Study Protocol and Statistical Analysis Plan

Title of Study: Effect of Cannabis and Endocannabinoids on HIV Neuropathic Pain

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GLOSSARY OF PROTOCOL-SPECIFIC TERMS

2AG: 2-arachidonoylglycerol
AE: adverse event
AEA: N-arachidonoylethanolamine
ARCI: Addiction Research Center Inventory
CBD: cannabidiol
CMCR: Center for Medicinal Cannabis Research
DEA: Drug Enforcement Administration
EC: endocannabinoid
EMA: Ecological momentary assessment
FAAH: fatty acid amide hydrolase
FDA: Food and Drug Administration
HAND: HIV-associated neurocognitive disorders
HIV-NP: HIV-associated neuropathic pain
HIV-SN: HIV sensory neuropathy
HNRP: HIV Neurobehavioral Research Program
HRV: heart rate variability
IMPACT: Individual Monitoring of Pain and Cannabis Taken
LC/MS: liquid chromatography-tandem mass spectrometry
MAGL: monoacylglycerol lipase
METH: methamphetamine
NNT: the number needed to treat
NP: neuropathic pain
NPRS: Numeric Pain Rating Scale
PBMC: peripheral blood mononuclear cells
PGIC: Patient Global Impression of Change
PLWH: persons living with HIV
SAE: serious adverse event
SMS: short message service
SUI: Substance Use Interview
THC: delta-9-tetrahydrocannabinol
TNFα: tumor necrosis factor alpha
TNS: Total Neuropathy Score
VAS: Visual Analog Scale

ABSTRACT

HIV-associated neuropathic pain (HIV-NP) affects a significant proportion of people living with HIV (PLWH) and has a major impact on everyday functioning and quality of life in this population. Over the past decade a growing number of preclinical studies and clinical trials have indicated that cannabis administration and manipulation of the endocannabinoid (EC) system may have therapeutic utility in addressing HIV-NP. EC CB2 receptor activation and inhibition of the EC deactivation enzymes fatty acid amide hydrolase (FAAH) and monoacyglycerol lipase (MAGL) have been shown to decrease pain in rodent models of HIV-NP, while acute exposure to cannabis reduces self-reported pain in PLWH with neuropathy. However, little work has been conducted to elucidate the effects of the two primary cannabinoids (delta-9-tetrahydrocannabinol, THC and cannabidiol, CBD) on the EC system or to assess EC function in PLWH. The objective of this application is to address these fundamental gaps in our knowledge by: 1) examining the acute effect of administering NIDA-prepared THC/CBD products on HIV-NP; 2) utilizing mHealth text messaging to monitor daily real-world self-administered cannabis effects on pain; 3) assessing the relationship between cannabinoids and EC biomarkers, including ligands, enzymes, and receptor expression; and 4) conducting exploratory analyses to evaluate the effect of longitudinal cannabinoid use on changes in HIV-NP over time. We will examine the effect of low (0.01%), medium (one vial of 5.1%), and high dose (two vials of 5.1%) CBD-containing cannabis on NP, the EC system, and heart rate variability (as a proposed objective biological measure of pain) in 100 PLWH who use cannabis to treat neuropathic pain. We will subsequently employ a mobile phone text messaging system, the Individual Monitoring of Pain And Cannabis Taken (IMPACT), to track cannabis exposure, CBD and THC consumption, and pain over a period of 6 months in these same PLWH participants. IMPACT will be used quantify the real-time effects of acute CBD/THC exposure on pain before and after cannabis self-administration, the real-time relationship between self-reported pain and changes in HRV, and to assess any longitudinal changes in NP, HIV clinical outcomes (viral load, CD4) and cognition during the 6-month period. This plan allows us to acquire data and compare the effects of cannabis product obtained from NIDA and self-administered cannabis obtained from local medicinal dispensaries. The overarching hypothesis is that CBD exposure and a higher CBD/THC ratio will exert beneficial effects both in the laboratory and during the observational study, including increasing EC biomarkers and reducing NP. In summary, this approach will advance our understanding about several key issues, including the interaction between cannabis constituents, the EC system and pain, the biological mechanisms that underlie these effects (EC enzymes, receptor), and the longer-term effects of cannabis use on health-related outcomes in HIV, including predictors of mortality (HRV, CD4, viral load) and neurocognition.

SCHEMA

Effect of Cannabis Administration and Endocannabinoids on HIV Neuropathic Pain

DESIGN: Our objective is to assess PLWH who have neuropathic pain and are currently using cannabis, including light use (1-4 times per month) and heavy use (5+ times per month). These participants will be enrolled in a study that consists of two stages: Stage 1) PLWH will participate in a 6-month mHealth text messaging program (Individual Monitoring of Pain and Cannabis Taken, or IMPACT) that will examine the association between cannabis obtained from local dispensaries and changes in daily pain. Participants will return for a visit after 6 months for follow-up testing so we can examine longitudinal changes in neuropathic pain, neuropsychological performance and EC function. Stage 2) A double blind crossover study where we administer three different doses of vaporized cannabis to each participant that contain THC and varying concentrations of CBD (low, medium, high), including: a) 2 vials of 0.01% CBD + 1.9% THC, b) 1 vial of 0.01% CBD + 1.9% THC and 1 vial of 5.1% CBD + 1.4% THC, and c) 2 vials of 5.1% CBD + 1.4% THC. Here we will examine the acute effects of cannabis obtained from NIDA on pain intensity, blood endocannabinoid (EC) levels, and the relationship of pain with heart rate variability (HRV).

