The Pharmacokinetics of Amprenavir, Zidovudine, and Lamivudine in the Genital Tracts of Men Infected with Human Immunodeficiency Virus Type 1 (AIDS Clinical Trials Group Study 850)

Arlene S. Pereira,[1] Laura M. Smeaton,[2] John G. Gerber,[3] Edward P. Acosta,[4] Sally Snyder,[5] Susan A. Fiscus,[1] Richard R. Tidwell,[1] Roy M. Gulick,[6] Robert L. Murphy,[7] and Joseph J. Eron, Jr.[1]

[1]Division of Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill; [2]Statistical and Data Analysis Center, Harvard School of Public Health, Cambridge, Massachusetts; [3]Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver; [4]Division of Clinical Pharmacology, University of Alabama at Birmingham; [5]Social and Scientific Systems, Silver Spring, Maryland; [6]Department of Medicine, Weill Medical College of Cornell University, New York, New York; [7]Division of Infectious Diseases, Northwestern University Medical School, Chicago, Illinois

The AIDS Clinical Trials Group Study 850 (ACTG 850) evaluated the penetration of zidovudine (ZDV), lamivudine (3TC), and amprenavir (APV), given alone and in combination with the 2 nucleoside analogues, into the male genital tract, because these factors may affect human immunodeficiency virus (HIV) type 1 suppression and transmission. Nineteen men receiving APV monotherapy and 12 men receiving triple therapy donated blood plasma (BP) and seminal plasma (SP) during therapy. Paired SP and BP were used to calculate compartmental concentration ratios. APV SP concentrations were consistently lower than BP concentrations, ZDV SP concentrations approximated BP concentrations early but became greater later in the dosing interval, and 3TC SP concentrations were substantially greater than BP concentrations throughout. Observed SP concentrations plotted with population BP concentration-time curves confirmed these findings, suggesting that passive diffusion (APV), slowed elimination (ZDV), and either active accumulation and/or inhibition of elimination (3TC) are responsible for SP concentrations of these agents. The antiretroviral effect of APV monotherapy was related to APV concentrations.

The level of human immunodeficiency virus (HIV) type 1 shed from the male genital tract is likely to correlate with the risk of transmission associated with sexual contact with an infected man [1–4]. The male genital tract is a separate compartment, isolated from the systemic circulation by anatomic and physiologic barriers (reviewed in [5]). Some antiretroviral agents that effectively suppress HIV-1 replication systemically may be impeded from entering the genital compartment; others may attain effective concentrations there. Antiretroviral agents active in the male genital tract may effectively suppress HIV-1 replication in that potential reservoir and may contribute to a decrease in sexual transmission and in transmission of resistant variants in a given population.

Seminal plasma (SP) concentrations of antiretroviral drugs have been reviewed recently elsewhere [6]. SP concentrations of the nucleoside analogues zidovudine (ZDV) [7–9] and lamivudine (3TC) [7, 10] are greater than blood plasma (BP) concentrations. Concentrations of stavudine are similar in BP and SP, but low [10]. Concentrations of the nonnucleoside reverse-transcriptase inhibitors nevirapine and efavirenz in SP are consistently measurable, although lower than in BP [10–13]. In contrast, SP concentrations of protease inhibitors (PIs) relative to blood vary by drug, seeming in part to parallel the level of protein binding. Saquinavir and ritonavir, both highly protein-bound, are difficult to detect in SP [14, 15]. Indinavir, which has more-limited protein binding, is readily detectable in SP [15, 16]. SP concentration of PIs may also be affected by the P-glycoprotein transporter (P-gp), an exit pump present in cells of the male genital tract [17, 18]. The SP concentrations of amprenavir (APV), which binds to plasma protein at a level intermediate to indinavir and the other PIs [19, 20], have yet

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Informed consent, approved by institutional review boards at each site, was obtained from all patients, and human experimentation guidelines of the US Department of Health and Human Services and of the participating institutions were followed in the conduct of the clinical trial.

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Drs. Eron and Gulick are ad hoc consultants to GlaxoSmithKline.

Reprints or correspondence: Dr. Joseph J. Eron, Jr., University of North Carolina School of Medicine, Div. of Infectious Diseases, 547 Burnett-Womack Bldg., CB 7030, Chapel Hill, NC 27599-7030 (joseph_eron@unc.edu).

