield and with excellent stereochemical integrity. Elaboration of the cyclohexane core into the natural product scaffolds was achieved over an additional 10 and 6 steps for (−)-Δ^9-THC and (−)-normethyl-Δ^9-THC analogues respectively. In these studies, all C8-subsittuted normethyl analogues showed reduced affinity for CB_1 (pK_i 5.6-6.2) compared to (−)-Δ^9-THC itself (pK_i 7.6), and generally functioned as CB_1 partial agonists akin to (−)-Δ^9-THC. Curiously, two analogues were devoid of CB_1 agonist activity, underscoring the importance of C8 and C9 sites to CB receptor binding and activity.

Conclusion: A small series of hitherto unknown C8-substituted (−)-normethyl-Δ^9-THC analogues were synthesised and assessed for binding and functional activity. These analogues exhibited similar, albeit reduced affinity for human CB_1 receptors whilst functioning as partial agonists. The absence of activity in two analogues highlights the sensitivity of CB_1 binding and affinity to C8 and C9 modified natural product scaffolds.

PHARMACOLOGICAL EVALUATION OF RECENT SYNTHETIC CANNABINOID RECEPTOR AGONISTS

Samuel Banister,*[1] Eric Sparkes,[1] Adam Ametovski,[1] Richard Kevin,[1] Elizabeth Cairns,[1] Katharina Grafinger,[2] Annelies Cannaert,[3] Iain McGregor,[1] Michelle Glass,[4] Mark Connor,[5] Volker Auwärter,[2] Christophe Stove[3] and Roy Gerona[6]

[1]The Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Sydney, NSW 2050, Australia;
[2]Frieburg
[3]Ghent
[4]Department of Pharmacology and Toxicology, The University of Otago, Dunedin 9016, New Zealand; [5]Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW 2109, Australia; [6]Clinical Toxicology and Environmental Biomonitoring Laboratory, UCSF, San Francisco, CA 94143, USA.

Introduction: More than 300 synthetic cannabinoid receptor agonists (SCRAs) have been identified in recreational drug markets across the globe since the first examples were detected a decade ago. Illicit manufacturers design new SCRAs using traditional medicinal chemistry techniques, and many recent and emerging SCRAs are unknown prior to their detection in illicit drug markets. Concerningly, SCRAs are increasingly associated with mass intoxications involving severe illness and death.

Methods: We have used a systematic combinatorial synthetic approach to rapidly and proactively develop a library of recent SCRAs and metabolites based on structural trends in the SCRA marketplace. The SCRA library was screened at human CB_1 and CB_2 receptors using radioligand binding assays, fluorescence-based membrane potential assays, nanoluciferase-based β-arrestin recruitment assays, and evaluated in mice using radiobiotelemetry. Through the Psychoactive Surveillance Consortium and Analysis Network (PSCAN), we have used LC-QTOF-MS to screen for all members of the library in patient samples from across the US.

Results: Many of the most recently detected SCRAs, such as 4CN-AB-BUTICA, 4CN-AMB-BUTINACA, 4F-MDMB-BINACA, and CUMYL-CBMICA showed nanomolar affinity for CB_1 and CB_2 receptors. Unlike Δ^9-THC, most recent SCRAs were found to act as high efficacy agonists of CB_1 and CB_2, with greater potency than Δ^9-THC itself. Many of these compounds demonstrated potent cannabimimetic activity in mice, inducing central CB_1-mediated hypothermia, hypolocomotion, and bradycardia with greater potency than Δ^9-THC. Several SCRAs showed potent proconvulsant activity in mice, consistent with reports of seizure in human users of these substances. Using this library of standards, PSCAN has identified many new SCRAs in patient samples for the first time, underscoring the value of a proactive approach to the synthesis and characterization of emerging SCRAs and their analogues, and the clinical confirmation of SCRAs in toxicology cases.

Conclusion: Structure-activity relationships for the CB_1 and CB_2 receptor binding and agonist activity of of the most recent and prevalent SCRAs, including 4CN-AB-BUTICA, 4CN-AMB-BUTINACA, 4F-MDMB-BINACA, 5F-AB-P7AICA, ADB-BUTINACA, AMB-4en-PINACA, and CUMYL-CBMICA and their analogues have been identified. Key structural features contributing to CB_1 agonist potency *in vitro* and *in vivo* were determined. Several SCRAs identified in illicit drug markets were observed to cause seizures in mice, consistent with clinically noted adverse effects in humans. The proactive generation of SCRA analogue libraries, and the utility of such libraries to the rapid detection of emerging drugs of abuse by clinical toxicologists, was demonstrated. The unprecedented potency and efficacy of many recently identified SCRAs at CB_1 receptors may contribute to the clinical toxicity of these substances.

FLUORINATED CANNABIDIOL DERIVATIVE PECS-101 EXERTS SUPERIOR ORAL BIOAVAILABILITY OVER CANNABIDIOL

Tamás Bíró*[1], Dalia Shabashov Stone[2], Ilan Winkler[2] and Gary Hiller[1]

[1]Phytecs Inc, Los Angeles, CA, USA and [2]Pharmaseed Ltd, Ness Ziona, Israel

Introduction: Cannabidiol (CBD) has been approved for the treatment of rare forms of childhood epilepsy (Epidiolex®) in many countries and is also a principal component in a drug approved for the treatment of spasticity associated with multiple sclerosis (Sativex®) in a smaller number of countries. Moreover, CBD as well as other phytocannabinoids are subjects of ongoing clinical trials for other indications. Notably, one of the major pitfalls of CBD applications is its relatively poor oral bioavailability. We have previously shown that among the several fluorinated CBD derivatives (F-CBDs), originally synthesized in the laboratory of Prof. Mechoulam, PECS-101 (previously named as HUF-101) exhibits 3-10-20 times greater efficacies/potencies, when compared to CBD, in multiple *in vivo* models of epilepsy; anxiety, depression, psychosis, and compulsive behavior (doi: 10.1371/journal.pone.0158779.); pain (doi: 10.1016/j.pnpbp.2017.07.012.); neuroprotection (doi: 10.1016/j.neuropharm.2018.08.009.), and inflammatory skin diseases. Here, we assessed whether the enhanced efficacies of F-CBD over CBD are, at least in part, due to better pharmacokinetic (PK) profile and characteristics of PECS-101.

Methods: PECS-101 was administered to SD rats (9 rats per group) at dose levels of 10 mg/kg orally (p.o.) or 1 mg/kg intravenously (i.v.). At predetermined time-points (5 min – 24 hrs, 9 points for each treatment) after administration, the animals were bled (3 rats per time-point) and plasma was collected and frozen. Bioanalytical analysis of blood samples was performed by using LC-MS-MS.

Results: Precision analytics of the blood samples, followed by conventional PK analysis, resulted in the following parameters:

Parameters	**PECS-101 10 mg/kg p.o.**	**PECS-101 1 mg/kg i.v.**
$t_{1/2}$ *(hr)*	3.3	5.8
$C_{initial}$ *(i.v.) (ng/mL)*		386.1
C_{max} *(obs) (ng/mL)*	814.0	269.1
T_{max} *(obs) (hr)*	0.5	0.08
$AUC_{(0\text{-}t)}$ *(obs area) (ng-hr/mL)*	1362.2	278.0
AUC_{∞} *(area) (ng-hr/mL)*	1369.6	286.4
Vd (obs area) (mL)		9057.6
CL (obs area) (mL/hr)		1079.213

Interestingly, p.o. administration led to piloerection 2 hrs after dosing whereas i.v. administration caused a transient decrease in motor activity 1 hr after dosing that disappeared after 2 hours. Notably, none of the animals died during the study, results that are consistent with those of a prior repeated dose 14-day oral toxicity study where p.o. administered PECS-101 (1, 10 or 50 mg/kg) did not cause any treatment-related and/or toxicologically significant effect in Balb/C mice.

Conclusions: PECS-101 exhibited more favorable PK profile (e.g. higher C_{max}, shorter T_{max}) than previously reported for CBD. Therefore, it can be postulated that the markedly higher efficacy of PECS-101 over CBD observed in various *in vivo* models is due, at least in part, to the significantly improved oral bioavailability of the F-CBD.

PHARMACOKINETIC PROFILE OF BIOACTIVE SUBSTANCES THC AND CBD AFTER ORAL CANNABIS EXTRACTS ADMINISTRATION

Cristiana Dumbrăveanu*[1,2], Kai Kummer[1], Katharina Strommer[2], Astrid Neumann[2] and Michaela Kress[1]

[1] Institute of Physiology, Medical University of Innsbruck, Innsbruck, Austria.
[2] Bionorica research GmbH, Innsbruck, Austria

Introducttion: Cannabis sativa contains a multitude of bioactive substances, predominantly delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) that are available as pure compounds for experimental and clinical use. In contrast, little is known on potential synergetic interactions of the other medical marihuana substances and whether they potentially affect absorption and bioavailability of THC or CBD. In this study, we aimed to explore and compare the pharmacokinetics of THC in two sets of plant extracts and semisynthetic (THC) or isolated (CBD) cannabinoids.

Methods: The pharmacokinetic profile of medical marihuana extract rich in cannabinoids (THC+), cannabinoid-depleted extract (THC-), THC single compound at corresponding concentration and CBD was investigated in C575BL/6J mice. Formulations were prepared in sesame oil and administered by oral gavage as a single dose. Concentrations of THC and its metabolites, 11-Hydroxy-THC (OH-THC) and 11-nor-9 carboxy-THC (THC-COOH), as well as CBD were evaluated in plasma, brain and spinal cord up to 6 hours after application.

Results: THC or CBD from all formulations administered were detectable in plasma with Cmax at 2 hours. THC+ yielded a faster rise and significantly higher THC levels already at 60 min in plasma and brain as compared to THC. As expected, THC was hardly detectable in plasma or nervous tissue after administration of cannabinoid-depleted extract (THC-). For THC+ the maximum concentration was reached faster than for THC in brain, but not spinal cord suggesting faster resorption of THC when applied as extract. This was supported by faster and higher peaks of THC metabolites in plasma and brain for THC+. Regarding THC metabolites, highest concentration levels in brain were reached for the psychoactive metabolite OH-THC, while in plasma COOH-THC showed highest concentrations. CBD single compound administration showed two times higher bioavailability in brain and spinal cord, in comparison to THC as a single compound.

Conclusion: Our results support improved bioavailability of THC from enriched medical marihuana extracts. Importantly, presence of CBD and THC in the spinal cord is reported for the first time.

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MULTITARGETING THE CANNABINOID RECEPTORS

Shayma El-Atawneh* and Amiram Goldblum

Molecular Modeling Laboratory, Institute for Drug Research,The Faculty of Medicine,
The Hebrew University of Jerusalem, Jerusalem 9112001, Israel

Introduction: Most diseases are multifactorial, and the design of effective therapeutics requires the consideration of many aspects like drug delivery, distribution, and elimination while balancing target selectivity and activity to achieve therapeutic efficacy and limit undesirable side effects. Most drug discovery programs prioritize high target affinity and selectivity. However, many diseases-including neuropsychiatric and metabolic disorders currently treated by drug combinations, have limited efficacies. This arises from the complexity and redundancy in key physiological responses and the disease's polygenic nature. A single multitargeted molecule has several advantages over combination therapy. Multitargeting is expected to reduce drug-drug interactions, provide a more straightforward pharmacokinetic profile, decreased toxicity and side effects, increased patient compliance, a more comfortable dosing regimen, and easier regulatory approval.

The cannabinoid receptors (CB1 and CB2) have different organs' distribution and are associated with numerous physiological processes. They are associated with many disorders, including obesity, pain, epilepsy, anxiety, depression, Parkinson's, Huntington's diseases, and others, making them highly popular targets for drug development. Combined treatment of peripheral CB1R antagonist and CB2R agonist was shown to abolish diabetes-induced albuminuria, inflammation, tubular injury, and renal fibrosis. Cannabinoid-2 (CB2) agonists (devoid of CB1 activity) have immunomodulatory roles in inflammatory systems: combined with activation of PPARγ and inhibition of TNF-alpha, they could be used as a potential therapeutics for "Inflammatory bowel disease" (IBD). Our computational methods enable searching and finding such multitargeted single candidates for various disorders.

Methods: Classification models are built by our in-house, a prize-winning generic algorithm for solving highly complex combinatorial problems, called "Iterative stochastic elimination" (ISE). It is used to construct multi-filter models that enable screening and scoring massive multi-million molecules from catalogs and pick the top-scored ones for in vitro and in vivo experiments. Screening by ISE models provides excellent sets of candidates. Docking is applied to further verification of top-scored candidates.

Results: Activity models of agonist and antagonist activity were built for CB1/CB2/PPARγ receptors (AUC= 0.85-0.95, mean MCC=0.55-0.98). These models were used for virtual screening for the desired target and anti-targets as well. Anti-targets will be considered to avoid side-effects like inhibition of CB1R in the CNS, which could lead to destructive psychoactive effects.

Conclusions: Our computational platform is an excellent tool for discovering novel bioactive multitargeted single molecules. Having a set of models enables to screen multi-million molecules quickly and predict the activity profile of ligands to a set of targets. ISE advantages are discovering new scaffolds, producing highly diverse sets of candidates that have never been tested for any bioactivity and therefore may be patented for any pharmacological action.

DISCOVERY OF NOVEL GPR55 MODULATORS USING LIGAND-BASED DRUG DESIGN STRATEGY

Laura Figuerola-Asencio*,[1] Dow P. Hurst,[2] Linda M. Console-Bram,[3] Pingwei Zhao,[3] Nadine Jagerovic,[1] Patricia H. Reggio,[2] Mary E. Abood[3] and Paula Morales[1]

[1]Instituto de Química Médica,
Consejo Superior de Investigaciones Científicas, Madrid, Spain
[2]Department of Chemistry and Biochemistry, UNC Greensboro, Greensboro, NC, USA
[3]Center for Substance Abuse Research, Department of Anatomy and Cell Biology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA

Introduction: GPR55, a cannabinoid-like receptor, is involved in various pathophysiological conditions. However, there is still a lack of potent and selective GPR55 ligands. These studies are focused on the thienopyrimidine scaffold based on the GPR55 antagonist ML192, previously discovered by high-throughput screening.[1] Using our GPR55 inactive state model, ML192 derivatives were designed, synthesized and evaluated using a β-arrestin recruitment assay in CHO cells overexpressing h-GPR55. These derivatives revealed efficacy as GPR55 antagonists. However, they did not show any increased potency compared with the hit ML192.[2,3]

Methods: *Modeling*: A fragment-based scaffold hopping approach was used to design new potent compounds based on the thienopyrimidine scaffold. A pharmacophore model developed upon previous SAR studies was used as core hopping input. The best results obtained from the Core-Hopping were filtered and analyzed according to different criteria including ADMET and PAINS properties. *Synthesis*: Selected candidates were synthesized and characterized by mass spectrometry, 1D and 2D NMR. The purity was determined by HPCL/MS and by elemental analysis. *Pharmacology*: Biological evaluation was realized using a β-arrestin recruitment assay in CHO cells overexpressing h-GPR55. Their selectivity vs other cannabinoid receptors has been determined.

Results: ML192 compound has been used as a starting point for the design of new GPR55 antagonists using a fragment-based scaffold hopping approach. These structures have been synthesized and are being testing in CHO cells overexpressing h-GPR55.

Conclusions. Combining modeling studies, pharmacology and synthesis allowed the discovery of new GPR55 ligand structures that may serve as research tools for studying GPR55.

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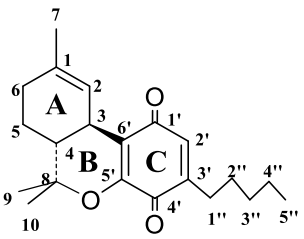
CANNABINOID QUINONES – SAR AND MECHANISM OBSERVATIONS

Natalya M. Kogan*, Maximilian Peters and Raphael Mechoulam

Institute for Drug Research, Hebrew University, Jerusalem, 91120, Israel

Introduction



A cannabinoid anticancer p-quinone, HU-331, was previously shown to be very effective against human cancer cell lines. The main mechanism of action of HU-331 in cancer cells was found to be inhibition of topoisomerase IIα, but it is also a PPARγ agonist. Now we report the syntheses of additional compounds in the cannabinoid quinone series, which help to shed more light on the SAR and the mechanism of action of HU-331 and related compounds.

Methods

The synthesis of cannabinoid quinones was performed by 5% aqueous KOH in ethanol, in O_2 atmosphere. The compounds were identified by MS and NMR. The anticancer activity was assayed by MTT test on Raji and Jurkat cancer cell lines. Thiol antioxidants binding was assayed by HPLC and NMR.

Results

13 new cannabinoid quinones were synthesized. From the structure-activity relationship in cannabinoid quinones series the following understandings regarding the influence of different parts of the molecule on its anticancer activity can be drawn:

- The double bonds on ring A have only a minor effect on the anticancer activity
- The position at which rings A and C are attached one to another is of importance – ring C has to be attached through the "normal", CBD-like position.
- The hydroxyl on ring C does not have to be free.
- Ring B has to be open.
- Ring A is of importance for the anticancer activity. Oxidation of the propylene group attached to this ring further improves the activity.
- The isopropenyl attached to position C4 of ring A is quite important for the anticancer activity; when absent the activity drops; the connection of isopropenyl to ring A as a bridge further improves the activity.
- Position C4' of ring C is crucial for anticancer activity. If blocked or hindered, the activity sharply drops. This is a position neutralized by thiol-containing antioxidants. This is probably a position through which a molecule binds to its intracellular target.

Conclusions

It seems that the topoisomerase-inhibiting activity (which needs the C4' position to be free) and the PPARγ agonism (for which this position is not important and can be blocked) can be present in parallel in these molecules. The research on cannabinoid quinones is far from complete and the possibility of targeting various diseases by acting on different mechanisms opens new horizons.

PHARMACOKINETIC ANALYSIS OF CANNABIDIOL AND MAJOR METABOLITES FOLLOWING ORAL ADMINISTRATION OF HEMP-DERIVED CANNABINOID CHEWS IN HEALTHY DOGS

Justyna Kulpa*, Graham Eglit and Dana Vaughn

Canopy Growth Corporation, Smiths Falls, ON, CA

Introduction: Evolving regulatory frameworks and social perceptions have renewed interest in the potential therapeutic properties of cannabinoids for companion animals in anxiety, epilepsy and pain/inflammatory conditions. To date, published pharmacokinetic literature in canines has suggested low bioavailability of CBD in the plasma following oral administration. However, dose linearity of CBD systemic exposure and production of CBD metabolites secondary to oral administration of soft chews has not been explored in dogs. Consequently, the objective of this study was to determine the pharmacokinetic profile of CBD and five major metabolites in dogs following a single oral administration of four different doses of soft chews formulated with hemp-derived CBD.

Methods: Fasted dogs received a single oral dose of one, three, five or ten soft chews (30.8 mg CBD/chew, n=4 males/group), for a total dose of 30.8, 92.4, 154 or 308 mg (~3, 9, 15 or 30 mg/kg) CBD. Blood was collected prior to dosing, and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours after test article administration. Plasma levels of CBD, 6α-OH-CBD, 6β-OH-CBD, 7-OH-CBD, 7-COOH-CBD and CBD-glucuronide were determined using LC-MS/MS. Pharmacokinetic parameters were calculated via a non-compartmental model. The association between dose (in mg/kg) and pharmacokinetic parameter values was evaluated using Spearman's rank correlation.

Results: Peak plasma concentration (C_{max}) of CBD (423.7 – 4998.1 ng/mL; 30.8 – 308 mg CBD administered) was positively correlated with dose administered (ρ=0.891, P <0.01), and was observed within 2 to 4 hours of dose administration in all dogs. Acute elimination half-life of CBD ($t_{1/2}$) was positively correlated with dose administered (ρ=0.550, P=0.027), and ranged from 20.3 ± 2.4 (30 mg) to 32.5 ± 11.7 (308 mg) hours. CBD systemic exposure (AUC_{last}) ranged from 1,666.9 ± 569.7 (30.8 mg) to 25,112.2 ± 1,446.9 (308 mg) ng/mL and had a positive dose correlation (ρ=0.962, P <0.01). Plasma CBD remained detectable at the last measured timepoint in all dogs (t_{last}=72 h) following a single administration.

In decreasing order, magnitude of both dose-normalized AUC_{last} and dose-normalized C_{max} for metabolites was 7-COOH-CBD > 6α-OH-CBD > 7-OH-CBD > CBD-glucuronide > 6β-OH-CBD. Metabolites followed a similar pharmacokinetic pattern to that of CBD, with peak levels ~2 to 4 hours following test article administration. For all metabolites, C_{max}, AUC_{last}, $t_{1/2}$, t_{last} and MRT_{last} were positively correlated with dose administered (ρ >0.5, P <0.05), while elimination rate constant (k_e) was negatively correlated with CBD dose (ρ <-0.5, P <0.05).

Conclusions: Plasma CBD levels were strongly positively correlated with dose administered following a single oral administration of hemp-derived soft chews. All measured CBD metabolites were detectable, with 7-COOH-CBD and 6α-OH-CBD being the major metabolites found in dogs. These findings demonstrate a favorable pharmacokinetic profile for CBD soft chews and provide support for continuing research on the potential therapeutic uses of this hemp formulation in dogs.

NOVEL SYNTHETIC COMPOUNDS WITH A CANNABIDIOL-LIKE SCAFFOLD AS GPR18 LIGANDS

Ana Lago-Fernandez*,[1] Pingwei Zhao,[2] Noori Sotudeh,[3] Luciana M. Leo,[2] Eugen Brailoiu,[2] Dow P. Hurst,[3] Paula Morales,[1] Patricia H. Reggio,[3] Mary E. Abood,[2] and Nadine Jagerovic[1]

[1]Instituto de Química Médica, Consejo Superior de Investigaciones Científicas, Madrid, Spain.
[2]Center for Substance Abuse Research, Temple University, Philadelphia, USA.
[3]Center for Drug Discovery, University North Carolina Greensboro, USA.

Introduction: While numerous cannabinoid ligands have been reported to stimulate the orphan GPCR receptor GPR18[1] – including NAGly, Abn-CBD and Δ^9-THC – few synthetic compounds have been designed to modulate this receptor. Based on an *in silico* model of GPR18[2] we have designed, synthesized and evaluated pharmacologically a new series of compounds targeting GPR18 with a cannabidiol-like scaffold.

Methods: *Synthesis.* A series of pyrazolyl-alkylresorcinols were synthesized following the procedure described in Lago-Fernandez *et al* 2017.[3] *Molecular modeling.* Docking studies in the active and inactive state GPR18 models[2] were performed using the OPLS3 force field as implemented in Macromodel (Schrödinger, LLC). *Pharmacological evaluation*. Intracellular calcium levels were determined in GPR18-transfected CHO-K1 cells by measuring fluorescence emissions from Fura-2 AM (510 nm) after alternating excitation at 340 and 380 nm at 0.25 Hz for 5 min. Images were analyzed in the NIS-Elements AR software (Nikon), and the fluorescence ratio at 340nm/380nm was converted to Ca^{2+} concentrations. Quantification of β-arrestin recruitment was accomplished using DiscoveRx PathHunter® CHO-K1 cells stably expressing GPR18 fused with a β-galactosidase enzyme fragment, and β-arrestin fused to an N-terminal deletion mutant of β-galactosidase.

Results: Several compounds in this family were able to either increase intracellular calcium in the GPR18 expressing cell line or completely block the rise of intracellular calcium induced by several agonists, including the putative GPR18 endogenous ligand NAGly. Furthermore, β-arrestin recruitment assays showed that these compounds had agonistic or inverse agonistic properties.

Conclusions: A new series of compounds targeting GPR18 with a cannabidiol-like scaffold has been successfully synthesized and evaluated *in vitro*. This novel GPR18 chemotype may serve as a basis for the development of pharmacological probes to study this orphan receptor.

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A NOVEL ROUTE TO METHYL SUBSTITUTIONS ON THE AROMATIC RING OF CANNABINOIDS

Lavi Y.[a,*], Kogan NM. [a,*], Breuer A. [a], Yekhtin Z. [b], Gallily R. [b] and Mechoulam R. [a]

[a] School of Pharmacy of The Hebrew University of Jerusalem
[b] Lautenberg Center for Immunology and
Cancer Research at The Hebrew University of Jerusalem
* Equally contributed

Introduction

In recent years, non-psychoactive Phyto-cannabinoids and especially cannabidiol (CBD) have received a growing interest as pharmaceutical candidates for inflammation and other illnesses. Although the synthesis of C-methylated cannabinoids has been previously reported by our group, these derivatives have not been tested for their anti-inflammatory activity. Moreover, the reported synthetic route, which we followed for C-methylated cannabinoids used expensive reagents and starting materials and the overall yields were low. In this study we report of a new method for the synthesis of mono and di-methylated cannabinoid derivatives and their evaluation for anti-inflammatory and anti-nociceptive activity.

Methods

In the new method for C-methylation, olivetol is first formylated using the Vilsmeier–Haack reagent to prepare an aldehyde, which is reduced to a methyl group using the Red-Al® reagent. The new derivatives were evaluated for anti-inflammatory and anti-nociceptive activity in-vivo in female Sabra mice. The mice were evaluated for edema formation and TNF-α plasma levels.

Results

Our new method for C-methylation represents a much more versatile route to C-methylated cannabinoids than previous methods. Starting from either mono or di-methylated olivetol derivatives, the new method allowed us to couple them with a terpene synthon of our choice to achieve the final C-methylated cannabinoids. We have successfully prepared C-methylated CBD derivatives as well as CBG and CBC derivatives. We have also synthesized new O-methylated derivatives of CBD and CBG. In the anti-inflammatory assays we saw that all these new compounds retain anti-inflammatory activity, comparable to that of the parent compounds. In one of the CBD derivatives, we observed a deviation from classical bi-phasic dose-response curve to a linear dose-response.

Discussion

In this study we report a new route to C-methylated cannabinoid derivatives, which is both versatile and robust. We were able to achieve through this method, in high yield and purity, an array of new compounds which were not accessible previously. Our results show that the C-methylated cannabinoids retain anti-inflammatory and anti-nociceptive activity when compared to the parent phyto-cannabinoid. The deviation from bi-phasic dose response to a linear dose-respond, might open new possibilities for further development of anti-inflammatory derivatives with a more favored pharmacological behavior.

SIMULTANEOUS EXTRACTION AND SYNTHESIS OF NEW CANNABINOIDS ACID ESTERS AND THEIR *IN VITRO* CYTOTOXICITY ASSESSMENT IN BREAST CANCER CELL LINES

Evangelos Dadiotis[1], Aikaterini Papakonstantinou[1,2], Vangelis Mitsis[3], Eleni Melliou[1], Haralabia Boleti[2] and Prokopios Magiatis*[1]

[1] Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece
[2] Intracellular Parasitism Laboratory, Department of Microbiology, Hellenic Pasteur Institute, 127 Vasilissis Sofias Avenue, Athens 11521, Greece
[3] Ekati Alchemy Lab SL, Barcelona, Spain

Introduction: Natural phytocannabinoids have a privileged pharmacophore scaffold and in addition to other activities present interesting antitumor properties. For this reason, development of new derivatives with simple preparation methods and increased activity are highly desirable. In this study we present the simultaneous extraction and synthesis of cannabinoid acid esters and their cytotoxicity assessment in breast cancer cell lines. The synthesis method was performed for the methyl, ethyl, isopropyl and butyl esters of cannabidiolic acid (CBDA), cannabigerolic acid (CBGA) and Δ9-tetrahydrocannabinolic acid (d9-THCA). Cannabinoid acids, their neutral forms (CBD, CBG and Δ9-THC) and their respective esters were evaluated for their cytotoxicity, to determine if esters are more potent than the acids and the neutral forms and how the activity is affected by the ester chain length.

Methods: The plant material used for the extraction was the inflorescences and/or the trichomes of different varieties of *Cannabis sativa* containing selectively high amounts of either CBDA or CBGA or THCA. Synthesis of each ester was achieved simultaneously with the extraction, using each time a different alcohol as extraction solvent and as a reagent for esterification. The extraction of the plant material was carried out in an ultrasonic bath. The extraction was performed in the presence of 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP). The cytotoxic activity of CBDA, CBGA and Δ9-THCA esters was studied *in vitro* by the MTT test in SK-BR-3 and MDA-MB-231 cells (human breast cancer cell lines). EC50s were calculated for all cell lines at 20% v/v O_2, in the presence of 10% FBS after 48h or 72h of incubation.

