HHS Public Access

Author manuscript

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2016 December 15.

Published in final edited form as:

J Acquir Immune Defic Syndr. 2015 December 15; 70(5): 510–514. doi:10.1097/QAI.0000000000000777.

Significant Decreases in both Total and Unbound Lopinavir and Amprenavir Exposures during Co-administration: ACTG Protocol A5143/A5147s Results

Julie B. DUMOND, PharmD, MS[a], **Joseph RIGDON, PhD**[b], **Katie MOLLAN, MS**[d,g], **Camlin TIERNEY, PhD**[e], **Angela D.M. KASHUBA, PharmD**[a,g], **Francesca AWEEKA, PharmD**[f], and **Ann C. COLLIER, MD**[c]

[a]UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC

[b]Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC

[c]University of Washington School of Medicine, Seattle, WA

[d]Center for AIDS Research, Lineberger Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

[e]Center for Biostatistics in AIDS Research, The Harvard T.H. Chan School of Public Health, Boston, MA

[f]UCSF School of Pharmacy, San Francisco, CA

[g]School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract

This secondary analysis explored changes in protein-unbound concentrations of lopinavir and amprenavir when co-administered in HIV-infected subjects. Total and unbound pharmacokinetic parameters were calculated and compared between subjects receiving each agent alone, and co-administration. When co-administered, unbound and total concentrations decrease. Co-administration significantly increased lopinavir unbound clearance, while significant changes in fraction unbound (f_u) were not detected. For amprenavir, significant increases in f_u and unbound clearance occurred with co-administration. This demonstrates the complex nature of drug-drug interactions between highly protein-bound, CYP-metabolized drugs, and the need to measure unbound concentrations in disease states like hepatitis C, where such agents are co-administered.

Keywords

dual protease inhibitor; lopinavir; ritonavir; amprenavir; unbound concentration; drug-drug interaction

Corresponding Author: Julie B. Dumond, PharmD, MS; 1093 Genetic Medicine Bldg, CB 7361, 120 Mason Farm Road, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7361; jdumond@unc.edu.

Introduction

HIV protease inhibitors (PIs) are highly bound to proteins such as albumin and alpha-1-acid glycoprotein. They have complex metabolism and transport profiles, and high potential for drug-drug interactions. For example, lopinavir (LPV) is a high-extraction drug (e.g. hepatic clearance is the major route of drug elimination), but it becomes low-extraction when co-administered with ritonavir due to inhibition of CYP3A-mediated clearance.[1] The ester pro-drug of amprenavir (APV), fosamprenavir (FPV), administered to improve oral absorption, tolerability and reduce pill burden, may be used with or without ritonavir. In either situation, FPV remains a high-extraction drug.[2] As previously shown, when FPV or APV is co-administered with lopinavir/ritonavir (LPV/r), decreases in total LPV area-under-the-concentration-time-curve (AUC) from 24-48% and decreases in total APV AUC from 53-67% are observed.[3-5]

The significance of changes in plasma protein binding due to drug-drug interactions is widely debated in the pharmacokinetics (PK) literature.[6,7] According to PK theory, only drugs that are high-extraction and administered intravenously, or those administered orally that are both high-extraction and non-hepatically eliminated should exhibit changes in unbound drug concentrations as a result of displacement of one drug by another on plasma proteins, i.e. due to a change in fraction unbound (f_u). Changes in the intrinsic clearance of unbound drug, whether due to changes in metabolism or transport, may affect the unbound concentrations of orally administered, low-extraction drugs or orally administered high-extraction drugs eliminated by hepatic mechanisms, such as LPV and APV, respectively.

Here, we expand upon previously reported decreases in total drug concentrations, with the observed decreases in unbound concentrations due to a drug-drug interaction between FPV and LPV when co-administered with ritonavir in HIV-infected subjects in AIDS Clinical Trials Group (ACTG) protocol A5143 and its pharmacology substudy, A5147s.

