Alterations in Brain Cannabinoid Receptor Levels Are Associated with HIV-Associated Neurocognitive Disorders in the ART Era: Implications for Therapeutic Strategies Targeting the Endocannabinoid System

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Abstract: HIV-associated neurocognitive disorders (HAND) persist despite the advent of antiretroviral therapy (ART), suggesting underlying systemic and central nervous system (CNS) inflammatory mechanisms. The endogenous cannabinoid receptors 1 and 2 (CB1 and CB2) modulate inflammatory gene expression and play an important role in maintaining neuronal homeostasis. Cannabis use is disproportionately high among people with HIV (PWH) and may provide a neuroprotective effect for those on ART due to its anti-inflammatory properties. However, expression profiles of CB1 and CB2 in the brains of PWH with HAND have not been reported. In this study, biochemical and immunohistochemical analyses were performed to determine CB1 and CB2 expression in brain specimens of HAND donors. Immunoblot revealed CB1 and CB2 were differentially expressed in frontal cortices from HAND brains compared to neurocognitively unimpaired (NUI) brains from PWH. CB1 expression levels negatively correlated with memory and information processing speed. CB1 was primarily localized to neuronal soma in HAND brains versus a more punctate distribution on neuronal processes of NUI brains. CB1 expression was increased in cells with glial morphology and showed increased colocalization with an astroglial marker. These results suggest that targeting the endocannabinoid system may be a potential therapeutic strategy for HAND.

Keywords: cannabinoid receptor; inflammation; astrocytes; immunohistochemistry

1. Introduction

Human immunodeficiency virus (HIV) in the antiretroviral therapy (ART) era has transformed from a terminal illness to a chronic disease with life expectancy approaching that of seronegative patients [1]. However, even despite effective virologic suppression with ART, HIV-associated neurocognitive disorders (HAND) persist, affecting up to 50% of people with HIV (PWH) [2]. HIV is known to seed the brain within days of infection, and while ART has proven effective at suppressing viral loads and reducing progression to acquired immunodeficiency syndrome (AIDS), it does not eradicate central nervous system (CNS) viral reservoirs [3, 4]. Persistent low-level HIV replication, chronic inflammation, ART neurotoxicity, and aging comorbidities are thought to contribute to the neuropathogenesis of HAND [5-8]. Important avenues for future investigation will involve optimizing HIV therapy within the CNS.

The mechanisms driving HAND are likely multifactorial but common prospective etiologies include disruptions in neuroinflammatory signaling and mitochondrial function [9], both pathways that are modulated by the endocannabinoid system (ECS) [10-
Indeed, impaired mitochondrial fission and fusion, dysregulated autophagy, premature apoptosis, and altered calcium homeostasis have all been implicated in the pathogenesis of HAND [9]. Postmortem brain studies of HAND decedents reveal persistent astrogliosis, microgliosis, inflammatory cytokines expression, and altered mitochondrial architecture [13-15]. Importantly, recent evidence has shown that the endocannabinoid system regulates neuronal and glial function in rodent brains [16]. Animal studies implicate the endocannabinoid system in brain functions that are commonly altered in HAND patients, including learning and memory, executive function, and reinforcement behavior [16].

The ECS represents a promising therapeutic target for increasing resilience to HAND. Rates of cannabis (i.e., marijuana) use are disproportionately high in this population with approximately 77% of HIV-infected adults reporting lifetime marijuana use compared to 44.5% in uninfected adults [17-19]. Many patients report that cannabis offsets symptoms of HIV and ART side effects, including neuropathic pain, nausea, myopathy, lipodystrophy, and mood problems [20, 21]. Cannabis may have promising utility in treating various neurodegenerative disorders with underlying inflammatory processes, and the expression of cannabinoid receptors is associated with Alzheimer’s disease [22], multiple sclerosis [23], Huntington’s disease [24], and Down’s syndrome [25]. The expression of cannabinoid receptors (CB₁ and CB₂) has been reported in HIV encephalitis [26], but the receptor expression in patients with a psychiatric diagnosis of HAND on ART remains unknown. A greater understanding of how cannabinoid receptor expression is associated with HAND is critical in evaluating the therapeutic potential of cannabinoid drugs, as well as their potential side effects.

