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Differential Extracellular and Intracellular Concentrations of Zidovudine and Lamivudine in Semen and Plasma of HIV-1–Infected Men

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Abstract

Objectives—To quantitate extracellular and intracellular zidovudine (ZDV) and lamivudine (3TC) concentrations in blood and semen of HIV-1–infected men.

Design—Nonblind, single-center, open-label pharmacokinetic (PK) study in 14 subjects receiving ZDV plus 3TC.

Methods—Paired blood and semen samples were obtained during 1 intensive visit and 3 single time point visits over 2 weeks. Extracellular ZDV and 3TC concentrations were measured in blood plasma (BP) and seminal plasma (SP), and intracellular ZDV and 3TC triphosphate (TP) concentrations were measured in isolated mononuclear cells using validated methods. HIV-1 RNA was measured in blood and semen. PK parameters were estimated using non-compartmental analysis.

Results—Median (interquartile range [IQR]) SP/BP area under the time-concentration curve over the 12-hour dosing interval ($AUC_{0–12h}$) ratios for ZDV and 3TC were 2.28 (1.48 to 2.97) and 6.67 (4.10 to 9.14), respectively, whereas individual SP/BP concentration ratios ranged from 1.9 to 91.4. Intracellular median (IQR) SP/BP $AUC_{0–12h}$ ratios for ZDV-TP and 3TC-TP were 0.36 (0.30 to 0.37) and 1.0 (0.62 to 1.30), respectively, whereas individual SP/BP concentration ratios ranged from 0.11 to 2.9. HIV-1 RNA was undetectable in both compartments.

Conclusions—ZDV and 3TC SP exposures are 2- to 6-fold greater than BP exposures. Seminal ZDV-TP exposures are ~40% of those found in peripheral blood mononuclear cells (PBMCs), whereas 3TC-TP exposures are similar to PBMC exposures. PK variability makes individual SP/BP ratios a suboptimal surrogate for genital tract exposure.

Keywords

HIV; pharmacokinetics; nucleoside triphosphate; antiretroviral therapy; genital tract

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Unprotected sexual intercourse is the predominant risk factor for acquiring HIV, with most transmission occur- ring from infected men to male and female partners.[1] The efficiency of HIV transmission depends on a variety of factors, including the type of sex act, the viral burden of the infected partner, and the susceptibility of the uninfected partner to HIV infection.[2–7] Although behavioral interventions, such as condom use, have been successful in reducing transmission,[8–10] approximately 4 million adults and children are infected globally on a yearly basis.[1] New and novel prevention methods are urgently needed to slow the spread of the epidemic.

One potential mechanism to slow HIV transmission is the use of antiretroviral drugs (ARVs) to reduce HIV RNA concentrations in infectious secretions. ARV therapy has been shown reliably to decrease HIV RNA concentrations in the genital tract (GT),11–14 and lower semen HIV RNA concentrations are expected to decrease the efficiency of sexual transmission. Chakraborty and colleagues4 predict that a semen HIV RNA concentration of 100,000 copies/mL would result in transmission in 1 per 100 episodes of heterosexual intercourse, whereas a seminal viral load of 1000 copies/mL would decrease the probability of transmission to 3 per 10,000 acts. The relation between HIV RNA concentrations in blood plasma (BP) and semen is imperfect,15–18 and careful examination of viral sequences demonstrates that BP and GT can be viewed as separate viral compartments.19–21 Persistent GT HIV RNA shedding in subjects receiving ARVs has been reported,[22] and long-lived resistant variants in the GT represent a particular problem for rebound viremia[23] and transmitted resistance.[19] It seems likely that poor penetration or altered metabolism of ARVs in the GT contribute to this problem.[24]

Nucleoside reverse transcriptase inhibitors (NRTIs) such as lamivudine (3TC) and zidovudine (ZDV) form the backbone of a typical ARV regimen, and this combination is currently recommended as an alternative option for initial treatment of HIV infection by the US Department of Health and Human Services.25 Random BP and seminal plasma (SP) concentrations of these drugs have been measured previously,14,26,27 and investigators have noted ZDV and 3TC concentrations to be 2 to 9 times higher in SP than in BP. As with all NRTIs, however, the active moiety is the triphosphate (TP) metabolite formed by intracellular enzymatic processes.28 Higher intracellular 3TC-TP and ZDV-TP concentrations in peripheral blood mononuclear cells (PBMCs) have been correlated with a faster decline in HIV RNA concentrations and an increase in CD4 T-cell counts.29 TP concentrations have not been previously characterized in GT mononuclear cells but are likely critical for NRTI efficacy locally. Here, we report on a comprehensive evaluation of extracellular and intracellular ZDV and 3TC concentrations in the male GT over a 12-hour dosing interval under steady-state conditions.

