Involvement of Intestinal Uptake Transporters in the Absorption of Azithromycin and Clarithromycin in the Rat

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Abstract:

Macrolide antibiotics azithromycin (AZI) and clarithromycin (CLARI) are large molecular weight compounds and are substrates for apically polarized efflux transporters such as P-glycoprotein, which can potentially restrict intestinal absorption. However, despite these undesired physicochemical and biopharmaceutical properties, AZI and CLARI exhibit moderate to excellent p.o. bioavailability in preclinical species and humans. Intestinal uptake transporters, such as organic anion transporting polypeptides (OATPs), can facilitate the uptake of drugs that are substrates and hence increase p.o. absorption. The present study was designed to determine whether the intestinal Oatps are involved in absorption of these macrolides. AZI or CLARI was dosed p.o. to Sprague-Dawley rats after p.o. administration with vehicle or rifamycin SV (RIF), an OATP inhibitor. The p.o. exposures of AZI and CLARI were reduced 65 and 45%, respectively, when coadministered with an optimized RIF regimen. The p.o. RIF had no affect on the total blood clearance of these macrolides and most likely did not cause induction of metabolizing enzymes and/or transporters. Therefore, the results suggest that inhibition of an RIF-sensitive uptake transporter such as Oatp along the rat gastrointestinal tract was responsible for reduced p.o. exposure of AZI and CLARI. In addition, AZI and CLARI caused inhibition of taurocholate uptake in rat Oatp1a5-transfected Madin-Darby canine kidney cell monolayers. The in vitro and in vivo results suggest that the intestinal Oatps are involved in the p.o. absorption of AZI and CLARI in the rat.

Macrolide antibiotics azithromycin (AZI) and clarithromycin (CLARI) are predicted by Lipinski’s “rule of 5” (Lipinski et al., 1997) to have poor permeation or absorption because of their large molecular weight and hydrogen bonding potential; however, these macrolides show moderate to excellent p.o. exposure in preclinical species and humans. For example, AZI exhibits 46, 97, and 37% p.o. bioavailability in rats, dogs (Shepard and Faulkner, 1990), and humans (Foulds et al., 1990), respectively, even though AZI does not conform to Lipinski rules with its molecular mass of 749 Da, 5 H-bond donors, and 14 H-bond acceptors (Lipinski et al., 1997). Lipinski et al. (1997) suggest that some therapeutic classes such as antibiotics contain orally active drugs because they are substrates for naturally occurring transporters. Both macrolides have been reported to be substrates for the apically polarized efflux transporter P-glycoprotein (P-gp), which can restrict intestinal permeation (Pachot et al., 2003; Sugie et al., 2004). The biliary and intestinal excretion of AZI in the rat has been reported to be mediated by P-gp and multidrug resistance-associated protein 2 (Sugie et al., 2004). CLARI exhibit moderate to excellent p.o. bioavailability in preclinical species and humans. Intestinal uptake transporters, such as organic anion transporting polypeptides (OATPs), can facilitate the uptake of drugs that are substrates and hence increase p.o. absorption. The present study was designed to determine whether the intestinal Oatps are involved in absorption of these macrolides. AZI or CLARI was dosed p.o. to Sprague-Dawley rats after p.o. administration with vehicle or rifamycin SV (RIF), an OATP inhibitor. The p.o. exposures of AZI and CLARI were reduced 65 and 45%, respectively, when coadministered with an optimized RIF regimen. The p.o. RIF had no affect on the total blood clearance of these macrolides and most likely did not cause induction of metabolizing enzymes and/or transporters. Therefore, the results suggest that inhibition of an RIF-sensitive uptake transporter such as Oatp along the rat gastrointestinal tract was responsible for reduced p.o. exposure of AZI and CLARI. In addition, AZI and CLARI caused inhibition of taurocholate uptake in rat Oatp1a5-transfected Madin-Darby canine kidney cell monolayers. The in vitro and in vivo results suggest that the intestinal Oatps are involved in the p.o. absorption of AZI and CLARI in the rat.
given its restricted substrate specificity (Hagenbuch and Meier, 2004); however, OATP2B1 is also believed to play a role in hepatic uptake of the statins (Poirier et al., 2007). To date, drug interactions with these OATPs have been primarily described at the hepatic level because of the potential for OATP inhibition to alter systemic and local concentrations of drugs, leading to altered pharmacokinetics and potential toxicity (Poirier et al., 2007). OATP1A2 (or OATP-A) also has relatively broad tissue distribution in humans, and although its distribution has been debated, it is believed to be expressed in the intestine (Kim, 2003) where it probably facilitates the intestinal uptake of structurally diverse drugs (Bailey et al., 2007). Recent studies have shown a clinical drug interaction between the flavonoid naringin and the antihistamine fexofenadine (an OATP1A2 substrate) that resulted in decreased fexofenadine p.o. bioavailability as an apparent result of OATP1A2 inhibition by naringin (Bailey et al., 2007; Glaeser et al., 2007). However, p.o. bioavailability modulated by substrate activity for intestinal OATPs has not been extensively characterized, presumably because of the emerging state of the OATP transporter field.