In this study, CB₁ and CB₂ receptor expression in postmortem brain specimens were investigated from a well-characterized cohort of HAND decedents on ART. CB₁ and CB₂ levels in the frontal cortex were assessed by immunoblot, and then CB₁ cellular localization was investigated using immunohistochemical techniques. Lastly, CB₁ and CB₂ levels were correlated with clinical covariates.

2. Materials and Methods

2.1 Study population

Brain specimens from a total of 24 HIV+ donors were acquired from the National NeuroAIDS Tissue Consortium (NNTC) (Institutional Review Board [IRB] #080323) (Table 1). All studies were conducted in accordance with the code of ethics of the National Institutes of Health and the University of California, San Diego. Neuromedical and neuropsychological examinations were performed on each case within a median of 12 months prior to death. Exclusion criteria for subjects included a history of CNS opportunistic infections or diagnoses unrelated to HIV infection that might impact CNS functioning, such as neurologic, psychiatric, or metabolic disorders. The most common pathologies described were systemic cytomegalovirus (CMV), Kaposi sarcoma (HHV-8), and hepatic disease. Diagnosis of HAND was made according to a standardized evaluation as described below [27].
Table 1. Clinical characteristics of study population. Abbreviations: NUI, Neurocognitively unimpaired; ANI, Asymptomatic neurocognitive impairment; MND, Minor neurocognitive dysfunction; HAD, HIV-associated dementia.

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<th>CD4 count</th>
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2.2 Neuromedical and neuropsychological evaluation

All participants underwent a comprehensive neuromedical assessment that included a detailed medical history and structured set of examinations for detecting lifetime and current diagnoses [27, 28]. For their baseline assessment, all subjects had venipuncture, cerebrospinal fluid (CSF), and urine samples collected. Clinical data (plasma viral load [VL], postmortem interval, CD4 count, and neuropsychological measures were obtained for the HIV+ donor cohorts.

Neuropsychological evaluation for HAND diagnosis was performed across seven neurocognitive domains including executive function, motor skill, processing speed, episodic memory, attention/working memory, language, and visual perception, as described by[27]. Raw test scores were transformed into normally-distributed T-scores adjusted for demographic variables, including age, education, gender, and race based on normative samples of HIV- participants and then averaged across all tests to obtain a global cognitive T-score and within domains to obtain cognitive domain-specific T-scores[29]. Functional impairments in everyday life were assessed using the Lawton and Brody Activities of Daily Living questionnaire[30] and Patient’s Assessment of Own Functional Inventory (PAOFI)[31]. HAND classifications, i.e., asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD), were assigned based on participant responses to the everyday functioning questionnaires and performances on the neuropsychological test battery according to established criteria[2].
2.3 ImmunoBlot

Tissues from the white matter (GM) and gray matter WM of the frontal cortices dissected at autopsy from HAND and neurocognitively unimpaired (NUI) brains were homogenized and fractionated, as described by[32], using a buffer that promotes separation of membrane and cytosolic fractions (1.0 mmol/l HEPES (Gibco, cat. no. 15630–080), 5.0 mmol/l benzamidine, 2.0 mmol/l 2-mercaptoethanol (Gibco, cat. no. 21985), 3.0 mmol/l EDTA (Omni pur, cat. no. 4005), 0.5 mmol/l magnesium sulfate, 0.05% sodium azide; final pH 8.8). Human brain tissue samples (0.1 g) were homogenized in 0.7 mL of fractionation buffer (1.0 mM HEPES, 5.0 mM Benzaidine, 2.0 mM b-mercaptoethanol, 3.0 mM EDTA, 0.5 mM magnesium sulfate, 0.05% sodium azide, pH 8.8) containing phosphatase (Millipore cat# 524624) and protease inhibitor (Millipore cat# 539131) cocktails. The tissue homogenate was centrifuged at 5000 x g for 5 min at room temperature, and the resulting supernatant was collected, placed in appropriate ultracentrifuge tubes, and centrifuged at 436,000 x g for 1 h at 44 °C in a TL-100 rotor (Beckman Coulter, Brea, CA). The supernatant was retained and represented the cytosolic fraction, and the pellets were resuspended in 0.2 mL of buffer and re-homogenized to obtain the membrane fraction.

