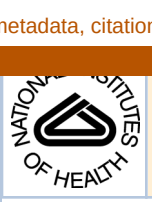

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Protein Binding of Lopinavir and Ritonavir During Four Phases of Pregnancy: Implications for Treatment Guidelines

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Abstract

Objective—To investigate the intraindividual pharmacokinetics of total (protein bound + unbound) and unbound lopinavir/ritonavir (LPV/RTV) and to assess whether the pediatric formulation (100mg/25mg) can overcome any pregnancy-associated changes.

Design—Prospective longitudinal pharmacokinetic (PK) study

Methods—HIV-infected pregnant antiretroviral therapy-naïve and experienced women receiving LPV/RTV 400mg/100mg tablets twice daily. Intensive PK evaluations were performed at 20–24 weeks (PK1), 30 weeks (PK2) followed by empiric dose increase using the pediatric formulation (100mg/25mg twice daily), 32 weeks (PK3), and 8 weeks postpartum (PK4).

Results—Twelve women completed pre-specified PK evaluations. Median (range) age was 28 (1–35) years and baseline BMI was 32 (19–41) kg/m^2. During pregnancy, total area under the time concentration ($AUC_{0–12hr}$) for LPV was significantly lower than postpartum [PK1, PK2 or PK3 vs. PK4, p= 0.005]. Protein unbound LPV $AUC_{0–12hr}$ remained unchanged during pregnancy [PK1: 1.6 (1.3–1.9) vs. PK2: 1.6 (1.3–1.9) μg*hr/mL, p=0.4] despite a 25% dose increase [PK2 vs. PK3: 1.8 (1.3–2.1) μg*hr/mL, p=0.5]. Protein unbound LPV predose concentrations (C_{12h}) did not significantly change despite dose increase [PK2: 0.10 (0.08–0.15) vs. PK3: 0.12 (0.10–0.15) μg/mL, p=0.09]. Albumin and LPV $AUC_{0–12h}$ fraction unbound were correlated (rs=0.3, p=0.03).

Conclusions—Total LPV exposure was significantly decreased throughout pregnancy despite the increased dose. However, the exposure of unbound LPV did not change significantly regardless of trimester or dose. Predose concentrations of unbound LPV were not affected by the

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Conflict of Interests:

The authors have no competing interests.

Author Contributions:

KBP and ADMK conceived and designed the experiments. HAP, AJJ, and KKS coordinated all specimen collection. RW and SM analyzed the specimens. KBP, JBD, MGH and ADMK analyzed the data. KBP and ADMK created the presentation of some of the data for the 18th Conference of Retrovirus and Opportunistic Infections February 28-March 2, 2011, Boston, MA. KBP, JBD and ADMK wrote the paper. All authors reviewed the paper.

additional dose and were 70-fold greater than the minimum efficacy concentration. These findings suggest dose adjustments may not be necessary in all HIV-infected pregnant women.

Keywords

HIV; pregnancy; lopinavir/ritonavir; protein unbound; pharmacokinetics

Background

HIV-infected pregnant women receive antiretroviral therapy for their own health and to prevent mother-to-child transmission of HIV. The US Department of Health and Human Services (DHHS) guidelines have recommended lopinavir/ritonavir (LPV/RTV, Kaletra®) as the first-line protease inhibitor for either goal (1). It has been previously demonstrated that standard dosing (400mg/100mg) using three LPV/RTV soft-gel capsules twice daily results in third trimester lopinavir area under the concentration-time curve (AUC) and trough concentrations below a target of 52ug*hr/mL and 1 μg /mL, respectively (2–5). This decrease in exposure was not overcome with dose increases to 533mg/133 mg (four capsules) twice daily (6). Exposure between the new Meltrex® tablet (200mg/50mg) formulation and standard-dose LPV/RTV soft-gel capsules are not significantly different, though over 17% of women receiving either formulation have lopinavir trough concentrations below 3 μg/mL (7). Dose increases to 600mg/150mg twice-daily results in third trimester concentrations similar to nonpregnant exposures at the standard 400mg/100mg dose (8). Based on these data, DHHS guidelines recommends increasing LPV/RTV dose to 600mg/150 mg twice daily in the third trimester. However, the increased bioavailability of the tablet formulation has the potential to decrease tolerability and increase toxicity at this higher dose.

Lopinavir and ritonavir are 98–99% bound to the blood plasma proteins albumin and alpha-1-acid-glycoprotein (AAG), and only protein-unbound drugs are available to traverse cell membranes and exert a pharmacologic effect (9). Due to technical complexity and the requirement for advanced analytical instrumentation, pharmacokinetic studies generally measure total drug concentrations (protein bound plus unbound) rather than unbound drug. Additionally, since plasma protein binding in most populations is consistent, measuring unbound drug is often unnecessary to assess efficacy(10). Pregnancy, however, is associated with alterations in plasma proteins (11–13), which may affect unbound drug concentrations. Consequently, total lopinavir and ritonavir exposures may not reflect drug available for antiviral activity; in this situation, protein unbound drug concentrations may be most relevant.