The present study was designed to determine whether the p.o. absorption of the macrolides AZI and CLARI are mediated by intestinal Oats in the rat. AZI or CLARI was dosed p.o. to rats after p.o. administration with vehicle or rifamycin SV (RIF), an OATP inhibitor (Fattinger et al., 2000; Vavricka et al., 2002). Previous studies conducted by our group showed reduction of the p.o. area under the absorption-concentration time curve (AUC) for fexofenadine [P-gp, OATP transporter field.]

Materials and Methods

Materials. AZI was purchased from Pliva Croatia Ltd. (Zagreb, Croatia), and CLARI was purchased from Apin Chemicals, Ltd. (Abingdon, Oxon, UK). Fexofenadine hydrochloride, RIF, sodium butyrate, and unlabeled taurocholate were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Taurocholate (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences and were purchased from Sigma-Aldrich (St. Louis, MO).

Animals. Male Sprague-Dawley rats (325–425 g) were purchased from Charles River Laboratories (Raleigh, NC) and were cannulated with Tygon tubing (Norton Performance Plastics, Akron, OH) femoral artery and vein catheters at least 2 days before study initiation. Animals were tethered, placed in an automated blood sampling system (Culex, Bioanalytical Systems Incorporated, West Lafayette, IN), and acclimated for a minimum of 24 h before dose administration. Catheter patency was maintained with regular flushing intervals with 20 IU/ml heparin derived from porcine intestinal mucosa (Abraxis Pharmaceutical Products, Schaumberg, IL) throughout the study and maintained under standard conditions with a 12-h light/dark cycle. All the animal protocols were approved by GlaxoSmithKline Animal Care and Use Committee and were conducted in accordance with International Animal Care and Use Committee policy.

Oral Administration of AZI or CLARI to Rats in the Absence and Presence of p.o. RIF. All of the animals were fasted overnight, and food (five pellets PMI-certified no. 5002 rodent diet; Purina Mills Incorporated, St. Louis, MO) was provided 4 h after dosing. AZI or CLARI was administered by p.o. gavage at 25 mg/kg, 16 ml/kg in a formulation composed of 1% (v/v) DMSO and 20% (w/v) hydroxypropyl β-cycloextrin in water; the selected dosage was equivalent or within 2 times the typical clinical dose for these macrolides. RIF was selected as the model intestinal Oatp inhibitor because it undergoes substantial first-pass metabolism (Fattinger et al., 2000); therefore, the effects of RIF should be seen at the intestinal level and much less in the liver. In these studies, RIF was administered by p.o. gavage at 88 mg/kg, 2.9 ml/kg per dose in 20% (w/v) hydroxypropyl β-cycloextrin in water under regimen 1 or 2 as six or eight doses, respectively (Fig. 1); repeat p.o. administration of RIF was selected to maintain RIF levels in the intestine during the macrolide absorptive phase.

Blood samples (75 µl) were collected from the femoral artery catheter into heparinized glass vials on the Culex fraction collector at 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 960, and 1440 min postdose and maintained at 8°C. Blood (25 ml) was hemolyzed with the addition of water (25 µl) and stored at approximately −80°C until subsequent liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis.

