ABSTRACT:
Pharmacokinetic coadministration experiments with atorvastatin (ATV) and rifampicin (RIF) in rats were performed to investigate the potential involvement of hepatic uptake transporters, Oatps (organic anion-transporting polypeptides), during hepatic drug elimination, as an in vivo extension of our recently published cellular and isolated perfused liver studies. ATV was administered orally (10 mg/kg) and intravenously (2 mg/kg) to rats in the absence and presence of a single intravenous dose of RIF (20 mg/kg), and pharmacokinetic parameters were compared between control and RIF-treatment groups. RIF markedly increased the plasma concentrations of ATV and its metabolites when ATP was administered orally. The area under the plasma concentration–time curve (AUCo–t) for ATV also increased significantly after intravenous dosing of ATV with RIF, but the extent was much less than that observed for oral ATV dosing. Significant increases in plasma levels were observed for both metabolites as well. The 7-fold higher AUC ratio of metabolites to parent drug following oral versus intravenous ATV dosing suggests that ATV undergoes extensive gut metabolism. Both hepatic and intestinal metabolism contribute to the low oral bioavailability of ATV in rats. In the presence of RIF, the liver metabolic extraction was significantly reduced, most likely because of RIF’s inhibition on Oatp-mediated uptake, which leads to reduced hepatic amounts of parent drug for subsequent metabolism. Gut extraction was also significantly reduced, but we were unable to elucidate the mechanism of this effect because intravenous RIF caused gut changes in availability. These studies reinforce our hypothesis that hepatic uptake is a major contributor to the elimination of ATV and its metabolites in vivo.

Bioavailability of orally administered drugs is often limited by first-pass effects because of metabolism and transporter-mediated processes. In clinical studies, significant interactions between the cholesterol-lowering agent, atorvastatin (ATV), and cytochrome P450 3A (CYP3A) inhibitors such as iraconazole (Kantola et al., 1998) and erythromycin (Stedl1k et al., 1999) were observed, with increased area under the plasma concentration–time curve (AUC) of ATV and reduction in the AUC values of its active metabolites ortho- or 2-hydroxy atorvastatin (2-OH ATV) and para- or 4-hydroxy atorvastatin (4-OH ATV). These results suggest extensive contribution of CYP3A toward ATV metabolism in vivo, even though other isozymes such as CYP2C8 have also been shown to metabolize ATV at a slower rate (Jacobsen et al., 2000). In humans, the absolute bioavailability of ATV is only 12% (Gibson et al., 1997), possibly as a result of substantial metabolism in both gut and liver by CYP3A4, the most abundant P450 enzyme expressed in both tissues. Our laboratory has noted that the two major active oxidative metabolites of ATV were formed during incubation with both human intestinal and hepatic microsomes (Christians et al., 1998; Jacobsen et al., 2000).

Various cell-based studies have suggested that multiple transporters (both uptake and efflux) located in the intestine and liver have the potential to affect the disposition of ATV in vivo (Hsiang et al., 1999; Wu et al., 2000; Hochman et al., 2004; Chen et al., 2005; Kameyama et al., 2005; Lau et al., 2006). As previously hypothesized and demonstrated by our laboratory, the interplay between transporters and metabolic enzymes may control the access of drug molecules to the enzymes, and changes in transporter function can modulate intestinal and hepatic metabolism without directly changing enzyme activity (Cummins et al., 2002, 2004; Wu and Benet, 2003; Lam and

ABBREVIATIONS: ATV, atorvastatin; P-gp, P-glycoprotein; MRP2/Mrp2, multidrug resistance-associated protein 2; Oatp, organic anion-transporting polypeptide; IPRIL, isolated perfused rat liver; P450, cytochrome P450; AUC, area under the plasma concentration–time curve; 2-OH ATV, ortho- or 2-hydroxy atorvastatin; 4-OH ATV, para- or 4-hydroxy atorvastatin; RIF, rifampicin; B/P, blood to plasma concentration ratio; Cl\text{iv}, total intravenous blood clearance; V\text{ss}, steady-state volume of distribution; F, oral bioavailability; F\text{abs} × F\text{G}, gut processes affecting availability; ER\text{H}, hepatic extraction ratio; F\text{vH}, hepatic availability; ER, extraction ratio.
Benet, 2004; Lau et al., 2004, 2006). It is likely that ATV may experience considerable transporter-enzyme interplay in vivo that could contribute to its low oral bioavailability.

