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Immune Activation Mediated Change in Alpha-1-Acid Glycoprotein: Impact on Total and Free Lopinavir Plasma Exposure

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Abstract

Background—Immune mediated changes in circulating α -1-acid glycoprotein (AAG), a type 1 acute phase protein, which binds protease inhibitors (PI), may alter protein binding and contribute to PI's pharmacokinetic (PK) variability.

Methods—In a prospective, 2-phase intensive PK study on antiretroviral naive human immunodeficiency virus (HIV)-infected subjects treated with a lopinavir-/ritonavir-based regimen, steady state PK sampling and AAG assays were performed at weeks 2 and 16 of treatment.

Results—Median entry age was 43 years ($n = 16$). Median plasma $\log_{10}$ HIV-1 RNA, CD4 T-cell counts, and AAG were 5.16 copies/mL, 28 cells/ μ L, and 143 mg/dL, respectively. The total lopinavir area under the concentration time curve (AUC_{12_total}) and maximum concentration (C_{max_total}) changed linearly with AAG at mean rates of 16 ± 7 mg*hr/L (slope $\pm$ SE); $P = .04$, and 1.6 ± 0.6 mg/L, $P = .02$, per 100 mg/dL increase in AAG levels, respectively ($n = 15$). A 29% drop in AAG levels between week 2 and week 16 was associated with 14% (geometric mean ratio [GMR] = 0.86; 90% confidence interval [CI] = 0.74-0.98) and 13% (GMR = 0.87; 90% CI = 0.79-0.95) reduction in AUC_{12_total} and C_{max_total} , respectively. Neither free lopinavir PK parameters nor antiviral activity (HIV-1 RNA average AUC minus baseline) was affected by change in plasma AAG.

Conclusions—Changes in plasma AAG levels alter total lopinavir concentrations, but not the free lopinavir exposure or antiviral activity. This observation may have implications in therapeutic drug monitoring.

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Keywords

antiretroviral drugs; pharmacokinetics; protein binding

Achieving and maintaining adequate antiretroviral drug concentrations is required to produce durable virologic response.^{1,2} The durability of an antiretroviral regimen is limited not only by the emergence of viral resistance but also by the development of drug toxicities, which may be related to supratherapeutic drug concentrations.³⁻⁵ Thus, the need to achieve a balance in drug concentrations where therapeutic effectiveness and tolerability are optimized underscores the desire for a better understanding of factors that influence plasma and target site drug exposure.

Most human immunodeficiency virus (HIV-1) protease inhibitors (PI) are lipophilic basic molecules with strong binding affinity (>90%) for α -1-acid glycoprotein (AAG), a type 1 acute phase protein whose circulating levels vary according to the inflammatory status of the individual. As such, HIV-1 induced immune activation up-regulates AAG synthesis. Conversely, down regulation in immune activation following antiretroviral therapy decreases AAG synthesis.^{6,7} Because PIs are highly bound to AAG in vivo, variation in plasma levels of this protein may contribute to inter- and intraindividual pharmacokinetic (PK) variability observed with the PIs.

While the impact of plasma binding protein on PK and pharmacodynamics (PD) has been described for various classes of pharmaceutical compounds in clinical use,⁸⁻¹⁰ such information is lacking for the antiretroviral drugs. Furthermore, in vitro data suggests reduced antiviral activity for many PIs in the presence of high AAG concentrations.^{11,12} Because antiviral effect in vivo depends on the penetration of free (active) drug into viral harboring cells, the concern has been expressed that elevated plasma AAG levels observed in advanced HIV infection may compromise treatment outcomes.

In a cohort of treatment naive HIV-infected patients, initiating antiretroviral therapy with lopinavir-/ritonavir-based regimen, we evaluated changes in plasma AAG over time following therapy, and examined the relationship between plasma AAG and total/free lopinavir PK parameters, and log₁₀ HIV-1 RNA average area under the curve minus baseline (AAUCMB), a measure of antiviral effect.