Intravenous Infusion of AZI or CLARI to Rats. The intravenous pharmacokinetics of AZI or CLARI was determined in the absence and presence of p.o. RIF regimen 2 (Fig. 1). This study was conducted as a crossover pharmacokinetic study, with the macrolide alone being dosed in the first session. After 144 h, the same rats were then dosed with macrolide in the presence of p.o. RIF regimen 2. The purpose of this study was to determine whether the blood clearance (CLb) of AZI or CLARI was affected by the presence of p.o. RIF. AZI or CLARI was administered via I-h infusion into the femoral vein of fed male Sprague-Dawley rats at a dose of 5 mg/kg, 4 ml/kg in 1% (v/v) DMSO and 20% (w/v) hydroxypropyl β-cycloextrin in saline. Blood samples were collected from the femoral artery at 20, 40, 60, 65, 75, 90, 120, 180, 240, 360, 480, 720, 960, 1440 min after initiation of the intravenous infusion. Samples were processed and stored as described above until concentration determination by LC/MS/MS analysis. Point estimates were used to determine whether p.o. administration of RIF regimen 2 resulted in CLb changes for AZI or CLARI. The point estimates were determined as follows: (CLb of macrolide + p.o. RIF regimen 2) divided by (CLb of macrolide alone).
Preparation of Blood Samples for LC/MS/MS Analysis. On the day of analysis, rat blood samples were thawed at room temperature, and fresh analytical standards were prepared and added to a 96-well plate. Analytical standards were prepared by back-spiking a known amount of drug into internal standard solution and adding 50:50% (v/v) heparinized rat blood/water keeping the same ratio as the samples. Tests were performed to ensure that backspiking standards and spiking standards directly into blood yielded the same response on the mass spectrometer. Proteins in the rat blood samples were precipitated with a 95:5% (v/v) mixture of acetonitrile/10 mM ammonium formate, pH 3.0, solution containing an internal standard. Samples and standards were mixed and centrifuged at 2250 relative centrifugal force for about 15 min. The LC autosampler syringe removed an aliquot (5–10 µl) from the supernatant for injection into the mass spectrometer.

LC/MS/MS Analysis of Rat Blood Samples. AZI, CLARI, and RIF were analyzed using atmospheric pressure chemical ionization or Turbo IonSpray ionization on an Applied Biosystems (Foster City, CA) triple quadrupole API 4000 mass spectrometer. Multiple reaction monitoring was used in positive-ion mode for AZI and CLARI and negative-ion mode for RIF. The precursor and product ions AZI, CLARI, and RIF were 749.5→591.5, 747.9→158.2, and 696.4→271.9, respectively. Sample was delivered to the mass spectrometer using a mobile phase composed of acetonitrile (with or without 0.05% formic acid) and either 10 mM ammonium formate, pH 3.0, or 0.05% formic acid in deionized water with a flow rate ranging from 0.45 to 1.25 ml/min under either isocratic or gradient conditions without any postcolumn split. Three different high-performance liquid chromatography systems were used to analyze different batches of samples, specifically a two-column Cohesive Aria TX2 turbulent flow system (Thermo Fisher Scientific, Waltham, MA), a single-column Cohesive Aria LX2 laminar flow system (Thermo Fisher Scientific), and a single-column Flux Instruments Rheos 2000 quaternary pumping system with degasser coupled with a CTC Analytics HTS PAL autosampler (Leap Technologies, Carrboro, NC). Chromatographic separation was achieved on the Phenomenex (Torrance, CA) Synergi Polar-RP (4 µm, 50 × 2.0 and 20 × 4.0 mm) column, the Phenomenex Synergi Gemini C18 (5 µm, 50 × 2.00 mm), and the Phenomenex Onyx Monolithic (50 × 4.6 and 25 × 4.6 mm) column. Peaks consisted of at least 10 data points with a minimal signal/noise ratio of 5:1. Standard curve ranges were linear over a range of 3 to 4 magnitudes with lower limits of quantification at 1 ng/ml or higher, depending on the expected concentration range of experimental samples.

