Polyinosinic/Polyctydlylic Acid–Mediated Changes in Maternal and FetalDisposition of Lopinavir in Rats

Vanja Petrovic and Micheline Piquette-Miller

Leslie Dan Faculty of Pharmacy, Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada

Received November 20, 2014; accepted April 17, 2015

ABSTRACT

Maintenance of optimal lopinavir (LPV) concentration is essential for effective antiretroviral therapy and prevention of mother-to-child transmission of human immunodeficiency virus. However, little is known about the effects of inflammation on the pharmacokinetics of this protease inhibitor and drug transporter substrate, particularly during gestation. Our objective was to study the effect of polyinosinic/polyctydlylic acid [poly(I:C)], a viral mimic, on key maternal drug transporters, and to examine the effect on maternal and fetal disposition of LPV in rats. Poly(I:C) (5.0 mg/kg i.p.) or saline vehicle was administered to pregnant Sprague-Dawley rats on gestational days 17–18. At 24 hours postinjection, all rats were administered LPV (10 mg/kg i.v.), and plasma and tissues were collected at 5–120 minutes postadministration. Plasma interferon-γ (IFN-γ) levels were measured by enzyme-linked immunosorbent assay, and transporter expression was measured via real-time polymerase chain reaction. Maternal plasma, hepatic, placental, and fetal LPV concentrations were determined by liquid chromatography–tandem mass spectrometry. Administration of poly(I:C) induced IFN-γ plasma levels and downregulated the expression of several important ATP-binding cassette (ABC) drug efflux transporters in the placenta and liver of pregnant rats, compared with controls ($P < 0.05$). Maternal LPV plasma concentration and area under the concentration-versus-time curve were significantly increased in the poly(I:C) group. Plasma protein binding was also significantly higher in poly(I:C)-treated rats. Pronounced increases in hepatic, placental, and fetal LPV tissue:unbound plasma concentrations were seen in the poly(I:C) group; however, absolute tissue concentrations were not changed. Since the majority of commonly used and clinically important antiretroviral drugs are known to be ABC transporter substrates, inflammation-mediated changes in transporter expression could affect their maternal disposition and fetal exposure.

Introduction

Prescription or over-the-counter medications are a necessity for as many as 80% of pregnant women (Anger and Piquette-Miller, 2008). Human immunodeficiency virus (HIV) infections are prevalent in 20 million women of child-bearing age worldwide. Clinically, antiretroviral (ARV) therapy is crucial for the prevention of mother-to-child transmission (MTCT) of HIV, but information on the disposition of ARVs in pregnancy or the effect of concomitant infections is still largely lacking. Being the front-line barrier against xenobiotics and blood-borne toxins between the mother and the fetus, the integrity of the placenta is of utmost importance. Evidence from the literature demonstrates that the placenta expresses a number of transporters, some of which function in the removal of endogenous compounds and xenobiotics. Specifically, several of the ATP-binding cassette (ABC) drug efflux transporters, including P-glycoprotein (P-gp/multidrug resistance protein 1/ABCB1), the multidrug resistance–associated protein (ABCC), and the breast cancer resistance protein (MRPs/ABCCs), and the breast cancer resistance protein (BCRP/ABCG2), are highly expressed in placental tissues and have been found to profoundly limit the in vivo passage of potentially toxic xenobiotics into the fetus (Petrovoulos et al., 2007; Zhou et al., 2008).

Although the role of ABC proteins in drug transport has been examined under normal physiologic conditions, the impact of maternal disease on drug transporter regulation and activity is still poorly understood. Infection and inflammation occur frequently in pregnancy, and induction of proinflammatory cytokines is seen with many prevalent obstetric complications, including preeclampsia, gestational diabetes, and various infections (Romero et al., 2007). Previous studies from our group and others have reported inflammation-mediated alterations in the expression and activity of ABC drug transporters in the biologically protective barriers of the liver, intestine, and brain, as well as the placenta in rodents (reviewed in Petrovic et al., 2007). Moreover, altered drug disposition due to the inflammation-mediated suppression of P-glycoprotein was previously observed in vivo in male mice (Hartmann et al., 2005).