After determination of the protein content of all samples by bicinchoninic acid assay (Thermo Fisher Scientific, cat. no. 23225), membrane fractions were resolved by SDS-PAGE and transferred onto PVDF membranes using the iBlot transfer system (Invitrogen, cat. no. IB24001) and NuPage transfer buffer (ThermoFisher Scientific, cat. no NP0006). The membranes were incubated for 1 h in 5% bovine serum albumin blocking solution and phosphate-buffered saline-tween 20 (PBST). Membranes were then incubated overnight at 4°C with primary antibodies against CB1 (Abcam, cat. no. ab23703) and CB2 (Abcam, cat. No. ab3561). Importantly, both of these antibodies were validated by the manufacturer for specificity in CB1 and CB2 knockout mice. Following visualization, blots were stripped and probed with a mouse monoclonal antibody against β-actin (ACTB; Sigma Aldrich, cat. no. A5441) diluted 1:2000 in blocking buffer as a loading control. All blots were washed in PBST, and then incubated with species-specific IgG conjugated to HRP (American Qualex, cat. no. A102P5) diluted 1:5000 in PBST and visualized with SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher Scientific, cat. no. 34096). Images were obtained, and semi-quantitative analysis was performed with the VersaDoc gel imaging system and Quantity One software (Bio-Rad).

2.4 Correlational Analyses between CB1 and CB2 protein levels and cognitive T-scores

We examined the relationship between global and domain-specific cognitive T-scores and CB1 and CB2 expression separately using Pearson’s R correlations.

2.5 Immunohistochemistry and double Immunofluorescence

Free-floating 40 μm thick vibratome sections of human brains were washed with PBS three times, pre-treated for 20 min in 3% H2O2, and blocked with 2.5% horse serum (Vector Laboratories, cat. no. S-2012) for 1 h at room temperature. Sections were incubated at 4°C overnight with the primary antibody, CB1 (Abcam, cat. no. ab23703) diluted in PBS. Sections were then incubated in secondary antibody, Impress HRP Anti-rabbit IgG (Vector, cat. no. MP-7401) for 30 min, followed by NovaRED Peroxidase (HRP) Substrate made with NovaRED Peroxidase (HRP) Substrate Kit as per manufacturer’s instructions (Vector, cat. no. SK-4800). Control experiments consisted of incubation with secondary antibody only. Tissues were mounted on Superfrost plus slides (Fisherbrand, cat. no. 12-550-15) and coverslipped with cytoseal (Richard Allen Scientific, cat. no. 8310-16). Immunostained sections were imaged with a digital Olympus microscope. For each
case (n=4 NUI and n=4 HAND) a total of 3 sections (10 images per section) were analyzed in order to quantify the average number of immunolabelled cells per field of view. CB$_1$+ cells of glial morphology and pyramidal neuronal bodies were counted manually. The specimens were blind coded and then broken after quantification. Background levels were obtained in tissue sections immunostained in the absence of primary antibody. Unfortunately, due to technical difficulties with eliminating background signal, tissue sections were not amenable to immunohistochemistry with the CB$_2$ antibody.

Double immunolabeling studies were performed to determine the percent colocalization of CB$_1$ receptors with GFAP+ (astroglia) and MAP2+ (neurons) cells in frontal cortices, as described[13, 33]. For this purpose, vibratome sections of human brains were immunostained with antibodies against CB$_1$ with GFAP (Sigma Aldrich, cat. no. G3893) for astrocytes and MAP2 (Santa Cruz Biotechnologies, cat# sc-32791) for neurons. Sections were then reacted with fluorescent secondary antibodies, goat anti mouse IgG 488 (Invitrogen, cat. no. A11011) and goat anti rabbit IgG 568 (Invitrogen, cat. no. A11036). Sections were mounted on Superfrost Plus slides and cover-slipped with vectashield (Vector, cat. no. 1000). Sections were imaged with a Zeiss 63× (N.A. 1.4) objective on an Axiovert 35 microscope (Zeiss) with an attached MRC1024 laser scanning confocal microscope system (BioRad, Hercules, CA). The percent colocalization was quantified using Image J and the SQUAASH method[34]. An examiner blinded to sample identification analyzed all immunostaining.

