Cumulative Antiretroviral Exposure Measured in Hair Is Not Associated With Measures of HIV Persistence or Inflammation Among Individuals on Suppressive ART

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Data on the relationship of antiretroviral exposure to measures of human immunodeficiency virus (HIV) persistence are limited. To address this gap, multiple viral, immunologic, and pharmacologic measures were analyzed from individuals with sustained virologic suppression on therapy (median 7 years) in the AIDS Clinical Trials Group A5321 cohort. Among 110 participants on tenofovir-(TFV)-disoproxil-fumarate (TDF)/emtricitabine (FTC)-containing regimens, we found no significant correlation between hair concentrations of individual antiretrovirals (ARVs) in the regimen and measures of HIV persistence (plasma HIV-1 RNA by single copy assay, cell-associated-DNA, cell-associated RNA) or soluble markers of inflammation. These findings suggest that higher systemic ARV exposure may not impact HIV persistence or inflammation.

Keywords. HIV; antiretroviral therapy; hair concentrations; measures of HIV persistence; HIV-related inflammation.

Among people living with human immunodeficiency virus (HIV) on treatment, strong relationships have been shown between systemic exposure to antiretroviral (ARV) therapy (ART) and virologic suppression in plasma (eg, HIV RNA < 40 copies/mL) [1, 2]. Lower levels of ARVs in sanctuary sites may be associated with persistent HIV-1 replication on ART [3], although maintenance of the reservoir may be due to proliferation of infected cells instead of ongoing replication [4].

Suboptimal ART adherence, even with suppression of viremia, has also been associated with higher levels of inflammation [5]. To date, however, studies have not examined the relationship between cumulative exposure to ARVs and measures of HIV-1 persistence or inflammation.

Plasma levels measure short-term exposure to ARVs and are susceptible to day-to-day variation [6]. Cumulative measures of ARV exposure (such as drug levels in peripheral blood mononuclear cells [PBMCs], dried blood spots, or hair) are increasingly used to quantify exposure, whether in the context of HIV treatment or pre-exposure prophylaxis. Drug concentrations in hair reflect uptake from the systemic circulation over weeks to months [7], and can be used to measure cumulative exposure to all ARVs, regardless of whether they are activated intracellularly (such as nucleoside/nucleotide reverse transcriptase inhibitors [NRTIs]) or not (eg, non-NRTIs [NNRTIs], protease inhibitors [PIs], and integrase strand transfer inhibitors [INSTIs]).

Assays have been developed and validated to measure hair concentrations for a number of ARVs. Multiple studies have shown strong relationships between various ARV levels in hair and suppression of plasma viremia [1, 8]. The AIDS Clinical Trials Group (ACTG) HIV Reservoirs Cohort Study (A5321) collected multiple samples for measures of HIV-1 persistence, inflammation, and ARV exposure among participants with sustained suppression of plasma viremia [9]. We present here the first analysis examining the relationship between long-term ARV exposure (using hair levels) and measurements of HIV-1 persistence and inflammation among individuals with long-standing virologic suppression.

METHODS

Study Population

ACTG A5321 is a longitudinal cohort that includes participants who started ART during chronic HIV infection in the context of ACTG clinical trials, achieved HIV RNA levels <50 copies/mL after 6 months, and had documented sustained virologic suppression with no documented breakthroughs [9]. Multiple viral, immunologic, and pharmacologic measures are collected prospectively. The current analysis examines participants who were on tenofovir (TFV) disoproxil fumarate (TDF)/emtricitabine (FTC)-based ART regimens at study entry. Participants provided written informed consent, with opt-in for hair collection, and ethics approval was obtained from all participating institutions.

Study Procedures

Hair Concentrations

Small samples of hair (approximately 50 strands) were cut from the scalp at the entry visit [1]. Hair concentrations of various

ARVs were measured via validated liquid chromatography/tandem mass spectrometry-based methods in the University of California San Francisco Hair Analytical Laboratory. Our assays have been peer reviewed and approved by the Division of AIDS' Clinical Pharmacology and Quality Assurance Program. Briefly, hair is cut into 1–2 mm length segments and 5 mg is weighed and processed. The ARV is extracted with an organic solvent, along with an internal standard, and then analyzed. For this analysis, ARV concentrations were measured in the 1.5 cm of hair closest to the participant's scalp, representing approximately 6 weeks' exposure.