Currently, the protease inhibitor lopinavir (LPV) is widely used in clinical regimens for pregnant HIV-positive women. LPV is a Biomolecules Classification System class II antiretroviral drug with a therapeutic concentration range of approximately 5–7 μg/ml (mean steady-state trough concentration) and peak plasma concentration of

ABBREVIATIONS: AAG, α1-acid glycoprotein; ABC, ATP-binding cassette; ARV, antiretroviral; AUC, area under the concentration-versus-time curve; BCRP, breast cancer resistance protein (ABCG2); HIV, human immunodeficiency virus; IFN, interferon; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LPV, lopinavir; MRP, multidrug resistance–associated protein (ABCC); MTCT, mother-to-child transmission; P-gp, P-glycoprotein (ABCB1); poly(I:C), polyinosinic/polyctydlylic acid; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; RTV, ritonavir.
about 10 μg/ml. Maintenance of optimal LPV concentration is essential for effective therapy and MTCT prevention, but little is known about the effects of inflammatory mediators on the pharmacokinetics of this protease inhibitor. Previous in vitro studies have established LPV as a substrate for P-gp and MRPP2 (Agarwal et al., 2007; Janneh et al., 2007). As HIV-positive pregnant women are susceptible to opportunistic infections (Watts et al., 2004), it is plausible that changes in drug transporters may occur and, in turn, mediate changes in LPV disposition. Indeed, we have previously shown that the inflammation-mediated downregulation of placental Bcrp led to the increased accumulation of the Bcrp substrate glyburide in rat fetus (Petrovic et al., 2008); therefore, changes in P-gp could alter fetal accumulation of LPV. Furthermore, LPV is extensively metabolized by CYP3A, a metabolizing enzyme that is sensitive to inflammation-mediated changes in expression and functionality (Renton, 2001; Kumar et al., 2004). LPV is also highly bound (≥99%) to plasma proteins that can fluctuate during infection, mainly α1-acid glycoprotein (AAG) and, to a lesser extent, albumin, thus having the potential to affect the concentration of free drug available for intracellular and transplacental passage (Kumar et al., 2004; Chandwani and Shuter, 2008).

Under the MTCT-prevention premise, the benefits of ARV therapy far outweigh the risks of fetal exposure to ARV drugs. However, the fetal safety of most ARV drugs has not been fully established, and long-term effects of increased exposure are still unknown (http://aidsinfo.nih.gov/contentfiles/PerinatalGL.pdf). On the other hand, underexposure may result in compromised prevention of virus transmission. Understanding disease-induced changes in the maternal and fetal disposition of the antiretroviral agents commonly prescribed in pregnancy is imperative. Therefore, we examined the effect of a viral-like infection, modeled by polyinosinic/polycytidylic acid [poly(I:C), a synthetic viral mimetic double-stranded RNA], on in vivo maternal and fetal disposition of lopinavir.

Materials and Methods

Animals and Experimental Design. Pregnant near-term Sprague-Dawley rats (gestational day 17–18; Charles River Laboratories, Senneville, QC, Canada) were injected i.p. with a single 5.0-mg/kg dose of poly(I:C) (Amersham Biosciences, Piscataway, NJ) dissolved in phosphate-buffered saline. Control pregnant rats (gestational day 17–18) were injected with sterile phosphate-buffered saline. Twenty-four hours after the initial injection, all animals received LPV (USP, Rockville, MD) through a tail vein injection (10 mg/kg, i.v.). LPV was dissolved (10 mg/ml) in 4:3:3 (v/v/v) ethanol/propylene glycol/5% dextrose in water. At various times (5, 25, 45, 60, and 120 minutes) after LPV administration, dams (n = 4 per time point) were sacrificed under anesthesia by exsanguination. Maternal blood was collected and plasma obtained via centrifugation (3000g, 15 minutes, 4°C) and preserved at −80°C. Maternal livers, placentas, and fetuses were immediately harvested, frozen in liquid nitrogen, and preserved at −80°C for further analyses. All animal studies were approved by the Office of Research Ethics at the University of Toronto and conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Cytokine Measurements. Maternal plasma concentrations of interferon-γ (IFN-γ) were determined via commercially available enzyme-linked immuno-sorbent assay kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. The sensitivity of this assay is 10 pg/ml. Each sample was examined in duplicate and results within the standard curve range reported. Results were pooled from all five time points in each study group (n = 20/group).