The role of hepatic transporters, organic anion-transporting polypeptides (Oatps), in modulating the disposition and biliary excretion of ATV and OH ATV has been examined previously in our laboratory by applying the isolated perfused rat liver (IPRL) system (Lau et al., 2006). Rifampicin (RIF), a known potent inhibitor of various Oatps (Fattinger et al., 2000; Shitara et al., 2002), effectively reduced the Oatp-mediated hepatic uptake of ATV in a dose-dependent manner, whereby lesser amounts of ATV were available for subsequent hepatic metabolism, as reflected by reduced metabolite formation detected in liver tissues and bile. However, inhibitory effects on hepatic Cyp3a and efflux transporter multidrug resistance-associated protein 2 (Mrp2) became measurable at high RIF concentrations.

It is well recognized that cytochrome P450 enzymes expressed in the intestine significantly contribute to oxidative gut wall extraction for many orally administered drugs such as sirolimus (Lampen et al., 1998) and cyclosporine (Gomez et al., 1995; Wu et al., 1995). These intestinal enzymes might act to reduce the oral bioavailability of ATV during the intestinal absorption phase before reaching the liver, where further first-pass hepatic metabolism can occur.

We present here a pharmacokinetic study performed in rats to examine the influence of hepatic uptake inhibition on ATV exposure and its metabolic extraction in vivo. The rat model was used since ATV undergoes metabolic pathways similar to that in humans; both oxidative metabolites and parent drug are predominantly excreted in the bile of humans (Lennernas, 2003) and rats (Black et al., 1999) and only minimally in urine (<2%). Here, ATV was administered to rats either as intravenous bolus doses or orally, with and without a concomitant intravenous dose of RIF. We expected to observe a lower hepatic metabolic extraction with concomitant RIF when the amount of ATV available for metabolism is reduced, leading to an increase in drug exposure. We also hypothesized that extensive intestinal extraction would occur for this highly permeable, poorly soluble class 2 drug (Wu and Benet, 2005), where the access of ATV to the enzymes would be enhanced because of efflux transporter-intestinal enzyme interplay. The purpose of this study was two-fold: 1) investigation of the contribution of Oatps toward hepatic clearance of ATV, and 2) differentiation of the extent of ATV first-pass metabolism of the intestine from that of the liver.

Materials and Methods

Materials. ATV, 4-OH ATV, and 2-OH ATV were kindly supplied by Parke-Davis (Ann Arbor, MI) and Bristol-Myers Squibb (Princeton, NJ). Fluvasatin (Novartis, Cambridge, MA) was a kind gift from the manufacturer. RIF powder for injection (Rifadin I.V.) was obtained from Sanofi-Aventis (Bridgewater, NJ) and was reconstituted with sterile saline for injection. All other chemicals and solvents for the analysis were of analytical grade and used as supplied commercially. Male Sprague-Dawley rats with jugular vein precannulation were purchased from Charles River Laboratories (Wilmington, MA).

Pharmacokinetic Experiments in Rats. All experiments with rats were performed in accordance with the National Institutes of Health guidelines and approved by the Committee on Animal Research, University of California San Francisco. Pharmacokinetic experiments with ATV, either alone or in combination with RIF, were performed in male Sprague-Dawley rats weighing 250 to 300 g with jugular vein precannulation (n = 5 per group). Access to water was maintained during the experiment, but animals were fasted beginning the night before the experiment and through the 4 h of the experiments. Rats were orally dosed with ATV that was formulated in 0.5% methylcellulose suspension, by gavage. Pilot studies indicated that dose-normalized ATV AUC values were unchanged for 1 mg/kg, 3 mg/kg, and 10 mg/kg oral doses. For intravenous application, ATV was dissolved in physiological saline with 20% methanol to obtain a concentration of 1 mg/ml and given by tail-vein injection. Pilot studies indicated that dose-normalized ATV AUC values were unchanged for 1 mg/kg, 2 mg/kg, and 4 mg/kg intravenous doses. The 10 mg/kg oral dose and 2 mg/kg intravenous dose were chosen for detailed study so as to yield quantifiable metabolite concentrations. RIF dissolved in saline was also given by tail-vein injection at a dose of 20 mg/kg, 5 min before the administration of ATV. Animals receiving ATV alone were injected with an equivalent volume of vehicle. Blood samples of approximately 300-μl volume were drawn at predefined time points over a period of 24 h. Plasma was generated by centrifugation at 10,000g for 5 min. Samples were stored at −20°C pending analysis.