Pharmacokinetic Analysis. AZI, CLARI, or RIF blood concentration-time data were analyzed by noncompartmental methods using the computer program WinNonLin Professional (version 4.1; Pharsight, Mountain View, CA). AUC was calculated using the linear trapezoidal rule for each incremental concentration range of experimental samples.

Cellular Uptake Assays in Stably Transfected MDCK Cells. Stable transfection and cloning of MDCK cells expressing rat Oatp1a5 (encodes Oatp3) have been described previously (Walters et al., 2000). MDCK-rat Oatp1a5 cells were maintained in culture with media composed of Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 350 µg/ml G418 (medium A). For the inhibition of [3H]taurocholate (Oatp substrate) uptake studies, MDCK or MDCK-rat Oatp1a5 cells were seeded onto 24-well plates at 1.4 × 10^5 cells/well in medium A. On day 2, the cells were refed the same media containing 10 mM sodium butyrate. On day 3, the cells were washed with prewarmed PBS and incubated in duplicate with Dulbecco’s modified Eagle’s medium containing 1 µM [3H]taurocholate and vehicle or the 100 µM concentration of cold taurocholate, fexofenadine, AZI, CLARI, or RIF. After 10 min at 37°C, the solution was removed, and wells were washed three times with ice-cold PBS and harvested to determine cell-associated radioactivity and protein as described previously (Walters et al., 2000).

Results

Macrolide p.o. Pharmacokinetics in the Absence and Presence of p.o. Rifamycin, an OATP/Oatp Inhibitor. The p.o. exposure of AZI or CLARI was determined in the rat in the presence or absence of one of two different regimens of p.o. RIF (Fig. 1), an OATP/Oatp inhibitor. The blood-concentration profiles for each of these macrolides in the absence and presence of RIF are displayed in Fig. 2. The p.o. pharmacokinetic parameters estimated for AZI or CLARI in the absence and presence of RIF regimen 1 or 2 are reported in Table 1 and Fig. 3.

For AZI, concomitant dosing of RIF regimen 2 reduced both the AUC and C_{max} of AZI more than that of RIF regimen 1 compared with AZI alone (Table 1). In addition, AZI T_{max} was unchanged in the presence of RIF regimen 1, and an apparent shift in T_{max} from 2.0 to 3.6 h was observed for AZI in the presence of RIF regimen 2 (Table 1); however, one of the rats in the study with RIF regimen 2 had a
The p.o. pharmacokinetic parameters for AZI or CLARI (25 mg/kg target dose) determined in the absence and presence of p.o. RIF (Oatp inhibitor) in the rat

AZI and CLARI pharmacokinetic parameters were estimated from blood-concentration data in Fig. 3. Each value represents the mean ± S.D. for the number of rats indicated next to each group.

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<th>C&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-3&lt;/sub&gt; (µg·h/ml)</th>
<th>AUC&lt;sub&gt;0-24&lt;/sub&gt; (µg·h/ml)</th>
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<tr>
<td>AZI (n = 16)</td>
<td>0.71 ± 0.22</td>
<td>2.0</td>
<td>1.00 ± 0.38</td>
<td>6.37 ± 1.92</td>
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<td>AZI + RIF regimen 1 (n = 8)</td>
<td>0.52 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9</td>
<td>0.74 ± 0.29</td>
<td>3.83 ± 1.48</td>
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<tr>
<td>AZI + RIF regimen 2 (n = 4)</td>
<td>0.14 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6</td>
<td>0.22 ± 0.16</td>
<td>1.99 ± 1.82</td>
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<tr>
<td>CLARI (n = 4)</td>
<td>1.70 ± 0.40</td>
<td>1.0</td>
<td>3.56 ± 0.61</td>
<td>6.48 ± 0.65</td>
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<tr>
<td>CLARI + RIF regimen 1 (n = 4)</td>
<td>0.70 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2</td>
<td>1.56 ± 0.51</td>
<td>3.03 ± 1.10</td>
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<tr>
<td>CLARI + RIF regimen 2 (n = 3)</td>
<td>1.12 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3</td>
<td>2.48 ± 1.04</td>
<td>5.28 ± 3.58</td>
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<sup>a</sup> ANOVA between AZI and CLARI group dose-normalized C<sub>max</sub> showed statistical significance (p < 0.009).