3. Results

3.1. CB$_1$ and CB$_2$ expression are increased in HAND brains on ART

To determine the expression levels of CB$_1$ and CB$_2$ in brains of HIV+ donors, we analyzed frontal lobe lysates generated from WM and GM from HAND cases as well as NUI cases (Table 1). Brain lysate membrane fractions were analyzed for CB$_1$, CB$_2$ and ACTB levels by immunoblot. In brain lysates from WM from HAND cases, CB$_1$ protein band intensity increased in MND and HAND compared to NUI and ANI (Fig. 1 A). The intensity of the band corresponding to CB$_2$ was similar in all groups (Fig. 1 A). Densitometry analyses of bands for CB$_1$ showed protein levels were significantly increased ~2-fold in MND and HAD when compared to NUI and ANI (Fig. 1 B). Densitometry analysis of the band corresponding to CB$_2$ revealed no significant difference between groups (Fig. 1 C). In brain lysates from GM from HAND cases, CB$_1$ protein band intensity increased in ANI, MND, and HAND compared to NUI (Fig. 1 D). The intensity of the band corresponding to CB$_2$ was less intense in ANI, MND, and HAND compared to NUI (Fig. 1 A). Densitometry analyses of bands for CB$_2$ showed protein levels were significantly increased ~1.7-, 1.9-, and 2-fold in ANI, MND, and HAD, respectively, when compared to NUI (Fig. 1 E). Densitometry analysis of the band corresponding to CB$_2$ revealed significant reduction (~40%, 60%, and ~50%, respectively) in ANI, MND, and HAD, respectively, when compared to NUI between groups (Fig. 1 F). These results suggest that CB$_1$ and CB$_2$ expression levels are differentially altered GM in brains of decedents that were diagnosed with HAND.
CB1 and CB2 expression are increased in HAND brains on ART. (A) Immunoblot of HAND donor WM frontal lobe lysates with antibodies specific for CB1 and CB2, and ACTB. (B and C) Quantification of normalized band intensity of CB1 and CB2 in WM stratified by HAND diagnosis. (D) Immunoblot of HAND donor GM frontal lobe lysates with antibodies specific for CB1 and CB2, and ACTB. (E and F) Quantification of normalized band intensity of CB1 and CB2 in GM stratified by HAND diagnosis. Statistical significance was determined by an one-way ANOVA followed by Tukey’s multiple comparisons test. *, p<0.05, **, p<0.01, ***p<0.001.

3.2. Elevated CB1 expression in brains of PWH may indicate poorer cognitive function

As an exporatory analysis, as data were available, CB1 and CB2 expression levels in GM and WM were correlated with global and cognitive domain-specific T-scores. CB2 expression levels in GM or WM did not relate to any cognitive outcome. Conversely, higher CB1 expression levels in GM significantly related to poorer memory T-scores (R=-0.45, p=.04; Figure 3A) and higher CB1 expression levels in WM significantly related to poorer speed of information processing (R=-0.49, p=.03; Figure 3B).
Greater CB₁ expression relates to worse cognitive function. (A) Correlation of CB₁ expression in GM with memory T-scores, \( R = -0.45; p = 0.04 \); \( n = 20 \). (B) Correlation of CB₁ expression in WM detected by immunoblot with speed of information processing T-scores, \( R = -0.49; p = 0.03 \); \( n = 20 \).

3.3 CB₁ expression and localization are altered in HAND brains on ART compared to NUI

To better understand the alterations in CB₁ levels in WM and GM of brains from HAND decedents, we performed immunolabelling for CB₁ in vibratome sections from the frontal cortex. First, we identified CB₁ expression in neurons in vibratome sections from the frontal cortex. The signal for CB₁ was clear and punctate throughout the neuronal processes in the GM in brains of HIV+ NUI decedents, however, CB₁ signal was very strong in soma of neurons in brains from HAND decedents (Fig. 2 A). Interestingly, the CB₁ dotting the neuronal processes throughout the GM was less common in brains from decedents diagnosed with HAND (Fig. 2 A). Next, to identify CB₁ co-localization with neurons, we double immunolabeled vibratome sections with antibodies against CB₁ and MAP2 and analyzed using confocal microscopy. In brains from decedents diagnosed as NUI, CB₁ stained strongly throughout the processes of MAP2+ cells (Fig. 2 B). However, the signal for CB₁ (red) was much more apparent in the soma of neurons in brains from decedents diagnosed with HAND when compared to CB₁ localization in brains from decedents diagnosed as NUI (Fig. 2 C). The quantification of the signal for CB₁ co-localizing with the signal for MAP2 was not significantly different in brains of decedents diagnosed as HAND when compared to CB₁ levels in brains of decedents diagnosed as NUI (Fig. 2 C). Next, we visualized CB₁ immunostaining in cells with glial morphology, which were apparent in WM but not GM. The signal for CB₁ was apparent in WM glial cells in brains of HIV+ NUI decedents. However, CB₁ signal was very strong in glial processes that extend from the soma more so in brains from HAND decedents than those from NUI decedents (Fig. 2 D). Strongly
stained glia cells were present in WM but not in GM. Next, to identify CB1+ astroglia, we double immunolabeled vibratome sections with antibodies against CB1 and GFAP and visualized using confocal microscopy. In brains from decedents diagnosed as NUI, CB1 stained lightly, with little colocalization with GFAP signal (Fig. 2E). CB1 stained more strongly in brains from decedents diagnosed with HAND (Fig. 2E). Quantification of the signal for CB1 co-localizing with the signal for GFAP increased by ~70% in brains of decedents diagnosed as HAND when compared to CB1 levels in brains of decedents diagnosed as NUI (Fig. 2F). These data suggest that astroglia increase expression of CB1 during HAND, while CB1 distribution in neurons may be abnormal in HAND, suggesting that astroglia may be an optimal therapeutic target using cannabinoids.

