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LOW CSF Concentrations of the Nucleotide HIV Reverse Transcriptase Inhibitor, Tenofovir

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Abstract

Tenofovir is a nucleotide HIV reverse transcriptase inhibitor whose chemical properties suggest that it may not penetrate into the central nervous system in therapeutic concentrations. The study objective was to determine tenofovir penetration into cerebrospinal fluid (CSF).

CHARTER is a multi-center, observational study to determine effects of antiretroviral therapy on HIV-associated neurological disease. Single random plasma and CSF samples were drawn within an hour of each other from subjects taking tenofovir between October 2003 and March 2007. All samples were assayed by mass spectrometry with a detection limit of 0.9 ng/mL.

183 participants (age 44 ± 8 years; 83 ± 32 kg; 33 females; CSF protein 44 ± 16 mg/dL) had plasma and CSF samples drawn 12.2 ± 6.9 and 11 ± 7.8 hours post-dose respectively. Median plasma and CSF tenofovir concentrations were 96 ng/mL (IQR 47 – 153) and 5.5 ng/mL (IQR 2.7 – 11.3), respectively. Thirty-four of 231 (14.7%) plasma and 9/77 (11.7%) CSF samples were below detection. CSF/plasma concentration ratio from paired samples was 0.057 (IQR 0.03 – 0.1; n=38). Median CSF/wild-type IC₅₀ ratio was 0.48 (IQR 0.24 – 0.98). Seventy-seven percent of CSF concentrations were below the tenofovir wild-type IC₅₀. More subjects had detectable CSF HIV with lower (≤ 7 ng/mL) versus higher (> 7 ng/mL) CSF tenofovir concentrations (29% vs. 9%; p=0.05).

Tenofovir concentrations in the CSF are only 5% of plasma concentrations, suggesting limited transfer into the CSF, and possibly active transport out of the CSF. CSF tenofovir concentrations may not effectively inhibit viral replication in the CSF.

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Keywords

tenofovir; CSF; pharmacokinetics

INTRODUCTION

Combination antiretroviral therapy (cART) can significantly reduce HIV replication, preserve immune function, decrease opportunistic infections and prolong survival.¹ cART may also reduce HIV replication in the central nervous system (CNS), contributing to declines in the incidence of neurologic disorders due to HIV infection.² Despite the success of cART in treating systemic HIV infection and reducing the incidence and severity of HIV-associated dementia (HAD), milder neurocognitive impairment remains prevalent.³⁻⁵

The CNS is one of the sanctuary sites for HIV.⁶ Several studies indicate that HIV replication may persist in the CNS, despite virologic suppression in blood and other tissues.^{7, 8} Long-term viral replication in the brain potentially may lead to damage, with symptoms of cognitive impairment and dementia. One reason for persistent viral replication in the brain may be poor CNS penetration of antiretroviral drugs. Optimal antiretroviral concentrations in the CNS are necessary to limit local HIV replication, contribute to preventing the development of HIV-associated neurocognitive disorders (HAND), and prevent drug-resistant viral strains in the CSF, which have the potential to re-infect the peripheral tissues, including blood, leading to overall treatment failure.⁹

Tenofovir is an FDA-approved nucleotide reverse transcriptase inhibitor used in combination drug treatment in HIV-infected patients.¹⁰⁻¹² Tenofovir disoproxil fumarate (tenofovir DF; formerly known as PMPA prodrug, 9-[(R)-2-[[bis[(isopropoxycarbonyl)oxy] methoxy]phosphinyl] methoxy] propyl]adenine fumarate) is an orally bioavailable prodrug of tenofovir, an acyclic nucleotide analog of adenosine monophosphate with activity *in vitro* against HIV type 1 (HIV-1) and HIV-2.^{13, 14} After oral absorption, tenofovir DF is rapidly converted to tenofovir by serum and tissue esterases in the intestine and systemic circulation.¹⁵ Intracellular phosphorylation yields the active metabolite, tenofovir diphosphate, which is a competitive inhibitor of HIV-1 reverse transcriptase and causes chain termination of the nascent viral cDNA.¹⁶ After oral administration of tenofovir DF, tenofovir is distributed to most tissues with the highest concentrations occurring in the kidney, liver and intestines.