<sup>b</sup> Comparison of dose-normalized C<sub>max</sub> between RIF regimens 1 or 2 to AZI or CLARI alone by t test: two-sample assuming unequal variances showed statistical significance (p < 0.05).

TABLE 1

The p.o. pharmacokinetic parameters for AZI or CLARI (25 mg/kg target dose) determined in the absence and presence of p.o. RIF (Oatp inhibitor) in the rat

The p.o. pharmacokinetic parameters for AZI or CLARI (25 mg/kg target dose) determined in the absence and presence of p.o. RIF (Oatp inhibitor) in the rat

$T_{\text{max}}$ of 8.0 h, which was delayed relative to the other three rats ($T_{\text{max}}$ ~1.5–3.0 h); therefore, the median $T_{\text{max}}$ for AZI is probably unaffected to a substantial degree by RIF regimen 2. The dose-normalized p.o. exposure of AZI, as expressed as a DNAUC<sub>0-24</sub>, was significantly reduced by p.o. RIF regimen 1 and regimen 2 by 38% (p < 0.01, ANOVA) and 65% (p < 0.01, ANOVA), respectively, compared with that of AZI alone (Fig. 3). The more frequent RIF dosing with regimen 2 caused a greater reduction (65%) in the p.o. exposure of AZI than that from regimen 1.

CLARI p.o. exposure was also attenuated in the presence of p.o. RIF. Both RIF regimen 1 and 2 caused reduction in CLARI $C_{\text{max}}$ and AUC, relative to that of CLARI alone, whereas $T_{\text{max}}$ appeared the same (Table 1). CLARI p.o. exposure, as expressed as a DNAUC<sub>0-24</sub>, was significantly reduced by p.o. RIF regimen 1 and regimen 2 by 53% (p < 0.01) and 45% (p < 0.001), respectively, compared with that of CLARI alone (Fig. 3). Thus, RIF regimen 1 and regimen 2 showed similar (~50%) reductions in CLARI p.o. DNAUC<sub>0-24</sub> relative to that of CLARI alone.

In an attempt to understand any possible differences in RIF systemic exposure after dosing of RIF regimen 1 and RIF regimen 2, the p.o. exposure of each RIF regimen was measured with concomitant dosing of AZI. Overall, RIF p.o. exposure was increased approximately 2-fold for RIF regimen 2 (AUC<sub>0-3</sub> ~0.28 µg·h/ml, AUC<sub>0-24</sub> ~0.67 µg·h/ml) compared with that of RIF regimen 1 (AUC<sub>0-3</sub> ~0.12 µg·h/ml, AUC<sub>0-24</sub> ~0.27 µg·h/ml). Thus, the more frequent dosing of RIF in regimen 2 gave a higher RIF exposure compared with that obtained from regimen 1. This higher exposure could explain, in part, the greater reduction in AZI p.o. AUC by RIF regimen 2 compared with that of RIF regimen 1.

Because RIF was dosed via repeat p.o. administration with concomitant p.o. macrolide dosing, potential vehicle (no RIF) effects on macrolide p.o. exposure were tested by determining AZI p.o. exposure with concomitant vehicle dosing for regimen 2 relative to that of AZI alone. The results from this study show similar AZI p.o. DNAUC<sub>0-24</sub> when AZI was dosed by itself or in the presence of the vehicle for RIF regimen 2 (Fig. 4), suggesting that the formulation volume administered in these RIF regimens did not affect macrolide p.o. exposure in rats.

Investigation of Possible Systemic Effects on p.o. RIF Dosing in Rats. To rule out possible systemic effects of p.o. RIF on macrolide disposition, the intravenous pharmacokinetics of AZI or CLARI were determined in the presence of p.o. RIF in rats. In these studies, either AZI or CLARI was administered via intravenous infusion by itself or with concomitant p.o. RIF regimen 2, and the mean macrolide intravenous CB<sub>b</sub> and point estimates were determined (Table 2). The mean CB<sub>b</sub> of AZI (23.2 ml/min/kg) was similar to AZI clearance in the presence of p.o. RIF regimen 2 (21.9 ml/min/kg). In addition, the mean CB<sub>b</sub> of CLARI (81.6 ml/min/kg) was similar to CLARI clearance in the presence of p.o. RIF regimen 2 (76.0 ml/min/kg). The mean point estimates for AZI and CLARI were 0.96 and 0.94,
The mean point estimate was determined by averaging and taking the S.D. of each test group. A t test: two-sample assuming unequal variances showed no significant differences (p = 0.3) between AZI and AZI + vehicle.

