Differential Effects of Rifampin and Ketoconazole on the Blood and Liver Concentration of Atorvastatin in Wild-Type and Cyp3a and Oatp1a/b Knockout Mice

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ABSTRACT

Atorvastatin is eliminated by CYP3A4 which follows carrier-mediated uptake into hepatocytes by OATP1B1, OATP1B3, and OATP2B1. Multiple clinical studies demonstrated that OATP inhibition by rifampin had a greater impact on atorvastatin systemic concentration than itraconazole-mediated CYP3A4 inhibition. If it is assumed that the blood and hepatocyte compartments are differentiated by the concentration gradient that is established by OATPs, and if the rate of uptake into the hepatocyte is rate-determining to the elimination of atorvastatin from the body, then it is hypothesized that blood concentrations may not necessarily reflect liver concentrations. In wild-type mice, rifampin had a greater effect on systemic exposure of atorvastatin than ketoconazole, as the blood area under the blood concentration-time curve increased 7- and 2-fold, respectively. In contrast, liver concentrations were affected more by ketoconazole than by rifampin, as liver levels increased 21- and 4-fold, respectively. Similarly, in Cyp3a knockout animals, 39-fold increases in liver concentrations were observed despite insignificant changes in the blood area under the blood concentration-time curve. Interestingly, blood and liver levels in Oatp1a/b knockout animals were similar to wild types, suggesting that Oatp1a/b knockout may be necessary but not sufficient to completely describe atorvastatin uptake in mice. Data presented in this work indicate that there is a substantial drug interaction when blocking atorvastatin metabolism, but the effects of this interaction are predominantly manifested in the liver and may not be captured when monitoring changes in the systemic circulation. Consequently, there may be a disconnect when trying to relate blood exposure to instances of hepatotoxicity because a pharmacokinetic-toxicity relationship may not be obvious from blood concentrations.

Introduction

Organic anion-transporting polypeptides (OATPs) are a family of drug transporters, encoded by SLCO genes that mediate uptake into various compartments within the body. One of the main physiologic function of OATPs is the elimination of bilirubin (Scharschmidt et al., 1975; Cui et al., 2001), but they also contribute to the disposition of a wide array of xenobiotics, including statins. OATPs are ubiquitously expressed (e.g., OATP4A1) or localized to a particular tissue such as OATP1B1 in the liver (Hagenbuch and Meier, 2004). In addition to OATP1B1, OATP1B3 and OATP2B1 are found at the sinusoidal membrane of hepatocytes (Kullak-Ublick et al., 2001). There is increasing evidence of drug interactions involving these liver-bound OATPs, as less of function due to chemical inhibition or genetic polymorphisms has been associated with clinically relevant drug interactions (Kalliokoski and Niemi, 2009; Shirata et al., 2013) and hyperbilirubinemia (van de Steeg et al., 2012; Chang et al., 2013).

Atorvastatin (Liptor) is a widely prescribed lipid-lowering statin that targets HMG-CoA reductase. Metabolism consists of hydroxyl- and beta-oxidation; and in particular, the role of CYP3A4 has been well-documented as it catalyzes the formation of two major active metabolites, 2-hydroxy- and 4-hydroxy-atorvastatin (Lea and McTavish, 1997). However, before atorvastatin can get metabolized, it needs to enter the liver, which is mediated by OATP1B1 (Kameyama et al., 2005; Lau et al., 2007), OATP1B3 (Schwarz et al., 2011), and OATP2B1 (Grube et al., 2006). Among them, OATP2B1 exhibits one of the highest affinities toward atorvastatin with K\textsubscript{m} of 200 nM (Grube et al., 2006).

