Improved Oral Bioavailability of Lopinavir in Melt-Extruded Tablet Formulation Reduces Impact of Third Trimester on Lopinavir Plasma Concentrations

L. J. Else,a,c M. Douglas,b L. Dickinson,a D. J. Back,a S. H. Khoo,a and G. P. Taylorb,d

Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom; St. Mary’s Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom; NIH Biomedical Research Centre, Royal Liverpool Hospital Trust, Liverpool, United Kingdom; and Section of Infectious Diseases, Imperial College London, London, United Kingdom

Lopinavir exposure was reduced during the third trimester in pregnant women receiving standard dosing of the soft-gel capsule (SGC; 400/100 mg twice daily [b.i.d.]). Pharmacokinetic data on the lopinavir tablet in pregnancy are limited. On the basis of the tablet’s improved bioavailability, standard dosing (400/100 mg b.i.d.) may provide adequate lopinavir exposure in pregnancy without a need for dose adjustment. Here we compared the total and unbound lopinavir pharmacokinetics throughout pregnancy in the second and third trimesters in HIV-infected women receiving standard dosing of the lopinavir SGC or tablet. Postpartum sampling was also performed in patients continuing therapy postdelivery. Blood samples were collected at 0 to 12 h postdosing, and lopinavir concentrations were determined by high-pressure liquid chromatography-tandem mass spectrometry. Nineteen patients were included: 8 received the SGC (cohort 1) and 11 received the tablet (cohort 2). Total lopinavir exposures in the third trimester were lower than those in the second trimester (35 and 28% for cohorts 1 and 2, respectively) and postpartum (35% for cohort 2). In the third trimester, the area under the concentration-time curve (AUC) from 0 to 12 h (AUC_0 –12) and maximum concentration were ~15% and 25% higher, respectively, for the lopinavir tablet than the SGC. One SGC patient had lopinavir concentrations of <1,000 ng/ml; all patients on the tablet had concentrations of >1,000 ng/ml. In cohort 2, the percentage of the AUC that was unbound was higher (nonsignificantly) in the second (1.28%) and third (1.18%) trimesters than postpartum (1.01%). Seventeen of 19 patients had an undetectable viral load at delivery. There were no HIV transmissions. Although lopinavir (tablet) exposures were reduced during the third trimester, the higher total and unbound concentrations achieved in women receiving the tablet than in women receiving the SGC suggest that the tablet’s improved oral bioavailability may partly compensate for the reduction in lopinavir exposure during the later stages of pregnancy.

Antiretroviral therapies (ARTs), with the exception of zidovudine, are unlicensed for use in pregnancy. However, they are widely used, in accordance with national and international guidelines, both for maternal treatment and for the prevention of HIV mother-to-child transmission (MTCT). Multiple physiological changes which can impact the pharmacokinetics (PKs) of these agents occur during pregnancy; therefore, studies in pregnancy are required to ensure appropriate dosing and avoid unnecessary toxicity or treatment failure. Protease inhibitors (PIs) are widely used during pregnancy for both treatment and prevention of MTCT due to their efficacy, lack of CD4 count-dependent toxicity, and short half-lives (t1/2s). However, plasma concentrations of certain protease inhibitors have repeatedly been shown to be significantly reduced during the third trimester, with some concerns over efficacy (37).

Lopinavir (LPV)-ritonavir (RTV), or LPV/r, is used during pregnancy, as it is potent and well tolerated and has no obvious human teratogenic effects (28). A number of studies reported reduced LPV exposure during the third trimester of pregnancy in patients receiving standard dosing of the LPV/r soft-gel capsule (3 SGC; 400/100 mg twice daily) than in the same subjects postpartum and nonpregnant historical controls (1, 22, 29, 30, 34). Subsequently, more favorable LPV exposures were demonstrated when the SGC dose was increased to 533/133 mg twice daily (25). The study authors recommended increasing the dose of LPV/r during the third trimester. Others, however, argued against such a global recommendation since plasma LPV concentrations at standard dosing were often above the accepted minimum effective concentration (MEC; 1,000 ng/ml) for treatment of naive patients (16, 21). Furthermore, there was considerable interpatient variability and no evidence that efficacy was compromised with reduced plasma LPV exposure in the third trimester.

Pharmacokinetic data on the LPV/r tablet formulation during pregnancy are limited. One study of 25 patients reported subtherapeutic plasma LPV concentrations in approximately 20% of women during pregnancy (14). Lambert et al. observed significantly reduced LPV concentrations in the second/third trimester; however, the majority of women did achieve concentrations above 1,000 ng/ml; exceptions were in cases where poor treatment adherence was suspected (20). Other studies, however, have reported no pregnancy-associated changes in LPV tablet pharmacokinetics (13, 17). The improved oral bioavailability and reduced intersubject variability of the tablet compared with the SGC (18) could indeed compensate for reduced LPV exposure during the later stages of pregnancy. However, because the tablet has a higher...
LPV is highly (98 to 99%) bound to plasma proteins, including alpha-1-acid glycoprotein (AAG) and albumin, but possesses a higher affinity for AAG (5). A number of studies have found that AAG concentrations are markedly decreased during the later stages of pregnancy (1, 15, 26). It is therefore possible that slight changes in plasma AAG levels may affect the concentration of free and active drug available for both intracellular and transplacental passage. Indeed, one study reported significantly reduced AAG concentrations during the third trimester which correspondingly resulted in decreased protein binding and a significantly (~17%) higher LPV unbound fraction during this period (1), although the authors concluded that the approximate 17% increase in unbound LPV concentrations during the third trimester would not have compensated for the overall reduction in total LPV exposure.