Methods

Study Protocol and Sub-study Inclusion Criteria

Details regarding A5143, A5147s, and main results have been published.[3,8] Briefly, treatment-experienced HIV-1 infected subjects were randomized 1:1:2 into 3 arms to compare the efficacy and PK of lopinavir/ritonavir 400/100 mg twice daily (LPV/r; Arm A), fosamprenavir/ritonavir 700/100 mg twice daily (FPV/r; Arm B), and the combination of fosamprenavir/lopinavir/ritonavir 700/400/100 mg twice daily (LPV/FPV/r; Arm C), all administered with tenofovir and 1-2 additional nucleoside agents. A planned PK substudy, A5147s, enrolled 8, 8, and 17 evaluable subjects in Arms A (LPV/r), B (FPV/r), and C (LPV/FPV/r), respectively; PK data from 10 additional subjects enrolled in A5143 became available after publication of interim A5147s analysis. Samples collected from these 43 subjects underwent analysis for unbound concentrations of LPV and APV. Fifteen subjects were further excluded from this analysis due to missing concentrations at pre-specified sampling points for PK parameter calculations[3]; 9 in the LPV/FPV/r arm, 3 in the LPV/r arm, and 3 in the FPV/r arm. In total, twenty-eight subjects (9, 9, and 10 in Arms A, B, and C, respectively) had complete and evaluable PK profiles for unbound LPV and/or APV. The

transfer of de-identified PK and demographic data from the ACTG to the University of North Carolina at Chapel Hill (UNC-CH) was approved by the ACTG and the UNC-CH Biomedical Institutional Review Board.

Unbound Lopinavir and Amprenavir Concentrations

The analytical methods for total drug concentrations have been previously reported.[3] Unbound concentrations of LPV and APV were determined by equilibrium dialysis using radiolabeled standards. The system was comprised of a Harvard apparatus multi-equilibrium dialyzer with Macro Teflon dialysis cells (1ml) and variable speed drive unit, a Beckman LS3801 Liquid Scintillation Counter, and Spectra/Por®RC membranes, using Dulbecco's phosphate-buffered saline as the dialysis buffer and 900 μL of sample plasma. Equilibrium time was 4-5 hours. For each drug, five samples of 4 different concentrations (LPV 0.0, 0.5, 6, and 12μg/ml; APV 0.0, 0.2, 2, and 8μg/ml) were analyzed on 5 separate days. The inter-day percent coefficient of variation (%CV) for LPV ranged from 9.9 to 12.1%. The average intra-day %CV for LPV ranged from 6.8% to 8.6%. The inter-day %CV for APV ranged from 6.4 to 9.5%. The average intra-day %CV for APV ranged from 1.8% to 6.6%. Recovery was 96.2% and 92.5% for LPV and APV, respectively.

Pharmacokinetic and Statistical Analyses

The area-under-the-concentration-time curve for the dosing interval (AUC_{tau}) was calculated in Phoenix Win Nonlin 6.3 (Pharsight, a Certara company, St. Louis, MO), using the linear-up/log-down method for both total and unbound concentrations. Apparent oral clearance at steady state (CL/F_{ss}) for total concentrations was calculated as dose/AUC_{tau}. Unbound clearance ($CL/F_{ss,u}$) was calculated for each subject by dividing estimated total CL/F_{ss} by the median fraction unbound (f_u) over the dosing interval for LPV and APV.[9]

While the study enrolled subjects with prior exposure to either LPV or APV, but not both, comparisons were restricted to subjects who were naïve to the drug under evaluation and thus eligible for randomization to either of the arms being compared (e.g. comparisons of LPV PK were limited to subjects who were LPV-naïve at randomization). Statistical comparisons of PK parameters were carried out on the natural logarithmic scale. Geometric mean ratio (GMR) estimates were calculated by exponentiation of the difference in means of log-transformed PK measurements, and 95% Wald-type confidence intervals were computed using unpooled variance estimates and Satterthwaite's approximate degrees of freedom. Exact Wilcoxon rank-sum tests were performed. A GMR below 1 indicates a lower geometric mean for APV/LPV combined (Arm C). Baseline demographics between subjects who did and did not have available unbound concentrations were compared using nonparametric tests (continuous data: Wilcoxon rank-sum test; categorical data: Fisher's exact test). Statistical analyses were conducted using a two-sided statistical significance level of 0.05, in SAS 9.3 (SAS Institute Inc., Cary, NC) or R version 3.1.2 (www.R-project.org). Comparisons were carried out on both total and unbound parameters; results for the total drug parameters presented here are not expected to provide the same results as those previously published, as this analysis is comprised of a set of subjects that does not completely overlap with the previous analysis set.[3]

Results

Subject Demographics

The mean and standard deviation (mean±SD) age of the 28 subjects with evaluable unbound and total PK was 44±8 years. All but two subjects were male, and 50% were Caucasian non-Hispanic. The mean±SD $\log_{10}$ copies/mL of viral load at enrollment was 4.61±0.66, with CD4+ cell counts of 211±167 cells/mm^3. No subjects in Arm A or Arm B had previous exposure to the randomized protease inhibitor; in Arm C, 9/10 subjects were naïve to both drugs, with 1 subject having previous LPV exposure. No significant differences in age, sex, race, baseline HIV RNA concentration, baseline CD4+ count, or previous drug exposure were observed between the 43 subjects with available total drug PK and the 28 with total and unbound PK (all p-values >0.2).