Quantitative Reverse-Transcription Polymerase Chain Reaction and mRNA Expression. Methods for RNA isolation, cdNA synthesis, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) have been previously described (Wang et al., 2005; Petrovic and Piquette-Miller, 2010). In brief, RNA was extracted from tissues (50–100 mg) using TRizol reagent (Invitrogen, Burlington, ON, Canada). RNA was quantified using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and then reverse transcribed to cDNA using the First Strand cDNA synthesis kit (Fermentas, Burlington, ON, Canada) according to the manufacturer’s protocol. Placental and hepatic mRNA expression levels of relevant genes were measured by qRT-PCR using the Roche LightCycler with the LC FastStart DNA Master SYBR Green I kit (Roche, Laval, QC, Canada). Oligonucleotides for previously reported primer sequences were synthesized at the Hospital for Sick Children (DNA Synthesis Centre, Toronto, ON, Canada) (Petrovic and Piquette-Miller, 2010). All mRNA levels were normalized to 18S ribosomal RNA, and the ratios are presented as the percentage of control values.

Lopinavir Liquid Chromatography–Tandem Mass Spectrometry Assay. Samples and standards were prepared as previously described (Anger and Piquette-Miller, 2011) using a liquid-liquid extraction adapted from Wang et al. (2006). In brief, fetuses, placentas, and maternal livers were homogenized in deionized water. One hundred microliters of thawed plasma or tissue homogenates comprised the samples. The same volume of drug-free plasma or tissue homogenate was used for constructing LPV standards by adding to tubes containing predried LPV. For plasma and hepatic samples, the final LPV standard concentrations ranged from 62.5 to 2000 ng/ml, whereas for fetal and placental samples, the standards measured 6.25–200 and 25–1000 ng/ml, respectively. Ritonavir (RTV) was used as the internal standard (final concentration, 200 ng/ml). For extraction, 50 μl of 500 mN Na2CO3 was mixed with the samples followed by adding 1.2 ml of hexane/ethyl acetate (1:1 v/v). The mixture was then vortexed (1 minute) and centrifuged (21,000 rpm, 5 minutes). The organic layer (700 μl) was transferred to a clean vial, evaporated under nitrogen gas, and the extract reconstituted in 200 μl of 80% methanol. The final extracts were aliquoted in autosampler vials, and 10 μl was used for sampling injection.

Plasma and tissue LPV concentrations were measured using high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS) as previously described (Anger and Piquette-Miller, 2011). In brief, for liquid chromatography, we used a CTC PAL autosampler (LEAP Technologies, Carrboro, NC) and an Agilent Technologies (Santa Clara, CA) 1100 series pump with a 50 × 4.6-mm, 5-μm LiChrosorb RP-8 column (Phenomenex, Torrance, CA), while MS/MS was performed on an API 4000 triple quadrupole mass spectrometer equipped with a TurbolonSpray source (Applied Biosystems/MDS Sciex, Foster City, CA). Analyst software, version 1.4.2, was used (Applied Biosystems/ MDS Sciex) for analysis of LPV and RTV peak areas and LPV quantification. The lower limit of detection was <3 ng/ml, and the lower limit of quantification was <10 ng/ml.

Pharmacokinetic Analysis. At each time point, plasma and hepatic tissue were obtained from four dams, while three fetuses and placentas were collected from each dam for a pooled analysis (per dam) of fetal and placental concentrations. Concentration-time curves were obtained by plotting the average of data points. Since this study design entailed one-point sampling data (one blood or tissue sample from each rat), a noncompartmental analysis program for sparse sampling (Phoenix WinNonlin 6.3; Certara, St. Louis, MO) was used to calculate key pharmacokinetic parameters derived from maternal plasma concentrations. Additionally, the Bailar method for destructive measurement sampling (Bailar, 1988; Hartmann et al., 2005; Zhou et al., 2008) was used to estimate the mean and S.E. of the maternal plasma area under the concentration-versus-time curve (AUC) and the fetal, placental, and hepatic tissue AUCs, from 5 to 120 minutes. The Bailar method was previously adapted by Zhou et al. (2008) to the following mathematical form:

\[
E(AUC) = \sum_{i=2}^{n} \left[ E(C_i) + E(C_{i-1}) \right] \times \left( t_i - t_{i-1} \right) / 2
\]

\[
V(AUC) = \sum_{i=2}^{n} \left[ V(C_i) + V(C_{i-1}) \right] \times \left( t_i - t_{i-1} \right) / 2
\]