Results: Depending on the plant material the extraction and synthesis method could lead up to 90% yield of each ester, without need for chromatographic or any other purification process. The study of the cytotoxic activity of CBGA, CBDA and Δ9-THCA and their respective esters by the MTT assay showed that CBG, CBGA and its respective esters were the most potent with $EC_{50} < 40$ μM in MDA-MB-231 cancer cells, after 48 hours of incubation. Specifically, CBGA butyl ester showed the best results with $EC_{50} < 10$ μM, while CBG had $EC_{50} = 33.7$ μM after 48 hours of incubation.

Conclusion: The synthesis of the esters described presently offers a new low-cost method for the simultaneous extraction and esterification of the cannabinoid acids from *C. sativa*. The prepared esters were found to be more potent than the corresponding cannabinoid acids and their neutral forms with the CBGA butyl ester, a new cannabigerolic acid derivative, showing the greatest activity and potential for further development.

DEFINING STERIC LIMITS IN A SERIES OF SYNTHETIC CANNABINOID RECEPTOR AGONISTS RELATED TO 5F-AB-PICA, 5F-ADB-PICA AND PX-1

Jack Markham,[1,2] Eric Sparkes,[1,2] Jia Lin Luo,[1,2,3] Richard C. Kevin,[1,3]
Christa Macdonald,[4] Rochelle Boyd,[1,2] Michelle Glass,[5] Mark Connor,[4]
Iain S. McGregor[1,3] and Samuel D. Banister[1,2]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, NSW, Australia
[2]School of Chemistry, The University of Sydney, NSW, Australia
[3]School of Psychology, The University of Sydney, NSW, Australia
[4]School of Medical Sciences, The University of Auckland, New Zealand
[4]Faculty of Medicine and Health Sciences, Macquarie University, NSW, Australia
[5]Department of Pharmacology and Toxicology, University of Otago, New Zealand

Introduction: The cannabinoid type 1 receptor (CB_1) is an attractive therapeutic target for the development of novel treatments for pain, epilepsy, and other conditions, with several synthetic CB_1 receptor agonists (SCRAs) approved by the FDA (e.g. nabilone). However, many unregulated SCRAs have also been identified in illicit drug markets. Many SCRAs such as AMB-FUBINACA and 5F-MDMB-PICA feature pendant amino acid side chains and are increasingly associated with acute toxicity and mass overdoses. There is a paucity of pharmacological data for the most recent SCRAs, and limited understanding of the relationships between structure, cannabinoid function, and toxicity.

Methods: We have explored the role of the pendant amino acid substituent in a series of SCRAs based on a 1-(5-fluoropentyl)indole-3-carboxamide scaffold. Analogues of SCRAs 5F-AB-PICA, 5F-ADB-BUTICA, and PX-1 featuring systematic modification of the amino acid side-chain were synthesised and screened at human CB_1 and CB_2 receptors using radioligand binding assays and fluorescence-based plate reader membrane potential assays. Additionally, selected analogues were subjected to radiobiotelemetric evaluation in mice to confirm *in vivo* cannabimimetic activity.

Results: *In vitro* CB_1 binding and potency was greatest for examples comprising a *tert*-leucinamide subunit (5F-ADB-PICA; K_i = 16.2 nM, EC_{50} = 0.77 nM), and decreased for the sterically smaller valinamide (5F-AB-PICA; K_i = 288 nM, EC_{50} = 5.2 nM), with further truncation (alaninamide, glycinamide) conferring micromolar CB_1 affinity and abolishing functional activity. Steric enlargement with leucinamide (K_i = 955 nM, EC_{50} = 137 nM) or phenylalaninamide (PX-1; K_i = 2630 nM, EC_{50} = 529 nM) conferred micromolar affinity and only moderate potency at CB_1. Although PX-1 was moderately potent *in vitro*, central CB1-mediated hypothermia was not observed *in vivo* at doses up to 30 mg/kg (i.p.). By comparison, 4CN-CUMYL-BINACA was potently hypothermic, causing a -7 °C body temperature change at doses up to 1 mg/kg (i.p.).

Conclusions: Amino acid-derived SCRAs demonstrate strict steric requirements for the amino acid side-chain (R group). Both truncation and expansion of the optimal *tert*-leucinamide group produced dramatic reductions in CB1 affinity and potency. Despite its prevalence in the illicit drug market, PX-1 was not cannabimimetic *in vivo*, potentially due to unfavorable physicochemical properties for central nervous system penetration, however, the possibility of psychoactivity in humans (via species differences, active metabolites, etc.) cannot be excluded. Further characterisation of the physicochemical properties conferring putative peripheral restriction in this series may inform the design of novel cannabinoid therapeutics devoid of psychoactivity for the treatment of pain.

CHROMENOPYRAZOLE-BASED CB2 BITOPIC LIGANDS

Paula Morales*,[1] Gemma Navarro,[2] Marc Gómez-Autet,[3] Laura Redondo,[1] Javier Fernández-Ruiz,[4] Laura Pérez-Benito,[3] Arnau Cordomí,[3] Leonardo Pardo,[3] Rafael Franco[2] and Nadine Jagerovic[1]

[1]Instituto de Química Médica, Consejo Superior de Investigaciones Científicas, Madrid, Spain.
[2]Dept. Biochemistry and Molecular Biology. Faculty of Pharmacy and Food Sciences. Universitat de Barcelona. Barcelona. Spain
[3]Laboratory of Computational Medicine, Biostatistics Unit, Faculty of Medicine, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[4]Dept. de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense de Madrid, Spain

Introduction: Bitopic ligands are emerging as promising approaches in GPCR drug discovery. These molecules, which can target both orthosteric and exosites, may improve binding affinity, subtype selectivity, receptors stability and reduce undesired side effects.[1] This strategy has not been yet attempted for the modulation of the endocannabinoid system. Therefore, we developed homobivalent bitopic ligands based on the chromenopyrazole scaffold.

Methods: *Synthesis.* A series of homobivalent chromenopyrazoles and their monovalent analogues were synthesized following the procedures previously described by us.[2] *Pharmacological evaluation*. In vitro binding affinities of the novel compounds were obtained from [^{3}H]-CP-55,940 competition-binding assays using membrane fractions from hCB_2-HEK293 cells. CB_2 functionality of selected bivalent ligands was evaluated by determination of their effect on forskolin-induced cAMP levels in hCB_2-HEK293 cells. *Molecular modeling.* Docking studies and molecular dynamics simulations were performed using the recently solved structure of CB_2.

Results: We present herein the design, synthesis and functional characterization of the first bitopic ligands for the CB_2 receptor. This compounds, which are based on the chromenopyrazole scaffold, are agonists that selectively target CB_2 versus CB_1 receptors. Molecular understanding of the binding mode was guided by molecular dynamic simulations and site-directed mutagenesis.

Conclusions: CB_2 receptor homobivalent bitopic ligands have been identified. These chromenopyrazoles interact concomitantly with the orthosteric site and an exosite located at the ligands entry portal and may serve to deepen in the pharmacological study of this receptor.

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CANNABINOID RECEPTORS EXPRESSED IN PROSTATE AND OTHER CANCER CELL LINES EXHIBIT ATYPICAL BINDING AND SIGNALING PROPERTIES: IMPLICATIONS FOR DRUG DEVELOPMENT

Amal M. Shoeib*, Lance N. Benson, Shengyu Mu and Paul L. Prather

Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Introduction: Synthetic and phytocannabinoids have been reported to produce tumor cell death in a variety of cancer types. However, the mechanisms mediating anti-cancer effects of cannabinoids have been incompletely characterized and may involve both cannabinoid-dependent and independent actions. Previous studies in our laboratory have shown Ewing Sarcoma cells express cannabinoid receptors (CBRs) with characteristics dissimilar to canonical receptors that might contribute to cannabinoid-induced decrease in cell viability. Therefore, the purpose of this study was to test the hypothesis that such "atypical" cannabinoid receptor expression is common in a variety of cancer cell types.

Methods: Cancer cell lines studied were DU-145 and PC-3 (human prostate), HeLa (human cervical), PC-12 (rat pheochromocytoma) and C6 glioma (rat glial). Cell lines were cultured in appropriate media supplemented with 10% FBS, 1% pen/strep. Cannabinoid receptor type-1 (CB1R) and cannabinoid receptor type-2 (CB2R) mRNA were detected in human cell lines by qRT-PCR. Presence of CBRs was determined by radioligand binding screens employing the well-characterized high affinity, non-selective CB1R/CB2R radioligands [^{3}H]WIN-55,212-2 and [^{3}H]CP-55,940. CBR affinity and density were assessed by competitive and homologous binding of [^{3}H]WIN-55,212-2, respectively. Functional activity of cannabinoids examined was evaluated by G-protein and adenylyl cyclase assays. Finally, effects of cannabinoids on cell viability were determined by 7-aminoactinomycin D (7-AAD) staining followed by flow cytometry.

Results: CB1R and CB2R mRNA were detected in human cancer cell lines by qRT-PCR. However, when radioligand binding screens to detect CBRs were employed, specific binding was observed only for [^{3}H]WIN-55,212-2, but not [^{3}H]CP-55,940 in all cancer cell lines examined. Next, homologous binding assays revealed that [^{3}H]WIN-55,212-2 bound to a single-site in all cancer cell lines with high affinity (4-16 nM) and was expressed at varying densities (0.5 to 3 pmole/mg). To focus studies, CBR characterization was compared between prostate cancer cell lines expressing low (PC-3) and high (DU-145) CBR density. In both cell lines, only 4 of 13 compounds with known high nanomolar affinity for CBRs were able to significantly displace [^{3}H]WIN-55,212-2. Furthermore, competition binding demonstrated that these 4 ligands exhibited high affinity with a rank order of affinity of WIN-55,212-2 > rimonabant > AM-251 > GW-405833. Functional assays revealed that these compounds did not modulate G-protein or adenylyl cyclase activity similar to that of well-established G_i/G_o-coupled CBRs. Finally, 3-day incubation with WIN-55,212-2 produced cell death of DU-145 cells and significant downregulation of CBRs.

Conclusions: Collectively, these results indicate that a variety of cancer cells express CBRs that exhibit unique binding and signaling properties and these "atypical" receptors might be potential targets for development of useful anti-cancer agents.

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CHEMISTRY AND PHARMACOLOGY OF SYNTHETIC CANNABINOID RECEPTOR AGONISTS ADB-BINACA APP-BINACA ADB-P7AICA AND THEIR ANALOGUES

Eric Sparkes*,[1,2] Monica Patel,[3] Katharina Elisabeth Grafinger,[4] Elizabeth Cairns,[1,5] Ross Ellison,[6] Iain S. McGregor,[1,5] Roy R. Gerona,[6] Volker Auwärter,[4] Michelle Glass[3] and Samuel D. Banister[1,2]

[1]The Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, The University of Sydney, NSW 2050, Australia
[2]School of Chemistry, The University of Sydney, NSW 2006, Australia
[3]Department of Pharmacology and Toxicology, University of Otago, Dunedin 9016, New Zealand
[4]Institute of Forensic Medicine, Forensic Toxicology, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany
[5]School of Psychology, The University of Sydney, NSW 2050, Australia
[6]Clinical Toxicology and Environmental Biomonitoring Laboratory, University of California, San Francisco, CA 94143, USA

Introduction: Synthetic cannabinoid receptor agonists (SCRAs) remain one of the most prevalent classes of new psychoactive substances (NPS), with little to no data available at the time of first detection. We have developed a proactive SCRA screening program using a combinatorial library generation approach to identify and characterise emerging compounds of concern. Recent SCRAs featuring a butyl substituent, including ADB-BINACA (ADB-BUTINACA) and APP-BINACA (APP-BUTINACA), as well as their systematic indole-, indazole-, and 7-azaindole analogues were synthesised and evaluated in vitro *via* radioligand binding and fluorescence-based functional assays at CB_1R and CB_2R.

Methods: All compounds were synthesized *via* alkylation of indole-, indazole-, and 7-azaindole precursors, followed by hydrolysis and amide coupling. Analytical characterisation was achieved by NMR, FTIR, and LC-QTOF-MS. Competitive binding experiments were performed at hCB_1 or hCB_2 derived from stably transfected HEK293 cell membranes using [^{3}H]SR141716A (CB_1) or [^{3}H]CP55,940 (CB_2). Functional assays were performed using stably transfected AtT20-CB_1 or -CB_2 lines *via* a FLIPR-like membrane potential assay (blue).

Results: ADB-BINACA (pK_i hCB_1= 8.40 M, -$pEC_{50}hCB_1$ = 9.08 M, E_{max} = 99%) was the most potent compound assessed, with the ADB (*tert*-leucinamide) group and indazole core conveying greater potency and efficacy in almost all cases. The rank order of potency and efficacy for amino acid groups was ADB > AB (valinamide) > APP (phenylalaninamide), and for heterocyclic cores was indazole > indole > 7-azaindole.

Conclusions: Chemical and pharmacological characterisation of recently detected butyl-substituted SCRAs and systematic analogues revealed clear structure-activity relationships for CB1 binding and activation. This study represents the first complete report of the synthesis, characterisation, and pharmacology of ADB-BINACA, APP-BINACA, and the hitherto unknown ADB-P7AICA recently detected in samples from an Alabama prison population. Such information is critical for informing appropriate health and legislative responses to these compounds.

NOT ALL ALLOSTERIC MOLECULES ARE CREATED EQUAL: EVIDENCE FOR SELECTIVE EFFECTIVENESS IN DOPAMINE-DYSREGULATED SYMPTOMS BY CB1 ALLOSTERIC MODULATORS

Kim S. Sugamori*[1], Catharine A. Mielnik[1], David B. Finlay[2], Mohammed Mustafa[3], Daniel J. Liput[4], Vincent M. Lam[1], Mostafa H. Abdelrahman[5], Laurent A. Trembleau[5], Ali Salahpour[1], Amy J. Ramsey[1], David Lovinger[4], Aron H. Lichtman[3], Michelle Glass[2], Iain R. Greig[5] and Ruth A. Ross[1]

[1]University of Toronto, Faculty of Medicine, Department of Pharmacology & Toxicology
[2]University of Otago, Department of Pharmacology & Toxicology
[3]Virginia Commonwealth University
[4]Laboratory for Integrative Neuroscience, NIAAA
[5]University of Aberdeen, UK.

Introduction: Dopamine controls cognitive and emotional aspects of goal-directed behaviour, with perturbations playing a role in a number of psychiatric disorders and their underlying symptoms. The endocannabinoid system (eCBS) serves as an important filter of afferent inputs in the dopamine system, helping shape how incoming information is conveyed onto dopamine neurons and to output targets. Therefore, we hypothesize that compounds targeting the eCBS could be candidates in treating positive and affective symptoms in psychiatric illness. We characterized two novel CB_1 allosteric modulators (ABM300 and ABD1085) both *in vitro* and *in vivo*; establishing *in vitro* and *in vivo* profiles and assessing therapeutic efficacy in mouse models of psychosis-like phenotypes, stemming from dopamine dysregulation.

Methods: *In vitro* characterization of both ABM300 and ABD1085 were completed, addressing allosteric effects on agonist binding to CB_1, eCB binding to CB_1 via an eCB biosensor, phosphorylation of ERK1/2, agonist-induced cAMP, and CB_1 agonist-induced arrestin recruitment. *In vivo*, the two compounds were characterized both for their effects on the CB_1-mediated classic tetrad paradigm, and therapeutically, for their effect on hyperdopaminergic phenotypes in two distinct transgenic mouse models: GluN1-Knockdown (GluN1KD) and Dopamine Transporter Knockout (DATKO).

Results: *In vitro*, ABM300 increases agonist (CP55,940) binding to CB_1 (E_{max} of 208.5% ± 21.6), while ABD1085 decreases binding (22.1% ± 31.51). The compounds further diverge when characterizing eCB binding to CB_1: ABD1085 inhibits eCB binding, whereas ABM300 has no effect. Both compounds decrease phosphorylation of ERK1/2 and decrease agonist-induced cAMP inhibition. They have similar potencies with IC_{50} values of 50nM and 10nM (ABM300 vs. ABD1085) as inhibitors of CB_1 agonist-induced arrestin recruitment. We observed striking differences in their effects *in vivo*. ABM300 decreased hyperactive exploratory phenotypes in both genetic models of hyperdopaminergia, with some effects in sensorimotor gating. ABD1085, on the other hand, had no effect on these dysregulated behaviours. It is important to emphasize, that while both compounds act as negative allosteric modulators of CB_1 signalling *in vitro*, only ABM300 had therapeutic efficacy *in vivo* in mouse models of hyperdopaminergia.

Conclusions: We have demonstrated that two CB_1 allosterics have very different *in vivo* profiles in genetic models of hyperdopaminergia. An important distinction between the two molecules is that ABD1085 may be defined as a negative allosteric modulator (NAM), while ABM300 may be categorized as a positive allosteric modulator (PAM)-antagonist. Perhaps this distinction allows for a more targeted approach; PAM-antagonist's potency potentially being dependent on the pathophysiological status of the eCBS in the disease model under investigation.

SUSTAINED-RELEASE OF WHOLE PLANT MEDICAL *CANNABIS* VIA MELT-PRINTED POLYMERIC MICRODEPOTS

Almog Uziel*[a,b], Anat Gelfand[b], Keren Amsalem[b], Paula Berman[b], Gil M. Lewitus[b], David Meiri[b] and Dan Y. Lewitus[a]

[a]Department of Polymer Engineering, Shenkar – Engineering Art Design, Ramat-Gan Israel.
[b]The Laboratory of Cancer Biology and Cannabinoid Research, Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel

Introduction: Currently, consumption of prescribed medical *Cannabis* is mostly limited to oral or respiratory pathways, impeding its duration of action, bioavailability and efficacy. In this work, we describe the prolonged administration of whole plant *Cannabis* medicinal extracts via injectable, long-acting polymeric microspheres, prepared via a novel melt-printing technique.

Methods: Cannabidiol (CBD)-enriched extract was mixed with a molten biodegradable polymer, polycaprolactone, then jetted using a jetting apparatus onto non-wetting surfaces to form discrete solid microspheres. In vivo dose response was measured by subcutaneous injection of microspheres into mice. Cannabinoids plasma concentrations were quantified over 14 days, compared to pure extract using LC/MS. The long-term therapeutic potential of a single administration of the microdepots compared to a single *administration of Cannabis extract* was performed using a pentylenetetrazol-induced convulsions model

Results: When injected subcutaneously in mice, the microdepots facilitate sustained release of the encapsulated extract over a two-week period. The prolonged delivery results in elevated serum levels of multiple, major and minor, phytocannabinoids for over 2 weeks, compared to a bolus injection of *Cannabis* extract. An empirical model for the release kinetics of the phytocannabinoids as a function of their physical traits was developed. One week following subcutaneous administration, the microdepots reduce the incidence of tonic-clonic seizures by 40%, increase the survival rate by 50%, and the latency to first seizures by 170%, compared to pure extract injection.

Conclusions: These results suggest that a long-term full-spectrum *Cannabis* delivery system may provide a novel path for *Cannabis* administration and treatments.

DECREASE IN OPIOIDS CONSUMPTION IN PATIENTS RECEIVING MEDICAL CANNABIS

Lihi Bar-Lev Schleider*[1,2] and Victor Novack[1]

[1] Clinical Cannabis Research Institute, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel.
[2] Research Department, Tikun Olam LTD.

Background: There is an increase in mortality in United States as a result of prescription opiates consumption. Recently, medical cannabis became a safe option for patients with pain symptoms. We hypothesized that medical cannabis treatment is associated with a decrease in opiates consumption.

Methods: We analyzed the data routinely collected as part of the treatment program on 2,848 patients consuming opioids when initiating cannabis treatment at Tikun-Olam clinic of which 1,532 responded both to the intake and 6-months follow-up questionnaire.

Results: The average patient age was 59.3±17.2 years and 52.0% of the patients were men. At baseline all patients were treated with opiates, mainly in the following medications: percocet (32.3%), targin (28.4%), oxycod (21.0%) and tramadex (19.2%). At six months 701 patients (24.6%) died, 312 (10.9%) stopped treatment, 92 (3.2%) switched to a different cannabis supplier and 1,743 patients (61.2%) continued treatment. Of the latter group, 1,532 (87.8%) responded to the questionnaire. 38.4% of them stopped taking opioids, 11.2% decreased dose, 48.7% continued to take the same dose and 0.7% increased the dose. Overall, median level of a pain decreased from 9 (IQR 8-10) to 5 (IQR 3-6) on 10 points scale ($p<0.001$). The observed infrequent adverse events were mild.

Conclusions: Initiation of cannabis treatment is associated with decrease in opiates consumption, while being a safe and effective option to treat pain symptoms.

EFFECTS OF CANNABIDIOL ON REWARDING AND AVERSIVE CONTEXTUAL MEMORIES INDUCED BY COCAINE AND LITHIUM

Rayssa Briânis* and Fabricio Moreira

Department of Pharmacology, Federal University of Minas Gerais, Brazil.

Introduction: Learning to associate contexts with potential threats or rewards is an adaptive process, which increases an individual's survival. However, inadequate expression of conditioned responses to aversive or rewarding stimuli may be a predisposing factor for anxiety and substance abuse disorders, respectively. Therefore, pharmacological modulation of contextual memories can be a useful approach in the treatment of certain psychiatric disorders. Considering evidence that the endocannabinoid system modulates conditioned contextual responses, compounds that facilitate endocannabinoid signaling are potentially useful for modulating both aversive and rewarding memories. Therefore, this work has been delineated to test the hypothesis that cannabidiol (CBD), a compound from Cannabis sativa, which facilitates the action of the endocannabinoid system, attenuates lithium-induced aversive contextual conditioning, and rewarding, induced by cocaine.

Methods: Male and female mice c57BL/6 (8–10 weeks old) received injections of Cocaine (15 mg/kg), Lithium Chloride (LiCl, 100 mg/kg) or vehicle for 3 days, each one paired with a specific chamber of the place conditioning box. On day 5 (test), the animals were placed in the central compartment and were allowed to explore the entire apparatus for 15 min. The time spent in each compartment was registered with the AnyMaze software (Stoelting Co®). The Place preference score was defined as the time spent in the paired compartment, subtracted by time spent in the unpaired compartment on day 5. To evaluate the effects of Cannabidiol (CBD), 3, 10, and 30 mg/kg on acquisition of reward memory, the compound was injected 30 min before cocaine on days 2,3 and 4. To evaluate their effects on expression, the animals received CBD injection 30 min before CPP exposition on day 5. Data were analyzed by two-way ANOVA followed by the Newman-Keuls test. The control experiments for CPP and CPA were subjected to one-sample t test, in which the CPP score of each group was compared to zero (neither preference or aversion). The significance level was considered as $p<0.05$. All experimental procedures were approved by Ethics Committee on the Use of Animals (CEUA-UFMG), protocol number 179/2020.

Results: The animals learned to associate the context with cocaine (reward) or LiCl (aversion) in the protocol proposed. We do not observed significative difference between the responses for male and female. CBD did not interfere with the acquisition or expression of contextual memories.

Conclusions: In the current study, behavioral data show that both male and female mice have learned to associate conditioned context to cocaine and lithium. On the other hand, the pharmacological modulation of contextual memories with CBD has so far shown no decrease in contextual reward or aversion.

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CANNABINOID-BASED MEDICINES FOR CHRONIC PAIN: FACTORS THAT IMPACT TREATMENT EFFECTIVENESS

Maria-Fernanda Arboleda*[1], Lucile Rapin[1], Cynthia El Hage[1], Michael Dworkind[1,2] and Erin Prosk[1]

[1]Santé Cannabis, Montreal, QC, Canada; [2]Department of Family Medicine, McGill University;

Introduction: It is widely cited that the most common clinical use of cannabinoid-based medicines (CBM) is for the treatment of chronic pain and related symptoms. However, there are still significant gaps in clinical research, and available guidelines cite insufficient evidence from randomized clinical trials. Of note, most clinical trials have used inhaled delta-9-tetrahydrocannabinol (THC) to control neuropathic pain, yet nociceptive and mixed pain, as well as effectiveness of other cannabis products remain grossly under-studied. In the light of these research gaps and the existing barriers to RCTs, real-world evidence may provide valuable insight, especially when collected prospectively in controlled clinical settings. The aim of this prospective study is to assess the overall effectiveness of CBMs and the impact of treatment and population-based factors including treatment formulation, method of administration and pain mechanism.

Methods: Participants are consenting adult patients treated with CBMs at a network of four medical cannabis clinics in Québec, Canada. Patients experienced pain as their primary symptom and had a 3-month follow-up visit (FUP), between July and November 2020. Complete medical history is documented at baseline, and effectiveness is assessed with the revised Edmonton Symptom Assessment Scale (ESAS-r) and the Brief Pain Inventory-short form (BPI-SF). Effectiveness between baseline and FUP is assessed using paired t-tests with a p-value set at $p = 0.05$. As the database increases, further analysis using linear mixed models will analyse the role of treatment plan and pain specificities factors on treatment effectiveness.

Results: The initial data extraction included 198 patients. Pain was predominantly non-cancer related, persistent and similarly distributed between neuropathic and nociceptive (Table 1). At initial visit, a large majority of patients were treated with plant-derived cannabis products, with 20% prescribed a combination of pharmaceutical cannabinoids and plant-derived medical cannabis. Overall, oral cannabis oil was the preferred formulation (78%) followed by a combination of oil and dried flower (17%). Cannabidiol (CBD)-rich products were predominantly prescribed (55.6%) followed by THC:CBD balanced products (41.4%). Average daily dosage of oral CBD was 12.6mg (range 2-63mg) and for oral THC 2.3mg (range 0.07-18mg). There was statistically significant (all ps <0.04) improvement between Baseline and FUP for all BPI-SF variables and for all ESAS-r variables except nausea (see Figure 1 and 2). BPI-Pain severity scores decreased from 5.83 to 4.75 and BPI-pain interference scores from 5.19 to 3.98. Improvement of clinical significance was also observed in pain, fatigue, depression, anxiety, drowsiness, well-being and other problems, assessed through the ESAS-r.

Conclusions: This study provides preliminary indication that CBMs may be considered as an adjunct for the treatment of various pain conditions besides neuropathic pain. Results are both novel and require further investigation. In contrast with current literature, oral CBD-rich products were the preferred product and method of administration. Accurate dosage of THC and CBD from clinical trials is often unclear and these study results may inform clinical guidelines and practice.

COMPREHENSIVE ANALYSIS OF THE IMPACT OF MEDICAL CANNABIS TREATMENT ON PTSD SYMPTOMS, ANXIETY AND DEPRESSION LEVELS, TOBACCO SMOKING, ALCOHOL ABUSE, THE USE OF PSYCHOACTIVE PHARMACEUTICALS, AND QUALITY OF LIFE

Alex Bibikau* and Aleks Petrova

REN Health, Milford, CT 06460

Introduction: A Prospective, Longitudinal, Open-Label, Observational Study (2016-2020) was conducted at REN Health involving 540 patients with diagnosed PTSD "Comprehensive Analysis of the Impact of Medical Cannabis Treatment on PTSD Symptoms, Anxiety and Depression Levels, Tobacco Smoking, Alcohol Abuse, the Use of Psychoactive Pharmaceuticals, and Quality of Life". 402 patients were included per inclusion criteria, 138 patients were excluded per exclusion criteria.

Demographics: 58% Males (n=234), 42% Females (n=168), Average Age 40 years old, 13% Veterans (n=52), 13% patients on Disability (n=53), 23% Healthcare workers (n=93).

Methods: PCL-5 (PTSD Checklist-5) developed by the National Center for PTSD with 0-80 Scale to assess severity of PTSD symptoms and 0-10 Scale for Average Anxiety and Depression levels for the month preceding initial evaluation and for the month preceding annual re-evaluation.

