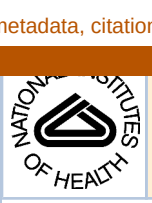

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The Pharmacokinetics and Viral Activity of Tenofovir in the Male Genital Tract

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Abstract

Objective—To measure tenofovir (TFV) concentrations in the male genital tract (GT) after single and multiple doses of tenofovir disoproxil fumarate (TDF) and evaluate the HIV-1 RNA response to monotherapy.

Design and Methods—A pharmacokinetic study of blood plasma (BP) and GT TFV concentrations in 9 men was conducted after 1 and ≥14 doses of TDF. TFV concentrations were measured by validated high-performance liquid chromatography–ultraviolet or tandem mass spectrometry methods, and HIV-1 RNA was measured using Roche (Roche Molecular Systems, Branchburg, NJ) or bioMerieux (bioMerieux, Durham, NC) kits.

Results—TFV GT concentrations were 4.4-fold ± 5.1-fold higher than BP after dose 1 and 5.1-fold ± 6.8-fold higher than BP after dose 14. Intracellular GT TFV-diphosphate concentrations were 9.4-fold higher than BP after dose 1 and 17.5-fold ± 22.6-fold higher after dose 7. After 14 days of TDF monotherapy, HIV-1 RNA decreased by 0.9 $\log_{10}$ copies/mL in blood and 1.0 $\log_{10}$ copies/mL in the GT.

Conclusions—High TFV concentrations were achieved rapidly in the GT of all subjects after single and multiple doses and potently reduced BP and GT HIV-1 RNA levels.

Keywords

antiretroviral therapy; HIV; genital tract; pharmacokinetics; sexual transmission

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Approximately 40 million people worldwide are currently living with HIV/AIDS, most of whom have been infected through sexual contact.[1] The risk of sexual transmission of HIV increases with increasing HIV RNA concentrations in the genital tract (GT) of men and women.[2-4] Evidence also suggests that the male GT may serve as a reservoir for HIV replication.[5] Thus, antiretrovirals (ARVs) that effectively penetrate into the genital compartment may reduce viral replication locally, reduce the risk of sexual transmission of HIV, and assist in decreasing the development of drug-resistant virus.

Tenofovir disoproxil fumarate (TDF) is the orally bioavailable form of tenofovir (TFV), which is converted intracellularly to TFV-diphosphate, a potent inhibitor of HIV reverse transcriptase. TFV has been shown to prevent simian immunodeficiency virus (SIV) infection in monkey models after vaginal inoculation. Early intervention with subcutaneous TFV (≤36 hours after vaginal inoculation with HIV-2) was effective in preventing infection in macaques.[6] These data suggest that TFV may have an active role in pre-exposure prophylaxis (PREP) and postexposure prophylaxis (PEP) regimens, which may be explained by TFV's ability to penetrate the female GT quickly and effectively.[7] However, the effectiveness of TFV to penetrate the male GT and its capacity to decrease viral shedding in semen are currently unknown.

In this study, we describe first-dose and steady-state extracellular drug exposure to TFV in the GT as compared with blood plasma (BP), evaluate intracellular drug exposure of TFV-diphosphate, and measure the ability of TFV to suppress GT HIV-1 RNA acutely in individuals on a short course of TDF monotherapy or using TDF to intensify an incompletely suppressive ARV regimen.

METHODS

Study Participants

Subjects were recruited from the Infectious Disease Clinic at the University of North Carolina (UNC) at Chapel Hill between January 2003 and May 2005. Men were eligible for enrollment if they were HIV-1 infected, ≥18 years of age, and had a plasma HIV-1 RNA level >200 copies/mL. Subjects were not currently taking any ARV medications or were on a stable yet incompletely suppressive ARV regimen with no plans for altering that regimen for at least 1 month. Potential participants were excluded if they were unable to abstain from sexual intercourse for 48 hours before study visits, had an estimated drug adherence rate of, <80%, had a creatinine clearance (Cl_{Cr}) <60 mL/min, weighed ≤50 kg, had an active genital or systemic infection, or had a hematocrit measurement <30%.

All participants gave written informed consent before study participation. This protocol was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill.