where \( E(AUC) \) and \( V(AUC) \) are the mean and variance of AUC, \( m \) is the number of time points of the time course experiment, and \( C_i \) and \( t_i \) are concentration and time, respectively. The time course was integrated by the trapezoidal formula without extrapolation. \( E(C_i) \) and \( V(C_i) \) are the mean and variance of concentration at time \( t_i \), defined as
where \( n_i \) is the number of the concentration data points at time \( t_i \). The variance of mean AUC is defined as

\[
V(AUC) = \frac{\sum_{i=1}^{n} [V(C_i)/n_i + V(C_{i-1})] \times [(t_i - t_{i-1})/2]^2}{n_i \times (n_i - 1)}
\]

The difference between groups is considered statistically significant if \( Z_i > 1.96 \) (95% confidence interval, \( P < 0.05 \)), where

\[
Z_i = \frac{[\phi_i - \phi_2]}{\sqrt{SE_1^2/2 + SE_2^2}}
\]

\( \phi_i \) and \( \phi_2 \) are the means of the AUC, and S.E.1 and S.E.2 are the standard errors of AUC for the two groups. S.E. of the AUC is defined as the square root of the variance of mean AUC.

**Protein Binding Assay.** LPV plasma protein binding was measured by ultrafiltration using Amicon Ultra-0.5 Centrifugal Filter Devices with a nominal molecular weight limit of 10,000 Da (Millipore, Billerica, MA). Plasma samples taken at 5 minutes were thawed and incubated at 37° C for 30 minutes, then transferred to filter devices and equilibrated at 30° C for 30 minutes. The samples were then centrifuged (14,000g, 15 minutes, 30°C) and the ultrafiltrate collected. Filtrate LPV concentrations were quantified using LC-MS/MS as described for plasma and tissues. For standard curve calibration, filtrate was collected from drug-free plasma samples. LPV concentrations in the filtrate samples were compared against the LPV concentrations in corresponding unfiltered plasma samples, and the percentage unbound (% unbound) was calculated as follows: % unbound = (Cfiltered /C unfiltered) - 100.

**Results**

**Interferon Response to Poly(I:C).** Interferon-\( \gamma \) levels were examined in maternal plasma samples from poly(I:C)-treated and vehicle control rats. Viruses or administration of double-stranded RNA such as poly(I:C) is known to induce type I interferons and an acute-phase response. Indeed, our enzyme-linked immunosorbent assay results demonstrated a significant induction of IFN-\( \gamma \) 24 hours after administration of 5.0 mg/kg poly(I:C), as compared with saline control (Fig. 1). Measurements of IFN-\( \gamma \) were below the detection limit in many control samples (11 out of 20) and were thus considered to be <10 pg/ml.

**Effect of Poly(I:C) on Maternal Drug Transporter and Cyp3a2 Expression.** The impact of poly(I:C) on the expression of transporters in placental and hepatic tissues was examined by qRT-PCR (\( n = 7–10/\) group) to confirm previously observed poly(I:C)-mediated changes in the expression of transporters that are reportedly implicated in LPV transport—namely, the two P-gp isoforms Abcb1a and Abcb1b, as well as Abcc2 (Mmp2) (Agarwal et al., 2017; Petrovic and Piquette-Miller, 2010).

In line with our previous observations, administration of poly(I:C) imposed significant changes in the expression of these transporters in the liver (Fig. 2). After administration of 5.0 mg/kg poly(I:C), Abcb1a was significantly reduced to 61% of control levels, whereas Abcb1b was drastically increased (\( P < 0.05 \)). Expression of hepatic Abcc2 (Mmp2) was significantly reduced to 61% of control levels (\( P < 0.05 \)). Additionally, expression levels of the metabolizing enzyme Cyp3a2 were examined, as downregulation has been reported in numerous models of acute and chronic inflammation. We found a significant downregulation of hepatic Cyp3a2 to 70% of the control mRNA value (\( P < 0.05 \)).