Results: The study demonstrated an average reduction of PTSD symptoms by 59% since Medical Cannabis Patient Registration. Average PTSD score at initial assessment was 53.57 out of 80 (21,534/402). Average PTSD score at reassessment was 22.04 out of 80 (8,861/402). The most significant improvement the patients demonstrated in the following PTSD symptoms: 1) 77.3% less Risky or Destructive Behavior; 2) 67.2% less Exaggerated Blame of Self or Others; 3) 66.7% less Difficulty Experiencing Positive Feelings. The least improvement the patients demonstrated in: 1) 50.2% less Emotional Reactivity to Reminders of Trauma; 2) 50.7% less Intrusive Thoughts about Trauma; 3) 50.9% less Difficulty Recalling Key Features of Trauma.

Among 138 excluded patients, 86% of patients either saw an improvement of PTSD Symptoms (49% of patients), or were stable with PTSD Symptoms (37% of patients), whereas only 14.5% of excluded patients demonstrated an increase in PTSD Symptoms (i.e. > 4 PCL points).

Other outcomes: Anxiety decreased by 57% on average. Depression decreased by 64% on average. 61% of patients who smoked Tobacco either quit (41%) or reduced Tobacco (20%). 93% of patients who abused Alcohol either quit (49%) or reduced Alcohol consumption (44%). The Use of Psychoactive Pharmaceuticals reduced ranging from the minimum of 37% (Antipsychotics, n=24, 33% stopped, 4% reduced) to the maximum of 90% (Benzodiazepines, n=106, 57% stopped, 33% reduced). Improvement in Quality of Life was reported by 99.25% of patients (n=399).

AN INITIAL ANALYSIS OF THE UK MEDICAL CANNABIS REGISTRY

Simon Erridge*[1,2], Oliver Salazar[1], Michal Kawka[1], Carl Holvey[2], Ross Coomber[2,3], Azfer Usmani[2,4], Mohammed Sajad[2,5], Sushil Beri[1,2], Jonathan Hoare[1,2], Shaheen Khan[2,6], Mark W Weatherall[2,7], Michael Platt[1,2], James J Rucker[2,8,9] and Mikael H Sodergren[1,2]

[1] Imperial College London, London, UK
[2] Sapphire Medical Clinics, London, UK
[3] St.George's Hospital NHS Trust, London, UK
[4] Dartford and Gravesham NHS Trust, Kent, UK
[5] Dudley Group of Hospitals NHS Trust, West Midlands, UK
[6] Guy's & St. Thomas' NHS Foundation Trust, London, UK
[7] Buckinghamshire Healthcare NHS Trust, Amersham, UK
[8] Department of Psychological Medicine, Institute of Psychiatry, Psychology & Neuroscience, Kings College London, London, UK
[9] South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Cannabis was rescheduled (from schedule 1 to schedule 2 of the Misuse of Drugs Act 1971) in 2018 in the United Kingdom to allow specialist doctors to initiate treatment for a range of conditions, with the vast majority of patients treated in the private sector. In order to assess the benefits of these treatments, it is important to assess clinical outcomes in a systematic way. The UK Medical Cannabis Registry is a comprehensive platform registry set up to capture longitudinal data following treatment with cannabis-based medicinal products (CBMPs), utilising remote data collection, and includes clinical efficacy measures, patient-reported outcomes measures (PROMS) and adverse drug reactions. The aim of this project is to analyse the characteristics and outcomes of the first cohort of patients prescribed CBMPs in the UK and to evaluate its effects on health-related quality of life and clinical safety.

Methods*:* A cross-sectional analysis was performed of the UK Medical Cannabis Registry. Primary outcomes were change in PROMS (EQ-5D-5L, General Anxiety Disorder-7 (GAD-7) and Single-Item Sleep Quality Scale (SQS)) at 1 and 3 months. Secondary outcome was the incidence of adverse reactions. Statistical significance was defined by p-value<0.050.

Results:129 patients were included in the final analysis with a mean age of 46.23 (± 14.51) years. The most common indication was chronic pain of undefined aetiology (n=48; 37.2%). The median initial cannabidiol and (−)-trans-Δ^9-tetrahydrocannabinol daily dose was 20.0mg (Range: 0.0 – 768.0mg) and 3.9mg (Range: 0.0 – 660.0mg), respectively. Statistically significant improvements in health-related quality of life were demonstrated at 1 and 3 months in GAD-7, SQS, EQ-5D-5L pain and discomfort subscale, EQ-5D-5L anxiety and depression subscale and EQ-VAS scores (p<0.050). There were 31 (24.03%) total reported adverse reactions.

Conclusions: This study suggests that CBMP therapy may be associated with an improvement in some specific health-related quality of life outcomes as self-reported by patients. CBMPs are also demonstrated to be relatively safe in the short to medium-term. Further research is required to understand these effects on a disease specific basis across longer-term follow up.

TRENDS IN CANNABIS USE FOR HYPEREMESIS GRAVIDARUM

Olivia First*[a], Kimber W MacGibbon[a], Catherine M Cahill[b], Ziva D Cooper[b]
Lillian Gelberg[b], Victoria Cortessis[c], Patrick M Mullin[c] and Marlena S Fejzo[c]

[a] Hyperemesis Education and Research Foundation, OR, USA
[b] David Geffen School of Medicine, University of California Los Angeles, CA, USA
[c] Keck School of Medicine, University of Southern California, CA, USA

Introduction: Nausea and vomiting during pregnancy (NVP) are common, and in the United States (US) medication is prescribed to alleviate these symptoms in 1/5 of all pregnancies. The most severe form, Hyperemesis Gravidarum (HG), is defined as a severe degree of NVP that strongly affects daily living activities and leads to an inability to eat and/or drink normally. HG accounts for nearly 400,000 emergency room visits/hospitalizations in the US annually and can cause esophageal rupture, post-partum PTSD, and rarely, Wernicke's encephalopathy and maternal/fetal death. It is associated with an increased risk of adverse fetal outcomes including preterm birth, fetal growth restriction, neurodevelopmental delay, and autism spectrum disorder. Antiemetic medication is often prescribed off-label with little information on effectiveness. Cannabis use for NVP is on the rise, but little is known about its use in HG pregnancies. The purpose of this study is to understand trends in cannabis use to treat HG.

Methods: A survey of patients on treatment for HG including cannabis use was posted on social media websites related to the Hyperemesis Education and Research Foundation between March 3 and March 11, 2021. Respondents were asked about self-reported effectiveness of cannabis for HG, type of cannabis product used (delta-9-tetrahydrocannabinol [THC] or cannabidiol [CBD] dominant), reason for use, frequency of use, and mode of administration.

Results: 458 people responded to the survey. Respondents were primarily white (79%), from the US (70%), and delivered in 2019-2020 (24%). The majority of participants lost weight during pregnancy, with over half reporting greater than 10% weight loss below pre-pregnancy weight. Approximately 12% of participants reported cannabis use for HG. The main reason for treating with cannabis (68%) was that prescribed antiemetics were ineffective. More than half the respondents used products that contain THC and reported primarily smoking them (56%). Respondents reported using THC only products (37%) more often than THC dominant products (24%). 29% of participants started using cannabis prior to pregnancy, while 44% started in the 1st trimester and 25% started in the 2nd trimester. The majority of participants (51%) reported using cannabis daily and 34% stopped in the 2nd trimester. 80% reported cannabis was effective in alleviating HG symptoms, compared to 62% for prescription antiemetics. 51% reported weight gain within 2 weeks of first cannabis treatment, compared to 23% for prescription antiemetics.

Conclusions: Respondents reported using cannabis products for HG primarily because prescribed medications were ineffective. Patients reported that cannabis was more helpful than prescription medications in alleviating HG symptoms and enabling pregnancy weight gain. Safe and effective use of cannabis products to treat HG pregnancies requires further research because undernutrition, lack of weight gain in pregnancy, HG, and cannabis use in pregnancy are all associated with adverse fetal outcomes.

THERAPEUTIC EFFICACY, REDUCED PRESCRIPTION DRUG USE, AND MINIMAL SIDE EFFECTS OF CANNABIS IN PATIENTS WITH CHRONIC ORTHOPEDIC PAIN

Ari Greis*[1], Eric Larsen[2], Conan Liu[3], Bryan Renslo[3], Anjithaa Radakrishnan[3] and Adrianne R. Wilson-Poe[4]

[1]Department of Rehabilitation Medicine,
Rothman Orthopedics at Thomas Jefferson University, Philadelphia, PA, USA
[2]Department of Neurology, St Luke's University Health Network, Bethlehem, PA, USA
[3]Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, USA
[4]Department of Neurobiology, Legacy Research Institute, Portland, OR, USA

Introduction: Although cannabis is widely used for the treatment of chronic pain, most research relies on patient self-report and few studies have objectively quantified its efficacy and side effects. Extant inventories for measuring cannabis use were not designed to capture the medically relevant features of cannabis use but rather to detect problematic use or Cannabis Use Disorder. Thus, we sought to capture the medically-relevant features of cannabis use in a population of patients with orthopedic pain and pair this data with objective measures of pain and prescription drug use.

Methods: Study participants included patients in a large orthopedic practice with chronic low back, neck, shoulder, hip, and knee pain. In this prospective observational study, patients were enrolled in Pennsylvania's medical cannabis program by their treating pain management physician, received cannabis education from their physician at the time of certification, and purchased products from state licensed cannabis retailers. The first follow-up was conducted at 3 months after the initiation of cannabis therapy and then at 6 and 12 months. The therapeutic efficacy of cannabis was assessed with the PROMIS (Patient-Reported Outcomes Measurement Information System), the EuroQol EQ-5D, and VAS (Visual Analog Scale). Participants' Pennsylvania Drug Monitoring Program data was collected over the 6 month period prior to and following their medical cannabis certification. Patients who met criteria for opioid and benzodiazepine prescription analysis included those who filled at least one opioid or benzodiazepine prescription within the six months prior to cannabis certification and did not have surgery within 6 months of certification.

Results: At the first follow up (~3 months), low back, neck, and joint pain was significantly decreased with improvements in function and quality of life with marginal improvement in pain scores at subsequent follow ups. In participants taking opioid pain medication prior to cannabis certification, 72.6% decreased opioid consumption and 38.5% ceased opioid consumption entirely. Patients taking 15+ morphine milligram equivalents per day were most likely to cease or decrease their opioid use. In patients taking benzodiazepines prior to having access to cannabis, 31% were able to discontinue. Intriguingly, 52% of patients did not experience intoxication as a side-effect of cannabis therapy. The vast majority (84%) of patients who did experience intoxication reported that it was either enjoyable or did not interfere with activities of daily life.

Conclusions: Medical cannabis use was associated with clinical improvements in pain, function, quality of life, and reductions in prescription drug use with minimal cannabis-induced intoxication in patients with chronic orthopedic pain. Enhancing the resolution of previous epidemiological studies, this work provides a direct relationship between the initiation of cannabis therapy and objectively fewer opioid and benzodiazepine prescriptions. Importantly, our work also identifies specific sub-populations of patients for whom cannabis may be most efficacious in reducing opioid consumption and highlights the importance of both physician involvement and patient self-titration in symptom management with cannabis.

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REAL-WORLD EVIDENCE DESCRIBING AUSTRALIAN MEDICINAL CANNABIS PATIENTS, SAFETY, AND TREATMENT OUTCOMES

Maja Kalaba*[1], Wendy Mak[1], Alistair Vickery[2], Tracie Ernenwein[2], Patrizia Washer[2], Graham Eglit[1] and Matthew Feldner[1]

Spectrum Therapeutics – A Canopy Growth Company, Montreal, QC, CAN[1]
Emerald Clinics – Specialist clinics owned by healthcare technology company, Emyria Limited, AUS[2]

Introduction: Following legislative changes in the regulatory framework for medicinal cannabis in Australia, there has been a steady growth in patient numbers with approximately 25,000 active patients at the end of 2020. Despite the growing demand, very little is known about the patterns of medicinal cannabis use among Australians, regulatory access remains difficult and there have been calls for more evidence in relation to medicinal cannabis by authoritative bodies. This analysis was designed to collect real-world data (RWD) primarily to examine characteristics of medicinal cannabis patients in Australia. Additional planned analyses that are underway include: long-term safety; changes in concomitant medications; and self-reported effectiveness.

Methods: A longitudinal RWD collection of patients who attended Emerald Clinics in Australia from January 2020 to January 2021 was conducted. Inclusion criteria were: patients who provided informed consent and were prescribed a product from Spectrum Therapeutics (ST), a Canadian licensed medicinal cannabis producer. Clinic follow-ups (FU) by phone or in-person were scheduled as follows: week-2, week-6, and then approximately every two months for the duration of the treatment period, or end of treatment. Demographics, primary indication for using medicinal cannabis, physician prescription [(product name, format, and dose (mgs of THC & mgs of CBD)], concomitant medications, adverse events, and patient-reported outcomes using the Brief Pain Inventory Scale (BPI), and RAND 36-item Short Form (SF-36) were collected. Descriptive statistics were applied to describe the patient cohort. Additional description of adverse events reported as well as mixed effects modeling to understand self-reported outcomes over time are currently underway.

Results: A total of 620 patients met inclusion criteria, of whom 484 (78%) completed FU1, 262 (42%) completed FU2, and 126 (20%) completed FU3. All 620 patients were included in the analysis of the primary objective. Mean age was 57.3 years ±18.1(range 7-94); 52.5% were female and 77.5% identified as Caucasian. Patients resided in Western Australia, New South Wales, and Victoria (66%, 30%, 4%, respectively). Seventy-five percent (n = 462) of patients were unemployed of whom 38% were unable to work due to pain or a condition other than pain, and 23% were retired. Fifty-six percent of patients have been living with pain for more than 5 years. A total of 13 primary indications were identified, the most prevalent in descending order were: chronic non-cancer pain (73.7%), cancer pain (9.7%), and insomnia (3.1%). Irrespective of the indication, Spectrum Blue Oil was prescribed in >75% of patients at initial appointment and each FU. The mean starting dose after initial 2-week titration was 1.2 ml (12mg THC: 18mg CBD), which increased to 1.4 ml by FU2 (14mg THC: 21mg CBD).

Conclusions: These initial findings describe a cohort of Australian medicinal cannabis patients who appear to be unemployed, and predominately presented with chronic non-cancer pain. Results will also elucidate long-term safety, changes in concomitant medications, and self-reported outcome measures over time.

SEX DIFFERENCES AMONG ADVERSE EVENTS TO CANNABIS

Maja Kalaba*, Erica Peters, Chanez N. Kebache and Mark A. Ware

Canopy Growth Corporation, Montreal, QC, CAN

Introduction: Although the prevalence of cannabis use has historically been higher in men, recent evidence suggests that the gender gap in cannabis use is narrowing. Data on sex differences in adverse events associated with cannabis use is lacking. These data are needed to understand whether women have unique responses to cannabis and may require sex-specific education on expected effects. The objective of this analysis is to describe differences observed between males and females among adverse events (AEs) collected globally by Canopy Growth Corporation (CGC) in 2020.

Methods: Between January and December 2020, AEs were collected from multiple solicited and unsolicited sources. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), and seriousness criteria were defined by the International Conference of Harmonization (ICH). Products consumed were classified by content of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Where an individual was consuming multiple products, the AE reported was counted for each product. For this analysis, we excluded cases where sex was unknown, and excluded AEs that were coded as "lack of efficacy", "product issue" and "invalid cases." All AEs included were verified by a healthcare professional.

Results: A total of 2125 individual case safety reports comprising 5488 AEs were reported in 2020, of which 70% were female. Across both males and females, 94% of AEs were reported as non-serious. Sex differences were observed across reported AEs by product profile and product formulation, such that AEs reported for CBD-dominant products were significantly more common among females than males (44.6% vs 28.3%, respectively; p= 0.001), while AEs reported for THC-dominant products were significantly more common among males vs females (34.6% vs 20.6%, respectively; p= 0.001). Females were more likely than males to report an AE with an oil (74.4 % vs 25.5%; respectively; p=0.001) and softgel (69.2% vs 30.8%; respectively; p=0.001).The most prevalent SOC category for both males and females was Nervous System disorders (21%, 22%, respectively), followed by Psychiatric disorders (20%) in males, and Gastrointestinal disorders (20%) in females, but no significant sex differences emerged across SOC categories.

Conclusions: Overall, cannabis products appeared to be safe and well tolerated by both sexes, but adverse events were more likely to be reported by females. The finding that AEs reported for CBD-dominant products were more common in women than men is consistent with a previous report that females have reported stronger subjective responses to vaporized (although not oral) CBD and CBD-dominant cannabis. While this analysis suggests that sex differences in adverse events associated with use of cannabis vary by cannabinoid and product formulation, findings are limited by potential confounders such as reporting bias (i.e. greater reporting in females vs. males) and unknown consumer preferences (i.e. females may be more likely to consume CBD-dominant products vs. males). Future research is needed to determine resolution status of AEs and to explore the influence of dose consumed, duration of use, and previous experience with cannabis use on sex differences in adverse events associated with cannabis consumption.

NANO-PROCESSED CBG/CBD: EFFECT ON PAIN, ATTENTION DEFICIT AND HYPERACTIVITY DISORDER, IRRITABLE BOWEL SYNDROME, AND CHRONIC FATIGUE

Robert Kaufmann* and Vince Sanders

Midwest Allergy Sinus Asthma, Springfield Illinois
Shaman Botanicals, Kansas City, Missouri

Introduction: Cannabigerol (CBG) and cannabidiol (CBD) have been shown to have anti-inflammatory effects, however, their effect on specific inflammatory compounds is variably similar, different, or additive. Previous studies have suggested that CBG may have a positive effect in patients with inflammatory bowel disease. Recently, a formulation of CBG and CBD was developed and distributed to the public with reports of improvement being found in individuals suffering from these and other conditions. This survey was conducted to determine the experiences of the users of this product were similar to those being reported and if any adverse side effects were experienced.

Methods: The water soluble product used was a combination 15 mg/ml of CBG and 5 mg/ml of CBD treated with nanotechnology to make it water soluble. Individuals using the product were surveyed via an on-line app with questions on their pre and post energy, attention, anxiety, fatigue, and calmness levels, thinking ability, and sleeping patterns. Additionally, they were asked if they had any of the following: pain, attention deficit and hyperactivity disorder (ADHD), chronic fatigue (CF), irritable bowel syndrome (IBS), ulcerative colitis (UC), and Crohn's disease (CD); and if they had them, their pre and post condition. All pre and post questions used an ordinal scale for rating their responses. Questions regarding adverse side effects were included.

Results: 220 subjects completed the survey. On the questions involving fatigue, energy, thinking, and attention, >70% of those surveyed reported positive improvement with using the product with calmness and anxiety positive improvement being 63.2% and 58.6%, respectively, while on 44.1 had a positive score for sleep. Pain was reported by 118 individuals with 57.6% reporting a reduction in pain with using the product with 57.6% being able to reduce their use of pain medications. ADHD was reported by 43 respondents of which 99.7% reported an improvement in their attention level, 63.5% and 62.8% reporting improvement in hyperactivity and impulsiveness, respectively. CFS was reported by 27.3% and 88.0% reported an improvement. IBS was reported by 46 respondents with 56.5% reporting their condition was improved with this product. Of those with pain, 61.5% reported less pain with 51.3% being able to reduce their use of pain medication. Only 4 subjects reported ICS and one reported an improvement with the use of the product. No adverse effects were reported by anyone using this product in this survey.

Conclusions: The nano processed CBG/CBD product used in this study was found to relieve pain and irritable bowel syndrome symptoms, enabling over 50% of individuals to decrease or stop their pain medication. In addition, it reportedly decreased fatigue, improved energy and thinking, improved attention levels, and may have some influence on anxiety, calmness, and sleep. These effects seemed to be useful for those with ADHD, especially in the aspects of improved thinking and attention. The findings of this survey warrant more definitive studies of this product, especially in the areas of pain management, IBS, CFS, and attention disorders.

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USE OF A WATER-SOLUBLE FORM OF CANNABINOL FOR THE TREATMENT OF SLEEPLESSNESS

Robert Kaufmann* and Vince Sanders

Midwest Allergy Sinus Asthma, Springfield Illinois
Shaman Botanicals, Kansas City, Missouri

Introduction: Sleeplessness, getting < 7 hours sleep/night (L7H), causes significant health and financial problems for individuals and industry. Sleeplessness is classified into 4 categories: Difficulty initiating sleep (DIS), Difficulty maintaining sleep (DMS), Early morning awakening (EMA), and Nonrestorative sleep (NRS). A water-soluble form of CBN, created using nano technology, has recently become available to the general public as a nutritional supplement. This study was undertaken to study the use and efficacy of this product in individuals with sleeplessness.

Methods: Individuals taking this product completed a self-evaluation survey of their sleep prior to and after taking this product for a $\geq$ 7 days. All individuals had the ability to self-adjust the amount of product being taken. The data was analyzed for changes in rate and severity of sleeplessness. Severity was access by the number of days/wk for all categories except NRS which had a symptom score with the range: -6 to +6. Non-parametric data was analyzed using paired Chi-squares and parametric data being analyzed using paired t-tests.

Results: Sixty individuals (aged 18-72) returned the survey with 36 being male and 24 female. The average amount of CBN taken/night was 2.5 $\pm$ 1.16 mg with males taking slightly more CBN (mean of 2.94 $\pm$ 1.04 mg) than females (mean of 2.02 $\pm$ 1.12, mg), $p<0.005$. Almost half (48%) of the individuals reported needing to adjust the CBN dosage to achieve optimal sleep, whether from not taking enough CBN (17) or taking too much (12). Use of this product significantly decreased the prevalence and the severity of sleeplessness in all categories. The prevalence of L7S decreased from 42% to 13%, $p < 0.001$. The percentage of individuals experiencing the different categories of sleeplessness significantly decreased from 42% to 2% for DIS, 57% to 22% for DMS, 42% to 13% for DMA, and 60% to 13% for NRS, $p < 0.001$ for all comparisons. The number of days/week that individuals had L7S decreased from 5.72 $\pm$ 1.46 to 1.88 $\pm$ 2.80, while the number of days/week decreased from 4.77 $\pm$ 2.12 to 0.38 $\pm$ 1.27 for DIS, 5.00 $\pm$ 1.89 to 2.21 $\pm$ 2.91 for DMS, and 4.96 + 1.82 to 1.7 $\pm$ 2.76 for EMS, $p < 0.001$ for all comparisons. In the individuals suffering from NRS prior to starting CBN their symptom scores improved from -2.42 $\pm$ 1.93 to 2.75 $\pm$ 3.01, $p < 0.001$. Thirteen individuals had an adverse side effect which resolved in all individuals after self-adjustment of their dose.

Conclusions This study found that low doses (1-4 mg) of a nano treated, water-soluble CBN product significantly improved the sleep of individuals who had been experiencing occasional sleeplessness. It rapidly induced sleep initially and after awakening during the night. It improved both the length and quality of sleep such that the individuals felt rested upon awakening in the morning. It appeared to produce restful sleep by enabling individuals to progress through the various stages of sleep normally.

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CANNABIS USE FOR ANXIETY DURING THE COVID-19 PANDEMIC IN THE UNITED STATES

Stephanie L. Lake*,[1,2] Ryan D. Assaf,[1,3] Pamina M. Gorbach,[1,3,4] Ziva D. Cooper[1,2,5]

[1]UCLA Cannabis Research Initiative, Jane and Terry Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, USA
[2]Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, USA
[3]Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, USA
[4]Division of Infectious Diseases, David Geffen School of Medicine, University of California, Los Angeles, USA
[5]Department of Anesthesiology and Perioperative Medicine, David Geffen School of Medicine, University of California, Los Angeles, USA

Background: The SARS-CoV2 (COVID-19) pandemic brought many changes related to elevated health concerns and social distancing regulations across the globe. In the United States (U.S.), anxiety is a leading mental health concern for which medical cannabis is used. Nearly 50% of Americans have struggled with related mental health issues, including anxiety during the COVID-19 pandemic. This cross-sectional study sought to determine if cannabis use for anxiety changed during the pandemic.

Methods: Between August 30, 2020 and September 10, 2020, an online cross-sectional survey was administered to adults (aged 18 and over) in the U.S. who used cannabis for medical and/or non-medical purposes in the previous year. We used McNemar's test to compare changes in prevalence and frequency of cannabis use for anxiety in the three months preceding the COVID-19 pandemic and for three months during the COVID-19 pandemic. Multivariable logistic regression was used to examine demographic characteristics, regulatory factors, and non-medical cannabis use patterns associated with increasing frequency of cannabis use for anxiety during COVID-19.

Results: In total, 1886 eligible respondents completed the survey. Of them, 603 (32.0%) reported medical cannabis use and 15.7% (n = 297; 49.3% of those who used medical cannabis) reported using cannabis for anxiety in at least one of the two assessment periods. The overall prevalence of cannabis use for anxiety did not change significantly from the pre-COVID assessment period (12.7% *vs.* 11.9% overall, or 39.8% *vs.* 37.1% among those who used medical cannabis; $p>0.05$). Among the sub-sample of respondents who used cannabis for anxiety, the frequency of use increased during COVID-19, with significantly more respondents reporting daily use of cannabis for anxiety in the period corresponding with COVID-19 (18.7% *vs.* 24.9%; $p=0.006$). The majority (>80%) of the sub-sample reported that cannabis made their anxiety "somewhat better" or "much better". In a multivariable model, participants who increased cannabis use for anxiety during COVID-19 were more likely to be women and living in a legal medical cannabis state. Compared to respondents in the Midwest, those from West and South census regions were significantly less likely to increase cannabis use for anxiety. Non-medical cannabis and CBD usage were not significantly associated with the outcome.

Conclusions: In this sample of adults in the U.S. who use cannabis, the prevalence of cannabis use for anxiety remained stable, but the frequency among those using for anxiety increased significantly during COVID-19. These findings may be indicative of heightened anxiety related to the pandemic, particularly among women and those living in the Midwest region of the US. Although anxiety is rarely an indication for medical cannabis use in states with medical cannabis regulatory frameworks, increased use of cannabis for anxiety management was more likely in states with medical cannabis legalization. Future research should monitor changes in mental health outcomes, including anxiety symptomology, associated with increases in cannabis use during COVID-19.

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USE OF CANNABIS TO SELF-MEDICATE FOR CHRONIC SPINE PAIN

Rachael Rzasa Lynn[1], Gregory L. Kinney[2], Rahwa Netsanet[3],
Vikas Patel[3], Michael Finn[4*] and Emily Lindley [3*]

University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
1. Department of Anesthesiology; 2. Department of Epidemiology;
3. Department of Orthopedics; 4. Department of Neurosurgery
* Shared Senior Authorship

Introduction: Chronic back and neck pain are highly prevalent and disabling musculoskeletal conditions that affect 70% to 85% of individuals at some point in their lives. When conservative care and non-steroidal anti-inflammatory drugs fail to provide relief, opioid pain medications are often prescribed. However, opioids are often ineffective at providing adequate relief and can result in frequent use of high doses that increases the potential for dependency and lethal overdose. As a result, there is a large international movement to reduce opioid use and find alternative analgesic treatments. One such treatment that has received a great deal of interest, from both the scientific community and patients, is cannabis. In the state of Colorado, cannabis was legalized for medical purposes in 2000 and then for recreational use in 2014. Pain relief is the most frequently cited benefit of medical cannabis by US adults and is one of the most common indications for medical cannabis card requests. The purpose of this study was to determine the patterns of cannabis usage among spine surgery patients presenting to a tertiary spine clinic in the state of Colorado, prior to legalization of recreational cannabis.

Methods: After local IRB approval, adult patients presenting for initial spine surgery evaluation at our institution were offered study enrollment. After informed consent, a brief survey regarding cannabis use was administered.