POPULATION: PLWH with neuropathic pain and current cannabis use.

STRATIFICATION: none

REGIMEN OR INTERVENTION
Three doses of cannabis (with varying concentrations of CBD) administered once per day on three separate days.

1.0 INTRODUCTION

HIV-associated neuropathic pain (HIV-NP) affects a significant proportion of people living with HIV (PLWH) and is one of the leading causes of disability in this population. Acute cannabis administration is reported to alleviate HIV-NP, but we have limited knowledge about the effects of cannabis constituents (THC and cannabidiol/CBD), the consequences of long-term cannabis use, and the impact of cannabis on endocannabinoid (EC) function in PLWH. Our objective is to address these three fundamental gaps in our knowledge by: 1) examining the acute effects of various CBD/THC products on HIV-NP, 2) utilizing a mHealth text messaging protocol (Individual Monitoring of Pain and Cannabis Taken or IMPACT) to monitor daily real-world cannabis use and changes in pain; 3) studying the relationship between cannabinoids, EC biomarkers, and chronic neuropathic pain.

1.1 Background

HIV-related neuropathic pain (HIV-NP) represents a significant problem for PLWH. HIV sensory neuropathy (HIV-SN), typically characterized by sensory deficits, numbness, tingling, or pain in feet or hands, is a common health concern in people living with HIV (PLWH)[1]. HIV-SN is often associated with neuropathic pain (NP) described as “stabbing”, “burning” or “aching” that is the most frequent source of HIV-SN disability[2], while the prevalence of reported pain in PLWH ranges from 27 to 97%[3]. A large scale study by our group indicated that 57% (881 out of 1539 PLWH) exhibited HIV-SN and 38% reported HIV-NP[4]. Treatment of HIV-NP remains difficult; a 2010 review indicated that only capsaicin, human nerve growth factor, and smoked cannabis showed superior pain reduction compared to placebo[5]. Studies at the University of California Center for Medicinal Cannabis Research (CMCR) indicate that 34-40% of patients exposed to cannabis cigarettes with delta-9-tetrahydrocannabinol (THC) exhibited significant pain reduction compared to 17-20% on placebo[6-8]. While THC doses in the 1.3% to 3.5% range alleviate pain for some participants, higher doses of THC (7-8%) did not provide additional benefit for pain reduction[8] or actually increased capsaicin-induced pain in healthy volunteers[9]. Importantly, combined cannabidiol (CBD)/THC extracts are reported to be more effective at treating pain compared to THC alone in human cancer-related pain and in rodents[10, 11]. CBD exhibits antioxidant and neuroprotective properties, inhibiting the release of inflammatory cytokines such as TNFα[12]. However, the optimal THC/CBD ratio for effective pain treatment is not yet known. There may also be discrepancies between data obtained from controlled clinical trials and the use of unregulated cannabis obtained from dispensaries. There is thus a clear need to close the gap between existing clinical data and real-life cannabis use from public sources, including the effects on the endocannabinoid (EC) system and NP.

Endocannabinoids reduce pain and modulate neuronal activity at synapses throughout pain processing pathways, but the effect of cannabis on the EC system is understudied [13]. The EC system includes two receptors, CB1 and CB2, and two primary ligands, N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), which are deactivated by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively[14, 15]. EC ligands act as a negative feedback system that inhibits the release of pro-inflammatory cytokines and reduces neuronal signaling in response to pain[13, 15, 16]. Elevated

peripheral levels of AEA and 2-AG occur in preclinical pain models[13] and FAAH and MAGL inhibitors produce antinociceptive effects in rodent models of acute and chronic pain[17, 18]. Mice lacking CB2 receptors also show enhanced pain responses to sciatic nerve ligation[19]. Despite the reported benefits of acute cannabis treatment for pain, chronic cannabis use may disrupt EC function[20]. Heavy cannabis use (several days per week) reduces EC ligand levels[21] and CB1 and CB2 expression in both humans and mice[22-25]. Psychotic patients with heavy cannabis use exhibited decreased CB2 and increased FAAH in peripheral blood mononuclear cells (PBMC) compared to healthy controls[22], while increased AEA in cannabis-naïve schizophrenia patients was completely blocked by cannabis use[20]. Cannabis use may disrupt EC function, but little is known about the specific effects of THC and CBD. One study in schizophrenia patients reported that 4 weeks of administered CBD increased plasma AEA and reduced FAAH[26]. Preclinical and *in vitro* data show that CBD may increase AEA expression by inhibiting both neuronal uptake and FAAH activity[27]. In contrast, THC, but not CBD, is a strong CB1 and CB2 receptor agonist[28, 29] and could represent the primary cannabis constituent that disrupts EC function. Overall, these data suggest that THC and CBD may have differential effects on EC activity, a question that requires investigation in a real-world setting of chronic cannabis use.