Lopinavir

The median AUC_{tau}, 12-hr concentrations (C12hr), and clearance values of total and unbound LPV when administered as LPV/r and LPV/APV/r (Arm A and C, respectively) and GMR 95% confidence intervals and p-value for their comparisons are presented in Table 1; concentration-time plots of total and unbound LPV, by arm, are shown in Figure 1a. Total LPV AUC_{tau} and C12hr were significantly lower when combined with FPV (Arm C) than without (Arm A; GMR for AUC_{tau} of 0.63 (95% CI: 0.46-0.85), p = 0.019, and GMR of 0.47 (0.28-0.8), p = 0.019 for C12hr). This was also true for the unbound LPV PK parameters (GMR for AUC_{tau} of 0.49 (0.33-0.72), p < 0.001 and 0.35 (0.18-0.65), p = 0.008 for C12hr). Total LPV apparent oral clearance at steady state (CL/F_{ss}) was not significantly different between arms (GMR of 1.34 (0.88-2.04), p = 0.14), although the median CL/F during co-administration (Arm C) was approximately 45% higher compared to single administration (Arm A). To assess potential causes of this drug-drug interaction, the distribution of the fraction unbound (f_u) within a subject was compared across arms; for LPV, the f_u was higher, but not significantly different between LPV alone and LPV/APV co-administration (median f_u of 0.011 vs 0.0088, p = 0.077). However, the LPV apparent oral clearance ($CL/F_{ss,u}$) for unbound drug concentration was significantly higher when co-administered with APV (671 L/hr) compared to being used alone (428 L/hr, p = 0.004).

Amprenavir

The median AUC_{tau}, 12-hr concentrations (C12hr), and clearance values of total and unbound APV when administered as FPV/r and LPV/APV/r (Arm B and C, respectively), and GMR analyses for their comparison are presented in Table 1; concentration-time plots of total and unbound APV, by arm, are shown in Figure 1b. As previously reported in the A5147s analysis, APV PK parameters were significantly lower in the LPV/FPV/r regimen (Arm C) than in the FPV/r regimen (Arm B; (GMR for AUC_{tau} of 0.45 (95% CI: 0.33-0.62), p < 0.001; GMR for C12 of 0.41 (0.26-0.65), p = 0.002; GMR for CL/F_{ss} of 2.11 (1.53-2.90), p = 0.002). This was also true for the unbound APV PK parameters (GMR for AUC_{tau} of 0.62 (0.39-0.97), p = 0.035 and 0.51 (0.28-0.94), p = 0.035 for C12hr). Here, for APV, the f_u and the $CL/F_{ss,u}$ were both significantly higher when dosed with LPV, with a median f_u of 0.067 and 0.079, p = 0.010 and a median $CL/F_{ss,u}$ of 231 and 475 L/hr, p = 0.037 for Arm B compared to Arm C.

Conclusions

These results illustrate the complex nature of drug-drug interactions between HIV PIs. For LPV, $CL/F_{ss,u}$ was significantly higher in the presence of APV, while a significant difference in f_u was not demonstrated, suggesting induction of hepatic metabolism or alteration of transport mechanisms. PIs are substrates for several drug transporters,[10] which may be altered and increase biliary elimination, increase efflux from intestinal cells, or decrease efflux in hepatic cells with resultant increased metabolism. Potentially, co-administration of multiple PIs may saturate transport mechanisms, resulting in altered intrinsic clearance.[10] For APV, when combined with LPV, alteration in $CL/F_{ss,u\ was}$ also observed, as well as a significantly higher f_u. These observed changes $CL/F_{ss,u}$ are consistent with pharmacokinetic theory, which predicts changes in unbound concentrations of these orally administered high-extraction drugs when metabolism or transport are altered. The significant difference in f_u for APV is not consistent with pharmacokinetic theory, but is consistent with results from ANRS 104, where APV was administered with LPV/r, rather than FPV here, but only unbound APV was measured.[5] Other potential mechanisms that could explain these APV changes, such as altered absorption or a physicochemical interaction in the gut are less likely, given that dose separation and increased ritonavir dosing still results in significantly lower LPV and APV concentrations when co-administered.[11] The complex interactions between co-administered PI that affect disposition may partially explain this phenomenon. Despite decreased unbound concentrations, however, in this and other reports, IQ ratios demonstrate unbound concentrations above the minimum needed for virologic effect and at least partial virologic efficacy was achieved.[4,5,12-14]

