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Efficacy of Δ-9-tetrahydrocannabinol for HIV-related Neuropathic Pain

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EFFICACY OF Δ-9-TETRAHYDROCANNABINOL FOR HIV-RELATED NEUROPATHIC PAIN

by
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A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the Sally McDonnell Barksdale Honors College.

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ABSTRACT

KAIA HORNE: EFFICACY OF Δ-9-TETRAHYDROCANNABINOL FOR HIV-RELATED NEUROPATHIC PAIN

(Under the direction of Dr. Jason Paris)

Despite the availability and success of antiretroviral therapeutics, ~30% of patients living with HIV experience neuropathic pain that is often intractable. The mechanisms are not known, but there is evidence to support a role for the HIV virotoxins, Tat and/or gp120, which can damage or degenerate neurons and peripheral nerves. One mechanism by which Tat and gp120 promote nerve damage involves the stimulation of proinflammatory cytokine production from immune cells which can damage or kill bystander cells. Notably, compounds found in *Cannabis* exert anti-inflammatory effects and many studies report HIV patients to consume more marijuana than seronegative individuals. When people living with HIV were asked about their pain management, many suggested that marijuana improved their symptoms. As such, we hypothesized that a component of cannabis, Δ-9-tetrahydrocannabinol (THC), exerts anti-inflammatory effects that can offset the proinflammatory profile of HIV gp120. THC was screened in human microglia for its capacity to reduce gp120-mediated inflammation. While THC may exert acute anti-inflammatory effects, long-term marijuana use is associated with cognitive perturbation and reduced immune function. THC contributes to the psychotropic activity of cannabis. Thus, the benefits must be weighed against the adverse effects. Future work will assess cannabis constituents that may exert anti-inflammation without psychotropic activity.

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LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
AEA	Anandamide or N-Arachidonoyl Ethanolamine
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BSA	Bovine Serum Albumin
cART	Combination Antiretroviral Therapy
CB1	Cannabinoid Type 1
CB2	Cannabinoid Type 2
CBD	Cannabidiol
CR	Chemokine Receptor
CD4+	Cluster of Differentiation 4
CDC	Centers for Disease Control and Prevention
CNS	Central Nervous System
Covid-19	Coronavirus Disease of 2019
CXCR4	C-X-C Chemokine Receptor Type 4
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DTI	Diffusion Tensor Imaging
EMEM	Eagle's Minimum Essential Medium
FAAH	Fatty Acid Amide Hydrolase
FBS	Fetal Bovine Serum

FDA	U.S. Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
Gag	Group-specific Antigen
GIRK 1/2	G Protein-Gated Inwardly Rectifying Potassium Channels 1 and 2
Gp41	Viral Envelope Glycoprotein 41
Gp120	Viral Envelope Glycoprotein 120
HAND	HIV-Associated Neurocognitive Disorders
HIV	Human Immunodeficiency Virus
HMC3	Human Brain Microglia Cells
HSV	Herpes Simplex Virus
Iba-1	Ionized Calcium Binding Adaptor Molecule 1
IL-β	Interleukin - Beta
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
NMDA	N-Methyl-D-Aspartate
NIH	National Institutes of Health
PrEP	Pre-Exposure Prophylaxis
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
Tat	Trans-Activator of Transcription
THC	Δ-9-Tetrahyrocannabinol
TLR4	Toll-Like Receptor Type 4
TNF-α	Tumor Necrosis Factor Alpha

UNAIDS	Joint United Nations Programme on HIV/AIDS

1. Introduction

Human Immunodeficiency Virus (HIV) is a sexually-transmitted disease that affects one's immune system for the rest of their life. As of 2020, approximately 37.7 million people around the world are living with HIV (UNAIDS, 2021). In the U.S., it was estimated that there were about 1,189,700 people infected with HIV in 2019 (CDC, 2021). This number included an approximation of U.S. citizens who were undiagnosed by a physician. Sexual transmission appears to account for about 70% of HIV transmission while drug use via injection and vertical transmission account for the remainder of cases (Shaw & Hunter, 2012). The risk of infection occurs through sharing bodily fluids with another person, sharing needles, or from maternal-infant transmission during any part of pregnancy or delivery. Socioeconomic factors of a society also play an important role in transmission. The most affected populations include Black/African American and Hispanic/Latino groups and those who identify as homosexual or bisexual men (CDC, 2021). The overall goal to end the HIV epidemic is to decrease the number of new HIV diagnoses (CDC, 2021).