METHODS

Study Population and Design

This was a single center, prospective, multiple dose, 2-stage intensive steady state PK study in which antiretroviral naive HIV-infected subjects were recruited from the Grady Infectious Diseases program (IDP) out-patient clinic in Atlanta, Georgia. Eligibility included male and female subjects age ≥ 18 years, CD4 T-cell counts ≤ 100 cells/ μ L, and HIV-1 RNA PCR ≥ 50 000 copies/mL. Subjects were not enrolled if they were on medications that could interact with the PIs, on investigational antiretroviral agents, had active opportunistic infection, had renal/hepatic impairment, or were pregnant. Sexually active females were required to have a negative pregnancy test and to use barrier contraception during the study.

At enrollment, demographic information and clinical laboratory data were collected, and antiretroviral adherence counseling was provided to all subjects. Baseline HIV-1 RNA PCR, CD4 T-cell counts, and serum AAG and C-RP measurements were performed. Subjects initiated therapy with lopinavir/ritonavir (LPV/r) 400/100 mg BID plus 2 nucleoside reverse transcriptase inhibitors at the standard doses recommended by the US Department of Health

and Human Services HIV Treatment Guidelines for Adults and Adolescents.¹³ Plasma HIV RNA, CD4 T-cell counts, AAG, and C-RP were measured again at weeks 2, 8, and 16. Intensive PK blood sampling was performed at weeks 2 and 16. All subjects provided written informed consent before undergoing any study procedures. This study was designed according to the ethical guidelines for human studies and approved by the Institutional Review Board of Emory University.

Pharmacokinetic Design

Baseline sampling for lopinavir PK profiling was performed at week 2 to ensure a steady state baseline parameter as the inhibitory effect of ritonavir on cytochrome P450 3A4 isoenzyme may not be optimal until about 10 to 14 days of antiretroviral treatment initiation. On intensive PK sampling days, subjects were admitted to the Emory University, Atlanta Clinical and Translational Science Institute (ACTSI), Clinical Interaction Network (CIN). At the CIN, a time 0 (baseline) blood draw was collected within 20 minutes prior to directly observed antiretroviral drugs administration with standard meals. Dedicated CIN personnel record the time of dosing, and postdose blood samples were collected at 1, 2, 3, 4, 6, 8, 10, and 12 hours. Blood samples were kept on ice until processed (within 60 minutes of collection). Plasma was separated by centrifugation at $900 \times g$ for 10 minutes, transferred to a polypropylene cryovial, and frozen at -70°C until analysis.

Bioanalytical and Pharmacokinetic Assays

Levels of AAG in the plasma were quantified using an enzyme-linked immunosorbent assay (ELISA) (AssayPro Human Alpha-1-Acid Glycoprotein ELISA Kit).¹⁴ This assay employs a quantitative competitive enzyme immunoassay technique. A polyclonal antibody specific for human AAG was precoated onto a 96-well microplate with removable strip. AAG in standards and samples was competed by biotinylated AAG sandwiched by immobilized antibody and streptavidin-peroxidase conjugate. All unbound material was then washed away and a peroxidase enzyme substrate was added. The color development was stopped and the intensity of the color was measured at a wavelength of 450 nm. The limit of quantification for this assay is 80 ng/mL, with intra-assay and interassay coefficient of variation of 4.3% and 7.0%, respectively. Total and free lopinavir plasma concentrations were measured by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection and ultrafiltration methods, respectively, as previously described.^{15,16}

Pharmacokinetic Analysis

PK parameters for total and free lopinavir were determined using noncompartmental methods (WinNonlin Pro, Version 5.2, Pharsight Corporation, Mountain View, CA.). Calculated PK parameters were as follows: area under the curve (AUC_{12}); maximum plasma concentration ($C_{\max}$); time to $C_{\max}$ ($T_{\max}$); minimum concentration ($C_{\min}$); oral clearance (CL/F); terminal apparent distribution volume (V_z/F); and elimination half-life ($t_{1/2}$). AUC_{12} was determined using the trapezoidal rule. $C_{\max}$, $C_{\min}$, and $T_{\max}$ were taken directly from the observed drug concentration-time data. CL/F was calculated as $\text{dose}/\text{AUC}_{12}$. V_z/F was calculated as dose divided by the product of λ_z and AUC_{12} . The elimination rate constant was determined by the linear regression of the terminal elimination phase concentration-time points; $t_{1/2}$ was calculated as $\ln(2)/\lambda_z$. Measured samples below the assay limit of quantitation (BLQ) were treated as 25 ng/mL (total) and 0.0125 ng/mL (free), halfway between zero and the lower limit of quantitation. Regression analysis was also used when practical to estimate the 12-hour concentration when the measured value was BLQ.