Calculation of Blood to Plasma Concentration Ratio (B/P) of ATV in Rats. Samples of rat blood were obtained from four rats weighing 250 to 300 g, placed in preheparinized tubes, and were used within 1 h after collection. Aliquots (1 ml) of blood were transferred to glass tubes and were spiked with ATV and placed in a 37°C shaking water bath for 30 min. Five hundred microliters were withdrawn from the incubation tubes and kept at −80°C for 5 min to achieve complete hemolysis. The remaining blood fraction was centrifuged for 5 min at 3000g to separate plasma and erythrocyte suspension. Calibration curves for ATV were prepared by spiking various concentrations of ATV to different matrices (whole blood, plasma, and erythrocyte). Four hundred microliters of acetonitrile were added to each sample (200 μl) for precipitation followed by 1 min of vortex mixing and centrifugation at 13,000g for 10 min. The supernatants were injected onto the LC/MS-MS apparatus for measurement as described below. Drug recovery from plasma and erythrocyte fractions should equal the amount of ATV detected in whole blood by mass balance, assuming hematocrit was 0.46 in rats (Davies and Morris, 1993), which was confirmed.

Metabolism by Rat Small Intestine Microsomes. Rat small intestinal microsomes were isolated and incubated as described previously by our laboratory (Lampen et al., 1998; Lau et al., 2006). In brief, microsome proteins (0.75 mg/ml), 10 mM MgCl2, 0.1 M phosphate buffer, pH 7.4, and ATV (in methanol, final concentration 0.1 μM) were preincubated for 5 min. The initial concentration of ATV was chosen at 0.1 μM so that it mimicked the in vivo ATV concentration following oral administration at 10 min, when RIF first caused an elevated increase in the plasma level of ATV. NADPH (1 mM) was added to start the reaction at 37°C. To assess the effects of RIF on ATV metabolism, RIF (in dimethyl sulfoxide, final concentration 10–50 μM) was added to the intestinal microsomal preparations. Metabolism blanks with no NADPH and heat-inactivated microsomes were run in parallel to serve as negative controls. The reaction was stopped by protein precipitation by the addition of an equal volume of ice-cold acetonitrile containing the internal standard fluvastatin (0.1 μM). In all assays, the final methanol and dimethyl sulfoxide concentrations were <0.5%. The supernatants were stored at −80°C for LC/MS-MS analysis.

LC/MS-MS Measurement of ATV, 2-OH ATV, 4-OH ATV, and RIF. The LC/MS-MS method for analyzing ATV and metabolites was described previously by Lau et al. (2006). Briefly, a Micromass Quattro Ultima instrument (Waters Corporation, Milford, MA) with electrospray-positive ionization was used. The multiple reaction monitor was set at 559.6 to 440.8 m/z for ATV, 575.2 to 440.5 m/z for 2-OH ATV and 4-OH ATV, and 412.3 to 265.9 m/z for the internal standard, fluvastatin. The cone voltage and collision energy were set at 30 V and 20 eV, respectively. The analytical column was an Agilent XDB C18 (4.6 x 50 mm, 5-μm particle size; Agilent Technologies, Palo Alto, CA). The mobile phase consisted of 46% acetonitrile containing 0.05% acetic acid and 5 mM ammonium acetate. Five-microliter aliquots were injected, and the flow rate was set at 1.0 ml/min with 1/4 split into the mass system. The quantitation limits for ATV and the metabolites (2-OH ATV and 4-OH ATV) were 5 nM and 1 nM, respectively.