Pain is typically assessed by self-report, but the field would benefit from the use of biological measures sensitive to HIV-NP. Common pain measures include the Visual Analog Scale (VAS), where individuals mark their level of pain on a 10 cm line from “no pain” to “worst pain” and the Numeric Pain Rating Scale (NPRS), a 0-10 scale with similar endpoints[5]. In contrast to self-report, suggested metrics of objective pain such as fMRI neuroimaging remain in development[30]. Another proposed option, heart rate variability (HRV), is a measure of autonomic function closely related to pain self-report and analgesic efficacy[31-34]. HRV quantifies the beat-to-beat variation in heart rate and provides a measure of sympathetic and parasympathetic tone. Healthy individuals exhibit higher HRV, but HRV is significantly lower in PLWH[35-37], correlates with the level of pain intensity in chronic fibromyalgia[38], and improved HRV after biofeedback is associated with less pain on VAS[39]. Persons with effective analgesic treatment of chronic pain exhibited improved HRV relative to pain sufferers with ineffective treatment[32], suggesting HRV may serve as a pain biomarker.

The effects of cannabis on health outcomes and cognition in HIV remain unclear. Despite prevalent cannabis use, there are significant concerns that drug exposure may impair cognition and affect everyday functioning[40-42]. Chronic cannabis use is associated with cognitive deficits across a wide range of domains (memory, attention, executive function)[43-45], but little is known about the potential neuropathology[46]. Preclinical studies indicate that CBD may have beneficial effects on cognition, including rescuing memory impairment in a mouse model of malaria[47], improving object recognition in a model of Alzheimer’s disease[48], and attenuating visuospatial deficits induced by THC in rhesus monkeys[49], but CBD exposure effects in PLWH are not known. Similarly, the effects of CBD and THC on measures of disease state, such as CD4 level and viral load, are not well understood. Rhesus macaques exposed to THC for 6 months exhibited lower viral load after infection with simian immunodeficiency virus[50], while daily cannabis use was associated with lower viral load among recently seroconverted PLWH when compared to less frequent use[51]. However, THC and CBD exposure was not reported in this study and remains an important and understudied issue in this field.

mHealth text messaging will serve as a useful tool to monitor the relationship between pain and

cannabis use. Text messaging is an effective method to modify health behaviors, monitor substance use, and track pain[52]. Our group has recently demonstrated the feasibility of using short message service (SMS) texting to promote ART adherence and monitor daily methamphetamine (METH) use in PLWH with bipolar disorder or METH dependence, but SMS has not been used to assess cannabis exposure in this population[53, 54]. There are a variety of cannabis mobile apps providing information on strain and dispensary information, but none that address abuse, addiction, or treatment[55]. Ecological momentary assessment (EMA), the technique of gathering data within a participant's environment using multiple assessments[56], has been applied to alcohol and cigarette use[57], but few studies have examined cannabis. We will utilize a novel text messaging protocol, Individual Monitoring of Pain And Cannabis Taken (IMPACT), to assess the relationship between real-world self-administered cannabis and changes in pain symptoms that precede and follow drug exposure.

2.0 STUDY DESIGN

Our objective is to assess 100 community-dwelling PLWH with neuropathic pain (PLWH-NP) who are recruited and tested at the HIV Neurobehavioral Research Program (HNRP)/Center for Medicinal Cannabis Research (CMCR) facilities located at the UCSD Medical Center. These participants will be enrolled in a study that consists of two stages: 1) administration of three different doses of vaporized cannabis obtained from NIDA (one dose per day on three separate days) that contain THC and varying concentrations of CBD, including: a) 2 vials of 0.01% CBD + 1.9% THC, b) 1 vial of 0.01% CBD + 1.9% THC and 1 vial of 5.1% CBD + 1.4% THC, and c) 2 vials of 5.1% CBD + 1.4% THC; 2) participation in a 6-month text message monitoring study, the Individual Monitoring of Pain and Cannabis Taken (IMPACT), where pain and cannabis use from local dispensaries is tracked. We will use a randomized crossover design where all participants receive each of the three cannabis doses at the HNRP and then are subsequently enrolled into IMPACT. We will recruit individuals who are currently using cannabis at least once a month to treat pain. The primary outcome measures of the study will examine: 1) the acute effects of NIDA-obtained cannabis on pain, EC levels, and the relationship with HRV (Aim 1); 2) the association between dispensary-obtained cannabis and changes in pain reported via IMPACT (Aim 2); 3) the effect of chronic THC and CBD exposure on longitudinal changes in pain, EC measures, and neurocognitive performance (Aim 3). Secondary and exploratory analyses will compare NIDA-obtained and dispensary-obtained cannabis effects on neuropathic pain and evaluate if there are different effects of reported cannabis strain (Sativa, Indica), specific cannabis products (e.g., OG Kush, Sour Lemon, etc.), or mode (smoking, vaporizing, ingesting food product) on pre-post drug exposure changes in pain

3.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

3.1 Inclusion Criteria

3.1.1 Ability of participant to provide informed consent.

3.1.2 Age 18 or older.

3.1.3 HIV-1 infection documented by any FDA licensed clinical test including HIV enzyme/antigen test or chemiluminescence immunoassay (E/CIA) or plasma

HIV-1 RNA viral load.