Multiple clinical studies have attempted to discriminate the relative contributions of CYP3A4 and OATPs to the disposition of atorvastatin and for assessing drug interaction liabilities. When rifampin was given as a single dose to inhibit OATPs, 10- to 12-fold increases in atorvastatin plasma concentrations were observed (Lau et al., 2007; Maeda et al., 2011). Interestingly, a CYP3A4 blocker itraconazole was less effective to yield only 2- to 3-fold increases (Kantola et al., 1998; Mazzu et al., 2000), and in one study no effect (Maeda et al., 2011), in atorvastatin plasma concentrations. These results suggest that hepatic uptake via OATPs may be the critical step in the disposition of atorvastatin. Indeed, studies have shown that uptake of atorvastatin into hepatocytes was rate-determining, and, therefore, limiting OATP function would have a bigger impact than inhibiting CYP3A4 on the systemic exposure of atorvastatin (Lau et al., 2007; Watanabe et al., 2010). However, although only modest changes were observed with atorvastatin in the plasma, modulating CYP3A4 activity may have a profound effect on liver concentrations because the liver is where atorvastatin is eliminated from the body (Watanabe et al., 2009).

Abbreviations: AUC, area under the blood concentration-time curve; HBSS, Hanks’ balanced salt solution; HEK, Human Embryonic Kidney; LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; OATP, organic anion-transporting polypeptide; PEG400, polyethylene glycol 400; UGT, UDP-glucuronosyltransferase.
concentrations would be higher than the vehicle-treated wild-type and in the Cyp3a knockout group (Fig. 1C), both the blood and liver concentrations would rise because Oatps directly modulate blood atorvastatin levels. Meanwhile, the liver concentrations would rise or fall depending on the magnitude of variation in the blood compartment, as atorvastatin will enter the liver via passive process. Overall, \( K_p \) would be reduced to a value less than 1. Conversely, in the presence of ketoconazole or in the Oatp1a/b knockout animals (Fig. 1B), blood concentrations would rise because Oatps directly modulate blood atorvastatin levels. Therefore, for compounds whose clearance is dependent on uptake into eliminating organs, drug interaction involving drug transporters and drug-metabolizing enzymes may manifest differently in the systemic circulation compared with the organs. Although atorvastatin is typically dosed as oral tablets, the current study used i.v. administration to focus the study to the liver. FVB mouse strains were employed because our preliminary data showed that rifampin and ketoconazole also showed differential effect on atorvastatin blood levels as observed in the clinic, and because FVBs were the background strain of Cyp3a and Oatp1a/1b knockout mice that were used to further investigate the varying contribution of metabolism and uptake in atorvastatin disposition.

Materials and Methods

Chemicals. Ketoconazole, rifampin, and cyclosporine were purchased from Fisher Scientific (Waltham, MA). Atorvastatin calcium was purchased from Crescent Chemical (Islandia, NY). Pitavastatin and rosuvastatin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). O-hydroxy atorvastatin lactone d5 was purchased from US Biologic (Salem, MA). Polyethylene glycol 400 (PEG400) was purchased from Avantor Performance Materials (Center Valley, PA). The 0.9% sodium chloride (saline) was purchased from Hospira (Providence, RI). Dulbecco’s modified Eagle’s medium was purchased from Invitrogen (Carlsbad, CA). All other chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO). Multiscreen HTS 96-well filter plates and HTS Vacuum Manifold were obtained from EMD Millipore (Darmstadt, Germany). Parent compound mutants were established by transfection and were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 400 \( \mu \)g/mL G418 at 37°C with 5% CO2 and 95% humidity. The inhibition studies were carried out between 48 and 72 hours after seeding the cells at a density of 5.0 \( \times \) 10^5 cells/well in 24-well tissue culture plates coated with poly-d-lysine and laminin. Cells were preincubated at 37°C in Hanks’ balanced salt solution (HBSS) for 10 minutes. The uptake inhibition studies were initiated with 0.1–200 \( \mu \)M ketoconazole or rifampin along with 0.1 \( \mu \)M atorvastatin. For the positive control, inhibition of uptake of 0.1 \( \mu \)M pitavastatin by rifampin was measured for OATP1B1 and OATP1B3 (Matsushima et al., 2008), whereas the inhibition of uptake of 0.1 \( \mu \)M rosuvastatin by cyclosporine was measured for OATP2B1 (Ho et al., 2006). Following 5-minute incubation at 37°C, the reaction was terminated by rinsing the cells twice with 1 mL ice-cold HBSS buffer. The cells were then sonicated in 200 \( \mu \)L HBSS for 5 minutes and precipitated with an equal volume of acetonitrile. Each sample was centrifuged, and the supernatant was injected onto liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) to monitor for atorvastatin.