RIF was detected with a multiple reaction monitor, which was set at 823.5 to 791.5 m/z. The sample cone voltage and collision energy were set at 30 V and 18 eV, respectively. The analytical column was a BDS C18 (50 x 4.6 mm) from Hypersil-Keystone (Bellefonte, PA). The mobile phase consisted of 36% acetonitrile containing 0.05% acetic acid and 5 mM ammonium acetate. The flow rate was 1.0 ml/min and 1/4 was split into the mass system. The lower limit of quantitation was 50 nM.
Pharmacokinetic Analysis. Peak plasma concentrations (C_{max}) and the time to reach peak plasma concentrations (t_{max}) were directly obtained by inspection. Pharmacokinetic parameters were estimated using the WinNonLin software package (Professional version 3.1; Pharsight, Mountain View, CA). Total intravenous blood clearance of ATV (CL_{inv}) was calculated as D/\left[\text{AUC}_{\text{inv}-\text{iv}} \times (B/P)\right] with D as dose and AUC_{inv-iv} as the area under the plasma concentration-time curve calculated by the linear/logarithmic trapezoidal rule (for up/down portions of the curve, respectively) and extrapolated to infinity using the apparent terminal disposition rate constant λ_z, determined by regression analysis of the linear terminal portion of the log plasma concentration-time curve. The apparent terminal half-life (t_{1/2}) was estimated from the terminal rate constant with t_{1/2} = ln 2/λ_z. For intravenous dosing, the steady-state volume of distribution (V_{ss}) was calculated as V_{ss} = MRT \times CL_{inv} with the mean residence time (MRT) being defined as the area under the first moment curve AUMC_{inv-iv} divided by AUC_{inv-iv} (Benet and Galeazzi, 1979). Because of assay limitations, ATV concentrations following oral dosing without RIF could only be measured up to 4 to 8 h, and half-life could not be accurately determined. Since, as will be discussed later, no significant changes in ATV terminal half-life (t_{1/2}) were observed between i.v. dosing with and without RIF and oral dosing with RIF, it appears that RIF and route of administration do not affect t_{1/2}. Therefore, for bioavailability calculations, AUC_{inv} values following oral dosing were all calculated by extrapolating from the last quantifiable concentration using a half-life of 245 min. This seemed to be a reasonable approach because extrapolated areas for ATV following oral dosing were less than 5% of total area. Comparisons of 2-OH ATV and 4-OH ATV metabolites to parent drug AUC ratios were only determined from 0 to 2 h and 0 to 4 h, respectively, since these were the time intervals at which both metabolites and parent ATV could be measured in all rats.

Oral bioavailability (F) was calculated by the ratios of dose-normalized AUC_{inv} after oral and intravenous dosing

\[ F = \frac{\text{AUC}_{\text{inv-oral}}}{\text{AUC}_{\text{inv-iv}}} \times \frac{\text{Dose}_{\text{oral}}}{\text{Dose}_{\text{iv}}} \] (1)

Hepatic blood clearance was assumed to equal CL_{inv}, that is, all ATV elimination following intravenous dosing is hepatic.

Hepatic extraction ratio (ER_{H}) was estimated as

\[ \text{ER}_{H} = \frac{\text{CL}_{H}}{Q_{H}} \] (2)

where rat hepatic blood flow (Q_{H}) was assumed to be 55.2 ml/min/kg (Naran- toni et al., 2001).

Since oral bioavailability is a function of F_{H} [hepatic availability (F_{H} = 1 - ER_{H}), F_{G} [gut availability (F_{G} = 1 - ER_{G})], and F_{abs} [fraction absorbed], as given in eq. 3 (Wu et al., 1995),

\[ F = F_{abs} \times F_{G} \times F_{H} \] (3)

it is possible to estimate the product of F_{abs} \times F_{G} as

\[ F_{abs} \times F_{G} = \frac{F}{F_{H}} \] (4)

Comparison of F_{abs} \times F_{G} calculated values give an estimate of the effects of RIF on the gut processes affecting ATV availability.