3.1.4 A diagnosis of HIV sensory neuropathy determined by a neurological exam performed by a nurse and defined per previous criteria[7, 59] as one or more clinical signs, including diminished or dull/sharp sensation in feet or reduced ankle reflexes.

3.1.5 Current use of cannabis

3.1.6 Ability to describe THC and CBD content in cannabis products

3.1.7 Ability to respond to text messages

3.2 Exclusion Criteria

3.2.1 Meeting criteria for current alcohol or substance dependence

3.2.2 Traumatic brain injury

3.2.3 Dementia or Alzheimer's disease

3.2.4 Psychosis

3.2.5 A respiratory condition, i.e., pulmonary disease, that would be exacerbated by inhaling vaporized cannabis.

3.2.6 History of cardiovascular disease, including myocardial infarction or stroke

3.2.7 Uncontrolled hypertension, defined as a systolic blood pressure greater than 150 mm Hg or a diastolic blood pressure greater than 100 mm Hg.

3.2.8 Pregnancy, breastfeeding, or unwillingness to prevent pregnancy during the cannabis administration portion of the study (using birth control in female participants of child-bearing age).

3.2.9 Unwillingness or inability to receive or respond to text messages.

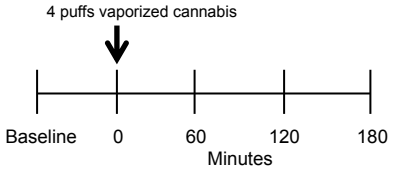
4.0 STUDY TREATMENT

4.1 NIDA Drug Supply Provision of Cannabis Product

We will be administering three cannabis doses to each participant using the vaporizer, including varying concentrations of CBD (low, medium, high): a) 2 vials of 0.01% CBD + 1.9% THC, b) 1 vial of 0.01% CBD + 1.9% THC and 1 vial of 5.1% CBD + 1.4% THC, and c) 2 vials of 5.1% CBD + 1.4% THC.

4.2 Cannabis Administration Protocol

Participants will receive vaporized cannabis using Volcano vaporizers (Storz and Bickel) with the aforementioned concentrations of CBD and THC at each of three visits. Allocation assignment of visits will be assigned using randomization schedule prepared by our statistician. The allocation schedule will be kept in the pharmacy and concealed from other study personnel. The cannabis will be stored in a freezer at 20°C until the day before use. At least 12 hours before each session, 800 mg of study cannabis will be thawed. The vaporization session will be performed in a HNRP negative pressure room designed to prevent the vapor from being transferred to rooms in which other occupants of the building are working. Puffs from the vaporizer balloon will be standardized so that a consistent amount is inhaled. This will be done according to a cued-smoking procedure shown to produce reliable increases in plasma THC levels[65]. A nurse will provide instructions to participants to 'inhale' (5 seconds), 'hold smoke in lungs' (10 seconds), and 'exhale' and to wait before repeating the puff cycle (40 seconds). Each participant will inhale 4 puffs at each session, an amount that was well tolerated during our previous human laboratory experiments [60]. We will measure vital signs (heart rate, respiration), pain intensity measured by a 0 to 10 numerical rating scale, and HRV at baseline (30 minutes before cannabis administration), at time zero (immediately after cannabis administration), and at 60, 120, and 180 minutes after drug administration. As a secondary measure of pain relief, we will use the Patient Global Impression of Change (PGIC) that will be measured hourly following administration of study medications. We will also conduct a von Frey assessment. The von Frey tool consists of monofilaments that are applied to the skin until bending of the filament occurs, calibrated to a specific pressure and designed to minimize movement artifacts[66]. Increased pain to von Frey stimulation occurs in NP and clinical trials indicate that cannabis treatment can reduce allodynia on this test[67]. We will include this measure in our battery and assess pain using the VAS as previously reported[67]; the von Frey filament will be applied on the dorsum of the more painful foot until bending is observed for 3 seconds, followed by a VAS pain rating.



To assess participant perception of drug effects, the Marijuana subscale (M-scale) of the Addiction Research Center Inventory (ARCI) will be administered[68]. The M-scale has 12 true/false statements describing the subjective effects of marijuana (see attached ARCI Marijuana subscale). We have elected not to include a placebo in the current design because participants with prior cannabis use are likely to be aware they are not receiving the drug, given the lack of psychoactive/physiological effects, and this may confound their report of pain. We have also chosen to administer one fixed dose (8 puffs) to all participants to avoid the confound of varying levels of drug intake. Participants will be allowed to engage in normal activities, such as reading or listening to music between assessments. Participants will remain in the laboratory under direct observation by nursing staff for 3.5 hours after the vaporization inhalations are completed (30 minutes after the 180-minute sample collection). At that time a final vital sign status check will be made and upon satisfactory readings, the subject will be released and driven back to his/her domicile by taxicab or prearranged transportation.