Mechanisms of HIV

HIV is a retrovirus disease that is made of a single stranded RNA genome. There are two types of HIV: HIV-1 and HIV-2. They are structurally similar, but this thesis will focus on HIV-1. HIV-1 can progress to acquired immunodeficiency syndrome (AIDS) if untreated. The HIV virion contains the *gag* gene which encodes for the structural proteins in its core, the *env* gene which encodes the viral envelope glycoproteins: gp120 and gp41, and the *pol* gene which encodes for the enzymes, reverse transcriptase, integrase, and protease, which are crucial for

viral replication (Fanales-Belasio, et al. 2010). Once HIV enters the body, T helper cells recognize the foreign virion and relocate to the site of initial infection and signal other cells to help rid the body of infection. The gp120 proteins on the HIV envelope attach to the $CD4^+$ molecules on the T-helper cells, macrophages, and monocytes to fuse the HIV particle to the host cell with the help of gp41 (Al-Jabri, et al. 2003). Once fused together, the HIV particle will shed its protein coat, activating the enzyme reverse transcriptase to transcribe the RNA to complementary DNA. Ribonuclease H then works to create a double strand DNA molecule by making a complementary DNA strand to the previous strand. The enzyme integrase will then incorporate the newly formed viral DNA into the host cell DNA. The viral DNA then undergoes transcription to form mRNA and regulatory proteins, Tat and Rev. Tat encourages transcription and formation of longer RNA strands while Rev facilitates the process and promotes the formation of mature viral particles. The viral mRNA that is created enters the cytoplasm to form new virions with the help of *pol*, *gag*, and *env* genes to create the structures of a new HIV particle (Fanales-Belasio, et al. 2010). The protease is important in cleaving the gag and pol proteins for viral assembly along with initiating apoptosis of the cell (Fanales-Belasio, et al. 2010; Mbita, et al. 2014). As HIV spreads throughout the body, it causes the loss of $CD4^+$ T lymphocytes through apoptosis (Stefanou, et al. 2019).

NeuroHIV and Pain

Approximately 15-55% of people living with HIV meet the criteria for neuroHIV (a constellation of neurological disorders that include cognitive impairment, mood disorders, and motor deficits) (Saylor, et al. 2016). Many individuals who are diagnosed with HIV experience intractable pain throughout their lives. Intractable pain is a chronic form of pain that is not

curable, so the focus of treatment is to manage pain (Roland & Sampson, et al. 2017). The HIV-1 proteins, Tat and gp120, promote neuropathic pain in via several mechanisms including the activation of immune cells and the stimulation of pro-inflammatory cytokines and chemokines. Tat is a neurotoxin that increases calcium levels, neuronal death, and dendritic degeneration (Hermes, et al. 2018). gp120 is an envelope protein of HIV can promote apoptosis of cells and prevents neurogenesis (Avraham, et al. 2014). As gp120 leaves the cell, it will interact with glutamate to activate the N-methyl D-aspartate (NMDA) receptors, which leads to neurotoxicity (Dawson, et al. 1993). gp120 activates C-X-C Chemokine Receptors Type 4 (CXCR4) on microglia, neurons, and astrocytes to release inflammatory cytokine IL-β which leads to the activation of a ubiquitin ligase and NMDA receptors through increased glutamate sensitivity (Kim, et al. 2011). This process can lead to inflammation and synapse loss. These proteins promote neuroinflammation by acting alone or in concert with each other and other HIV-related virotoxins. The net effect of these actions can result in chronic pain due in part to nerve and neuron damage. In looking for a solution, many patients reported the use of cannabis to alleviate pain. In a study conducted to determine the effectiveness of cannabis, it was seen that many participants experience pain relief in addition to an improvement in mood and daily functioning (Ellis, et al. 2009).