**TABLE 2**

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<th>Point Estimates b</th>
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<tr>
<td>0.96 ± 0.25</td>
<td>22.3 ± 3.7</td>
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<tr>
<td>0.94 ± 0.07</td>
<td>21.9 ± 4.7</td>
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<td>0.76 ± 0.53</td>
<td>81.6 ± 10.8</td>
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* Each value represents the mean ± S.D. for the number of rats indicated next to each group.

respectively (Table 2), demonstrating that p.o. RIF regimen 2 had no substantial effect on AZI or CLARI CLb in rats.

### Investigation of Potential Time-Dependent Effects of p.o. RIF Administration on the Metabolism and/or Transport of AZI and CLARI in Rats

Additional studies were conducted to determine whether RIF induction of metabolizing enzymes and/or transporters in rats—because induction has been reported for the structurally related analog rifampicin (Fromm et al., 1996; Greiner et al., 1999; Hamman et al., 2001)—also caused similar to that previously observed (Figs. 2 and 3) in rats concomitantly dosed with RIF regimen 2. In the second dosing session, the blood concentrations and p.o. exposures of AZI and CLARI were decreased in a similar fashion to that previously observed (Figs. 2 and 3) in rats concomitantly dosed with RIF regimen 2. In the second dosing session, the blood concentrations and p.o. exposure of AZI and CLARI were increased (Fig. 5) with mean ± S.D. DNAUC 0–24 for AZI or CLARI of 0.22 ± 0.04 and 0.19 ± 0.06 μg · h/ml/mg/kg, respectively. These p.o. exposures for AZI and CLARI were very similar to those observed in previous studies where these macrolides were dosed alone to rats that were not pre-exposed to RIF (mean ± S.D. DNAUC 0–24 for AZI and CLARI was 0.22 ± 0.07 and 0.25 ± 0.03 μg · h/ml/mg/kg, respectively) (Fig. 3).

**In Vitro Uptake Inhibition Studies in MDCK Cells Transfected with Rat Intestinal Oatp1a5 (Encodes Oatp3).** The uptake of [3H]-taurocholate, an endogenous substrate for rodent intestinal Oat (Kim, 2003), was studied using Oatp1a5 (encodes Oatp3)-transfected MDCK cells in the presence of 100 μM cold taurocholate, RIF, fexofenadine, AZI, or CLARI. Inhibition of [3H]-taurocholate uptake in the presence of each compound was then determined as a percentage of the [3H]-taurocholate uptake in control cells (Fig. 6). [3H]-Taurocholate (5 μM) uptake in the cells was inhibited 60% by cold taurocholate (100 μM). RIF, the model Oatp inhibitor used in the in vivo rat studies, inhibited [3H]-taurocholate uptake by 86% (Fig. 6); this inhibition of taurocholate uptake by RIF was similar to that observed (~87%) for the well known and characterized OATP1A2/Oatp1a5 substrate and inhibitor fexofenadine. At the concentration tested, AZI and CLARI also caused substantial inhibition of [3H]-taurocholate uptake in Oatp1a5-MDCK cell monolayers with ~99 and 90% inhibition, respectively (Fig. 6).

### Discussion

Uptake and efflux drug transporters play a significant role in the absorption, distribution, metabolism, and excretion properties of xenobiotics, as they often work in concert with metabolizing enzymes to be the key determinants of p.o. bioavailability. Intestinal uptake transporters, such as OATPs, can facilitate the uptake of drugs that are substrates and hence increase p.o. absorption. Macrolide antibiotics AZI and CLARI exhibit undesirable physicochemical (e.g., large molecular weight) and biopharmaceutical (e.g., substrates for efflux transporter P-gp) properties, yet show moderate to excellent p.o. bioavailability in preclinical species and humans; therefore, the present study was designed to determine whether the p.o. absorption of AZI and CLARI is mediated by intestinal Oatps.