Although dual PI treatment within the context of HIV infection is not a recommended treatment strategy, the dawn of hepatitis C virus (HCV) PIs, which share metabolism and transporter pathways with HIV PIs, introduce similar conundrums when treating HIV/HCV co-infected patients.[15] Ritonavir, which has complex effects on CYP450 enzyme and drug transporter inhibition and induction, is also used in HCV treatment as a pharmacokinetic enhancer.[16-18] Decreases in unbound darunavir concentrations when co-administered with the HCV drug telapravir in a co-infected patient have recently been reported.[19] Understanding the mechanisms behind these changes is critical to efficacious co-administration of these drugs. Increases in unbound clearance result in lowered unbound concentrations, as demonstrated here, with potential detrimental outcomes depending on the efficacy target of the drug.[6,20] therefore, studying unbound concentrations, which are the determinants of efficacy, is worthwhile when drugs with complex metabolic and transport profile, and thus, unpredictable interactions, are co-administered.

Acknowledgments

The authors would like to thank the A5143/A5147s study participants, study sites, and research personnel. Phoenix WinNonlin software was provided to the Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, by Certara as a member of the Pharsight Academic Center of Excellence Program. Radiolabeled standards were generously provided to the Drug Research Unit at San Francisco General Hospital by GlaxoSmithKline (Research Triangle Park, NC) and Abbott Laboratories (North Chicago, IL). The study drugs were provided to the AIDS Clinical Trials Group by Gilead Sciences (Foster City, CA) and GlaxoSmithKline (Research Triangle Park, NC).

Disclaimers: Dr. Collier is a member of a Data and Safety Committee for a Merck & Co.-sponsored study; she has received research support from Schering-Plough, Roche Molecular Systems, and Merck & Co; she or immediate family members formerly owned stock in Abbott Laboratories, Bristol-Myers-Squibb, Johnson & Johnson, and Pfizer.

KM has received research support to University of North Carolina at Chapel Hill from Merck, AbbVie, and Gilead.

Funding Sources: UM1AI068636 from the National Institute of Allergy and Infectious Diseases and supported by National Institute of Mental Health (NIMH), National Institute of Dental and Craniofacial Research (NIDCR).

JD is supported in part by K23AI093156.

JD, JR, KM, and ADMK are supported in part by the University of North Carolina at Chapel Hill Center for AIDS Research (CFAR), an NIH funded program P30 AI50410.

AC is supported in part by UM1 AI069481.

CT is supported by NIH/NIAID grant AI068634 for the Statistical and Data Management Center for the Adult AIDS Clinical Trials Group