IC50 values are presented as mean ± S.D. with a minimum of three wells used to generate each data point. The IC50 was estimated by a sigmoidal inhibition model and was fit to:

\[
V = V_0/(1 + Log(I/IC50)^n)
\]

where \( V \) is the uptake of atorvastatin (or pitavastatin and rosuvastatin for the positive control) in the presence of the test inhibitors, \( V_0 \) is the uptake of atorvastatin (or pitavastatin and rosuvastatin for the positive control) in the absence of the inhibitor, \( I \) is the inhibitor concentration, and \( n \) is the hillslope.

In Vivo Pharmacokinetics Studies. Female FVB (wild-type) Cyp3a knockout, and Oatp1a/b knockout mice (Taconic Farms, New York, NY) were housed at controlled temperature and humidity in an alternating 12-hour light/dark cycle with free access to food and water before the study. All in vivo studies were performed in accordance with Institutional Animal Care and Use Committee guidelines and in harmony with the Guide for Laboratory Animal Care and Use. Six animals from each mouse model were randomly divided into two groups. One group was dedicated to collecting blood pharmacokinetics, whereas the other group was used to collect blood and liver at 15 minutes postdose. Six animals from each mouse model were pretreated either with 35 mg/kg ketoconazole (60% PEG400/40% saline) given orally 30 minutes prior to atorvastatin administration (Lau et al., 2006b), or with 20 mg/kg rifampin (10% DMSO/10% PEG400/80% saline) given i.v. via tail vein 5 minutes prior to atorvastatin administration (van Waterschoot et al., 2009), or with the appropriate vehicle control. Following pretreatments, all animals were given a single 5 mg/kg i.v. dose of atorvastatin (25% PEG400/75%). In the blood pharmacokinetics group, each animal was serially bled via tail nick at 0.033, 0.083, 0.25, 0.5, 1, 2, and 4 hours postdose. A total of 15 \( \mu \)L blood was collected from each time point and diluted with 60 \( \mu \)L water containing 1.7 mg/mL EDTA. In the second group, blood was collected and the livers were excised at 15 minutes postdose. This time point was chosen because previous work with pravastatin showed that liver levels may quickly decline and \( K_p \) ratios may plateau after 30 minutes in some strains (Iusuf et al., 2012). The 15-minute time point was the earliest at which it was technically feasible. All blood and liver samples were stored at approximately −80°C until thawed for LC-MS/MS analysis.

Sample Analysis. Atorvastatin concentrations in blood and liver were determined by LC-MS/MS. The LC-MS/MS system includes a Shimadzu SMB-20A controller with LC-30AD pumps, a Shimadzu Nexera XC18 column (50 × 2 mm, 2.7 \( \mu \)m particle size). The aqueous mobile phase was water with 0.1% formic acid (A), and the organic mobile phase was acetonitrile with 0.1% formic acid (B). The gradient was the following: 20% B at 0.0 minutes, increased to 80% B from 0.2 to 2 minutes, maintained at 80% B for 0.5 minutes, and decreased to 20% B within 0.03 minutes. The total run time was 3 minutes with flow rate at 1 mL/min. The ionization was conducted in the positive electrospray ionization ion mode using m/z 559.1–440.1 for atorvastatin and 562.1–453.1 for 2-hydroxy atorvastatin lactone d5 as the internal standard, the supernatant was injected onto a Phenomenex Kinetex XC18 column (50 × 2 mm, 2.7 \( \mu \)m particle size). The aqueous mobile phase was water with 0.1% formic acid (A), and the organic mobile phase was acetonitrile with 0.1% formic acid (B). The gradient was the following: 20% B for the first 0.2 minutes, increased to 80% B from 0.2 to 2 minutes, maintained at 80% B for 0.5 minutes, and decreased to 20% B within 0.03 minutes. The total run time was 3 minutes with flow rate at 1 mL/min. The ionization was conducted in the positive electrospray ionization ion mode using m/z 559.1–440.1 for atorvastatin and 562.1–453.1 for 2-hydroxy atorvastatin lactone d5 as the internal standards for quantitation. The injection volume was 10 \( \mu \)L.