Intensive (total and unbound) pharmacokinetic studies on the LPV/r tablet are needed, as there are currently insufficient data on LPV exposure (area under the concentration-time curve [AUC]) in plasma and discrepancies concerning dosing during pregnancy. In the current analysis, LPV/r pharmacokinetics were compared throughout pregnancy (first, second, and third trimesters) and postpartum in HIV-infected women receiving standard dosing of the LPV/r SGC (3 SGC; 400/100 mg twice daily) or tablet formulation (2 tablets; 400/100 mg twice daily).

MATERIALS AND METHODS

Subjects. HIV-infected pregnant women were recruited from a dedicated HIV antenatal clinic at St. Mary’s Hospital, London, United Kingdom, between September 2005 and January 2010. The studies were approved by the Medicines and Healthcare Products Regulatory Agency and a local research ethics committee and conducted in accordance with International Conference on Harmonization (ICH) good clinical practice (GCP) standards. Pregnant women receiving LPV/r for their own immunological well-being or to prevent MTCT were eligible and were informed of the study. Written informed consent was obtained prior to enrollment into the study, and recruitment varied by gestational age, depending on the study. Patients received a triple-ART regimen containing either the LPV/r SGC (3 SGC; 400/100 mg twice daily; cohort 1) or the LPV/r tablet (2 tablets; 400/100 mg twice daily; cohort 2) for those recruited after June 2006.

Study design. Blood sampling was undertaken in the second and third trimesters and at approximately 4 to 6 weeks postpartum in women conceiving while they were on an LPV-based regimen. For women who commenced ART during pregnancy, steady-state plasma concentrations were measured during the third trimester and postpartum where applicable. Postpartum pharmacokinetic sampling was not undertaken in women who discontinued treatment postdelivery, in which the only indication for therapy was to prevent MTCT. In addition, time-matched maternal blood and umbilical cord blood samples were collected at delivery in order to ascertain the transplacental penetration of LPV/r. Demographic and clinical parameters were collected. HIV load (determined using a branched DNA HIV assay [Bayer Diagnostics] with a limit of detection of 50 HIV RNA copies per ml of plasma), CD4 lymphocyte count, a full blood count, and a routine biochemical profile were determined at baseline and at each study time point using standard accredited methods.

Analytical and pharmacokinetic methods. Pharmacokinetic evaluations were conducted at steady state following a morning dose (taken with a standardized breakfast) in patients stable on LPV/r for at least 2 weeks. Blood samples were collected predosing (0 h) and 1, 2, 4, 6, 9, and 12 h postdosing. Patients also received a lunch after 4 h and dinner at between 6 and 9 h postdosing. The times of drug intake on the day of delivery and the evening before were recorded, as were the times that the maternal and cord blood samples were taken. Blood was collected in heparin tubes and immediately centrifuged (1,000 × g, 10 min, 4°C), and the plasma was removed and stored at −30°C. Prior to analysis the plasma was heat inactivated (58°C, 40 min).

Total plasma LPV and RTV concentrations were determined at the Liverpool Pharmacology Research Laboratories using a validated high-pressure liquid chromatography-tandem mass spectrometry methodology (12). The laboratory complies with ICH good clinical laboratory practice standards and participates in an external quality assurance program (KKGT, Radboud University Medical Centre, Nijmegen, The Netherlands) (10). The assay lower limits of quantification (LLQ) were 16 ng/ml (LPV) and 5 ng/ml (RTV).

Unbound (ultrafiltrate) LPV concentrations were quantified using an adapted version of this method (using a spiked ultrafiltrate standard curve) in order to account for differential matrix effects. LPV calibration curves were linear over the range of 5.45 to 421 ng/ml, and inter- and intra-assay variations (expressed as the coefficient of variation [CV]) ranged from 2 to 8%. Ultrafiltration was used to separate total and unbound LPV. Centrifree microparticulation filter devices filters (maximum volume, 1 ml; Millipore Corporation, Bedford, MA) were incubated with Tween 20 (500 μl; 5%; Bio-Rad Laboratories Inc.) at room temperature for 24 h to limit nonspecific binding (adsorption) of free drug to the surface of the device. The filters were subsequently washed with deionized water (500 μl) and centrifuged (1,500 × g, 10 min, 37°C). This step was repeated, and the filters were then inverted and centrifuged (1,000 × g, 3 min, 37°C) to remove excess water. Patient plasma (500 μl) was then injected, and the devices were centrifuged (1,500 × g, 60 min, 37°C). The resultant ultrafiltrate (~170 μl per sample) was retained for drug analysis. The percent recovery of lopinavir using this technique was assessed using drug-free ultrafiltrate spiked with [14C]lopinavir and was 69% ± 4.1% (mean ± standard deviation [SD]) and constant over a range of LPV concentrations (1,000, 5,000, 10,000, and 15,000 ng/ml).

