

THE PHARMACOKINETICS (PK) OF AMPRENAVIR (APV), LAMIVUDINE (3TC) AND ZIDOVUDINE (ZDV) IN THE MALE GENITAL TRACT OF HIV-1 INFECTED MEN

A Pereira^{*1}, J Gerber², L Smeaton³, E Acosta⁴, R Gulick⁵, R Murphy⁶, J Eron¹

¹Univ. of North Carolina School of Medicine, Chapel Hill, NC ²Univ. of Colorado Health Sciences Center, Denver, CO ³Statistical and Data Analysis Center, Harvard School of Public Health, Cambridge, MA ⁴Univ. of Alabama at Birmingham, Birmingham, AL ⁵Weill Medical College of Cornell Univ., NY, NY ⁶Northwestern Univ. School of Medicine, Chicago, IL



Contact Information:

Arlene Pereira
807 Brinkhous-Bullitt Bldg.
UNC-CH CB# 7525
Chapel Hill, NC 27599
argoyle@isis.unc.edu
phone (919) 966-4294
fax (919) 966-0704

ABSTRACT

BACKGROUND Penetration of antiretroviral drugs into the male genital tract may be important for optimal HIV suppression in that compartment. Because drugs may distribute differently into the seminal and systemic compartments, a better understanding of the seminal PK of antiretrovirals is needed. **METHODS** 31 men enrolled in ACTG 850, a substudy of a 24wk, randomized, placebo controlled study. Men received either APV (n=19) or APV/3TC/ZDV (n=12) and donated blood (BP) and semen (SP) at known times following an observed medication dose at wks 8 and 24. Timed SP concentrations of APV, 3TC, and ZDV were plotted against the described population BP PK of these drugs. The first paired SP and BP collected from study subjects within 1-hr of each other were used to calculate SP:BP ratios (n=19, 8, and 7 for APV, 3TC, and ZDV; respectively). These data were used to examine the correlation between the two compartments. **RESULTS** The drugs demonstrated distinct SP PK compared to population BP PK. APV SP were < BP population PK concentrations; 3TC SP were > BP; and SP of ZDV approximated BP early in the dosing interval, but was > BP later. This was also true for the paired SP and BP study samples (p=0.0005, APV SP:BP, p=0.002 3TC SP:BP Wilcoxon signed rank test). Median APV SP:BP ratios from 0-2-hr and 2-12-hr post dose were both ≤ 1. The respective ratios for 3TC and ZDV were 1.8 and 1.0 (0-2-hr), and 5.3 and 12.9 (2-12-hr). Spearman's ranked correlation test revealed no correlation between paired SP and BP of 3TC (r=0.36). ZDV and APV SP and BP were weakly correlated (r=0.7 and 0.7, respectively). **CONCLUSIONS** Both APV monotherapy and APV/3TC/ZDV combination therapy reduce seminal shedding of HIV-1. All 3 drugs penetrate the seminal compartment, but with apparently distinct SP PK. 3TC has a flat SP PK suggesting active accumulation or inhibition of elimination; ZDV appears to be concentrated at later time points suggesting slowed elimination, while APV SP follows the BP PK but at lower concentrations.

INTRODUCTION

In 1997, there were an estimated 16,000 new infections of HIV-1 per day worldwide. It is likely that in 2001, the rate of infection has increased. Sexual contact with infected men is the most common route of viral transmission. Therefore, we have chosen to study the PK of antiretroviral drugs and viral distribution in SP. Based on clinical evidence, it is likely that sexual transmission of the virus will be reduced by reducing the concentration of HIV-1 in SP. However, the male genital tract is a separate compartment, detached from the systemic circulation by anatomic and physiologic barriers. These barriers impede antiretroviral drug distribution between the two compartments, possibly leading to unique selective pressures on HIV-1 replication. Phenotypic, tropic, and genotypic differences between seminal and blood isolates exist. If we can determine which antiretroviral drugs are active in the male genital tract, we can potentially develop therapies that not only increase the quality of life of the infected host, but impact sexual transmission as well.