Atorvastatin concentrations were determined by using a 12-point calibration curve. Peak area ratio of parent/internal standard was plotted against nominal analyte concentrations, which was fit with quadratic regression using 1/x weighting. The \( R^2 \) was between 0.98 and 0.99, with nominal concentration range between 0.193 and 11940 nM for blood and 0.609 and 3974 nM for liver.

The limit of quantitation was 0.193 nM for blood and 0.609 nM for the liver.

Data Analysis. Area under the blood concentration-time curve (AUC) was determined by noncompartmental methods using Phoenix WinNonlin, version 6.3 (Pharsight, Mountain View, CA). AUCs were transformed to natural logarithm, and analysis of variance (GraphPad Prism 5, San Diego, CA) was used to analyze differences between the vehicle-, ketoconazole-, and rifampin-treated groups.
within a particular animal model. The significance of difference was determined using Tukey’s post hoc test, and Student’s paired t test was used to determine the differences between rifampin- and ketoconazole-treated groups with vehicle. Mean (±S.D.) was reported, and P < 0.05 was considered statistically significant.

Results

Pharmacokinetics of Atorvastatin. Atorvastatin was rapidly eliminated from the blood following i.v. administration with an apparent half life ranging between 30 and 50 minutes. Figure 2 shows that, in the wild-type and Oatp1a/1b knockout groups, atorvastatin levels were below the limit of quantitation at 2 hours, whereas atorvastatin was measurable out to 4 hours for the remaining groups.

Table 1 summarizes the AUC of atorvastatin in the absence or presence of rifampin and ketoconazole to inhibit Oatps and Cyp3a, respectively. AUC of atorvastatin in the vehicle-treated animals was 0.957 ± 0.385 μM × hour. When coadministered with rifampin, the exposure of atorvastatin increased 7.3-fold to 7.01 ± 4.89 μM × hour. Changes associated with ketoconazole were not significant with AUC of 2.07 ± 1.47 μM × hour. This differential modulation of atorvastatin systemic concentration was consistent with multiple observations in the clinic (Kantola et al., 1998; Mazzu et al., 2000; Lau et al., 2007; Maeda et al., 2011).

To further probe the contribution of uptake and metabolism, the disposition of atorvastatin was evaluated in Oatp1a/b and Cyp3a knockout mice. The Cyp3a knockout mice are deficient for all eight-
cluster Cyp3a genes, which most likely represent all the human CYP3A in the liver, kidney, and intestines (van Herwaarden et al., 2007). Further analyses have shown that Cyp3a and UGT mRNA levels were not different than the wild-type animals (L. Salphati, personal communication). In the vehicle-treated Oatp1a/b knockout animals, the AUC was 1.66 ± 0.56 μM × hour. As observed in the Cyp3a knockout group, a modest elevation of atorvastatin exposure was observed with ketoconazole at 3.13 ± 0.36 μM × hour. Interestingly, although there was little change in atorvastatin exposure in the Oatp1a/b knockout compared with vehicle-treated wild-type animals, rifampin enhanced atorvastatin levels by 5.2-fold to 8.56 ± 6.3 μM × hour, suggesting that atorvastatin uptake into the liver may be mediated by additional transporters such as Oatp2b1.