Total drug blood intrinsic clearance (f_{o} \times CL_{inv}) was calculated based on the well-stirred hepatic clearance model (Wilkinson, 1983),

\[ f_{o} \times CL_{inv} = CL_{inv} \times Q_{H}/(Q_{H} - CL_{inv}) \] (5)

Student’s t test was used to analyze the difference between two groups. The p value for statistical significance was set at <0.05.

Results

Effect of RIF on the Pharmacokinetics of ATV under Intravenous Dosing. ATV was detectable in plasma up to 12 h after dosing, although sampling was carried out for 24 h. The two active metabolites reached the 1 nM limit of detection at even earlier times (Fig. 1, B and C). Single intravenous doses of RIF increased the AUC_{inv} of ATV and its two metabolites (Table 1). The AUC_{inv} of ATV was increased by 36% (p < 0.05) with concomitant RIF compared with ATV alone. The AUC values of the active metabolites for 2-OH ATV and 4-OH ATV were, respectively, 4-fold greater (p < 0.01) and 3.5-fold greater (p < 0.001) for the RIF-treatment group than the control group over comparable time periods. C_{max} of 2-OH ATV was increased by 3.1-fold (p < 0.01), whereas that of 4-OH ATV was increased by 6.8-fold (p < 0.01).

The intravenous clearance (CL_{inv}) and steady-state volume of distribution (V_{ss}) in blood were calculated using the measured blood to plasma ratio (B/P) of 1.47. There was a 28% significant decrease in CL_{inv} (p < 0.05). The 26% decrease in V_{ss} by RIF treatment did not reach statistical significance (Table 2). Parallel changes in CL_{inv} and V_{ss} often result from changes in protein binding, but here this would suggest that RIF increased protein binding, a highly unlikely outcome, and our in vitro measures showed no change of RIF on ATV binding (data not shown). The calculated total drug intrinsic clearance (f_{o} \times CL_{inv}) decreased significantly by 53% from 88.0 ± 42.6 to 41.4 ± 11.6 ml/min/kg for this intermediate extraction ratio drug. There was

![FIG. 1. Mean (± S.D.) plasma concentrations of ATV (A), 2-OH ATV (B), and 4-OH ATV (C) in rats (n = 5) after a single intravenous dose of 2 mg/kg ATV with and without RIF given as a bolus intravenous dose (20 mg/kg). Solid circles indicate the ATV-alone control group; open circles indicate the RIF-treatment group. Data are depicted on a semilogarithmic scale.](image-url)
ERH calculated in the presence of RIF was significantly decreased by Metabolic Extractions of ATV.

AUCP) was virtually unchanged in the absence or presence of RIF was maintained when calculating ERH, dilutions following intravenous dosing with RIF were within the 2-fold dilutions measured following intravenous control dosing. ATV concen-

trations following intravenous dosing with RIF did not exceed concen-
trations measured following intravenous parent ATV dosing, the first measured time point was at 5 min.

For intravenous parent ATV dosing, the first measured time point was at 5 min. Blood values following intravenous dosing. a

N.D., not determinable due to assay limits of quantitation.

* p < 0.05; ** p < 0.01; *** p < 0.001 compared with values for the same dosing route in the absence of RIF.

† Significantly different from comparable intravenous ratio (p < 0.05; ** p < 0.01) in the AUC ratio of metabolites

The inhibitory effects of RIF (0 –50 M) by rat small intestinal microsomes were also examined (Table 4), since we had not previ-

ously reported these values (Lau et al., 2006). RIF did not alter the subsequent metabolism. Both 2-OH ATV and 4-OH ATV were

indicating a reduced partition of ATV into the liver compartment for

blocker, RIF, significantly increased the AUCs of ATV and OH ATVs

The estimated ERH of ATV was 0.58 ± 0.13. Therefore, F_H = 0.42 ± 0.13. From eq. 4, mean (F_abs × F_G), which is 71% greater than the control (p < 0.05) (Table 3), suggesting that intravenous RIF coadministration in the rat can affect both gut and liver bioavailability of ATV.