Nucleosides seem to penetrate into the CNS better than protease inhibitors and have been useful in treating patients with HAD.¹⁷ However, the physicochemical properties of tenofovir are different from those of other nucleosides, and suggest that penetration in the brain may be poor.^{18, 19} A study in guinea pigs found that tenofovir was transported across the blood-CSF barrier, but not the blood-brain barrier (BBB) as evidenced by the significantly higher tenofovir concentration in the choroid plexus and CSF as compared to that of the cerebrum, cerebellum, pituitary gland and cerebral capillary endothelial cells.²⁰ This differential transport may be related to the limited transport of tenofovir by P-glycoprotein²¹ and more significant transport of tenofovir by the multidrug resistance associated protein family and organic anion transporters,²²⁻²⁵ as P-glycoprotein is minimally expressed at the blood-CSF barrier.²⁶ The limited inhibition of P-glycoprotein by tenofovir may also explain the limited BBB penetration compared to other antiretrovirals with inhibitory activity on P-glycoprotein.²⁷ Moreover, tenofovir has physicochemical similarities to foscarnet and adefovir, which penetrate the CNS poorly.¹⁸

The entry of an antiretroviral drug into the brain is dependent upon its ability to cross the BBB as tight junctions between brain endothelial cells restrict entry by paracellular diffusion.¹⁹ Tenofovir is 99% unbound to plasma proteins,¹⁶ and thus should penetrate across membranes, as only unbound drug is available to passively diffuse. However, highly polar drugs with relatively low lipid solubility—such as tenofovir—do not readily undergo passive diffusion through the endothelial cell membranes. Thus, its movement across the BBB is dependent on active transport systems.²⁸

Tenofovir is a widely used component of current antiretroviral regimens. In the United States, treatment guidelines recommend the use of tenofovir plus lamivudine or entricitabine as the preferred option for the double nucleoside/tide component of initial antiretroviral therapy.²⁹ Because tenofovir concentrations in CSF of humans have not been evaluated, we measured plasma and CSF concentrations of tenofovir in HIV infected subjects to explore its CNS pharmacokinetics and pharmacodynamics.

METHODS

Participants

The CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study is a six-center, observational cohort study designed to determine the effects of potent antiretroviral therapy on HIV-associated neurological disease. All research was approved by institutional review boards at each site. As part of the CHARTER study, single plasma and CSF samples were drawn at biannual study visits between October 2003 and March 2007. Plasma/CSF sample pairs were drawn within an hour of each other. The analysis described here involved 183 HIV-infected subjects randomly selected from the CHARTER cohort who were taking tenofovir and had plasma and/or CSF samples stored in the sample repository. All the participants were taking tenofovir disoproxil fumarate 300 mg by mouth once daily for a median (interquartile range, IQR) of 8.5 (IQR 2.5–18.4) months at the time of first sampling. Data from one to three study visits were included for each subject in the analysis. Fifty-five pairs of CSF and plasma samples, and an additional 176 plasma and 22 CSF samples were analyzed.

Measurements

Samples were assayed by liquid chromatography-mass spectrometry. Validation of the plasma assay using calibration standards showed precision, with a less than 8% coefficient of variation between different assay runs, and accuracy, with a less than 6% deviation from the known standard concentrations. Calibration standards ranged from 0.9 to 500 ng/mL, with an assay quantitation limit of 0.9 ng/mL. CSF validation inter-assay precision and accuracy were within 7% at 0.9 ng/mL, and within 8% for other controls.