Few studies have longitudinally and comprehensively measured unbound lopinavir and ritonavir concentrations throughout pregnancy. Investigations with sparse sampling have had mixed results: either an 18% increase in the fraction of unbound lopinavir in third trimester compared with postpartum (14), or no significant changes (15).

To address the hypothesis that the physiological changes associated with pregnancy will decrease the total (protein bound plus unbound) but not the protein-unbound (active) lopinavir and ritonavir concentrations, this study longitudinally evaluates total and unbound lopinavir and ritonavir exposures in HIV-infected pregnant women in the second and third trimesters of pregnancy and postpartum. The pediatric formulation of LPV/RTV (100mg/25mg twice daily), rather than an additional adult tablet (200mg/50mg), is empirically added to standard dosing (400mg/100mg twice daily) in the third trimester. The study is designed to investigate whether LPV/RTV 500mg/125mg is able to overcome any pregnancy-

associated changes in total and free drug exposure while minimizing toxicity and maximizing tolerability.

Methods

Study Design and Subject Selection

HIV-infected pregnant women were enrolled in this open-label, longitudinal pharmacokinetic (PK) study to assess the total (protein-bound plus unbound) and protein-unbound exposure of fixed-dose combination lopinavir/ritonavir tablets (LPV/RTV, Kaletra®, Abbott Laboratories, Abbott, IL USA). Subjects were recruited from the Infectious Diseases Clinics at the University of North Carolina (UNC) at Chapel Hill, North Carolina and Northwestern University (NU), Chicago, Illinois. The Biomedical Review Boards from each individual institution approved the study, and all subjects provided written informed consent prior to study procedures. Subjects were enrolled from October 2007 to September 2009 (National Clinical Trial Registry No. 00766818).

Subjects were eligible to participate if they were less than 20 weeks gestation and receiving or planning to initiate LPV/RTV tablets (400 mg/100 mg twice daily). Subjects were required to be ≥18 years of age with a current singleton and uncomplicated pregnancy. Subjects were excluded if they were being treated for an opportunistic infection; had prior obstetrical complications or preterm delivery; had a hemoglobin < 9.0 gm/dL; were unable to complete a dose record card at home; were <80% adherent to the current antiretroviral regimen as determined by dosing card, provider or self report; or were receiving other protease inhibitors or concurrent medications known to interact with LPV/RTV. Subjects with documented virologic failure of a LPV/RTV regimen or who had a genotype that would predict failure to LPV/RTV were excluded.

Two to four weeks prior to enrollment, subjects underwent screening in which informed consent, physical examination and routine safety laboratory testing (complete blood count, serum chemistries, liver function tests, urinalysis, and absolute CD4+ cell count and HIV RNA) were performed, and medical and obstetrical histories were obtained. Subjects agreed to allow investigators to capture any laboratory results, clinical events or sonograph results that occurred between study visits. HIV DNA results for the infants were obtained at 0–2 days, 2–4 weeks, 4–6 weeks and 4–6 months of life as part of routine infant care. All infants received oral zidovudine 2mg/kg every 6 hours for 6 weeks postpartum in addition to intravenous zidovudine at delivery.

Study Visits

Subjects underwent four intensive pharmacokinetic (PK) visits with observed dosing within 15 minutes of a standard meal in the clinical research units at the following time points: 20–24 weeks (PK1), 30 weeks (PK2), 32 weeks (PK3) gestation, and 8 weeks post partum (PK4). Subjects were contacted one week prior to each PK visit to reinforce compliance, to record the time of each dose on provided cards, and to bring cards to study visit. Adherence was also assessed by pill counts at each study visit. Safety labs (hemoglobin, chemistries) were obtained at all visits before blood samples for PK analysis were initiated. At each PK visit, blood samples were obtained 30 minutes prior to dosing and 2, 4, 6, 8, 10 and 12 hours postdose. The LPV/RTV dose was 400mg/100mg twice daily at PK1 and PK2. Immediately following completion of PK2, the dose was increased to 500/125mg twice daily by adding a 100mg/25 mg tablet (pediatric formulation) to standard-dose in order to determine possible effects on drug exposure using a smaller dose as an alternate dosing strategy. PK 3 occurred two weeks later. The 500mg/125mg dose was continued for the remainder of pregnancy

through 2 weeks postpartum, at which time the dose was decreased to 400mg/100mg twice daily for the duration of the study.

Sample Processing

Whole blood was obtained using K2-EDTA-containing collection tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 3000g for 15 minutes at 4°C within 30 minutes of collection. The resulting plasma was divided into labeled cryovials and stored at −80°C until analysis. Total drug concentrations in plasma for lopinavir and ritonavir were measured using validated and Division of AIDS CPQA-approved high performance liquid chromatography (HPLC) method with ultraviolet detection. Intraday and interday variability was <10% and the dynamic range was 10–10,000 ng/mL (16).