METHODS

Study Design and Population

HIV-infected adult male subjects receiving ZDV/3TC twice daily as part of their ARV regimen were enrolled in a nonblind, open-label, descriptive pharmacokinetic (PK) study to assess the relation between parent drug concentration, intracellular drug metabolite concentrations, and HIV-1 RNA concentrations in BP and SP from May 2000 to June 2003. Subjects were recruited from the Infectious Disease Clinic at the University of North Carolina at Chapel Hill. Exclusion criteria included: age ≤17 years; vasectomy; active bacterial, fungal, or opportunistic GT or systemic infection at the time of enrollment; unwilling or unable to donate semen; abnormal blood chemistries or blood cell counts; or unable to receive ZDV/3TC for any reason. This protocol was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill.

In addition to 300 mg of ZDV and 150 mg of 3TC dosed twice daily, subjects could receive protease inhibitors or nonnucleoside reverse transcriptase inhibitors as part of the ARV regimen prescribed by their clinic provider. Subjects received the study regimen for 4 to 48 months before protocol enrollment. Before the PK visit, subjects completed a 7-day dosing card to ensure steady-state conditions; for the visit to continue, subjects must have achieved at least 90% adherence overall and have taken all doses in the preceding 3 days. Subjects were then admitted to the Verne S. Caviness General Clinical Research Center for an overnight stay for intense BP sampling and 2 semen samples. Matching blood and semen samples were also obtained at 3 outpatient visits over 2 weeks to complete the PK sampling from the seminal compartment and to measure HIV-1 RNA response in BP and SP.

Sample Collection and Processing

At the steady-state PK visit, blood samples were obtained immediately before the next dose (time = 0), and at 0.5, 1, 2, 4, 6, 8, and l2 hours after an observed dose of ZDV/3TC. Semen samples were obtained by masturbation immediately before dosing at time = 0, and again 12 hours after dosing. Paired BP and SP samples were obtained at 3 outpatient visits over 2 weeks at 3, 6, and 9 hours after a dose of ZDV/3TC for extracellular concentrations and intracellular TP concentrations. The time interval between semen collections was designed to avoid potential effects of repeated sampling on drug concentrations in this compartment. Subsequent formal study of the effect of sampling interval of ARV concentrations in semen demonstrates that short sampling intervals have no significant effects on measured concentrations.[30]

Whole blood was obtained using ethylenediaminetetraacetic acid (EDTA)–containing collection tubes (BD Diagnostics, Franklin Lakes, NJ) and was centrifuged at 1400 g (2800 rpm) for 15 minutes at 4°C. The resultant BP was aliquoted into labeled cryovials and stored at −80°C until analysis of extracellular ZDV/3TC concentrations and HIV-1 RNA concentrations. PBMCs were obtained using 10-mL cell preparation tube (CPT) tubes with sodium citrate (BD Diagnostics) for intracellular ZDV/3TC concentration analyses. After collection and centrifugation at 300 g (1300 rpm) for 30 minutes, the resultant plasma and buffy coat were transferred to a 15-mL Falcon tube and washed with phosphate-buffered saline (PBS). After an additional centrifugation step, the cell pellet was resuspended and viable PBMCs were counted with a hemocytometer using the trypan blue exclusion method. After the cells were repelleted and lysed with methanol, the cellular debris was removed in a final centrifugation step and the methanolic supernatant containing the TP was transferred to a labeled cryovial and stored at −80°C until analyses.

Semen samples were collected into a standard specimen collection cup. After collection, samples were left at room temperature to liquefy for 45 minutes and then centrifuged at 1400 g (2800 rpm) for 15 minutes at room temperature to separate SP from the cellular fraction. SP aliquots were transferred to labeled cryovials and stored at −80°C until analysis of extracellular ZDV/3TC concentrations and HIV-1 RNA concentrations. To isolate seminal mononuclear cells, the cell pellet was resuspended and layered on a Percoll gradient before centrifugation at 1400 g (2800 rpm) for 20 minutes. The cells were removed from the interface, washed with PBS, and resuspended. Cells were then counted and processed for storage using the same method as described previously for PBMCs.