In placenta, we also observed significant changes in the mRNA expression of examined transporters (Fig. 2). Placental Abcb1a and

![Fig. 1. IFN-\( \gamma \) concentration in maternal plasma. Pregnant rats were injected with poly(I:C) and sacrificed 24 hours later. IFN-\( \gamma \) plasma concentrations were determined via enzyme-linked immunosorbent assay and are presented as picograms per milliliter of plasma. Data represent the mean ± S.E. (\( n = 20/\)group) and statistics calculated by t test. ***\( P < 0.001 \), as compared with saline control.](image1)

![Fig. 2. Effect of poly(I:C) on the hepatic and placental mRNA expression. Hepatic and placental tissues were collected from near-term pregnant rats 24–26 hours after poly(I:C) administration (5 mg/kg, i.p.). Analysis of mRNA expression was performed via qRT-PCR and gene expression normalized to 18s, as described in Materials and Methods. Data represent the mean ± S.E. as a percentage of control value (\( n = 7–10/\)group) with statistics calculated by t test. *\( P < 0.05 \), as compared with saline control.](image2)

![Fig. 3. Time course of LPV concentration in maternal circulation. Pregnant rats were administered LPV (10 mg/kg, i.v.) 24 hours after exposure to poly(I:C) (5 mg/kg, i.p.). Maternal blood samples were collected at 5, 25, 45, 60, and 120 minutes post-LPV injection (\( n = 4/\)dams/group/time point). Total drug concentrations in maternal plasma were determined via LC-MS/MS, as described in Materials and Methods. Results are presented as the mean ± S.D. Statistical differences between the two groups at each time point were calculated by t test. *\( P < 0.05 \), **\( P < 0.01 \).](image3)
Bailer significantly higher in the poly(I:C) group (Fortier et al., 2004; Petrovic and Piquette-Miller, 2010). Likewise, the maternal plasma AUC5-120min of LPV was significantly increased by more than 2-fold in the poly(I:C) group, suggesting that inflammation imposes changes in the pharmacokinetics of LPV. It is well recognized that induction of inflammation by bacteria or virus can cause changes in drug disposition (Moran et al., 2008).

Increased LPV levels in pregnant poly(I:C)-treated rats could occur through a number of mechanisms, such as altered plasma protein binding, metabolism, and distribution. LPV is highly bound (≥99%) to plasma proteins (Kumar et al., 2004; Chandwani and Shuter, 2008). An unbound fraction of 0.89% has been reported for LPV in serum from healthy pregnant women (Gulati et al., 2009), which is comparable to the 0.85% we saw in the control pregnant rats. A significant correlation of LPV protein binding with AAG concentrations has been reported in pregnant and postpartum HIV-infected women (Awecka et al., 2010). It is well known that inflammation can cause an induction of AAG (Schreiber et al., 1989). In humans, the serum concentration of AAG rises 2–5 times during an acute phase response (Hoçekpi̇ et al., 2003). Various models of acute inflammation in rats have also demonstrated an increase in AAG levels, resulting in altered pharmacokinetics of highly bound drugs (Murai-Kushiya et al., 1993; Laethem et al., 1994). Consistent with reported increases in AAG, we found a significant increase in plasma protein binding of LPV in poly(I:C)-treated rats, as compared with the control group. Generally, increased protein binding of a drug is associated with a decrease in the steady-state volume of distribution (Vd or Vss). The calculated values for control and poly(I:C)-treated dams were determined by a validated LC-MS/MS assay, as described in Materials and Methods. Overall, our data showed that the fetal and placental LPV tissue concentrations were much lower than the maternal total plasma LPV concentrations, indicating limited fetal distribution. Plasma levels in the pregnant rats were in line with the typical clinical therapeutic range of LPV in HIV-infected patients. A significant increase in maternal plasma concentrations of LPV was seen in rats administered poly(I:C), as compared with vehicle control, with the most pronounced differences seen at 25–60 minutes after LPV administration (Fig. 3). Table 1 summarizes the pharmacokinetic parameters calculated for both groups of pregnant rats. We obtained a terminal half-life of approximately 1.5 hours, which is in line with previous reports (Kumar et al., 2004). Terminal half-life was unchanged in the poly(I:C)-treated group. However, large differences in time-zero concentration, clearance, and volume of distribution are evident between the treatment groups. As expected, LPV was highly bound to plasma proteins (>99%) and significantly different between treatment groups. The unbound fraction in the control rats was 0.85 ± 0.33%, compared with 0.1 ± 0.01% in the poly(I:C) group (P < 0.05).