Protein binding quantification was performed by rapid equilibrium dialysis as previously described (17). Briefly, plasma was incubated at 37°C for 18 hours in rapid equilibrium dialysis cartridges (Thermo Scientific, Pittsburg, PA) followed by liquid-liquid extraction with methyl *tert*-butyl ether (MTBE) (Fisher Scientific, Norcross, GA, USA). Darunavir (in 50/50 methanol: water) was used as the internal standard. An Agilent 1200 series HPLC System and an Agilent 1100 MSD (Agilent Technologies, New Castle, DE) in positive ESI mode were used. Analytes were separated on an Agilent Zorbax Eclipse XDB-C8 (3.0 × 50mm, 1.8m) column. For both lopinavir and ritonavir, assay sensitivity was 2 ng/ml, precision was within 15%, and accuracy was 95–110% for lopinavir and 90–107% for ritonavir. Recovery for both drugs was >90%.

Statistical Analyses

Pharmacokinetic parameters were estimated using noncompartmental methods (Phoenix WinNonlin Pro 5.2; Certara, L.P., St. Louis MO). Maximum concentration (Cmax), time at maximum concentration (Tmax), and concentration 12 hours after dosing (C_{12h}) were determined by visual inspection of the subject profiles, and used the log-linear trapezoidal method to calculate the area-under-the-time-concentration-curve over the 12-hour dosing interval ($AUC_{0–12h}$). Total drug apparent oral clearance was calculated as dose/$AUC_{0–12h}$. Detectable concentrations that were below the limit of quantification were imputed as 50% of the lower limit of quantification. Descriptive statistics using Phoenix WinNonlin Pro 5.2 were performed. Pharmacokinetic parameters are presented in median (interquartile range (IQR)) unless otherwise noted. Differences in pharmacokinetic parameters at different time points were assessed using the Wilcoxon signed rank test. No adjustments were made for multiple comparisons.

The percent protein unbound of lopinavir was calculated by subtracting percent protein bound from 100%. Correlation between albumin or α-1-acid glycoprotein (AAG) and unbound drug concentrations were assessed using Spearman rank correlation coefficients.

Results

Patient Characteristics

Twelve HIV-infected pregnant women from two academic institutions (11 UNC, 1 NU) were enrolled, and all completed the study as designed. Demographic information is presented in Table 1. The median age of the participants was 28 (range 18–35) years; median body mass index (BMI) was 32 (range 19–41) kg/m^2 at enrollment. Nine (75%) were Black. Eight subjects were antiretroviral naïve and initiated therapy at a median of 12 (7–18) weeks gestation, consisting of lopinavir/ritonavir 400mg/100mg (LPV/RTV) twice daily in addition to the nucleoside reverse transcriptase inhibitor (NRTI) fixed-dose combinations of either zidovudine 300mg/lamivudine 150mg (ZDV/3TC) twice daily or

tenofovir disoproxil fumarate 300mg/emtricitabine 200mg (TDF/FTC) once daily. The remaining 4 subjects had received 1 or 2 prior antiretroviral regimens. One subject was on ZDV/3TC plus an unboosted protease inhibitor prior to pregnancy and was switched to LPV/RTV after pregnancy was confirmed; and the other three were not on antiretroviral therapy and initiated LPV/RTV (plus ZDV/3TC or TDF/FTC) after 12-weeks gestation for fetal protection.

Study enrollment occurred between 16–20 weeks gestation. At enrollment, eleven (92%) subjects were receiving ZDV/3TC and one (8%) TDF/FTC. One subject developed hemolytic anemia at 24 weeks gestation, which had occurred in a pregnancy 12 years prior when the subject was HIV-negative. As a result, ZDV/3TC was discontinued and the subject was changed to TDF/FTC for the remainder of the pregnancy. Therefore, two subjects received TDF/FTC for the majority of gestation. The median absolute CD4 count was 509 (116–880) cells/mm^3 at 20–24 weeks. HIV RNA was < 48 copies/mL in all 12 women at delivery. There were no perinatal transmissions.

Pharmacokinetic (PK) Results

Lopinavir Exposure

Total lopinavir: Total lopinavir $AUC_{0–12h}$ did not change between the second and third trimesters (PK1 vs. PK2, p=0.58) (Figure 1a). The increased dose also did not result in a significant difference in total lopinavir $AUC_{0–12h}$ [PK2: median (IQR) 64.1 (51.3–69.7) vs. PK3: 69.1 (55.2–78.2) μg*hr/mL; p=0.27] (Table 2). Despite a 25% increase in dose, total lopinavir $AUC_{0–12h}$ increased by only 8% (p=0.37). The median total lopinavir $AUC_{0–12h}$, regardless of dose or gestation, was significantly lower than postpartum (PK1, PK2 or PK3 vs. PK4, p=0.005). The apparent oral clearance of lopinavir was significantly higher during pregnancy than post-partum: 3.1 (2.2–4.2) L/hr in the second trimester, 3.4 (3.1–4.2) in the third trimester, 3.6 (3.1–3.9) L/hr following the dose increase, and 1.3 (0.6–1.8) L/hr, postpartum, (PK1, PK2, or PK3 vs. PK4, respectively; $p < 0.03$).