Metabolic Studies of ATV by Rat Small Intestinal Microsomes. The inhibitory effects of RIF (0–50 μM) on the in vitro metabolism of ATV (initial concentration is 0.1 μM) by rat small intestinal microsomes were also examined (Table 4), since we had not previously reported these values (Lau et al., 2006). RIF did not alter the metabolism of ATV up to a concentration of 35 μM and reduced it significantly, but less than 20%, at 50 μM as reflected by the increase in ATV concentrations. As depicted in Fig. 3, RIF plasma concentrations following intravenous dosing were less than 50 μM at all time points.

Discussion

Recently, we evaluated the interaction of ATV with hepatic uptake transporters, Oatps, in vitro in cellular systems and ex situ using the IPRL model (Lau et al., 2006). Hepatic uptake inhibition by an Oatp blocker, RIF, significantly increased the AUCs of ATV and OH ATVs with reduced amounts of all compounds in RIF-treatment livers, indicating a reduced partition of ATV into the liver compartment for subsequent metabolism. Both 2-OH ATV and 4-OH ATV were proven to be substrates of Oatp1b2, using in vitro assays, thereby explaining their increased AUC values by RIF (Lau et al., 2006).

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intravenous</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{max} (μM)</td>
<td>0.81 ± 0.41*</td>
<td>0.011 ± 0.055</td>
</tr>
<tr>
<td>t_{max} (min)</td>
<td>10 (10–30)</td>
<td>10 (10–30)</td>
</tr>
<tr>
<td>F_{rat} (μM min)</td>
<td>2.22 ± 0.52***</td>
<td>0.14 ± 0.13**</td>
</tr>
<tr>
<td>AUC_{c,a} (μM min)</td>
<td>0.015** 0.021</td>
<td>0.011 0.055</td>
</tr>
</tbody>
</table>

N.D., not determinable due to assay limits of quantitation.

* p < 0.05; ** p < 0.01; *** p < 0.001 compared with values for the same dosing route in the absence of RIF.

† Significantly different from comparable intravenous ratio (p < 0.01).

Data are mean values ± S.D. (n = 5); the level of significance was set at p < 0.05.

**Table 2**

Summary of derived pharmacokinetic parameters of ATV in rats (n = 5) after a single oral dose (10 mg/kg) or intravenous dose (2 mg/kg) of ATV with and without RIF given as a bolus intravenous dose (20 mg/kg)

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>With RIF</th>
<th>Without RIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATV</td>
<td>10 (10–30)</td>
<td>10 (10–30)</td>
</tr>
<tr>
<td>F_{rat} (μM min)</td>
<td>2.22 ± 0.52***</td>
<td>0.14 ± 0.13**</td>
</tr>
<tr>
<td>AUC_{c,a} (μM min)</td>
<td>0.015** 0.021</td>
<td>0.011 0.055</td>
</tr>
</tbody>
</table>

N.D., not determinable due to assay limits of quantitation.

* p < 0.05; ** p < 0.01; *** p < 0.001 compared with values for the same dosing route in the absence of RIF.

† Significantly different from comparable intravenous ratio (p < 0.01).

Data are mean values ± S.D. (n = 5); the level of significance was set at p < 0.05.
FIG. 2. Mean (± S.D.) plasma concentrations of ATV (A), 2-OH ATV (B), and 4-OH ATV (C) in rats (n = 5) after a single oral dose of 10 mg/kg ATV with and without RIF given as a bolus intravenous dose (20 mg/kg). Solid circles indicate the ATV-alone control group; open circles indicate the RIF-treatment group. Data are depicted on a semilogarithmic scale.

FIG. 3. Mean (± S.D.) plasma concentration of RIF after a single intravenous dose (20 mg/kg) in an ATV rat pharmacokinetic study with RIF coadministration (n = 5).