Potential Mechanisms of HIV-mediated Pain

Inflammation occurs when the immune system responds to tissue injury with the use of cell recruitment and mediator release (Ellis, et al. 2013). Neuroinflammation is largely mediated by activation of astrocytes and microglia (the macrophage of the brain). Pro-inflammatory cytokines contribute to pain experienced by HIV^+ individuals. Glia cells might also be a cause

for HIV-mediated pain through the release of glutamate (Cairns, et al. 2015). Peripheral sensitization can occur after nerve lesion in the absence or presence of tissue inflammation (von Hehn, et al. 2012). Central sensitization occurs when the brain experiences a strong pain signal in the absence of inflammation in the periphery (Merlin, et al. 2014). A lower $CD4^+$ T cell count is associated with pain in individuals (Aouizerat, et al. 2010).

Current HIV Therapeutic Treatments

Pre-exposure prophylaxis (PrEP) can prevent HIV infection and combination antiretroviral therapy (cART) is used to decrease the viral replication after HIV infection has occurred. cART attenuates some associated comorbidities. This is accomplished by combining multiple antiretroviral agents that will interact well together to attack multiple symptoms (Maenza & Flexner, et al. 1998). When patients were treated with antiretroviral agents for greater than 12 months, they were less likely to show multinucleated giant cells or diffuse myelin pallor (Glass, et al. 1993). PrEP is a preventative drug that is prescribed to individuals who have been exposed to HIV or have a greater chance of contracting it, but have not yet been diagnosed with the disease (NIH, 2021). While these therapies have proven effective, they do not cure the disease. They also lack the ability to target some HIV neurotoxic proteins. cART has allowed people diagnosed with HIV to live longer lives, but it does not alleviate pain associated with HIV rather it has turned it into a chronic condition (Yuan & Kaul, et al. 2019).

Palliative care is an approach to improve the overall quality of life of a patient by treating physical, psychosocial, and spiritual problems (Rasche, et al. 2019). HIV is associated with many other infections and illnesses as it will lower an infected person's immune system as

their white blood cells are affected. Cannabis and cannabinoids are being considered as a therapeutic to resolve chronic pain and other intense symptoms (Rasche, et al. 2019).

HIV is Associated with Cannabis Use/Abuse

Approximately 77% of HIV infected adults report lifetime marijuana use (Montgomery, et al. 2019). There are many contradictions as to whether cannabis use is helpful or harmful to individuals with the disease. Cannabis overuse or abuse can often occur with the ingestion of edibles as many individuals underestimate the effects of cannabis in their body (Kelly, et al. 2021). New synthetic drugs have a stronger binding affinity to CB1 than Δ-9-tetrahydrocannabinol (THC) and can lead to symptoms such as hallucination and paranoia (Heinbockel, et al. 2018). Some articles suggest that use of cannabis can increase the chances of HIV progressing to AIDS diagnosis (Tindall, et al. 1988).

Potential Therapeutic Effects of Cannabinoids

There are many mechanisms of Tat and gp120 that can be associated with causing pain in HIV+ individuals. One way in which Tat causes pain is by affecting the length, volume, and process filaments of dendritic cells. A study found that pretreating cells with Fatty acid amide hydrolase (FAAH) enzyme inhibitor PF3845 can counteract the effects of Tat by maintaining normal Ca^{2+} levels and dendritic volume and preventing neuronal cell death (Hermes, et al. 2018). Endocannabinoids, N-arachidonoyl ethanolamine (anandamide/AEA) and 2-arachidonoylglycerol (2-AG), use the CB1 receptor to protect cells against Tat (Xu, et al. 2017). One way to inhibit the activity of gp120 may be to target the chemokine receptor binding sites on the host cell (Caffrey, et al. 2011). Mannose binding lectin and silver nanoparticles are capable of attaching to the binding sites to block the entry of gp120 from multiple strains of HIV

(Caffrey, et al. 2011; Ezekowitz, et al. 2003). 12-residue peptide 1 is also shown to block gp120 at $CD4^+$ and co-receptor binding sites (Umashankara, et al. 2010).