The p.o. exposure of AZI or CLARI was determined in the rat after concomitant p.o. administration of vehicle control or a regimen of RIF, an OATP/Oatp inhibitor (Fattinger et al., 2000; Vavricka et al., 2002). Despite the repeated p.o. administration of RIF in the rat under regimen 1 or 2, the maximal blood concentration of rifamycin determined after p.o. administration was only 0.4 μg/ml (1 μM). The low systemic blood concentration is consistent with reports of high first-
pass elimination (Fattinger et al., 2000), making RIF an ideal tool compound for preferential inhibition of intestinal Oatps in the rat.

Overall, treatment with p.o. RIF significantly decreased the p.o. absorption of AZI and CLARI in the rat, suggesting Oatp-mediated absorption of these macrolides along the gastrointestinal tract. The dose-normalized p.o. exposure of AZI was reduced 38 and 65% (Fig. 3) after p.o. administration of RIF regimens 1 and 2 (Fig. 1), respectively, compared with that of AZI alone in rats. The dose-normalized p.o. exposure of CLARI was reduced 53 and 45% (Fig. 3) after dosing with p.o. RIF regimens 1 and 2 (Fig. 1), respectively, compared with that of CLARI alone in rats. The increased dosing frequency of RIF regimen 2 had a pronounced effect on the absorption of AZI but not on CLARI, relative to that of RIF regimen 1. One possible explanation may be the higher absorptive intrinsic permeability of CLARI compared with that of AZI (data not shown), suggesting RIF can inhibit carrier-mediated absorption of CLARI but that more frequent RIF dosing does not further reduce the absorption of CLARI in vivo. However, drug-transporter interactions are quite complex, so the contribution of other factors such as inhibition and/or substrate affinity for other transporters makes it difficult to fully explain these observations because AZI, CLARI, and RIF are also substrates of P-gp (Pachot et al., 2003; Sugie et al., 2004) (internal report data not shown). One alternate hypothesis is that RIF is also a P-gp inhibitor. For example, the unchanged p.o. exposure for CLARI with more extensive RIF dosing with regimen 2 may not have further lowered CLARI exposure because of possible inhibition and/or saturation of P-gp, given higher levels of RIF along the gastrointestinal tract and the high intrinsic apparent permeability of CLARI. Therefore, it is possible that RIF-mediated inhibition of CLARI absorption by intestinal Oatp/OATP occurs but is countered somewhat by P-gp inhibition with higher doses of RIF. However, the local gastrointestinal concentrations and relative in vivo affinities to intestinal Oatp versus that of P-gp are not known for AZI or CLARI; therefore, further exploration of this hypothesis is warranted.

Additional rat pharmacokinetic studies were conducted to explore whether the attenuated exposure of these macrolides in the presence of p.o. RIF was caused by inhibition of intestinal uptake and not by other factors such as altered macrolide clearance, induction by RIF, or possible vehicle effects on repeat dosing. The intravenous pharmacokinetics of AZI and CLARI were determined in the absence or presence of RIF regimen 2 to the rat. The p.o. RIF had no effect on the CLb of AZI or CLARI in rats (Table 2), ruling out possible systemic effects such as alteration of hepatic macrolide clearance by RIF. Thus, it is unlikely that macrolide interaction with hepatic Oatp(s) is responsible for the reduction of macrolide systemic exposure with p.o. RIF administration in rats. In addition, the possibility of RIF induction of transporters and/or metabolizing enzymes was investigated by administering p.o. RIF regimen 2 with the macrolide in the first dosing session and then 48 h later in the same rats, administering the macrolide alone. The results from this rat crossover pharmacokinetic study with each of these macrolides showed similar, if not slightly higher, macrolide p.o. exposure in the second dosing session (Fig. 6), suggesting that p.o. RIF did not show any evidence of time-dependent effects on metabolism and/or transport of AZI or CLARI in rats. Finally, potential effects of the dosing vehicle were tested in rats because relatively large volumes of this vehicle were administered p.o., and it was uncertain whether such volume might affect the exposure of the macrolide. In this rat pharmacokinetic study, either AZI was administered alone or was dose with the regimen 2 vehicle control. Results from this study showed similar p.o. DNAUCs for AZI when either administered alone or with concomitant dosing of the vehicle control for regimen 2 in rats (Fig. 4), suggesting no vehicle effects on macrolide p.o. exposure. Taken together, the results from these in vivo pharmacokinetic studies show that an intestinal Oatp and/or other RIF-sensitive uptake transporter plays a significant role in the p.o. absorption of AZI and CLARI in the rat.