Measures of HIV-1 Persistence

Residual low-level plasma viremia using a single copy assay (iSCA) was measured using real-time PCR with a probe for a highly conserved region of integrase in the HIV-1 *pol* gene [10]; the lower limit of quantification of this assay for a 4-mL plasma sample is 0.4 copies/milliliters (mL). Cell-associated RNA (CA-RNA) and cell-associated DNA (CA-DNA) were measured via published methods at the University of Pittsburgh ACTG Virology Specialty Laboratory [9].

Immunologic Measures

To evaluate levels of soluble biomarkers of inflammation and immune activation, frozen plasma samples from each participant were thawed and analyzed. Plasma concentrations of interleukin (IL)-6, high-sensitivity CRP (hsCRP), soluble CD14 (sCD14), soluble CD163 (sCD163), interferon gamma-induced protein (IP)-10, and tumor necrosis factor (TNF)-α were quantified using enzyme-linked immunosorbent assay (ELISA) kits (R&D, Minneapolis, MN) at an ACTG Immunology Specialty Laboratory [9].

Statistical Analysis

Rank-based (Spearman) correlation coefficients assessed relationships between hair levels of ARVs (the average rank of TFV/FTC levels and concentrations of each individual drug) at study entry with HIV-1 persistence measurements (iSCA, CA-RNA, and CA-DNA) and soluble inflammatory markers. Hair levels, reservoir measures, and soluble biomarkers were each assessed as continuous measures. For HIV-1 DNA values <3 and CA-RNA values <19 copies/10^6 CD4+ T cells (below assay limits based on the number of cells analyzed), and iSCA <0.4 copies/mL, results were imputed to a lower number and analyzed as the lowest rank. The relationship between exposure reservoir measures was examined separately by sex. Differences in TFV concentrations with an accompanying pharmacoenhancer, cobicistat or ritonavir, in the regimen (versus an unboosted anchor ARV) were assessed by the Wilcoxon rank sum test; the rank sum test also evaluated differences in drug concentrations by iSCA <0.4 versus ≥0.4 copies/mL. Data analyses were performed using SAS 9.4.

RESULTS

Demographics

Hair samples at A5321 entry were analyzed for 110 participants on TDF/FTC (Table 1); besides TDF/FTC, 49% were on an NNRTI, 25% on an INSTI, and 26% on a boosted PI. The median age of participants was 48 years (range 23–69) and 24% female. The distribution of self-reported race/ethnicity was 15% black, 59% white, 23% Hispanic, and 3% Asian. The median duration on ART prior to entry was 7 years (range 4–16). Although all participants had sustained virologic suppression via commercial HIV-1 RNA assays, 48% had residual low-level viremia (≥0.4 copies/mL) by iSCA. Median CA-DNA and CA-RNA levels were 48 (interquartile range [IQR], 14–142) and 564 (IQR, 229–1236) copies/10^6 CD4+ cells, respectively (Table 1).
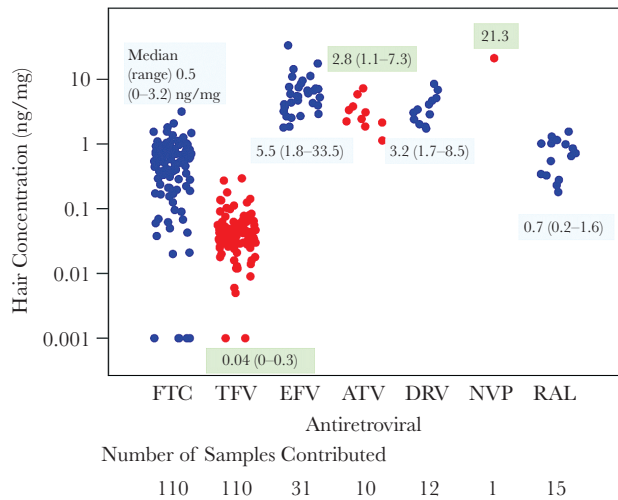
Hair ARV Concentrations

Figure 1 shows the distribution of TFV and FTC hair concentrations among the 110 participants and the distribution of each anchor drug in hair for the subset of participants on each ARV. In participants with measureable TFV and FTC, concentrations varied over 100-fold. Concentrations of third agents were detectable in all participants and varied over a narrower range. Median concentrations of TFV in hair were 25% higher in participants on cobicistat or ritonavir than in those on an unboosted anchor ($P = .015$).

