Poster #787

Lopinavir Protein Binding During Pregnancy

Aweeka FT[1], Stek A[2], Best B[3], Hu C[4], Holland D[3], Hermes A[5], Burchett S[6], Read J[7], Mirochnick M[8], Capparelli EV[3] for the P1026s team

[1]University of California, San Francisco, San Francisco, CA; [2]University of Southern California, Los Angeles, CA; [3]University of California, San Diego, San Diego, CA; [4]Statistical and Data Analysis Center, Harvard School of Public Health, Boston, MA; [5]Abbott Laboratories, Abbott Park, IL; [6]Children's Hospital Boston, Boston, MA; [7]NICHD, Bethesda, MD; [8]BU School of Medicine, Boston, MA

Contact:
Francesca Aweeka
faweeka@sfghsom.ucsf.edu
(415)476-0339

Abstract

Background. Pregnancy may lead to alterations in protein binding (PB) of drugs due to changes in the plasma concentration (conc) of albumin (Alb), alpha-1 acid glycoprotein (AAG), and steroid and placental hormones. In nonpregnant adults, the PB of the protease inhibitor lopinavir (LPV) is ≥99%, and small changes in PB could have a large effect on the fraction of drug unbound (FU), which exerts pharmacological effect. We have previously reported a significant reduction in total plasma LPV conc with standard dosing during pregnancy. We now report LPV PB during the 3rd trimester (AP) and at 2–6 weeks postpartum (PP).

Methods. P1026s is an ongoing, prospective, non-blinded study of antiretroviral (ARV) pharmacokinetics (PK) in HIV-1–infected pregnant women receiving one or more ARV for routine clinical care, and has enrolled 2 cohorts of women receiving LPV/r (Kaletra) 400/100mg or 533/133mg bid. LPV PK, AAG, and Alb were determined AP and PP. Plasma samples from each PK study day were pooled into low conc (0 and 12 h post dose) and high conc (1–8 h post dose) for LPV PB determination via ultrafiltration using radiolabeled LPV. Linear models were used to correlate LPV FU with covariates.

Results. AP and PP samples were available from 28 and 25 women respectively (10 black non-Hispanic, 13 Hispanic, 3 white, 3 other; median age=31 years). Mean plasma protein concentrations were reduced AP (AAG=477 mg/L/Alb=3.28 g/dL) vs. PP (AAG=1007 mg/L/Alb=3.85 g/dL) (p<0.0001). LPV plasma binding was reduced during pregnancy, resulting in higher FU (mean 0.96 ± 0.16% AP vs 0.82±0.21% PP, p=0.001); Alb conc had no effect on LPV binding. AAG concentration was significantly correlated with the extent of LPV binding: each 100 mg/L increase in AAG was associated with an increase in LPV binding of 0.07% AP and 0.05% PP. Higher LPV conc (after adjustment for AAG) was also associated with reduced binding and higher FU PP. Time PP had no impact on the FU comparing measurements ≤4 wks to those >4wks PP.

Conclusions. AAG conc was lower and LPV FU higher during late pregnancy compared to PP. The 17% relative increase in LPV FU during late pregnancy is much smaller than the reduction in total LPV conc. Therefore, reduced protein binding will only compensate for a portion of the decrease in LPV exposure associated with pregnancy.

Background

Treatment of HIV Disease in Pregnant Women

- Many pregnant women with HIV infection receive combination antiretroviral regimens including a protease inhibitor for maternal control of HIV and to prevent mother to child transmission. The current US Public Health Service Perinatal Guidelines recommend HAART for most pregnant women.[1]
- Lopinavir/ritonavir is one of the most common protease inhibitor regimens used by pregnant women in the United States.

Protein Binding of Anti-HIV Protease Inhibitors

- Most of the HIV-1 protease inhibitors are highly bound to plasma proteins (>99% bound) and bind to both alpha-1 acid glycoprotein (AAG) and albumin.
- The free drug fraction or unbound fraction (FU) is the percentage of total drug that is free to exert its pharmacological effect, traverse cellular membranes, and penetrate into target tissues.
- For lopinavir/ritonavir, less than 1 percent is unbound, and small changes in protein binding can result in substantial changes in the fraction of drug that is "active."
- If changes in FU occur, this will impact the **interpretation in total drug measurements** (i.e., changes in total drug measurements during pregnancy compared to postpartum).
- Changes in FU usually do not impact the actual amount of free drug available or the net pharmacological effect of the drug. This is due to the re-equilibration of drugs in terms of distribution into tissues and drug clearance. But what may change is the total drug concentration measured.