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to be described. To our knowledge, the relationship between PI concentrations and antiretroviral effects in seminal fluid is unexplored. AIDS Clinical Trials Group Study (ACTG) 850, a substudy of ACTG 347 [21], was designed to study the antiretroviral activity, pharmacokinetics, and pharmacodynamics of APV monotherapy and APV/ZDV/3TC combination therapy in the male genital tract. The antiretroviral activity results of this substudy have been described elsewhere [22]; the pharmacokinetic and pharmacodynamic results are presented here.

Subjects, Materials, and Methods

Study design and study subjects. The study design and the entry criteria for ACTG 347 have been described elsewhere [21]. Subjects were 3TC and PI naive, were >13 years old, and had HIV-1 RNA levels of >5000 copies/mL and CD4 cell counts of >50 cells/mm^3. Subjects in the parent study were randomly assigned to receive either 1200 mg APV (Agenerase; Vertex Pharmaceuticals, GlaxoSmithKline [formerly GlaxoWellcome]), 300 mg ZDV (Retrovir; GlaxoSmithKline), and 150 mg 3TC (Epivir; GlaxoSmithKline), given twice a day; or 1200 mg APV given twice a day as monotherapy.

Subjects in ACTG 850 were men willing to donate timed SP samples at baseline (for HIV-1 RNA analysis) and at weeks 8 and 24 (for RNA and pharmacokinetic analyses) [21]. The protocol specified that SP and BP samples obtained during therapy be collected 6 h after an observed medication dose. The times of most recent medication dose, semen sampling, and blood sampling were recorded. Subjects were asked to abstain from sexual activity for 48 h before semen donation

Quantifying HIV-1-RNA, APV, ZDV, and 3TC in SP and BP. Concentrations of ZDV and 3TC were measured in SP and BP by using validated high-performance liquid chromatography (HPLC)–mass spectroscopy (MS)/MS methods described elsewhere [23, 24]. Lower limits of quantification of these drugs were 5 ng/mL in both SP and BP. APV concentrations in SP and BP were determined by using a validated HPLC-MS/MS method with stable, isotopically-labeled $^{13}C_6$-APV as the internal standard. The lower limit of quantification was 10 ng/mL in both matrices [25].

HIV-1 RNA concentrations in SP and BP were determined by the NucliSens assay (Organon-Teknika), as described elsewhere [22]. The lower limit of quantification was 400 HIV-1 RNA copies/mL.

Statistical analysis. Despite stipulation in the study protocol that specimens be obtained 6 h after a dose, sample collection during therapy occurred over a wide range of times, allowing for basic pharmacokinetic assessments in BP and SP. SP:BP drug compartmental concentration ratios were calculated only with matched paired samples, which were semen and blood specimens collected within 1 h of each other (inclusively). These ratios were grouped according to the time at which the BP donation occurred during the 12-h dosing interval. Matched paired samples obtained within 0–2 h of drug administration were considered to be representative of the absorption phase of drug distribution. Those collected after 2 h were assumed to be representative of the elimination phase of systemic drug. These samples were collected predominantly 8–12 h after a dose, during the drug-elimination phase. In addition, to take advantage of the scatter in the timed SP collections from study subjects over the dosing intervals, BP concentration-time points for the 3 drugs were simulated for comparison with SP concentrations. The ADAPT II package of programs (Biomedical Simulations Resource, University of Southern California, Los Angeles) was used to conduct the simulations [26]. In brief, pharmacokinetic parameters for each drug were taken from the literature and used to simulate plasma concentration-time data, following the same doses used in this study [27–29].

Statistical analyses were predominantly descriptive. Distributions of drug concentrations in each compartment were summarized with medians and interquartile ranges (IQRs). Semen:blood concentration ratios were calculated among matched pairs. Rank-based (Spearman) correlations were used to describe the association between SP and BP concentrations among matched pairs. Distributions of matched SP and BP concentrations were compared by means of the Wilcoxon signed rank test. Distributions of SP and BP concentrations (or ratios) were each compared for early versus late phase with the Wilcoxon rank sum test.