4.3. IMPACT

All participants will be instructed on how to use the IMPACT text messaging/website survey program, practice the SMS interface with study staff, and set up personalized preferences (e.g., optimal times to receive messages). If they do not have a cell phone capable of receiving and sending text and picture messages, one will be provided to them at the baseline session and we will purchase bulk 'minutes' in order to provide the text message intervention. Participants will also receive digital milligram scales that they can use to weigh out cannabis products, including joints, blunts, and bowls. Participants will not be required to use the scales, but they will be provided as an optional tool to improve the accuracy of reporting cannabis quantity. Participants will not be responsible should they lose or break the devices. We will also ask participants to identify the source of their cannabis products, including the name of the medical dispensary where the product was obtained. If the participant obtained cannabis from another source, such as a neighbor or friend, this source will be anonymously identified as "friend". We will not collect any names or any identifying information about any specific individuals who provide cannabis to the participant; only the names of medical dispensaries in San Diego will be collected. Participants will also not be required to disclose the source of the cannabis and may select an option of "decline to provide" or "I do not know" or "unknown" when asked about this information. (45 minutes). After the 6-month IMPACT is completed, participants will return for a visit that will include the same assessments during the pre-entry visit, including a medical exam with neuropathy testing, assessment of substance use, administration of pain rating scales, neurocognitive testing, and a blood draw to repeat baseline evaluations including THC, CBD, and EC biomarkers and check hepatic/renal function. We will not administer cannabis at this session, and similar to the pre-entry visit, request that participants abstain from cannabis for 24 hours before this visit.

5.0 CLINICAL MANAGEMENT ISSUES

5.1 Adverse Events

The medical response to adverse events (AE) or serious adverse events (SAE) will be managed and assessed under the on-site direction of our medical team. An AE is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure. An SAE is defined as the following: an AE that results in death, is life-threatening (even if temporary in nature), results in a permanent impairment of body function or permanent damage to body structure, hospitalization, prolongation of hospitalization, or medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure, or represents a serious medical event.

AE associated with acute cannabis exposure may include the following: 1) likely effects: difficulty with balance, eye irritation, throat irritation, increased heart rate, possible low blood pressure, reversible problems with appetite; 2) less likely effects: changes in mood (euphoria or dysphoria), loss of memory, decreased ability to concentrate or think properly; 3) rare but

serious effects: dizziness, head and chest pressure, disorientation, agitation, combativeness, incoherence, or visual hallucinations.

If requested by the research team, a personal assessment of the participant will be performed. Participants will be followed by the research team until such time as the adverse event resolves. A Registered Nurse and/or research associate will consult with study PIs to discuss each adverse event. In the event of any uncertainty, the decision as to whether or not the adverse event is to be labeled a SAE will be made by a Medical Officer. If deemed not to be a SAE, the AE will be reported at the time of the UCSD IRB continuing review or annual review by the FDA and NIDA.

All AEs will be collected on an Adverse Event log which will include the Patient ID, date, visit number, time in 24-hour format, description of adverse event, date of resolution if applicable, determination if related to the study, and date reported to UCSD IRB and study sponsor (NIDA). All AEs experienced by the participant, i.e., from the time of study drug administration through the end of the study, will be reported on this log. Any AE or SAE so recorded will be reported annually to the Human Research Protection Program (HRPP) at UCSD. In the event of an SAE, the PIs will report SAE to the UCSD IRB within 10 days as required by the HRPP. Such data will also be promptly reported to NIDA. Any AE and SAE reported may result in revisions to the study protocol. If so, these revisions will be submitted to the UCSD IRB for review. Any unexpected fatal or life threatening experiences associated with the use of the drug (21 CFR 312[.32[c] [ii] [2]) will be reported to the FDA via telephone or facsimile transmission within 7 calendar days after NIDA's initial receipt of the information. In accordance with FDA Code of Federal Regulations, the investigators will be responsible for reporting of SAE's directly to the FDA using the mandatory reporting form FDA 3500A.

5.2 Toxicity

Our group has previously administered a similar dose of THC (3.5%) to participants in prior studies using both cigarettes (Wilsey B, Marcotte T, Tsodikov A, et al. A randomized, placebo-controlled, crossover trial of cannabis cigarettes in neuropathic pain. J Pain. Jun 2008;9(6):506-521) and vaporized cannabis (Wilsey B, Marcotte T, Deutsch R, Gouaux B, Sakai S, Donaghe H. Low-dose vaporized cannabis significantly improves neuropathic pain. J Pain. Feb 2013;14(2):136-148). In both studies his findings indicated that none of the participants receiving 3.5% THC exhibited any AE or SAE due to drug exposure. Based on the data he has collected, we do not anticipate AE or SAE due to acute THC exposure in the current study (1.4% to 1.9%). In addition, we will be recruiting participants with a history of cannabis use rather than cannabis-naïve individuals; therefore, there is also lower risk of a novel AE or SAE occurring in these participants.