Analyses

Samples drawn more than 48 hours after a reported tenofovir dose were excluded from the analysis (2 CSF and 3 plasma samples). Non-parametric statistics were used to summarize the data. Pearson's correlation measured the association between plasma and CSF tenofovir concentrations. Associations between CSF tenofovir concentration and CSF protein were explored as a crude surrogate for disruption of the blood-CSF barrier. CSF and plasma concentrations were compared to the lowest reported tenofovir 50% inhibitory concentration (IC₅₀), defined as the drug concentration at which wild-type HIV viral replication is inhibited to 50% of the replication levels seen in drug-free media (11.5 ng/mL).¹⁶ Wilcoxon rank-sum tests compared tenofovir plasma or CSF concentrations in subjects with and without detectable plasma or CSF HIV RNA. In subjects with detectable CSF HIV RNA (> 50 copies/mL), Fisher Exact Test assessed the proportion of subjects with tenofovir ≤ 7 ng/

mL in the CSF versus the proportion of subjects with tenofovir > 7 ng/mL in the CSF. The cut-off value of 7 ng/mL was selected post-hoc by recursive partitioning.

RESULTS

Subjects were mostly male [150/183 (82%)], averaged 44 ± 8 years of age, weighed a mean of 81 ± 16 kg, and had a mean CSF protein level of 44 ± 16 mg/dL. The median (IQR) plasma HIV RNA and CD4 cell counts were <50 (<50 – 340) copies/mL and 426 (252 – 616) cells/mm³. CSF HIV RNA levels were suppressed to <50 copies/mL in 146 of 183 persons (80%) and plasma levels were similarly suppressed in 94 of 183 (51%). Ninety-four subjects took either lamivudine or emtricitabine, 42 were taking efavirenz, and 122 were on a ritonavir-boosted protease inhibitor, most commonly atazanavir (n=73) and lopinavir (n=43). All of the subjects who had CSF samples measured were taking a protease inhibitor-based regimen.

The median plasma concentration of tenofovir in 231 plasma samples was 96 ng/mL (IQR 47 – 153) at a mean post dose interval of 12.2 ($\pm$ sd 6.9) hours (Table 1 and Figure 1). The median CSF concentration of tenofovir in 77 CSF samples was 5.5 ng/mL (IQR 2.7 - 11.3) at a mean post dose interval of 11 ($\pm$ sd 7.8) hours. Tenofovir concentrations in 9 out of 77 total (11.7%) CSF and in 34 out of 231 total (14.7%) plasma samples were undetectable (< 0.9 ng/mL). Increasing CSF tenofovir concentrations were not strongly correlated with increasing CSF protein concentrations ($r^2=0.05$, $p=0.07$).

For the 55 paired CSF and plasma samples, the CSF concentration was below detection in 6 samples (with measurable concentrations in the plasma ranging from 26.1 to 319.8 ng/mL), the plasma concentration was undetectable in 10 samples (with measurable concentrations in the CSF ranging from 1.2 to 32 ng/mL), and one subject had undetectable concentrations in both plasma and CSF. The median CSF to plasma tenofovir concentration ratio in the 38 paired specimens with measurable tenofovir in both plasma and CSF was 0.057 or 5.7% (IQR 0.03 – 0.1) after a median of 6.1 months of therapy. The median (IQR) time between plasma and CSF sample draw times was 0.4 (0.3 – 0.7) hours. The ratio did not change with time post-dose (Figure 2). One subject had a CSF concentration approximately 84% of the corresponding plasma concentration (CSF = 13.2 ng/mL and plasma=15.7 ng/mL). The next highest ratios were in the 30 – 40% range (n=2), and all other subjects had ratios of CSF to plasma concentrations of less than 21%. Plasma and CSF concentrations were not significantly correlated ($r^2=0.04$, $p=0.2$). Subjects with CSF HIV RNA > 50 copies/mL (n=8) had median (IQR) CSF to plasma concentration ratios of 0.045 (0.027 – 0.123). Subjects with undetectable CSF HIV RNA (n=30) had CSF to plasma concentration ratios of 0.064 (0.034 – 0.098, $p>0.1$).