Table 1. Demographics of Participants in the A5321 Hair Study (n = 110)

Participant Characteristic	N or Median (%, range or IQR)
Age at study entry (years)	48 (range 23–69)
Sex	
Male	84 (76%)
Female	26 (24%)
Race/ethnicity	
White non-Hispanic	65 (59%)
Black non-Hispanic	17 (15%)
Hispanic	25 (23%)
Asian/Pacific Islander	3 (3%)
Years of ART at study entry	7 (range 4–16)
CD4+ cell count (cells/mm³)	
Pre-ART	250 (IQR, 101–359)
Study entry	661 (IQR, 494–839)
Pre-ART plasma HIV-1 RNA (log₁₀cps/mL)	4.6 (IQR, 4.2–5.1)
ART regimen at A5321 entry (all TDF-FTC based)	46 (42%) EFV; 7 (6%) RPV; 1 (1%) NVP; 11 (10%) ATV/r; 18 (16%) DRV/r; 5 (5%) EVG/cobi; 22 (20%) RAL
Measures of viral persistence	
Plasma HIV-1 RNA (iSCA, copies/mL)	52% < 0.4; 48% ≥ 0.4
Cell associated HIV-1 RNA (copies/10^6 CD4+ cells)	48 (IQR, 14–142)
Cell associated HIV-1 DNA (copies/10^6 CD4+ cells)	564 (IQR, 229–1236)

Abbreviations: ART, antiretroviral therapy; ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; EFV, efavirenz; EVG/cobi, elvitegravir/cobicistat; IQR, interquartile range; iSCA, single copy assay; NVP, nevirapine; RPV, rilpivirine.

Figure 1. Interindividual variability in antiretroviral (ARV) concentrations seen even among virologically suppressed individuals. ARV concentrations in hair below the lower limit of quantification were set to 0.001. Abbreviations: ATV, atazanavir; DRV, darunavir; EFV, efavirenz; FTC, emtricitabine; NVP, nevirapine; RAL, raltegravir; TFV, tenofovir.

Correlation Between Hair Concentrations of ARVs and HIV-1 Persistence

A significant correlation was not detected between TFV and FTC concentrations in hair and low-level viremia measured by iSCA ($r = 0.09$ and $r = -0.04$, respectively); results were comparable when examining iSCA <0.4 versus ≥0.4 copies/mL ($P = .18$ and $P = .88$ for TFV and FTC, respectively). Similarly, a significant correlation was not detected between rank-based TFV concentrations in hair and either CA-RNA ($r = -0.02$) or CA-DNA ($r = -0.1$), or between FTC concentrations in hair and either CA-RNA ($r = 0.01$) or CA-DNA ($r = -0.05$). Finally, significant correlations were not found between hair concentrations of any of the NNRTI, PI, or INSTI (efavirenz, raltegravir, atazanavir, or darunavir) and the 3 measures of HIV-1 persistence (Supplementary Table 1). When stratifying analyses by sex, no significant correlations were observed between hair levels and HIV-1 persistence measures in either men or women.

Correlation Between Hair Concentrations of ARVs and Soluble Markers of Inflammation

Significant correlations were not detected between ranked TFV and FTC concentrations in hair and the 6 soluble biomarkers (Supplementary Table 2).

DISCUSSION

This is the first study to report the relationship between cumulative measures of ARVs and measures of HIV-1 persistence or inflammation. We found no significant correlation among ARV hair levels and 3 different measures of HIV-1 persistence and 6 soluble markers of inflammation in participants on suppressive ART. We observed marked interindividual variability in

ARV exposure, which has been reported previously [11], but not among individuals with sustained virologic suppression. Finally, we confirm previous findings [12] that concomitant pharmacoenhancers result in higher TFV levels.