Positive virologic response was defined as either a 1-$\log_{10}$ decrease from baseline in HIV-1 RNA level or an HIV-1 RNA level of <400 copies/mL at first study follow-up (week 8). Compartmental (SP and BP) responses were determined, and concordance and discordance in virologic response between the 2 compartments were recorded for each study arm. For each study arm, the Wilcoxon rank sum test was used to determine whether a significant difference in the distribution of SP APV concentrations existed either between those who achieved a positive response in SP (responders) and those whose SP HIV-1 RNA level remained >400 copies/mL (nonresponders), or between those who responded in BP and those who did not respond in that compartment. To evaluate whether time elapsed since dosing was a confounding factor, we also compared this variable between compartmental responders and nonresponders by the Wilcoxon rank sum test.

Results

Thirty-seven of the 81 men enrolled in ACTG 347 agreed to participate in ACTG 850; baseline characteristics of the study participants have been reported elsewhere [22]. The 31 men who returned to donate samples for pharmacokinetic analysis during the follow-up period (participating in the study) constitute the study population reported here. Participants were predominantly (61%) white, non-Hispanic, with a median age of 37 years. Baseline median BP RNA level was 4.2 $\log_{10}$ copies/mL (IQR, 3.7–4.7 $\log_{10}$ copies/mL), SP RNA level was 3.5 $\log_{10}$ copies/mL (IQR, <2.6–4.08 $\log_{10}$ copies/mL), and CD4 cell count was 304.5 cells/mm^3 (IQR, 169–402 cells/mm^3), with no significant difference between the 2 treatment groups. Twelve of the 31 men received APV/ZDV/3TC, and the remaining 19 received only APV. All 31 subjects donated BP, and 30 subjects donated SP at the first follow-up visit, with 1 sample missing from a subject in the triple-therapy study arm. Despite early discontinuation of the APV monotherapy arm in the parent study, 14 subjects also donated SP and/or BP samples at a second follow-up visit, which occurred on or before the 24th week of the study. In all, 41 BP and 43 SP samples were collected

while subjects were receiving study medications. Fourteen matched paired samples were collected from each study arm: 17 in the absorptive (early) phase and 11 in the elimination (late) phase.

All APV concentrations in BP were above the lower quantification limit; one in SP was below the limit. All 3TC concentrations in both SP and BP were above the lower quantification limit. One concentration in SP and 2 concentrations in BP for ZDV were below the lower quantification limit. Values below the limit of quantification were set to the lower limit for calculations.

The median absorptive-phase SP and BP APV concentrations were 741.2 ng/mL ($n = 20$) and 5212.7 ng/mL ($n = 18$), respectively, and median elimination-phase concentrations were 217 ng/mL ($n = 23$) and 339.3 ng/mL ($n = 23$), respectively. Median SP and BP ZDV concentrations were 459.4 ng/mL ($n = 9$) and 476.7 ng/mL ($n = 10$), respectively, in the early phase and 127.4 ng/mL ($n = 10$) and 5.1 ng/mL ($n = 8$), respectively, in the late phase. Median 3TC concentrations were 2305.8 ng/mL in SP ($n = 9$) and 880.5 ng/mL in BP ($n = 10$) early and 2497.7 ng/mL in SP ($n = 10$) and 168.4 ng/mL in BP ($n = 8$) late (figure 1*A*). All samples collected during the study were used to calculate these values.

When we compared the concentrations in BP and SP for each agent, using matched paired samples, SP concentrations were significantly lower than BP concentrations for APV ($P = .002$), significantly higher for 3TC ($P = .002$), and not significantly different for ZDV ($P = .76$). Using only first follow-up matched paired samples (11 from the APV monotherapy arm and 8 from the APV/ZDV/3TC arm), median SP:BP drug-concentration ratios were 0.14 ($n = 19$), 2.16 ($n = 7$), and 3.16 ($n = 7$) for APV, ZDV, and 3TC, respectively. These values were similar when all matched paired samples were considered. There was a positive correlation between paired APV and ZDV semen and blood concentrations ($r = 0.53$, $P = .004$, and $n = 28$; and $r = 0.70$, $P = .006$, and $n = 14$, respectively). A correlation could not be demonstrated for 3TC ($r = 0.046$; $P = .88$; $n = 14$).