From the 77 subjects with measured CSF tenofovir concentrations, CSF HIV RNA concentrations were available for 72. Fifty-six of these 72 subjects (78%) had CSF HIV RNA $\leq$ 50 copies/mL, while the remaining 16 of 72 (22%) had detectable CSF HIV. Rates of detectable CSF HIV were increased in those with tenofovir concentrations in the CSF below 7 ng/mL [14/49 (28.6%)] compared to those with higher concentrations [2/23 (8.7%)], $p=0.05$ by a one-tailed test and $p=0.07$ by a two-tailed test].

Fifty-nine of 77 (77%) CSF concentrations did not exceed the 50% inhibitory concentration (IC₅₀) for tenofovir (11.5 ng/mL for wild-type virus).¹⁶ The tenofovir CSF concentration/IC₅₀ ratio was a median of 0.48 (IQR 0.24 – 0.98). In subjects with CSF HIV RNA > 50 copies/mL (n=16), median CSF tenofovir concentrations were 3.5 ng/mL (IQR 1.6 – 6.1). In subjects with undetectable CSF HIV RNA (n=56), median CSF tenofovir concentrations were 5.6 ng/mL (IQR 2.9 – 11.5 ng/mL, $p=0.097$).

Forty of 231 (17%) plasma tenofovir concentrations were below the IC₅₀ value of 11.5 ng/mL. The median plasma tenofovir concentration/IC₅₀ ratio was 8.3 (IQR 4.1 – 13.3). The median plasma concentration in the 101 samples with plasma HIV RNA > 50 copies/mL was 77.5 ng/mL (IQR <0.9 – 125.8). The median tenofovir concentration in the 130 plasma samples with undetectable plasma HIV RNA was higher (104 ng/mL, IQR 61.1 – 175, p=0.002).

DISCUSSION

In this cohort of subjects, the median concentration of tenofovir in CSF was only 5% of that in plasma, levels of penetration (CSF to plasma concentration ratio) that are much lower than those observed for the nucleoside reverse transcriptase inhibitors. Nucleoside antiretroviral drugs such as abacavir, didanosine, lamivudine and stavudine have CSF-to-plasma concentration ratios of approximately 15 – 30%.³⁰⁻³⁴ Similarly low CNS penetration of tenofovir was found in guinea pigs, who had penetration in the brain tissues of less than 5%, although CSF penetration in guinea pigs was higher than that seen in humans in this study.²⁰ Zidovudine has a median 65% penetration, although variability between patients is high.^{30, 35} Tenofovir does show higher penetration than most protease inhibitors studied, with the exception of indinavir (which has lower protein binding than most protease inhibitors).³⁶ Protease inhibitor CSF/plasma concentration ratios are usually in the range of 1% or less.^{37, 38} Thus, the penetration of this nucleotide agent appears to be intermediate between the penetration of protease inhibitors and nucleoside agents.

More than three-quarters of tenofovir concentrations in the CSF were below the lowest reported tenofovir IC₅₀ of 11.5 ng/mL.¹⁶ These low concentrations were unable to reliably suppress HIV replication in the CNS. Patients with CSF tenofovir concentrations of 7 ng/mL or less were more likely to have 50 HIV copies or more in CSF compared to those with CSF tenofovir levels higher than 7 ng/mL. Lower plasma tenofovir concentration was associated with significantly increased likelihood of detectable viral replication in the plasma. While higher plasma and CSF concentrations are associated with suppression of viral replication in the corresponding compartments, plasma and CSF concentrations were not strongly associated with one another. This finding suggests that processes other than simple passive diffusion govern the penetration of tenofovir into the CSF.