It is hypothesized that THC may ameliorate gp120-induce inflammation. If true, this may be one mechanism by which cannabis can alleviate HIV-related pain. While this thesis was interrupted by the SARS-CoV-2 pandemic, some pilot data were collected to assess the influence of THC and/or gp120 on a human microglial cell line grown in culture. Given that there is higher affinity to CB1 and CB2 at nanomolar concentrations (Stella, et al. 2010), a medial concentration of 100 nM was used as well as a higher concentration of 1000 nM. It was expected that gp120 would promote microglial morphology consistent with proinflammation and THC would attenuate this.

2. Materials and Methods

2.1 Chemicals

THC was obtained from the Marijuana Research Laboratory at the University of Mississippi (University, MS, USA) and was diluted to stock concentration in DMSO (10 µM). Recombinant $gp120_{ADA}$ was purchased from ImmunoDx (Woburn, MA, USA; #1081) and was diluted to a concentration in ddH_2O.

2.2 Cell Culture

Human brain microglia cells (HMC3) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA; #CRL-3304). HMC3 cells were seeded onto 24-well plates at a density of 5×10^3/well. Cells were maintained at a temperature of 37℃ (5% CO_2) in growth medium: 89.5% EMEM (Life Technologies, Carlsbad, CA), 10% heat-inactivated fetal bovine serum (FBS; Thermo Scientific Hyclone, Logan, UT), and 0.5% antibiotic/antimycotic mixture (Life Technologies). Cells were not used beyond passage 8.

2.3 Procedure

Twenty-four hours after seeding, cells were treated with THC (diluted in media to a final concentration of 100 nM or 1,000 nM) and/or gp120 (diluted in media to a final concentration of 500 pM) and incubated (37℃, 5% CO_2) for 24 h. Cells were fixed with paraformaldehyde (4%) and processed for immunocytochemistry as described below.

2.4 Immunocytochemistry

Fixed cells were permeabilized (Triton X - 100 0.1%) and blocked (BSA 0.1%) for 30 mins and incubated overnight (4℃) with primary antibodies to ionized calcium-binding adaptor

protein (Iba-1, rabbit, 1:250). Following this, cells were incubated with an anti-rabbit secondary antibody (AlexaFluor 594, 1:500). Morphology was used to measure microglial activation (Davis, et al. 1994; Yoichi, et al. 1999). Microglia was scored on a scale of 1-3, based on the established scoring system for microglia morphology and phenotypes (Davis, et al. 1994; Yoichi, et al. 1999; Ladeby, et al. 2005): 1 = resting state with long processes extending from a circular cell body, 2 = active/reactive state with shorter and thicker processes and increased immunoreactivity in the cell body, 3 = reactive microglia that are phagocytic and show a cell body that is absent of extended processes.

2.5 Statistical Analyses

Data were analyzed via repeated measure ANOVA with gp120 exposure as the between-subjects factor and THC concentration as the within-subjects concentration. If present, main effects were to be delineated by Fisher's PLSD *post hoc* test and interactions were to be delineated by simple main effects and main effect contrasts with alpha corrected for familywise error. Alpha was set at 0.05 for all analyses.

3. Results

3.1 - Finding 1: There was no significant effect of THC on gp120 infected cells.

HMC3 cells were classified based on their morphology. Each morphological state was independently graphed to determine the percent of each phenotype in the environment after treatment. No significant effects of gp120 nor THC were observed on the proportion of cells that were resting (Fig. 1), activated (Fig. 2), ameboid (Fig. 3), or on the activation scale of HMC3 cells (Fig. 4).

