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[Cecile Lahiri](#), *Emory University*

[Kedria Reed-Walker](#), *University of Alabama at Birmingham*

[Anandi Sheth](#), *Emory University*

[Edward P. Acosta](#), *University of Alabama at Birmingham*

[Aswani Vunnava](#), *Emory University*

[Ighoverha Ofotokun](#), *Emory University*

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Cerebrospinal Fluid Concentrations of Tenofovir and Emtricitabine in the Setting of HIV-1 Protease Inhibitor-Based Regimens

Cecile D. Lahiri, M.D, M.Sc¹, Kedria Reed-Walker, B.S², Anandi N. Sheth, M.D, M.Sc¹, Edward P. Acosta, Pharm.D², Aswani Vunna, M.S¹, and Ighovwerha Ofotokun, M.D, M.Sc¹

¹Emory University School of Medicine, Department of Medicine, Division of Infectious Diseases

²University of Alabama-Birmingham School of Medicine, Department of Pharmacology and Toxicology, Division of Clinical Pharmacology

Keywords

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Introduction

In the combination antiretroviral therapy (cART) era, prevalence of HIV-associated neurocognitive disorders (HAND) remains high: 36.2-44.8%¹, contributing to morbidity¹ and mortality². Suboptimal control of HIV in the central nervous system (CNS) likely plays a role in this phenomenon, as supported by elevated neopterin and detectable HIV-1 RNA in cerebrospinal fluid (CSF) of patients on long term cART³. Studies have shown that limited CNS penetration by antiretroviral (ARV) drugs is associated with HAND^{4,5}.

Multiple factors impact CNS drug penetration, including molecular size, lipophilicity, plasma protein binding, and affinity for efflux pump transporters⁶. For example, the ATP-binding cassette (ABC) superfamily of efflux transporters, including p-glycoprotein and multidrug-resistance associated proteins (MRPs), are expressed at blood-brain and blood-CSF barriers and can limit drug disposition⁷. Because many ARVs are substrates and/or inhibitors of ABC transporters, complex drug-drug interactions can occur. Specifically, protease inhibitors (PIs) atazanavir (ATV) and darunavir (DRV) are common in cART regimens when given with ritonavir (RTV) plus a backbone of tenofovir disoproxil fumarate (TDF), the tenofovir (TFV) pro-drug, and emtricitabine (FTC)⁸. Both ATV and DRV

Correspondence and request for reprints: Ighovwerha Ofotokun, 49 Jesse Hill Jr Dr. SE, Atlanta, Georgia 30303, (p) 404-616-0659, (f) 404-616-9702, iofotok@emory.edu.

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Compliance with Ethical Standards: The Emory University Institutional Review Board and Grady Research Oversight Committee approved this study, and it was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The authors have full control of the primary data and agree to allow the journal to review the data if requested.

inhibit p-glycoprotein and MRP-1⁹⁻¹¹; these transporters are found at blood-brain and blood-CSF barriers and are important for TFV¹² and FTC transport¹³.

We compared CSF TFV and FTC concentrations between patients receiving two different RTV-boosted PI regimens: ATV vs DRV. We also examined associations between CSF drug concentrations and CSF HIV-1 RNA detection.

Methods

Study population and specimens

Plasma and CSF specimens were obtained from a cross-sectional study conducted at the Grady Health System-affiliated Ponce de Leon Center in Atlanta, Georgia from May to December 2012. Data and specimen collection methods have been previously described¹⁴. Briefly, plasma and CSF were collected at drug trough times in thirty (30) HIV-1 infected patients receiving daily TDF/FTC (300 mg/200 mg) plus RTV (100 mg)-boosted daily ATV (300 mg) or DRV (800 mg). Eligibility criteria included documented adherence to their current boosted-PI regimen and undetectable plasma HIV-1 RNA (<40 copies/mL) for 6 months preceding study entry. The Emory University Institutional Review Board and Grady Research Oversight Committee approved this study; written informed consent was obtained from all study participants.

Plasma and CSF drug concentrations

Blood was collected from venipuncture and spun at 2300 rpm for 10 minutes to eliminate erythrocytes from the resulting plasma specimen. CSF was collected via lumbar puncture using sterile technique a median 35 minutes following blood draw (interquartile range, IQR, 30-45). Plasma and CSF aliquots were stored at -80°Celsius until analysis. Plasma and CSF TFV and FTC were quantitated using a modified previously published methodology¹⁵. The calibration curve was linear over the range of 5 to 1000 ng/mL for TFV and 5 to 5000 ng/mL for FTC using a 50 µL aliquot of human plasma. Validation of the plasma assay showed precision < 8% coefficient of variation between calibration standards on different assay runs, and accuracy < 6% deviation from known concentrations. Plasma quality controls showed overall precision < 7% and accuracy within 10%. CSF samples were extracted and analyzed with plasma samples. Cross-validation using CSF quality controls showed overall precision < 7% and accuracy within 13%. CSF and plasma quality controls showed < 5% difference for both analytes, demonstrating equivalence of the two matrices.