References

1. Corbett AH. Kaletra (lopinavir/ritonavir). The Annals of pharmacotherapy. Jul; 2002 36(7-8):1193–1203. [PubMed: 12086554]
2. Wire M, Shelton M, Studenberg S. Fosamprenavir. Clin Pharmacokinet. 2006 Feb 01; 45(2):137–168. [PubMed: 16485915]
3. Kashuba AD. Combining fosamprenavir with lopinavir/ritonavir substantially reduces amprenavir and lopinavir exposure: ACTG protocol A5143 results. AIDS (London). Jan 28; 2005 19(2):145–152.
4. Pham PA, Hendrix CW, Barditch-Crovo P, et al. Amprenavir and lopinavir pharmacokinetics following coadministration of amprenavir or fosamprenavir with lopinavir/ritonavir, with or without efavirenz. Antiviral therapy. 2007; 12(6):963–969. [PubMed: 17926651]
5. Taburet A-M. Interactions between amprenavir and the lopinavir-ritonavir combination in heavily pretreated patients infected with human immunodeficiency virus. Clinical pharmacology and therapeutics. Apr; 2004 75(4):310–323. [PubMed: 15060509]
6. Benet LZ. Changes in plasma protein binding have little clinical relevance. Clin Pharm Ther. 2002; 71(3):115–121.
7. Heuberger J, Schmidt S, Derendorf H. When is protein binding important? Journal of Pharmaceutical Sciences. 2013; 102(9):3458–3467. [PubMed: 23650013]
8. Collier AC, Tierney C, Downey GF, et al. Randomized study of dual versus single ritonavir-enhanced protease inhibitors for protease inhibitor-experienced patients with HIV. HIV clinical trials. Mar-Apr;2008 9(2):91–102. [PubMed: 18474494]
9. Banfield C, O'Reilly R, Chan E, Rowland M. Phenylbutazone-warfarin interaction in man: further stereochemical and metabolic considerations. British journal of clinical pharmacology. Dec; 1983 16(6):669–675. [PubMed: 6661352]
10. Griffin L, Annaert P, Brouwer KL. Influence of drug transport proteins on the pharmacokinetics and drug interactions of HIV protease inhibitors. J Pharm Sci. Sep; 2011 100(9):3636–3654. [PubMed: 21698598]
11. Corbett AH, Patterson KB, Tien H-C, et al. Dose Separation Does Not Overcome the Pharmacokinetic Interaction between Fosamprenavir and Lopinavir/Ritonavir. Antimicrobial Agents and Chemotherapy. Aug 1; 2006 50(8):2756–2761. [PubMed: 16870769]
12. Arvieux C, Tribut O. Amprenavir or fosamprenavir plus ritonavir in HIV infection: pharmacology, efficacy and tolerability profile. Drugs. 2005; 65(5):633–659. [PubMed: 15748098]
13. Raguin G, Chene G, Morand-Joubert L, et al. Salvage therapy with amprenavir, lopinavir and ritonavir 200 mg/d or 400 mg/d in HIV-infected patients in virological failure. Antiviral therapy. Aug; 2004 9(4):615–625. [PubMed: 15456093]

14. Raguin G, Poizat G, Morand-Joubert L, et al. Salvage therapy with amprenavir, lopinavir and ritonavir is durably potent in HIV-infected patients in virological failure: 1-year results. AIDS (London, England). Jan 11; 2007 21(2):241–243.
15. Karageorgopoulos DE, El-Sherif O, Bhagani S, Khoo SH. Drug interactions between antiretrovirals and new or emerging direct-acting antivirals in HIV/hepatitis C virus coinfection. Current opinion in infectious diseases. Feb; 2014 27(1):36–45. [PubMed: 24305043]
16. Kwo PY, Mantry PS, Coakley E, et al. An Interferon-free Antiviral Regimen for HCV after Liver Transplantation. The New England journal of medicine. Nov 11.2014
17. Lawitz E, Sullivan G, Rodriguez-Torres M, et al. Exploratory trial of ombitasvir and ABT-450/r with or without ribavirin for HCV genotype 1, 2, and 3 infection. The Journal of infection. Sep 22.2014
18. Feld JJ, Jacobson IM, Jensen DM, et al. Randomised study of danoprevir/ritonavir-based therapy for HCV genotype 1 patients with prior partial or null responses to peginterferon/ribavirin. Journal of hepatology. Sep 16.2014
19. Curran A, Guiu JM, Ribera E, Crespo M. Darunavir and telaprevir drug interaction: total and unbound plasma concentrations in HIV/HCV-coinfected patients with cirrhosis. The Journal of antimicrobial chemotherapy. 2014; 69(5):1434–1436. [PubMed: 24363317]
20. Gonzalez D, Schmidt S, Derendorf H. Importance of relating efficacy measures to unbound drug concentrations for anti-infective agents. Clinical microbiology reviews. Apr; 2013 26(2):274–288. [PubMed: 23554417]