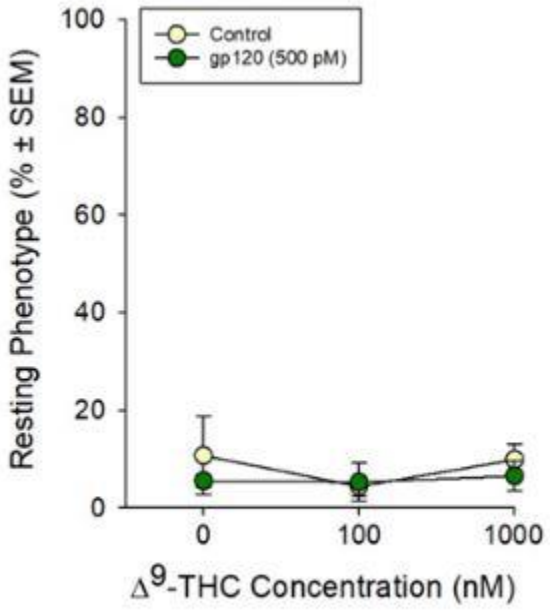

Figure 1: Percent resting phenotype after exposure to gp120 and/or THC

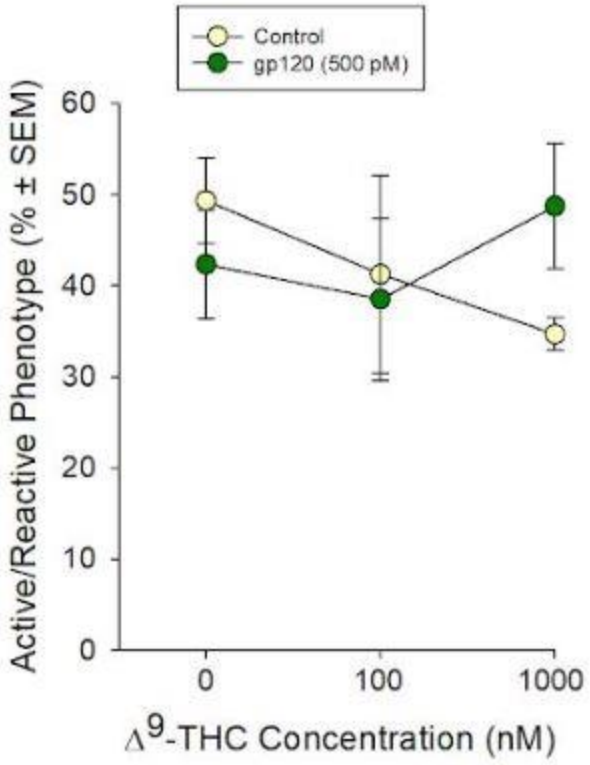

Figure 2: Percent active/reactive phenotype after exposure to gp120 and/or THC

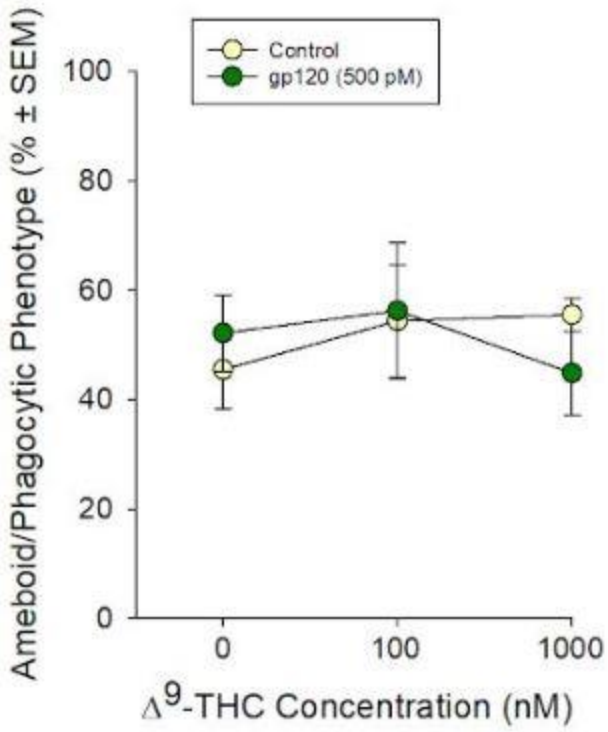

Figure 3: Percent phagocytic phenotype after exposure to gp120 and/or THC

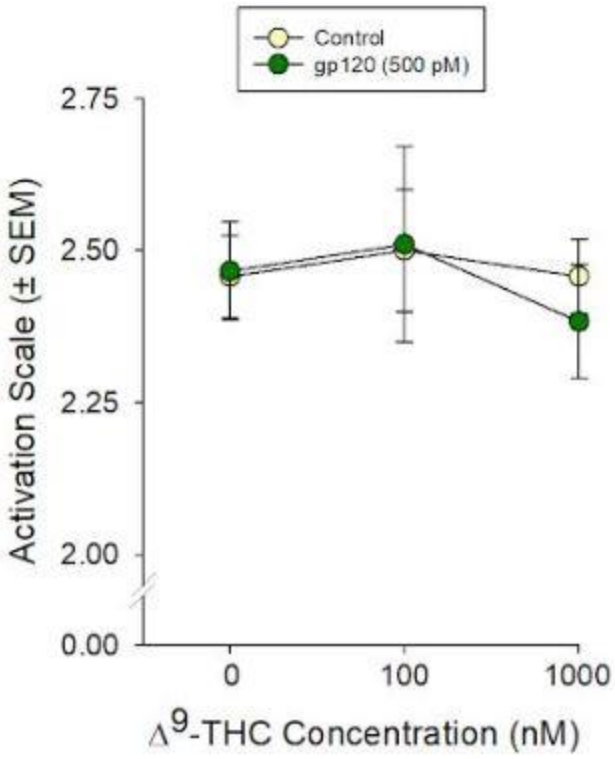

Figure 4: Activation scale of HMC3 cells after exposure to gp120 and/or THC

4. Discussion

It was hypothesized that administering THC on to cells that had been exposed to gp120 would ameliorate its effects. gp120 is critical for HIV infection of a cell. It was believed that targeting gp120 can attenuate HIV replication as is the therapeutic strategy behind some novel antiretrovirals such as Maraviroc. In the experiment performed, THC did not decrease the negative effects of gp120. This outcome could be due to ineffective binding of THC to cannabinoid receptor, CB2, and was driven by high variance. Expanding the dose-response concentration curve would assess whether concentrations with greater CB2 binding affinity would yield more consistent results. As well, replication of the current experiment, beyond what could be conducted given the SARS-CoV-2 pandemic, is expected to reduce variance. Further, a lack of protection by THC could have also been due to actions at receptors associated wuth psychoactive effects, rather than anti-inflammatory effects.

Cannabis and Cannabinoids

Cannabinoids can be placed into three different groups: phytocannabinoids (or natural cannabinoids), synthetic cannabinoids, and endocannabinoids. Synthetic cannabinoids, or artificial cannabinoids, are made in a lab for therapeutic purposes. Natural cannabinoids can be found in plants and wildlife as they grow naturally in our ecosystem. Cannabis is made up of the phytocannabinoids, Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Both components have shown to be capable of positive