The greatest changes in lopinavir C_{12h} were between the third trimester (PK2) and postpartum (PK 4) [4.0 (3.4–5.4) vs. 7.2 (6.1–9.3) μg/mL; p=0.001] (Figure 2A). Postpartum C_{12h} was 38–80% higher than at any point during pregnancy. The increased dose increase to 500mg/125mg resulted in a 23% increase (4.0 vs. 4.9 μg/mL; p=0.03) in total lopinavir C_{12h} as measured at 30 and 32 weeks, respectively.

Protein unbound lopinavir: Figure 1b highlights that neither dose nor gestation had an effect on unbound lopinavir median $AUC_{0–12h}$.. Table 2 more specifically outlines that lack of significant changes in median unbound lopinavir $AUC_{0–12h}$ following the increased dose (PK2 vs. PK3, 1.6 (1.3–1.9) vs. 1.8 (IQR 1.3–2.1) μg*hr/mL, respectively; p=0.47). Similarly, gestation period did not significantly alter unbound lopinavir exposure before the dose increase (PK1 vs. PK2; p=0.41), or following the dose increase (PK1 vs. PK3; p=0.76). Compared to the postpartum $AUC_{0–12h}$, unbound lopinavir exposure was significantly lower throughout pregnancy regardless of dose (PK1, PK2 or PK3 vs. PK4, p=0.05, p=0.01, and p=0.03, respectively).

The increased dose also did not significantly alter the median unbound lopinavir C_{12h} concentration: (PK3 vs. PK2: 0.12 (0.10–0.15) vs. 0.10 (0.08–0.15) μg/mL, respectively; p=0.09) (Figure 2b). Unbound lopinavir C_{12h} concentrations were highest in the second trimester [PK1: 0.15 (0.08–0.16) μg/mL], and did not significantly change following delivery [0.16 (0.14–0.27) μg/mL; p=0.09)]. Only the C_{12h} at 30 weeks gestation prior to dose escalation (PK2) was significantly different from the postpartum C_{12h} [PK2: 0.10 (0.08–0.15) vs. PK4: 0.16 (0.14–0.27) μg/mL; p=0.05)]. Less than 2% of all concentrations

were below the wild type IC_{50} (50% inhibitory concentration) for unbound lopinavir of 0.00064 μg /mL(18).

Ritonavir Exposure

Total ritonavir: Total ritonavir median $AUC_{0–12h}$ was lower throughout pregnancy, regardless of dose increase, compared to postpartum $AUC_{0–12h}$ (Figure 1c). The median total ritonavir $AUC_{0–12h}$ in the second trimester (PK1), the third trimester (PK2) and following the dose increase (PK3) were 2.5 (1.7–3.0), 2.3 (1.7–3.0), and 2.4 (1.9–3.3) μg*hr/mL, respectively, compared with 5.2 (2.9–5.8) μg*hr/mL postpartum (PK4); (p=0.002, p=0.002 and p=0.002, respectively). Overall, total ritonavir exposure was not significantly changed during pregnancy. Apparent oral clearance of ritonavir (Table 2) was greater during pregnancy than the postpartum period; (PK1, PK2, or PK3 vs. PK4, $p< 0.01$).

Total ritonavir C_{12h} were similar between the second and third trimesters [0.12 (0.09–0.20) vs. 0.12 (0.08–0.14) μg /mL, respectively; p=0.5] (Figure 2c). The higher dose did result in a significant increase in median C_{12h} to 0.15 (0.11–0.18) μg /mL, p=0.02. Total ritonavir C_{12h} was significantly lower during pregnancy, despite the additional dose, compared to postpartum C_{12h} [0.28 (0.2–0.4) μg /mL; (PK1, PK2, or PK3 vs. PK4, $p\leq0.01$].

Protein unbound ritonavir: Similar to lopinavir, the protein unbound ritonavir median $AUC_{0–12h}$ did not change regardless of gestation or dose (Figure 1d). The dose increase, (PK2 vs. PK3, 0.3 vs. 0.3 μg*hr/mL, p=1.0) did not alter the unbound ritonavir exposure. Nor was there a difference in exposure in the second or third trimesters before (PK1 vs. PK2, p=0.12), or after (PK1 vs. PK3, p=0.97) the dose increase. Postpartum exposure was significantly higher than during pregnancy regardless of dose (PK1, PK2 or PK3 vs. PK4, p=0.01, p=0.01, and p=0.03, respectively).

The increased dose did not significantly alter the median unbound ritonavir C_{12h} (PK3 vs. PK2, p=1.0). The unbound ritonavir C_{12h} was highest in the second trimester (PK1) (0.02 μg/mL) and was the only time in which a significant difference in postpartum C_{12h} was found (0.02 vs. 0.03 μg/mL, p=0.01).

Less than 1% of all unbound ritonavir concentrations were under 1 mg/mL. A single subject had concentrations below this threshold at the beginning of her postpartum visit, suggesting a missed dose or a delay in dosage.