CBD does not bind to the known cannabinoid receptors and its mechanism of action is yet unknown[86]. The administration of CBD appears to be much less problematic compared to THC. In a recent study comparing oral THC, CBD, and placebo in healthy volunteers[87], CBD was well tolerated and there were no differences between oral CBD and placebo on symptomatic or physiological variables. Other work suggests that CBD may in fact attenuate some of the psychoactive effects of THC[88-90]. After an extensive literature review, Bergamaschi and

colleagues concluded that CBD is a substance with low toxicity[91]. Based on these findings, we do not anticipate that there will be AE or SAE due to acute CBD administration in the study. However, no specific research on vaporized combinations of THC/CBD has been performed and we will collect data at every visit on any adverse effects that occur.

5.3 Monitoring Participant Safety

Ongoing medical treatment for HIV will not be altered or modified in any way for research purposes. During the initial enrollment and screening process, participants will be informed about potential events that may occur as a result of cannabis exposure and will be asked to contact the staff if any adverse events occur during study participation. Vital signs will be monitored during the cannabis administration procedure at hourly intervals to monitor the participant's health status. At any sign of an adverse reaction (e.g. a change in blood pressure or heart rate or development of psychological distress), medical staff will be contacted. Participants will be monitored by medical staff until such time as the adverse event resolves. In the event of a medical emergency, participants can be transported to the UCSD Medical Center Emergency Room. Participants will remain in the laboratory under direct observation by nursing staff for 3.5 hours after the cannabis vaporization inhalations are completed. At that time a final vital sign and self-report status check will be made and upon satisfactory readings, the subject will be released and driven back to his/her domicile by taxicab or prearranged transportation. The return transport procedure also will be observed directly by staff to ensure compliance. In an emergency situation that occurs outside of the university where participants are in need of immediate help as a result of an adverse event, the participants will be informed that they should first call 911 and then report the incident to the study staff as soon as possible. During IMPACT participants will also receive regular text messages that will solicit information about any adverse events associated with their self-administered cannabis use. Participant text logs will be monitored on a daily basis to ensure that no serious problems have occurred and to supervise each individual's continuing participation in the study. Participants are informed that they may withdraw from the research at any time if they find any aspect objectionable.

6.0 CRITERIA FOR DISCONTINUATION

6.1 Criteria for Discontinuation of Cannabis Administration

All participants will be informed of the risks of the study and the anticipated and potential effects of exposure to vaporized cannabis. We will monitor participant status throughout the day of the cannabis administration sessions, including assessing blood pressure at regular intervals (60, 120, 180 minutes after drug administration).

Stopping rules for study subject discontinuation include:

Pulse: pulse rate > 130 beats per minute or < 50 beats per minute

Blood Pressure: systolic blood pressure > 160 mmHg or < 80 mmHg, diastolic blood pressure > 100 mmHg or < 40 mmHg

Respiratory: shortness of breath, respiratory rate > 22 breaths per minute, or oxygen saturation less than 92%

Cardiac: chest pain

Neuro: confusion, loss of coordination, falling down, fainting
Psych: panic attack, psychotic reaction (hallucinations, psychotic depression)

6.2 Premature Study Discontinuation

8.2.1 Pregnancy or breast feeding.

8.2.2 Request by the subject to withdraw.

8.2.3 At the discretion of the investigators, UCSD IRB, NIDA, or other agencies providing oversight, i.e., Research Advisory Panel of California.

8.2.4. Rare but serious side effects of cannabis administration as listed in Section 6.1.

7.0 STATISTICAL ANALYSIS PLAN

7.1 Aim 1 Measures and Analyses

Data will be assessed for normality and transformations applied as needed (e.g., log). Neuropathic pain before and after acute cannabis administration will be assessed by NPRS and the von Frey test. Plasma AEA and 2-AG will be measured with LC/MS. Flow cytometry will quantify the percentage of PBMC that express CB2, FAAH, and MAGL, including monocytes, CD4+ T-cells, and CD8+ T-cells. To examine the effect of different CBD/THC products on NP and EC biomarkers, we will use a linear mixed model with subjects treated as a random effect to model the response measures. This methodology takes into account the repeated measures aspect of the within-subjects crossover study design, incorporating information from observations for each subject at different treatment doses and multiple time points within each dose. For initial modeling, terms will include dose (0.01%, 5.1% - 4 puffs, and 5.1% - 8 puffs) CBD treated as a categorical variable), time (0, 60, 120 and 180 minutes after exposure treated as a continuous variable), and dose x time interaction. Additional terms will also be included for the sequence in which the treatments were administered (e.g., low CBD vs. medium CBD) and for second-order time ($time^2$). The quadratic term is intended to model a U-shaped response curve if responses initially increase (or decrease), reach a maximum (or minimum), then decrease (or increase) back to baseline levels. For each outcome measure, each of these last 2 terms will be omitted from subsequent models and not reported if nonsignificant. Any potential factors that associate with outcome variables at the $\alpha = 0.20$ level in single-predictor analyses (history of light vs. heavy cannabis use, mode of prior cannabis use – i.e., engaging in smoking, or vaporizing, or both), time since last cannabis use before baseline, duration of cannabis use over lifetime, presence of adolescent cannabis use, duration of HIV infection, nadir CD4, viral load, gender, the presence of other non-cannabis analgesic medications, history of non-cannabis substance use disorders) will be included in the linear model. Dose effects at each time point will be assessed with mixed modeling after re-coding time as a categorical factor and including dose and dose x time terms (plus a term for sequence if this is significant in the initial model). Differences among doses will be evaluated using the Tukey honestly significant difference (HSD) comparison tests for differences of effects over all time points and contrasts within each