Analytic Methods

Extracellular and intracellular concentrations in blood and semen were measured using validated and previously published liquid chromatography tandem mass spectrometry (LC/MS/MS) methods.[31,32] The dynamic range for extracellular concentrations of ZDV and 3TC was 5 to 5000 ng/mL, with a minimum of 90% accuracy, and interday and intraday variability of 3% to 14% and 1% to 7.6% relative standard deviation (RSD), respectively. The lower limits

of quantitation (LLQs) for intracellular ZDV-TP and 3TC-TP were 0.10 and 10.5 pmol, respectively. For concentrations up to 150 pmol, recovery was ≥93%, and the coefficient of variation (CV) for interday variability ranged from 4.5% to 8.4%. The CV for intraday variability ranged from 1% to 8%.

HIV-1 RNA concentrations in BP were determined using the Roche Amplicor Monitor kit (Pleasanton, CA; LLQ = 50 copies/mL) and in SP using the Organon Teknika Nuclisens assay (Durham, NC; LLQ = 400 copies/mL).

PK parameters, including the area under the time-concentration curve over the 12- hour dosing interval ($AUC_{0–12h}$), were estimated for BP and SP using WinNonlin Professional (Version 4.1; Pharsight, Inc., Mountain View, CA). Half-lives were calculated from λ_z values obtained from the noncompartmental analysis. For all analyses, concentration measurements lower than the lower limit of detection were imputed as 0 and those lower than the LLQ were imputed as ½ LLQ. Concentration ratios were calculated to describe differences between drug exposure in BP and SP for extracellular ZDV and 3TC and intracellular ZDV-TP and 3TC-TP.

RESULTS

Demographic data for the 14 men enrolled in the study are presented in Table 1. These men ranged in age from 26 to 48 years; 50% were African American and 50% were white. In addition to ZDV/3TC, most (64%) were also receiving a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor. At baseline, the median HIV RNA concentration in BP was 2.6 $\log_{10}$ copies/mL and <400 copies/mL in SP; the median CD4 T-cell count was 512 cells/mm^3. Men were followed in this study for 2 to 44 weeks. Over this period, HIV RNA levels declined to undetectable concentrations in blood and semen in all but 1 participant. This subject had been receiving a stable regimen of ZDV/3TC for 3.5 years before enrollment and had undetectable HIV RNA in BP up to 32 weeks after enrollment, with a single detectable HIV RNA measurement of 880 copies/mL at 2 weeks of enrollment.

Table 2 presents median (interquartile range [IQR]) $AUC_{0–12h}$ in SP and BP for ZDV and 3TC and their intracellular TP metabolites. Five of 112 total ZDV BP samples were lower than the LLQ, and imputed values were used for these samples; all other samples were within the quantitation limits for each assay. For ZDV, the $AUC_{0–12h}$ in SP was 3790 (IQR: 2481 to 4783) h·ng/mL, whereas the $AUC_{0–12h}$ in BP was 1479 (IQR: 1175 to 1748) h·ng/mL. By dividing each subject's SP $AUC_{0–12h}$ by their respective BP $AUC_{0–12h}$, the SP/BP exposure ratio was calculated. Using this AUC ratio approach, rather than using the SP/BP ratios of concentrations obtained at individual time points, provided a robust overall estimate of GT drug penetration. For ZDV, the median (IQR) SP/BP $AUC_{0–12h}$ ratio was 2.28 (1.48 to 2.97), indicating that ZDV exposure in SP was twice that of BP. For 3TC, the median (IQR) SP/BP $AUC_{0–12h}$ was 6.67 (4.10 to 9.14), indicating that 3TC exposure in SP was >6 times that of BP.

In Figure 1A, the median (IQR) time-concentration profile for ZDV depicts higher exposures in SP than in BP over the dosing interval. The maximal concentration (C_{max}) observed in each compartment was similar (median SP C_{max} = 561 ng/mL, median BP C_{max} = 785 ng/mL). Using the available data, the median ZDV half-lives in SP and BP were 6.9 hours and 2.7 hours, respectively.

Figure 1B illustrates 3TC concentrations in SP consistently higher than in BP throughout the dosing interval. Generally, SP concentrations remained constant across the dosing interval. The median 3TC half-lives in SP and BP were calculated to be 7.1 hours and 3.8 hours, respectively.

Figure 2 depicts the median (IQR) TP PK in blood and semen for ZDV-TP (see Fig. 2A) and 3TC-TP (see Fig. 2B). These concentrations were more consistent across the dosing interval than extracellular ZDV and 3TC. For ZDV-TP, the PBMC and seminal mononuclear cell median (IQR) half-lives were 25.0 (5.5 to 28.5) and 14.2 (12.9 to 14.3) hours, respectively. For 3TC-TP, PBMCs and seminal mononuclear cell median (IQR) half-lives were 10.4 (3.3 to 23.1) and 10.2 (1.2 to 22.2) hours, respectively.