**Liver Concentration of Atorvastatin.** Atorvastatin concentrations in the liver following i.v. administration were monitored at 15 minutes postdose. Table 2 summarizes the liver concentrations of atorvastatin in the absence or presence of rifampin and ketoconazole in various animal models. Overall, unlike the systemic circulation, blocking metabolism had a bigger impact than uptake on the liver levels. In the wild-type animals, atorvastatin liver concentration was 1.15 ± 0.61 nmol/g tissue. This was associated with liver-to-blood concentration ratio (Kp) of 3.3, as shown in Figure 3, suggesting that active uptake allows atorvastatin to preferentially cross the sinusoidal barrier to reside in hepatocytes. In the presence of rifampin, the Kp value fell below unity to 0.33, as 3.7-fold increase in liver concentration was accompanied by 37-fold increases in the blood, supporting the view that active uptake is required for atorvastatin to cross the blood-hepatocyte barrier. Conversely, Kp increased to 34 with ketoconazole, which is probably driven by the 21-fold boost in the liver concentration to 24.3 ± 5.5 nmol/g tissue. In the vehicle-treated Cyp3a knockout group, considerable elevations in liver concentration were observed at 44.5 ± 15.8 nmol/g tissue, resulting in Kp value of 82. Coadministration of rifampin or ketoconazole did not have a significant impact on the liver levels, and the Kp values ranged between 13 and 82. In the vehicle-treated Oatp1a/b knockout group, liver levels were unchanged compared with the vehicle-treated wild-type group. However, rifampin did augment the liver levels by 2-fold to 3.72 ± 0.30 nmol/g tissue and Kp dropped below 1, which supported the observation that atorvastatin uptake into the liver may be mediated by additional transporters sensitive to rifampin. In the presence of ketoconazole, Kp increased as the liver concentration reached 31.6 ± 8.2 nmol/g tissue.

**Inhibition of Atorvastatin Uptake of OATP1B1, OATP1B3, and OATP2B1.** The inhibition of atorvastatin uptake by rifampin or ketoconazole was assessed in HEK cells expressing OATP1B1, OATP1B3, and OATP2B1. Inhibition, measured as IC50, is summarized in Table 3. Rifampin block of 0.1 μM pitavastatin uptake was run as a positive control for OATP1B1 and OATP1B3 to yield IC50 values of 1.4 ± 0.1 μM and 1.2 ± 0.6 μM, respectively, which are within range of published Ks values (Matsushima et al., 2008). Although rifampin was more potent than ketoconazole, the uptake of atorvastatin by OATP1B1 and OATP1B3 was also reduced at higher ketoconazole concentrations. Specifically, rifampin IC50 values against atorvastatin uptake in OATP1B1 and OATP1B3 were 2.0 ± 0.6 μM and 1.4 ± 0.1 μM, respectively, whereas ketoconazole IC50 values in OATP1B1 have shown that Cyp3a and UGT mRNA levels were not different than the wild-type animals (L. Salphati, personal communication). In the vehicle-treated Oatp1a/b knockout animals, the AUC was 1.66 ± 0.56 μM × hour. As observed in the Cyp3a knockout group, just a modest elevation of atorvastatin exposure was observed with ketoconazole at 3.13 ± 0.36 μM × hour. Interestingly, although there was little change in atorvastatin exposure in the Oatp1a/b knockout compared with vehicle-treated wild-type animals, rifampin enhanced atorvastatin levels by 5.2-fold to 8.56 ± 6.3 μM × hour, suggesting that atorvastatin uptake into the liver may be mediated by additional transporters such as Oatp2b1.
respectively (data not shown). The Ki of ketoconazole against rosuvastatin was run as a positive control for OATP2B1 to yield values of 0.10 ± 0.01 μM, which is consistent with published values (Ho et al., 2006). Both rifampin and ketoconazole were less effective in inhibiting OATP2B1 uptake of atorvastatin, as the IC50 values were 160 ± 48 μM and 211 ± 58 μM, respectively. When administered to mice in vivo, the blood concentration of rifampin and ketoconazole at 15 minutes postdose was approximately 19 μM and 4 μM, respectively (data not shown). The Ki of ketoconazole against CYP3A4 was previously reported at 14.9 nM (Gibbs et al., 1999), whereas rifampin did not have an effect on atorvastatin metabolism (Lau et al., 2006a). These data suggest that rifampin may be affecting only OATP1B1 and OATP1B3 activity, whereas ketoconazole may be specific to only blocking CYP3A.