Pharmacokinetic analysis. Pharmacokinetic parameters of total and unbound drug, including the trough concentration at 0 h (Cmin) and 12 h (Cmax) maximum concentration (Cmax), and time to maximum concentration (Tmax), were determined by visual inspection of individual concentration-time curves. Estimates of drug exposure (AUC from time zero to 12 h [AUC0–12]) and the apparent elimination half-life (t1/2) were calculated using noncompartmental analysis (WinNonlin software, version 6.1; Pharsight Corporation, Mountain View, CA). The MEC (KKGT, Radboud University Medical Centre, Nijmegen, The Netherlands) (5) was previously found to be associated with a high response rate in nonpregnant and treatment-naive adults with wild-type HIV-1 infection (16, 21). Intra- and interindividual variations in pharmacokinetic parameters were expressed in terms of the percent CV. The percentage of unbound drug exposure in plasma (AUCunbound) was determined by (unbound AUC0–12/total AUC0–12) · 100. The placental transfer of LPV was assessed using nonparametric analyses, including the Mann-Whitney test and Kruskal-Wallis test for multiple comparisons. All statistical analysis was performed and analyzed using the Statistica program (version 2.6.8, 2008; Biomedical Software, Statsdirect Ltd., Cheshire, United Kingdom). P values were two-sided at the 0.05 significance level.

RESULTS

Subjects. A total of 21 women were enrolled in the study: 9 patients received the LPV/r SGC (cohort 1) and 12 patients received the tablet formulation (cohort 2). Two patients were excluded...
TABLE 1 Clinical and demographic characteristics of the study population at baseline for all subjects and cohorts 1 and 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects (n = 19)</th>
<th>Cohort 1 (n = 8)</th>
<th>Cohort 2 (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26.4 (17.4–41.6; 19)</td>
<td>25.3 (17.4–32.6; 8)</td>
<td>31.6 (19.3–41.6; 11)</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>67.8 (50.4–116;19)</td>
<td>58.7 (50.4–84.0; 8)</td>
<td>77.0 (55.0–116;11)</td>
</tr>
<tr>
<td>CD4 count (no. of cells/μl)</td>
<td>380 (40–1020; 19)</td>
<td>395 (40–730; 8)</td>
<td>460 (80–1020;11)</td>
</tr>
<tr>
<td>HIV RNA load (no. of copies/ml)</td>
<td>3633 (&lt;50–83856; 18)</td>
<td>5519 (751–83856;8)</td>
<td>3362 (&lt;30–65129)</td>
</tr>
</tbody>
</table>

Race/ethnicity

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>Black</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>No. (%) naive patients initiating ART in pregnancy</td>
<td>12 (63)</td>
<td>6 (75)</td>
<td>6 (55)</td>
</tr>
<tr>
<td>Gestational age at ART initiation (wk)</td>
<td>26.6 (14.9–29.9; 12)</td>
<td>26.8 (24–28.6; 6)</td>
<td>25.1 (14.9–29.9; 6)</td>
</tr>
<tr>
<td>Gestational age at time of first PK sampling (wk)</td>
<td>28.6 (13–35.9; 19)</td>
<td>29.7 (13.7–34.1; 8)</td>
<td>26 (13–35.9; 11)</td>
</tr>
<tr>
<td>T2</td>
<td>24 (13–26)</td>
<td>19.5 (13.7–25.7)</td>
<td>24 (13–26)</td>
</tr>
<tr>
<td>T3</td>
<td>34 (28.6–36.3)</td>
<td>32.9 (28.6–34.1)</td>
<td>34.3 (30–36.3)</td>
</tr>
<tr>
<td>PP</td>
<td></td>
<td>7.1 (2.6–8.9)</td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients receiving co-ART</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zidovudine-lamivudine</td>
<td>11 (58)</td>
<td>6 (75)</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Abacavir-lamivudine</td>
<td>5 (26)</td>
<td>0 (0)</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Tenofovir-emtricitabine</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Abacavir-zidovudine-lamivudine</td>
<td>1 (5)</td>
<td>1 (13)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Zidovudine-tenofovir</td>
<td>1 (5)</td>
<td>1 (13)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Values are given as median (range; number of patients).

a Values are given as mean (range; number of patients).

b In patients initiating ART during pregnancy.

c, T2, second trimester; T3, third trimester; PP, postpartum.

from the final analysis. One patient (cohort 1) was receiving the oral solution of LPV/r in combination with nevirapine (a potential inducer of lopinavir metabolism) (2), and there were concerns over her adherence (first-trimester pharmacokinetic sampling revealed an LPV AUC₀–₁₂ of only 5,076 ng · h/ml). Another patient was excluded (cohort 2), as she received the LPV/r tablet at the higher dose of 600/150 mg twice daily.