Both the blood-CSF and the blood-brain barriers restrict passage of many small molecules into the CNS. The tight junctions between epithelial cells of the choroid plexus and the brain capillaries largely prevent paracellular diffusion, although the tight junctions of the blood-CSF barrier are quantitatively leakier than those of the blood-brain barrier. Physicochemical characteristics of the parent compound, tenofovir, may prevent transcellular passage (either passive or facilitated) into the CSF. The molecule has two hydroxyl groups, which confer two negative charges at physiologic pH. These negative charges may repel the molecule from the negatively charged surface of the plasma membrane. Further, tenofovir does not have adequate lipophilicity to passively diffuse through the lipophilic components of cell membranes. Because of its high polarity and low lipophilicity, tenofovir likely needs to rely on active transport processes to enter the CSF or CNS.

Tenofovir has been shown to be a substrate for transporter enzymes that contribute to the blood-brain or blood-CSF barriers, including organic anion transporters (OAT) 1 and 3, and multidrug resistance associated proteins (MRP) 2 and 4 but not permeability glycoprotein (P-glycoprotein).^{21-24, 39} These transporters may exclusively efflux tenofovir out of the CSF or brain, or may carry tenofovir both into and out of the central compartment, but with efflux processes being the dominant pathways. A limitation of this study was that the penetration of tenofovir across the blood-brain barrier was not measured. Rather, the CSF

penetration was used as an accessible surrogate marker to estimate brain penetration. This may be important due to the differential expression of P-glycoprotein and other transporters at those different interfaces and the fact that tenofovir is not a P-glycoprotein substrate.^{21, 26} This differential expression may lead to limited efflux at the blood-brain barrier interface and more efflux at the blood-CSF barrier interface resulting in relatively higher concentrations in brain parenchyma, which unfortunately cannot be measured *in vivo* in humans.

CSF tenofovir concentrations in this study were measured in subjects taking concomitant protease inhibitors, particularly ritonavir-boosted protease inhibitors. Ritonavir and several other protease drugs are known inhibitors of P-glycoprotein. Protease inhibitor effects on other transport enzymes, such as OAT 1 and 3 and MRP 2 and 4 which are important for tenofovir transport, are less well-characterized. Ritonavir may inhibit MRP2⁴⁰ and OAT3⁴¹, and does not appear to affect OAT1 or MRP4⁴¹, although data are not conclusive and are limited to mainly *in vitro* studies. If protease inhibitors do affect the transport proteins for which tenofovir is a substrate, then concomitant administration of tenofovir with protease drugs may affect the penetration of tenofovir into the central nervous system.

Interestingly, one subject with paired plasma and CSF samples had nearly equal tenofovir concentrations in the plasma and in the CSF. One potential explanation could be that the subject has a genetic alteration in the active transport process(es) governing the transport of tenofovir into or out of the central nervous system. Also noted among the subjects with paired CSF and plasma samples were undetectable concentrations in either the plasma or the CSF compartments individually, highlighting the high variability of drug exposure between subjects. One subject had undetectable concentrations in both fluids, suggestive of poor adherence. Subjects with undetectable CSF HIV RNA had ~ 40% higher CSF to plasma ratios (6.4%) than subjects who had CSF HIV RNA > 50 copies/mL (4.5%), though these values were not significantly different.

Tenofovir has been measured in other physiologic compartments. Plasma tenofovir concentrations derived from cord blood in women at steady-state on standard tenofovir doses was 96% of maternal plasma concentrations.⁴² Tenofovir freely distributes across the placenta. Tenofovir has also been measured in semen and cervicovaginal fluid. The median semen-to-plasma concentration ratio in 15 subjects was 3.3 in a study by Lowe and colleagues.⁴³ The median plasma tenofovir concentrations in that study were 112 ng/mL, similar to the value found in this study of 96 ng/mL, while the semen concentrations were 250 ng/mL. In cervicovaginal fluid, concentrations range from 75% to over 500% of plasma concentrations.^{44, 45} Several possible explanations could account for the much better penetration of tenofovir into genital tract and fetal compartments. Tenofovir may be able to better diffuse paracellularly in those anatomic sites that do not have the tight junctions between endothelial cells. Also, these other anatomic sites may have different distributions or activity of transporter enzymes that affect tenofovir disposition.^{46, 47}