time point. All response observations, including information from subjects who do not complete all experimental sessions, will be included in the analyses. Similar mixed-model analyses will be performed on the primary pain outcome after adjustment for psychomimetic side effects (measured with the ARCI scale) to assess for effects of the study drug on pain independent of subjective responses. The proportion of subjects with a 30% pain reduction, a standard for effective pain reduction[60] will be compared between each of the doses with chi-square tests; we will also calculate the number needed to treat (NNT) for a 30% pain reduction rate. We will also conduct analyses to examine the association between pain and EC biomarkers, including AEA, 2-AG, FAAH, and MAGL expression. A 5% significance level will be used for all testing. The association of pain levels with HRV will use a linear mixed-effects model, adjusting for potential confounders as above. Within-subject correlation will be accounted for using subject-specific random intercepts and slopes.

7.2 Aim 2 Measures and Analyses

Based on our existing data for PLWH SMS compliance, we anticipate that participants will respond to 80-90% of the daily text messages during IMPACT. To address the issue of missing data, we will incorporate questions that allow participants to indicate their pain and cannabis use for the prior day if they did not respond to the earlier message. For days that have completely missing data, we will treat the intermittently missing data as missing completely at random. However, this assumption will be checked in sensitivity analyses by comparing the outcomes of participants based on the amount of missing data. Study dropout will be examined separately using statistical methods in current use [Diggle, Heagerty, Liang, and Zeger, Analysis of Longitudinal Data, 2nd ed., Oxford University Press, 2013]: The relationship between CBD, THC, and the CBD/THC ratio during a cannabis exposure event to changes in pain before and after the event will be examined using longitudinal data methods – e.g., mixed-effects linear models, controlling for potential confounders including baseline pain, in addition to those listed under Aim 1. The total CBD and THC consumed during the exposure will be calculated using the SUI method, estimating total grams of cannabis consumed multiplied by the percentages of CBD and THC content. Subject-specific random intercepts will be included in the model in order to account for the within-subject correlation of the outcome. Exploratory analyses will examine if there are different effects of reported cannabis strain (Sativa, Indica), individual types (e.g., OG Kush, Sour Lemon, etc.), or mode (smoking, vaporizing, ingesting food product) on pre-post changes in NP. We will also conduct exploratory analyses to examine if there is an interaction between reported levels of THC (low, < 1%, medium, 1-6%, high, > 10%) and CBD (low, < 1%, medium, 1-6%, high, > 10%) on pain change after drug use. The association of daily measures of pain levels with HRV will use a linear mixed-effects model, adjusting for potential confounders. The within-subject correlation will be accounted for using subject-specific random intercepts. Longitudinal changes in HRV between the first and last week will be assessed with a linear model that includes cannabis use (light vs. heavy) and CBD/THC intake, as predictors. Regression analyses will also examine the association between HRV and self-report pain measures quantified at the baseline and final visits.

Comparison of NIDA-obtained and dispensary-obtained cannabis effects on NP. We will compare the effect of laboratory cannabis administration and IMPACT self-report data on changes in pain (both measured with NPRS) before and after drug exposure. We will assess pain change scores for the laboratory study and IMPACT measured as the difference between

baseline (before drug) and 60 and 120 minutes after drug, time points that correspond with pain reduction in our prior THC work[60]. We will use the following analyses: 1) plot the distribution of the CBD/THC ratio (x- axis) against pain change (y-axis), calculate the slope of pain change scores for both the lab data and IMPACT, and determine if the slopes are similar or significantly different (a mixed effect model that will estimate changes in outcomes/slopes). 2) If the slopes are nonlinear, we will calculate the area under the curve (AUC) – a measure of total pain change – up to a CBD/THC ratio of 10 (our high CBD dose in the lab). 3) It is possible that our IMPACT data show that many participants only use one or two types of cannabis (e.g., with only a single CBD/THC ratio), thus it might be difficult to plot a CBD/THC ratio distribution. In that event, we will classify IMPACT cannabis use into 3 categories (low CBD/THC ratio, < 1; matched CBD/THC 1:1; high CBD/THC ratio, >1, and examine the correlation between the pain change in each category (averaged over the IMPACT study) with the pain change scores we observe for our low, medium, and high CBD laboratory doses. If our findings indicate a higher slope or greater AUC for NIDA cannabis vs. dispensary cannabis, this would suggest less efficacious pain relief from the local cannabis sources. In contrast, a strong correlation between CBD pain change scores for NIDA and dispensary product in one or more CBD/THC ratio categories would support a consistent finding for the effectiveness of different CBD amounts. In summary, either agreement or discrepancy between NIDA and dispensary data would be highly informative and would help guide future policy recommendations for cannabis-based pain treatment.