Matched paired samples were used to calculate early and late SP:BP drug-concentration ratios (figure 1*B*). The median early and late ratios for APV were both <1, as concentrations declined over time in both compartments (figure 1*A*). There was a positive correlation between paired early APV SP and BP concentrations ($r = 0.81$; $P = .0001$; $n = 17$). The SP:BP concentration ratios for ZDV and 3TC were higher in the elimination phase, compared with the absorption phase ($P = .09$ for ZDV; $P = .02$ for 3TC) (figure 1*B*), predominantly because, during the elimination phase, BP concentrations were substantially lower for both agents ($P = .09$ for ZDV, and $P = .01$ for 3TC for the comparison of BP concentrations between absorption and elimination phases). SP concentrations decreased less or not at all ($P = .61$ for both the ZDV and 3TC absorption vs. elimination comparisons) (figure 1*A*). In all but one of the late matched paired samples, SP ZDV concentrations were greater than the corresponding BP concentrations. All late 3TC SP concentrations were higher than matching concentrations in BP (figure 1*C*).

Simulated BP concentration-time profiles of APV, ZDV, and 3TC generated from published pharmacokinetic data [27–29] were plotted beside measured SP concentrations of APV, ZDV, and 3TC (figure 2). Measured BP levels of all 3 drugs varied but fell within the range of the simulated curves (data not shown). The simulations demonstrated that the 3 drugs exhibit early BP peaks ~2 h after a dose, followed by an extended elimination phase. The measured SP APV concentrations were similar to or less than the simulated BP APV concentrations throughout the dosing interval. The measured SP ZDV concentrations approximated simulated BP concentrations early but were higher than the simulated BP concentrations toward the end of the dosing interval. In contrast, the measured SP 3TC levels demonstrated a flat concentration-time relationship that was substantially higher than the simulated BP concentrations throughout the dosing interval.

Responses to antiretroviral therapy in BP and SP were compared with APV concentrations in each compartment. All men receiving combination therapy had an HIV-1 RNA response in the seminal compartment. In the APV monotherapy group, 8 subjects responded in both BP and SP, 3 subjects did not respond in either compartment, and 8 subjects responded in SP but not BP. The median SP APV concentration in responders ($n = 16$) was 626.6 ng/mL; in nonresponders, it was 77.7 ng/mL ($n = 3$; $P = .31$). The median BP APV concentration in responders ($n = 8$) was 4664.2 ng/mL; in nonresponders, it was 576.8 ng/mL ($n = 11$; $P = .05$). Sample collection times were not significantly different between responders and nonresponders in either compartment. Results were similar when median APV concentrations and HIV-1 RNA responses BP from both the monotherapy and combination therapy arms were compared. The comparison between distributions of SP APV concentrations in responders versus nonresponders (medians, 741.2 vs. 77.7 ng/mL) when both arms were considered was still not significant ($P = .16$). In the 8 men who received APV alone and responded in SP but not BP, the median seminal fluid concentration was 419 ng/mL, and the median BP APV concentration was 597.2 ng/mL. However, the SP:BP concentration ratios in matched paired samples from the men with discordant responses ($n = 5$) did not significantly differ from those with concordant responses ($n = 6$; $P = .86$ for the comparison of distribution of ratios).

Discussion

ACTG 850 was the first study to assess the pharmacokinetics of APV, ZDV, and 3TC in both SP and BP. The use of APV monotherapy, a strategy under study in 1997 but now not recommended for the treatment of HIV-1 infection [30], provided the opportunity to assess the pharmacodynamics of treatment