Statistical Analysis

Demographic and clinical characteristics were summarized by descriptive statistics. Statistical analyses of the PK parameters (AUC_{12} , C_{max} , C_{12} , and CL/F) by period were performed on a logarithmic scale so that the data distribution would be roughly Gaussian. Exponentiation of the difference between period means of the log-transformed values provides the geometric mean ratio ($GMR = \log[\text{period 2}] - \log[\text{period 1}] = \log[\text{period 2}/\text{period 1}]$). To assess the significance of period differences in the PK parameters for lopinavir and ritonavir, 90% confidence intervals (CIs) were constructed for the GMR. Significant period PK differences were found if the 90% CI for the GMR did not contain 1.

Repeated measures analyses for total and free lopinavir concentrations were performed with a means model with SAS Proc Mixed (Version 9) providing separate estimates of the means by the targeted blood sampling times (0, 1, 2, 3, 4, 6, 8, 10, and 12 hours) and period (1 or 2). Unstructured variance-covariance form among the repeated measurements was assumed for each outcome and robust estimates of the standard errors of parameters were used to perform statistical tests and construct 95% CIs. Statistical tests were 2-sided. A P value $\leq .05$ was considered statistically significant. Similar repeated measures analyses were performed for AAG (natural log), C-reactive protein (natural log), $\log_{10}$ HIV-1 viral load, and CD4 T-cell counts.

Rates of increase for total lopinavir area under the concentration time curve (AUC_{12_total}) and free lopinavir area under the concentration time curve (AUC_{12_free}) and maximum concentration (C_{max_total} and C_{max_free}) were obtained using a mixed effects linear model specifying that each dependent variable follows a linear regression over serum AAG, with a random intercept for each patient.

AAUCMB was calculated for $\log_{10}$ HIV-1 RNA PCR based on measurements over 16 weeks using previously described approach.¹⁷ The rates of change in plasma AAG, AUC_{12_total} , AUC_{12_free} , C_{max_total} , and C_{max_free} for period 1 regressed on $\log_{10}$ HIV-1 RNA AAUCMB were obtained with simple linear regression.

RESULTS

Demographic and Baseline Clinical Data

Of the 16 enrolled subjects, 88% were African American, 75% were male, and the median age was 43 years. The median entry $\log_{10}$ HIV RNA PCR and CD4 T-cell counts were 5.16 copies/mL and 28 cells/ μ L, respectively, reflecting the HIV disease severity of the cohort. Detailed demographic characteristics, baseline clinical and laboratory profile, and antiretroviral regimens are summarized in Table I.

Plasma AAG, C-RP, and Lopinavir PK Profile

The estimated mean pretreatment serum AAG level was 156 mg/dL, a value 2-fold higher than the mean previously reported for healthy HIV-1 sero-negative individuals.^{18,19} An initial 9% rise in serum AAG levels at week 2 of antiretroviral therapy corresponded to a 56.6% rise in C-RP (a marker of immune activation) from 0.11 mg/dL to 0.39 mg/dL, as shown in Figure 1A and 1B. Subsequently, with progressive reduction in plasma HIV-1 viral loads and rise in CD4 T-cell counts following successful therapy, plasma AAG levels fell from a value of 170 mg/dL at week 2 to 121 mg/dL at week 16, corresponding to a drop of 29% ($P = .01$). The AAG decline of 49 mg/dL is not statistically significant after a Bonferroni multiple comparisons adjustment.

Complete PK data were available for 15 of the 16 subjects. Figure 2A and 2B and Table II describe the PK profiles for both total and free lopinavir at week 2, ie, period 1 (when plasma AAG was markedly elevated), and week 16, ie, period 2 (when AAG levels were relatively lower). Large interpatient variability existed for both the total and free lopinavir PK parameters, for example, at period 1 the percent coefficient of variation (%CV) for AUC_{12_total} and AUC_{12_free} were 58.84% and 46.94%, respectively. The ratios of free to total drug concentration profile were 0.30%, 0.20%, and 0.24%, respectively, for period 1 C_{max} , C_{12} , and AUC_{12} , and 0.33%, 0.22%, and 0.25%, respectively, for period 2 C_{max} , C_{12} , and AUC_{12} . Overall, the estimated lopinavir percent protein binding was high ($99.75\% \pm 0.15\%$).