ACTG 347 [1], was a 24-week, double blind, randomized, placebo-controlled study of the safety and efficacy of two therapeutic regimens: APV/ZDV/3TC combination antiretroviral therapy or APV monotherapy. ACTG 850 [2], a substudy of ACTG 347, was designed to study both the effects of these two therapies on HIV-1 shedding in SP and the distribution of APV, ZDV, and 3TC into the male genital tract. Preliminary analysis of all data collected from ACTG 850 revealed the following median SP and BP concentrations [3]:

- ♦ Median APV concentrations in SP and BP were 319.8 ng/mL and 1013.6 ng/mL, respectively.
- ♦ Median ZDV concentrations were 259.0 and 261.7 ng/mL in SP and BP, respectively.
- ♦ Median 3TC concentrations were 2206.6 and 591.2 ng/mL in SP and BP, respectively.

However, until now, no attempt has been made to determine the kinetics of drug disposition into the male genital tract or the effect on viral load of APV with or without ZDV/3TC.

The goals of ACTG 850 were to:

1. Determine the kinetics of APV, 3TC, and ZDV distribution into the male genital tract.
2. Describe the effect of APV monotherapy on SP and BP viral loads.

STUDY DESIGN AND SUBJECTS

Inclusion requirements for ACTG 347/850 involved being HIV-1 seropositive, 3TC and protease inhibitor naive, and ≤ 13 years old. The only additional criterion for enrollment into ACTG 850 was that subjects must be men willing to donate timed SP samples. Subjects were excluded from ACTG 347/850 if they were previously unable to tolerate ZDV. Upon enrollment into ACTG 347/850, subjects discontinued their current antiretroviral therapy, if any. Subjects were randomized to receive either 1200 mg APV (Agenerase®; Vertex Pharmaceuticals, Cambridge, MA; GlaxoSmithKline (formerly GlaxoWellcome), Research Triangle Park, NC), 300 mg ZDV (Retrovir®; GlaxoSmithKline, formerly GlaxoWellcome), and 150 mg 3TC (Epivir; GlaxoSmithKline, formerly GlaxoWellcome) given once bid as combination antiretroviral therapy or 1200 mg APV given bid as monotherapy. Subjects of ACTG 850 donated SP and BP before receiving any study medications for baseline viral RNA analysis. Follow-up SP and BP were donated at wks 8 and 24. Samples were to be collected 6-hr post dose, with the times of most recent medication dose, semen sampling, and blood sampling recorded. Subjects were asked to abstain from sexual activity for 48-hr prior to semen donation.

Table 1. Patient Characteristics:

Race/Ethnicity of Subjects		
	Number	Percentage of Total
White Non-Hispanic	23	61
Black Non-Hispanic	5	16
Hispanic (Regardless of Origin)	3	10
Age of Subjects (Median = 37)		
	Number	Percentage of Total
Under 30	7	23
30-39	12	39
40-49	11	35
50-59	1	3
Characteristics of Each Study Arm [Median (IQR)]		
	Monotherapy n = 19	Combination Therapy n = 12
SP VL	3.48 (2.6-4.08)	3.89 (2.6-5.11)
BP VL	4.14 (3.69-5.04)	4.23 (3.71-4.72)
CD4+	229 (169-356)	336 (162-416)

METHODS

1. Kinetics of APV, 3TC, and ZDV distribution into the male genital tract.

Concentrations of ZDV and 3TC in SP and BP were measured using validated HPLC-MS/MS methods described elsewhere [4,5]. Lower limits of quantitation of these drugs were 5 ng/mL in both SP and BP. APV concentrations were determined using a validated HPLC-MS/MS method with isotopically stable *d*₆-amprenavir as the internal standard. The lower limit of APV quantification in both matrices was 30 ng/mL. Times of most recent medication dose, semen sampling, and blood sampling were recorded and utilized for analysis.

2. Effect of APV monotherapy on SP and BP viral loads.

HIV-1 RNA concentrations in SP and BP were determined by the NucliSens assay (Organon-Teknika, Durham, NC). The lower limit of quantitation was 400 copies/mL in both SP and BP. Positive virologic response, defined as an HIV-1 RNA level < 400 copies/mL or a > 1.0 log₁₀ decline from baseline to first sample, was determined independently in the BP and SP of subjects in the two study arms.