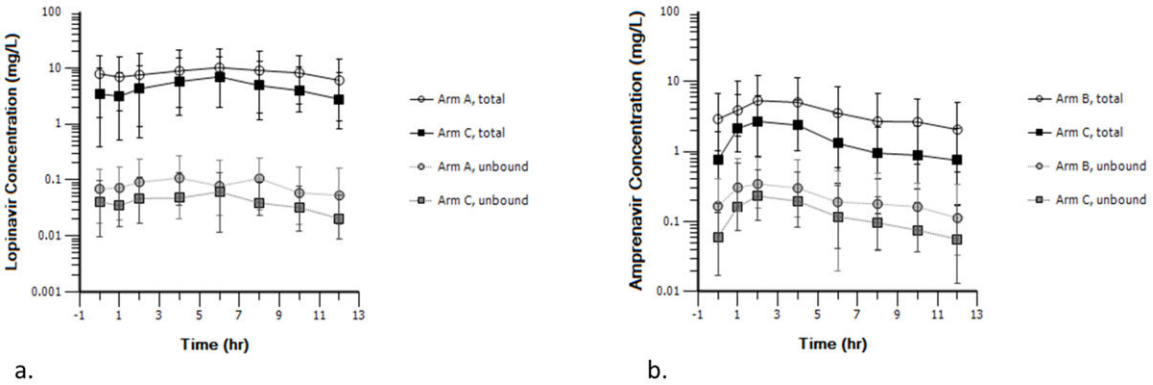

Figure 1.

Total and unbound lopinavir (a) and amprenavir (b) concentration-time plots, by study arm. Data are shown as median with interquartile range. For both graphs, Arm C is the combined treatment arm; Arm A is lopinavir/ritonavir alone (a); Arm B is fosamprenavir/ritonavir alone (b).

Table 1

Total and unbound lopinavir (LPV) and amprenavir (APV) pharmacokinetic parameter and statistical comparison results.

Analyte	Parameter	Arm	Median (Q1, Q3)	Arm C:A GMR/CI	p-value
Total LPV	AUC_{tau} (mg*hr/L)	A	89.4 (86.8, 112)	0.63 (0.46-0.85)	0.019*
		C	51.4 (42.9, 65.6)		
	C12hr (mg/L)	A	5.98 (5.67, 7.26)	0.47 (0.28-0.80)	0.019*
		C	2.76 (1.73, 4.28)		
	CL/F_{ss}, (L/hr)	A	4.38 (3.51, 4.51)	1.34 (0.88-2.04)	0.14
		C	6.36 (4.18, 7.94		
Unbound LPV	AUC_{tau} (mg*hr/L)	A	0.905 (0.885, 1.03)	0.49 (0.33-0.72)	<0.001*
		C	0.589 (0.426, 0.730)		
	C12hr (mg/L)	A	0.053 (0.040, 0.093)	0.35 (0.18-0.65)	0.008*
		C	0.021 (0.014, 0.027)		
	$CL/F_{ss,u}$ (L/hr)	A	428 (390, 460)	1.88 (1.27-2.80)	0.004*
		C	671 (552, 1005)		
LPV Fraction unbound		A	0.011 (0.0097, 0.012)	0.78 (0.62-0.99)	0.077
		C	0.0088 (0.0073, 0.010)		
Analyte	**Parameter**	**Arm**	**Median (Q1, Q3)**	**Arm C:B GMR/CI**	**p-value**
Total APV	AUC_{tau} (mg*hr/L)	B	42.5 (34.9, 52.6)	0.45 (0.33-0.62)	<0.001*
		C	16.9 (14.8, 22.3)		
	C12hr (mg/L)	B	2.05 (1.65, 2.73)	0.41 (0.26-0.65)	0.002*
		C	0.745 (0.577, 1.33)		
	CL/F_{ss}, (L/hr)	B	16.5 (13.3, 20.0)	2.11 (1.53-2.90)	0.002*
		C	41.6 (30.5, 47.3)		
Unbound APV	AUC_{tau} (mg*hr/L)	B	3.04 (2.16, 3.97)	0.62 (0.39-0.97)	0.035*
		C	1.63 (1.28, 2.10)		
	C12hr (mg/L)	B	0.112 (0.091, 0.205)	0.51 (0.28-0.94)	0.035*
		C	0.056 (0.044, 0.112)		
	$CL/F_{ss,u}$ (L/hr)	B	231 (184, 330)	2.17 (1.00-4.68)	0.037*
		C	475 (399, 696)		

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2016 December 15.

Analyte	Parameter	Arm	Median (Q1, Q3)	Arm C:A GMR/CI	p-value
APV Fraction unbound		B	0.067 (0.056, 0.070)	1.31 (1.07-1.59)	0.010*
		C	0.079 (0.075,0.092)		

*A Wilcoxon rank-sum test p-value < 0.05 was considered statistically significant (denoted with).

AUC_{tau}: area under the concentration-time curve over the dosing interval; CL/F_{ss}: apparent oral clearance at steady state, total drug; $CL/F_{ss,\ u}$: apparent oral clearance at steady state, unbound drug; GMR/CI: geometric mean ratio and 95% confidence interval; Q1: 25^{th} percentile; Q3: 75^{th} percentile.