Relationship Between Plasma AAG Levels and Lopinavir Pharmacokinetic Profile

The geometric mean ratio of the relative plasma exposure and the 90% CI for total and free lopinavir AUC_{12} , C_{max} , and C_{12} comparing period 2 with period 1 are presented in Table III. A 29% difference in plasma AAG level observed between the 2 periods was associated with statistically significant reduction in total lopinavir AUC_{12} and C_{max} of 14% (GMR = 0.86, 90% CI = 0.74-0.98) and 13% (GMR = 0.87, 90% CI = 0.79-0.95), respectively. Lopinavir C_{12} was reduced by 17% (GMR = 0.83, 90% CI = 0.63-1.07), however, this change was not statistically significant. Significant differences were not observed for free lopinavir PK profile between the study periods. Similarly, no change was observed in total ritonavir plasma exposure between the 2 periods (data not shown).

Furthermore, a linear relationship was found between plasma AAG and total lopinavir AUC_{12_total} (Figure 3A; slope $\pm$ (SE) = 0.16 ± 0.07 , $P = .04$) and C_{max_total} (Figure 3B; slope = 0.016 ± 0.006 , $P = .02$). For every 100 mg/dL increase in plasma AAG, total lopinavir AUC_{12_total} and C_{max_total} changed by 16 mg*h/L and 1.6 mg/L, respectively. No such relationship was observed for the free lopinavir PK profile as shown in Figure 3C and 3D. The slope for the plot of AUC_{12_free} against plasma AAG was 0.076, $P = .76$, while that of C_{max_free} against plasma AAG was 0.004, $P = .89$.

Impact of Plasma AAG on Antiviral Effect of Lopinavir

Mean reduction in $\log_{10}$ HIV-1 RNA PCR of 3.12 ± 0.20 copies/mL was observed between baseline and week 16 of antiretroviral therapy. At the end of the 16-week follow-up period, 50% (8/16) of the subjects had achieved complete virologic suppression (HIV-1 RNA < 50 copies/mL). All but 1 subject had > $\log_{10}$ 2.0 copies/mL reduction in HIV RNA by week 16. Immune reconstitution during the study period was reflected by a significant ($P < .001$) increase in mean CD4 T-cell counts from 40 cells/ μ L at baseline to 164 cells/ μ L at week 16.

Total and free lopinavir plasma exposure and AAG levels regressed on the antiviral activity of the treatment regimen (HIV-1 RNA AAUCMB) are depicted in Figure 4. As anticipated, higher drug exposure was associated with lower AAUCMB, however, none of the relationships were statistically significant.

DISCUSSION

It has long been recognized that circulating levels of AAG, the plasma protein to which HIV PIs bind, varies according to patients' inflammatory status. The synthesis of this type 1 acute phase protein rises significantly in response to continuous stress, as encountered in chronic inflammatory diseases, infections, and malignancies.⁶ Likewise, on recovery or remission of these conditions, AAG concentrations have been shown to decrease toward normal levels.⁷ Based on these observations, and because HIV infection results in a state of chronic immune activation and inflammation, it is expected that plasma AAG levels will be elevated in

individuals with untreated HIV infection, particularly those with advanced disease. Therefore, the high level of pretreatment serum AAG (mean of 156 mg/dL) observed in this cohort of treatment naive subjects with advanced disease (mean $\log_{10}$ HIV-1 RNA of 5.27 copies/mL, and mean CD4 T-cell counts of 40 cells/ μ L) was consistent with expectation. Furthermore, observed change in plasma AAG with antiretroviral therapy in this cohort followed a similar pattern as change in C-RP, an inflammatory marker suggesting the observed elevation in AAG level, was mediated by HIV-induced immune activation.