and negative effects. THC is a psychoactive ingredient that can cause euphoria, relaxation, anxiety/paranoia, impairments in attention and memory, and many more effects (D'Souza, et al. 2008). CBD is a non-psychoactive ingredient that produces an anti-inflammatory response through the reduction of cytokines (Petrosino, et al. 2018).

Endocannabinoids are produced by an individual's neuronal cells to defend against foreign agents. The endocannabinoids, N-arachidonoyl ethanolamine (anandamide/AEA) and 2-arachidonoylglycerol (2-AG), are ligands for G-protein coupled receptors, CB1 and CB2 (de FONSECA, et al. 2005). CB1 receptors are mainly expressed in the central and peripheral neurons while CB2 receptors are mainly expressed in the immune cells (Pertwee, et al. 2008). CB1 receptors were associated with increased calcium levels in astrocytes (Navarette, et al. 2008). This suggests that CB1 receptors may play a role in cannabinoid addiction and pain. CB1 controls neurotransmitter release in order to maintain homeostasis and prevent excessive neuronal activity in the central nervous system (CNS) (Walter, et al. 2004).

Cannabinoids that are Used Clinically

Dronabinol, Cannabidiol, and Nabilone are approved drugs by the U.S. Food and Drug Administration (FDA) that treat nausea and appetite loss associated with HIV. The FDA has approved the use of drug brands, marinol (active ingredient dronabinol) and cesamet (active ingredient nabilone) (FDA, 2021). Nabilone is prescribed as 1 mg capsules (Sheikh, et al. 2021). Dronabinol is administered in doses ranging from 2.5 mg-10 mg to be most effective (Sheikh, et al. 2021). Low doses of dronabinol significantly increase food and caloric intake and higher doses improve weight gain (Haney, et al. 2007). There seemed to be no effect in cognitive function associated with this drug (Haney, et al. 2007). Dronabinol is also associated with an improvement in mood and nausea in most patients (Beal, et al. 1995).