Study Design and Procedures

All inpatient and outpatient visits were performed at the Verne S. Caviness General Clinical Research Center located within the UNC Memorial Hospital. Subjects were required to continue their current stable ARV therapy, with the addition of TDF at a dosage of 300 mg/d for 28 days or to initiate TDF monotherapy at a dosage of 300 mg for 14 days before being placed on highly active antiretroviral therapy (HAART). For the duration of the study, subjects recorded the exact time of TDF administration on a study drug diary card and were interviewed as to their medication adherence at each study visit.

All subjects were advised to abstain from sexual intercourse for 48 hours before each visit. Subjects were expected to donate semen with a matching blood sample at each of the outpatient

visits. On days 1, 3, 5, and 7, 24-hour postdose blood plasma (BP) and GT samples were taken. During the intensive pharmacokinetic (PK) visit on day 14, blood samples were taken at 0, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after dosing, whereas GT samples were taken at 0 and 12 hours. Men who were receiving other ARVs in addition to TDF also had PK samples taken at times 0, 4, 10, and 24 hours after dosing on 4 separate outpatient visits anytime from days 14 to 28. At the end of the dosing interval, and just before administering the dose within which a seminal sample would be collected, men were asked to empty and discard the contents of the seminal compartment by masturbation and ejaculation.

Sample Processing

Blood samples used for extracellular analysis were collected in ethylenediaminetetraacetic acid (EDTA)–containing tubes and were kept on ice for no more than 60 minutes before plasma was separated by centrifugation at 2600 rpm for 15 minutes at 4°C. Plasma was aliquoted into cryovials and frozen at −80°C until analysis. Peripheral blood mononuclear cells (PBMCs) were isolated using CPT (Becton-Dickinson, Franklin Lakes, NJ) tubes containing sodium citrate. The CPT tube, containing 8 mL of whole blood, was centrifuged at 2700 rpm for 20 minutes at room temperature. The contents of the CPT tube, isolated above the gel, were poured into a 15-mL conical tube. Approximately 2 mL of phosphate-buffered saline (PBS) was added to the CPT tube to resuspend any remaining monocytes and was then added to the 15-mL conical tube. PBS was added to the conical tube to bring the total volume to 15 mL. The conical tube was then centrifuged at 1300 rpm at room temperature for 10 minutes. The supernatant was discarded, and the cell pellet was resuspended in 100 μL of PBS and placed on ice. Mononuclear cells were counted using a hemocytometer with trypan blue exclusion. Remaining cells were lysed for approximately 15 minutes with 200 μL of 100% methanol. Cell debris was removed by centrifugation at 4°C for 15 minutes at 3000 rpm. The supernatant was transferred to a cryovial and stored at −80°C until analysis.

Semen samples were left to liquefy at room temperature for 30 minutes and then centrifuged for 20 minutes at between 2000 and 2600 rpm in a conical tube at 4°C. Seminal plasma was separated into 75-μL and 200-μL aliquots for HIV-1 RNA and drug concentration analyses and stored at −80°C. The cell pellet was resuspended in 8 mL of PBS, layered onto 4 mL of Percoll (Sigma-Aldrich, St. Louis, MO), and centrifuged at 2000 rpm for 20 minutes at room temperature. The seminal mononuclear cells found at the interface between the PBS and Percoll (top 2 layers) were carefully removed, placed in 10 mL of PBS, and centrifuged, and the cell pellet was resuspended to 100 μL. Mononuclear cells were counted using a hemocytometer and trypan blue exclusion. Cells were lysed for approximately 15 minutes with 200 μL of 100% methanol. Cell debris was removed by centrifugation for 15 minutes at 3000 rpm at 4°C. The supernatant was aliquoted into cryovials and stored at −80°C until analysis.

Tenofovir Quantification

Extracellular TFV concentrations in BP and seminal plasma (SP) were quantitated at the UNC Center for AIDS Research (CFAR) Clinical Pharmacology and Analytical Chemistry Core using validated liquid chromatography (LC)/ultraviolet (UV)[8] methods. Intraday and interday precision were 3.7% and 5.2%, respectively, and the lower limit of quantification (LLOQ) was 10 ng/mL. Cellular lysates were sent to Gilead Sciences (Durham, NC) for quantification of TFV-diphosphate using a validated LC–tandem mass spectrometry (MS/MS) method.[9] Within-run accuracy (% error) values ranged from 1.3% to 14.5%, and precision (% relative standard deviation [RSD]) values ranged from 0.01% to 10.9% for TFV-diphosphate, carbovir triphosphate, and lamivudine triphosphate. Between-run accuracy (% error) values ranged from 20.7% to 13.9%, and precision (% RSD) values ranged from 2.5% to 7.9% for the 3 compounds. All calculated values for quality control samples fell within industry guidelines

of 20% for the LLOQ and 15% for the upper limit of quantitation. The LLOQ was 15 fmol/10^6 cells.