The clinical and demographic characteristics of the study population (n = 19) are shown in Table 1. Of these, 12/19 patients were treatment naive and initiated an LPV/r-based regimen during pregnancy. Two of 8 patients in cohort 1 were treatment naive or off treatment at the time of conception and commenced/recommended LPV/r at the end of the first trimester, and 5/11 patients (cohort 2) were already receiving ART preconception. The nucleoside reverse transcriptase inhibitor backbone primarily consisted of zidovudine-lamivudine (Combivir) in 11 (58%) patients and abacavir-lamivudine (Kivexa) in 5 (26%). Eleven of 19 women delivered by caesarean section (CS; 7 with prelabor CS [PLCS], 4 with emergency CS). Seventeen of the 19 (89%) women had an undetectable viral load at delivery, and 14 stopped treatment after delivery. There were no HIV transmissions. One woman (cohort 1) had a detectable plasma viral load (pVL) at the time of delivery (374 copies/ml; delivered via emergency CS); her plasma LPV concentrations in the third trimester (36 weeks) were adequate (>1,000 ng/ml; AUC₀–₁₂, 47,483 ng · h/ml).

Lopinavir (total and unbound) pharmacokinetics. Full pharmacokinetic profiles of LPV (total and unbound) and RTV (total) concentrations were determined in the second trimester in 9/19 (47%) patients (3/8 in cohort 1, 6/11 in cohort 2) and in the third trimester in 17/19 (89%) patients (6/8 in cohort 1, 11/11 in cohort 2). Two women (cohort 1) did not undergo pharmacokinetic sampling during the third trimester due to preterm delivery (PTD) at 32 and 33 weeks, respectively.

The median gestational ages at the time of antepartum pharmacokinetic sampling were 24 weeks (range, 13 to 26 weeks) in the second trimester and 34 weeks (range, 28.6 to 36.3 weeks) in the third trimester (Table 1). The median gestational age at the time of delivery was 39 weeks (range, 32 to 41 weeks), with six (32%) preterm deliveries (<32 weeks) (median gestational age at the time of delivery, 33.5 weeks; range, 32.0 to 36.2 weeks; n = 6). In addition, 6/19 patients (5/8 in cohort 1 and 1/11 in cohort 2) had paired maternal blood and umbilical cord blood samples taken at delivery to ascertain LPV and RTV transplacental transfer.

LPV (total and unbound) and RTV (total) pharmacokinetics in the second and third trimesters of pregnancy and postpartum for pregnant women receiving either the LPV/r SGC (cohort 1) or tablet (cohort 2) are summarized in Table 2. For the patients receiving the SGC formulation, geometric mean total (unbound) LPV AUC₀–₁₂, Cₚₚₜₜ , and Cₘₚ were 76,921 ng · h/ml (618 ng · h/ml), 4,169 ng/ml (30 ng/ml), and 8,198 ng/ml (73 ng/ml), respectively, in the second trimester (n = 4) and 50,391 ng · h/ml (492 ng · h/ml), 2,364 ng/ml (26 ng/ml), and 6,031 ng/ml (58 ng/ml), respectively, in the third trimester (n = 6). Total LPV exposure in the third trimester was, on average
Total and unbound LPV pharmacokinetic parameters during the second and third trimesters of pregnancy and postpartum (where applicable) in HIV-infected pregnant women receiving LPV/r SGC (cohort 1) or LPV/r tablet (cohort 2)\(^a\)