Results: Of the 200 patients offered enrollment, 184 (92%) agreed to participate: 83 males (45%) and 101 females (55%). Of the 184 participants, 35 patients (19%) reported self-medicating with cannabis to treat their chronic spine pain. Cannabis users were significantly younger than non-users (44 vs. 54, $p<0.001$), but the overall age ranges were similar between groups (users 18-84, non-users 18-95). Patients using cannabis reported higher NRS pain scores than non-users: back pain (users 7.2 vs non-users 6.1), neck pain (6.6 vs 5.4), leg pain (6.0 vs 5.6), and arm pain (7.4 vs 4.2). Users had a longer history of spine pain than non-users (49% of users had experienced pain for >3 years vs 33% of non-users). Cannabis users also had higher incidences of depression (43% vs 19%), anxiety (29% vs 14%), and other diagnosed chronic pain syndromes (29% vs 10%). Users were also more likely to be using opioids than non-users (57% vs 42%). Patients typically used cannabis at least once a day (63%) with the most common administration route being inhalation of smoked plant material (45%), followed by a combination of smoked and oral ingestion (19%), and a combination of both smoked and vaporized plant material (10%). Of the patients who reported cannabis use for their pain, 43% had a Medical Marijuana Card. When asked about their pain relief from cannabis, 74% of users reported that cannabis "very much" or "moderately" relieved their pain, and 81% felt that cannabis worked better than or equal to their opioid medications. In addition to pain relief, 77% of users reported at least one other positive effect of cannabis, including sleep (63%), decreased anxiety (57%), and mood (17%). However, 46% reported at least one negative side effects of cannabis use, including increased appetite/weight gain (29%), difficulty concentrating (23%), increased anxiety/paranoia (14%), memory problems (11%), and depressed mood (6%).

Conclusion: Nearly 20% of surveyed patients with chronic spine pain reported current use of cannabis for pain management. The majority of these patients indicated that cannabis provided at least moderate alleviation of their chronic spine pain. Further, most felt that cannabis worked better than or equal to their opioid medications. However, 46% of the patients reported negative side effects, with about half of these patients indicating the side effects are "important." Less than half of the cannabis users had a legal card for medical cannabis, and were thus using illegal channels to obtain cannabis. With increased access to cannabis following the recreational legalization in Colorado, we anticipate the number of patients self-medicating with cannabis in 2020 is likely much higher. We are currently planning a repeat survey to directly compare current use rates with those seen prior to legalization of recreational sales.

SEX DIFFERENCES IN SAFETY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF TWO ORAL MEDICAL CANNABIS PRODUCTS AMONG HEALTHY ADULTS

Laura MacNair*[1], Erica N. Peters[1], Graham M.L. Eglit[1], Irina Mosesova[1], Ryan Vandrey[2], Hunter Land[1], Mark A. Ware[1], Cynthia Turcotte[1] and Marcel O. Bonn-Miller[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario Canada
[2]Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Few studies have examined sex differences with respect to medical cannabis products. This is a secondary data analysis from two double-blind, placebo-controlled, multiple-dose studies investigating Spectrum Yellow oil (20 mg/mL cannabidiol [CBD]/0.9 mg/mL Δ^9-tetrahydrocannabinol [THC]) and Spectrum Red softgels (0.03 mg CBD/ 2.5 mg THC) wherein safety, pharmacokinetic (PK), and pharmacodynamic (PD) sex differences were examined.

Methods: In both studies, participants (males, n = 38; females, n = 46) were randomized to 1 of 5 THC dose conditions in which they received approximately 5, 10, 15, or 20 mg THC (and corresponding levels of CBD) daily, or placebo, every 12 hours for seven days. Outcomes included treatment-emergent adverse events (TEAEs), plasma concentrations of CBD, THC, and primary metabolites (7-OH-CBD, 7-COOH-CBD, 11-OH-THC, and 11-COOH-THC) on Days 1 and 7, and 12 hour changes in a 20-item measure of subjective drug effects on Days 1, 3, and 7. Principal component analysis was applied to each item at hour 4 on Day 1 and generalized estimating equations with mean centered time were conducted. Subjective drug effects did not differ between the two studies; hence, data on subjective effects were pooled.

Results: In both studies, females had more TEAEs overall (15, Spectrum Yellow; 20, Spectrum Red) than males (6, Spectrum Yellow; 7, Spectrum Red), and TEAEs reported by females were of greater severity (p< .05 in each study). After controlling for body weight, PK parameters for CBD, THC, and metabolites did not differ by sex. Principal components analysis of the 20 items of subjective drug effects yielded four components: Bad Effects, Good Effects, Energy, and Distraction. On Day 1, females reported significantly greater acute increases in Bad Effects relative to men, and this difference was more pronounced at the 15 and 20 mg THC daily doses. However, the sex-related disparity in Bad Effects dissipated by Day 7. Both males and females had a dose-dependent increase in drug response to Good Effects on Day 1 and Day 3; however, on Day 7, females in the 15 and 20 mg THC dose groups continued to show a significant increase from pre-drug in Good Effects, while males' showed minimal increases in Good Effects from pre-drug at any dose on Day 7. Ratings of Energy and Distraction did not differ by sex.

Conclusions: Healthy females had a higher incidence and severity of TEAEs over 7 days of dosing of oral medical cannabis products as compared with males. Sex differences in subjective effects varied by both dose and dosing day. PK may not completely explain observed sex differences in adverse and subjective effects, and future studies are needed to elucidate other mechanisms (e.g., endocannabinoid tone) that could explain sex differences in adverse and subjective response to cannabinoids.

Acknowledgements: This study was funded by Canopy Growth Corporation.

EFFECTS OF CANNABIDIOL (CBD) ON SIMULATED DRIVING AND COGNITIVE PERFORMANCE: A RANDOMISED CONTROLLED TRIAL

Danielle McCartney*, Anastasia S. Suraev, Richard C. Kevin, Christopher Irwin, Melissa J. Benson, Ronald R. Grunstein, Camilla M. Hoyos and Iain S. McGregor

Lambert Initiative for Cannabinoid Therapeutics,
University of Sydney, Sydney, NSW, Australia
Woolcock Institute of Medical Research, Sydney, NSW, Australia

Objective: Cannabidiol (CBD) use is increasing worldwide as its therapeutic effects are better established and legal availability improves. While CBD is not generally considered intoxicating, a more robust understanding of its effects on cognitively demanding day-to-day activities, such as driving, would be prudent given its growing popularity. This study investigated the effect of acute oral CBD treatment on simulated driving performance, cognitive function and subjective experiences in healthy volunteers.

Methods: Eighteen healthy individuals (8 female; median [IQR] age, 28.5 [8.5] years) **who** had held an unrestricted driver's license for >1 year and had not used cannabis in >3 months participated in this randomised, double-blind, crossover trial. Participants completed four experimental sessions each separated by a washout period ≥7 days. During each session, they were administered a placebo, or 15, 300, or 1500 mg CBD in a high fat (50 g) dietary supplement (Calogen®, Nutricia) designed to increase its bioavailability. Driving performance was measured 45- and 210-minutes post-treatment using a 30-minute simulated scenario incorporating a ~7-minute Car Following Task (CF) and a ~25-minute Secondary Task (along highway and rural roads). Standard deviation of lane position (SDLP), a well-established marker of impairment, was measured throughout. Cognitive function and subjective experiences (stoned, sedated, anxious, alert, and sleepy) were also measured, and blood sampled, at regular intervals. We hypothesised that CBD would not increase SDLP by more than the non-inferiority margin (Δ) compared to placebo. Δ was defined as Cohen's d_z effect of 0.50 based on research suggesting a 0.05% blood alcohol concentration (BAC) has an effect of this magnitude SDLP (McCartney et al., Hum Psychpharm Clin, 35 (2020) e2749). Secondary outcomes were analysed using linear mixed-effect models.

Results: Non-inferiority (Upper 95% CI for SDLP <0.50) was established during the Secondary component of both drives under the 15 and 300 mg treatments and during the CF component of Drive 2 under the 300 mg treatment. The 1500 mg treatment also demonstrated non-inferiority during the Secondary component of Drive 1 and CF component of Drive 2, but increased SDLP during the CF component of Drive 1 (d_z=0.48; 95% CI's: 0.11, 0.86). The remaining comparisons were inconclusive (95% CI's included zero and 0.50). CBD did not impair cognitive performance on the paced serial addition, divided attention or digit symbol substitution tasks (*p*'s>0.05). However, participants tended to report feeling less alert with 1500 mg than placebo (*p*=0.086).

Conclusion: When consumed orally, lower doses of CBD (up to at least 300 mg) do not appear to affect driving performance. Higher doses may, however, increase SDLP in the absence of any marked feelings of intoxication or cognitive impairment. Further research is required to clarify the clinical significance of this increased SDLP, which could be comparable to that observed at a 0.05% BAC.

MORE CANNABIS-INDUCED HYPODOPAMINERGIC ANHEDONIA AND COGNITIVE DECLINE FROM LEGALIZATION REQUIRING PUTATIVE INDUCTION OF DOPAMINE HOMEOSTASIS AND POTENTIAL OPIOID RESTORATION MODELING?

Kenneth Blum*,[1] Joseph Morgan[2*], Jean Lud Cadet[3], David Baron[1], Paul R. Carney[4], Jag Khalsa[5] and Mark S. Gold[6]

[1]Western University Health Sciences, Pomona, CA, USA; [2] Substance Use Disorders Institute, University of the Sciences, Philadelphia, PA., USA; [3] Molecular Neuropsychiatry Research Branch, DHHS/NIH/NIDA Intramural Research Program, NIH, Baltimore, MD; [4] Neuroscience Program, University of North Carolina at Chapel Hill, Chapel Hill, NC; [5] Department of Microbiology, Immunology, & Tropical Diseases, Georgetown University School of Medicine, Washington, DC., USA; [6]Department of Psychiatry, Washington University School of Medicine, St. Louis. MO., USA

Introduction: Regular use of marijuana has substantially increased among younger adults resulting in the development of cannabis use disorder (CUD) with an estimated prevalence of 8.3% in the United States. Heavy use of marijuana (unanalyzed plant material), especially if contaminated with neurotoxic pesticides or solvents, is associated with hypodopaminergic anhedonia (depression), cognitive decline, poor memory, inattention, impaired learning performance, reduced dopamine brain response-associated emotionality, and increased severity of addiction. There is increasing concern by the addiction medicine community that because of contaminants and high THC content in some oral/vaped cannabis products, adverse cognitive effects of marijuana may become more pronounced in young adults who abuse these products. However, therapies may be able to clinically restore the dopaminergic dysfunction by inducing 'dopamine homeostasis' and reduce cannabis use and neurocognitive adverse events in heavy and very heavy chronic users. Methods: Blum's group developed the first patented genetic addiction risk severity (GARS) test as evidenced from studies linked to clinical predictive outcomes using the Addiction Severity Index (ASI). Including testing over 1000 patients each for at least 11 genetic variants associated with increased addiction risk. Understanding the common neuromodulating aspects of neurotransmission and its disruption via chronic exposure of drugs like cannabis and or even opioids and behavioral addictions, requires a known approach involving "dopamine homeostasis". Specifically, published studies illustrate the coupling of GARS with KB220Z variants utilizing a semi-customized precision Pro- Dopamine Regulation (PDR) matched to one's GARS. Results: The same group showed many beneficial effects of KB220 variants (i.e., attenuation of anhedonia) in over 48 peer reviewed published clinical trials including triple blind placebo control. Blum's group developed the brain reward cascades (BRC) showing the interaction of many neurotransmitters leading to the neuronal release of dopamine at the N. Accumbens (NAc)[1]. In other studies, KB220z induces Bold activation and increases resting state functional connectivity in naive rodents specific to the brain reward circuitry[2]. rsfMRI in abstinent heroin addicts following one-hour post KB220z oral administration revealed induction of "Dopamine Homeostasis."[3] Conclusions: While there is plethora of research supporting high dose THC and alterations of both anatomical and neurophysiological unwanted brain changes, there are some positive effects of CBD with or without low doses of THC to help attenuate alcohol/heroin abuse. Certainly, based on what we know about the co-localization of CB1 and delta/mu opioid receptors, when activated by for example THC and subsequent enkephalin induced inhibition of GABA leads to required dopamine release at NAc. Early (70s) studies from Blum's group[4] showed the importance of THC in reducing alcohol withdrawal and confirmed by more recent published reports, provide rational to carefully investigate CBD with THC to potentially help reduce unwanted aberrant heroin seeking behavior. Understanding these psychological, neurobiological, anatomical, genetic/epigenetic data provides indisputable evidence that must be utilized in decisions concerned with cannabis legalization and its medical utilization.[4,5]

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EFFECT OF FULL SPECTRUM CANNABIDIOL ON HEART RATE VARIABILITY AND PSYCHOLOGICAL STRESSORS IN MILD TRAUMATIC BRAIN INJURY: CASE SERIES

[1]J. Patrick Neary*, [1]Jyotpal Singh, [1]Taylor A. Teckchandani and [2]Lanishen Bhagaloo

[1]Faculty of Kinesiology & Health Studies, University of Regina, Regina, SK, Canada
[2]College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Introduction: Cannabinoids have been known to have physiological and psychological protective properties. There are suggestions that an ensemble effect of all the properties of the Cannabis plant can work synergistically to improve neurological functioning following traumatic brain injury. However, there has been rising interest in full spectrum cannabidiol (CBD), without the presence of Δ9-tetrahydrocannabinol (THC). Therefore, we present novel results analysing the effects of different dosages of full spectrum CBD on heart rate variability (HRV) parameters and psychological stressors in three female individuals suffering from medically diagnosed post-concussion syndrome.

Methods: Each participant completed the Patient Health Questionnaire-9, Generalized Anxiety Disorder-7 Questionnaire, and the Positive and Negative Affect Schedule. Participants were then attached to a 3-lead electrocardiograph and the raw electrocardiogram waveform was used to assess HRV. Following 5 minutes of seated rest, the participant underwent guided breathing at 6 breaths per minute for 5 minutes. Psychological and HRV data was recorded every 10 to 20 days for up to 69 days. CBD was self-administered by the participants with dosage ranging from 25mg/mL to 400mg/mL per day. Individual correlations between HRV responses with respect to scores on the questionnaires were completed using Pearson correlation quotient (r).

Results: All three participants displayed significant correlations between HRV parameters and the psychological questionnaires ($p<0.05$) but demonstrated individual differences. Participant 1 showed improvements in spectral HRV (ms^2) ($r= -0.90$ to -0.97) and Poincaré plot SD1 (ms) ($r= -0.92$) as Generalized Anxiety Disorder-7 scores decreased; Participant 2 showed increases in Poincaré plot parameters SD1 (ms) and SD2 (ms) ($r= 0.84$ and 0.89), total power (ms^2) ($r= 0.85$) and low frequency (ms^2) ($r= 0.88$) as the positive scores from Positive and Negative Affect Schedule increased; Participant 3 showed decreases in the very low frequency (ms^2) spectral component as the negative scores from Positive and Negative Affect Schedule decreased ($r= 0.95$).

Conclusion: Full spectrum CBD can therapeutically target the cardiovascular system in individuals suffering from post-concussion syndrome, and measuring HRV can provide greater insight as to the ability of CBD to aid with psychological stressors such as anxiety. Therefore, we suggest that more studies are needed to observe changes in HRV when using cannabidiol to reduce psychological stressors. This would provide additional research data to investigate the underlying mechanism(s) for the improved psychological profile.

WORRY AMONG PREGNANT AND BREASTFEEDING WOMEN CANNABIS CONSUMERS IN THE UNITED STATES DURING THE COVID-19 PANDEMIC

Natalia Parraguez*[1], Cynthia Lebron[1], Scylla Blervacq[1],
Sarah E. Messiah[2] and Denise C. Vidot[1]

[1]School of Nursing and Health Studies, University of Miami, Miami, FL, USA; [2]University of Texas Health Science Center, Dallas, TX, USA.

Introduction: Cannabis use has doubled in the past decade in the United States. Increasingly, pregnant women are self-medicating or following health care provider recommendations to use cannabis to treat nausea, pain and mental health symptoms such as depression and anxiety. Worry is a trait within general anxiety disorder and can have physical health manifestations; yet is understudied among pregnant and breastfeeding women. There has been an increased prevalence of anxiety and depression among pregnant and breastfeeding women amid the COVID-19 pandemic; however, little is known about the impact among those who consume cannabis. The objective of this study was to describe the patterns of cannabis use and worry due to the COVID-19 pandemic among currently or previously breastfeeding and pregnant women.

Methods: Mothers who self-reported cannabis use were recruited to complete an anonymous internet survey through social media using hashtags. The survey inquired about cannabis use prior to the COVID-19 pandemic and administered the COVID-19 Cannabis Health Questionnaire. Participants self-reported the cannabinoid ratios of the most frequently consumed cannabis. The sample consisted of 164 women living in the United States between the ages of 18 and 43 who reported being currently or previously breastfeeding and/or pregnant.

Results: The majority of the sample of women reported use of cannabis for anxiety (66.5%) and depression (54.3%), followed by treating nausea (36%). Most women indicated using THC as the dominant cannabinoid, followed by varied CBD/THC ratio. During COVID-19, 68.3% of pregnant women were increasingly worried about their pregnancy and 54.7% of all women worried about infecting their child. Thirty-one (31.1%) percent of women reported an increased frequency of cannabis use since COVID-19 was declared a pandemic.

Conclusion: These results suggest the prevalence of cannabis use among current and previously pregnant and breastfeeding women prior to the COVID-19 pandemic that has increased since COVID-19 was declared a pandemic. Women report the use of cannabis to relieve anxiety and depressive symptoms. In this sample, there was increased apprehension regarding COVID-19 and risking their newborns to exposure.

SAFETY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF SPECTRUM RED SOFTGELS IN HEALTHY PARTICIPANTS

Erica N. Peters*[1], Irina Mosesova[1], Laura MacNair[1], Ryan Vandrey[2], M. Hunter Land[1], Mark A. Ware[1], Cynthia Turcotte[1] and Marcel O. Bonn-Miller[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario, Canada
[2]Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction: Due to a lack of published pharmacokinetic (PK) and/or pharmacodynamic (PD) data, informed physician and patient decision-making surrounding appropriate dosing of cannabis used for medical purposes is limited. This Phase 1, multiple-dose study evaluated the safety, tolerability, PK, and PD of Spectrum Red softgels (2.5 mg Δ^9-tetrahydrocannabinol (THC) and 0.03 mg cannabidiol (CBD)), a cannabis-based product commercially available for medical use in Canada and Australia.

Methods: Participants (N = 41) were randomized to one of five groups: 5 mg THC and 0.06 mg CBD daily (Treatment A), 10 mg THC and 0.12 mg CBD daily (Treatment B), 15 mg THC and 0.18 mg CBD daily (Treatment C), 20 mg THC and 0.24 mg CBD daily (Treatment D), or placebo. Participants were confined to a research facility and received study medication every 12 hours, approximately 60 minutes after a standardized meal for 6 days, plus a single dose in the morning of Day 7. Participants were discharged after a 32-hour post-dose blood draw on Day 8, and returned to the research facility on Days 9, 10, 11, and 13 for blood draws and study assessments. Treatment-emergent adverse events (TEAEs); plasma and urine concentrations of THC, CBD, and metabolites; and self-reported subjective effects were collected.

Results: All TEAEs (65/65) were of mild to moderate severity; none was serious. The highest number of TEAEs (30/65) occurred on the first day of treatment. The most common TEAEs included somnolence, lethargy, and headache (reported by 8, 7, and 5 participants, respectively). On Day 7, maximum observed plasma concentration of 11-carboxy-THC increased by 2.0- and 2.5-fold as the dose doubled between Treatments A and B and between Treatments B and D, respectively. Quantifiable concentrations of THC and 11-hyroxy-THC were infrequent on both Days 1 and 7; those concentrations that were quantifiable were observed between 1 and 4 hours post-dose. Mean peak post-treatment ratings of self-reported subjective effects of "feeling the effects," "bad effects," and "dazed" differed between Treatment D and placebo on Days 1 and 7.

Conclusions: Over a week of twice-daily dosing of Spectrum Red softgels, daily doses of THC up to 20 mg and of CBD up to 0.24 mg were generally safe and became better tolerated after the first day of treatment. A prudent approach to improve tolerability with Spectrum Red softgels might involve initial daily doses no higher than 10 mg THC and 0.12 mg CBD in divided doses, with titration upwards over time as needed based on tolerability. This study advances beyond single-dose studies because its multiple-dose design and broad dose-ranges more closely approximate real-world conditions in individuals who consume non-pharmaceutical cannabis products.

This study was funded by Canopy Growth Corporation.

CANNABICHROMENE: PHARMACOKINETICS IN HEALTHY ADULT PARTICIPANTS

Erica N. Peters*[1#], Laura MacNair[1], Irina Mosesova[1], Uwe Christians[2], Cristina Sempio[2], Jost Klawitter[2], M. Hunter Land[1], Mark A. Ware[1], Cynthia Turcotte[1] and Marcel O. Bonn-Miller[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario, Canada
[2]iC42 Clinical Research and Development, Department of Anesthesiology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA

Introduction: Cannabichromene (CBC) is a phytocannabinoid commonly found in cannabis, yet its acute post-dose pharmacokinetics (PK) have not been examined in humans. This is a secondary data analysis from a trial investigating Spectrum Yellow oil, an oral cannabis product used for medical purposes that contained 20 mg cannabidiol (CBD) and 0.9 mg Δ^9-tetrahydrocannabinol (THC), as well as 1.1mg CBC, per 1 mL of oil. We have previously reported that Spectrum Yellow oil was well-tolerated.

Methods: Participants (*N*=43) were randomized to one of 5 groups: 120 mg CBD, 5.4 mg THC, and 6.6 mg CBC daily; 240 mg CBD, 10.8 mg THC, and 13.2 mg CBC daily; 360 mg CBD, 16.2 mg THC, and 19.8 mg CBC daily; 480 mg CBD, 21.6 mg THC, and 26.4 mg CBC daily; or placebo. Study medication was administered approximately every 12 hours for seven consecutive days. Plasma CBC concentrations were collected after a single dose and after the final dose, and analyzed by a validated two-dimensional high-performance liquid chromatography - tandem mass spectrometry assay (lower limit of quantification = 0.78 ng/mL).

Results: Within each treatment group, there was notable variability between participants in mean concentrations of CBC at each timepoint. After single and multiple doses, the maximum observed plasma concentration (C_{max}) of CBC increased by 1.3-1.7-fold for each 2-fold increase in dose, and the t_{max} range was 1.6-4.3 h. Based on the ratio of administered CBD, THC, and CBC to the plasma concentration as observed in the parent study, the dose of CBD was 18 times higher than the dose of CBC, yet the area under the curve from time zero to last measurable concentration (AUC_{0-t}) of CBD was only 6.6-9.8 fold higher than the AUC_{0-t} of CBC; the dose of THC was similar to the dose of CBC, yet THC was quantifiable in fewer plasma samples than was CBC.

Conclusions: At daily doses up to 26.4 mg, CBC in the presence of CBD and THC appears to be well-tolerated and is quantifiable in plasma in humans. CBC may have preferential absorption over CBD and THC when administered together.

This study was funded by Canopy Growth Corporation.

CANNABIS USE AND THE INCIDENCE OF MENTAL HEALTH DIAGNOSES: A 12-YEAR STUDY OF PEOPLE WHO USE DRUGS IN A CANADIAN SETTING

Hudson Reddon*, Cameron Grant, Ekaterina Nosova, Kora DeBeck and M-J Milloy

BC Centre on Substance Use, University of British Columbia, Vancouver, Canada

Introduction: Despite the expansion of recreational cannabis use policies, the impact of cannabis use on the development of mental health disorders continues to be an area of scientific controversy. We undertook the present study to analyze the association between cannabis use on the incidence of mental health disorders among people who use drugs (PWUD).

Methods: The data for this investigation was collected from two prospective cohorts of PWUD in Vancouver, Canada between September 2005 and November 2017. Study participants completed follow-up visits biannually after baseline and extended Cox regression models were used to estimate the association between cannabis use and the diagnosis of any mental health disorder.

Results: A total of 1,940 participants were enrolled in this study, including 704 (36.3%) females and 194 (10.0%) reported a diagnosis of a mental health disorder at baseline. In the multivariable analysis, cannabis use was not significantly associated with mental health disorder diagnosis over follow-up (adjusted hazards ratio [AHR] = 0.89, 95% CI 0.50–1.61). Childhood trauma (AHR = 2.14, 95% CI 1.30–3.50) and recently accessing social services (AHR = 2.06, 95% CI 1.09–3.90) were positively associated with mental health disorder diagnosis.

Conclusions: Frequent use of cannabis was not significantly associated with incident mental health disorder diagnosis over a 12-year follow-up period. These findings contribute to a large discordant body of evidence surrounding the mental health consequences of cannabis use. Further analysis using long-term prospective designs and integrating genetic risk for mental health will be needed to characterize this relationship with more certainty.

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LONG-TERM FOLLOW-UP OF MEDICAL CANNABIS THERAPY IN LOW BACK PAIN RESULT OF A SEQUENTIAL 500-PATIENT COHORT

Dror Robinson

Department of Orthopedics, Hasharon Hospital
and Tel Aviv University

Introduction: During the last 10 years medical cannabis therapy (MCT) has been used extensively in Israel. One of the approved indications is neuropathic pain including radicular pain emanating from the lower back. The following data includes a sequential cohort of patients. These were prospectively evaluated patients first treated at a single cannabis-oriented orthopedic clinic between Dec 2013 and Dec 2015 (average follow-up 65±3 months, range 60 to 72 months) . This cohort represents real world experience with cannabis as a therapeutic in radicular pain.

Methods: All patients were evaluated by a single physician according to a standardized protocol including anamnesis, SF12, concomitant medications as issued by HMO's, and invasive procedures performed. The degree of pain was scored according to the Brief Pain Inventory (BPI). The disability degree was scored using the Oswestry Pain Index (ODI). Patients with a minimal five-year follow-up period were included in the study

Results: Discontinuance rate of cannabis therapy was less than 1 percent (4 of 500). Pain intensity was 9±1 and Pain interference was 7.5±2 prior to MCT initiation. SF12 PCS was 43±8 and MCS was 38±9. Patients were treated by 20 grams per month initially and dose was adjusted as needed every few months. After a minimal 5 year follow-up period dose averaged 45±20 grams per month. Narcotic usage declined in all patients. 83% stopped using narcotics altogether. 11% decreased their usage by one half morphine equivalent units. 6% percent did not significantly decrease their narcotic consumption. Pain intensity at last follow-up was 4±2 and pain interference was 2±2. SF12 PCS was 75±12, SF12 MCS was 67±15. Work participation increased from 15±8% to 53±11% and ODI decreased from 88±10 to 54±21.

Discussion: The current study indicates that in a real-life scenario patients with chronic radicular pain improve both in degree of pain intensity and in degree of pain interference by the use of MCT. This improvement allows decrease in opiate consumption and increase in work participation. The ameliorative effects of MCT persist for a minimum period of five years. The down-side is the hesitancy of patients to stop MCT therapy, making long-term treatment with its attendant costs and risks, highly likely.

THE EFFECT OF MEDICAL CANNABIS TREATMENT ON POLYPHARMACY IN OLDER ADULTS

Addie Ron*[1], Vered Hermush[2,3] and Lihi Bar-Lev Schleider[4,5].

[1]Geriatric Section, Sharon District, Maccabi Helath Services, Israel;
[2]Geriatric Wing, Laniado Hospital, Netanya, Israel.
[3]Technion School of Medicine, Haifa, Israel.
[4]Research Department, Tikun Olam -Cannbit Pharmaceuticals.
[5]Clinical Research Center, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel

Background: The use of medical cannabis in recent years is growing substantially in all age groups, but especially in older adults. Most of them use cannabis for medical purposes. Polypharmacy is of particular concern in older people and half of them receive five or more medications. The use of greater numbers of drug therapies has been independently associated with worst outcomes such as increased risk for adverse drug events, hospital admissions, falls, hip fractures, and more. Addressing polypharmacy can identify drug-related problems and improve older adult's medical status. We aim to investigate the effect of medical cannabis treatment on polypharmacy in older adults.

Methods: A prospective study that included all patients 65 years of age or older who received medical cannabis from January 2015 to May 2018 in a specialized medical cannabis clinic. We included specifically those who reported five or more prescribed medications. We analyzed those who used medical cannabis for at least six months and were willing to answer the initial questionnaire and the six months follow-up questionnaire.

Results: During the study period, 1,765 patients above 65 years of age with more than five medications began cannabis treatment and answered the intake questionnaire. 919 patients continued treatment for at least 6 months, from them 724 answered the follow up questionnaire. 57% of responders were women and 50% were 75 years of age or older. The most common indications for cannabis treatment were pain and cancer. 80% of patients consumed 20-30 grams of cannabis per month. After six months of treatment 42.4% of patients discontinued at least one medication with averaged number of medication 8.4 at initiation and 5.8 after six months respectively. Another 6% lowered medication dosage. 32% of responders had mild adverse events such as dizziness and dry mouth.