3. Statistical Analysis

Statistical analyses are mostly descriptive because of the small sample size and limited follow-up. Concentrations were divided into early (0-2-hr) and late (>2-hr) post dose groups. The division at 2-hr was based on the simulated population PK profile of these 3 drugs. SP and BP samples were considered matched pairs if they were obtained (inclusively) within 1-hr of one another. Rank-based (Spearman) correlations describe the association between the concentrations in the two compartments among the first matched pair per subject, separately by early or late dosing group. Compartmental ratios of SP to BP were also calculated among quantifiable concentrations.

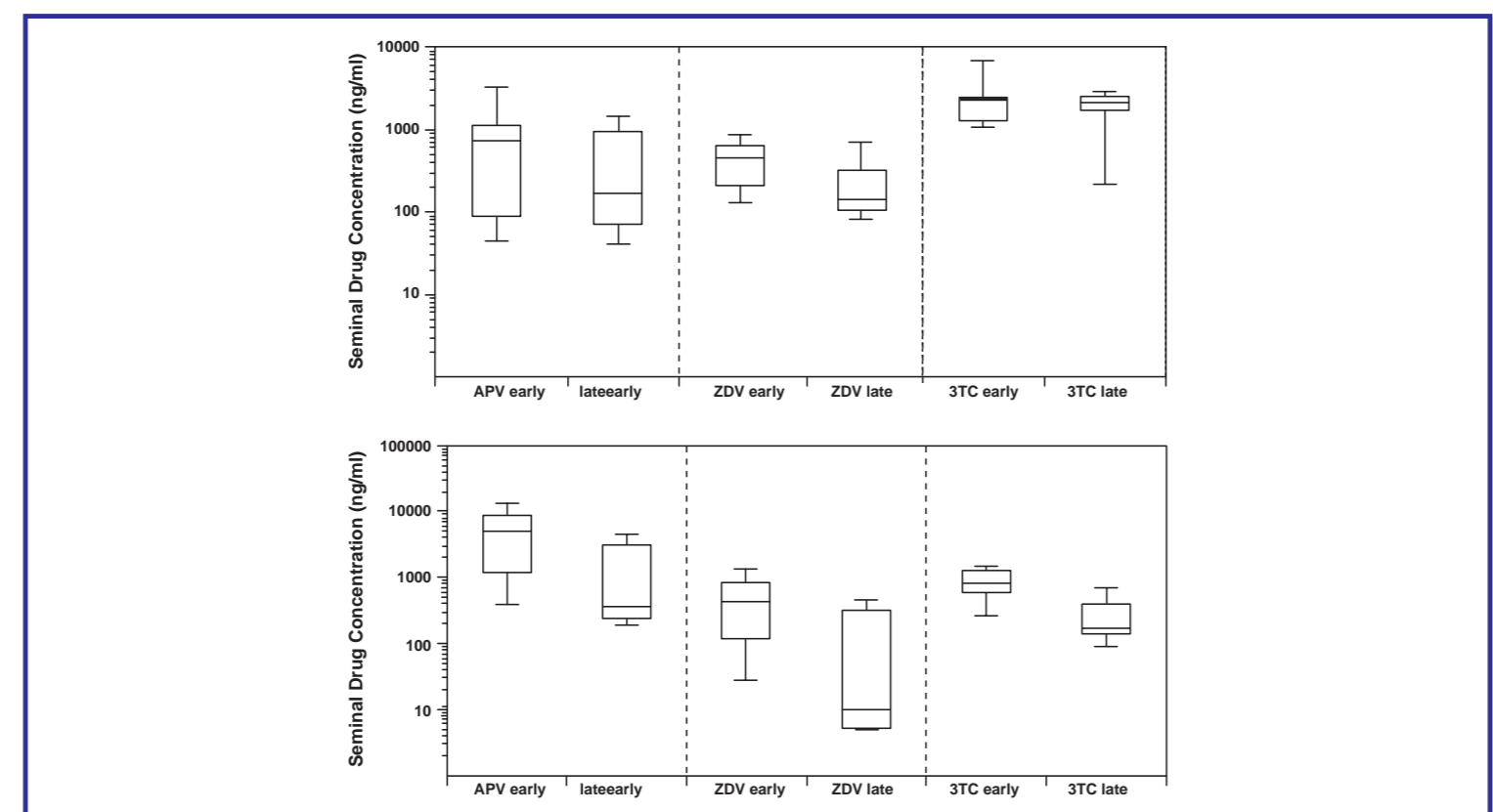
In the monotherapy arm, APV concentrations were compared between RNA response groups, RNA responders or non-responders. Responders were those with <400 copies/mL and or > 1 Log decrease VL. APV concentrations were compared by a Wilcoxon rank sum test. Time post dose for these samples was compared in a similar manner. Distributions were summarized with medians and interquartile ranges (IQR).