<table>
<thead>
<tr>
<th>Drug and PK parameter</th>
<th>Cohort 1 (n = 8)</th>
<th>Cohort 2 (n = 11)</th>
<th>PP (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2 (n = 3)(^b)</td>
<td>T3 (n = 6)(^d)</td>
<td>T2 (n = 6)(^h)</td>
</tr>
<tr>
<td><strong>Total LPV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC(_{0–12}) (ng · h/ml)</td>
<td>76,921 (47,176–124,081)</td>
<td>50,391 (36,493–75,726)</td>
<td>80,940 (70,712–94,656)(^a)</td>
</tr>
<tr>
<td>C(_{\text{trough}}) (ng/ml)</td>
<td>4,169 (2,598–6,858)</td>
<td>2,364 (1,570–4,092)</td>
<td>4,590 (4,090–5,258)(^b)</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/ml)</td>
<td>8,198 (4,661–13,684)</td>
<td>6,031 (4,595–8,362)</td>
<td>9,228 (7,939–11,026)(^c)</td>
</tr>
<tr>
<td>C(_{\text{predose}}) (ng/ml)</td>
<td>5,735 (3,799–8,955)</td>
<td>3,383 (2,321–5,308)</td>
<td>5,681 (4,738–7,112)</td>
</tr>
<tr>
<td>(t_{1/2}) (h)</td>
<td>7.54 (4.81–11.04)</td>
<td>6.19 (5.34–7.23)</td>
<td>8.28 (6.35–11.39)</td>
</tr>
<tr>
<td><strong>Unbound LPV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC(_{0–12}) (ng · h/ml)</td>
<td>618 (204–1,371)</td>
<td>492 (306–822)</td>
<td>1,036 (864–1,293)</td>
</tr>
<tr>
<td>C(_{\text{trough}}) (ng/ml)</td>
<td>30 (11–61)</td>
<td>26 (15–48)</td>
<td>125 (109–148)</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/ml)</td>
<td>73 (24–161)</td>
<td>58 (35–93)</td>
<td>150 (119–178)</td>
</tr>
<tr>
<td>C(_{\text{predose}}) (ng/ml)</td>
<td>45 (19–90)</td>
<td>27 (18–50)</td>
<td>76 (57–117)</td>
</tr>
<tr>
<td>(t_{1/2}) (h)</td>
<td>6.38 (6.11–6.66)</td>
<td>7.56 (6.31–9.05)</td>
<td>4.91 (3.99–6.55)</td>
</tr>
<tr>
<td>% AUC(_{\text{unbound}})</td>
<td>0.80 (0.54–1.14)</td>
<td>0.80 (0.5–1.29)</td>
<td>1.28 (1.05–1.63)</td>
</tr>
<tr>
<td><strong>RTV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC(_{0–12}) (ng · h/ml)</td>
<td>2,804 (1,640–3,057)</td>
<td>1,918 (993–3,708)</td>
<td>3,570 (2,866–4,617)(^f)</td>
</tr>
<tr>
<td>C(_{\text{trough}}) (ng/ml)</td>
<td>108 (48–153)</td>
<td>77 (17–207)</td>
<td>125 (90–179)</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/ml)</td>
<td>401 (239–420)</td>
<td>298 (196–458)</td>
<td>549 (451–705)</td>
</tr>
<tr>
<td>C(_{\text{predose}}) (ng/ml)</td>
<td>144 (10–364)</td>
<td>144 (72–191)</td>
<td>206 (121–368)</td>
</tr>
<tr>
<td>(t_{1/2}) (h)</td>
<td>5.00 (3.09–4.49)</td>
<td>4.76 (3.97–5.75)</td>
<td>3.77 (3.31–4.41)</td>
</tr>
</tbody>
</table>

\(^a\) Third trimester significantly lower than second trimester \((P = 0.008)\).
\(^b\) Third trimester significantly lower than postpartum \((P = 0.002)\).
\(^c\) Third trimester significantly lower than second trimester \((P = 0.001)\).
\(^d\) Third trimester significantly lower than postpartum \((P = 0.013)\).
\(^e\) Second trimester significantly lower than second trimester \((P = 0.009)\).
\(^f\) Second trimester significantly lower than postpartum \((P = 0.029)\).
\(^g\) Third trimester significantly lower than postpartum \((P = 0.007)\).
\(^h\) Third trimester significantly lower than postpartum \((P = 0.026)\).
\(^i\) Values are given as geometric means (95% CIs).
\(^j\) Numbers of samples. Number of samples = 4; 1 patient had two samples taken during the second trimester.

\(^k\) Number of samples = 9; 3 patients had two samples taken during the second trimester.

\(^\dagger\) Third trimester was significantly lower than second trimester \((P = 0.026)\). LPV C\(_{\text{trough}}\) in the third trimester was also lower \((P < 0.013)\) than that in the second trimester and postpartum. In addition, of the 5 patients that continued treatment postdelivery, all experienced an increase in LPV C\(_{\text{trough}}\) postpartum (geometric means, 2,665 ng/ml in the third trimester \([n = 5]\) and 4,653 ng/ml postpartum \([n = 5]\)). All patients receiving the tablet had LPV concentrations of >1,000 ng/ml throughout pregnancy and postpartum. One patient was dose adjusted to 3 tablets (600/150 mg) twice daily after pharmacokinetic sampling in the third trimester; her viral load was undetectable and she discontinued treatment postdelivery.

The average (geometric mean) total LPV AUC\(_{0–12}\) and C\(_{\text{max}}\) were approximately 15% and 25% (nonsignificantly) higher, respectively, in patients receiving the tablet \((n = 11)\) than in those on the SGC \((n = 6)\) during the third trimester, whereas the LPV C\(_{\text{trough}}\) and C\(_{\text{predose}}\) were similar \((-6%)\) between the two cohorts (Fig. 1a). Intersubject variation of plasma LPV concentrations over each time point of the 12-h dosing interval was similar between the two regimens (29 to 56% for cohort 1, 28 to 56% for cohort 2).