Serum Proteins: The median (IQR) albumin and AAG are shown in Table 1. There were no significant changes in albumin between the first 3 PK evaluations (PK1 vs. PK2: p=0.2; PK1 vs. PK3: p=0.8, PK2 vs. PK3: p=0.9). There were also no significant changes in AAG (PK1 vs. PK2: p=0.4; PK1 vs. PK3: p=0.2, PK2 vs. PK3: p=0.5). Both albumin and AAG were significantly greater at postpartum PK4 ($p<0.001$). The drug binding proteins albumin and AAG were modestly correlated (rs=0.48, p=0.0006), as were the pharmacokinetic parameters LPV $AUC_{0–12h}$ and C_{12h} (rs=0.47, p=0.0009). However, in this dataset, only albumin significantly correlated with LPV $AUC_{0–12h}$ fraction unbound (rs=0.3, p=0.03).

Safety

The additional 100mg/25mg LPV/RTV tablet was tolerated without any complaints of nausea or vomiting. There were no symptomatic or biochemical adverse events above Grade 1 that were related to LPV/RTV. No subject required premature discontinuation or dosing alterations.

Discussion

The widespread use of antiretroviral therapy has resulted in reduction of perinatal HIV transmission rates to <1–2% in developed countries (19). However, the pregnancy-associated variations in drug binding proteins may alter antiretroviral bioactivity (20, 21), resulting in decreased efficacy, suboptimal maternal viral suppression, and ultimately perinatal transmission. Conversely, overdosing unnecessarily may increase maternal and fetal toxicity (22). Because of such risks, understanding the pharmacokinetics of LPV/RTV is paramount to provide dosing guidance for LPV/RTV in HIV-infected pregnant women.

Unlike previous evaluations (5, 6, 8, 23, 24), this study employed a unique design in which comprehensive pharmacokinetic measurements of both total (protein bound and unbound) and protein unbound lopinavir and ritonavir were performed in the same 12 women at predetermined gestational ages. The intra-subject comparison strategy has not been previously employed, but is essential in assessing the impact of dose escalation on both the total drug and protein unbound drug concentrations. Moreover, intensive pharmacokinetic assessments were performed before *and* after a 100mg/25mg (or 25% of standard dosing) pediatric tablet was empirically added to standard dosing (400mg/100mg) as alternative to "recommended" third trimester dose increases (600mg/150mg or 50% dose increase). Our *a priori* hypothesis was that dose increases were not necessary as protein unbound (or active) drug concentrations were not expected to significantly change despite previously reported changes in total drug concentrations (3, 5, 6, 8, 15). The ability to assess tolerability and potential toxicity of a 25% rather than a 50% dose increase would provide clinically useful information as an alternate strategy for women who may require a dose adjustment. Two weeks post-partum all women return to standard 400mg/100mg dosing. Pharmacokinetics were subsequently performed 8 weeks postpartum consistent with normalization of plasma concentrations in other studies (15), although the precise timing of normalization of pregnancy-related changes has not been determined. These factors provide the most comprehensive pharmacokinetic analysis of LPV/RTV in HIV-infected pregnant women to date.

Our study found that a 25% increase in LPV/RTV dose results in <10% increase in total lopinavir exposure, which is not clinically relevant as concentrations remain above the minimum effective concentration (1 μg/mL for wild-type virus) (25). One explanation for the decrease total lopinavir concentrations is an increase in total lopinavir clearance. Our estimate of lopinavir clearance during pregnancy is between 120–216% higher than postpartum estimates, potentially due to changes in enzymatic processes (26). The relative consistency of Cmax would suggest that oral absorption remains stable.

Higher total lopinavir C_{12h} (4 μg/mL) (27, 28) are targeted in protease inhibitor treatment-experienced patients. Of the 12 participants in this study, standard-dose LPV/RTV results in total lopinavir C_{12h} below this threshold in 33% (n=4) of women in the second trimester and 50% (n=6) in the third trimester. Following the dose increase, 91% (n=11) of women achieve C_{12h} above 4 μg/mL with C_{12h} of 2.9 μg/mL in a single naïve subject. All participants remain virologically suppressed before and after dose changes despite the decrease in $C_{12h.}$ However, our study population, by circumstance, had received two or less prior regimens. Our study suggests that evaluating C_{12h} in the later part of the second trimester may be beneficial in women who are treatment-experienced to determine whether pre-dose concentrations are below target and therefore require a dose increase. This strategy would diminish the risk of potential toxicities when a dose increase is not necessary. The use of the pediatric formulation (100mg/25mg) provides an alternative dosing strategy when the C_{12h} falls slightly below the targeted threshold, further minimizing the risk of toxicities.

The pharmacological effects of LPV/RTV are determined by the protein-unbound (or active) drug concentration, as only the unbound drug is able to transverse biological membranes and exert antiviral activity. In contrast to previous investigations, full pharmacokinetic profiles of unbound lopinavir and ritonavir were generated with our methodology. Using a protein-unbound IC_{50} (50% inhibitory concentration) for lopinavir of 0.00064–0.00077 μg/mL (18), unbound C_{12h} exposures in all women were greater than 70-fold this threshold in the third trimester prior to the dose increase. The increased dose resulted in <15% increase in protein-unbound $AUC_{0–12h}$ and <12% increase in C_{12h}. The unbound fraction of lopinavir did not significantly change during the second or third trimesters and postpartum regardless of dose, and $AUC_{0–12h}$ modestly correlated with physiological changes in albumin. The addition of a standard LPV/RTV tablet (200mg/50mg) could possibly have resulted in higher protein-unbound C_{12h}, which may have been unlikely to benefit our study participants and could have resulted in toxicity or intolerability.