Virologic suppression and immune reconstitution following antiretroviral therapy is often accompanied by a reduction in the level of immune activation and inflammation.²⁰ As such, serum AAG level should decrease with antiretroviral therapy. While this was the case in the current study, the pattern of AAG decline with therapy was intriguing. An initial 9% rise in AAG level was observed from baseline to week 2 of therapy followed by a subsequent 29% reduction from week 2 to week 16. The AAG rise at week 2 corresponded with an unanticipated sharp rise in C-RP during the same period. A plausible explanation for this pattern of change in AAG and C-RP levels could be up regulation of the immune system during early phase of antiretroviral initiation with activation of the acute phase reactant pathway followed subsequently by gradual down regulation of immune activation as treatment progresses, a form of paradoxical reaction to antiretroviral therapy.

Marked interindividual as well as intraindividual variability has been observed in the plasma PK of the PIs.²¹ The sources of such variability are multi-factorial and include pharmaceutical formulation, genetics, sex, age, and concomitantly administered drugs through drug interactions, etc.²² However, the findings that a majority of PIs exhibit a high degree of protein binding raises the possibility that protein binding could also have some impact on the plasma concentrations of these agents. Whereas data for various classes of therapeutic agents seem to show that protein binding has a clinically significant effect on plasma PK,⁸⁻¹⁰ similar data for the antiretroviral drugs are limited. Our data demonstrates modest changes in plasma steady state total lopinavir PK parameters following changes in AAG levels. Statistically significant aggregate changes of 14% and 13%, respectively, in lopinavir AUC_{12_total} and C_{max_total} following a 29% reduction in plasma AAG level from week 2 to week 16 were observed for the cohort. Notably, a direct relationship with statistically significant slopes was noted between total lopinavir AUC_{12_total} and AAG levels, and between C_{max_total} and AAG levels. The slopes of these relationships were 0.16 and 0.016, respectively, for AUC_{12_total} and C_{max_total} . Assuming linearity in these relationships, these observations imply that for a 100 mg/dL change in plasma AAG level, lopinavir AUC_{12_total} will vary by 16 mg*h/L, while the C_{max_total} will vary by 1.6 mg/L. This magnitude of change in total lopinavir plasma exposure with AAG levels could account for some of the inter- and intraindividual variability observed in the plasma concentrations of this drug and possibly other PIs.

It has been recognized that the scope of the interaction between ritonavir (RTV) and concomitantly administered PIs may extend beyond the inhibition of the cytochrome P-450 3A4 isoenzyme. Although the affinity of lopinavir for AAG relative to that of RTV was not directly assessed in the current study, RTV had been shown to alter the intrinsic clearance and induce displacement from protein binding sites of concomitantly administered PIs,²³ characteristics that could influence the unbound component of highly protein bound drugs administered together with RTV. Furthermore, the question often arises whether an immune mediated change in plasma AAG levels has pharmacodynamic implications. In vitro data suggest a reduced viral inhibitory effect of many PIs in the presence of high AAG concentrations.^{11,12} Because antiviral activity depends on intracellular penetration of free (unbound) drug into viral harboring cells, there is concern that a similar effect could occur in vivo in the setting of elevated plasma AAG, particularly for a PI-based regimen

coadministered with RTV. Our data appear to allay some of this concern. While total lopinavir concentrations varied with plasma AAG levels, such variation was not observed with the free drug concentrations, the component that is largely responsible for the antiviral effect of the drug. Indeed, the relationship between AAG levels and HIV-1 RNA AAUCMB was weak and not statistically significant. Thus, free lopinavir plasma exposure and antiviral activity of a PI-based regimen may not be influenced by plasma binding protein levels.

The observation that variations in plasma binding protein levels have little or no impact on free lopinavir PK profile or antiviral effect should not be completely surprising. In general, except in rare cases of drugs with a high extraction ratio and narrow therapeutic index administered parenterally, changes in plasma protein binding will usually not influence the free drug exposure.²⁴ As plasma free lopinavir concentration presumably rises with drop in plasma bind protein levels, so does the free drug clearance. Thus, the net effect for most orally administered drugs at a steady state would be a stable unbound drug concentration even in the face of a wide variation in plasma binding protein levels. Notwithstanding, changes in plasma levels of binding protein may influence the total drug concentrations in manners that may have clinical implications if, as is often the practice, the total rather than the free drug concentrations are measured during therapeutic drug monitoring (TDM). The less than optimal outcomes in several clinical trials in which the utility of TDM were assessed may be due in part to failure to account for an HIV-induced variation in plasma AAG levels and its influence on the total drug concentrations such as observed in the current study.^{3,25,26} Furthermore, variability in PIs plasma concentrations observed in women during different trimesters of pregnancy could partly be related to changes in plasma AAG levels as the plasma levels of this protein have been shown to vary widely in this setting.^{27,28} Thus, incorporating variation in plasma binding protein into the interpretation of total drug concentrations in general and during TDM deserves re-evaluation in the context of a larger study. Better still, measurement of free in addition to total drug concentrations may lead to improved interpretation of plasma drug concentrations. It should be noted, however, that methods for measuring free drug concentrations are cumbersome, expensive, and not widely available. Our findings could also have implications in the timing of steady state plasma PK sampling. Should changes in total plasma concentrations of highly bound PIs with alteration in binding protein levels, as observed in our cohort, be validated in a larger study to minimize variability, it would make sense to delay the collection of steady state PK samples in the setting of acute or chronic inflammatory state until AAG levels are normalized or stabilized following appropriate therapeutic intervention.