For ZDV-TP, the median (IQR) $AUC_{0–12h}$ in SP was 646 (361 to 1029) h·fmol/10^6 cells, whereas the median (IQR) $AUC_{0–12h}$ in BP was 1460 (1241 to 2172) h·fmol/10^6 cells. The SP/BP ratio for ZDV-TP was 0.36 (IQR: 0.30 to 0.37). For 3TC-TP, the median (IQR) $AUC_{0–12h}$ in SP was 82,068 (64,342 to 139,404) h·fmol/10^6 cells, whereas the $AUC_{0–12h}$ in BP was 108,600 (IQR: 78,687 to 164,984) h·fmol/10^6 cells, yielding an SP/BP ratio of 1.0 (IQR: 0.62 to 1.30). Although extracellular GT concentrations were higher than BP, the intracellular TP concentrations in seminal mononuclear cells were similar to, or lower than, PBMC concentrations. For ZDV-TP, the SP/BP $AUC_{0–12h}$ of 0.36 indicates that the seminal cell exposure was approximately 40% that of PBMCs. The SP/BP $AUC_{0–12h}$ ratio of 1.0 for 3TC-TP indicates equivalent exposure, however.

Figure 3 compares SP/BP concentration ratios at each collection point for the 4 analytes. The concentration ratios for ZDV-TP and 3TC-TP did not differ appreciably over time, whereas they were highly dependent on the time of sampling for parent ZDV and 3TC. For ZDV and 3TC, individual SP/BP concentration ratios ranged from 1.9 to 91.4 and from 1.9 to 30.5, respectively, with CVs ranging from 60% to 285%. In comparison, parent drug $AUC_{0–12h}$ ratio CVs ranged from 48% to 87%. Samples obtained early in the dosing interval gave lower SP/BP ratios, whereas those obtained later in the dosing interval gave higher ratios. This is particularly evident for ZDV, which maintains high concentrations in SP over a dosing interval, whereas BP concentrations decline quickly. For ZDV-TP and 3TC-TP, individual SP/BP concentration ratios ranged from 0.11 to 2.9 and from 0.14 to 2.5, respectively.

Correlation analysis to investigate potential relations between extracellular parent drug and the TP metabolite exposures in SP and BP did not provide strong evidence of a direct relation for ZDV or 3TC ($r < 0.6$, $P > 0.07$ for all analyses; data not shown).

DISCUSSION

This is the first study designed to provide full extracellular and intracellular PK profiles in blood and semen for ZDV and 3TC. Using calculated AUCs in this investigation, ZDV SP exposure was approximately 2 times greater than BP exposure, and 3TC SP exposure was approximately 6 times greater than BP exposure. Three previous reports of ZDV and 3TC concentrations in SP using isolated time points reported ZDV and 3TC SP exposures ranging from 2.16- to 9.1-fold greater than BP exposures.[14,26,27] By obtaining several timed samples over 2 weeks, this investigation was able to calculate an $AUC_{0–12h}$ in SP, which, when compared with the $AUC_{0–12h}$ in BP, provides a more accurate estimate of drug penetration over a dosing interval.

Different ARV exposures in the GT relative to BP have been reported for men and women, 14·24·33[–40] and the exact physiochemical properties that govern drug passage into GT secretions are currently unknown. Differences exist between classes of agents and between specific agents within each class. This behavior in the male GT is consistent with steady-state GT behavior in women (3TC 4-fold higher in GT, ZDV 2-fold higher in GT).[24]

Similar to our previous report for the nonnucleoside agent efavirenz,[33] extracellular ZDV and 3TC individual SP/BP concentration ratios change considerably in a single subject over the dosing interval because of different rates of drug penetration into the GT. Therefore, the use

of $AUC_{0–12h}$ ratios provided a more robust measure of overall drug exposure in SP relative to BP. This can be seen by the lower CVs for $AUC_{0–12h}$ ratios (range: 48% to 87%) compared with individual concentration ratios (range: 60% to 285%). Given the logistics of seminal sample collection for constructing an $AUC_{0–12h}$, however, there is some increased variability around the estimates of individual PK parameters (particularly C_{max}).

Unexpectedly, this investigation found that elevated extracellular ZDV and 3TC exposures in semen did not result in elevated intracellular TP exposures. Although 3TC exposure was approximately 6-fold higher in semen than in blood, 3TC-TP exposure in seminal mononuclear cells was similar to that in PBMCs. Despite 2-fold higher ZDVexposure in SP compared with BP, ZDV-TP exposure in seminal mononuclear cells was only approximately 40% of that measured in PBMCs.