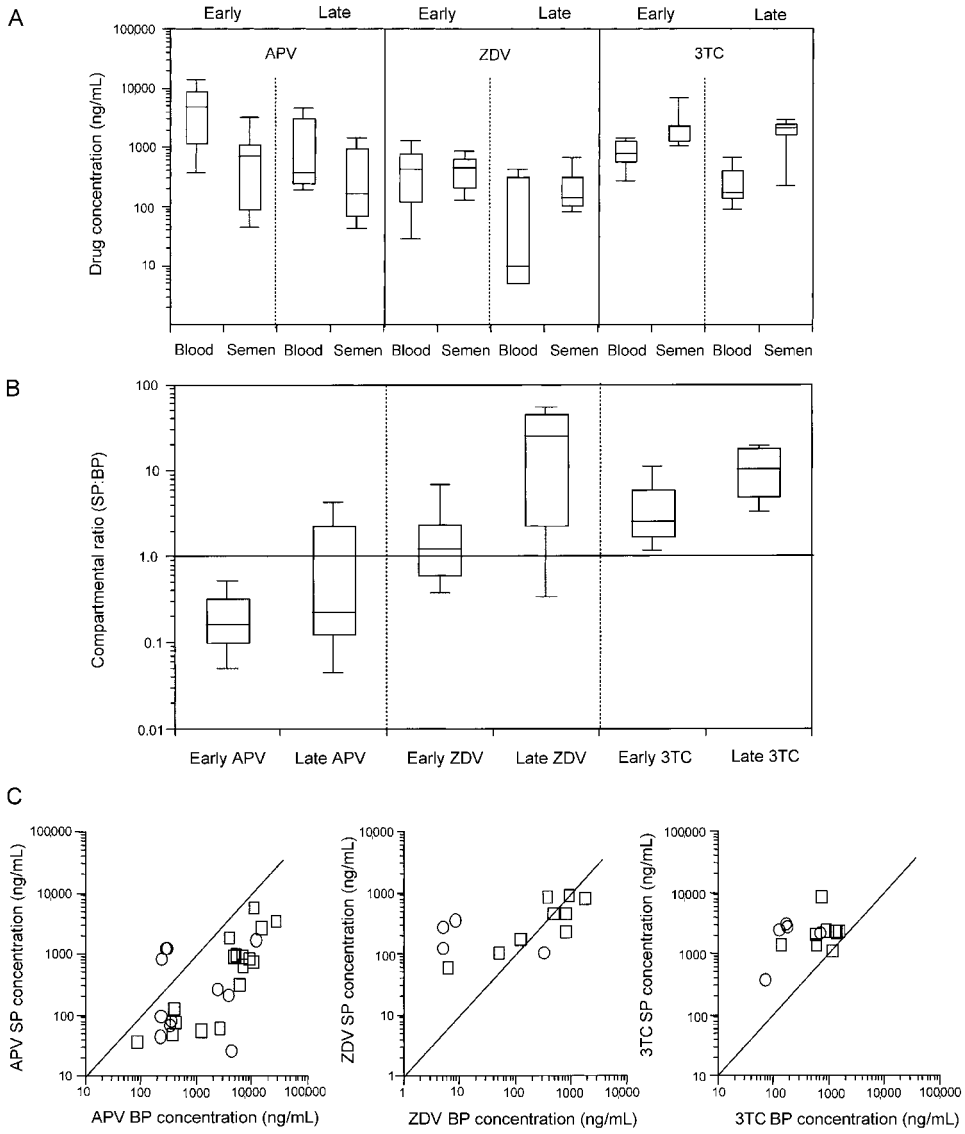

Figure 1. *A,* Drug concentrations in seminal plasma (SP) and blood plasma (BP). SP and BP samples are paired together for each drug and separated into early and late collections, as displayed in box-and-whiskers plots on a logarithmic scale. The ends of the whiskers designate the 5th–95th percentiles, the sides of the box represent the 25th and 75th percentiles, and the horizontal line within the box is the median. *B,* Median, interquartile range, and 5th–95th percentile range of calculated compartmental ratios for matched paired samples are shown. The median early and late amprenavir (APV) concentration ratios were 0.14 (n = 17 samples) and 0.21 (n = 11 samples), respectively. The median early and late zidovudine (ZDV) concentration ratios were 1 (n = 9 samples) and 33.8 (n = 4 samples), respectively. The median early and late lamivudine (3TC) concentration ratios were 2.39 (n = 9 samples) and 15.7 (n = 5 samples), respectively. *C,* Concentrations of APV, ZDV, and 3TC in matched paired semen and blood samples collected early (0–2 h after dosing; □) and late (>2 h after dosing; ○) in the dosing interval are shown. The solid line represents the line of agreement between SP and BP concentrations.

response to this PI in the systemic and genital tract compartments without the confounding effects of other antiretroviral agents.