Plasma AUC between 5 and 120 minutes (AUC5-120) was significantly higher in the poly(I:C) group [126 ± 13 μg*min/ml in controls vs. 283 ± 28 μg*min/ml in poly(I:C)], as calculated by Bailler’s method (Table 2). The AUC5-120 for fetal, placental, and hepatic tissue concentrations did not differ significantly between the two groups (Table 2), reflecting similar absolute tissue concentrations (Supplemental Fig. 1). However, when normalized to unbound LPV concentrations, higher levels of accumulation were seen in these tissues at all time points, with significantly higher levels at 1–2 hours (P < 0.05) (Fig. 4).

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Poly(I:C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC5-120 minutes (μg*min/ml)</td>
<td>126.06 ± 13.530</td>
<td>282.550 ± 27.730</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>220.085</td>
<td>482.860</td>
</tr>
<tr>
<td>Vss (ml/kg)</td>
<td>45</td>
<td>21</td>
</tr>
<tr>
<td>Co (ng/ml)</td>
<td>4567</td>
<td>1803</td>
</tr>
<tr>
<td>Ctr (ng/ml)</td>
<td>6199</td>
<td>17.329</td>
</tr>
<tr>
<td>Fu (%)</td>
<td>0.85 ± 0.33</td>
<td>0.10 ± 0.01</td>
</tr>
</tbody>
</table>

### Table 2

Lopinavir plasma and tissue AUC5-120 min

<table>
<thead>
<tr>
<th>AUC5-120 min</th>
<th>Control</th>
<th>Poly(I:C)</th>
<th>Z0</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal plasma (ng*min/ml plasma)</td>
<td>126.060 ± 13.530</td>
<td>282.550 ± 27.730</td>
<td>5.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fetus (ng*min/g fetus)</td>
<td>2900 ± 400</td>
<td>2050 ± 420</td>
<td>1.45</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Placenta (ng*min/g placenta)</td>
<td>26,830 ± 2240</td>
<td>30,200 ± 5470</td>
<td>0.82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Liver (ng*min/g liver)</td>
<td>82,660 ± 11,820</td>
<td>83,330 ± 8510</td>
<td>0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

### Discussion

Lopinavir is commonly used in HIV-infected pregnant women as part of highly active antiretroviral therapy; however, the impact of coexisting conditions such as infection or inflammation on disposition is largely unknown (Watts, 2006; Lambert et al., 2011). Maintaining adequate drug concentrations is necessary as subtherapeutic concentrations can increase the risk of MTCT, whereas supratherapeutic concentrations can increase the risk of drug toxicities (Ofotokun et al., 2011).

In the present study, we examined the impact of an acute inflammatory response elicited by poly(I:C) on the disposition of LPV in pregnant rats. Consistent with induction of inflammation following a viral infection, we saw a dramatic increase in plasma concentrations of IFN-γ in the poly (I:C) group (Fortier et al., 2004; Petrovic and Piquette-Miller, 2010). Likewise, the maternal plasma AUC5-120min of LPV was significantly increased by more than 2-fold in the poly(I:C) group, suggesting that inflammation imposes changes in the pharmacokinetics of LPV. It is well known that induction of inflammation by bacteria or virus can cause changes in drug disposition (Moran et al., 2008).

Increased LPV levels in pregnant poly(I:C)-treated rats could occur through a number of mechanisms, such as altered plasma protein binding, metabolism, and distribution. LPV is highly bound (≥99%) to plasma proteins (Kumar et al., 2004; Chandwani and Shuter, 2008). An unbound fraction of 0.89% has been reported for LPV in serum from healthy pregnant women (Gulati et al., 2009), which is comparable to the 0.85% we saw in the control pregnant rats. A significant correlation of LPV protein binding with AAG concentrations has been reported in pregnant and postpartum HIV-infected women (Awecka et al., 2010). It is well known that inflammation can cause an induction of AAG (Schreiber et al., 1989). In humans, the serum concentration of AAG rises 2–5 times during an acute phase response (Hoçekpi̇ et al., 2003). Various models of acute inflammation in rats have also demonstrated an increase in AAG levels, resulting in altered pharmacokinetics of highly bound drugs (Murai-Kushiya et al., 1993; Laethem et al., 1994). Consistent with reported increases in AAG, we found a significant increase in plasma protein binding of LPV in poly(I:C)-treated rats, as compared with the control group. Generally, increased protein binding of a drug is associated with a decrease in the steady-state volume of distribution (Vd or Vss). The calculated values for control and poly(I:C)-treated dams were determined by a validated LC-MS/MS assay, as described in Materials and Methods. Overall, our data showed that the fetal and placental LPV tissue concentrations were much lower than the maternal total plasma LPV concentrations, indicating limited fetal distribution. Plasma levels in the pregnant rats were in line with the typical clinical therapeutic range of LPV in HIV-infected patients. A significant increase in maternal plasma concentrations of LPV was seen in rats administered poly(I:C), as compared with vehicle control, with the most pronounced differences seen at 25–60 minutes after LPV administration (Fig. 3). Table 1 summarizes the pharmacokinetic parameters calculated for both groups of pregnant rats. We obtained a terminal half-life of approximately 1.5 hours, which is in line with previous reports (Kumar et al., 2004). Terminal half-life was unchanged in the poly(I:C)-treated group. However, large differences in time-zero concentration, clearance, and volume of distribution are evident between the treatment groups. As expected, LPV was highly bound to plasma proteins (>99%) and significantly different between treatment groups. The unbound fraction in the control rats was 0.85 ± 0.33%, compared with 0.1 ± 0.01% in the poly(I:C) group (P < 0.05).