Our subjects were patients on therapy for HIV infection, and as such, sampling was limited to within a dosing interval. Although this approach can underestimate half-life, the long intracellular half-life estimates of ZDV-TP and 3TC-TP reported here (median values of 10.2 to 25.0 hours) are similar to those reported elsewhere in PBMCs.[32,41,42] The more stable TP concentrations observed over the dosing interval suggest that a single SP/BP concentration ratio may serve as a reasonable proxy for overall intracellular drug exposure in the male GT. Because collection, processing, and analysis of PBMCs and seminal mononuclear cells are complex procedures, using single time points rather than multiple sampling strategies could facilitate research in this area.

The differential TP exposures between PBMCs and seminal mononuclear cells observed here may be attributable to differential cellular activation or different cell populations between the 2 compartments. 3TC (a cytidine analogue) and ZDV (a thymidine analogue) have different intracellular metabolic pathways for activation.[28] The enzymes involved in these pathways can be affected by cellular factors, such as growth phase and activity.28 ZDV is preferentially phosphorylated in activated cells,[43,44] whereas 3TC is preferentially phosphorylated in quiescent cells.[45] These subjects had been on stable therapy for at least 6 months and had been screened for sexually transmitted diseases (STDs) before participating in this study. Therefore, fewer activated mononuclear cells in the male GT could account for the low SP/BP ratio of ZDV-TP. There are currently no data on intracellular drug concentrations in the GT of men with ongoing HIV replication or an active STD, however.

Differential phosphorylation between SP and BP may also be explained by diverse cell populations in the 2 compartments. The distribution of mononuclear cell subsets in semen is unknown. It has recently been observed in PBMCs that different cell types may phosphorylate nucleosides to varying extents, however.[46]

At the end of the 2-week treatment period, all subjects had undetectable HIV RNA concentrations in BP and all but 1 subject had undetectable HIV RNA in SP. It is notable that this subject had the lowest intracellular seminal exposure for ZDV-TP and 3TC-TP. Unfortunately, viral sequencing could not be performed on his samples.

The small number of men in this cohort precluded meaningful statistical evaluations of drug concentrations with HIV RNA and/or CD4 T-cell response. Other researchers have reported correlations between TP concentrations in PBMCs and drug efficacy,[29] with higher TP concentrations leading to faster declines in HIV-1 RNA and quicker improvements in CD4 cell counts. Future PK-pharmacodynamic modeling with these data to explore the highly complex intracellular-extracellular drug concentration relation in BP and SP is planned but is outside the scope of this article.

The GT pharmacology data presented here are important for the health of the HIV-infected individual and to gain insight into the development of drug resistance in the GT. Understanding the pharmacology of nucleoside analogue reverse transcriptase inhibitors, the backbone of ARV therapy, is critical for choosing agents that minimize the development of resistance in the GT. Lower active ZDV concentrations in seminal cells may provide a pharmacologic basis for the male GT as a sanctuary site for HIV replication. Sexual transmission of resistant HIV is becoming increasingly common as ARV treatment becomes more widely available,[19,47–53] and the choice of drug regimen clearly drives HIV resistance patterns in the male and female GT compartments.[54–56] In addition to these seminal TP data for 3TC and ZDV, tenofovir diphosphate concentrations in seminal mononuclear cells have recently been reported to be 5 times higher than in PBMCs at steady state.[34] The ability of tenofovir diphosphate to be equally phosphorylated in activated and quiescent cells may partially explain the high intracellular concentrations,[44] although other factors currently under investigation may contribute to these differences.

For prevention of HIV transmission, these data are most critical for elucidating the influence of local drug concentrations on HIV shedding and the development of drug resistance. To assess the ability of drugs to act locally and protect HIV-negative individuals from infection, tissue concentrations of ARVs in the rectum, penile foreskin, and vaginal/cervical mucosa would be the most relevant to evaluate.

HIV is found in all male GT compartments, including organs that contribute to the generation of semen (ie, prostate, seminal vesicles, testicles). Some differences may be observed in the penetration of ARVs into each of these compartments, but they are unlikely to be clinically relevant.[57] Recent developments in techniques to measure the fraction of the total drug concentration contributed by seminal vesicle–derived fluid and prostatic fluid should continue to advance the understanding of GT pharmacology and implications for local drug resistance.[58]

In conclusion, this is the first study to report ZDV, ZDV-TP, 3TC, and 3TC-TP exposures over a dosing interval in SP and seminal mononuclear cells in comparison to BP and PBMCs. These data have implications for development of compartmental resistance and further assist us in understanding ARV activity in the male GT.