RESULTS

Table 2. Samples Obtained During Study

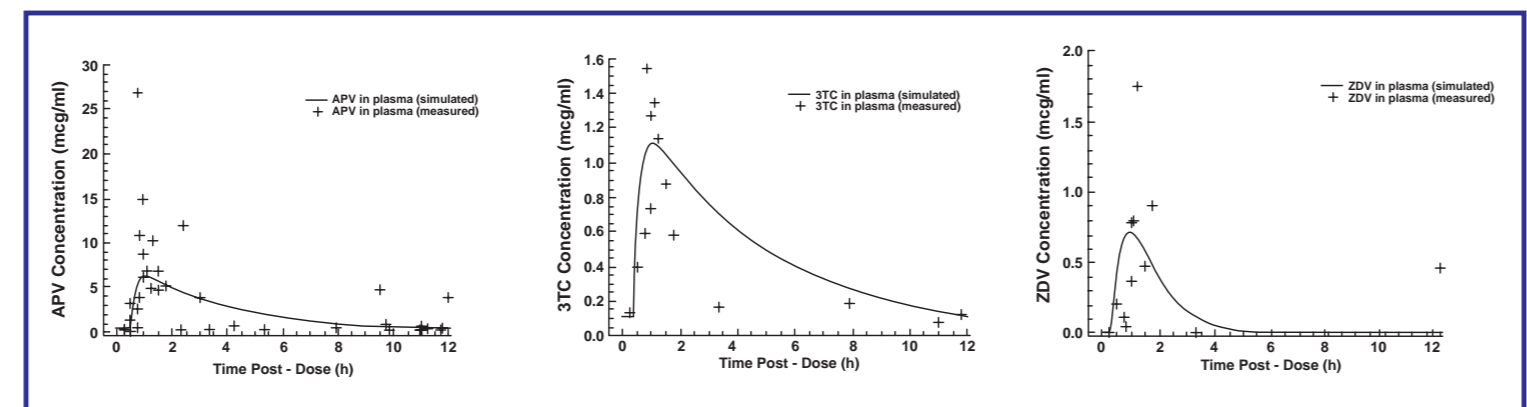
	Monotherapy		Combination Therapy	
	0-2-hr	>2-hr	0-2-hr	>2-hr
SP	8	16	10	9
BP	8	15	10	8
Paired Samples	8	6	9	5

Figure 1. Median Early and Late SP and BP Concentrations of Amprenavir, Zidovudine, and Lamivudine



Median, IQR and 5th-95th percentile range of drug concentrations in SP and BP from samples collected early (first 2-hr of dosing interval) vs. late (>2-hr after dose). All samples collected during the study were used to construct this figure.