Conclusion: Our study demonstrates that the therapeutic use of cannabis is safe in older adults. Initiating medical cannabis may decrease the use of other prescription medicines and therefore might reduce worst outcomes of polypharmacy.

RISKS FACTORS FOR POTENTIAL ADDICTION AMONG CANCER PATIENTS WHO USE MEDICAL MARIJUANA

Yakir Rottenberg

Faculty of Medicine, Hebrew University of Jerusalem, Israel;
Department of Oncology, Hadassah Medical Center, Jerusalem, Israel.

Background: Marijuana preparations are frequently used by cancer patients. In Israel, marijuana is being given to cancer patients after being prescribed by their oncologist. However, marijuana use can lead to addiction. This study aimed to find risk factors associated with higher doses of marijuana prescriptions among cancer patients.

Methods: In the current study, all cancer patients who received marijuana prescriptions between 08/2013 and 06/2015 at the Hadassah−Hebrew University Medical Center were included. The association between higher doses of marijuana prescriptions and demographic data, socioeconomic and smoking statuses were assessed using an adjusted model.

Results: The final study population included 276 patients. The highest doses of marijuana were prescribed for patients with head & neck, gynecological, and colorectal cancers. In the adjusted model, lower socioeconomic status and current smoking were associated with higher doses of marijuana. However, no association between age, sex, and/or past smoking and higher dose of marijuana were found.

Conclusion: There is evidence of an increased risk of addiction among cancer patients who have lower socioeconomic status and are currently smoking. The current study emphasizes the need for special attention in prescription marijuana for these populations.

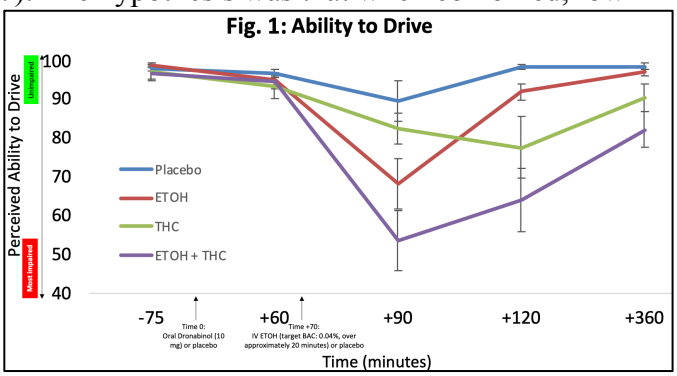
ETHANOL AND THC EFFECTS ON SIMULATED DRIVING

Ashley M. Schnakenberg Martin*[1,2], Jose Cortes-Briones [1,2], Patrick Skosnik. [1,2], L. Taylor Flynn [1,2], Esra Sefik[1,2], Christina Luddy[1,2], Mohini Ranganathan[1,2] and Deepak Cyril D'Souza[1,2]

1) Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA
2) VA Connecticut Healthcare System, West Haven, CT, USA

Introduction: Motor vehicle accidents are among the top ten leading causes of morbidity and mortality worldwide. While the effects of alcohol on driving are well-known and have been widely studied, the effects of cannabis and its constituent cannabinoids on driving are less clear, and there is even less known about the effects of the combination of alcohol and cannabinoids on driving.

Methods: In a within-subject, randomized, placebo-controlled, double-blind, 2 x 2 design (ethanol: active/placebo x THC: active/placebo), the interactive effects of low dose ethanol (i.v ethanol clamped at BAC 0.04%) and oral THC (10 mg) on subjective effects and simulated driving were studied in healthy humans (n=17). The hypothesis was that when combined, low dose ethanol and low dose THC would have synergistic effects. Road tracking error was indexed by the standard deviation of lateral position (SDLP). Self-reported ability to drive, other subjective effects associated with cannabis, and heart rate were measured. Repeated measures general linear models (GLMs) were used to assess differences in SDLP, heart rate, and subjective feeling states.



Results: At doses of THC, ethanol, and the combination that significantly produced feeling states consistent with intoxication, subjects reported reduced ability to drive (placebo > THC > ethanol > combination). The combination of ethanol and THC produced a greatest decrease in self-reported ability to drive compared to placebo (40% decrease), and the sum of the effects of either THC (8% decrease) or ethanol (24% decrease) alone (Fig.1). The drugs also increased heart rate relative to placebo ($p < .05$). However, there were no significant drug effects on SDLP.

Conclusions: Low doses of THC, ethanol and their combination produced drug effects consistent with intoxication. Participants reported being aware of their ability to drive being impaired by ethanol, dronabinol and their combination. The combination produced synergistic decreases in self-reported capacity to drive, whereas the combination produced additive tachycardia. That participants were aware that their ability to drive was compromised but the driving simulator did not detect such effects, is interesting and needs further study. Given the objective and subjective findings, the driving simulator may not have been sensitive enough to detect study drug effects. The findings of this study are relevant to the not uncommon practice of combining alcohol and cannabinoids and the effects on driving.

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HAIR REGROWTH WITH TOPICALLY APPLIED CANNABIDIOL (CBD) IN HEMP EXTRACT - A CASE SERIES

Gregory L. Smith

Department of Cannabinoid Research, Medical Life Care Plans, St. Pete Beach, FL, USA

Introduction: Cannabidiol (CBD) has novel therapeutic effects, different from and synergistic with current hair regrowth therapies. CBD via topical application easily reaches hair follicles where it is acts as negative modulator of the CB1 receptor and agonist of vanilloid receptor-1 (TRPV1) and vanilloid receptor-4 (TRPV4). All these ECS receptors relate to hair follicle function. Negatively modulating CB1 receptor activity on the hair follicle with CBD has been shown to result in hair shaft elongation; in addition, the hair follicle cycle (anagen, catagen, telogen phases) is controlled by TRPV1. The effects of CBD on hair growth are dose dependent and higher doses may result in premature entry into the catagen phase via a different receptor known as TRPV4. CBD has also been shown to increase Wnt signaling, which causes dermal progenitor cells to differentiate into new hair follicles and maintains anagen phase of the hair cycle. This study was conducted to determine if topical application of a hemp-oil high in CBD would result in hair regrowth in the area of the scalp most affected by androgenetic alopecia.

Methods: A study was done of 35 (28 men and 7 women, ages 46-76) subjects from a "Hair and Scalp" clinic with androgenetic alopecia (AGA). They used a once daily topical hemp-extract high in CBD for 6 months. The subjects were given a topical paste in a 2oz jar and advised to apply a thin layer once each morning to the areas of baldness. Each 2oz jar contained 1,000mg of the whole plant hemp extract, or 108mg of CBD. A 2oz jar lasted approximately one month, which is an average daily dose of 3-4mg of topically applied CBD.A hair count of the greatest area of alopecia was carried out before treatment and again after 6 months of treatment. To facilitate consistent hair count analysis, a clear acrylic mold, containing a one-centimeter cutout, was made of each subject's head. No subjects had been using hair regrowth products and used hair regrowth treatments during the trial.

Results: The results revealed that men did slightly better than women, and the vertex area did better than the temporal areas. There were statistically significant increases ($p < 0.01$) in hair counts in men and women in the vertex and temporal arears when studied independently. The hair count increased 93.5%, from 18.5 to 32.7 ($p < 0.001$), when temporal and vertex areas were combined. On average, there was a statistically significant 93.5% increase in nonvellus hair after six months of once daily use. All subjects had some regrowth. There were no reported adverse effects.

Conclusions: Though the exact mechanism of therapeutic effects is not known, CBD is most likely functioning as negative allosteric modulator of the CB1 receptor and TRPV1 agonist affecting hair shaft elongation, matrix cell function and the hair follicle cycle. It may also be acting via Wnt messaging. Since the CBD works through novel mechanisms entirely different from finasteride and minoxidil, it can be used in conjunction with these current drugs and would be expected to have synergistic effects.

MEDICAL CANNABIS USE AND PAIN: AN EXPERIENCE SAMPLING STUDY

Sharon Nznitman*[a], Dennis Rosenberg[b], Simon Vulfsons[b], David Meiri[c] and Talya Greene [d]

[a]School of Public Health, University of Haifa;
[b]Institute for Pain Medicine, Rambam Health Care Campus;
[\c]Faculty of Biology, Technion-Israel Institute of Technology;
[d]Department of Community Mental Health, University of Haifa

Introduction: Randomized control trials show that medical cannabis (MC) has pain-reliving effects, but very little research has tested associations of pain and MC use after long-term treatment and through methods that have external validity outside of the experimental setting. This study examines associations of pain, associated painful experiences and MC use in the daily lives of chronic pain patients that have been using MC for extended periods.

Methods: Seventy-eight chronic pain MC patients provided data on momentary experiences of pain, negative affect, fatigue and MC use three times daily over a 10 day period ($n_{observations}$ = 1688). Mixed effects models examined within- and between-person experiences of pain, affect and fatigue and associations with MC use.

Results: Within persons, elevated experiences of pain intensity and interference with activities were associated with greater intention to use MC within the next hour. No evidence was found that time elapsed since last MC use was associated with levels of pain, negative affect or fatigue.

Conclusions: After long-term use, chronic pain patients intend to use MC in response to pain experiences. Yet, they may not actually achieve the pain relief. More research is needed to examine whether continued MC use despite lack of pain relief is related to relief of other symptoms (e.g. dependence, withdrawal) or positive benefits (e.g. general sense of well-being).

ACUTE INTOXICATION BY A SYNTHETIC CANNABINOID (JWH-018): COGNITIVE, PSYCHOMOTOR AND PSYCHOTOMIMETIC EFFECTS

Eef Theunissen*, Johannes Reckweg, Nadia Hutten, Kim Kuypers and Johannes Ramaekers

Department of Neuropsychology and Psychopharmacology
Maastricht University, The Netherlands

Introduction: Synthetic cannabinoids are being used for more than ten years, but placebo-controlled studies into their effects are still scarce. SC's are much more potent than natural cannabis, and as a result, many surveys report more psychological problems. In the current study, we investigated the cognitive, psychomotor and subjective response to an acute dose of the synthetic cannabinoid JWH-018.

Methods: In this placebo-controlled, within-subjects study, 24 healthy cannabis-experienced participants inhaled the vapor of 75µg JWH-018/kg bodyweight and placebo on two separate occasions. To induce a minimum level of intoxication, a booster dose was given on an if-needed basis. Participants were subsequently monitored for four hours, during which psychomotor, cognitive and psychotomimetic effects were measured.

Result: Data were analyzed using a GLM Univariate ANOVA with Drug (placebo and JWH-018) as a within-subject factor. Subjective intoxication was significantly increased after the JWH-018 administration and reached a maximum (average 64%) at 30 minutes after inhalation. JWH-018 impaired motor coordination (CTT), attention (DAT and SST), memory (SMT), speed-accuracy efficiency (MFFT), and response speed (DAT), and this was most strongly demonstrated within the first 2.5 h after administration. Psychedelic symptoms (Bowdle) and dissociative states (CADSS) were significantly increased after JWH-018.

Conclusion: It is concluded that healthy volunteers who are acutely intoxicated with JWH-018, show clear cognitive and psychomotor impairment. In addition, JWH-018 was found to induce psychotomimetic effects in healthy participants with no history of mental illness.

CANNABIS PRODUCT PREFERENCES IN U.S.-BASED BREAST CANCER PATIENTS

Marisa Weiss*[1,2,3,4], Katherine Aliano-Ruiz[4], Meghan Buckley[5], Sam Meske[4], Stephanie Kjelstrom[5], Sherry Danese[6], Adam Leitenberger[1], Melissa Bollmann-Jenkins[1], Julianne Hibbs[4], Sharon Larson[5], Nancye Green[7] and Diana Martinez[8]

1 Breastcancer.org, Ardmore, PA
2 Lankenau Medical Center, Radiation Oncology, Wynnewood, PA
3 Socanna for Medical Cannabis Research, Narberth, PA
4 Main Line Health, Lankenau Institute for Medical Research, Wynnewood, PA
5 Main Line Health, Center for Population Health Research, Wynnewood, PA
6 Outcomes Insights, Agoura Hills, CA
7 Donovan/Green, New York, NY
8 Columbia University, Department of Psychiatry, New York, NY

Introduction: Increasingly, breast cancer patients are using cannabis to relieve disease symptoms and treatment side effects, however, little is known about the preferred cannabis product content and methods of delivery. The goal of this study was to better understand breast cancer patients' cannabis product preferences.

Methods: U.S.-based members of Breastcancer.org and the Healthline.com communities were invited to participate in an anonymous survey between 12/16/2019 and 1/19/2020. Subjects confirmed they were age ≥18 and diagnosed with breast cancer within 5 years. After informed consent, non-identifiable data were collected and analyzed in aggregate.

Results: Among the 612 respondents who met the eligibility criteria and completed the survey, 42% reported using cannabis to treat breast cancer symptoms or treatment side effects. Most preferred cannabidiol (CBD) containing products: 22% preferred CBD only, 21% preferred mostly CBD, and 19% preferred 1:1 delta-9-tetrahydrocannabinol (THC) to CBD. Only 26% preferred THC-dominant products. No preference was reported by 7%. An average of 3.7 (median = 2, SD = 4.2) products were used.

The most popular delivery methods were edibles (70%); and liquids or tinctures (65%). Two-thirds of the participants (68%) used inhalation products (51% smoking; 45% vaping pens, and 12% vaping "volcano" device).

Despite respondents' stated preference for medical cannabis, 77% of medical cannabis users had also utilized recreational sources.

Conclusions: The majority of U.S.-Based breast cancer patients prefer products containing mostly cannabidiol, although many also consumed THC products. Various methods of delivery were commonly used: edibles, liquids/tinctures and inhalation products were each preferred by 65-70% of users. Over 75% of medical cannabis users also utilized recreational cannabis.

A SURVEY: ATTITUDES TOWARDS CANNABIS USE IN PEOPLE WITH BREAST CANCER

Marisa Weiss*[1,2,3,4], Meghan Buckley[5], Katherine Aliano-Ruiz[4], Stephanie Kjelstrom[5], Sam Meske[4], Sherry Danese[6], Adam Leitenberger[1], Melissa Bollmann-Jenkins[1], Julianne Hibbs[4], Sharon Larson[5], Nancye Green[7], Paul Gilman[4] and Diana Martinez[8]

I[1]Breastcancer.org, [2]Lankenau Medical Center, Radiation Oncology, [3]Socanna for Medical Cannabis Research, [4]Main Line Health, Lankenau Institute for Medical Research, [5]Main Line Health, Center for Population Health Research, [6]Outcomes Insights, [7]Donovan/Green, [8]Columbia University, Department of Psychiatry

Background: Over 20+ years, medical cannabis has become sequentially legalized in 33 states of the U.S. for people with serious medical conditions. Yet, it is unknown how attitudes towards cannabis have evolved in people with breast cancer.

Methods: Members of Breastcancer.org and Healthline were invited to complete a survey between 12/16/2019 and 1/19/2020. Eligibility criteria included age ≥18 years, resident of the US and a breast cancer diagnosis within the past 5 years. The data were analyzed for attitudes about cannabis before and after diagnosis, in users and nonusers, and by age. The survey was led by Socanna, conducted by Outcomes Insights, and supported by a grant from Ananda Health.

Results: Of the 832 respondents who completed screening, 725 met eligibility criteria, and 612 completed the survey. Respondents had a median age of 57 and were from 46 states, reflecting the U.S. population density. Although 87% of respondents were at least somewhat interested in learning about medical cannabis, a majority (58%) stated that they have not used it to treat breast cancer symptoms or treatment side effects.

Regardless of age: most breast cancer patients believe that cannabis should be viewed similarly to other natural products (78%), they prefer natural products to treat their symptoms (76%), and believe the benefits of medical cannabis outweigh the risks (71%). Only 11% were worried about addiction to cannabis; 6% were concerned about addiction to CBD. Users are strongly motivated by a desire to avoid opioids (47%) and because they felt they had no other way to effectively treat their symptoms (41%). Medical cannabis is perceived to be more pure, clean, safe, private and socially acceptable than recreational cannabis. Virtually all (98%) of users found medical cannabis to be helpful; 75% describe it as extremely helpful. Both users and non-users claimed to be markedly more in favor of medical cannabis than they were 10 years ago, citing these top reasons for changing their opinions; learning more about the science behind medical cannabis (27%), deriving a personal benefit from cannabis (31%), and seeing someone else benefit (18%).

Conclusions: Perceptions on cannabis have changed significantly. Among breast cancer survey respondents, cannabis is viewed as a natural product for which the benefits outweigh the risks, regardless of prior cannabis use and age. Many participants also believed that cannabis use was the only effective means for treating their symptoms and felt that cannabis serves as an alternative to opioids. However, there is a lack of research supporting many of these beliefs and few participants understood the potential risks of cannabis use, especially in the setting of treatment for breast cancer. Thus, while cannabis likely has some benefits to cancer patients, there is a need to educate patients on its limitations.

A SURVEY OF PATIENTS WITH CANCER WHO REPORT ANTI-CANCER BENEFITS OF CANNABIS USE

Dylan Zylla[1]*, Grace Gilmore[1], Justin Eklund[1], Jordan Guggisberg[1] and Sara Richter[2]

[1]Cancer Research Center, HealthPartners/Park Nicollet, Minneapolis, MN;
[2]Professional Data Analysts, Minneapolis, MN

Background: Patients with cancer are increasingly using cannabis to alleviate symptoms of pain, nausea, insomnia, anxiety and appetite loss. Preclinical studies suggest possible anti-tumor effects of cannabinoids. Nearly one in four patients use cannabis with an expectation that it may improve their cancer, at times forgoing more traditional treatments.

Methods: From June 2020-January 2021, we shared a 30 question online survey (www.catasurvey.com) with cannabis research collaborators, online patient groups, and social media platforms. We aimed to find patients with stage IV cancer using cannabis who reported anti-cancer benefits to recruit for a follow-up study. Descriptive analyses for each question were provided for all survey respondents.

Results: 730 patients with a cancer diagnosis from 14 different countries and 38 different states (US) completed the survey, of whom 522 (72%) indicated they were currently using cannabis. Among cannabis using patients, 206 (39%) indicated that cannabis helped treat/control their cancer in addition to alleviating symptoms. Patients reporting an anti-cancer benefit were predominately aged 41-60 y.o. (54%), female (65%), and Caucasian (89%). Cancers of breast (28%), prostate (14%), gynecological system (13%), lymphoma (11%), and lung (10%) were most common. 77 patients (37%) had stage IV disease. Cannabis use details for patients who reported an anti-cancer benefit can be seen in Table 1. Only 8% of patients reported never receiving traditional anti-cancer therapy, while 41% reported using cannabis concurrent with at least one other traditional systemic anti-cancer therapy (e.g., chemotherapy, immunotherapy, hormone-therapy). Despite claiming an anti-cancer benefit from cannabis, 15% of patients reported their cancer was progressing on most recent cancer assessment (e.g., imaging or tumor marker).

Conclusions: Nearly 4 in 10 patients with cancer believe their cannabis use led to an anti-cancer impact, yet no clinical trials have been completed to test this theory. Cannabis type and dosing varies greatly amongst patients reporting anti-cancer benefits. Validating these claims through surveys was not feasible. We plan to compile a case series from a subset of survey respondents after a thorough review of medical records. Ultimately, prospective clinical trials will be required to provide direct evidence of any anti-cancer impact cannabis may have.

Cannabis Use Frequency	N (%)	Route of Administration[a]	N (%)	Lab Tested for Purity/Potency[a]	N (%)
Daily	168(82)	Vape/smoke	115(56)	Yes	132(64)
Almost every day	20(10)	Oral[b]	173(84)	No	87(42)
Once a week	4(2)	Topical	75(36)		
THC/CBD Ratio[a]	**N (%)**	**Use with Food**	**N (%)**	**Money Spent on Cannabis in Average Month in USD**	**N (%)**
High THC/Low CBD	124(60)	Always	37(18)	$0-$99	64(31)
Balanced THC/CBD	103(50)	Sometimes	113(55)	$100-$199	46(22)
High CBD/Low THC	53(26)	Never	55(27)	$200-$299	28(14)
CBD-only	38(18)			$300+	67(33)

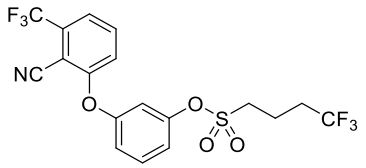
Table 1. Cannabis use summary among patients with cancer who are currently using cannabis and report cannabis had an anti-cancer benefit (reported that cannabis helped treat/shrink/control their cancer) (n=206). [a]Answers are not mutually exclusive. [b]Includes oral capsule, tincture, oil, edibles, lozenges

SAR EFFORTS TOWARDS PERIPHERALIZATION OF THE CANNABINOID RECEPTOR PARTIAL AGONIST BAY 59-3074

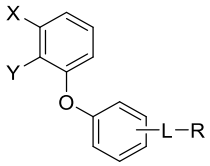
George Amato*, Ann Decker, Danni Harris, Scott Runyon and Rangan Maitra

Center for Drug Discovery, RTI International, Research Triangle Park, NC, USA

Introduction: Selective modulation of peripheral cannabinoid receptors (CBRs) has potential therapeutic applications in medical conditions including obesity, diabetes, liver diseases, GI disorders, and pain. While there have been considerable efforts to produce selective antagonists or full agonists of CBRs, there has been limited reports on partial agonists. This is important because Δ^9-Tetrahydrocannabinol (THC) is a partial agonist of both CBRs with a high degree of brain penetration. Partial agonists of CBRs with limited brain penetration will enable studies aimed at better understanding peripheral effects of these types of compounds on various physiological processes independent of their CNS-effects. BAY 59-3074 (**1**) is a centrally penetrating partial agonist of both human and rat CB1 and CB2 receptors with efficacy in rat models of neuropathic and inflammatory pain. We report herein our efforts to synthesize analogs of **1** that would favor peripheral selectivity, while maintaining partial agonism of CBRs.



1, BAY 59-3074



2, Compounds Prepared

L = NHCO, $NHSO_2$, $NHCO_2$, NHCONR, $NHSO_2NH$, meta or para substituted
R = Alkyl, Cycloalkyl, Aryl
X = CF_3, H
Y = CN, Cl

Methods: More polar analogs of **1** that contain at least one H-bond donor (**2,** ~25 compounds) were synthesized and evaluated for potency and efficacy using a cAMP assay for hCB1. Select compounds were also evaluated using a hCB1 binding assay.

Results: Analogs of **1** were sought with structural features that would favor peripheral selectivity. While direct replacement of the sulfonate ester linker of **1** with a sulfonamide linker resulted in a full agonist with similar potency, two partial agonists were identified that have moderate hCB1 potency in the cAMP assay (**21**, EC_{50} 830 nM, 17% E_{max}; **24**, EC_{50} 370 nM, 36% E_{max}). These two compounds have unique structural features. Compound **21** has a meta substituted core with a sulfamide linker, while compound **24** has a para substituted core with an amide linker.

Conclusions: The search for a peripheral analog of **1** revealed a complex interdependence of efficacy and potency on the linker (**L**), the group (**R**) attached to the linker and the substitution pattern (meta or para) of the phenyl core. Despite these challenges, this early research showed that it is possible to develop partial agonists of hCB1 with properties favoring peripheral restriction.

Acknowledgements: This work was funded by R01DK124615 from NIDDK/NIH to R.M.

BRAIN PENETRANT, BUT NOT PERIPHERALLY RESTRICTED, SYNTHETIC CANNABINOID 1 RECEPTOR AGONISTS PROMOTE MORPHINE-MEDIATED RESPIRATORY DEPRESSION

Beth M. Wiese*[1], Erika Liktor-Busa[1], Sarah A. Couture[1], Spyros P. Nikas[2], Lipin Ji[2], Yingpeng Liu[2], Alexandros Makriyannis[2], Igor Spigelman[3], Todd W. Vanderah[1] and Tally M. Largent-Milnes[1]

[1]Department of Pharmacology, University of Arizona, 1501 N Campbell Ave., Life Sciences North Rm 621, Tucson, AZ, USA
[2]Chemistry and Chemical Biology, Bouve College Health Sciences – Center for Drug Discovery, 116 Mugar Life Sciences Building, Northeastern University, 360 Huntington Ave, Boston, MA, USA
[3] Division of Oral Biology and Medicine, School of Dentistry, University of California, 10833 Le Conte Ave, Box 951668, Los Angeles, CA, USA

Introduction: Cannabis use and acceptance continues to rise despite the gaps in knowledge in regard to the mechanisms of the endocannabinoid system in many physiological functions, especially respiratory influence. There are no reports of a fatal cannabis overdose compared to hundreds of thousands who have succumbed to a lethal accidental opioid overdose. Fatal opioid overdoses are typically attributed to respiratory depression. As more and more people continue to use cannabis alone and as an adjunct therapy, it is essential to understand the impact this has on respirations.

Methods: With recent evidence of cannabinoid receptor 1 (CB1R) presence in the brain stem respiratory centers, as well as in the peripheral lung tissue, it is vital that the mechanisms involved in central and peripheral CB1R modulation of respiratory function be delineated. In this study, utilizing whole body plethysmography, we sought to define the roles of central versus peripheral CB1R activation on respiratory function alone and in combination with morphine.

Results: As previously shown the synthetic cannabinoid, AM356 10 mg/kg, induced respiratory depression on its own; however, here we show the peripherally restricted CB1 agonist (PrNMI 0.3 and 0.6 and 1 mg/kg) did not. In contrast, the combination of this peripherally restricted CB1 agonist, PrNMI 0.3 and 0.6 mg/kg, with morphine significantly prevented morphine-induced respiratory depression, while the AM356 combination with morphine enhanced respiratory depression.

Conclusions: These studies support prior literature that centrally mediated synthetic CB1 activation causes respiratory depression, while adding to the field of knowledge that utilization of a peripherally restricted CB1R agonist may be a unique strategy to attenuate the respiratory depression associated with opioid therapy.

INHALED CANNABIS DELIVERY DURING PREGNANCY: MATERNAL-FETAL TRANSMISSION AND EFFECTS ON FETAL AMYGDALA DEVELOPMENT

Samantha L. Baglot*[1], Catherine Hume[1], Robert J. Aukema[1], Gavin N. Petrie[1], Jack Bieber[1], Ryan J. McLaughlin[2] and Matthew N. Hill[1]

[1]Department of Cell Biology and Anatomy, University of Calgary, Calgary, AB, Canada
[2]Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA, USA

Introduction: Cannabis use during pregnancy is increasingly common, as high as 10-20%. Evidence from clinical studies suggests that prenatal cannabis exposure (PCE) may lead to growth retardation and alterations in neurobehavioral trajectories. However, little is known about the pharmacokinetics or maternal-fetal transmission of cannabis, or how THC alters fetal brain endocannabinoid (eCB) function or neurodevelopment. Human studies of PCE are often confounded by unknown timing and amount of exposure, whereas animal models allow examination of these questions with control over these variables. Rodent studies have shown that PCE results in altered locomotion, social interaction, and cognitive functioning in offspring; however, to date most of this work has utilized injectable Δ^9-tetrahydrocannabinol (THC), which presents complications given that there are dramatic differences in the pharmacokinetics of THC dependent on route of administration. Thus, to perform translational work that is relevant to humans, it is imperative to utilize an animal model of inhaled cannabis delivery during pregnancy. The overall aim of this study is to employ a validated vapor chamber system to administer THC during pregnancy to determine: 1) maternal-fetal transmission of THC based on length of exposure (acute versus chronic) and tissue type (maternal plasma, placenta, and fetal brain); and 2) effects of chronic THC on fetal amygdala development.

Methods: Aim 1: pregnant rats were exposed to THC via inhalation (10% THC distillate; 15-min session) either chronically (from gestational day [GD] 7 through 21) or acutely (on GD21). Dams and their fetuses were euthanized immediately or 6 hours following the last exposure. Aim 2: pregnant rats were exposed to THC via inhalation beginning from GD1; and dams and their fetuses were euthanized on GD15, 17, or 19 to examine fetal brain development across various timepoints. Maternal blood, placenta, and fetal brains were collected for both aims via caesarean section surgery. All samples from aim 1 were analyzed for levels of THC and metabolites, and global fetal brain eCB levels, through mass spectrometry (MS). Fetal amygdala samples from aim 2 will also be analyzed for eCB levels via MS, as well as eCB and immune-related gene expression via qPCR, and cytokine levels via multiplex assay.

