Effect of Cannabis Use on Human Immunodeficiency Virus DNA During Suppressive Antiretroviral Therapy

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Cannabis use is frequent among people living with human immunodeficiency virus (HIV) and is associated with reduced systemic inflammation. We observed a faster HIV DNA decay during antiretroviral therapy among cannabis users, compared to those with no drug use. No cannabis effect was observed on cellular HIV RNA transcription.

Keywords. MSM; cannabis; HIV DNA; cellular transcription.

Cannabis is widely consumed in the United States [1], particularly among people living with human immunodeficiency virus (HIV) [2–4], and this has fostered an important debate regarding the impact of cannabis on virologic and inflammatory biomarkers. On one hand, a recent study reported reduced adherence to antiretroviral therapy (ART) and retention in care in cannabis users [5], and 2 other studies found no effects of cannabis on viral suppression [4, 6]. On the other hand, 1 study has demonstrated beneficial impacts of cannabis on plasma HIV RNA among recently infected people living with HIV (PLWH) [7]. More recently, Manuzak et al [8] evaluated the impact of cannabis use on inflammation and immune activation in ART-treated PLWH and reported that cannabis use was associated with (1) reduced activation of CD4⁺ and CD8⁺ T cells and (2) a shift in the composition of the monocyte populations in peripheral blood.

Here, we investigated the effect of cannabis use (with or without other drugs) on the decay and transcriptional activity of the HIV reservoir during suppressive ART.

METHODS

Ethics Statement

The study was approved by the University of California San Diego Human Research Protections Program. All adult participants provided written informed consent.

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Study Cohort and Clinical Data

There were 48 men who have sex with men (MSM) coenrolled in the San Diego Primary Infection Resource Consortium [9] and in the Translational Methamphetamine Acquired Immunodeficiency Syndrome (AIDS) Research Center cohort [10]. The estimated duration of infection (EDI) was determined using serologic and virologic parameters [9]. A median of 4 (interquartile range [IQR] 1–5) longitudinal peripheral blood mononuclear cells (PBMCs) and plasma samples per individual were collected over a median of 22 months (IQR 12–31) following ART initiation. Absolute CD4⁺ and CD8⁺ T cell counts, plasma HIV RNA levels (Amplicor, Roche), and self-reported ART adherence were measured.

To ascertain substance use (not including alcohol), we utilized 3 validated questionnaires, including the World Health Organization Composite International Diagnostic Interview–Substance Abuse Module 2.1 [10, 11, 12]. We were primarily interested in the effect of cannabis on cellular HIV DNA and HIV RNA. Due to the small sample size and to reduce the false discovery rate, we combined all other drugs (except cannabis). This yielded 4 groups, based on self-reported drug use: (1) no drug use; (2) cannabis use only; (3) use of drugs other than cannabis (“other drugs”); and (4) use of both cannabis and other drugs (“both drugs”).

Total Cellular Human Immunodeficiency Virus DNA and RNA

DNA was extracted from 5 million PBMCs for each time point using an AllPrep DNA/RNA Mini Kit (Qiagen, CA). Total HIV DNA (polymerase) was quantified by droplet digital polymerase chain reaction and normalized to the housekeeping gene ribonuclease P/MRP Subunit P30 [13]. Levels of all fully elongated and correctly processed HIV mRNA molecules were quantified [14] and normalized to total RNA concentration [15].

Flow Cytometry

Markers of T cell activation (CD38⁺HLA-DR⁺) and monocyte activation (CD16⁺) were assessed by flow cytometry [16].

Statistical Analyses

We developed (generalized) linear mixed-effects regression models to investigate the relationship of cannabis and other drugs on 2 outcomes during ART: (1) changes in HIV DNA levels, and (2) cell-associated HIV RNA transcripts (detectable or undetectable). For each model, we included a random intercept for subject and the following fixed effects: (1) time from ART initiation (ie, slope); (2) drug use; and (3) time by drug use (ie, the difference in slopes). To determine which fixed effects to include in the final model, we tested whether the outcome changed over time among all participants. If it did change at

the 10% significance level, we subsequently tested whether the drug user groups differed in the rate of change and in the outcome level at ART initiation; if not, we tested whether the groups differed in the overall outcome level. To control for other sources of variability, we added relevant covariates (ie, age, time from EDI to ART, CD4 count, CD4/CD8 ratio, mean % activated [CD38⁺HLA-DR⁺] of CD4⁺ and CD8⁺ T cells, and mean % of CD14⁺CD16⁺ monocytes), if associated with the outcome at $P < .10$ in individual models. Our final models include all predictors and covariates that were significant at $P < .05$. Because 34% of all time points exhibited levels of cellular HIV RNA below the limits of detection, we used mixed-effects logistic regression to analyze this outcome. Data are expressed as median (IQR).

RESULTS

Study Cohort and Samples

This study evaluated 206 blood samples from 48 MSM who initiated ART within a median of 4 months (IQR 1–6) from EDI and achieved suppressed HIV RNA within a median of 5 months (IQR 3–8), without any documented blips of HIV RNA replication. Of the 48 individuals, 10 (21%) reported no drug use, 5 (11%) reported use of cannabis only, 16 (33%) reported use of drugs other than cannabis, and 17 (35%) reported use of cannabis and other drugs. Summaries of cohort

characteristics and drug self-reports at ART initiation are provided in [Supplementary Tables 1 and 2](#).