HIV-1 RNA Determinations

BP HIV-1 RNA was quantified with the Roche Amplicor HIV-1 Monitor UltraSensitive assay (version 1.5; Roche Molecular Systems, Branchburg, NJ). The quantification of HIV-1 RNA in the SP was performed with the bioMerieux NucliSens HIV-1 QT assay (bioMerieux, Durham, NC).[10] The limits of quantitation for the Roche Amplicor HIV-1 Monitor UltraSensitive and the bioMerieux NucliSens HIV-1 QT assay are 50 copies/mL and 400 copies/mL, respectively. All BP and GT samples that were determined as having an HIV-1 RNA level >1000 copies/mL at day 14 were genotyped to assess whether the K65R mutation was present. The determination of HIV-1 RNA and viral genotype was performed by the UNC CFAR Virology Core.

Data Analysis and Statistical Methods

Blood and GT results were grouped according to time after TDF ingestion. Mean extracellular and intracellular GT/BP concentration ratios were calculated among matched pairs in all subjects. The area under the time-concentration curve ($AUC_{0-\tau}$) was calculated for BP using WinNonlin (version 4.0.1; Pharsight, Mountain View, CA). A comparison of BP and GT results was calculated using STATA (version 8.0; StataCorp LP, College Station, TX). HIV-1 RNA values below the limit of quantification in GT and BP were assigned values of 399 copies/mL and 49 copies/mL, respectively. The mean changes in BP and GT HIV-1 RNA level and blood CD4 cell count were calculated between day 14 and baseline. TFV extracellular and intracellular concentrations are expressed as mean (ng/mL for extracellular concentrations and fmol/10^6 cells for intracellular concentrations) with the corresponding SD, unless otherwise stated.

RESULTS

Study Population

Nine men were enrolled in the study. Eight men received TDF as monotherapy, and 1 man received TDF intensification added to a nonsuppressive ARV regimen. Non-Hispanic blacks accounted for most of the subjects (67%). Subject demographics are described in Table 1.

Extracellular Tenofovir Concentrations in the Genital Tract

Figure 1 displays the BP and SP TFV concentrations from a first-dose to steady-state condition. After a single dose, TFV rapidly and effectively penetrated into SP. On day 1, the extracellular TFVGT/BP concentration at 24 hours (C_{24h}) ratio was 4.4 ± 5.1. At steady state, TFV continued to penetrate into SP effectively. The extracellular TFV GT/BP concentration ratio at the end of the dosing interval was 5.1 ± 6.8.

Intracellular Tenofovir Concentrations in the Genital Tract

Seminal mononuclear cell yields ranged from 1.0×10^5 to 5.2×10^6. Because of low mononuclear cell yields (<10^6 cells per sample), TFV-diphosphate concentrations could not be detected in 6 of the 9 men. Although an association was noted between frequency of semen sample collection and seminal volume ($r^2 = 0.75$), no association was found between seminal volume or frequency of semen collection and seminal leukocyte yield ($r^2 = 0.04$) that might explain these undetectable concentrations. For those in whom intracellular TFV-diphosphate could be detected, mean GT and PBMC concentrations were 1544 ± 2079 and 118 ± 59 fmol/10^6 cells, respectively. Mean GT/BP C_{24h} ratios after the first, fifth, and seventh doses of TDF (the first week of therapy) were 9.4, 8.0, and 17.5, respectively.