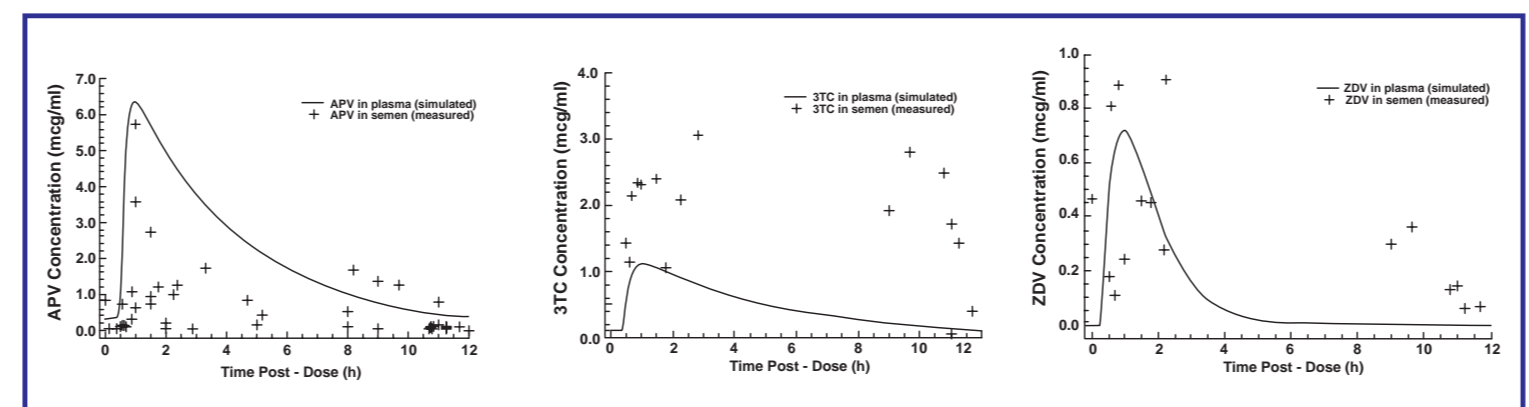
Figure 2A. Concentration-Time Profile of Amprenavir, Zidovudine and Lamivudine in BP



A simulated BP concentration-time profile was generated based on population BP PK data for comparison to samples donated during ACTG 850. ZDV and 3TC parameters used for the PK simulations were taken from the literature [6,7]. APV data were provided by the manufacturer. A one-compartment, oral absorption, linear elimination model was used to generate the simulated BP concentration-time curves. BP collected 0-12-hr post dose appear on these graphs as +.

Amprenavir, zidovudine, and lamivudine: There is a lot of variability in the measured BP levels, however, all three drugs are consistent with the simulated curves—early peak at approx. 2-hr followed by an extended trough.

Figure 2B. Concentration-Time Profile of Amprenavir, Zidovudine and Lamivudine in SP