In conclusion, this study demonstrates the pharmacokinetic effects of pregnancy on total and unbound lopinavir and ritonavir concentrations before and after empiric dose escalation. For many pregnant women, especially treatment naïve or women with wild type virus, LPV/RTV dose increases during pregnancy may not be necessary since the protein-unbound drug exposure is not altered. If drug exposure in women with resistance mutations is of concern, a dose increase of 100mg/25mg could be utilized in order to minimize toxicity. Future studies aimed towards providing accurate dosing recommendations in pregnancy of any antiretroviral agent will be best achieved through a longitudinal evaluation of total and unbound drug exposures throughout pregnancy in the same women.

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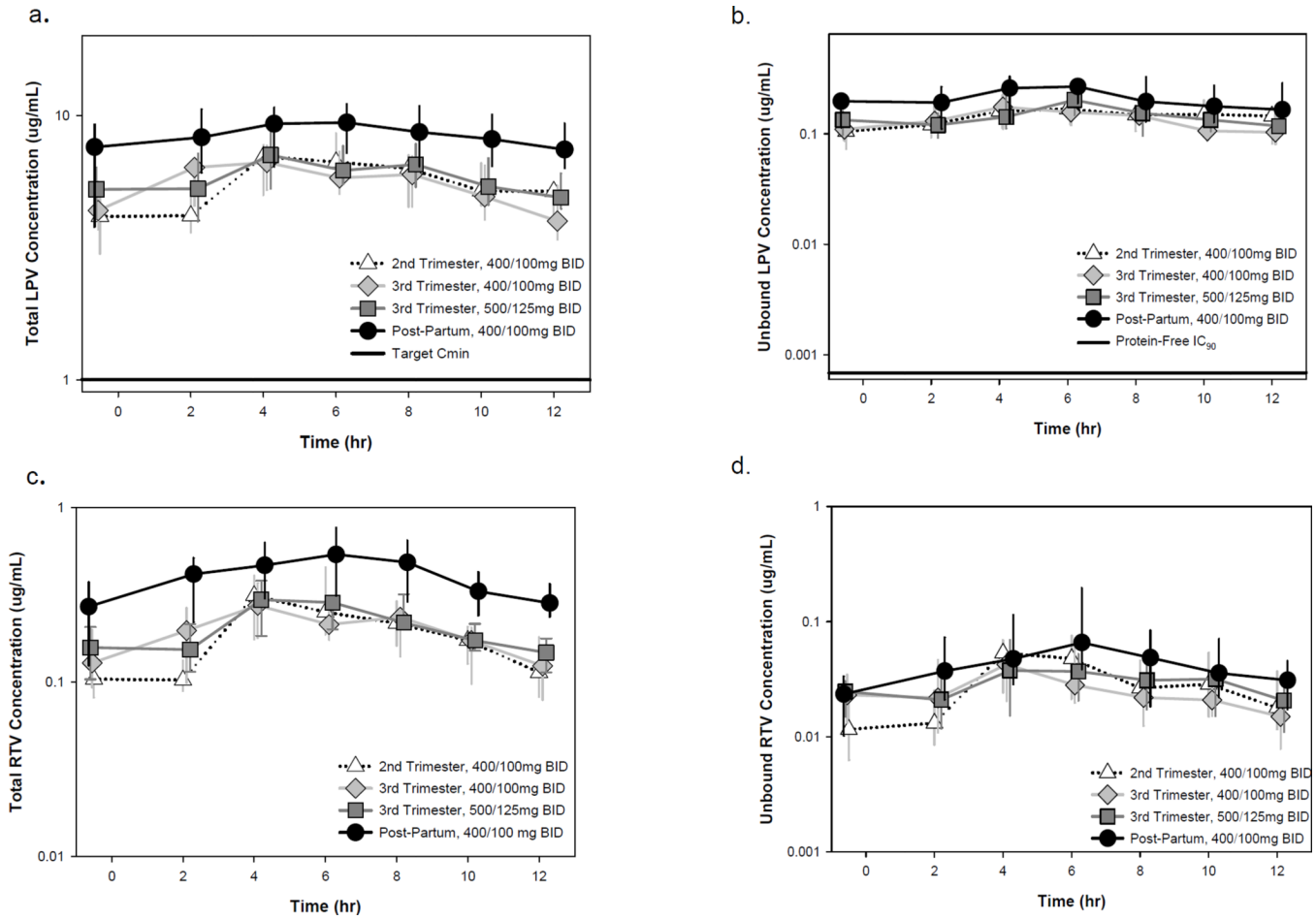

Figure 1. Concentration-Time Profiles of Lopinavir (LPV) and Ritonavir (RTV)

- **a.** Total (Protein Bound and Unbound) Lopinavir Concentrations
- **b.** Protein Unbound Lopinavir Concentrations
- **c.** Total (Protein Bound and Unbound) Ritonavir Concentrations
- **d.** Protein Unbound Ritonavir Concentrations

Data are presented as median (25^{th}, 75^{th} percentile) (ug/mL)
Reference lines indicate minimal efficacy concentrations.