CSF HIV-1 RNA

CSF HIV-1 RNA was measured with COBAS[®] Ampliprep/COBAS[®] Taqman[®] version 2.0 HIV-1 assay (Roche Molecular Systems, Inc, Branchburg, NJ, USA); this assay detects 20 to 10,000,000 copies/mL, with 98.3% sensitivity and 99.4% specificity.

Statistical analyses

Demographic characteristics were summarized by descriptive statistics. Differences between ATV and DRV arms were examined using Chi Square or Fisher's exact tests for categorical variables and Wilcoxon rank sum tests for continuous variables. Drug concentrations were

reported as geometric means with 95% confidence intervals (CI) and medians with IQR in ng/mL. D'Agostino and Pearson normality tests were performed to assess for normality of distribution. Differences between drug concentrations were assessed using parametric unpaired two-sided two-sample t tests with an alpha level of 0.05. Associations between plasma and CSF drug concentrations were evaluated using Spearman rank correlation tests. To assess variables predictive of mean CSF drug concentrations, univariate and multiple linear regression modeling with forward selection was utilized. Relationships between CSF drug concentrations and detection of CSF HIV-1 RNA were assessed using point biserial correlation tests. Analyses were performed using SAS[®] 9.3 (SAS Institute, Cary, NC) software.

Results

Study Demographics

Thirty HIV-infected persons were enrolled: 15 were receiving ATV and 15 were receiving DRV. Plasma samples were available for analysis from all 30 participants, and CSF was available for all (15/15) participants in the ATV arm and 14/15 in the DRV arm. Demographic characteristics have been previously described and were similar between treatment arms ¹⁴; briefly, most were males (23/30, 76.7%), black (26/30, 86.7%), and median age was 46.9 years (interquartile range, IQR, 37.9-51.9). Five of the 7 females were in the ATV arm compared to 2 in the DRV arm (p = 0.4).

Plasma and CSF TFV and FTC concentrations

Plasma TFV trough concentrations were similar in both arms: geometric mean was 48.4 ng/mL (95% CI, 35.4-66.2) in the ATV group and 53.2 ng/mL (95% CI 39.5-71.5) in the DRV group (Table 1). CSF TFV concentrations were above the limit of quantitation (5 ng/mL) in only 4 participants: 1 (6.7%) in the ATV arm and 3 (21.4%) in the DRV arm. Three of these 4 participants were black (75%) and male, consistent with the overall study population. However, participants with detectable CSF TFV were older than those with undetectable TFV (median age 52.0 years [IQR 45.3-61.1], versus 45.7 years [IQR 33.7-50.9]), had lower creatinine clearance (median 84.5 mL/min [IQR 58.8-123] versus 100.5 mL/min [IQR 83.8-119.8]), and had higher median plasma TFV concentrations (142.55 ng/mL [IQR 73.9-214.6] versus 52.0 ng/mL [IQR 43.0-65.3]). Only 1 of these 4 participants achieved a CSF TFV concentration above 11.5 ng/mL, the lowest reported IC₅₀ for wild-type HIV-1 ¹⁶.

Compared with the ATV arm, mean plasma FTC concentrations were almost doubled in the DRV arm: 108.3 ng/mL (95% CI 65.7-178.5) and 56.7 ng/mL (95% CI 42.3-76.0), respectively (p = 0.02), Table 1. Using linear regression, other statistically significant predictors of plasma FTC concentrations in univariate analyses included creatinine clearance (p = 0.04) and age (p = 0.05). However, treatment arm was the only variable that remained significant in multiple linear regression analyses, p = 0.04. In addition, mean FTC CSF concentrations were higher in the DRV group compared to ATV: 53.5 ng/mL (95% CI 35.3-81.2) versus 32.2 ng/mL (95% CI 22.7-45.5), p = 0.05 (Table 1). CSF:Plasma FTC concentration ratios were similar in both arms: 0.6 (95% CI 0.4-0.7) for ATV and 0.5 (95%

CI 0.4-0.6) for DRV, $p = 0.4$ (Table 1). Plasma and CSF FTC concentrations were significantly correlated in both DRV and ATV arms, $r = 0.9$, $p < 0.0001$ and $r = 0.6$, $p = 0.02$, respectively.