Cannabidiol (CBD) is a common substance that is available over the counter in many forms including oils and is an inverse agonist drug at the CB2 receptor that has been found to cause an anti-inflammatory response. It is an inverse agonist drug at the CB2 receptor that can

inhibit immune cell migration (Pertwee, et al. 2008). There has been difficulty administering CBD orally so alternative forms are being considered. A new technique using self-emulsifying drug delivery system improved the bioavailability of CBD to avoid build-up in the gastrointestinal tract (Knaub, et al. 2019).

Potential Mechanisms of Cannabis to Alleviate HIV-neuropathic Pain

The cannabinoid agonist, WIN55,212-2, caused concentration dependent inhibition of nitrite production in addition to protecting cells from Tat-induced cytotoxicity (Esposito, et al. 2022). It also protects the brain tissue against ischemic injury, but is not effective at higher concentrations (Nagayama, et al. 1999). Activation of the CB1 receptor decreases the amount of glutamate released which inhibits excitatory synaptic responses in the striatum (Gerdeman, et al. 2001). CB1 is also shown to provide protection against acute excitotoxic seizures associated with glutamatergic cortical neurons (Monory, et al. 2006). THC can bind to CB1 or CB2, but has a higher affinity of CB1 mechanisms to mediate its effects (Pertwee, et al. 2008).

Cannabis Use on Cognitive Function

Chronic or long-term use of cannabis can have negative effects on cognitive function. Moderate to heavy use of marijuana is also associated with neurocognitive decline (Thames, et al. 2016). There is an association of early marijuana use with memory impairment (Skalski, et al. 2018). Another study showed delayed memory associated with marijuana use rather than complete memory loss (Cristiani, et al. 2004). THC can impair basic motor functions and executive cognitive functions (Crean, et al. 2011). It was also found that HIV^+ light marijuana users performed better at verbal fluency tests than HIV- light marijuana users (Thames, et al.

2016). By targeting the endocannabinoid system, there is a possibility to suppress the neuronal damage that occurs in HIV-associated neurocognitive disorders (HAND) (Wu, et al. 2019).

Cannabis may be helpful for other comorbidities - HIV wasting

Some of the most common comorbidities are heart failure, chronic pulmonary disease, and renal disease (Boersma, et al 2014). HIV is also associated with a loss in appetite and decline in mental health and associated psychological symptoms. In a study, participants with multiple HIV-related symptoms were more likely to develop a psychiatric disorder or drug dependence disorder (Bing, et al. 2001). There was a significant association of HIV encephalitis that was seen by the presence of giant cells in white matter (Bell, et al. 1998). Another study showed the effects of administering dronabinol to HIV^+ individuals. It found that there was a great improvement in appetite, mood, and nausea in patients with AIDS-related anorexia (Beal, et al. 1995).

HIV viremia

The placenta is responsible for delivering nutrients and providing protection against HIV through cytokines and chemokines. It is believed that drugs of abuse such as cannabis may affect the expression of cytokines and chemokines to stimulate vertical transmission (Wang, et al 2011). The synthetic cannabinoid CP55,940 inhibits activity through CB1 or CB2 in a concentration dependent manner (Rock, et al. 2007). Synthetic cannabinoid, WIN55,212-2, binds with low affinity to CB1 and CB2 receptors and selectively blocks G protein-gated inwardly rectifying potassium channels 1 and 2 (GIRK1/2) neuronal excitability in the brain (An, D, et al.

2021). Higher viremia at cART initiation is associated with slower viral suppression (Krastinova, et al. 2015).

Biological Mechanisms vs. Behavioral Mechanisms for adverse HIV outcomes and poor cART response with cannabis use

There are some cases in which cART is unable to restore CD4+ T cell levels to normal (Palmer, et al. 2016). It is suggested that starting cART during primary HIV infection can have a negative impact on long-term immune response if interruptions occur (Krastinova, et al. 2015). It would be better to start cART therapy in the chronic phase of HIV to avoid a greater loss of CD4+ T cells (Krastinova, et al. 2015).