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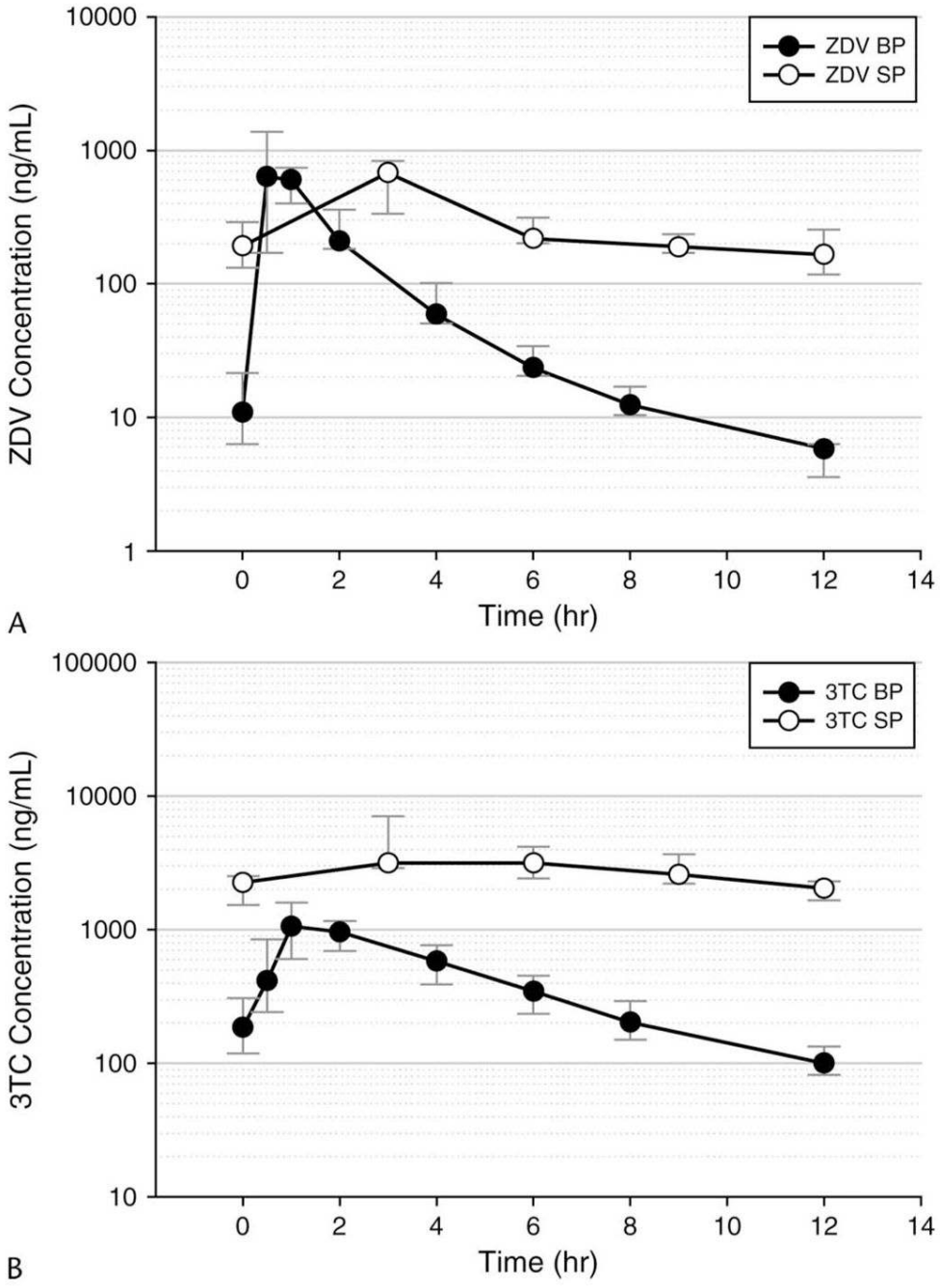

FIGURE 1.

A, Median (IQR) extracellular ZDV parent concentrations in BP and SP over the 12-hour dosing interval. Semen collection occurred longitudinally at steady state to construct a composite concentration-time profile. B, Median/IQR extracellular 3TC parent concentrations in BP and SP over the 12-hour dosing interval. Semen collection occurred longitudinally at steady state to construct a composite concentration-time profile.