Predictors of Human Immunodeficiency Virus DNA Levels and Decay

The median unadjusted HIV DNA level at ART initiation was 2.4 log₁₀ copies/10⁶ cells (IQR 1.0–3.1) with a significant decrease of HIV DNA over time among all participants ($P < .01$; [Supplementary Table 3](#)). Cannabis-only users had significantly greater HIV DNA levels at the time of ART initiation ($P = .04$), but subsequently experienced a faster HIV DNA decay, compared to participants with no drug use ($P < .01$; [Figure 1](#)). Participants who used other drugs (with or without cannabis) had similar levels of HIV DNA at ART initiation ($P = .41$ and $P = .66$, respectively) and during ART ($P = .43$ and $P = .76$, respectively), compared to those who used no drugs. The 4 groups did not differ in baseline CD4 counts ($P = .80$) or EDIs ($P = .46$).

Regarding markers of cellular activation, study participants taking other drugs had a persistent, higher proportion of activated (CD38⁺HLA-DR⁺) CD8⁺ T cells, as compared to those who used no drugs, even after controlling for significant covariates. There were no differences by groups related to CD38⁺HLA-DR⁺CD4⁺ T cells or CD14⁺CD16⁺ monocytes. Overall, the mean percentage of CD38⁺HLA-DR⁺CD8⁺ T cells was positively associated with HIV DNA levels in the individual model ($P < .02$), but this was no longer significant when drug groups were included ($P = .08$).

Figure 1. Observed HIV DNA (log₁₀ copies/10⁶ CD4+) in cohort during ART as a function of time (Ln months since ART Initiation) and drug use. The thin lines and dots indicate individual data points. Thick lines and shaded areas indicate model-estimated values and their 95% confidence intervals. * $P < .05$: the mean HIV DNA level at ART initiation and slope differences compared to the no-drug group. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; Ln, natural log.

Cellular Human Immunodeficiency Virus RNA Transcription

The median levels of cellular HIV RNA at baseline were 4.9 log₁₀ copies/ml (IQR 3.8–5.7; [Supplementary Table 1](#)), and all of the participants had detectable levels at ART initiation. Participants who reported use of cannabis (with or without other drugs), did not exhibit significantly different proportions of detectable cellular HIV RNA ($P = .59$ and $P = .28$, respectively). However, participants who used other drugs were significantly more likely to have detectable cellular HIV RNA levels during ART ($P = .01$). Similarly, participants with higher peak HIV RNA levels (before ART initiation) and higher mean percentages of activated CD8⁺ T cells had more detectable cellular HIV RNA levels during ART ($P < .05$). Participants with higher CD4/CD8 ratios had less detectable cellular HIV RNA levels ($P < .10$). Interestingly, only the other drug effect remained significant for cellular HIV RNA when included in a final multivariate model ($P = .01$; other effects, $P > .4$; [Supplementary Table 4](#)).

DISCUSSION

In this longitudinal study of PLWH with well-characterized histories of substance use, we found that exclusive use of cannabis was associated with a faster decay of HIV DNA, but had no impact on cell-associated HIV RNA transcription or cellular activation during suppressive ART. However, the consumption of other drugs was associated with increased CD8⁺ T cell activation and with increased cellular HIV RNA transcription during ART. Our results are consistent with a previous study reporting that cannabis use was associated with lower plasma HIV RNA levels among recently infected PLWH [7], and with reports of reduced HIV replication and cellular infection rates in the presence of cannabinoids in vitro [17, 18]. Further, cannabis use has been previously associated with decreased frequencies of interleukin 23 and tumor necrosis factor α [8] and, more recently, with the reduction of interferon- γ inducible protein 10 levels in plasma [19]. Altogether, these published findings point to a potential anti-inflammatory effect of cannabis that, in turn, might positively impact HIV replication and persistence [20], although another study has suggested that tetrahydrocannabinol may enhance HIV replication by suppressing immune functions in mice [21], and this possibility should be evaluated in future, larger clinical studies. In our study, the effect of cannabis on HIV DNA was not evident when cannabis was used in combination with any other drugs, highlighting the complex interplay between drug use and viral infections.

The small sample size and the multiple potential confounding factors among participants with a complex history of drug use are major limitations to this report. Furthermore, cannabis use was not confirmed by the direct measurement of cannabis metabolites, and only relied on self-report. While inaccurate self-reporting could confound our observations, studies have demonstrated the reliability of self-reported drug use [22].

The mechanisms by which cannabis impacts the HIV reservoir—whether directly or indirectly by limiting immune

activation, the infection of CD4⁺ T cells, and macrophages and inflammation—remain uncertain. Further work is necessary to explore these mechanisms and the interactions with other drugs, which have been associated with contradictory effects on the immune system. These findings may have implications for and reaffirm the need for regular screening for substance use in HIV care settings and for a holistic approach to HIV cures from healthcare providers, physicians, epidemiologists, and researchers.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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