Results: Results of aim one indicate highly correlated levels of THC and metabolites in maternal blood, placenta, and fetal brain. Interestingly, chronic exposure of THC during pregnancy produced altered metabolism compared to acute exposure in dams and their fetuses, suggesting metabolic tolerance to chronic cannabis exposure. Furthermore, our results indicate that ~30% of circulating maternal THC reaching the fetal brain. We found no impact of prenatal exposure to THC on fetal whole brain eCB levels. Analysis of fetal amygdala development is ongoing.

Conclusions: These results highlight the importance of an animal model of inhaled THC delivery. Further, this research is important to establish placental ability to breakdown cannabis and determine the level and timeframe of fetal exposure, as well as the effects of PCE on fetal neurodevelopment.

SYNTHETIC CANNABINOIDS AND $^{9}\Delta$-THC DIFFERENTIALLY MODULATE MITOCHONDRIAL FUNCTION IN NORMAL RAT KIDNEY (NRK) CELLS

Christian V. Cabanlong*, Paul L. Prather and Lee Ann MacMillan-Crow

Department of Pharmacology and Toxicology, College of Medicine,
University of Arkansas for Medical Sciences, Little Rock, AR, USA

Introduction: Synthetic cannabinoids (SCs) are a large class of novel psychoactive compounds often referred to as "fake marijuana". Although these compounds produce psychoactive effects similar to $^{9}\Delta$-THC, SC use is unpredictable and can be toxic, often resulting in admission to emergency departments, intensive care units and/or death. In particular, initial reports indicate that the SC XLR-11 produces renal toxicity and may do so by modulation of mitochondrial function. To provide additional mechanistic insight underlying SC-induced renal toxicity, this study tested the hypothesis that structurally distinct high affinity full CB1R agonists modulate mitochondrial activity differentially in normal renal kidney (NRK) cells.

Methods Cannabinoids examined in this study were $^{9}\Delta$-THC, XLR-11, 4F-MDMB-BUTINACA and its commercially available metabolites: 4-fluoro MDMB-BUTINACA 3-carboxyindazole metabolite "M1," 4-fluoro MDMB-BUTINACA 3-carboxy-2'-indazole metabolite "M2," 4-fluoro MDMB-BUTINACA butanoic acid metabolite (CRM) "M3," and 4-fluoro MDMB-BUTINACA N-(4-hydroxybutyl) metabolite (CRM) "M4". CB1R affinity and activity of cannabinoids examined were assessed in mouse brain homogenates by radioligand binding and G-protein activation assays, respectively. JC-1 staining was employed to monitor mitochondrial membrane potential in NRK cells cultured in 6, 12, and 24-well plates in DMEM containing 5% fetal calf serum maintained in a humidified incubator with 5% CO_2 and 95% air.

Results: Receptor binding assays determined that all cannabinoids, except M1 and M2, exhibit high CB1R affinity with a rank order of affinity of 4F-MDMB-BUTINACA >> M4 > Δ9-THC > M3. Functional studies found that all high affinity CB1R ligands, except M3, act as full agonists. M3 exhibits partial agonist activity. In NRK cells, XLR-11 produces mitochondrial membrane hyperpolarization, $^{9}\Delta$-THC induces depolarization, and neither 4F-MDMB-BUTINACA nor M4 alter mitochondrial membrane potential. In NRK cells overexpressing superoxide dismutase (SOD), hyperpolarization induced by XLR-11 was completely reversed, while depolarization resulting from $^{9}\Delta$-THC was unaffected. Studies are ongoing to determine if the observed modulation of mitochondrial function by these cannabinoids is mediated by CB1Rs.

Conclusions: These data demonstrate that although all cannabinoids examined exhibit high affinity and activity at CB1Rs, they produce markedly different modulation of mitochondrial membrane potential in NRK cells. Furthermore, the mechanism(s) of action underlying the observed effects on mitochondrial function are also distinct, involving production of reactive oxygen species for XLR-11, but not for $^{9}\Delta$-THC. Such distinct modulation of mitochondrial membrane potential in NRK cells may explain why some SCs are more nephrotoxic than others.

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EFFECTS OF CHRONIC EXPOSURE TO LOW DOSE OF Δ^9- TETRAHYDROCANNABINOL IN ADOLESCENCE AND ADULTHOOD ON SEROTONIN/NOREPINEPHRINE NEUROTRANSMISSION AND EMOTIONAL BEHAVIORS

Danilo De Gregorio*[1], Joshua Dean Conway[2], Martha-Lopez Canul[1], Luca Posa[1], Francis Rodriguez Bambico[2] and Gabriella Gobbi[1]

[1]Department of Psychiatry, McGill University, Montreal, QC, Canada
[2]Department of Psychology, Memorial University of Newfoundland, St. John's NL, Canada

Introduction: Chronic exposure to the Δ^9-tetrahydrocannabinol (THC), the main cannabis component, during adolescence has been shown to be associated with an increased risk of depression and suicidality in humans. Little is known about the impact of the long-term effects of chronic exposure to low doses of THC in adolescent compared to adult rodents.

Methods: THC (1mg/kg i.p., once a day) or vehicle was administered for 20 days in both adolescent (post-natal day, PND 30-50) and young adult rats (PND 50-70). After a washout period (20 days), behavioral paradigms and electrophysiological recordings of serotonin (5-HT) and norepinephrine (NE) neurons were carried out.

Results: Adolescent THC exposure resulted in depressive behavior: a significant decrease in latency to first immobility in the forced swim test, increased anhedonia in the sucrose preference test. Decrease entries in the open arm were observed in the elevated plus maze after adolescent and adult exposure, indicating anxious phenotype. A reduction in dorsal raphe 5-HT neural activity without changing locus coeruleus NE activity was found in THC adolescent and adult exposure.

Conclusions: Altogether, these findings suggest that low doses of chronic THC exposure during the developmental period and adulthood could result in increased vulnerability of the 5-HT system and anxiety symptoms; however, depressive phenotypes occur only after adolescent, but not adult exposure, underscoring the higher vulnerability of young ages to the mental effects of cannabis.

EFFECTS OF PRENATAL NABILONE EXPOSURE ON MOUSE GESTATION AND EMBRYO MORPHOLOGY USING MICRO-CT IMAGING

Megan Drupals*[1,2], Janice Hicks[1], Shoshana Spring[3], Tina Weng[3], Ben Darwin[3], Lindsay Cahill[3], Brian Nieman[3], John Sled[3], Ameet Sengar[1], Benjamin Steinberg[1,2] and Michael Salter[1,2]

[1]Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada.
[2]Department of Physiology, University of Toronto, Toronto, ON, Canada.
[3]Mouse Imaging Centre, The Hospital for Sick Children, Toronto, ON, Canada

Background: There is little known about the potential consequences of maternal cannabis use during pregnancy, despite increasing consumption rates. Early evidence from human studies points to long-lasting neurodevelopmental problems in children prenatally exposed to cannabis. To study the effects of prenatal cannabis use on development, we developed a murine maternal cannabinoid exposure model using nabilone, a synthetic CB1/CB2 agonist.

Methods: Female CD1 mice were treated once daily with subcutaneous nabilone (0.012mg per dose) or vehicle beginning 7 days prior to timed mating until day 15.5 of gestation. At E15.5, embryos and placentae were collected, weighed, and fixed for micro-CT imaging to assess structural organ changes. Morphological changes were detected using a voxel-based approach.

Results: There were no differences in gestation time, litter sizes or proportions of sex of offspring. Analysis of 3-6 embryos per dam at E15.5 showed no significant difference in embryo weight, however placentae were significantly smaller in the drug group, by approximately 10% ($p<0.05$ by unpaired T-test). Detailed voxel-based analysis of micro-CT images of E15.5 embryos did not reveal any gross anatomical developmental effects following prenatal nabilone exposure in sex-aggregate data.

Conclusions: Prenatal nabilone exposure did not have any effect on embryo or litter size, or gestation time. Placenta from the drug-exposed group weighed less than those from vehicle treated animals. Quantitative analyses did not find structural brain or other organ differences in nabilone-exposed embryos. Next, our group will pursue imaging experiments using THC and CBD. This research enables us to explore specific effects of prenatal cannabinoid exposure to further our knowledge and provide accurate information surrounding cannabis use during pregnancy.

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REPEATED LOW DOSES OF Δ^9-TETRAHYDROCANNABINOL AFFECTS MEMORY PERFORMANCE THROUGH SEROTONERGIC SIGNALING IN MICE

L. Galera-López*[1], V. Salgado-Mendialdúa[1], I. Manzanares[1], A. Bergadà-Martínez[1], E. Moreno[2], V. Casadó[2], A. Hoffman[3], C. Lupica[3], R. Maldonado[1,4] and A. Ozaita[1,4]

[1] University Pompeu Fabra, Barcelona, Spain
[2] University of Barcelona, Barcelona, Spain
[3] National Institute on Drug Abuse Intramural Research Program, Baltimore, MD, USA
[4] IMIM-Hospital del Mar Medical Research Institute, Barcelona, Spain

Introduction: Cannabis is the most widely used illicit drug worldwide. Its principal psychoactive component, Δ9-tetrahydrocannabinol (THC), acts as a partial agonist of the main cannabinoid receptor in the brain, the CB1 receptor. Indeed, CB1 receptors are the main responsible for the central effects of THC including memory impairment. CB1 receptors may form heterodimers with the serotonin 5-HT2A receptors. CB1/5-HT2A heterodimers were found responsible for the memory impairment produced by acute high dose of THC in mice. In this study we investigated whether a repeated exposure to a low dose of THC could affect memory performance and synaptic plasticity in mice.

Methods: Mice were treated i.p. with THC at 1 mg/kg (THC 1) for 7 days and the effects of the treatment on non-emotional and emotional long-term memory were evaluated using the novel object-recognition memory test and the trace fear conditioning paradigm, respectively. Hippocampal synaptic plasticity alterations were studied by evaluating long-term potentiation (LTP) recall and spine density analysis. Cellular effects of THC 1 were assessed studying c-Fos expression in brain areas relevant for learning and memory. Moreover, the 5-HT2A specific antagonist MDL100907 at 0.01 mg/kg (MDL) was used to reveal the involvement of serotonergic signalling through 5HT2A receptors.

Results: We found that THC 1 acutely administered does not affect memory performance. Instead, THC 1 repeated administration significantly impairs novel object-recognition and fear memory. This deficit was also detected when the training for memory assessment was carried out 24 h after the last THC 1 administration. At that time, a general enhancement of c-Fos expression was observed in several brain regions of THC 1 treated animals compared to control. In addition, THC 1-treated mice showed a decreased spine density at CA1 pyramidal neurons and reduced LTP at Schaffer collateral-CA1 synapses. Interestingly, repeated THC 1 modified the expression of *Htr2A*, pointing to a potential deregulation of CB1/5-HT2A heterodimers. Moreover, both sub-chronic (15 min before each THC 1 administration) and acute (15 min before the last THC 1 administration) pre-treatment with MDL completely prevented the THC-associated memory impairment in both non-emotional and emotional long-term memory.

Conclusions: Together, these results reveal the significance of serotonergic signalling through 5HT2A receptors in the amnesic effects of a low dose of THC.

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AN INVESTIGATION OF PPARA AND PPARΓ IN THE CNS EFFECTS OF Δ^9-THC IN MICE

Briana J. Hempel*, Guo-Hua Bi, Madeline E. Crissman, Sruti Pari and Zheng-Xiong Xi

Molecular Targets and Medication Discovery Branch, National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD USA.

Introduction: Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors that regulate gene expression by activating transcription factors. Three receptor subtypes have been isolated: PPARα, PPARγ, and PPARβ/δ. *In vitro* assays indicate that Δ^9-tetrahydrocannabinol (THC) is a PPARγ agonist and the endocannabinoids (AEA, 2-AG, OEA, PEA) may be activators of PPAR*α* and PPARγ. To date, it is unknown whether PPARs play a role in the pharmacological effects of cannabinoids *in vivo*. Here we explored this issue by using selective PPARα and PPARγ antagonists prior to THC administration to observe whether inhibition of these receptors alters THC-induced classical tetrad effects as well as its subjective effects as assessed by optical intracranial self-stimulation (oICSS) in mice.

Methods: In Experiment 1, subjects were pretreated with GW6471 (a selective PPARα antagonist, 3 and 5 mg/kg) or GW9662 (a selective PPARγ antagonist, 2 and 5 mg/kg) and THC-induced tetrad effects – analgesia, hypothermia, catalepsy and immobility were measured. In Experiment 2, GW6471 (3 mg/kg) and GW 9662 (2 mg/kg and 5 mg/kg) were administered prior to THC (3 mg/kg, i.p.) and DAT-cre mice underwent oICSS maintained by optical stimulation of midbrain dopamine neurons. Experiment 3 is ongoing, and we are using RNAscope *in situ* hybridization to examine the expression of PPARα and PPARγ on dopamine, GABA and glutamate neurons in the ventral tegmental area (VTA) and nucleus accumbens (NAc).

Results: Neither PPARα nor PPARγ inhibition altered THC's effects in the tetrad at high doses (10, 30 mg/kg). However, an intermediate dose of THC (3 mg/kg) attenuated oICSS, shifting the stimulation frequency-response curve downward. This effect was blocked by antagonism of PPARα, but not PPARγ receptors. Preliminary findings indicate that a subset of PPARα receptors are found on VTA dopamine neurons.

Conclusions: Our results suggest that, in addition to CB_1 and CB_2 receptor mechanisms, an additional dopaminergic PPARα receptor pathway may be involved in THC's subjective (aversive-like) effects in mice.

CHARACTERISING 'THE MUNCHIES'; EFFECTS OF TETRAHYDRO-CANNABINOL (THC) VAPOUR EXPOSURE ON RAT FEEDING BEHAVIOURS

Catherine Hume*, Samantha Baglot and Matthew Hill

Department of Cell Biology and Anatomy, University of Calgary, Alberta, Canada

Introduction: With medical and recreational cannabis being legalised in many parts of the world, there is an urgent drive to investigate the physiological effects of cannabis to determine if its use is associated with certain health-related effects. It is well established that cannabis acutely drives food intake, commonly referred to as 'the munchies', and that tetrahydrocannabinol (THC), the main psychoactive compound in cannabis, is responsible for driving these feeding effects. Cannabis induced feeding has been modelled in rodent studies. However, the majority of these studies systemically or orally administer high doses of cannabinoids. As the most common route of cannabis use is inhalation (via smoking or vaping), and with the pharmacological properties of inhaled cannabis being drastically different to injected or consumed cannabis, there is a great need to study cannabis induced feeding behaviours using more translational inhalation animal models. Furthermore, the effects of cannabis on daily homeostatic feeding patterns or macronutrient specific food preferences have not been well characterised in the literature. Therefore, the aim of this project was to use a translational THC vapour inhalation rat model to characterise the effects of cannabis on; *study 1)* normal daily feeding patterns, *study 2)* feeding in the satiated state, and *study 3)* food preference.

Methods: Adult male and female Sprague Dawley rats were exposed to THC (100mg/ml in vehicle, n=7) or vehicle (polyethylene glycol, n=7) vapour for 15min (10s hit every 2min) and subsequent food intake measured. This exposure protocol induces high blood and brain THC levels. For *study 1* (THC effects on normal feeding patterns), post-vapour regular chow feeding was measured. For *study 2* (THC effects on feeding when satiated), rats were given a 10% sucrose chow mash 'preload' to eat prior to vapour exposure and post-vapour chow feeding measured. For *study 3* (THC effects on food preference), post-vapour intake of two different foods (80% carbohydrate (high-carb) and 80% fat (high-fat) food) was simultaneously measured. Differences between treatment group and sex were determined using a two-way ANOVA with Bonferroni post-hoc testing ($p<0.05$ considered significant).

Results: Prior to vapour exposure, there was no difference in 24hr feeding patterns between THC and vehicle groups for all studies. In *study 1*, THC disrupted normal feeding patterns by significantly increasing chow intake within the first hour following vapour exposure, a result of increased feeding bout number. Furthermore, THC exposed rats were able to compensate for acute increased food intake by reducing subsequent food intake 3-4hr post-vapour exposure so that 24hr energy intake and body weight was no different from the vehicle group. In *study 2*, despite rats being pre-satiated, THC significantly increased chow intake within the first 30min following vapour exposure. THC exposed rats in *study 2* then followed similar feeding patterns to *study 1* THC exposed rats, resulting in no differences in 24hr energy intake and body weight between THC and vehicle groups. In *studies 1* and *2*, there were no major sex differences in post-THC feeding patterns. In *study 3*, THC exposed rats had increased preference for high-fat food in the first 30min following vapour exposure, with males showing a 21% higher preference and females showing a 54% higher preference for the high-fat food than the vehicle group. *Study 3* THC exposed rats also reduce subsequent food intake (both high-fat and carb) to compensate for acute increases in high-fat food consumption following vapour exposure.

Conclusions: THC acutely disrupts homeostatic feeding patterns by increasing food intake directly after vapour exposure. This effect can also be seen in pre-satiated animals, demonstrating how robust THC driven feeding is. However, rats are able to homeostatically compensate for acute THC-induced feeding by reducing subsequent food intake, to prevent energy overconsumption. This suggests that with a regular diet, acute cannabis exposure may not disrupt overall energy balance. Furthermore, THC vapour exposure acutely influences food preferences, an effect which is particularly apparent in females, suggesting that cannabis may promote the consumption of 'unhealthy' high-fat foods. This data sheds light on how cannabis use influences energy balance, information which is critical for the health and well-being of regular cannabis users. The mechanisms by which THC drives feeding, alters food choice, and potential sex differences will be the focus of future studies.

LOW-DOSAGE THC TREATMENT DIFFERENTLY ALTERS SPINE DYNAMICS IN OLD AND YOUNG MICE

Joanna A. Komorowska-Müller*[1,4], Anne-Kathrin Gellner[3,2], Kishore A. Ravichandran[2,1], Andreas Zimmer[1] and Valentin Stein[2]

[1] Institute of Molecular Psychiatry, Medical Faculty, University of Bonn, Germany
[2] Institute of Physiology II, Medical Faculty, University of Bonn,
[3] Klinik und Poliklinik für Psychiatrie und Psychotherapie, University of Bonn,
[4] International Max Planck Research School for Brain and Behavior, Bonn, Germany

Introduction: Studies have proven that the ECS undergoes age-dependent changes, with an overall activity decrease in old individuals. We recently reported that a long-term treatment of old mice with a low-dosage of Δ9-tetrahydrocannabinol (THC), a cannabinoid receptor agonist, rejuvenates their gene expression patterns and improves their cognitive abilities to the level of young mice. These changes were accompanied by an increase in Synapsin I and possibly in spine numbers.

Methods: The purpose of this study was to evaluate the effects of low-dosage THC treatment on dendritic spine dynamics using in vivo imaging technique. We thus imaged the same dendritic segments in the somatosensory cortex in order to asses spine dynamics (formation, stability and spine loss) before, during and after the 28-days, 3 mg/kg THC treatment in 3- and 18-month old mice for up to 10 weeks.

Results: We show that the THC treatment effect is age-dependent. In 3-month old mice THC treatment dramatically increased spine dynamics for up to 2 weeks, while not effecting significantly the overall spine density. On a contrary, in 18-month-old mice THC treatment decreased spine dynamics, especially loss of dendritic spines, which resulted in significantly increased spine density. Also, the timing of the treatment differed with age as the first effects in 18-month old mice were detected after 2-3 weeks into the treatment and lasted minimum 28 days after the treatment.

Conclusions: We have conclusively shown that even low-dosage THC treatment can affect spine dynamics in the mice somatosensory cortex and that this effect is age-dependent. The change in spine dynamics correlates with previously discovered improvement of cognitive abilities in old mice providing more insight into the possible mechanism of brain rejuvenation by THC.

INVESTIGATING STRESS REACTIVITY IN RATS FOLLOWING ACUTE THC VAPOUR EXPOSURE

Savannah H. Lightfoot*, Samantha L. Baglot, Catherine Hume,
Ryan J. McLaughlin[1] and Matthew N. Hill

Hotchkiss Brain Institute, University of Calgary, Calgary, AB, CA
[1]Washington State University, Pullman WA USA

Introduction: Management of stress and anxiety is often listed as the primary motivation behind cannabis use, yet the understanding of how acute and chronic exposure to cannabis modulates the neurobehavioral and endocrine response to stress is not well characterized. Human research has found that chronic cannabis use is associated with increased basal cortisol levels yet blunted responses to stress. Preclinical research has demonstrated mixed effects of THC, much of which is suggestive of dose-dependent effects; however the predominance of this work has employed an injection method in order to deliver THC. As inhalation is the primary form of consumption in humans, modelling this approach in rodents is important given the robust impact route of administration has on the pharmacokinetics and biodistribution of THC and its metabolites. With advancements in drug delivery for rodent studies, basic science approaches can now be employed to better establish the impact of cannabis use on stress reactivity in a highly translational manner, as has been recently demonstrated with respect to the effects of chronic self-administration of cannabis vapor on stress reactivity in rodents. The current study aims to examine the impact of acute, controlled passive delivery of THC to male and female rats to determine how this impacts neural and endocrine responses to acute stress.

Methods: Thirty-two male adult rats were habituated to the THC vapour delivery system for 3 days, following habituation (day 0), a baseline blood sample was taken. Following this period, animals received 9 days of vehicle vapour exposure, where a blood sample was taken on the first day of vehicle exposure (day 1) to determine any impact on stress hormone levels. On day ten, rats were randomly assigned to one of two vapor conditions: 1) control (n = 12); and 2) THC (10 sec puff every 30 sec for 15 mins; n = 20) where immediately following exposure blood samples were collected and animals were then divided into one of two stress conditions: 1) naïve; and 2) stress (30 min restraint stress) and blood samples were additionally collected in the stress-exposed rats to determine the impacts of THC on stress reactivity. Plasma corticosterone (cort) were quantified using ELISA, THC levels were quantified using mass spectrometry and brains were collected to measure activation of neurons using c-fos.

Results: The initial exposure to vehicle vapour increases cort levels in all animals, however this habituated by day 9. Acute exposure to THC immediately elevated cort level compared to vehicle exposure controls, however THC had no impact on the magnitude of stress. The impact of THC vapor on females is currently underway, as is the processing of brains for c-fos.

Conclusions: These data indicate that acute exposure to THC vapor is sufficient to produce elevations in the stress hormone cort, consistent with previous work, but does not impact stress-induced changes in cort. As much of the previous work has found larger effects in females then males, our ongoing sex difference studies will help to determine if these acute effects are different in females.

DETECTION OF Δ^9-TETRAHYDROCANNABINOL AND METABOLITES IN THE MEIBOMIAN LIPIDS OF TEAR SAMPLES THROUGH LC-MS/MS

Allen Mello*, Denise A. Valenti and Sabra Botch-Jones

Biomedical Forensic Sciences, Boston University, Boston, MA, USA

Introduction: With an increased in legal adult use Marijuana, the risk of Driving Under the Influence of Drugs is steadily increasing. However, there exists limitations with current methods of detection of drug analyte. This research explores the use of meibomian tear fluid as a novel matrix to detect THC and its accompanying analytes. We will illustrate the use of the approach by describing a single case.

Methods: Δ9-Tetrahydrocannabinol (THC), 1-Hydroxy- THC, and 11-nor-carboxy-THC, were used in detection because these analytes are produced in the metabolism of THC. Since THC is very lipid soluble, it is present in lipid rich environments in the body. This project optimized a collection of tear fluid, along with a simple buffer extraction, to create a method suitable for direct injection. Collection was completed by BVI Weck-Cel® Sterile Cellulose strips, measuring approximately 2 x 20 mm, and placed in Thompson eXtreme PVDF 0.2 μm, pre-slit, red cap, filter vials containing Quantisal buffer solution. Tear samples were collected from the volunteer participant according to Institutional Review Board (IRB) standards before and after administration of Marijuana. Samples were collected approximately 30 minutes post administration in order to capture tears when the analyte is most potent in the body. Samples and calibration standards were analyzed using Liquid Chromatography Tandem Mass Spectrometry (LC/MS-MS) with the QSight® 220 CR LC-MS/MS and the Halo® C18 3.0x50 mm (2.7 μm) column.

Results: We identified the cannabinoids; Δ9-Tetrahydrocannabinol, 11-Hydroxy- THC, and 11-nor-carboxy-THC in the meibomian tear fluid of a chronic, long term marijuana user. The cannabinoids were detected in the meibomian tear fluid collected prior to the participant having consumed their legal adult use marijuana using inhalation. The volume of cannabinoids detected post consumption were considerably greater and had relationship to the blood sample obtained post consumption. We did not measure cannabinoids in the blood prior to consumption. The data obtained related to the blood and the meibomian tear fluid were in agreement with the reported extent of marijuana use as described by the participant in the standardized marijuana consumption survey administered during the initial undosed visit.

Conclusion: Final analysis of patient samples dictated that THC, and its metabolites, could be detected and quantitated in collected tear fluid. The use of the Meibomian Tear Fluid as a matrix for the presence of cannabinoids will likely be an effective and efficient means of identifying marijuana use roadside by law enforcement officers.

PHARMACOLOGICAL CHARACTERIZATION AND BIASED SIGNALING OF THC, THCA AND THCV IN CANNABINOID CB_1 AND CB_2 RECEPTORS

[1]Iu Raïch, [1]Rafael Rivas-Santisteban, [1]Catalina Pérez-Olives, [2]Eddy Sotelo, [3]María Teresa García-Valverde, [3]Carlos Ferreiro-Vera, [1]Gemma Navarro*, [4]Xavier Nadal-Roura, [3]Verónica Sánchez de Medina and [5]Rafael Franco

[1]Barcelona University
[2]Santiago de Compostela University
[3]Phytoplant Research S.L
[4]Ethnophytotech Research & Consulting S.L.U
[5]CIBERNED

Introduction: The binding of (-) trans-delta-9-tetrahydrocannabinol (THC), and of two related compounds that are present in plant material and extracts from Cannabis sativa varieties, tetrahydrocannabinolic acid (THCA) and tetrahydrocannabivarin (THCV), to the orthosteric center of cannabinoid CB_1 or CB_2 receptors.

Methods: This binding was assessed using the SNAP-Tag technology and fluorophore-conjugated selective agonists. Signaling in terms of forskolin-induced modification of cAMP levels, mitogen activated protein kinase (MAPK) pathway activation, ß-arrestin recruitment and dynamic mass redistribution was assessed in a heterologous expression system. Assays were performed in cells expressing the human version of the CB_1 receptor, the human version of the CB_2 receptor or coexpressing the two receptors, which are able to form CB_1-CB_2 heteromers.

Results: The results demonstrate differential effects depending on the chemical structure of the phytocannabinoid and on the receptor and a particular biased signaling using as a reference either THC, or a selective agonist, arachidonyl-2'-chloroethylamide (ACEA), in the case of the CB_1 receptor, or JWH-133 (6aR,10aR)-3-(1,1-Dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran) in the case of the CB_2 receptor.

Conclusions: THC and THCV present binding at orthosteric CB1 and CB2 receptor hydrophobic pocket at less than 1 microM concentrations. THCV does not reduce the forskolin activated cAMP via CB1 or CB2 receptors and acts as an antagonist at both Cannabinoid receptors.

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SEX-DEPENDENT EFFECTS OF ADOLESCENT THC EXPOSURE ON COGNITIVE PERFORMANCE IN A MOUSE MATERNAL IMMUNE ACTIVATION MODEL OF SCHIZOPHRENIA

Todd M. Stollenwerk* and Cecilia J. Hillard

Department of Pharmacology and Toxicology, Neuroscience Research Center, Medical College of Wisconsin, Milwaukee, WI, USA

Introduction: Cognitive deficits contribute greatly to the long-term burden of schizophrenia. It has been shown that maternal immune activation is associated with the development of schizophrenia in offspring. Epidemiological studies suggest that adolescents with exposure to high amounts of THC at early ages have a greater risk for developing schizophrenia. However, the causal nature of this association is not well understood. In the present study, we have investigated the role of adolescent THC exposure in a maternal immune activation model of schizophrenia to determine if there are synergistic effects on cognitive impairment.