### Discussion

As combination drug therapy becomes more common, the need to better understand drug interactions is growing. Once believed to be most applicable for drug-metabolizing enzymes such as CYP3A4, interpretation of drug interactions has more frequently included drug transporters. Consequently, Food and Drug Administration guidance on drug interaction encompasses scenarios for both metabolizing enzymes and drug transporters (FDA, 2012). It is assumed that the systemic concentration will mirror changes in the organs and tissues. However, depending on the mechanisms controlling drug disposition, drug interactions may manifest differently in the blood compared with other compartments within the body (Watanabe et al., 2009). The objective of this study was to investigate changes in blood and liver concentrations of atorvastatin when blocking uptake (via single-dose rifampin administration and with Oatp1a/b knockout mice) or metabolism (via ketoconazole administration, which is also a CYP3A inhibitor, and with Cyp3a knockout mice).

Atorvastatin is extensively metabolized with contributions from CYP3A4 (Lea and McTavish, 1997), but a prerequisite to metabolism is active uptake into the liver, which is mediated by OATP1B1 (Kameyama et al., 2005; Lau et al., 2007), OATP1B3 (Schwarz et al., 2011), and OATP2B1 (Grube et al., 2006). Multiple clinical studies have shown that rifampin had a larger effect on atorvastatin plasma concentration than itraconazole (Kantola et al., 1998; Mazzu et al., 2000; Lau et al., 2007; Maeda et al., 2011). These observations may initially indicate that atorvastatin drug interactions involving OATPs may be of greater consequence than with CYP3A. In fact, the prescribing recommendation to avoid the risk of myalgia and rhabdomyolysis (LIPITOR, 2012), which is driven by systemic concentrations, is more cautious for OATP inhibitors such as cyclosporine (Ho et al., 2006) and protease inhibitors (Chang et al., 2013) than for itraconazole. However, if it is assumed that the blood and the hepatocyte compartments are differentiated by the concentration gradient that is established by OATPs, and if the rate of uptake into the hepatocyte is rate-determining to the elimination of atorvastatin from the body, it is hypothesized that the systemic concentrations may not necessarily reflect liver concentrations, especially in the presence of various inhibitors. Specifically, big increases in the systemic concentration are expected with rifampin because active uptake directly influences the depletion of atorvastatin from the blood (Fig. 1B). When OATPs are removed, atorvastatin will rely on passive diffusion to enter the hepatocytes, and, therefore, liver levels would depend on the concentration gradient that is established between the blood and hepatocyte compartment. In this study, Kp value would decrease and fall below unity. Conversely, big increases in the liver are expected with itraconazole or ketoconazole to yield a higher Kp value because metabolism directly influences the depletion of atorvastatin in the liver (Fig. 1C). Although the systemic concentration may rise if there is a big enough modulation in the liver, elevation of atorvastatin levels in the liver does not necessarily imply that equivalent changes will be observed in the blood compartment because the concentration gradient established by OATPs remains intact. This effect

![Fig. 3](image-url)  
Fig. 3. Summary of liver-to-blood concentration ratio (Kp) of atorvastatin following 5 mg/kg i.v. administration (N = 3). Wild-type FVBS are represented by solid black bars, Cyp3a knockouts are represented by checkered bars, and Oatp1a/b knockout mice are represented by solid gray bars. Each animal group were treated with either vehicle (Veh), rifampin (Rif) or ketoconazole (Keto). Data are presented as mean ± S.D. *P < 0.05.

### Table 2

Summary of mean atorvastatin liver and blood concentration (±S.D.) when administered 5 mg/kg i.v. in the absence and presence of 20 mg/kg i.v. rifampin or 35 mg/kg ketoconazole in various animal models

<table>
<thead>
<tr>
<th></th>
<th>Liver Concentration (nmol/g tissue)</th>
<th>Blood Concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild Type</td>
<td>Cyp3a Knockout</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.15 (0.61)</td>
<td>44.5 (15.8)</td>
</tr>
<tr>
<td>Rifampin</td>
<td>4.31 (0.54)*</td>
<td>40.6 (10.0)</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>24.3 (5.5)*</td>
<td>64.0 (12.7)*</td>
</tr>
</tbody>
</table>

*P < 0.05

and OATP1B3 were 23.9 ± 3.2 μM and 28.4 ± 5.7 μM, respectively. IC50 of cyclosporine against the uptake of 0.1 μM rosvuastatin was run as a positive control for OATP2B1 to yield values of 0.10 ± 0.01 μM, which is consistent with published values.