In univariate linear regression models, treatment arm ($p = 0.05$), plasma FTC concentration ($p < 0.0001$, Figure 1a) and sex ($p = 0.03$, Figure 1b) were significant predictors of CSF FTC concentration. Because treatment arm and plasma FTC concentrations exhibited features of collinearity, only plasma FTC concentrations were chosen for multiple linear regression modeling. A two predictor model with plasma FTC concentration and sex explained 70% of the variance in FTC CSF concentrations, $R^2 = 0.7$, ($F = 31.9$), $p < 0.0001$. When holding sex constant, a 10% increase in FTC plasma concentration was associated with a 6.6% increase in CSF FTC concentrations. Holding plasma FTC concentrations constant, female sex was associated with a 30% decrease in CSF FTC concentrations.

CSF HIV-1 RNA

Of the 30 study participants, 6 (20%) had detectable CSF HIV-1 RNA: 2 in the ATV arm and 4 in the DRV arm ($p = 0.7$). Detection of HIV-1 RNA in the CSF was not associated with CSF FTC concentrations in either group (ATV arm: $r = 0.05$, $p = 0.9$; DRV arm: $r = 0.4$, $p = 0.2$).

Discussion

Plasma and CSF drug trough concentrations of FTC and TFV were compared in HIV-1 infected individuals receiving two different PIs: ATV or DRV. In contrast, prior literature has reported random NRTI concentrations in populations receiving different ARV classes.

Plasma and CSF TFV concentrations in this study are consistent with those published previously^{17,18}. Specifically, both Calcagno et al and Best et al reported low CSF TFV concentrations, median 6 ng/mL (IQR 2-8) and 5.5 ng/mL (IQR 2.7-11.3), respectively. TFV may have difficulty penetrating the CSF-brain barrier due to low lipophilicity or affinity for efflux membrane transporters such as MRP 4¹⁹. In addition, mean plasma and CSF FTC concentrations in our study were slightly lower than those reported by Calcagno et al: median 212 ng/mL (IQR 86.5-445.5) and 68 ng/mL (IQR 2.5-98.0), respectively. This may be explained by variation in post-dose sampling: Calcagno et al sampled a median of 15 hours post-dose (IQR 13.8-19.4) while our study sampled at trough times (median 23.8 hours [IQR 23.5 - 24.3]).

Participants in the DRV arm had significantly higher plasma FTC concentrations compared to the ATV arm, even when controlling for possible confounders. One possible explanation is drug-drug interactions. For example, plasma TFV concentrations are increased with boosted PIs: pharmacokinetic studies showed 29% increase in TFV C_{\min} with boosted ATV²⁰ and 37% increased C_{\min} with boosted DRV²¹. While mechanisms for these drug-drug interactions have not been fully elucidated, PI inhibition of efflux transporters on apical renal tubule membranes, including p-glycoprotein, MRP2 and MRP4, is thought to play a role^{22,23}. A similar mechanism may explain the higher plasma FTC concentrations in the DRV arm although little is known regarding effect of DRV on MRP2 or MRP4 transporters.

Our data showed significantly higher CSF FTC concentrations in patients receiving DRV compared to ATV, however this difference was likely driven by high plasma FTC concentrations in the DRV arm. CSF FTC concentrations were strongly associated with plasma FTC concentrations, consistent with the literature¹⁸. Female sex was negatively associated with CSF FTC concentrations. When we compared females (n = 7) to males (n = 23), we found no significant differences in age, race, creatinine clearance, or BMI to explain this finding, however our sample size of females was small, thus limiting our analyses. One theory to explain this sex discrepancy is differential expression of transporters on the brain-CSF and blood-brain barriers between males and females. For example, Schuetz et al²⁴ found decreased p-glycoprotein activity in females compared to males; sex differences in other transporter activity that may influence FTC movement into the CNS, such as MRP-1, have not been studied.

Detection of CSF HIV-1 RNA was not associated with CSF FTC concentrations: all 6 participants with detectable CSF HIV-1 had corresponding CSF FTC concentrations over the *in vitro* IC₅₀ upper limit for FTC antiviral activity in cell culture (18.5 ng/mL)²⁵. However, CSF drug concentrations may not accurately represent brain parenchyma penetration, as drugs can exhibit heterogeneous distribution in the brain due to factors such as changes in the functionality of barriers from HIV infection.

In summary, TFV CSF concentrations were below the limit of quantitation for most participants; it is unlikely that TFV plays a prominent role in virologic control of HIV-1 in the CNS. While 3 of the 4 participants with detectable TFV CSF concentrations were taking DRV, a larger dataset is needed to determine whether TFV CSF concentrations actually differ between arms. In contrast, FTC CSF concentrations differed significantly between treatment arms, largely due to higher plasma FTC concentrations in the DRV arm. To a lesser extent, sex was also a predictor of FTC CSF concentrations. Small sample size and cross-sectional study design were limitations of this study. Longitudinal studies adequately powered to examine the impact and mechanisms of drug-drug interactions and sex differences in ARV reservoir penetration are warranted and could impact clinical practice.