7.3 Aim 3 Measures and Analyses

We will use a linear model to examine the association between the total CBD, THC, and CBD/THC ratio during IMPACT and the change in NP, disease markers (CD4, viral load, Total Neuropathy Score, and neurocognitive global T-score), and EC biomarkers (AEA, 2-AG, FAAH, MAGL, CB2) from baseline to the final visit after month 6. We will control for confounders as in Aims 1 and 2, including any CBD and THC levels recorded at the baseline and final sessions. Linear models will compare the month 6 – baseline change in pain and in EC biomarkers between the heavy- and light-cannabis groups. One exploratory analysis will test for an interaction between group (light vs. heavy) and CBD and THC levels on change in NP and EC biomarkers. We will assess if exposure mode (smoking/vaporizing/ingestion) predicts pain outcome. We will examine if there is an association between pain change over time and changes in EC biomarkers. During the 6-month IMPACT, alterations may occur in cannabis use or pain medication (e.g., a person enrolled as a light cannabis user may start to engage in heavy use or begin using non-cannabis medication for pain, i.e., an opiate, which we will record with IMPACT). To address these issues we will conduct light vs. heavy use analyses based both on the baseline group classification and the actual use during IMPACT, include a cannabis use ‘change’ factor in the analyses (going from light to heavy use, or vice versa), and include the initiation of any non-cannabis pain medication as a factor in the model.

7.4 Power Analyses

A recent study indicated a medium effect size (Cohen’s d = 0.46) for CBD treatment that increased plasma AEA levels compared to an antipsychotic drug control condition[26]. Preclinical models of neuropathic pain in mice demonstrate that FAAH inhibitors decrease hyperalgesia

and allodynia with large effect sizes (Cohen's d = 4-5)[18]. Our pilot HRV data indicate a medium to large effect size (Cohen's d = 0.8) for reduced RMSSD in PLWH with neuropathic pain compared to PLWH without neuropathy. We conducted power calculations with the G*POWER software[92] comparing the area under the curve (AUC) of the change from baseline in pain levels between the three doses of cannabis. The actual analyses will have higher power, since they rely on a longitudinal mixed-effects model. With n=100 participants completing the cross-sectional baseline visits, and measured in a within-participant design, as described above, we have 80% power to detect an effect size Cohen's f=0.180. This Cohen's f corresponds to a range of group means for the three groups corresponding to a small-to-medium Cohen's d = 0.382-0.441. We have 0.61-0.76 power to detect such a pairwise difference as statistically significant in post-hoc analyses (e.g., with Bonferroni correction for post-hoc comparisons). If the completion rate at this stage is 95% (90%) of the initial enrollment of n=100, the detectable effect size is Cohen's f=0.185 (0.190). These correspond to a range of group means for the three groups corresponding to small-medium Cohen's d = 0.403-0.465.

For Aim 2, with 20% attrition over the 6 months of IMPACT we have n= 80 participants with 6 months (26 weeks) of data. We conservatively ignore the drop-out participants (who nonetheless contribute partial data). Assume, conservatively, that participants will use cannabis on average 2 times/week. This gives n=80 and k=52 observations/participant. If ICC is the intra-class, or within-participant correlation, the effective sample size (number of equivalent independent observations) is $n_{eff} = n/(ICC+(1-ICC)/k) \approx n/ICC$ [e.g., Killip, Mahfoud, Pearce. What is an intracluster correlation coefficient? Crucial concepts for primary care researchers, Ann Fam Med, 2004; 2:204-208.]. Assuming conservatively ρ=0.5 (or lower), we get $n_{eff} = 2n = 200$ [$n_{eff} = 160$]. We have 80% power to detect a (partial) correlation R between the individual predictors (Hypothesis 1: CBD, THC, and the CBD/THC ratio; Hypothesis 2: pain levels and HRV) and change in pain of R = 0.218 (or semi-partial $R^2 = 0.0475$), after adjusting for confounders. Calculations used G*Power.

For Aim 3, assuming n = 80 participants complete the 6-month follow-up, we have 80% power to detect an association between an individual predictor and response (e.g., total CBD and changes in neuropathic pain levels) of R = 0.302 (or semi-partial $R^2 = 0.092$), after adjusting for confounders. The calculations used G*Power.

8.0 PARTICIPANTS

8.1 Monitoring

The study team will review all adverse events during the study by cumulative reports on a monthly basis. A Data Monitoring Committee (DMC) will review the study for safety yearly. The study will only be stopped early if there are serious safety concerns.

The following items will be included in the yearly DMC meetings:

- Progress of the study, including participant recruitment, accrual and retention.
- Number of premature discontinuations and reasons for discontinuation

- Compliance with study treatment and adherence to IMPACT
- Safety and treatment toxicity, including summary of any AE and SAE
- Any protocol violations

8.2 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form will be obtained from the participant (or parent, legal guardian, or person with power of attorney for participants who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant, parent, or legal guardian, and this fact will be documented in the participant's record.

8.3 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the, IRB and/or DMC.

8.4 Study Discontinuation

The study may be discontinued at any time by the IRB and/or DMC as part of their duties to ensure that research participants are protected.

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