### Table 3

Summary of mean IC50 ± S.D. of ketoconazole or rifampin against atorvastatin uptake in HEK-transfected OATP1B1, OATP1B3, and OATP2B1 cells (N = 3)

<table>
<thead>
<tr>
<th></th>
<th>OATP1B1</th>
<th>OATP1B3</th>
<th>OATP2B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 (μM)</td>
<td>2.0 ± 0.6</td>
<td>1.4 ± 0.1</td>
<td>160 ± 48</td>
</tr>
<tr>
<td>Rifampin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>23.9 ± 3.2</td>
<td>28.4 ± 5.7</td>
<td>211 ± 58</td>
</tr>
</tbody>
</table>

*Positive control of OATP1B1: IC50 of rifampin against 0.1 μM rosvuastatin was 2.0 ± 0.6 μM.

*Positive control of OATP1B3: IC50 of rifampin against 0.1 μM rosvuastatin was 1.4 ± 0.1 μM.

*Positive control of OATP2B1: IC50 of rifampin against 0.1 μM rosvuastatin was 23.9 ± 3.2 μM.

*Positive control of OATP1B1: IC50 of ketoconazole against 0.1 μM rosvuastatin was 28.4 ± 5.7 μM.

*Positive control of OATP1B3: IC50 of ketoconazole against 0.1 μM rosvuastatin was 28.4 ± 5.7 μM.

*Positive control of OATP2B1: IC50 of ketoconazole against 0.1 μM rosvuastatin was 28.4 ± 5.7 μM.
of reducing metabolism is in contrast to compounds like midazolam whose elimination is not limited by uptake into an organ of elimination, and whose systemic concentration would reflect the liver concentration. Therefore, for compounds whose clearance is dependent on uptake into eliminating organs, drug interaction involving drug transporters and drug-metabolizing enzymes may manifest differently in the systemic circulation than in organs and tissues.

Overall, Figure 2 shows that the elimination of atorvastatin was rapid, as evidenced by concentrations falling below the limit of quantitation after 4 hours postdose for most groups of animals, which was consistent with previous observations (DeGorter et al., 2012). Following concomitant administration of rifampin or ketoconazole, Tables 1 and 2 showed that these inhibitors produced differential effects on the blood and liver concentrations of atorvastatin in the wild-type animals. Specifically, rifampin had a greater effect on atorvastatin systemic exposure than ketoconazole. In contrast, ketoconazole had a greater effect on the liver levels than with rifampin, as hypothesized. The divergent effect in the blood and the liver is apparent in the Kp ratio, as shown in Figure 3. In the vehicle-treated wild-type group, Kp ratio was greater than unity at 3.3, confirming that atorvastatin requires active uptake into the hepatocytes. This value is within range of what was observed previously (Chen et al., 2007; DeGorter et al., 2012; Higgins et al., 2014). Kp values declined to 0.33 with rifampin, further supporting the claim that uptake is required for atorvastatin. Meanwhile, ketoconazole augmented the Kp value to 34, which corresponds to the large accumulation of atorvastatin observed in the liver.