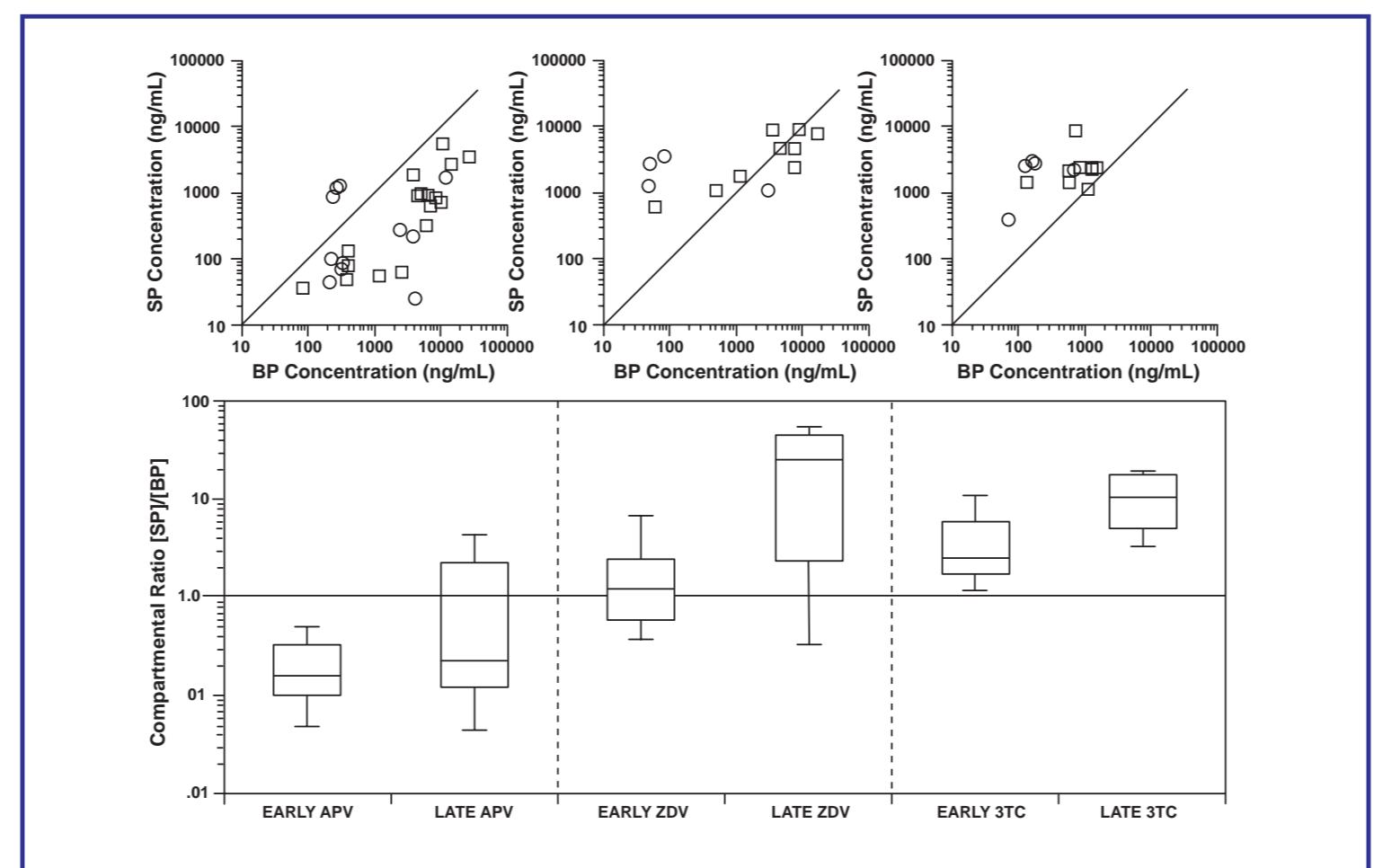
SP collected during this study appear on these graphs as +.

Amprenavir: The shape of the APV SP concentration-time curve follows the BP curve but at lower concentrations.

Lamivudine: 3TC has a flat SP concentration-time curve that is all significantly higher than the BP concentration.

Zidovudine: ZDV, in SP appears to become concentrated only late in the dosing interval when compared to BP concentrations.

Figure 3. Concentration of Amprenavir, Zidovudine, and Lamivudine in Paired Semen and Blood Samples Collected Early and Late in the Dosing Interval