It should also be noted that lopinavir, administered as LPV/r, has been shown to exhibit concentration dependent protein binding,²⁹ an observation that could further complicate the interpretation of plasma free lopinavir concentration, especially if a single sample taken at a random time point during the dosing interval is utilized. However, that was not the case in the current study. Overall, lopinavir protein binding was very high at 99.75%, and was relatively stable between and within the 2 study periods (week 2 and week 16). A plausible explanation for this discrepancy could be the severity of HIV disease in the current study with the resulting high plasma AAG levels (mean of 156 mg/dL) and corresponding high lopinavir protein binding. Whereas all the subjects in the Boffito et al study were virologically suppressed with mean CD4 T-cell counts of 290 cells/ μ L, in the current study the mean entry CD4 T-cell counts were 40 cells/ μ L, and the median entry plasma log₁₀ HIV-1 RNA were 5.27 copies/mL.

While the relationship between plasma AAG levels and lopinavir plasma concentrations as observed in the current study is intriguing, the data should be interpreted in the context of a pilot study with a small sample size. The impact of other covariates such as sex, age, and genetics that are known to influence AAG levels and drug concentrations could not be

assessed. Although a majority of subjects experienced greater than 2 log₁₀ reduction in plasma HIV-1 RNA, and CD4 T-cell recovery was robust, the 16 weeks' study duration was probably too short to fully evaluate the influence of antiretroviral therapy on AAG changes over time. Larger studies with a more diverse population and longer follow-up period may be needed to exclude the possibility that immune mediated changes in plasma AAG level affect the PIs' PK profile.

CONCLUSION

Our data demonstrate that total plasma lopinavir PK parameters are influenced by immune-mediated changes in plasma AAG in subjects with advanced HIV disease. Lopinavir exhibited high protein binding in this cohort with elevated plasma AAG and its total plasma exposure varied in direct relationship with circulating AAG levels. However, changes in circulating AAG levels did not affect free lopinavir PK parameters nor were they related to changes in AAUCMB, a measure of antiviral effect, thus allaying the concern that plasma protein binding levels may influence therapeutic outcomes. Importantly, this finding may have implications in the interpretation of PI plasma drug concentrations during TDM, and suggest that measurement of both the total and free drug concentrations may provide optimal information during TDM.

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Figure 1. Longitudinal change in mean serum α -1-acid glycoprotein (AAG) (A), and mean C-reactive protein (B), in 16 antiretroviral naïve HIV-infected patients treated with a lopinavir-/ritonavir-based regime. Vertical bars = 95% confidence intervals (CIs).

Figure 2. Longitudinal change by period in mean total and mean free lopinavir concentration time profiles in 15 antiretroviral naive HIV-infected patients treated with a lopinavir-/ritonavir-based regime. Vertical bars = 95% confidence intervals (CIs).

Figure 3.

(A) Estimated mean linear increase in total lopinavir area under the concentration time curve (AUC_{12}) by serum α -1-acid glycoprotein (AAG) using data from periods 1 and 2 ($P = .04$ when comparing the estimated AUC_{12} slope to zero). (B) No statistically significant linear relationship when free lopinavir AUC_{12} was regressed on serum AAG ($P = .76$). (C) Estimated mean linear increase in total lopinavir C_{max} by serum AAG ($P = .02$). (D) No statistically significant linear relationship when free lopinavir C_{max} was regressed on serum AAG ($P = .89$).