LPV was highly protein bound during pregnancy: the average (geometric mean) LPV unbound concentrations and percentage unbound in cohort 1 (second and third trimesters) and cohort 2 (second trimester) were 48% and 28%, respectively. In the second trimester, pregnancy was associated with a 16% increase in plasma LPV protein binding, but this was not statistically significant \((P = 0.257)\).
(second and third trimesters and postpartum) are shown in Fig. 2a and b, respectively. In cohort 1 in both the second and third trimesters, the LPV percent $AUC_{unbound}$ values were equal at $\sim 0.80\%$, with little fluctuation in the unbound fraction over the course of the dosing interval (Fig. 2a). In cohort 2, the percent $AUC_{unbound}$ was higher antepartum: 1.28% in the second trimester, 1.18% in the third trimester, and 1.01% postpartum. The fractions of unbound LPV (geometric mean) were approximately 27% and 17% higher in the second and third trimesters, respectively, than postpartum (Fig. 2b). Women receiving the tablet formulation of LPV (400/100 mg twice daily) did not have significantly higher percent $AUC_{unbound}$ values than women receiving the standard dosing of the SGC (400/100 mg twice daily) in the third trimester ($P = 0.221$).

**Ritonavir pharmacokinetics.** Calculated RTV pharmacokinetic parameters are presented in Table 2. When administered as part of the SGC formulation, RTV $AUC_{0-12}$, $C_{trough}$, and $C_{max}$ were lower in the third trimester than the second trimester, but this was not statistically significant. In those receiving the LPV/r tablet, the differences in RTV pharmacokinetics between the second and third trimesters were small; however, significantly lower RTV exposures and trough concentrations were observed in the second and third trimesters compared with those observed postpartum ($P < 0.029$). The average (geometric mean) total RTV $AUC_{0-12}$ in the third trimester was approximately 56% higher in patients receiving the tablet than in those receiving the SGC ($P = 0.216$) (Fig. 1b).

**Placental transfer.** Time-matched maternal and umbilical cord blood samples were obtained from 6/19 women at delivery: 5/8 patients in cohort 1 and a single maternal blood and two cord blood samples from a mother who gave birth to twins (in cohort 2). The median times between the last dose and collection of maternal and fetal blood at delivery were 3.7 h (range, 0.5 to 10.4 h) and 3.7 h (range, 1.5 to 10.1 h), respectively. Total LPV was detectable in all cord-maternal sample pairs. Geometric mean total LPV concentrations were 4,483 ng/ml (95% CI, 3,049 to 6,714 ng/ml) in maternal blood and 625 ng/ml (95% CI, 128 to 1,680 ng/ml) in cord blood. The mean $\pm$ SD $C/M$ ratio of total LPV was 0.17 $\pm$ 0.09. All patients had measurable unbound LPV in both maternal and cord plasma. The geometric mean unbound LPV concentrations in maternal and cord samples were 37.7 ng/ml (28 to 52.5 ng/ml) and 11.2 ng/ml (7.87 to 17.63 ng/ml), respectively,
resulting in a mean ± SD unbound C/M ratio of 0.31 ± 0.09. The percentage of unbound LPV was significantly higher (2.5-fold) in the cord compartment than the maternal compartment (2.22% ± 1.48% versus 0.89% ± 0.32%; P = 0.033, paired t test), although high intersubject variability in LPV unbound fractions was apparent in both compartments. Total (low-dose) RTV was detectable in all 6 women at delivery; geometric mean RTV concentrations in maternal and umbilical cord blood were 320 ng/ml (95% CI, 175 to 465 ng/ml) and 30.7 ng/ml (95% CI, 19.6 to 41.7 ng/ml), respectively, resulting in a mean ± SD total RTV C/M of 0.13 ± 0.08.

**DISCUSSION**

Here we describe the total and unbound pharmacokinetics of LPV in two cohorts of pregnant women: one cohort receiving the standard LPV/r SGC dose of 400/100 mg twice daily and the other receiving standard dosing of the LPV tablet (400/100 mg twice daily). Although LPV exposure was reduced during the third trimester in both cohorts, concentrations were adequate (>1,000 ng/ml) in the majority of women studied.

In cohort 1, none of the women had pharmacokinetic samples taken postpartum. Most received short-term ART (START) during pregnancy and therefore discontinued treatment shortly after delivery. Likewise, fewer patients underwent pharmacokinetic sampling in the second trimester, mainly due to late presentation and because early commencement of START in asymptomatic mothers was deemed to be necessary only in women with high baseline plasma pVLs.