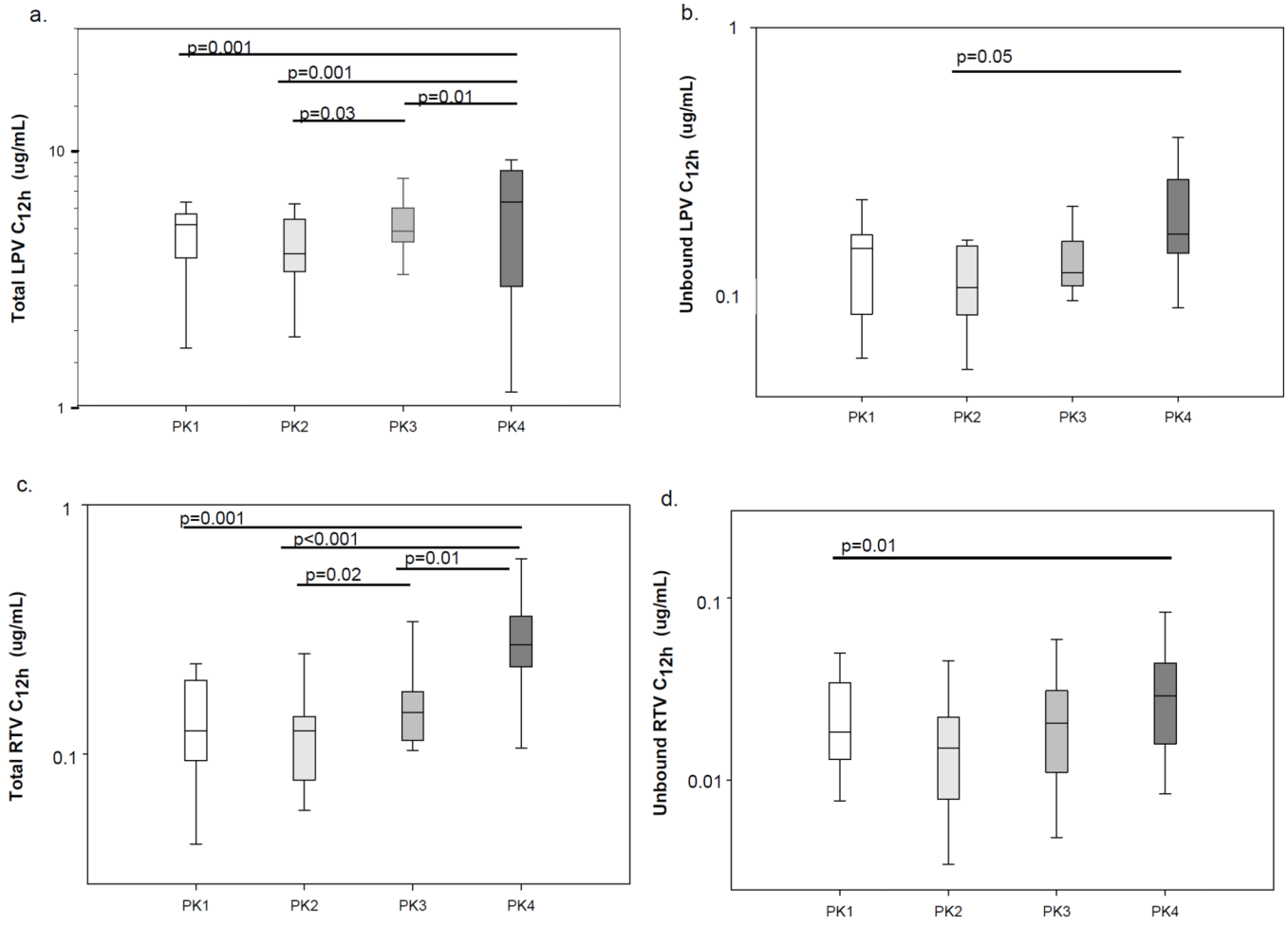

Figure 2. Lopinavir and Ritoniavir C_{12h} Comparisons

- **a.** Total (Protein Bound and Unbound) Lopinavir C_{12h}
- **b.** Protein Unbound Lopinavir C_{12h}
- **c.** Total (Protein Bound and Unbound) Ritonavir C_{12h}
- **d.** Protein Unbound Lopinavir C_{12h}

C_{12h}: concentration 12 hours after dosing
Data are reported as median (25^{th}, 75^{th} percentile) (μg/mL)
(Only statistically significant differences are identified)