Drug Metabolism of Anti–HIV-1 Protease Inhibitors

- The HIV-1 protease inhibitors are metabolized by cytochrome p450 isozymes. Lopinavir and ritonavir are metabolized primarily by CYP3A4.

Impact of Pregnancy on Protein Binding and Drug Metabolism

Protein Binding:

- Changes in plasma binding proteins are associated with changes in plasma volume, so as pregnancy advances and plasma volume expands, a dilutional hypoalbuminemia occurs.[2]
- Steroid and placental hormones displace drugs from their protein-binding sites, resulting in a decrease in binding capacity for albumin and an increase in the unbound (free) drug fraction.
- Alpha-1 acid glycoprotein (AAG), the principal binding protein for basic drugs (i.e., protease inhibitors), decreases over the course of pregnancy.[3,4]
- In addition, changes in drug protein binding capacity may occur with some reports of a steady increase in production of endogenous ligands, such as free fatty acids, that can compete for drug binding sites other than their own on albumin.[5,6]
- Furthermore, alterations in protein structure may also impact binding capacity.[7] The effects can alter binding to albumin and AAG.

Drug Metabolism:

↑ CYP 3A4 and 2D6

↓ CYP1A2, xanthine oxidase, and N-acetyl transferase, e.g., caffeine CL ↓ 70%

Fetal-placental unit has metabolic potential

CYP3A4 increases 35% throughout pregnancy[8]

- It is common for protease inhibitor exposure to be altered due to physiological changes in drug distribution and metabolism associated with pregnancy.

Previous Finding and Study Hypothesis

- We recently reported that the pharmacokinetic exposure of lopinavir (as estimated by the area under the total drug concentration versus time curve, AUC) was reduced 28% at an average gestational age of 35 weeks, compared to the same women postpartum.[9]
- Hypothesis: A potential increase in unbound fraction (FU) could partially offset a decline in total drug AUC so that dosage adjustments during pregnancy may be less critical than previously suggested.

Study Objectives

1. To measure the protein binding of lopinavir during third-trimester pregnancy and 2–6 weeks postpartum
2. To determine the unbound fraction (FU) of lopinavir during pregnancy and compare to postpartum estimates
3. To assess whether AAG or albumim concentration correlate with FU
4. To assess whether lopinavir total drug concentrations influence FU

Study Design

Enrollment

- P1026s is an on ongoing, prospective, non-blinded study of antiretroviral (ARV) pharmacokinetics in HIV-1–infected pregnant women receiving one or more ARV for routine clinical care.
- P1026s has enrolled two cohorts of women receiving lopinavir/ritonavir (LPV/r) 133/33 mg soft gel capsules. The first cohort used the standard dose of 400/100 mg throughout pregnancy. 80% of these women had PK parameters below the target 10th percentile in late pregnancy; therefore the 2nd cohort used 533/133 mg BID.
- Enrollment included pregnant women ≥13 yrs who initiated lopinavir as part of clinical care before 35 weeks gestation with intent to continue the current regimen until at least 6 weeks postpartum.
- Exclusion criteria included concurrent use of medications known to interfere with absorption, metabolism, or clearance of LPV/r, multiple gestation, or clinical or laboratory toxicity likely to require a change in medications.

Study Procedures

- Pharmacokinetic evaluations occurred at 36 weeks gestation (antepartum, AP) and 2–6 weeks postpartum (PP).
- Subjects included in the pharmacokinetic analysis were on the same dosages for at least 2 weeks to assure steady-state conditions.
- Pharmacokinetic sampling occurred serially over a 12-hour dosing interval (0, 2, 4, 6, 8, &12 hours).

Pharmacokinetic analysis

- Sample analysis: LPV and RTV measurements were analyzed by the Pediatric Clinical Pharmacology Laboratory at the University of California, San Diego.
- Total LPV/RTV concentrations were estimated using a validated reversed-phase multiplex high-performance liquid chromatography method.
- For LPV protein binding determinations, plasma samples from each PK study day were pooled into low concentration (0 and 12 hours post-dose) and high concentration (1–8 hours post-dose) for both total concentration and FU determinations.
- The protein binding method employed ultrafiltration and radiolabeled drug (^{3}H) supplied by Abbott Laboratories.