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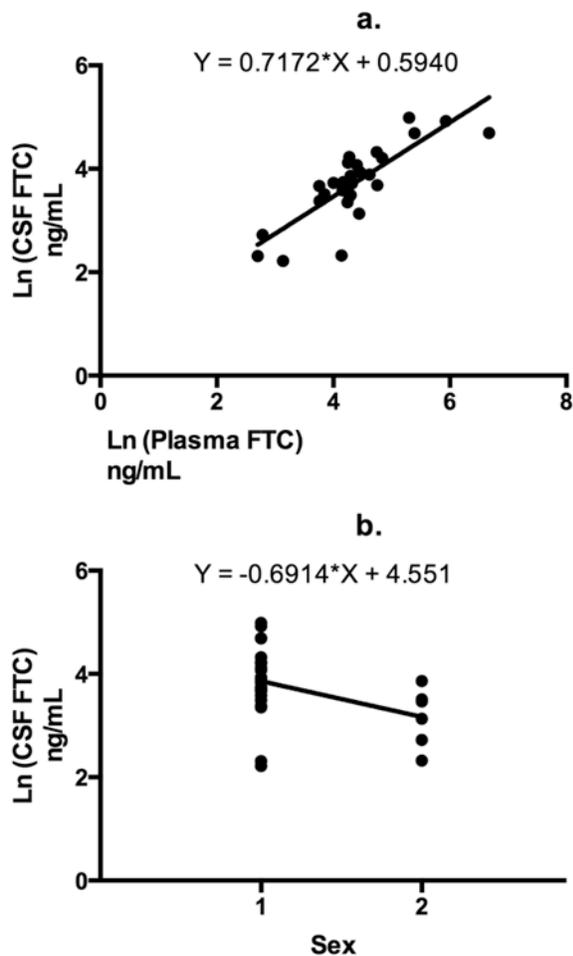


Fig 1. Univariate linear regression analyses are shown for the dependent variable Ln (cerebrospinal fluid [CSF] emtricitabine [FTC] concentration) and significant predictor variables **a)** Ln (Plasma FTC concentration) was a significant predictor of Ln (CSF FTC concentration), $R^2 = 0.7$, $p < 0.0001$ **b)** Sex (1 = male and 2 = females) was a significant predictor of Ln (CSF FTC concentration). There is a negative association between female sex and Ln (CSF FTC concentrations), $R^2 = 0.2$, $p = 0.03$.

Table 1
Drug trough concentrations for tenofovir and emtricitabine

Tenofovir and emtricitabine trough concentrations (C_{min}) in ng/mL			
	Atazanavir arm (n = 15)	Darunavir arm (n = 15)	P value ^a
Tenofovir			
<u>CSF^b</u>			
GM ^c (95% CI ^d)	n/a ^e	n/a	n/a
Median (IQR) ^f	n/a	n/a	
<u>PLASMA</u>			
GM (95% CI)	48.4 (35.4-66.2)	53.2 (39.5-71.5)	ns ^g
Median (IQR)	51.5 (32.7-62.1)	53.1 (47.4-76.0)	
<u>CSF:PLASMA</u>			
GM (95% CI)	n/a	n/a	n/a
Median (IQR)	n/a	n/a	
Emtricitabine			
<u>CSF</u>			
GM (95% CI)	32.2 (22.7-45.5)	53.5 (35.3-81.2) ^h	0.05
Median (IQR)	33.4 (28.6-47.5)	50.0 (39.9-108.9)	
<u>PLASMA</u>			
GM (95% CI)	56.7 (42.3-76.0)	108.3 (65.7-178.5)	0.02
Median (IQR)	69.6 (43.1-73.3)	86.3 (73.7-200.0)	
<u>CSF:PLASMA</u>			
GM (95% CI)	0.6 (0.4-0.7)	0.5 (0.4-0.6)	ns
Median (IQR)	0.7 (0.4-0.9)	0.6 (0.5-0.7)	

^aDrug concentrations and CSF:Plasma Ratios compared between groups using parametric unpaired two-sided two- sample t tests. P values > 0.05 were considered non-significant (ns);

^bCSF = cerebrospinal fluid;

^cGM = geometric mean;

^dCI = confidence interval;

^en/a = not applicable: CSF tenofovir was below limit of quantitation (<5 ng/mL) in 25/29 available samples;

^fIQR = interquartile range, 25th -75th percentile;

^gns = non significant, p > 0.05;

^h14/15 participants in darunavir arm had CSF available for analysis