Figure 4.

No significant linear relationship was identified between the total and free lopinavir pharmacokinetic (PK) parameters, nor between serum α -1-acid glycoprotein (AAG) regressed on HIV-1 RNA average AUC minus baseline (AAUCMB).

Table I

Baseline Demographic and Clinical Characteristics (n = 16)

Characteristics	n (%) ^a	Median (25th, 75th) ^b
Gender		
Male	12 (75)	
Female	4 (25)	
Race		
African American	14 (88)	
White (non-Hispanic)	1 (6)	
Hispanic	1 (6)	
Others	0 (0)	
Antiretroviral regimen		
Lopinavir/ritonavir + zidovudine + lamivudine	13 (81)	
Lopinavir/ritonavir + stavudine + lamivudine	1 (6)	
Lopinavir/ritonavir + abacavir + lamivudine	2 (12)	
Age (yr)		43 (29, 50)
Weight (kg)		64 (57, 70)
Body mass index (kg/m ²)		22 (19.7, 23.3)
Log ₁₀ HIV RNA PCR (copies/mL)		5.16 (4.3, 5.6)
Plasma α -1-acid glycoprotein (AAG) (mg/dL)		143 (106, 226)
CD4 + T-cell counts (cells/ μ L)		28 (15, 62)
Creatinine clearance ^c [ml/min] ^a		97 (68, 112)
Alanine amino transferase [mg/dl]		31 (27, 40)
Aspartate amino transferase [mg/dl]		28 (25, 58)

^aNumber.^b25th and 75th percentile.^cCreatinine clearance calculated by the Cockcroft-Gault equation.

Table II

Total and Free Lopinavir Pharmacokinetic Profile (n = 15)

Total Profile	Period 1 (week 2)			Period 2 (week 16)		
	C _{max} (mg/L)	C ₁₂ (mg/L)	AUC ₁₂ (mg* ^h /L)	C _{max} (mg/L)	C ₁₂ (mg/L)	AUC ₁₂ (mg* ^h /L)
Mean	12.11	7.07	114.58	10.44	5.62	95.82
SD	4.66	4.46	53.78	3.66	3.15	37.49
GM	11.34	5.89	104.64	9.84	4.86	89.42
CV (%)	38.49	63.08	46.94	35.04	55.98	39.12

Free Profile	Period 1 (week 2)			Period 2 (week 16)		
	C _{max} (ng/mL)	C ₁₂ (ng/mL)	AUC ₁₂ (ng* ^h /mL)	C _{max} (ng/mL)	C ₁₂ (ng/mL)	AUC ₁₂ (ng* ^h /mL)
Mean	36.65	14.26	277.75	33.95	12.39	242.46
SD	18.75	9.16	163.41	15.65	8.35	116.10
GM	32.80	11.22	241.68	30.85	7.78	217.47
CV (%)	51.16	64.21	58.84	46.09	67.37	47.88
F/T (%)	0.30	0.20	0.24	0.33	0.22	0.25

C_{max}, maximum concentration; C₁₂, concentration at the end of dosing interval; AUC, area under the concentration time curve; SD, standard deviation; GM, geometric mean; CV, coefficient of variation; F/T, free to total ratio.

Table III

Relative Plasma Exposure and 90% Confidence Intervals for Total and Free Lopinavir Pharmacokinetic Profile, Comparing Period 2 (week 16) and Period 1 (week 2), n = 15

Pharmacokinetic Parameters	Relative Plasma Exposure (Total Concentration) GMR	Relative Plasma Exposure (Free Concentration) 90% CI	Pharmacokinetic Parameters	Relative Plasma Exposure (Total Concentration) GMR
AUC ₁₂ (ng*h/L)	0.86 ^a	0.74-0.98	AUC ₁₂ (ng*h/L)	0.86 ^a
C _{max} (ng/mL)	0.87 ^a	0.79-0.95	C _{max} (ng/mL)	0.87 ^a
C ₁₂ (ng/mL)	0.83	0.64-1.07	C ₁₂ (ng/mL)	0.83
CL/F (L/h)	1.21	0.91-1.61	CL/F (L/h)	1.21

GMR, geometric mean ratio.

^a Statistically significant.