Statistical Analysis

- The Wilcoxon signed rank test was used to compare AP to PP results.
- Linear models were used to correlate LPV FU with covariates.

Subject Demographics at 3rd Trimester PK Sampling*

	Median (min, max)*
Age (yrs)	31.4 (18.2, 40.9)
CD4+ count (cells/mm^3)**	446 (126, 1049)
Weight (kg)	86.3 (64, 130)
HIV-1 RNA level**	<71 (<20, 41853)***
Gestational Age (wks)	33.9 (30.4, 37.4)
Race/Ethnicity	
White (non-hispanic)	10%
Black (non-hispanic)	35%
Hispanic	45%
Asian-Pacific Islander	3%
Other	7%

** Median (Min, Max) for continuous variables and percentages for categorical variables*
*** Closest measurement within eight weeks from the 3rd trimester PK evaluation*
**** 27/28 (96.4%) subjects had viral load suppressed below 400 copies/mL.*

Results

Antepartum (AP) and postpartum (PP) samples were available from 28 and 25 women, respectively.

	AP	PP	% Change PP vs AP	p value
AAG conc (mg/L)*	477 (145)	1007 (374)	↑111	<0.0001
Albumin conc (mg/dL)	3.28 (0.41)	3.85 (0.49)	↑17	<0.0001
LPV FU (%) (0+12h)	0.91 (0.14)	0.78 (0.18)	↓14	0.005
LPV FU (%)(1-8 h)	1.02 (0.22)	0.87 (0.25)	↓15	0.002
LPV FU (%) (average)	0.96 (0.16)	0.82 (0.21)	↓14	0.002

** Mean (SD)*

Figure 1. Correlation of AAG with Fraction of Drug Unbound

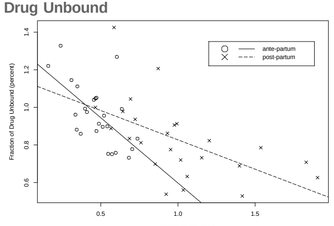
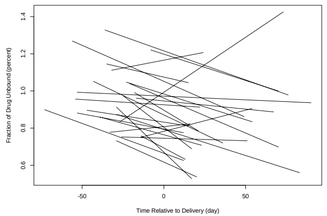


Figure 2. Fraction of Drug Unbound AP vs PP



- AAG concentration was significantly correlated with the extent of LPV binding. At the third trimester PK evaluation each 100 mg/L increase in AAG was associated with a decrease in LPV unbound fraction (FU) of 0.07%, and at the postpartum PK evaluation each 100 mg/L increase in AAG was associated with a decrease of 0.05% in LPV unbound fraction after adjustment for total LPV.
- Albumin was not significantly correlated with LPV unbound fraction (FU).
- Total LPV alone was not significantly correlated with LPV unbound fraction (FU) at both AP and PP. But at PP after adjusting for AAG concentration, higher LPV concentration was significantly correlated with reduced LPV binding and higher FU: each increase of 1µg/mL in total LPV concentration was associated with an increase of 0.02% in LPV unbound fraction (FU).
- Women receiving high-dose LPV (533/133) did not have significant difference in LPV unbound fraction (FU) compared to women receiving standard dose (400/100), with or without adjustment for other parameters.
- The time PP did not have an impact on LPV unbound fraction (FU) when comparing measurements ≤4 weeks to those >4 weeks PP.

Conclusions

- LPV unbound fraction (FU) was higher during late pregnancy compared to postpartum, increasing by 17%.
- This correlated with the lower AAG concentration observed in these subjects (antepartum versus postpartum) and reported previously for pregnant women.
- The 17% relative increase in LPV unbound fraction (FU) during late pregnancy as compared to postpartum is smaller than the 28% reduction in LPV AUC found in pregnant women on the standard dose of lopinavir within this study.
- Reduced protein binding will only compensate for a portion of the decrease in LPV exposure associated with pregnancy.

Acknowledgments

The P1026s team wishes to thank the volunteers participating in this study and the study coordinators at the participating sites. This study was supported in part by the Pediatric AIDS Clinical Trials Group (currently IMPAACT) of the National Institute of Allergy and Infectious Diseases, the General Clinical Research Center Units funded by the National Center for Research Resources, and by the Pediatric/Perinatal HIV Clinical Trails Network of the National Institute of Child Health and Human Development. We thank Abbott Laboratories, Abbott Park, Illinois, for their support of this work.

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University of California San Francisco
UCSF
AIDS Research Institute