TABLE 3

Measures of ATV bioavailability and calculated hepatic and gut metabolic extraction based on eqs. 1 to 4

<table>
<thead>
<tr>
<th>ATV</th>
<th>F</th>
<th>ER_{H}</th>
<th>F_{H}</th>
<th>(F_{abs} \times F_{C})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alone</td>
<td>0.052 ± 0.020</td>
<td>0.58 ± 0.13</td>
<td>0.42 ± 0.13</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>With RIF</td>
<td>0.14 ± 0.03***</td>
<td>0.42 ± 0.07*</td>
<td>0.58 ± 0.07*</td>
<td>0.24 ± 0.04*</td>
</tr>
</tbody>
</table>

* p < 0.05; *** p < 0.001 compared with ATV alone.

Here, we studied the pharmacokinetics and metabolism of ATV under both intravenous and oral administration in rats. We had hoped that intravenous administration of RIF would have no gut effect, thereby allowing us to investigate the in vivo effect of hepatic Oatp on the drug-drug interaction between ATV and RIF following intravenous administration of both drugs.

As shown in Fig. 1 (A–C), intravenous coadministration of ATV and RIF led to a significant 4-fold increase in the AUC values for ATV’s metabolites, consistent with our IPRL data (Lau et al., 2006). We also observed a significant 36% increase in ATV AUC after 1 μM RIF was coadministered with 1 μM ATV in our previous IPRL study (Lau et al., 2006). As depicted in Fig. 3, the plasma concentration of RIF reached a value of ~35 μM at 5 min after ATV dosing. At this concentration, RIF should be effective in inhibiting the hepatic uptake of ATV, considering that the K_{i} values of RIF inhibition on ATV’s uptake obtained from in vitro cellular uptake assays were 2.88 μM for Oatp1a4 and 0.79 μM for Oatp1b2 (Lau et al., 2006). As indicated in Table 3, ATV has an ER_{H} value of 0.58, which would classify ATV as an intermediate hepatic extraction ratio drug (i.e., 0.3 ≤ intermediate ER_{H} ≤ 0.7), where blood flow considerations can modify the effect of changes in intrinsic clearance on total clearance. That is, upon RIF treatment, total drug intrinsic clearance (f_{a} \times CL_{int}) decreased significantly by 53%, almost twice that observed for the 28% decrease in total hepatic clearance (CL_{H}) (Table 2).

When ATV was administered orally at 10 mg/kg, there was a marked 3.5-fold increase in AUC_{0-\infty} in the presence of RIF (Fig. 2A), which was more extensive than for (f_{a} \times CL_{int}) observed after intravenous dosing. Since clearance following oral dosing (CL/F) is not affected by hepatic blood flow [i.e., CL/F = (f_{a} \times CL_{int})/(F_{abs} \times F_{C})], the marked change in oral clearance suggests that (F_{abs} \times F_{C}) must also have been increased following intravenous RIF dosing.

It is likely that some hydroxy metabolites are formed by intestinal wall Cyp3a during the absorption phase when ATV is dosed orally. This can be examined by comparing AUC ratios of metabolites to parent ATV. The AUC_{0-\infty} to AUC_{0-\infty} ratio was 7-fold higher for oral control versus i.v. control (Table 2), indicating a greater portion of metabolism under oral administration, as a result of first-pass metab-
olism from both intestine and liver, as opposed to that under the i.v. route, where metabolism is assumed to occur only (or predominantly) in the liver. To obviate saturation of either transport or metabolic processes, the chosen doses for both routes of administration were within the linear dose-exposure range found in preliminary studies. As shown in Table 3 for ATV-alone dosing, the estimated gut availability ($F_{abs} \times F_G$) was one-third of the liver availability ($F_L$). Given that ATV has rapid and almost complete intestinal absorption (Lennernas, 2003), we suspect that the contribution of $F_{abs}$ to the low value of estimated gut availability should be relatively minor compared with that of $F_G$. The significant gut metabolism is probably due to the high capacity of intestinal enzymes to metabolize ATV and/or increased drug exposure to metabolizing intestinal enzymes from the contribution of intestinal efflux transporters at the apical domain of the enterocyte, such as P-glycoprotein (P-gp), leading to recycling of drugs between the gut lumen and the enterocytes, thereby causing significant enzyme-transporter interplay (Benet et al., 1999; Johnson et al., 2001; Cummins et al., 2002, 2004). Since ATV has the characteristics of a class 2 compound according to the Biopharmaceutics Drug Disposition Classification System proposed by Wu and Benet (2005), its high permeability will allow ready access to the gut membrane, but the low solubility will limit the concentrations coming into the enterocytes, thereby preventing saturation of efflux transporters.