Each of these drugs appears to have different seminal pharmacokinetic profiles relative to blood. Evidence presented here suggests that the entry of APV into SP is likely driven by concentration-dependent passive diffusion. This evidence includes a relatively constant ratio of APV SP to BP concentrations throughout the dosing interval (figure 1*B*) and the fact that APV SP concentrations over time follow the shape of the simulated APV concentration-time curve in BP (figure 2) [29]. These data would not support the hypothesis that PI influx and efflux from the male genital tract may be governed by active transport pumps, such as P-gp [18]. The hypothesis that the degree of penetration of PIs into the male genital tract is more dependent on the degree of PI BP protein binding, with less-

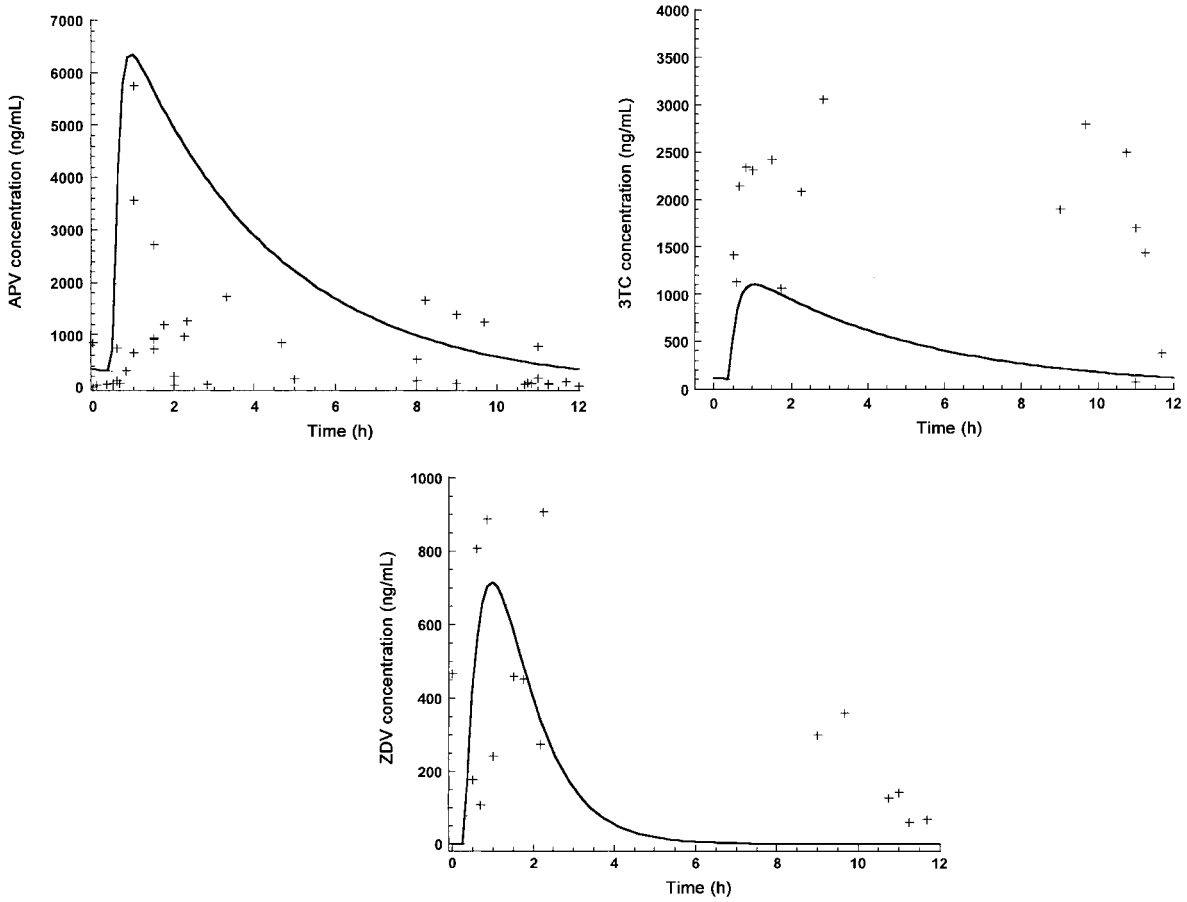

Figure 2. Simulated blood plasma (BP) concentration-time profiles and measured seminal plasma (SP) concentrations for amprenavir (APV), lamivudine (3TC), and zidovudine (ZDV). Simulated BP concentration-time profiles were generated on the basis of population BP pharmacokinetic data taken from the literature [27–29]. Measured SP concentrations for APV, 3TC, and ZDV collected 0–12 h after dosing are also plotted (+) on the graph.

bound drugs (APV and indinavir) having higher concentrations, is supported by these results and by the work of others [6, 14, 16]. Median SP APV concentrations approached or exceeded estimated protein-binding corrected concentrations required to inhibit wild-type virus. Of note, the 3 nonresponders in SP had a median concentration well below the estimated inhibitory concentration [31]. The extent of protein binding of any PI in SP is not known.