While the ability of tenofovir to penetrate the CSF is limited, the concentration of the active moiety of tenofovir (intracellular tenofovir diphosphate), was not measured in the cells of the CSF or plasma in this study. Because tenofovir is activated intracellularly, the measurement of the plasma or CSF IC₅₀ of the inactive compound, tenofovir, may be less strongly correlated with viral suppression than are the IC₅₀s of drugs that are directly active such as protease inhibitors. However, the tenofovir IC₅₀ can be assumed to approximate drug potency, recognizing that a certain amount of extracellular inactive drug is in equilibrium with the amount of intracellular drug, which is converted to the active compound. This is supported by our findings in both plasma and CSF of a positive

relationship between increasing tenofovir concentrations and a higher likelihood of viral suppression.

While seemingly ineffective tenofovir CSF concentrations were observed in a majority of subjects, only 20% had detectable CSF viremia. This can be explained by several reasons. First, subjects were taking other antiretrovirals that may have been effective in the CSF. Second, HIV in CSF derives from sources both within and outside of the central nervous system. Tenofovir's effectiveness outside the nervous system could then still be contributing to suppression of that source of HIV. Finally, the IC_{50} in macrophages, the primary target cell for HIV in the brain, may be lower than the IC_{50} in lymphocytes.

A limitation of this study is that the CSF/plasma concentration ratio is a dynamic measure, and single ratio measurements may not accurately estimate exposure over time in this sanctuary site. Drugs with very different elimination half-lives in the plasma and CSF will have CSF/plasma ratios that differ markedly depending on time post dose. Therefore, paired samples must be drawn in close time proximity to one another to estimate an accurate ratio at that particular time post-dose. In this study, paired plasma and CSF samples were all drawn within 42 minutes or less of one another to obtain an accurate ratio at that particular time post-dose. The pairs of samples from this study were drawn at a wide range of post-dose sampling times between subjects up to 48 hours after a dose. The ratio did not vary with time-post dose between subjects. Only a modest change in plasma concentrations and very little change in CSF concentrations over the dose interval were observed, coinciding with the lack of correlation between the ratio and time post-dose. However, paired samples within a subject at different post-dose times may provide a more accurate estimate of tenofovir CSF to plasma ratios.

Despite these limitations, the low tenofovir CSF penetration observed in these subjects is consistent with CNS animal data and with predictions based on physicochemical characteristics of tenofovir. Our finding that higher tenofovir concentrations occurred in samples with suppressed virus indicates that tenofovir CSF penetration (or lack thereof), may be important to antiviral activity and clinical effects. Because plasma and CSF tenofovir concentrations are not related, increasing plasma exposure (by increasing dose, for example), may not reliably increase tenofovir CSF concentrations. Our data suggest that tenofovir taken concomitantly with protease inhibitors may not provide protection against viral replication in the CSF.

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Figure 1. Tenofovir Concentrations versus Time after Dose
Closed circles are tenofovir plasma concentrations, and open circles are tenofovir CSF concentrations.

Figure 2. CSF/Plasma Ratios versus Time after Dose

Ratios of detectable concentrations of paired plasma and CSF concentrations versus plasma sample time after dose, with linear regression line. Median (IQR) time between plasma and CSF sample collection was 0.4 (0.3 – 0.7) hours. Ratios varied widely among patients and showed no clear change over the dosing interval.

Table 1

Tenofovir Concentrations

	Plasma	CSF	CSF/Plasma Ratio ^I
Number of Samples	231	77	38
Tenofovir Concentration (ng/mL)			
Median	95.5	5.5	0.057
IQR	46.9 – 153.2	2.7 – 11.3	0.03 – 0.1
Range	< 0.9 – 859.7	< 0.9 – 38.5	0.004 – 0.84

^I Ratios are reported only for subjects with tenofovir above the detection limit in both plasma and CSF samples; this subset was selected from a total of 55 pairs.