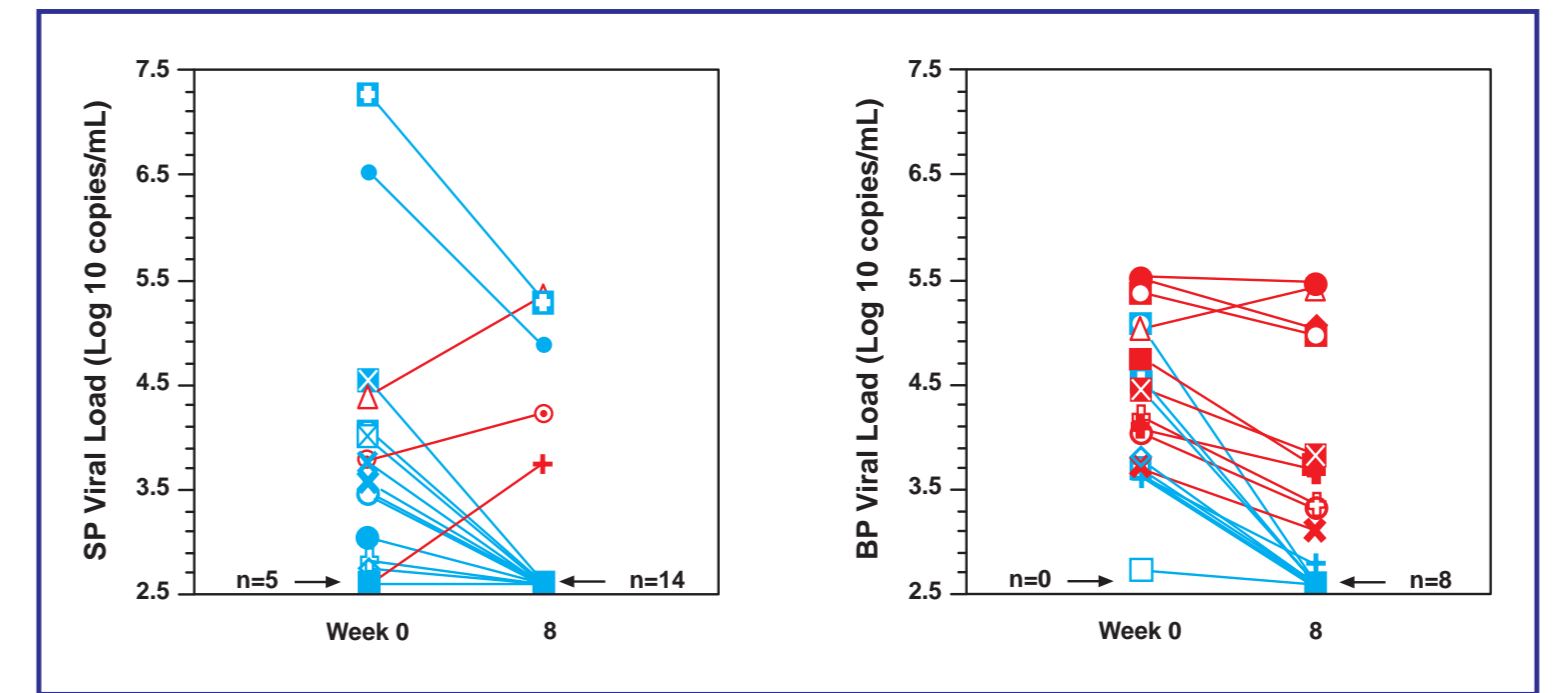
To better understand the PK of drug distribution, only matched paired samples (SP and BP collected within 1-hr of each other) were used to construct the scatter and box-and-whiskers plots. In the scatter plot, samples collected 0-2-hr post dose are early and are displayed with □, those collected after 2-hr are late and are displayed with ○. Median, IQR, and 5th-95th percentile range of calculated compartmental ratios are depicted in the box-and-whiskers plots.

Amprenavir: All early APV samples have BP>SP. Median early and late compartmental ratios are similar and <1. There is a positive correlation between early SP and early BP concentrations (r=0.70, p=0.007, n=13) suggesting similar absorption rates in the systemic and seminal compartments. There is no correlation between late SP and late BP concentrations, however. This may be due to variability in APV elimination from SP, as evidenced by the broad late APV box and whisker plot above.

Zidovudine: 5/12 (4/8 early and 1/4 late) samples have BP>SP. Neither early nor late SP and BP concentrations are correlated. The median compartmental ratio increases from early to late suggesting the ZDV's removal from SP is slower than from BP.

Lamivudine: 1 early and all late 3TC samples have SP>BP, and neither early nor late SP and BP concentrations are correlated. Compartmental ratios remain significantly ≥1 at both early and later collections suggesting that 3TC remains trapped in the SP at high concentrations even when BP is being cleared of the drug.

Figure 4. SP and BP Viral Load at Enrollment, First Follow-up, and Second Follow-up in the Monotherapy Therapy Study Arm



The blue lines show instances of positive virologic response, red show instances of no response. n = number of patients at the limit of quantitation at weeks 0 and 8 in SP and BP. The symbols are matched between seminal and systemic compartments.