Virology and Immunology

For men receiving 14 days of TDF monotherapy and who had complete paired BP and GT samples, HIV-1 RNA levels were reduced by a geometric mean of 0.9 $\log_{10}$ copies/mL (BP) and 1.0 $\log_{10}$ copies/mL (GT), respectively. For these men, the median HIV-1 RNA levels after 14 days in BP and GT were 866 copies/mL and <400 copies/mL, respectively. A mean increase in CD4 count of 31 ± 89 cells/μL (range: 62 to 150 cells/μL) was observed over 14 days in subjects receiving TDF monotherapy. The HIV-1 RNA in the GT was initially suppressed (<400 copies/mL) and remained so for the duration of the study in the 1 man on TDF intensification. His HIV-1 RNA level in BP was 366 copies/mL at baseline and 709 copies/mL by day 28 of TDF intensification. No K65R mutations were seen in any of the BP (n = 4) or GT (n = 1) samples having an HIV-1 RNA level >1000 copies/mL at the end of the study period.

DISCUSSION

The effectiveness of ARV therapy for the prevention of sexual transmission of HIV may rely on the capacity of ARVs to accumulate in sanctuary sites, such as the GT,[11,12] allowing for maximal suppression of HIV RNA replication. In this study, TFV successfully and rapidly penetrated the GT with concentrations reaching up to 19.1 times those concurrently found in BP. In the 3 subjects in whom intracellular concentrations of TFV-diphosphate, TDF's active metabolite, were successfully measured, these concentrations were also higher in seminal mononuclear cells compared with PBMCs. This is an important finding and not uniform across the nucleoside/nucleotide reverse transcriptase inhibitors that have been investigated to date.

Only zidovudine and lamivudine triphosphate have been previously measured in seminal mononuclear cells. Although zidovudine and lamivudine achieve extracellular GTexposures 4- to 6-fold higher than BP, intracellular triphosphate concentrations are lower (zidovudine) or similar (lamivudine) to concentrations in PBMCs.[13] This divergence in exposure may be attributable to different kinase activities or cellular activation in the 2 compartments, since zidovudine is preferentially phosphorylated in activated cells and lamivudine is preferentially phosphorylated in quiescent cells.[14] In contrast, TFV is able to be phosphorylated in activated and resting cells,[15] which may result in higher TFV-diphosphate concentrations in the male GT. The extracellular and intracellular GT concentrations for TFV are the highest, relative to BP, among the ARVs investigated to date.[13,16-21]

Two important characteristics are questioned when evaluating an ARV agent's potential role in decreasing the infectiousness of HIV-1: the antiviral effect and the evolution of resistance within the GT. This is the first study to evaluate the antiviral response in the human GT with TDF monotherapy. We found an equally potent virologic response (approximately a 1.0-log decline in HIV-1 RNA) in the GT and BP in subjects after receiving 14 days of TDF monotherapy. This potent and rapid compartmental virologic response suggests that the probability of further sexual transmission of HIV from men to their partners is reduced, because HIV-1 RNA concentrations within the GT serve as a surrogate marker for infectiousness.[2,3,16] Second, ARV monotherapy is traditionally associated with the development of resistance. The 4 patients with BP HIV-1 RNA levels >1000 copies/mL and the 1 patient with a GT HIV-1 RNA level >1000 copies/mL after 14 days of monotherapy did not develop the K65R mutation, however, which is consistent with a previous investigation that dosed TDF monotherapy for 21 days.[22]

Only 1 other study (ACTG 850) has characterized drug concentrations and viral response to ARV monotherapy in BP and GT secretions.[16] This evaluation of 19 men receiving amprenavir monotherapy at a dose of 1200 mg twice daily obtained single random samples of BP and GT secretions for drug concentration analysis. Eight men responded in BP and SP, 8 of 19

responded in SP only, and 3 of 19 did not respond in SP or BP. Differences in sampling strategies for PK and HIV-1 RNA response preclude making comparisons with the current study, however.

In summary, the increased concentration of TFV in its active form may explain the potent and rapid reduction of HIV-1 concentration observed in the male GT. Because HIV-1 RNA concentrations in BP may not be as reliable a predictor of RNA concentrations in the GT,[23] we believe these results to be important as they relate to the potential of TDF to reduce the sexual transmission of HIV.