Figure 3: CB1 expression and localization is altered in HAND brains on ART compared to NUI. (A) Immunostaining of CB1 in the frontal cortices of NUI and HAND brain tissue. (B) Double-immunostaining of CB1 and MAP2 in frontal cortices of HAND and NUI brain tissue. (C) CB1 signal %colocalization of CB1/MAP2. (D) Immunostaining of CB1 in the frontal cortices of NUI and HAND brain tissue and the quantification of CB1+ glia. (E) Double-immunostaining of CB1 and GFAP in frontal cortices of HAND and NUI brain tissue. (F) Quantification of %colocalization of CB1/GFAP. Statistical significance was determined by an unpaired t test. *, p<0.05.

4. Discussion

The current study provides evidence that the ECS is altered in brain tissues of HIV+ decedents that were diagnosed with HAND while on ART. This study is the first to analyze CB1 and CB2 receptor expression in HAND brains on ART. We identify pathological changes in CB1 localization in neurons and astroglia that may reflect an attempt by brain cells to restore neuronal homeostasis. Correlational analyses between CB1 levels and specific neurocognitive domains suggest that ECS changes associated with HIV and ART may be related to the development of HAND. These findings are consistent with previous studies that reported increases in CB1 levels in PWH with HIV encephalitis and may implicate the ECS as a promising therapeutic target in PWH with HAND in the ART era[26] and in neuroinflammatory diseases in general[35].
PWH use cannabis at higher rate than the general population, with 14-56% of PWH using cannabis compared to <10% in the general population[19, 36-40]. The higher rates of use in PWH may be due to cannabis ability to alleviate anxiety, depression, nausea, sleep disorders and other symptoms associated with HIV infection[20, 41]. While the evidence that cannabis effectively eliminates such symptoms in all patients is variable, our data suggest that the ECS is upregulated in response to HIV infection and ART, possibly as a compensatory mechanism to restore brain homeostasis. Future studies of how cannabis use affects the ECS in the brain in PWH are needed to better understand the therapeutic implications of cannabis.

Alterations in CB1 and CB2 expression levels may constitute a compensatory response to neuroinflammation that persists in PWH on ART despite low or undetectable viral loads [6, 7, 42]. In particular, increases in WM CB1 may compensate for the observed decreases in WM CB2 levels. Indeed, chronic inflammation in PWH on ART likely contributes to comorbidities such as HAND and depression. Several studies suggest that cannabinoids are anti-inflammatory and neuroprotective[10, 43-45]. Our recent studies showed that a cannabinoid receptor agonist, WIN55,212-2, may be neuroprotective by reducing neuroinflammatory gene expression in reactive astroglia, although we found that WIN55,212-2 was acting through peroxisome proliferator-activated receptors (PPAR) [13, 46]. Importantly, there is evidence that the phytocannabinoids isolated from cannabis, Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), activate PPAR α and γ via signaling pathways that could be downstream of and also independent of CB1 and CB2 [47-49]. Other studies have shown that THC and CBD are neuroprotective in animal models for neurodegenerative diseases [45, 50]. Thus, THC and CBD may mimic endocannabinoid signaling mediated down modulation of neuroinflammation, a process that may be perturbed in PWH. These findings warrant further studies to delineate the mechanisms of cannabis mediated neuroprotection and the role of the ECS in these processes to better understand mechanisms of action underlying the therapeutic effects.