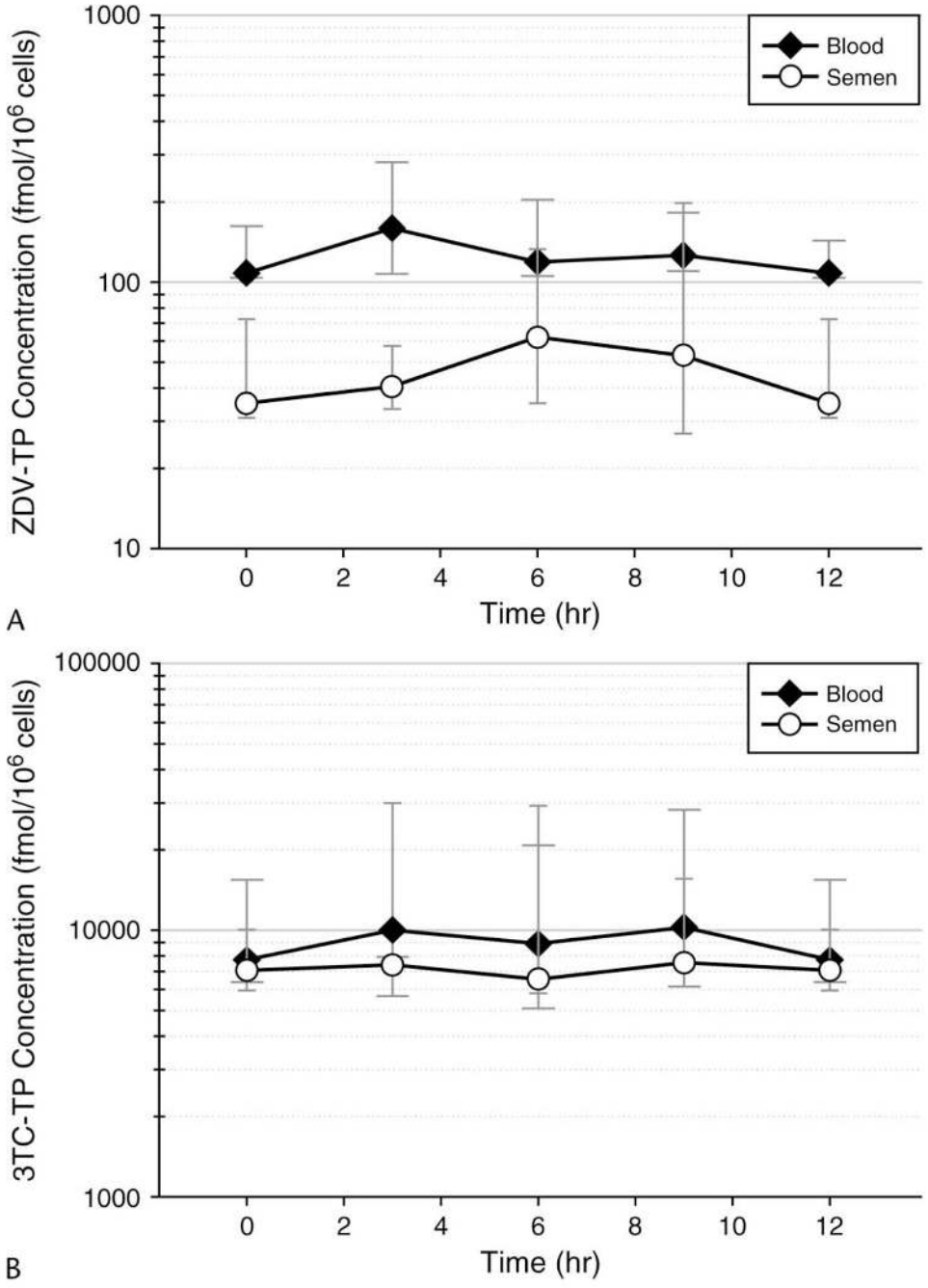

FIGURE 2.

A, Median (IQR) ZDV-TP concentration-time profile in blood (◆) and semen (○) over the 12-hour dosing interval. Subjects received 300 mg of ZDV twice daily. Semen collection occurred longitudinally at steady state to construct a composite concentration-time profile. B, Median/IQR 3TC-TP concentration-time profile in blood (◆) and semen (○) over the 12-hour dosing interval. Subjects received 150 mg of 3TC twice daily. Semen collection occurred longitudinally at steady state to construct a composite concentration-time profile.