Although chemical inhibitors are valuable tools to investigate the function of a specific enzyme or transporter, data interpretation and analysis can be confounded by the possibility of cross-reactivity due to nonspecificity. Table 3 shows that, whereas rifampin was at least 10-fold more potent than ketoconazole against OATP1B1 and OATP1B3, higher concentrations of ketoconazole blocked the uptake of atorvastatin. To further differentiate contributions from uptake and metabolism for atorvastatin disposition, Cyp3a and Oatp1a/b knockout animals were used. As observed in the ketoconazole-treated wild-type animals, Cyp3a knockout group exhibited extensive changes only in the liver. Rifampin increased the blood exposure, but ketoconazole did not produce additional effects. As illustrated in Figure 1C, these data suggest that there is a substantial drug interaction when opposing atorvastatin metabolism, but this is only manifested in the liver and may not be captured if monitoring changes in the systemic concentration. Therefore, there may be disconnects when trying to relate exposure to instances of hepatotoxicity, because defining a pharmacokinetic-toxicity relationship may not be obvious when using plasma concentrations. It should be noted that the translatability of the aforementioned drug interaction in the preclinical models to the clinic is unknown, especially because the metabolism profile may be different between mice and humans (Black et al., 1998). Moreover, the implication of such drug interaction in the liver may seem inconsequential because the frequency of reported hepatotoxicity with atorvastatin is rare considering its prevalent use. However, this may be attributed to the fact that atorvastatin is already contraindicated with strong CYP3A4 inhibitors (LIPITOR, 2012); and indeed, hepatotoxicity was observed when atorvastatin was administered with CYP3A4 inhibitor diltiazam (Lewin et al., 2002; Liu et al., 2010). In addition, data presented in this study provide the first set of evidence on the potential risk of hepatotoxicity when blocking metabolism, despite only small changes in the plasma compartment. Nonetheless, experiments in humanized CYP3A animal models to monitor for both atorvastatin and its metabolites are planned to further investigate CYP3A-atorvastatin drug interaction.

There are differing reports with atorvastatin disposition in Oatp1a/b knockout animals. Following i.v. administration, one study saw no alterations of atorvastatin plasma levels (DeGorter et al., 2012), whereas another showed 17-fold increase in blood AUC (Higgins et al., 2014). These discrepancies may be attributed to differences in study design such as dose and sex/strain differences. Data presented in this work are more aligned with DeGorter et al. (2012), because no differences were observed between wild-type and Oatp1a/b knockout animals. It is not clear why there are discrepancies between various laboratories, but it is noteworthy to mention that, in addition to atorvastatin, data in Higgins et al. (2014) were different for simvastatin (DeGorter et al., 2012) and also for pravastatin in the FVB mice (Iusuf et al., 2012). Interestingly, coadministration of rifampin in the Oatp1a/b knockouts generated an additional increase in atorvastatin concentration, as illustrated in Figure 1B, suggesting that removing Oatp1a/b may be necessary but not sufficient to prevent atorvastatin uptake. Although atorvastatin is also a good substrate for OATP2B1 (Grube et al., 2006), Table 3 shows that rifampin is unlikely to impact Oatp2b1 activity, suggesting that there may be additional rifampin-sensitive transporters in the mice that can modulate the uptake of atorvastatin into liver. These data with atorvastatin show that, unlike the Cyp3a knockout models, the Oatp1a/b knockout models were not able to mimic the observed clinical interaction. These results demonstrate the need to further characterize and validate the drug transporter mouse models. In particular, for well-studied drugs such as simvastatin, which is not a substrate for Oatp2b1 (Grube et al., 2006), the Oatp1a/b knockout or the humanized OATP1B1/OATP1B3 models may be useful tools to understand human disposition and to assess clinical drug interactions. However, the utility of animal models focusing on a specific transporter may be limited for compounds whose distribution depends on multiple transporters such as with atorvastatin.

In summary, atorvastatin concentrations in the blood and liver were investigated when uptake mediated by OATP or metabolism catalyzed by CYP3A was blocked. Both chemical inhibitors and knockout animal models were used to better understand the disposition and drug interaction liability of atorvastatin. This is one of the first studies demonstrating that there are differential effects in the blood and liver concentration of atorvastatin, depending on which pathway is obstructed. Specifically, blood levels were most affected when the uptake pathway was restrained, whereas the liver levels were most affected when the metabolism pathway was reduced. These data indicate that important drug interaction may transpire in tissues and organs that may not necessarily be described by the systemic circulation.

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