Plasma AUC between 5 and 120 minutes (AUC5-120) was significantly higher in the poly(I:C) group [126 ± 13 μg*min/ml in controls vs. 283 ± 28 μg*min/ml in poly(I:C)], as calculated by Bailler’s method (Table 2). The AUC5-120 for fetal, placental, and hepatic tissue concentrations did not differ significantly between the two groups (Table 2), reflecting similar absolute tissue concentrations (Supplemental Fig. 1). However, when normalized to unbound LPV concentrations, higher levels of accumulation were seen in these tissues at all time points, with significantly higher levels at 1–2 hours (P < 0.05) (Fig. 4).
half-life is a function of clearance and volume of distribution, and these were both decreased after treatment with poly(I:C).

It is well established that inflammation can lead to changes in drug disposition through downregulation of drug-metabolizing enzymes (Morgan, 1997; Renton, 2001). For example, impaired theophylline clearance during confirmed viral infection episodes is attributed to an impaired theophylline disposition through downregulation of drug-metabolizing enzymes (Morgan, 1997; Renton, 2001). For example, impaired theophylline clearance during confirmed viral infection episodes is attributed to an impaired theophylline disposition through downregulation of drug-metabolizing enzymes (Morgan, 1997; Renton, 2001). For example, impaired theophylline clearance during confirmed viral infection episodes is attributed to an impaired theophylline disposition through downregulation of drug-metabolizing enzymes (Morgan, 1997; Renton, 2001). For example, impaired theophylline clearance during confirmed viral infection episodes is attributed to an impaired theophylline disposition through downregulation of drug-metabolizing enzymes (Morgan, 1997; Renton, 2001).
blood ranging from 5 to 25% (Gingelmaier et al., 2006). Likewise, cord-to-maternal ratios ranging from 7 to 43% are reported for nelfinavir (van Heeswijk et al., 2004). As both P-gp expression and high plasma protein binding play important roles in restricting placental transfer of these agents, it is plausible that disease or inflammation-mediated changes could contribute to clinical variability. The observed decrease in placental expression of P-gp in the poly(I:C)-treated rats would lead to increased accumulation of drug substrates in fetal tissues. On the other hand, the observed inflammation-mediated increase in LPV plasma protein binding would lead to decreased transplacental transfer, as only free drug is able to cross cell membranes. Overall, we observed significant increases in LPV accumulation in placenta and fetal tissues of poly(I:C)-treated rats when normalized to unbound plasma concentrations. This is in line with other studies that have shown an increase in accumulation of P-gp substrates attributed to decreased P-gp expression and activity. For instance, a markedly higher accumulation of the P-gp substrate 99mTc-sestamibi was seen in fetal tissues isolated from endotoxin-treated dams (Wang et al., 2005). Decreased P-gp expression and increased protein binding are opposing factors that attenuate the impact of inflammatory response on absolute tissue accumulation. To this point, we examined the total (free and bound) concentrations of LPV in fetal tissues, the levels in poly(I:C) and control groups were not significantly different, suggesting there is likely no appreciable difference in pharmacodynamics. However, the fact that increased levels of tissue accumulation are seen when normalized to unbound LPV plasma concentrations indicates that transfer of free drug is greater in the poly(I:C) group. This suggests that, in addition to altered plasma protein binding, decreased expression of the efflux transporters plays a role in LPV disposition. Although LPV is clinically administered orally as a coformulation with low-dose ritonavir to increase bioavailability, it has been reported that LPV binding to AAG is not affected by RTV at therapeutically relevant concentrations (Gulati et al., 2009).