In contrast to APV, ZDV SP concentrations are similar to or higher than concentration in BP (figure 2), with concentrations diverging over time, suggesting extended ZDV elimination from SP. These results are consistent with a recent small study of 4 HIV-1–infected men receiving ZDV, in which SP concentrations increased rapidly and were followed by a long elimination phase [8]. ZDV may enter the genital compartment by passive diffusion, but either active transporters [32, 33] or the chemical properties of ZDV (reviewed in [5]) may enhance accumulation or slow elimination.

The pharmacokinetics of 3TC in the male genital tract demonstrate that this agent reaches high levels in SP, and the shape of 3TC SP pharmacokinetic curve appears flat, with no identifiable peak or trough (figures 1*A* and 2), although sampling was sparse over the middle range of the dosing interval. Concentrations of 3TC in SP are consistently greater than corresponding blood concentrations, strongly suggesting that there is active transport, potentially by nucleoside transporters, against a concentration gradient and/or sequestration of this compound in the male genital tract.

Our pharmacokinetic data show that higher APV concentrations were associated with virologic response in the monotherapy arm in both compartments, though statistically significant only in blood. APV clearly penetrates the male genital tract and, when adequate concentrations are reached, virologic responses are observed. Discordant compartment responses were seen, but in each example there was an HIV-1 RNA response in SP but not in BP. In general, SP virus loads are lower

than BP virus loads [34–37]. The numbers of infected cells producing virus are likely to be lower, resulting in a greater likelihood of suppression. However, there are examples of men with very high levels of HIV-1 RNA in semen [38], which may be more difficult to suppress with antiretroviral therapy. If in some circumstances antiretroviral penetration into this potential sanctuary site is not adequate, then HIV-1 replication in the genital tract might contribute to preservation of HIV reservoirs or might be a source of resistant virus. Given that APV concentrations in semen appear to reflect those in blood, enhancement of APV BP concentration with ritonavir might lead to more complete suppression of virus replication in the genital tract. Seminal mononuclear cells with replication-competent HIV-1 have been demonstrated, despite suppression of HIV RNA levels in blood and semen by antiretroviral therapy [39]. Whether higher drug concentration in SP could lead to decay in the size of this pool of infected cells should be further studied.

There are limitations to the observations presented here. Semen formation is dynamic, and seminal fluid is composed of distinct secretions from organs such as the seminal vesicles and prostate gland. Drug concentrations may vary among these different secretions, as shown for indinavir and nevirapine [12]. Furthermore, ejaculation may temporarily deplete the seminal fluid of drug, affecting drug concentrations or virus load in subsequent ejaculations. Our data may not reflect SP pharmacokinetics of men with a higher frequency of ejaculation. In addition, traditional frequent pharmacokinetic sampling of this compartment in humans is not possible. In this study, timed SP concentrations were plotted beside population-based BP concentration-time curves to obtain a reasonable estimate of the relationship of blood and semen drug concentrations over time because the number of paired samples was somewhat limited. Protein binding of any drug in SP is likely to be difficult to measure; therefore, the free fraction of APV in semen and the relationship between free concentrations in blood and semen remains unknown, although the antiviral effect seen in SP with APV monotherapy suggests an adequate free fraction of APV.

In summary, this study describes 3 different patterns of antiretroviral distribution over time into the male genital tract, including the marked accumulation and limited fluctuation of 3TC concentrations in this compartment. This study clearly documents penetration of the PI APV into the male genital tract over a broad range of concentrations—typically lower but correlated with systemic concentrations. Antiretroviral responses in semen were observed in the majority of men who received APV monotherapy and in all men who received combination therapy. Overall, significant progress has been made toward understanding penetration of antiretrovirals into the male genital tract. However, additional well-designed studies are needed to explore the associations between total and free drug concentrations in SP and antiretroviral responses, persistent HIV-1 replication, and emergence of resistant virus in this compartment.

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