The AUCG/AUCP ratio was significantly increased by RIF following i.v. dosing (Table 2). RIF caused a 310% increase in the total metabolites AUCt-$\text{Cl}_{\text{int}}$, in contrast to the 36% increase in ATV AUC (and the 56% decrease in $f_{\text{u}} \times \text{Cl}_{\text{int}}$). This finding is consistent with metabolite uptake also being inhibited by RIF. However, RIF has no significant effect on the AUCG/AUCP ratio following oral dosing. That is, RIF caused a parallel increase in metabolites and parent ATV. A number of hypotheses could be put forward to explain these results, but here we only report the observation, since we were unsuccessful in avoiding RIF gut effects and, therefore, cannot experimentally differentiate liver versus gut inhibitory effects.

RIF is conventionally known for its induction effect on several drug-metabolizing enzymes such as CYP3A4 and 2C9 as well as P-gp and MR2P when given following a multiple dosage regimen (Schuetz et al., 1996; Fromm et al., 2000). More recently, Kajosaari et al. (2005) reported that RIF also exhibits an enzymatic inhibitory effect toward pagipladin P450-mediated metabolism in hepatic microsomal studies. However, the potency of RIF’s inhibitory effect toward P450 enzymes is probably substrate-specific because RIF did not exhibit substantial inhibition on the metabolism of ATV as reported in our rat hepatic microsomal studies (Lau et al., 2006). We show here that RIF also had no effect on intestinal microsomal metabolism (Table 4) at RIF concentrations comparable to those measured in vivo. We believe that the RIF effect on reducing hepatic metabolic extraction is most likely due to its inhibition on hepatic Oatps, which in turn led to reduction in the amount of parent drug getting into the hepatocytes for metabolism. Moreover, RIF could contribute to increased gut availability through inhibition of intestinal efflux transporters following oral dosing of ATV. Upon RIF treatment, ($F_{abs} \times F_G$) significantly increased from 0.14 ± 0.08 to 0.24 ± 0.04 in the present study. However, our previous work does not provide an adequate explanation of this finding. As shown by our IPRIL and transporter studies, ATV is subject to efflux transport by MR2P in addition to P-gp (Lau et al., 2006). RIF was able to exhibit inhibitory effects on efflux of ATV (5 μM) mediated by MR2P, but not P-gp. But these effects were only observed at concentrations of 50 μM or higher using the human MR2P- or P-gp-overexpressing cell lines. Ongoing study using CYP3A4-transfected Caco-2 cells (Cummins et al., 2002, 2004) did suggest a significant reduction in the metabolic extraction ratio from the apical to basolateral direction when the efflux of ATV was inhibited by the concomitant GG918, a potent P-gp inhibitor with no inhibitory effect on CYP3A4-mediated metabolism (data not shown).

In conclusion, these pharmacokinetic experiments reinforce our hypothesis that hepatic Oatp is one of the major determinants for drug elimination, especially for drugs that are mainly eliminated by the liver, and/or undergo hepatic metabolism. We have also shown that a single bolus dose of RIF might cause significant drug-drug interaction by impairing hepatic uptake. In addition, ATV undergoes extensive gut metabolism besides hepatic metabolism and exhibits low oral bioavailability in rats, possibly because of transporter-enzyme interplay at both the intestinal and hepatic level, but because intravenous RIF administration caused obvious changes in gut bioavailability, we were unable to differentiate intestinal enzymatic and transporter effects in vivo.

Acknowledgments. We thank Lance Chou for excellent technical assistance in the rat pharmacokinetic studies.

References


Address correspondence to: Dr. Leslie Z. Benet, Professor, Department of Biopharmaceutical Sciences, 533 Parnassus, Room U-68, University of California San Francisco, San Francisco, CA 94143-0446. E-mail: leslie.benet@ucsf.edu