Methods: Pregnant C57B6/J dams were injected i.p. with 5 mg/kg Poly(I:C) or saline on GD12. The offspring were treated with 3 mg/kg THC or vehicle control daily through voluntary consumption of cereal from PND35-39. After PND100, cognitive performance was tested using Morris water maze (MWM) and fear conditioning. For the MWM, mice were trained to locate a submerged platform in 4 daily sessions consisting of 4 trials per day. On day 5, a probe trial consisted of removing the platform and tracking the mice for 60 s. On day 6, the platform was placed in a new quadrant, and mice were subjected to 4 trials to locate the new platform position. In the fear conditioning assay, mice were exposed to a training session of 7 tones paired with foot shocks. On the following 5 days, mice were exposed to 20 tones without foot shocks. In all sessions, freezing time during the tone was measured. Data were analyzed by 3-way ANOVA.

Results: There were no differences between treatment groups or sexes during the training phase in the MWM. During the probe trial, there was a trend towards decreased time in the correct quadrant in female but not male mice treated with THC during adolescence. During the spatial reversal phase, female but not male mice treated with THC found the new platform more quickly for both saline and Poly(I:C) groups. In the fear conditioning assay, females but not males treated with prenatal Poly(I:C) showed more rapid learning of the cue-conditioned freezing response. Additionally, female but not male mice treated with Poly(I:C) had delayed extinction learning. There were no effects of adolescent THC exposure in the fear conditioning assay.

Conclusions: In the measures of cognitive performance measured, female but not male mice were most affected by the prenatal Poly(I:C) or adolescent THC treatment. We found that in female mice, adolescent THC exposure may not only decrease spatial memory but also alter the searching strategy to be more effective at finding a platform in a new location. We also found that, relative to saline-treated mice, female mice treated with prenatal Poly(I:C) had enhanced fear learning while extinction of the fear memory was impaired. We found no interaction of prenatal Poly(I:C) with adolescent THC exposure in any of these studies.

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EFFECTS OF *CADM2* VARIATION ON PREFERENCE FOR AND CONSUMPTION OF THC- AND CANNABIS OIL-INFUSED COOKIE DOUGH

Hayley H. A. Thorpe*, M. Asfandyaar Talhat, Sandra Sanchez-Roige, Abraham A. Palmer, and Jibran Y. Khokhar

Department of Biomedical Sciences, University of Guelph, Guelph, ON, CA

Introduction: It is estimated that 3.9% of the global population uses cannabis annually. Recent genome-wide association studies suggest that polymorphisms in *Cell Adhesion Molecule 2* (*CADM2*), a gene associated with impulsive behaviours and substance use, confers risk for the ever-use of cannabis (Stringer et al., Transl. Psychiatry 6 (2016) e769; Pasman et al., Nat. Neurosci. 21 (2018) 1161-1170). However, the causal relationship between this gene and vulnerability to use cannabis has yet to be elucidated. In the present study, we used a novel method of determining edible THC and cannabis preference with a *Cadm2* null mouse line to investigate the role of *CADM2* in motivation for THC and cannabis oil edibles.

Methods: Male and female *Cadm2*$^{-/-}$, *Cadm2*$^{+/-}$, and *Cadm2*$^{+/+}$ mice were presented with two cookie dough balls containing pure THC or vehicle for 30 minutes daily. In a separate study, mice were delivered cannabis oil (~24mg/mL THC, <0.1mg/mL CBD) in place of THC. Escalating doses of THC were delivered between 0.002-0.02mg/gram of dough for 3-4 consecutive days per dose. Preference was calculated as amount of THC-containing dough consumed over the total amount of dough consumed. Data were analyzed by RM-ANOVA and Bonferroni post-hoc testing.

Results: *Cadm2*$^{-/-}$ mice showed the lowest preference for THC-containing dough. A similar trend was observed in the total amount of THC dough consumed, and vehicle dough consumption was inversely highest in *Cadm2*$^{-/-}$ mice. In contrast, *Cadm2*$^{+/+}$ mice showed higher preference for cannabis oil-containing dough than *Cadm2*$^{-/-}$ mice. Irrespective of genotype or dough type, drug dough preference dose-dependently decreased with escalating doses. Drug dough consumption was lowest and vehicle dough consumption highest in *Cadm2*$^{-/-}$ relative to *Cadm2*$^{+/-}$ and *Cadm2*$^{+/+}$ mice. Significant sex- and sex-by-dose effects were observed in vehicle dough consumption and total dough consumption during cannabis oil-containing dough administration.

Conclusions: The novel edible preference test utilized by this study can be used to determine cannabis oil and THC edible preference in mice according to genotype. Lower drug dough preference and THC consumption in *Cadm2*$^{-/-}$ mice suggests that *Cadm2* is important in the motivation to consume THC-containing edibles. These findings support evidence that high-expression *CADM2* polymorphisms may increase risk for lifetime cannabis use and are in line with the proposed role of *CADM2* in impulsivity; absence of the *Cadm2* gene may reduce engagement in risky behaviours such as self-administering cannabis and other psychotropic substances.

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COMPARATIVE PHARMACOKINETICS OF Δ^9-TETRAHYDROCANNABINOL IN ADOLESCENT AND ADULT MICE AND RATS

Alexa Torrens*[1], Valentina Vozella[1], Cindy Vu[1], Dakota Grimes[1], Faizy Ahmed[1], and Daniele Piomelli[1]

[1]Department of Anatomy and Neurobiology, University of California, Irvine, CA, USA

Introduction: There is an overall consensus across epidemiological surveys that adolescent-onset use of cannabis is associated with impairments in cognition and affect that continue into adulthood (Tapert et al., Current Drug Abuse Rev. 1 (2008) 99-111). Supporting a causal link between cannabis use and changes in brain function, studies showed that exposing adolescent rodents to the psychotropic constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), causes persistent dysregulations in affect, memory and reward-seeking behavior (Rubino and Parolaro, Bio Psych. 79 (2016) 578-585). Despite their mechanistic value, these animal experiments have generally discounted the possible pharmacodynamic consequences of age and sex-related changes in the absorption, distribution and metabolism of Δ^9-THC. To fill this knowledge gap, we investigated the pharmacokinetic properties of Δ^9-THC in adolescent and adult mice and rats of both sexes.

Methods: Δ^9-THC was administered (0.5 or 5 mg/kg, intraperitoneal) to pubertal adolescent (33-37-day old) and adult (70-day old) C57BL/6 male mice and male and female Long-Evans rats. Δ^9-THC and its first-pass metabolites – 11-hydroxy-Δ^9-THC (11-OH-THC) and 11-nor-9-carboxy-Δ^9-THC (11-COOH-THC) – were quantified in plasma and brain by liquid chromatography/tandem mass spectrometry. Data were analyzed by Student's unpaired *t*-test and $P < 0.05$ was considered statistically significant.

Results: In mice, Δ^9-THC administration (5 mg/kg) produced higher circulating concentrations of Δ^9-THC and its metabolites in adolescents than in adults. Similar changes or trends were also seen at lower dosages. Conversely, lower brain concentrations and brain-to-plasma ratios for Δ^9-THC and higher brain concentrations for Δ^9-THC metabolites were measured in adolescent relative to adult male mice. In striking contrast with mice, in rats Δ^9-THC administration produced lower circulating and higher brain concentrations of Δ^9-THC in adolescents than in adults. Although metabolite concentrations were elevated in the plasma and brain of adolescent males, no age-related differences were seen in females. Strikingly, in female rats, the concentration of the active metabolite, 11-OH-THC, was three times higher than the concentration observed in males, regardless of tissue or age.

Conclusions: Our experiments reveal the existence of significant differences in the distribution of Δ^9-THC between adolescent and adult male mice. These age-dependent differences are reversed in the rat. Moreover, there are dramatic sex-dependent differences in the metabolism of Δ^9-THC– a result with significant implications for the interpretation of studies comparing Δ^9-THC effects in male and female rodents. The human relevance of these developmental variations in Δ^9-THC absorption and metabolism remains to be established, but the PK properties of Δ^9-THC across the lifespan clearly deserve further study.

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AN UPDATED ANALYSIS OF GENERAL CLINICAL OUTCOME MEASURES ACROSS PATIENT GROUPS IN THE UK MEDICAL CANNABIS REGISTRY

Mehmet Ergisi*[1], Simon Erridge[1,2], Michael Harris[1], Michal Kawka[1], Devaki Nimalan[1], Oliver Salazar[1], Katerina Loupasaki[1], Rayyan Ali[1], Carl Holvey[2], Ross Coomber[2,3], Azfer Usmani[2,4], Mohammed Sajad[2,5], Sushil Beri[1,2], Jonathan Hoare[1,2], Shaheen Khan[2,6], Mark W Weatherall[2,7], Michael Platt[1,2], James J Rucker[2,8,9], and Mikael H Sodergren[1,2]

[1] Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
[2] Sapphire Medical Clinics, London, UK
[3] St.George's Hospital NHS Trust, London, UK
[4] Dartford and Gravesham NHS Trust, Kent, UK
[5] Dudley Group of Hospitals NHS Trust, West Midlands, UK
[6] Guy's & St. Thomas' NHS Foundation Trust, London, UK
[7] Buckinghamshire Healthcare NHS Trust, Amersham, UK
[8] Department of Psychological Medicine, Institute of Psychiatry, Psychology & Neuroscience, Kings College London, London, UK
[9] South London & Maudsley NHS Foundation Trust, London, UK

Introduction: There is a growing body of literature supporting the efficacy of cannabis-based medicinal products (CBMPs) across numerous medical conditions. However, there is a paucity of clinical data on the efficacy of CBMPs in conditions where they are prescribed. The UK Medical Cannabis Registry collects longitudinal data following treatment with CBMPs in UK-based patients, including validated measures of clinical outcomes and quality of life through remote collection of clinical efficacy measures, patient-reported outcomes measures (PROMS) and adverse drug reactions. This study aims to detail the changes in health-related quality of life following CBMP treatment and clinical safety, across a spectrum of clinical indications.

Methods: A prospective cohort analysis of the UK Medical Cannabis Registry was performed. Primary outcomes included change from baseline in PROMs collected across all patients (the General Anxiety Disorder Scale (GAD-7), EQ-5D-5L, and Sleep Quality Scale (SQS)) at 1, 3 and 6 months. Secondary outcomes included the self-reported incidence, severity, and duration of adverse events. Statistical significance was defined as $p<0.050$.

Results: Three hundred and twelve patients were included in the final analysis, with an average age of 44.8. The most common primary diagnoses were chronic pain of undefined aetiology (n=102, 32.7%), neuropathic pain (n=43, 13.8%) and fibromyalgia (n=31, 9.9%). Prior to enrolment 112 (35.9%) patients consumed cannabis daily. The median cannabidiol and (−)-trans-Δ^9-tetrahydrocannabinol doses prescribed at baseline were 20.0mg (0.0–510.0mg) and 3.0mg (0.0–660.0mg), respectively. Statistically significant improvements were observed in GAD-7, EQ-5D-5L Index, EQ5D VAS and SQS scores at 1, 3 and 6 months ($p<0.050$). There were 123 (39.4%) reported adverse events, of which nausea (n=13, 4.17%), dry mouth (n=12, 3.85%), somnolence (n=10, 3.21%) and dizziness (n=9, 2.88%) were the most reported.

Conclusion: This study demonstrated CBMP treatment to be associated with a relatively low incidence of severe adverse events in the medium-term. Positive changes following treatment were observed in general, as well as anxiety and sleep-specific, health-related quality of life outcomes. Randomised controlled trials are still awaited in the study of the clinical benefits of CBMPs, however real-world evidence can help inform the prescribing of clinicians currently, whilst also looking to provide insights on the safety and outcomes of long-term prescribing.

UK MEDICAL CANNABIS REGISTRY: AN ANALYSIS OF CLINICAL OUTCOMES OF MEDICINAL CANNABIS THERAPY FOR ANXIETY

Mehmet Ergisi*[1], Simon Erridge[1,2], Michael Harris[1], Michal Kawka[1], Devaki Nimalan[1], Oliver Salazar[1], Katerina Loupasaki[1], Rayyan Ali[1], Carl Holvey[2], Ross Coomber[2,3], Michael Platt[1,2], James J Rucker[2,4,5] and Mikael H Sodergren[1,2]

[1] Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
[2] Sapphire Medical Clinics, London, UK
[3] St. George's Hospital NHS Trust, London, UK
[4] Department of Psychological Medicine, Kings College London, London, UK
[5] South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Anxiety disorders are amongst the most prevalent psychiatric illnesses in the UK. Whilst there are several pharmacological treatments for anxiety disorders there is variation in efficacy and acceptability profiles of commonly prescribed medications. Further identification of novel targets for the treatment of anxiety disorders for those with resistant disease or unable to tolerate currently available medications is an area of unmet need. The endocannabinoid system has been identified as a potential pharmacological target in the management of these conditions. However, there is a paucity of high-quality clinical data on the efficacy of Cannabis-based medicinal products (CBMPs). Here we analyse clinical outcomes in patients with a diagnosis of anxiety disorder treated with CBMPs.

Methods: This study details a prospective case series of patients enrolled in the UK Medical Cannabis Registry with a diagnosed anxiety disorder treated with CBMPs. Validated patient reported outcome measures (PROMS), including the General Anxiety Disorder Scale (GAD-7), EQ-5D-5L, and Sleep Quality Scale (SQS), were collected and analysed at baseline and at 1-month, 3-month and 6-months. Secondary outcomes included the self-reported incidence, severity, and duration of adverse events. All PROMs data were deemed as non-parametric using the Shapiro Wilk test, thus the Wilcoxon rank sum was used for statistical analysis. Statistical significance was defined as $p<0.050$.

Results: Sixty-four patients were included in the final analysis with a diagnosis of anxiety. Thirty (46.9%) patients consumed cannabis daily or every other day prior to the baseline assessment. The median cannabidiol and (−)-trans-Δ^9-tetrahydrocannabinol doses prescribed at the baseline assessment were 16.3mg (0.0–188.0mg) and 13.0mg (0.0–440.0mg) respectively. Twenty-five (39.1%) adverse events were recorded, of which dry mouth (n=5, 7.81%), somnolence (n=4, 6.25%), and headache (n=2, 3.13%) were the most common. There was a statistically significant improvement in the 1-month and 3-month follow-up scores for the GAD-7, EQ-5D-5L Index, EQ5D VAS, EQ5D Anxiety and Depression and SQS compared to the baseline scores ($p<0.050$). Further, there was a statistically significant improvement in the 6-month follow-up scores for the GAD7, EQ5D VAS, EQ5D Anxiety and Depression and SQS compared to the baseline scores ($p<0.050$).

Conclusion: This study suggests that CBMP therapy in this patient cohort of patients diagnosed with anxiety disorder is associated with a relatively low frequency of adverse events and a favourable safety profile. We observed a longitudinal improvement in anxiety-specific and general health-related quality of life. Whilst randomised controlled trials with placebo comparators are still required, these data may help inform future clinical studies and practice.

MEDICAL CANNABIS, OVER-THE-COUNTER CBD AND PUBLIC OPINION IN THE UNITED KINGDOM

Simon Erridge*[1,2], Ross Coomber[2,3] and Mikael H Sodergren[1,2]

[1] Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
[2] Sapphire Medical Clinics, London, UK
[3] St. George's Hospital NHS Trust, London, UK

Introduction: In the UK there has been rapid transformation of the regulatory landscape of cannabis in recent years. In November 2018, cannabis was moved to schedule 2 of the Misuse of Drugs Act 1971 legalising the medical cannabis prescriptions. Whilst medical cannabis must adhere to good manufacturing practice guidelines ensuring consistent cannabinoid concentrations, over-the counter (OTC) cannabidiol (CBD) oils were widely available with limited regulation. An analysis of OTC CBD in the UK has previously demonstrated that products often contained variable CBD concentrations compared to those advertised and many also contained detectable levels of (−)-trans-Δ^9-tetrahydrocannabinol. Since March 31st 2021, all OTC CBD shall require a Novel Foods application requiring consistency in product manufacturing and stability. This study aims to assess the awareness and opinion of the UK population regarding access to and differences between medical cannabis and OTC CBD.

Methods*:* A cross-sectional survey analysis was performed in the UK between March 22nd and March 31st 2021 through YouGov®. The respondents were weighted to be representative of the UK adult population. Questions were administered in series using a branching logic on topics including medical cannabis legality, barriers to medical cannabis in the UK, personal use of CBD, desire for access to CBD in the future for medical purposes, awareness of the Novel Food status change, desire to continue using OTC CBD after this change, and knowledge of cost difference between medical cannabis grade CBD and OTC CBD.

Results: In total 10,684 participants completed the survey. The weighted population included 5,502 (51.5%) female participants. There were 6,090 (57.0%) were upper or middle class and 4,595 (43.0%) were working class. Participants were predominantly from England (n=9,231; 86.4%). Only 5,494 (51.4%) respondents were aware that medical cannabis is legal in the UK. When asked about the main barrier to medical cannabis access, 1,863 (17.4%) respondents said people were not aware of what conditions it would be used for, 1,210 (11.3%) answered that people did not know about medical cannabis more broadly and 2,686 (25.1%) thought that the association with recreational use would prevent people wanting access. CBD was used for wellness purposes and medical conditions by 684 (6.4%) and 286 (2.7%) respondents respectively. For those who did not use CBD in any capacity, 2,933 (31.0%) would like access for medical reasons if available at a reasonable price. Most respondents were unaware of the recent change in regulation of OTC CBD (n=10,076; 94.3%). After being made aware of this change 119 (17.4%) of those who currently use OTC CBD said they would consider switching to CBD obtained via prescription, whilst 273 (39.9%) would source another OTC CBD preparation. Most participants did not know whether a price difference existed between medical cannabis grade CBD and OTC CBD (n=7,261; 68.0%). Of the remaining respondents, the majority thought OTC CBD was either the same cost or more expensive (n=2,586; 24.2%).

Conclusions: This study demonstrated a lack of awareness within the UK population of the legality and uses of medical cannabis as a treatment modality. Stigma was still thought to play a role in the public attitudes to medical cannabis. Similarly, there was a lack of awareness of the changing regulation of OTC CBD. Further education of the public, in addition to healthcare professionals, are required to allow patients access medical cannabis when appropriate

PILOT STUDY REVEALS SEX DIFFERENCES IN T CELL STIMULATED MOUSE SPLENOCYTES PRE-TREATED WITH CANNABIDIOL

Christa M. Frodella* and Barbara L.F. Kaplan

College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA

Introduction: Cannabidiol (CBD) has been shown to have anti-inflammatory effects in autoimmune disorders such as multiple sclerosis (MS) and its counterpart murine model, experimental autoimmune encephalomyelitis (EAE). A significant point of interest in the initial pathogenesis of MS and EAE is the impact that T-cell stimulation has on other cell populations (i.e., B cells and macrophages), cytokines, and pro-inflammatory and anti-inflammatory genes in the spleen, a peripheral lymphoid organ. Since CBD has been shown to be anti-inflammatory, the purpose of this study was to determine the anti-inflammatory effect of CBD on T-cell stimulation in splenocytes over time and elucidate its effectiveness in both males and female mice.

Methods: Male and female mouse splenocytes were pre-treated in the presence of vehicle (ethanol 0.1% v/v), cannabidiol (10uM), or left untreated with and without anti-CD3/CD28 (T cell) stimulation. Cells were cultured for seven (male) and nine (female) days and analyzed on D1, D2, D4, D7, and D9 (female only). Cells were stained extracellularly for the following makers CD4 and CD8 (T cells), F4/80 (macrophages), and CD19 (B cells) and detected with flow cytometry. TNFα was quantified from supernatant using an ELISA kit. RNA was isolated from cells and the following genes were quantified using qPCR: *Tnfa*, *Noxo1*, *Sema4d*, and *Ctsb*. Data were analyzed using two-way ANOVA.

Results: CBD significantly prevented an increase in stimulated female CD4 cells on D4, D7, and D9 ($p<0.02$), CD8 cells on D9 ($p<0.0001$), and macrophages on D2 ($p=0.0052$). CBD had no significant impact on stimulated male CD4 and CD8 T cells and macrophages. CBD increased B cell percentage in stimulated female cells on D7 ($p=0.0001$) and D9 ($p=0.0119$) but exhibited no significant impact on stimulated male B cells. CBD did not significantly impact TNFα levels or transcription of all genes in stimulated male and female splenocytes.

Conclusion: Splenocytes treated with CBD and that were T-cell stimulated exhibited a sex difference between males and females in macrophage, CD4/CD8 T cell, and B cell percentages over time. In females, CBD protected against CD4/CD8 and macrophage expansion but did not protect against B cell expansion.

EXPLORING QUALITY OF LIFE OUTCOMES OF CHRONIC PAIN PATIENTS FROM THE UK MEDICAL CANNABIS REGISTRY

Michael Harris*[1], Simon Erridge[1,2], Mehmet Ergisi[1], Devaki Nimalan[1], Michal Kawka[1], Oliver Salazar[1], Rayyan Ali[1], Katerina Loupasaki[1], Carl Holvey[2], Ross Coomber[2,3], Azfer Usmani[2,4], Mohammed Sajad[2,5], James J Rucker[2,6,7], Michael Platt[1,2], and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Dartford and Gravesham NHS Trust, Kent, UK
5. Dudley Group of Hospitals NHS Trust, West Midlands, UK
6. Department of Psychological Medicine, Kings College London, London, UK
7. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Up to 28 million people in the UK are affected by chronic pain. Cannabis-based medicinal products (CBMPs) have been associated with promising improvements regarding chronic pain outcomes, yet current evidence regarding the safety and effectiveness of CBMPs for treating chronic pain is limited. Naturalistic data from registries can provide evidence of patient outcomes in a real-world setting. The aim of this study is to explore the outcomes of the initial subgroup of patients from the UK Medical Cannabis Registry who were prescribed CBMPs for chronic pain conditions, including effects on health-related quality of life, their adverse event profile, and changes in opioid prescribing.

Methods: A prospective case series of data from the UK Medical Cannabis Registry was performed of chronic pain patients. The primary outcome consisted of changes in patient-reported outcome measures, including EQ-5D-5L, General Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), Visual Analog Scale (VAS) Pain, Brief Pain Inventory short form (BPI), and the McGill Pain Questionnaire (MPQ), at 1, 3, and 6 months compared to baseline. Secondary outcomes included the incidence and severity of adverse events, and the change in oral morphine equivalent dosage. Statistical significance was defined at p-value<0.050.

Results: 190 patients were included in the final analysis. The median initial THC & CBD daily doses were 2.0mg (Range:0.0–442.0mg) and 20.0mg (Range:0.0–188.0mg) respectively. Statistically significant improvements were observed within GAD-7, SQS, EQ-5D-5L usual activities; pain and discomfort subdomains, EQ-VAS, EQ-5D-5L index, BPI, MPQ, and VAS Pain validated scales at 1, 3, and 6 months ($p<0.050$). Significant reductions in oral morphine equivalent doses were seen at 3 and 6 months, where this data was available ($p<0.050$). Seventy-four adverse events (n=39; 20.5%) were reported, of which 37 (n=25; 13.2%) were rated as mild, 23 (n=17; 8.9%) as moderate, and 13 (n=6; 3.2%) as severe; the most common adverse event was nausea (n=11; 5.8%).

Conclusions: This data suggests an association between short to medium-length CBMP treatment and improvements in health-related quality of life for chronic pain patients. CBMPs were generally well tolerated, with most adverse events reported as mild to moderate in severity. Future studies, which should include placebo-control, are still required to improve the collective knowledge on safety and efficacy for CBMP treatment for those with chronic pain.

THE INFLUENCE OF CANNABIDIOL ON FATTY ACID TRANSPORTERS AND LIPID ACCUMULATION DURING NONALCOHOLIC FATTY LIVER DISEASE DEVELOPMENT

Karolina Konstantynowicz-Nowicka*, Klaudia Sztolsztener and Ewa Harasim-Symbor

Department of Physiology, Medical University of Białystok, Białystok, Poland

Introduction: Nonalcoholic fatty liver disease (NAFLD) is an important health problem in the Western society, whose occurrence is strongly associated with obesity. The elevated levels of circulating FFAs reaching liver override its oxidation capacity and excessive energy is stored as triacylglycerols (TAG). This lipid fraction may be a precursor for other more biologically active lipids such as diacylglycerols (DAG), which increased deposition may cause NAFLD deterioration. The storage of lipids depends on the efficiency of available fatty acids transport into the cell. It is known that a major role in the regulation of lipid transport is played by protein transporters such as membrane fatty acid binding protein (FABPpm) and fatty acid transport protein (FATP). Although there are studies confirming fatty acid transporters role in NAFLD development, the possibility of their expressions' regulation by natural compounds like cannabidiol (CBD) do not exist in literature. Thus, in the present study we evaluated the effect of CBD on lipid accumulation and total fatty acid transporters expression.

Methods: The experiments were conducted on male Wistar rats receiving standard rat chow or high fat diet (HFD) for 7 weeks. In each of the two experimental groups half the animals were obtaining intraperitoneal CBD injections (10 mg/kg-1 body weight) for last 14 days (5th-7th week of experiment). The liver was isolated and the tissue was snap frozen in liquid nitrogen and stored at−80°C. The Western Blot technique was used to determine the expression of fatty acid transporters (FATP5, FATP2 and FABPpm). Free fatty acids (FFA), triacylglycerols and diacylglycerols content was assessed by gas liquid chromatography. Data were analysed by two-way ANOVA followed by an appropriate post-hoc test ($P < 0.05$ considered significant).

Results: In our study we indicated considerable decrease in DAG concentration after CBD as well as CBD and HFD treatment compared to the control and HFD group respectively. Furthermore, we observed significant increase in TAG content after CBD alone treatment as well as CBD with HFD as this fraction is the only one, among accumulated lipids, which seems to be rather safe for proper liver function. The expression of fatty acid transporters: FATP 2 and FATP 5 were significantly decreased after CBD and high fat diet administration in rats liver in comparison with the HFD group.

Conclusions: Our data clearly showed that the main cause of DAG accumulation diminishment caused by CBD under high availability of fatty acids was the reduction in lipid uptake facilitated by fatty acid transporters. Moreover, CBD treatment also caused the redirection of lipids into excessive accumulation of TAG as it is said to be livers' protective mechanism against excessive deposition of other more biologically active lipids. Thus, CBD may be used as protective agent against NAFLD deterioration.

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THE IMPACT OF THE CORONAVIRUS PANDEMIC ON CANNABIS USE PATTERNS IN THE NEW YORK METROPOLITAN AREA: A LONGITUDINAL SURVEY

Sean P. Madden*, Cameron A. Haslip and Nehal P. Vadhan

Feinstein Institute for Medical Research and the Center for Addiction Services and Personalized Interventions Research, Great Neck, NY, USA

Introduction: The COVID-19 pandemic has been associated with increased substance use in general, but understanding of its effects specifically on cannabis use is limited. The present study tracked changes in cannabis use and assessed their psychosocial sequelae during the pandemic.

Methods: 710 cannabis users from the NY metropolitan area who had completed an original online survey on cannabis use patterns and related variables (via REDCap) in the winter of 2018 were recruited via email to complete a follow-up survey. This survey assessed the same variables as the previous study, as well as cannabis use changes specifically associated with the pandemic (defined as March 2020 – present). 146 adults (age 21-72; M=34.5, SD=8.6) completed the survey. Participants were compensated for completing each survey with digital gift cards ($15/$30).

Results: On average, participants reported currently using cannabis 6.3 (SD = 1.7) days per week and spending $90.8/wk (SD = 81.0) on cannabis, both of which were similar ($p>0.05$) to their use in 2018 (6.3 days/wk [SD = 1.5] and $80.9/wk [SD = 75.0]). Despite the above data, 73 (50.0%) reported an increase in cannabis use during the pandemic, 55 (37.7%) reported no change, and 18 (12.3%) reported a decrease. Reported reasons for increased use included increased time spent at home (82.0%), boredom (79.5%), anxiety (65.8%), social distancing (53.4%), unemployment (43.8%), and decreased social interaction (38.4%). Consistent with these data, participants reported increasing the recreational nature of their cannabis use relative to the medicinal use, during the pandemic compared to 2018 ($p<0.05$). Lastly, more participants reported using cannabis alone than with others or both alone/with others during the pandemic relative to 2018 ($p<0.05$).