The neuronal pathology observed in HAND brains compared to NUI may reflect altered distribution of mitochondria throughout neuronal soma and processes. The CB1 receptor has been shown to be located at mitochondria as well as on the plasma membrane of cells [51]. CB1 has also been reported to be upregulated in neurodegenerative diseases [26]. Cannabinoid receptor agonists have shown promising therapeutic effects in multiple animal models for neurodegenerative diseases. Additionally, previous studies have shown that cannabinoid receptor agonists alter inflammatory gene expression and mitochondrial metabolic processes in multiple cell types[13, 20, 46, 47, 51-57]. Astroglia and neurons are highly involved in endocannabinoid signaling[54, 56, 58, 59]. Moreover, endocannabinoid signaling regulates neurotransmission and metabolism in and between the two brain cell types[12, 45, 49, 58-62]. These studies, for the first time, identify a shift in localization of CB1 receptors on neurons from punctate distribution to localization to the soma of neurons, which may be associated with a shift in mitochondrial fusion and fusion processes in neurons as we reported in HAND brains[32]. Indeed, our findings in NUI brains are consistent with previous findings that show a subset of CB1 receptors dot the outer membranes of mitochondria where they alter function of the electron transport chain among other pathways [53, 55]. However, more studies are needed to confirm this hypothesis. In the least, the alterations in CB1 and CB2 expression and localization in HAND may indicate the ECS as a promising target for therapeutic intervention. This assertion is consistent with recent studies showing that PWH that use cannabis may demonstrate improved cognitive function compared to PWH not using cannabis[44, 63]. Future studies are needed to develop therapies aimed at astroglia to restore immunometabolic balance in the brain.

While important, relevant, and timely observations are presented, this study has several noteworthy limitations. A potential caveat in this study was the limited cohort size, which probably is not fully representative of the whole population of PWH. Future studies using brain specimens from a larger cohort that is demographically representative of PWH in regard to race, sex, age and other characteristics may more accurately
elucidate the effects of HIV on CB₁ and CB₂ expression in the brain. Including HIV-controls in future analyses will be important to understand how HIV infection affects CB₁ and CB₂ expression independent of HAND status. Important specimen data on recency and frequency of exposure to cannabis and other drugs of abuse were not available for all participants and therefore limited the study to only correlations with HIV clinical data and neuropsychological measures of HAND. The analyses here are limited to only frontal cortex tissues, while CB₁ and CB₂ are expressed throughout the brain and in regions such as the hippocampus and striatum, which are implicated to be involved in HAND phenotypes. CB₁ and CB₂ are expressed in brain cell-types other than astroglia and neurons, including microglia and possibly oligodendroglia and it will be important to perform future studies that determine CB₁ and CB₂ levels in these cell types in specimens from PWH with HAND on ART. The ECS is composed of endocannabinoid-producing and -degrading enzymes as well as receptors aside from CB₁ and CB₂; none of which were examined in this investigation. CB₁ and CB₂ expression levels could not be related to markers of neuroinflammation or neurodegeneration as these data are currently unavailable; future studies are necessary to assess these correlations. Finally, the data presented here are observational and associative, lacking mechanistic links between HIV, ART, and the changes in CB₁ and CB₂. This work lays the foundation for future studies to identify HIV and ART-associated factors leading to alterations in CB₁ and CB₂ in the brain as well as a better understanding of the potential ameliorative role of cannabis in PWH.

5. Conclusions

Overall, this study identified alterations in CB₁ and CB₂ expression as potential mechanisms contributing to, or, alternatively, a consequence of the neuropathogenesis of HAND during the ART era. Importantly, these shifts in CB₁ and CB₂ expression may be dependent on cell-type and therefore could indicate that therapies designed to target these receptors or other ECS proteins in neurons or glial cells may be beneficial for HAND patients. These findings further support the recent interest in astroglia as mediators of neurodegenerative diseases and studies that show promising effects of cannabinoids in combatting such diseases [58, 64]. Future studies are needed to develop therapies aimed at astroglia and neurons to restore brain homeostasis.

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Data Availability Statement: All data will be available by reasonable request and will also be deposited with the National NeuroAIDS Tissue Consortium.

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