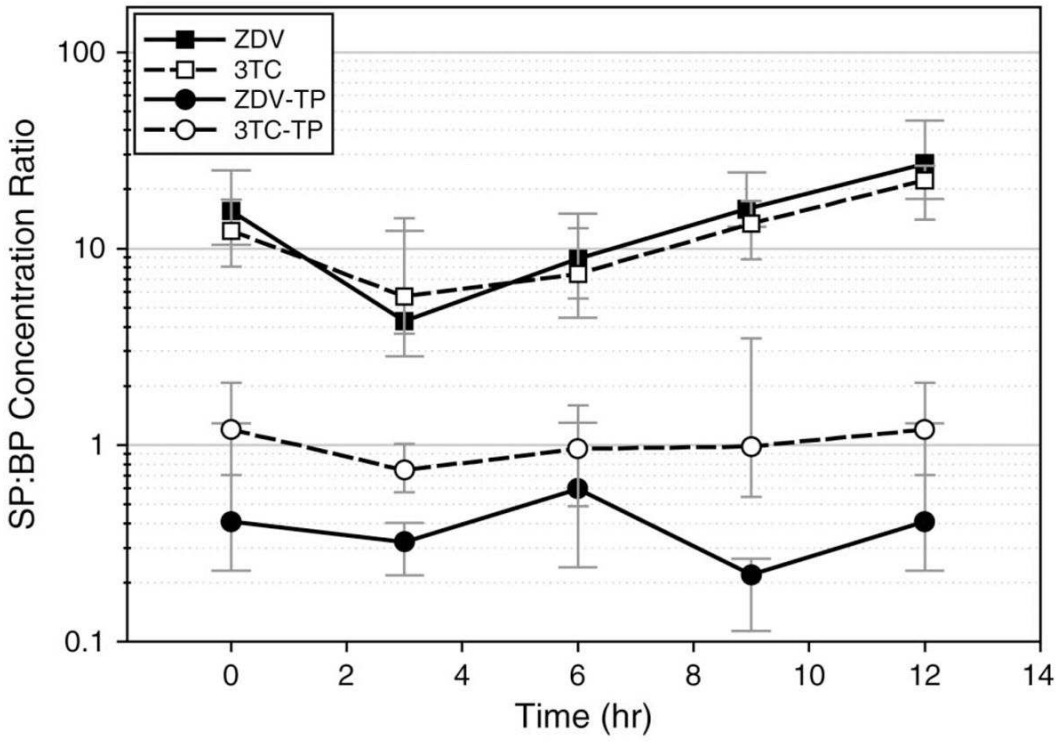

FIGURE 3.
Median (IQR) SP/BP concentration ratios at each collection point for ZDV, 3TC, ZDV-TP, and 3TC-TP. Ratios were obtained by dividing the SP concentration at each time point by the corresponding BP concentration.

TABLE 1

Demographic and Drug Regimen Information for Study Subjects

Parameter	
Age (y)	36 (34 to 40)
Blood HIV RNA (log_{10} copies/mL)	2.6 (1.87 to 2.67)
CD4 T-cell count (cells/μL)	512 (388 to 773)
Length of follow up (wk)	4 (2 to 33)
Race	
African American	7 (50%)
White	7 (50%)
Drug regimen (n = 14)	
ZDV/3TC	5 (36%)
ZDV/3TC + NNRTI*	4 (28%)
ZDV/3TC + PI†	5 (36%)

Data are presented as median (IQR) or number (percent).

*Three men received efavirenz; 1 man received nevirapine.

†Three men received nelfinavir; 2 men received indinavir.

TABLE 2

Median (IQR) $AUC_{0–12h}$ Values for ZDV and 3TC in BP and SP and ZDV-TP and 3TC-TP

	ZDV (h·ng/mL)	ZDV-TP (h·fmol/10^6 cells)	3TC (h·ng/mL)	3TC-TP (h·fmol/10^6 cells)
$AUC_{0–12h}$ BP	1479 (1175 to 1748)	1460 (1241 to 2172)	4924 (4373 to 6237)	108,600 (78,687 to 164,984)
$AUC_{0–12h}$ SP	3790 (2481 to 4783)	646 (361 to 1029)	31,084 (28,071 to 44,184)	82,068 (64,342 to 139,404)
SP/BP ratio	2.28 (1.48 to 2.97)	0.36 (0.30 to 0.37)	6.67 (4.10 to 9.14)	1.0 (0.62 to 1.30)

Median (IQR) of the SP/BP ratios of each provides an estimate of SP exposure relative to BP.