In cohort 2, only five patients continued treatment and underwent PK sampling postpartum. As a result, the power of the study to show differences attributable to pregnancy was limited, since statistical techniques analyzing the data as antepartum-postpartum pairs (which exclude the effect of intersubject variability) could not be performed. Nonetheless, LPV antepartum and postpartum predose concentrations (0-h morning concentration, equivalent to a therapeutic drug monitoring [TDM] C_{trough})
were comparable to those in studies investigating LPV tablet pharmacokinetics in pregnant women undergoing routine TDM (20). In the only other intensive pharmacokinetic study, Best and colleagues (3) reported LPV pharmacokinetics in HIV-infected pregnant women receiving an increased tablet dose (600/150 mg twice daily) in the third trimester and standard dosing (400/100 mg twice daily) in the second trimester and at 2 weeks postpartum. The LPV AUC\textsubscript{0\textendash}12 in the second trimester was significantly reduced (median, 72 \textmu g \cdot h/ml) compared with that in patients receiving an upward dose adjustment in the third trimester (median, 96 \textmu g \cdot h/ml), and both second and third trimester AUC\textsubscript{0\textendash}12\textsubscript{S} were significantly lower than the AUC\textsubscript{0\textendash}12\textsubscript{postpartum} (median, 133 \textmu g \cdot h/ml). The authors concluded that the higher tablet dose should be used in both the second and third trimesters, on the basis of the finding that subjects had LPV exposures in the third trimester similar to those of nonpregnant adults receiving the standard 400/100-mg tablet dose (∼98 \textmu g \cdot h/ml) (3). Interestingly, however, LPV exposures at 2 weeks postpartum were relatively high, despite the fact that subjects were reverted back to standard (400/100-mg twice daily) dosing postdelivery. This may have, in turn, accounted for the statistical differences between antepartum and postpartum pharmacokinetics reported in this study.

One limitation is that postpartum pharmacokinetics may not necessarily reflect true exposure in nonpregnant women, as, depending on the time of pharmacokinetic sampling postdelivery, the physiological changes associated with pregnancy that result in decreased plasma concentrations may not have fully resolved. On the basis of LPV/r (tablet) pharmacokinetic data from a study of Boffito et al. (6a) performed in 16 (6 female) HIV-negative healthy volunteers, LPV postpartum pharmacokinetics in the current study were comparable to those of nonpregnant adults and may therefore provide a more reasonable estimation of reduced LPV exposure during pregnancy. Comparison of these data is justified since there are no reported differences in LPV pharmacokinetics between healthy volunteers and HIV-infected subjects (9) or any known gender-related differences in LPV pharmacokinetics (27).

Previous studies had found no significant difference in LPV trough concentrations between pregnant patients receiving standard doses of the SGC and patients receiving the tablet formulations (17). Equally, we observed equivalent LPV trough concentrations in both cohorts; however, total LPV exposure and maximum concentrations were nonsignificantly higher in patients taking the tablet (cohort 2; \(n = 11\)) than those receiving the SGC (cohort 1; \(n = 6\)). We recognize that there are statistical limitations in comparing independent cohorts, especially given that the intersubject variability in protease inhibitor concentrations invariably exceeds the intrasubject variability. Nonetheless, the approximate 15% and 25% increases in LPV exposure and maximum concentrations, respectively, in cohort 2 were consistent with the reported 18% increase in oral bioavailability of the LPV/r tablet described in healthy volunteers (18).

In addition, a relative 27% increase of the percent AUC\textsubscript{unbound} LPV in the second trimester and 17% increase in the third trimester compared with that postpartum in patients receiving the LPV tablet (cohort 2) may compensate, to a certain extent, only for the overall decrease in total LPV exposure seen during pregnancy. These data are consistent with those reported by Aweeka and colleagues (1). Previous evaluations by Lambert et al. (20) also revealed no significant changes in the fraction of unbound LPV antepartum versus postpartum in patients receiving standard dosing of the LPV tablet. Interestingly, the percent unbound values reported were lower (first/second trimester = 1.01% [\(n = 16\)]; third trimester = 0.83% [\(n = 43\)]; postpartum = 0.96% [\(n = 12\)]) than the percent AUC\textsubscript{unbound} values described here. One explanation is that Lambert et al. (20) measured only LPV trough concentrations, taken at approximately 12 to 14 h postdosing (roughly equivalent to a \(C_{\text{predose}}\) [0-h] sample) as part of routine TDM. Upon analysis of the pharmacokinetic profiles in the current study (cohort 2; Fig. 2b), it is apparent that LPV exhibits concentration-dependent binding, a phenomenon that has been observed previously (6), in which unbound concentrations and the percentage of unbound LPV are higher during the absorptive and maximum phases of the dosing interval, thereby accounting for a higher overall percent AUC\textsubscript{unbound}. These data highlight the importance of intensive pharmacokinetic studies, which provide a more meaningful representation of drug distribution over the course of an entire dosing interval.