Conclusions: These data suggest that a majority of the sample perceived an increase in their cannabis use during the pandemic, but the actual reported frequency/amount indicators of use were not different from pre-to-post. Examination of more granular data, such as cannabis types, routes of administration and use occasions per day, may shed greater light on this apparent contradiction. However, it seems likely that an increase in time at home and boredom led to increased recreational over medicinal cannabis use.

CERAMIDE SYNTHASE ISOFORMS ARE UPREGULATED BY CANNABIDIOL RESULTING IN CYTOTOXIC EFFECTS IN PANCREATIC DUCTAL ADENOCARCINOMA

Nagina Mangal[1,2], Vikash Reebye[1], Barbara Pacchetti[3], Anguraj Sadanandam[2] and Mikael H Sodergren[1]

[1] Imperial College Medical Cannabis Research Group, Department of Surgery & Cancer, Imperial College London, UK
[2] Systems and Precision Cancer Medicine Team, Division of Molecular Pathology, Institute of Cancer Research
[3] EMMAC Life Sciences, London, UK

Introduction: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most aggressive malignancies with a median 5 year-survival rate of 8%. Cannabidiol (CBD) has been found to exhibit antineoplastic potential and may potentiate the anticancer effects of gemcitabine. The biological mechanism for this synergy remains elusive but in other tumour types, CBD therapy has been linked to *de novo* synthesis of ceramide involving a variety of ceramide synthases (Cers1-6). The sphingolipid ceramide is a potent tumour suppressor lipid with roles in apoptosis and autophagy. The aim of this study was to establish an *in vitro* platform to investigate the cytotoxic mechanism of action of CBD in PDAC, evaluate the potential synergy with gemcitabine therapy, and describe the effect on ceramide induction in this pathway.

Methods: Three PDAC cell lines (Panc03.27, Panc1 and Capan2) with differing gemcitabine sensitivities were procured for this study. Drug treatments included CBD (Adven150, EMMAC Life Sciences, London, UK) and Gemcitabine (MedChemExpress). IC_{50} values were calculated on GraphPad and synergy using the excess over bliss equation. Unpaired t-test with Welch's correction was used to compare vehicle and treated groups. A p-value of $p<0.05$ was considered statistically significant. QRT-PCR data was performed by the relative cycle threshold (Ct) method, and all samples were examined in triplicate. mRNA expression levels were calculated by the relative quantification method using the $2^{-(\Delta\Delta Ct)}$ method.

Results: CBD reduced cell viability in all cell lines with IC_{50} values in the range of 15uM-25uM and gemcitabine IC_{50} values in 6.7nM-500nM with Panc1 exhibiting highest resistance to gemcitabine and CBD whereas Panc03.27 was most sensitive to both drugs. Panc1 was further investigated using the excess over bliss score showing moderate synergy with a score of 0.6 (>0=synergy, <0= antagonism and =0 determines independence). Furthermore, we observed upregulation of mRNA levels of ceramide synthase (CerS) isoforms 1,2,4,5 and 6 in both CBD monotherapy and CBD & gemcitabine combination treatment. Significant mRNA fold changes were exhibited for CerS4 (2.1), CerS5 (1.75) and CerS6 (1.98) in CBD monotherapy compared to control. Combination treatment exhibited statistical significance of upregulation for CerS isoforms 1,2,4,5 and 6 with mRNA fold changes of 2.24, 1.21, 2.33, 2.18 and 2.63 respectively ($p<0.0001$).

Conclusions: We have established an *in vitro* platform to investigate the cytotoxic mechanism of action of CBD in PDAC. We have observed an upregulation of ceramide following CBD therapy which is further amplified by gemcitabine providing a cytotoxic mechanism of action in PDAC. These data contribute to our understanding of the role of CBD in treatment of PDAC and reveals potential targets for combination therapies.

ANALYSIS OF PALLIATIVE CARE PATIENTS FROM THE UK MEDICAL CANNABIS REGISTRY: INITIAL EXPERIENCE AND OUTCOMES

Devaki Nimalan[1], Michal Kawka[1], Simon Erridge[1,2], Mehmet Ergisi[1], Michael Harris[1], Oliver Salazar[1], Rayyan Ali[1], Katerina Loupasaki[1], Carl Holvey[2], Ross Coomber[2,3], Michael Platt[1,2], James J Rucker[2,4,5], Shaheen Khan[2,6], Mikael H Sodergren[1,2]

[1] Imperial College Medical Cannabis Research Group, Dept of Surgery and Cancer, Imperial College London, UK
[2] Sapphire Medical Clinics, London, UK
[3] St. George's Hospital NHS Trust, London, UK
[4] Department of Psychological Medicine, Kings College London, London, UK
[5] South London & Maudsley NHS Foundation Trust, London, UK
[6] Guy's & St. Thomas' NHS Foundation Trust, London, UK

Introduction: Cannabis-based medicinal products (CBMPs) have a proven role in the treatment of chemotherapy-induced nausea and vomiting. However, there is a paucity of high-quality evidence with regards to the optimal therapeutic regimen, safety, and effectiveness of CBMPs in palliative care, as existing clinical trials are limited by methodological heterogeneity. Observational data from patient registries can provide an alternative data source. The aim of this study is to summarise the outcomes of the initial subgroup of patients from the UK Medical Cannabis Registry who were prescribed CBMPs for a primary indication of palliative care, including effects on health-related quality of life and clinical safety.

Methods: A naturalistic, observational case series of data from the UK Medical Cannabis Registry was undertaken. The primary outcome consisted of changes in patient-reported outcome measures including EQ-5D-5L, General Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), Visual Analog Scale (VAS) and the Australia-Modified Karnofsky Performance Scale (AKPS), at 1 and 3 months compared to baseline. Secondary outcomes included the incidence and characteristics of adverse events. Statistical significance was defined by $p\text{-value}<0.050$.

Results: 16 patients were included in the final analysis, with a mean age of 63.25 years. Patients were predominantly prescribed CBMPs for cancer-related palliative care (n=15, 94%). The median initial CBD and THC daily doses were 32.0mg (Range: 20.0 – 384.0mg) and 1.3mg (Range: 1.0 – 16.0mg) respectively. Improvements in patient reported health outcomes were observed according to SQS, EQ-5D-5L mobility, pain and discomfort, and anxiety and depression subdomains, EQ-5D-5L index, EQ-VAS and Pain VAS validated sales at both 1-month and 3-month. No changes were statistically significant. Three adverse events (18.75%) were reported, of which two were rated as mild, one as moderate.

Conclusion: This small study provides an exploratory analysis of the role of CBMPs in palliative care in the first cohort of patients since the legalisation of CBMPs in the UK. CBMPs were well tolerated with few adverse events, all of which were mild to moderate in severity and resolved spontaneously. A post-hoc power calculation using data from this case series determined a sample size of 33 would be required to determine a significant difference at 3 months in reported AKPS score. An updated analysis shall be performed when this is available. Further long-term safety and efficacy studies involving larger cohorts are needed, with comparisons with placebo and standard treatments for cancer pain and palliative symptoms.

EVALUATING THE CB_1 RECEPTOR AS A THERAPEUTIC TARGET FOR UVEAL MELANOMA

Marzia Pendino*[1,2], Sandra Garcia Mulero[3,4], Rebecca Sanz Pamplona[3], Simone Marcone[5], Kayleigh Slater[1,2], Josep Piulats [4] and Breandan N. Kennedy[1,2]

[1]UCD School of Biomolecular and Biomedical Science and [2]UCD Conway Institute, University College Dublin, Dublin, Ireland.[3]Unit of Biomarkers and Susceptibility, Cancer Prevention and Control Program, Catalan Institute of Oncology (ICO), Barcelona, Spain, [4]Clinical Research in Solid Tumors Group (CREST), Barcelona, Spain [5]Trinity Translational Medicine Institute, Department of Surgery, Dublin, Ireland

Introduction: Anti-proliferative, anti-metastatic and anti-angiogenic effects of cannabinoids are reported. This research evaluates the therapeutic potential of cannabinoids in uveal melanoma (UM) in relation to the viability, long-term proliferation, and cancer secretome of UM cell lines. UM is a rare cancer, but the most common intraocular malignancy in adults. It arises from melanocytes within the uveal tract. Unfortunately, up to 50% of patients develop liver metastases that rapidly progress to mortality. There are no effective therapies available for metastatic UM.

Methods: Cannabinoid pathway gene expression, for 80 primary UM samples within The Cancer Genome Atlas, was analysed for association with disease free survival and overall survival. Gene set variation analysis enriched molecular functions and biological pathways linked to high or low cannabinoid receptor expression from the Molecular Signatures Database. The association between cannabinoid signalling gene expression and prognosis was adjusted by sex and age. *In vitro* assays utilised Mel285 and OMM2.5 human UM cell lines derived from a primary UM of the eye and a UM liver metastasis, respectively. Cell viability was examined at 96 hours after treatment with the synthetic cannabinoid HU210 by measuring metabolic activity. Colony formation assays assessed long-term UM cell proliferation, quantified by a GelCount™ system. Multiplex ELISA determined the secreted levels of 10 inflammatory factors at 4 and 24 hours after treatment with HU210 in the OMM2.5 cell line. For Western Blot analysis, transferred protein was probed with primary antibodies to CB_1. In zebrafish, developmental angiogenesis of the hyaloid vasculature was assessed in 5 day old *Tg(fli1:EGFP)* fixed larvae treated with 20μM HU210.

Results: Kaplan-Meijer survival curves demonstrate a significant corelation between high CB_1 expression and disease-free survival in UM patients. The CB_1 agonist HU210 appears to result in a dose-dependent reduction in Mel 285 and OMM2.5 cell viability with 50 μM HU210 reducing cell viability by 96% after 96 hours treatment. Western blot analysis confirmed the expression of CB_1 in Mel285, Mel290, OMM2.5 cells and an orthologous protein in the adult zebrafish eye.

Conclusion: Significant correlations between high CB_1expression and disease-free survival in UM patients was identified. HU210 reduces viability of UM cell lines. The effects of HU210 on UM cellsis linked to modulating inflammatory pathways. Future directions are to evaluate the roles of CB_1 and CB_2 receptors using antagonists in viability and proliferation assays.

Acknowledgments: Founded by IRC Enterprise Partnership Scheme with Breakthrough Cancer Research. (Grant. No. EPSPG/2020/160)

THE EFFECT OF CANNABIDIOL (CBD) ON EXERCISE PHYSIOLOGY AND BIOENERGETICS: A RANDOMISED CONTROLLED PILOT TRIAL

Ayshe Sahinovic*, Christopher Irwin, Peter T. Doohan, Richard C. Kevin, Amanda J. Cox, Namson S. Lau, Ben Desbrow, Nathan A. Johnson, Angelo Sabag, Matthew Hislop, Paul S. Haber, Iain S. McGregor and Danielle McCartney

Lambert Initiative for Cannabinoid Therapeutics, University of Sydney, Sydney, Australia
School of Health Sciences and Social Work, Griffith University, Gold Coast, Australia
NICM Health Research Institute, Western Sydney University, Sydney, Australia

Introduction: The non-intoxicating cannabinoid, cannabidiol (CBD), has demonstrated anti-inflammatory, analgesic, anxiolytic and neuroprotective effects that have the potential to benefit athletes. However, if CBD is to have utility in the sporting context, understanding how it affects physiological performance and psychological responses to exercise is critical. This pilot study investigated the effects of CBD on key parameters of submaximal and exhaustive running exercise.

Methods: Nine endurance-trained males (VO_{2max}: 57.4±4.0 $mL \cdot min^{-1} \cdot kg^{-1}$), who reported not using cannabinoids in >3 months, participated in this randomised, double-blind, crossover trial. Participants completed two experimental sessions, separated by a ≥7 day washout, during which they were administered a single oral dose of CBD (300 mg) or placebo 1.5 hours before undertaking a submaximal run (60 minutes at 70% VO_{2max}) (RUN 1) and an incremental run to exhaustion (RUN 2). Heart rate (HR), ratings of perceived exertion (RPEs), ratings of pleasure–displeasure, blood glucose (BG) and lactate (BL) concentrations were measured at 20-minute intervals throughout RUN 1. Respiratory gases were also sampled continuously between 24–32, 37–45 and 50–58 minutes of RUN 1. VO_{2max} and time to exhaustion (TTE) were recorded during RUN 2. Venous blood was drawn at Baseline, Pre- and Post-RUN 1, Post-RUN 2 and 1-hour Post-RUN 2. Data were synthesised using Cohen's d_z effect sizes and 85% confidence intervals (CIs), given that this pilot study was not formally powered to assess effect. CBD's effects were deemed worthy of further investigation ('*possibly moderate'*) if the 85% CI included 0.5 (or –0.5) but not zero.

Results: Submaximal oxygen consumption (37 minutes: +38±48 $mL \cdot min^{-1}$, d_z= 0.802, 85% CIs: 0.786, 0.818; 50 minutes: +26±55 $mL \cdot min^{-1}$, d_z= 0.464, 85% CIs: 0.439, 0.490) and ratings of pleasure (20 minutes: +0.7±0.9, d_z=0.770, 85% CIs: 0.529, 1.011; 40-minutes: +0.8±1.1, d_z=0.712, 85% CIs: 0.416, 1.007) appeared *moderately* elevated with CBD. No accompanying changes in HR, RPE, BG and BL were observed. VO_{2max} (+119±206 $mL \cdot min^{-1}$, d_z= 0.578, 85% CIs: 0.521, 0.635) and TTE (+40±85 sec, d_z=0.461, 85% CIs: 0.374, 0.548) also appeared *moderately* elevated with CBD. Exercise increased serum interleukin (IL)-6, IL-1β, tumor necrosis factor-α, lipopolysaccharide and myoglobin concentrations (i.e., Baseline vs. Post-RUN 1, Post-RUN 2 and/or 1-hour Post-RUN 2, *p*'s<0.05). However, these changes were too small to reliably evaluate the effect of CBD (where an effect appeared to be present). Plasma CBD concentrations were 0±0, 3±2, 77±18, 164±35 and 99±26 $ng.mL^{-1}$ at each respective time point.

Conclusion: These preliminary findings suggest that CBD has potential to alter physiological and psychological responses during exercise. Further research involving a larger participant sample is required to confirm these findings.

CANNABINOID SYNTHASES GENE COPY NUMBER AND EXPRESSION ANALYSIS IN CANNABIS VARIETIES FOUND IN URUGUAY

Lucía Vignale*, Lucía Malta and Astrid Agorio

Molecular Biology Department,
Clemente Estable Institute of Biological Research (IIBCE),
Montevideo, Uruguay

Introduction: The plant *Cannabis sativa* (*C. sativa*) has been used by many civilizations for thousands of years and it is mostly known for its capacity to produce certain compounds called cannabinoids, being delta-9-tetrahidrocannabinol (THC) and cannabidiol (CBD) the most studied to date. We know nowadays that *C. sativa* can produce more than 120 cannabinoids depending on their genetics and environment, but most of their synthetizing enzymes have not yet been described. In this study, we try to shine a light on the different cannabinoid synthases and their copy number that can be found on varieties that have been circulating in Uruguay in recent years.

Methods: The cannabinoid synthases gene copy number was estimated through quantitative PCR analyses. Specific primers were designed to recognize each of the enzymes coding genes: tetrahydrocannabinolic acid synthase (THCAS), cannabichromenic acid synthase (CBCAS), cannabidiolic acid synthase 1 and 2 (CBDAS1 and CBDAS2), and chalcone synthase (CHS) used as a control for single copy gene. The same primers were used to study gene expression in flowers. The analyses were performed on fresh plant material donated by registered Uruguayan self-cultivators; varieties corresponded to chemotypes one, two, and three.

Results: We found out that the THCAS and CBDAS1 genes appear in zero or one loci in the genomes analyzed, whereas the copy number of CBCAS and CBDAS2 genes were much more diverse, and covered between zero and fourteen copies. THCAS and CBDAS1, when present, were expressed in flower tissue; CBCAS and CBDAS2's expression were extremely low or undetectable under the same conditions. We also obtained full length sequences for copies of these two genes in the genomes analyzed that could codify for putatively functional enzymes, but according to expression studies were little or not expressed in the analyzed flowers.

Conclusions: Cannabinoid synthases gene copy number appear to be fixed to an absence-presence manner for canonic THCAS and CBDAS1, while it explores a vast diversity when it comes to CBCAS and CBDAS2's genes. More studies should be carried out to explore when these different copies are expressed, study the type of cannabinoid they produce, and understand the role they might have related to the other enzymes expression patterns.

Acknowledgements: Project financed by the National Research and Innovation Agency (ANII) (FCE_3_2018_1_148016) and the Basic Science Development Program (PEDECIBA).

DIFFERENTIAL ENANTIOMER-SPECIFIC SIGNALING OF CANNABIDIOL AT CB1 RECEPTORS

Taryn Bosquez*[1], Sierra Wilson[1], Christos Iliopoulos-Tsoutsouvas[2,3], Shan Jiang[3], Spyros P. Nikas[2], Alexandros Makriyannis[2,3], Ken Mackie[1] and Alex Straiker[1]

[1]Gill Center for Biomolecular Science, Department of Psychological and Brain Sciences, Program in Neuroscience, Indiana University, Bloomington IN USA 47405
[2]Center for Drug Discovery and Department of Pharmaceutical Sciences, Northeastern University, Boston MA USA 02115
[3]Center for Drug Discovery and Department of Chemistry and Chemical Biology, Northeastern University, Boston MA USA 02115

Introduction: The two main cannabinoid constituents of cannabis are Δ^9-THC and cannabidiol (CBD). Δ^9-THC pharmacology has been studied extensively over several decades. CBD, long considered inactive, is now the subject of vigorous research related to epilepsy, pain and inflammation and has enjoyed an enthusiastic popular embrace as a virtual cure-all. However, our understanding of CBD pharmacology is still limited. We and others have shown that CBD inhibits cannabinoid CB_1 receptor signaling, likely as a negative allosteric modulator. The cannabis plant makes (-)-CBD, but CBD can also exist as an enantiomer, (+)-CBD.

Methods: We tested and compared both CBD enantiomers in a reliable *in-vitro* cAMP-based assay that used a fluorescent binding protein to measure cAMP accumulation in CB_1-expressing CHO-K1 cells. When CB_1 is activated, its coupling to the $Ga_{i/o}$ G protein subunit leads to an inhibition of cAMP accumulation. Each CBD enantiomer was tested in a concentration series against CB_1 agonist, 2-AG, to measure cAMP accumulation in CHO-K1 cells expressing CB_1 receptors. Area under the curve analysis allowed us to compare the signaling characteristics of the two enantiomers. In addition to this, (+)-CBD was tested in wild-type HEK293 cells, as preliminary data indicated the enantiomer to target additional receptor types endogenously present in the cell line.

Results: The two CBD enantiomers similarly interfered with 2-AG-induced CB_1 singling, by increasing cAMP production following their application to CHO-K1-CB_1 cells ((+)-CBD IC_{50}:150nM; (-)-CBD IC_{50}: 290nM). In addition to these findings, we have also determined (+)-CBD, but not (-)-CBD, to induce an inhibitory effect on cAMP accumulation in wild-type HEK293 cells. We report this inhibition to be the result of (+)-CBD activating two subtypes of endogenous sphingosine-1 phosphate (S1P) receptors, $S1P_1$ and $S1P_3$. Activation of each of these receptors partially contributes to the full inhibitory effect of (+)-CBD seen in the HEK293 cells.

Conclusion: These results indicate not only (-)-CBD, but also (+)-CBD, have the potential to be considered negative allosteric modulators, as they attenuate CB_1 activation in the presence of an agonist. These results also suggest CBD enantiomers have their own unique and interesting signaling properties, as (+)-CBD was seen to have a modulatory effect at certain S1P receptors, which could have therapeutic implications.

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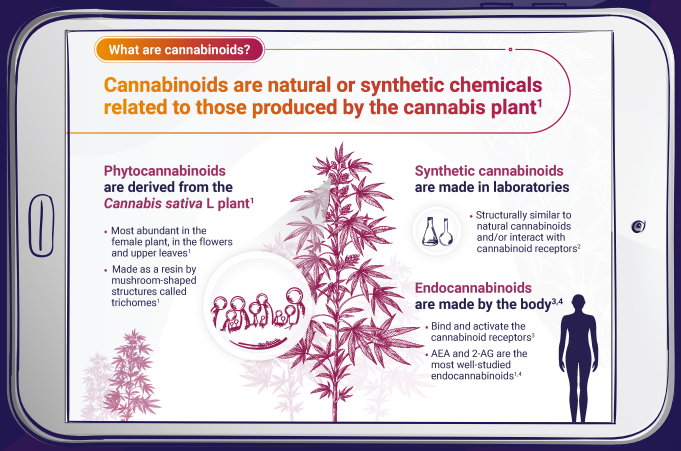

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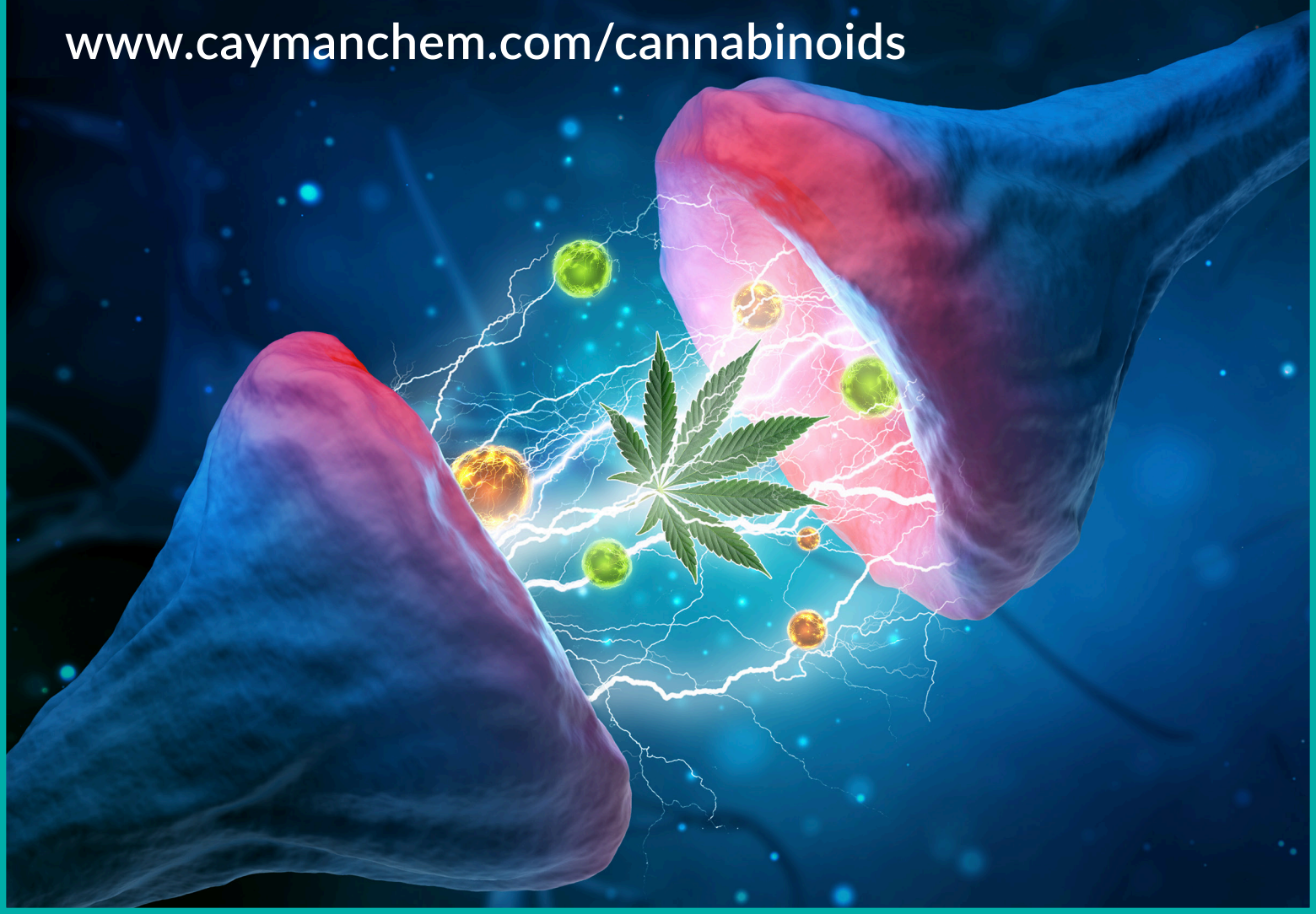

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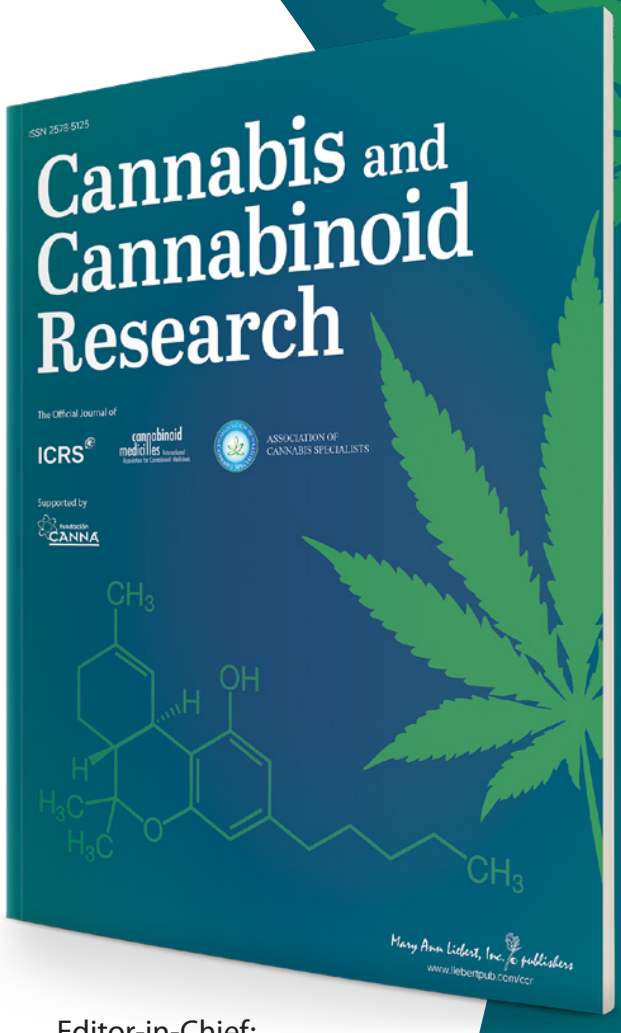

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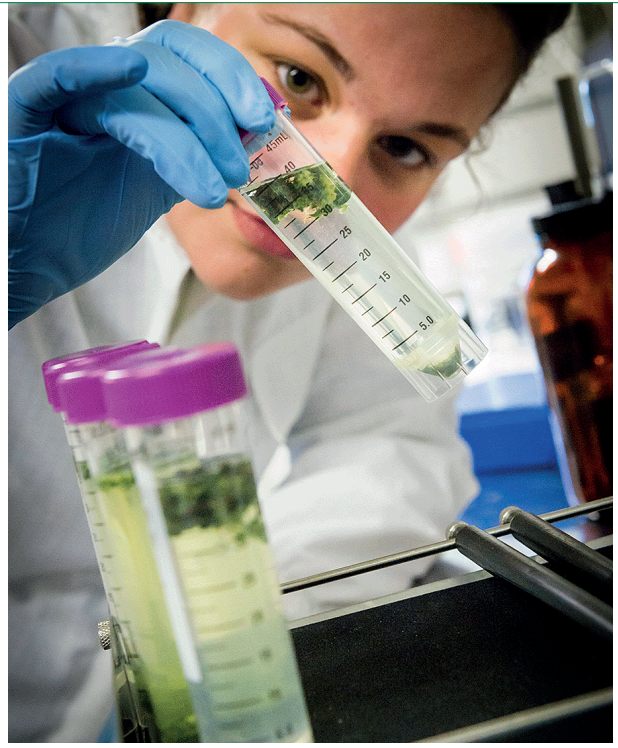

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