Although macrolide uptake by hepatic OATP1B1 and OATP1B3 has been shown (Seithel et al., 2007), the interaction of intestinal Oats has not been investigated until the present study. The major Oats expressed in the intestine include Oatp1a5 (originally called Oatp3) (Walters et al., 2000) and Oatp2b1 (Choudhuri et al., 2003). In rats, Oatp1a5 mRNA is expressed at low but fairly constant levels (approximately 0.04 pg/g of total RNA) down the length of the small intestine to the most distal ileum, which expresses lower levels of Oatp1a5 mRNA (Walters et al., 2000). As a first step toward identifying the specific intestinal transporters responsible for macrolide uptake, competition studies were conducted using an Oatp1a5 stably transfected MDCK cell line. The ability of unlabeled taurocholate, RFX, fexofenadine, AZI, or CLARI to inhibit rat Oatp1a5-mediated [3H]taurocholate uptake (Oatp1a5 substrate; Kim, 2003) was determined and reported as a percentage of control. Fexofenadine is a well documented Oatp1a5/OATP1A2 substrate (Cvetkovic et al., 1999; Shimizu et al., 2005; Kikuchi et al., 2006; Glaeser et al., 2007), and many laboratories in addition to ours have observed OATP/Oatp inhibition by RIF (Fattinger et al., 2000; Vavricka et al., 2002; Hagenbuch and Meier, 2004). Overall, at the concentration (100 μM) tested in this study, substantial (~90% or greater) inhibition of [3H]taurocholate uptake was seen for fexofenadine, RFX, AZI, and CLARI (Fig. 6).

Although the competition results are suggestive, additional direct transport studies will be required to determine whether the macrolides are Oatp1a5 substrates. As a result of gene duplication and divergence, significant species differences exist between the human and rodent Oatp/OATP superfamily of transporters (Kikuchi et al., 2006).
However, rat Oatp1a5 is generally believed to be an ortholog of human OATP1A2 based on a combination of amino acid identity, substrate similarity, and syntenic chromosomal localizations (Kim, 2003; Hagenbuch and Meier, 2004). Additional in vitro studies will be required to further characterize macrolide interaction with individual rodent and/or human intestinal Oatp/OATPs including Oatp1a5/ OATP1A2 and Oatp2b1/OATP2B1. It should also be noted that although suggestive, these results do not exclude the possibility that a RIF-sensitive transporter other than an Oatp/OATP is responsible for the intestinal uptake of these macrolides.

The majority of OATP drug-drug interaction studies have been evaluated at the hepatic level with statins because the statins are substrates for hepatic OATPs, their site of action is in the liver (Poirier et al., 2007), and these drugs are commonly prescribed. However, OATPs are also expressed in the intestine, and there is considerable debate in the literature regarding their potential contribution to solute uptake by the human intestine (Kim, 2003; Marzolini et al., 2004).

Drug-food interactions have been reported for the antihistamine fexofenadine and the flavonoid naringin (isolated from grapefruit juice), and the decreased fexofenadine p.o. bioavailability has been attributed to intestinal OATP1A2 inhibition by naringin (Bailey et al., 2007; Glaeser et al., 2007). Therefore, it is possible that similar drug-drug interactions exist in humans as a result of interactions with intestinal OATPs such as that described herein for rats.

In conclusion, these studies suggest that p.o. absorption of AZI and CLARI is mediated via facilitated uptake by Oatp and/or other RIF-sensitive intestinal transporter in rats. The interaction of macrolides with intestinal uptake transporters may explain how such large molecules achieve good p.o. bioavailability in preclinical species and humans.

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