The implication of lower LPV exposure during pregnancy and the drug’s limited placental transfer in terms of both the maternal virologic response and the risk of intrapartum MTCT remains undefined. A degree of assurance can be gained from the low rates of virologic failure and vertical HIV transmission among pregnant women stable on LPV/r-based regimens, with many achieving full viral suppression at the time of delivery and in certain cases being able to deliver vaginally. It is also likely that a substantial prophylactic benefit to the fetus occurs through coadministered nucleoside analogues, which are known to achieve high concentrations in the placenta (8) and female genital tract (11). In the current study (with ~63% treatment-naive patients), despite a reduction in LPV exposure during the third trimester in both cohorts, LPV concentrations were adequate (>1,000 ng/ml) in the majority of women studied. However, this proposed efficacy target relates only to naïve patients without PI mutations. The impact of low protease inhibitor concentrations upon viral resistance (systemic and compartmentalized) and response in treatment-experienced patients remains a concern, especially as lower LPV concentrations have been associated with the development of resistance in experienced subjects (7, 24). For protease inhibitor-experienced patients, an efficacy target of 4,000 ng/ml has been proposed, as this was shown to be associated with achieving and maintaining an undetectable viral load in this population (7). In cohort 1 (SGC), 33% and 83% of patients had LPV plasma concentrations below 4,000 ng/ml in the second and third trimesters, respectively. In cohort 2 (tablet), 33%, 91%, and 20% had LPV plasma concentrations below this target in the second and third trimesters and postpartum, respectively.

In previous cohorts, rates of detectable viral loads at delivery ranged from 6 to 16%; however, most present only isolated cases of detectable viremia or virologic failure specifically associated with subtherapeutic LPV concentrations during pregnancy (17, 22, 34); in certain cases, these may have been due to poor treatment compliance (20). However, Peytavin et al. found in a large patient cohort (\(n = 101\)) that LPV trough concentrations in the second and third trimesters were statistically correlated with pVL at delivery (29). It is worth noting that an inability to achieve viral suppression at delivery may be more closely associated with a patient’s pretreatment viral load and/or the timing of short-course ART initiation in naïve subjects than a reflection of LPV/r efficacy or pharmacokinetics per se. For instance, Read et al. found that in
patients with high baseline pVLs (>100,000), early initiation of treatment (<20 weeks) increased the likelihood of achieving an undetectable viral load at delivery (31).

On the other hand, unnecessary upward dose adjustments of the tablet formulation could result in excessive exposure of the fetus to the drug, which may have implications upon fetal organogenesis, growth, and development. Indeed, despite the obvious benefits of ART in preventing MTCT, concerns remain over an association between PI-containing ART and the prevalence of preterm delivery, low birth weight, and fetal abnormalities. While LPV/r is classified in pregnancy category C by the Food and Drug Administration (28), the European Medicines Agency-approved label rightly highlights the lack of any teratogenicity signal in more than 600 prospective reports to the Antiretroviral Pregnancy Register (APR). The potential association of protease inhibitor-based ART use at conception and during early pregnancy with the risk of other adverse pregnancy outcomes remains controversial, with many studies yielding discordant results. In collated data from all LPV/r-exposed pregnancies enrolled with the APR from September 2000 to July 2007, 13.4% of infants were born prematurely (<37 weeks gestation) and infants exposed in the first trimester had an increased likelihood of PTD and low birth weight than infants whose earliest exposure occurred later in the second/third trimester (32); other studies have reported similar findings (4, 19, 23, 36). However, this has not been consistently proven, with some authors reporting no differences in PTD risk between women initiating ART later in pregnancy and those already receiving ART preconception (35). Discrepancies between studies may be linked to patient and practice heterogeneity, as HIV disease stage, HIV-1 RNA load, and CD4 count are all potential confounders of timing of ART initiation in pregnancy and adverse pregnancy outcomes. For instance, women with advanced HIV infection are more likely to deliver prematurely and to receive ART early during pregnancy (38). In the current study, there were six (32%) preterm deliveries, which is consistent with other reports (23, 36). Three mothers were treatment naïve and commenced LPV/r treatment in the late second or early third trimester. Interestingly, recent data have suggested that RTV-boosted PI, compared with nonboosted PI, was associated with an increased incidence of complications during pregnancy and induced premature births (33). It is unclear whether such an association, if confirmed, would be driven by RTV per se or by higher concurrent PI concentrations; nonetheless, caution should be exercised, particularly with upward dose adjustments of the LPV/r tablet, given that the RTV boosting dose will also be increased. More studies specifically powered to evaluate a possible association between LPV (and low-dose RTV) pharmacokinetics following early and late LPV exposures during pregnancy and risk of PTD (as well as other adverse pregnancy outcomes) in HIV-infected mothers receiving LPV/r based ART are needed.

In conclusion, despite a significant reduction in LPV exposure during the later stages of pregnancy and, in turn, reduce the concern surrounding the need to adjust the dose in naïve patients during the third trimester. Nonetheless, the concern over poor treatment compliance or subtherapeutic (<4,000 ng/ml) LPV concentrations and associated viral resistance in treatment-experienced patients justifies TDM and adjustment of the LPV/r dose accordingly. We await pharmacokinetic data from patients receiving the LPV/r tablet at a dosage of 500/125 mg twice daily by substitution of a pediatric LPV/r 100/25-mg tablet, which may provide a viable alternative for certain cases.

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**REFERENCES**