Table 3. Virologic Response at Week 8 in the Monotherapy Arm

Compartmental Virologic Response in Patients Receiving APV Monotherapy (n=19)						
Positive Virologic Response: BP and SP		9				
Positive Virologic Response: BP Only		1				
Positive Virologic Response: SP Only		7				
No Virologic Response		1				
		SP Response			BP Response	
		Positive Virologic Response	No Virologic Response	Sig. Dif. Btw. Responders and Non-Responders	Positive Virologic Response	No Virologic Response
Median Compartmental [APV]		825.6 ng/mL	76.8 ng/mL	p=0.05	4664.2 ng/mL	576.8 ng/mL
Median (IQR) Time Post Dose (hr)		2.83 (1.5-8.2)	2 (0.7-9)	p>0.6	6 (1-11.75)	3 (0.75-11)
						Sig. Dif. Btw. Responders and Non-Responders
						p>0.6

DISCUSSION

1. Kinetics of APV, 3TC, and ZDV distribution into the male genital tract.

Ours is the first study that has attempted to look at the PK of APV, ZDV, and 3TC in the seminal compartment in a dynamic manner. Since we did not have enough BP samples to obtain our own population PK we had to rely on the literature. However it is encouraging to see that the timed BP samples that we obtained in this study fit well into the generated population curves. In addition, the nucleoside SP and BP concentrations and compartmental ratios determined in the present study correlate with previously published data [8,9].

The phenomenon of time-dependent drug disposition of antiretrovirals into the male genital tract has already been described for ZDV [10], 3TC and stavudine [11], and nelfinavir and indinavir (reviewed, [12]). APV appears to be unique in that its concentration ratios remain approximately 0.2 throughout the dosing interval. By comparing the measured SP concentrations to the simulated population PK curves and measured BP, we were able to formulate hypotheses as to how these drugs behave in this sanctuary compartment.

FUTURE STUDIES

- ♦ To describe mechanisms of drug transport and sequestration in the male genital tract.
- ♦ To study antiretroviral protein binding in SP.
- ♦ To measure intra-cellular concentrations of antiretroviral drugs in SP.
- ♦ To assess the effect of frequent ejaculation on antiretroviral drug distribution in to the male genital tract.
- ♦ To co-localize drug and virus in specific tissues and compartments within the male genital tract.

REFERENCES

1. R. Murphy, R. Gulick, V. DeGruttola, R. D'Aquila, J. Eron, J.-P. Sommadossi, J. Currier, L. Smeaton, I. Frank, A. Caliendo, J. Gerber, R. Tung, D. Kuritzkes, The Journal of Infectious Diseases 179 (1999) 808.
2. J. Eron, L. Smeaton, R. Gulick, J. Currier, J. Lennox, R. D'Aquila, M. Rogers, R. Tung, R. Murphy, The Journal of Infectious Diseases 181 (2000) 162.
3. A. Pereira, J. Eron, J. Dunn, J. Gerber, R. Tidwell, K. Kenney, S. Fiscus, J.-P. Sommadossi, R. Tung, R. Gulick, R. Murphy, 7th Conference on Retroviruses and Opportunistic Infections (2000) Abstract 317.
4. A. Pereira, K. Kenney, M. Cohen, J. Hall, J. Eron, R. Tidwell, J. Dunn, Journal of Chromatography B 742 (2000) 173.
5. K. Kenney, S. Wring, R. Carr, G. Wells, J. Dunn, Journal of Pharmaceutical and Biomedical Analysis (2000) 967.
6. E. Acosta, K. Henry, L. Page, A. Erice, H.J. Balfour, C. Fletcher, Pharmacotherapy 17 (1997) 424.
7. K. Moore, G. Yuen, E. Hussey, G. Pakes, J.J. Eron, J. Bartlett, Antimicrobial Agents and Chemotherapy 43 (1999) 3025.
8. A. Pereira, A. Kashuba, S. Fiscus, J. Hall, R. Tidwell, L. Troiani, J. Dunn, J. Eron, M. Cohen, Journal of Infectious Diseases 180 (1999) 2039.
9. K. Henry, B. Chinock, R. Quinn, C. Fletcher, K. deMiranda, H. Balfour, JAMA 20 (1988) 3023.
10. P.L. Anderson, S.E. Noormohamed, K. Henry, R.C. Brundage, H.H. Balfour, C.V. Fletcher, Pharmacotherapy 20 (2000) 917.
11. S. Taylor, R. vanJeesswijk, R. Hoetelmans, J. Workman, S. Drake, D. Pillay, 39th International Conference on Antimicrobial Agents and Chemotherapy (1999).
12. S. Taylor, A. Pereira, HIV Medicine 1 (2000) 18.