Structural Changes by HIV and or Cannabinoids

Reverse transcription-polymerase chain reaction (RT-PCR) was done on the brain tissue of HIV patients and it found a correlation of expression for mRNA for tumor necrosis factor-alpha (TNF-α) with cognitive impairment and patterns of neurological changes (Glass, et al. 1993). Changes in grey matter have been detected through the use of magnetic resonance imaging (MRI; with contrast). In a functional magnetic resonance imaging (fMRI) scan, individuals who are using cannabis showed lower neural activity in the dorsal prefrontal cortex, dorsal and ventral striatum, amygdala, and parahippocampal gyrus (Kober, et al. 2014). Heavy marijuana use alone was associated with smaller volumes in the entorhinal cortex and fusiform gyrus (Thames, et al. 2016). Participants diagnosed with HIV had a reduced thickness of the cingulate gyrus (Thames, et al. 2016). Abnormalities in white matter could be seen with Diffusion Tensor Imaging (DTI). For Barratt Impulsivity Scale Total and Attention, in the left

and right frontal regions, higher fractional anisotropy was associated with higher levels of impulsivity for chronic marijuana users (Gruber, et al. 2011).

Sex Differences

In men, there was evidence that those who used marijuana at the time of the study or previously in their life were at higher risk of mortality (Sidney, et al. 1997). Toll-like receptor 4 (TLR4) agonist lipopolysaccharide was found to cause allodynia only in male mice (Sorge, et al. 2011). Compared to men, women have higher levels of innate immune activation markers and neopterin and coagulation makers (Cohn, et al. 2020). The activation of innate immune markers, soluble CD163 and soluble CD14, suggested that monocyte or macrophage activation in the CNS is associated with neuronal injury (McGuire, et al. 2015).

Effects of Cannabinoids on HIV progression

There are varying results of whether cannabinoids have an effect on the progression of HIV to AIDS. In a study done by the Sydney AIDS project it was concluded that individuals who progressed to AIDS and had smoked marijuana three months before the study often exhibited lower percentage of CD4+ cells and a higher percentage of CD8+ cells than those who had not used marijuana (Tindall, et al. 1988). This suggests that marijuana use is not associated with reducing pain levels. In another study, participants reported cannabis use 2-3 times per week and there was no significant association with HIV progression found in the group (Coates, et al. 1990). There was no significant association between age or the amount of drinking and smoking done by an individual previous to the study (Coates, et al. 1990).

Potential Limitations of Cannabinoids as HIV therapeutic

There can be negative effects associated with cannabinoids or cannabis use. The use of THC may weaken the immune system and lead to less resistance to bacterial and viral infections (Friedman, et al. 2003). THC is associated with an increased risk of herpes simplex virus-2 and often resulted in more severe herpes genitalis, higher mortality rates, and high mean titers of virus shed from the vagina (Cabral, et al. 2006). CBD increases chances of cellular death when exposed during neuronal differentiation (Schönhofen, et al. 2015).

Limitations of the Experiment

For this experiment, we considered the effects of targeting gp120 with THC to ameliorate the effects of HIV-related neuropathic pain. Our results showed no significant effect of THC compared to controls. If we were to replicate this study, we would perform additional experiments and a wider range of concentrations. The timing of incubation with THC and gp120 may also be important. Given that gp120 can be cytotoxic, longer durations of exposure may have killed cells that would otherwise contribute to the dynamic range of the assay. A live/dead assay will be included in future experiments to rule this out. Non-psychoactive, potentially anti-inflammatory constituents of cannabis, such as CBD, should also be examined.

In conclusion, pain in HIV^+ individuals may occur in part due to proinflammtory actions of gp120. In the literature, cannabis and cannabinoids are suggested as a possible analgesic. Current medications include dronabinol, CBD, and nabilone. THC was tested to determine its effect on the mechanisms of gp120, but the concentrations assessed yielded variable results and

need to be replicated. Further testing should be completed to determine if additional components of cannabis can serve as potential therapeutics for HIV-associated neuropathic pain.

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