In conclusion, our study in a rat model of viral infection demonstrated that inflammation-mediated changes in plasma protein binding, drug metabolism, and drug transport significantly alter maternal and fetal disposition of LPV. It remains to be elucidated whether a similar trend would be seen in clinical cases of coinfection in pregnant women. As LPV metabolism and P-gp–mediated transport appear highly comparable between rodents and humans (Kumar et al., 1999), further clinical investigation of inflammation-mediated effects on LPV is warranted. Moreover, as LPV is generally administered in combination with other antiretrovirals that are substrates of P-gp, MRP2, BCRP, and CYP3A (Kis et al., 2010), the effects of inflammation on combination ARV therapies remain to be elucidated.

Acknowledgments

The authors thank Michelle Young and Dr. Matthew Forbes at the Advanced Instrumentation for Molecular Structure Laboratory, Department of Chemistry, University of Toronto, for providing their invaluable expertise and technical assistance with our LC-MS/MS experiments. The authors thank Dr. Sarabjit Gahir for the provision of Phoenix WinNonlin software and pharmacokinetic expertise. The authors also thank Ragi Ghoneim, Alex Cressman, and Nick Zhiakov for their technical assistance with animal studies.

Authorship Contributions

Participated in research design: Petrovic, Piquette-Miller.
Conducted experiments: Petrovic.
Performed data analysis: Petrovic.
Wrote or contributed to the writing of the manuscript: Petrovic, Piquette-Miller.

References

Chandwani A and Shuter J (2008) Lopinavir/ritonavir in the treatment of HIV-1 infection: a re-
chem Pharmacol 44:680–690.
Morgan ET (1997) Regulation of cytochromes P450 during inflammation and infection.
ing enzymes and transporters in infection, inflammation, and cancer. Drug Metab Dispos 36:205–216.
vention on hepatic cytochrome P450A mRNA levels of tissue accumulation are seen when normalized to unbound LPV plasma concentrations indicates that transfer of free drug is greater in the poly(I:C) group. This suggests that, in addition to altered plasma protein binding, decreased expression of the efflux transporters plays a role in LPV disposition. Although LPV is clinically administered orally as a coformulation with low-dose ritonavir to increase bioavailability, it has been reported that LPV binding to AAG is not affected by RTV at therapeutically relevant concentrations (Gulati et al., 2009).

In conclusion, our study in a rat model of viral infection demonstrated that inflammation-mediated changes in plasma protein binding, drug metabolism, and drug transport significantly alter maternal and fetal disposition of LPV. It remains to be elucidated whether a similar trend would be seen in clinical cases of coinfection in pregnant women. As LPV metabolism and P-gp–mediated transport appear highly comparable between rodents and humans (Kumar et al., 1999), further clinical investigation of inflammation-mediated effects on LPV is warranted. Moreover, as LPV is generally administered in combination with other antiretrovirals that are substrates of P-gp, MRP2, BCRP, and CYP3A (Kis et al., 2010), the effects of inflammation on combination ARV therapies remain to be elucidated.

Acknowledgments

The authors thank Michelle Young and Dr. Matthew Forbes at the Advanced Instrumentation for Molecular Structure Laboratory, Department of Chemistry, University of Toronto, for providing their invaluable expertise and technical assistance with our LC-MS/MS experiments. The authors thank Dr. Sarabjit Gahir for the provision of Phoenix WinNonlin software and pharmacokinetic expertise. The authors also thank Ragi Ghoneim, Alex Cressman, and Nick Zhiakov for their technical assistance with animal studies.

Authorship Contributions

Participated in research design: Petrovic, Piquette-Miller.
Conducted experiments: Petrovic.
Performed data analysis: Petrovic.
Wrote or contributed to the writing of the manuscript: Petrovic, Piquette-Miller.


Address correspondence to: Dr. Micheline Piquette-Miller, Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, ON M5S 3M2, Canada. E-mail: m.piquette.miller@utoronto.ca