Previous studies have examined the impact of ART *intensification* (adding agents to currently suppressive ART) on persistent viremia and inflammation. In general, studies adding raltegravir and/or maraviroc to suppressive ART regimens early in HIV infection or in the context of chronic suppressive therapy [13, 14] have failed to show consistent or sustained impacts on markers of HIV-1 persistence. Similarly, in this cohort of individuals with sustained virologic suppression on ART at traditional doses, those exhibiting higher ARV concentrations in hair did not demonstrate lower measures of HIV persistence.

An ongoing debate has focused on whether low-level replication of HIV continues in tissues of individuals with suppression of viremia on ART. The fact that higher ARV concentrations in our study did not correlate with lower levels of viremia by iSCA (or lower levels of HIV transcription by CA-RNA) suggests that residual proviral transcripts and viremia are produced by stable reservoirs of infected cells that are not influenced by higher exposure to ARVs. However, it is not known whether higher hair ARV concentrations are associated with higher local levels of ARVs in sanctuary sites, such as the lymph node, where ongoing replication has been postulated to occur. Previous studies have demonstrated that ARV concentrations in sanctuary sites (lymphatic tissues [3], genital tissues, the central nervous system [CNS]) are lower (when normalized by weight of tissue or number of cells) than in the plasma. Ongoing work in A5321 will define the relationship between local concentrations of ARVs in various reservoir sites (CNS, genital tract, rectal tissue) and local reservoir measures (eg, cell-associated RNA in the CNS) to help resolve this debate.

Of note was the wide interindividual variability in ARV hair concentrations seen among participants with sustained virologic suppression (over 100-fold for TFV and FTC) (Figure 1), indicating that current ART regimens can be highly effective despite variable cumulative exposure. Such variability has been noted previously [11], but these studies were performed in cohorts with a range of virologic outcomes, whereas A5321 participants had sustained, well-documented virologic suppression. Prior studies have not observed variability in hair ARV levels by hair color or melaninization [14]. The variability in ARV levels seen among A5321 participants is likely due to interindividual variability in ARV pharmacokinetics, but also may reflect variability in adherence over the 6 weeks of exposure measured by hair levels. A prior study examined the association between self-reported suboptimal adherence and a variety of soluble biomarkers of inflammation and immune activation, finding an inverse relationship between adherence and some biomarkers [5]. Our analysis, which assessed actual drug exposure and not adherence, did not duplicate this finding in

this highly selected population. Therefore, further study of the relationship between adherence, drug exposure, and inflammation is needed in a more routine clinic population, including examining correlates of poor adherence such as stress and substance use. Finally, as observed in previous analyses, cumulative TFV concentrations are higher when regimens are boosted with ritonavir or cobicistat than without pharmacoenhancement, a finding of increasing concern because higher ARV levels may lead to greater toxicity [12].

Our study has several limitations, including the small numbers of participants examined using individual ARVs other than FTC and TFV. In addition, at the time of the A5321 entry, the most commonly used INSTI was raltegravir, so data on either elvitegravir- or dolutegravir-based therapy is unavailable. In addition, the HIV persistence metrics were measured in peripheral samples (plasma and PBMCs) only. Because A5321 enrolled participants with sustained virologic suppression, we cannot assess whether drug levels are associated with HIV persistence in patients with more variable virologic outcomes. Finally, we did not collect data on self-reported adherence in A5321, which may have helped to elucidate some of the between-patient variability observed in hair concentrations.

In conclusion, we found no significant relationship between cumulative ARV exposure in chronically suppressed HIV-infected individuals on ART, as quantified by ARV concentrations in hair spanning a 100-fold range, and measures of either viral persistence or inflammation. This lack of correlation suggests that increasing exposure to currently available ARVs would not have a significant impact on HIV-1 persistence or inflammation. However, it is not known whether ARV concentrations in hair adequately reflect ARV concentrations in tissues. Future studies need to investigate this question and the impact of higher systemic exposure to currently available INSTIs and newer ARVs on HIV persistence and inflammation. Such data are required to support future cure strategies focused on discovering more potent ARV or ARV combinations, rather than approaches that reverse HIV-1 latency and promote the clearance of stable reservoirs of HIV-infected cells [15].

Supplementary Data

Supplementary materials are available at *The Journal of 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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