Table 1

Characteristics and Pregnancy Outcomes of Study Participants

	Baseline	20–24 wks	30 wks	32 wks	8 wks postpartum
Age (years)	28 (18–35)				
BMI (kg/m^2) *		32 (19–41)	34 (21–40)	34 (21–41)	32 (19–39)
Ethnicity (N/%)					
Black	9 (75%)				
Hispanic	3 (25%)				
NRTI (N/%)					
ZDV/3TC	11 (92%)	11 (92%)	10 (83%)	10 (83%)	10 (83%)
TDF/FTC	1 (8%)	1 (8%)	2 (17%)	2 (17%)	2 (17%)
ART Exposure (N/%)					
Naïve	8 (67%)				
Experienced	4 (33%)				
Albumin * (g/dL)		3.3 (3.2–3.5)	3.25 (3.2–3.5)	3.4 (3.1–3.6)	4.2 (4.1–4.4)
α-1acid glycoprotein * (mg/dL)		54 (50–66)	57 (47–68)	64 (53–74)	82 (72–116)
AST * (U/L)	21 (13–29)	20 (10–35)	28 (15–57)	22 (15–57)	24 (18–160)
ALT * (U/L)	20 (<8–38)	22 (<8 –35)	20 (<8–35)	20 (<8–35)	23 (12–60)
CD4 cell count * (cells/μL)	443 (116–880)	509 (116–880)	783 (594–959)	537 (96–1020)	827 (404–1436)
HIV RNA * (copies/mL)	278 (<48–12600)	1576 (<48–12,600)	<48 **	<48 **	<48 **

* Median (range)

** All Subjects

NRTI=nucleoside reverse transcriptase inhibitor
ART= antiretroviral therapy
AST= aspartate aminotransferase
ALT= alanine aminotransferase

Table 2

Pharmacokinetic Parameters for Lopinavir and Ritonavir.

		Time of visit (PK Visit)	Cmax *(ug/mL)*	C_{12h} *(ug/mL)*	$AUC_{0–12h}$ (u*g*hr/mL)*	Cl *(L/hr)*
Lopinavir	Total (Bound + Unbound)	20–24 wks (PK1)	7.4 (6.6–8.5)	5.2 (3.9–5.7)	61.3 (57.7–73.0)	3.1 (2.3–4.2)
		30 wks (PK2)	7.5 (6.7–7.9)	4 (3.4–5.4)	64.1 (51.3–69.7)	3.4 (3.1–4.2)
		32 wks (PK3)	8.2 (6.7–9.3)	4.9 (4.4–6.0)	69.1 (55.2–78.2)	3.6 (3.1–3.9)
		Postpartum (PK4)	9.6 (7.2–11.4)	7.2 (6.1–9.3)	98 (67.1–115.1)	1.3 (0.6–1.8)
	Unbound	20–24 wks (PK1)	0.18 (0.16–0.24)	0.15 (0.08–0.16)	1.6 (1.3–1.9)	123 (42–192)
		30 wks (PK2)	0.21 (0.16–0.23)	0.1 (0.08–0.15)	1.6 (1.3–1.9)	155 (115–217)
		32 wks (PK3)	0.24 (0.16–0.28)	0.12 (0.10–0.15)	1.8 (1.3–2.1)	131 (108–205)
		Postpartum (PK4)	0.28 (0.20–0.37)	0.16 (0.14–0.27)	2.6 (1.6–3.6)	92 (63–94)
Ritonavir	Total (Bound + Unbound)	20–24 wks (PK1)	0.35 (0.19–0.44)	0.12 (0.093–0.20)	2.5 (1.7–3.0)	29 (18–38)
		30 wks (PK2)	0.3 (0.24–0.39)	0.12 (0.08–0.14)	2.3 (1.7–3.0)	33 (25–46)
		32 wks (PK3)	0.36 (0.29–0.48)	0.15 (0.11–0.18)	2.4 (1.9–3.3)	37 (27–42)

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	Time of visit (PK Visit)	Cmax *(ug/mL)*	C_{12h} *(ug/mL)*	$AUC_{0–12h}$ (ug*hr/mL)	Cl *(L/hr)*
	Postpartum (PK4)	0.62 (0.38–0.77)	0.28 (0.22–0.36)	5.2 (2.9–5.8)	12 (10–17)
Unbound	20–24 wks (PK1)	0.06 (0.29–0.95)	0.02 (0.01–0.03)	0.4 (0.2–0.6)	169 (97–261)
	30 wks (PK2)	0.05 (0.03–0.09)	0.02 (0.01–0.02)	0.3 (0.2–0.5)	261 (160–398)
	32 wks (PK3)	0.05 (0.04–0.09)	0.02 (0.01–0.03)	0.3 (0.3–0.5)	306 (192–476)
	Postpartum (PK4)	0.08 (0.05–0.20)	0.03 (0.02–0.04)	0.5 (0.2–1.1)	127 (71–215)

C_{12h} concentration 12 hours after dosing;

$AUC_{0–12h}$ area-under-the-concentration-time curve over the 12 hour dosing interval.

Data are reported as median (25^{th}, 75^{th} percentile).