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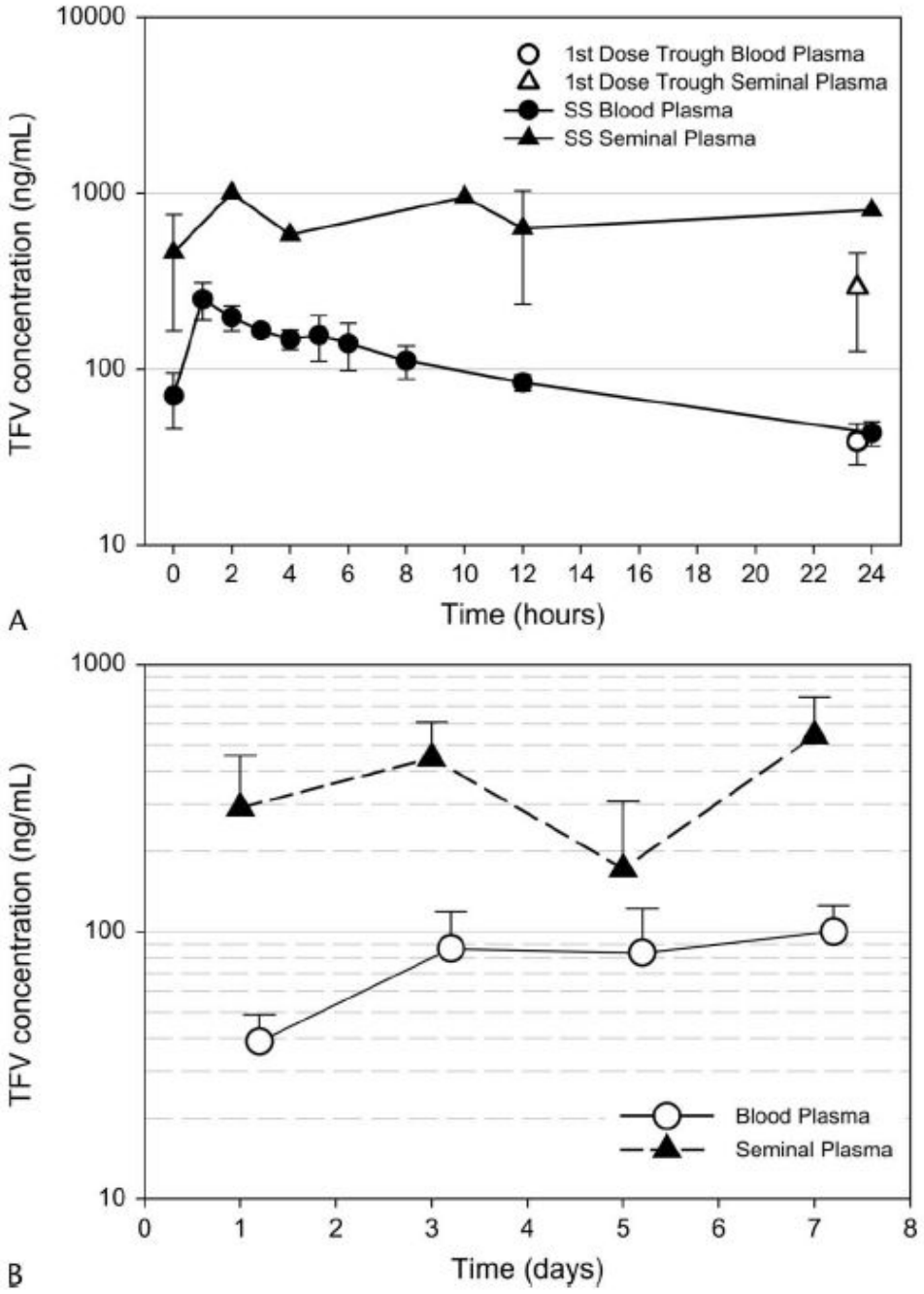

FIGURE 1.

A, TFV concentrations in SP and BP on day 1 and steady state (mean ± SE). B, Trough TFV concentrations in SP and BP on days 1, 3, 5, and 7 (mean + SE).

TABLE 1

Patient Demographics at Baseline

Demographics n = 9 Men	Mean	SD
Age (y)	39.9	6.0
Plasma HIV RNA level (log_{10} copies/mL)*	4.4	0.6
GT HIV RNA level (log_{10} copies/mL)*	3.2	0.7
CD4 count (cells/μL)*	353	403
Race/ethnicity	6 (66.7%) African American 3 (33.3%) white	

*Male patient on TDF intensification not included.