Hepatic Basolateral Efflux Contributes Significantly to Rosuvastatin Disposition II: Characterization of Hepatic Elimination by Basolateral, Biliary, and Metabolic Clearance Pathways in Rat Isolated Perfused Liver

Nathan D. Pfeifer, Arlene S. Bridges, Brian C. Ferslew, Rhiannon N. Hardwick, and Kim L. R. Brouwer

Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, Chapel Hill, North Carolina (N.D.P., B.C.F., K.L.R.B.); and Department of Pathology (A.S.B.) and Curriculum in Toxicology (R.N.H., K.L.R.B.), School of Medicine, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Received July 30, 2013; accepted September 30, 2013

ABSTRACT

Basolateral efflux clearance (CL_{BL}) contributes significantly to rosuvastatin (RSV) elimination in sandwich-cultured hepatocytes (SCH). The contribution of CL_{BL} to RSV hepatic elimination was determined in single-pass isolated perfused livers (IPLs) from wild-type (WT) and multidrug resistance-associated protein 2 (Mrp2)-deficient (TR^{-}) rats in the absence and presence of the P-glycoprotein and breast cancer resistance protein (Bcrp) inhibitor, elacridar (GF120918); clearance values were compared with SCH. RSV biliary clearance (CL_{Bile}) was ablated almost completely by GF120918 in TR^{-} IPLs, confirming that Mrp2 and Bcrp primarily are responsible for RSV CL_{Bile} . RSV appearance in outflow perfusate was attributed primarily to CL_{BL} , which was impaired in TR^{-} IPLs. CL_{BL} was ~6-fold greater than CL_{Bile} in the linear range in WT IPLs in the absence of GF120918. Recovery of unchanged RSV in liver tissue

increased in TR^{-} compared with WT (~25 versus 6% of the administered dose) due to impaired CL_{BL} and CL_{Bile} . RSV pentanoic acid, identified by high-resolution liquid chromatography-tandem mass spectroscopy, comprised ~40% of total liver content and ~16% of the administered dose in TR^{-} livers at the end of perfusion, compared with ~30 and 3% in WT livers, consistent with impaired RSV excretion and “shunting” to the metabolic pathway. In vitro-ex vivo extrapolation between WT SCH and IPLs (without GF120918) revealed that uptake clearance and CL_{BL} were 4.2- and 6.4-fold lower, respectively, in rat SCH compared with IPLs; CL_{Bile} translated almost directly (1.1-fold). The present IPL data confirmed the significant role of CL_{BL} in RSV hepatic elimination, and demonstrated that both CL_{BL} and CL_{Bile} influence RSV hepatic and systemic exposure.

Introduction

The role of hepatic transport in the pharmacokinetics and pharmacodynamics of rosuvastatin (RSV) has long been

This research was supported by the National Institutes of Health National Institute of General Medical Sciences [Grant R01 GM41935] (to K.L.R.B.); and National Institutes of Health National Institute of Environmental Health Sciences [Grant T32 ES007126] (training grant to R.N.H.). This work is based upon research supported in whole or in part by the North Carolina Biotechnology Center [Institutional Development Grant 2012-IDG-1008] (to the UNC Eshelman School of Pharmacy). N.D.P. was supported, in part, by the University of North Carolina Royster Society of Fellows.

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dx.doi.org/10.1124/jpet.113.208314.

recognized (Nezasa et al., 2002; Ho et al., 2006; Zhang et al., 2006; Kitamura et al., 2008). Mechanisms mediating hepatic uptake [organic anion transporting polypeptides (OATPs), sodium-taurocholate cotransporting polypeptide (NTCP)], and biliary excretion [multidrug resistance-associated protein (MRP)2, breast cancer resistance protein (BCRP)] have been well characterized (Ho et al., 2006; Huang et al., 2006; Zhang et al., 2006; Kitamura et al., 2008; Keskitalo et al., 2009; Hobbs et al., 2012). Recently, a novel uptake/efflux protocol in sandwich-cultured hepatocytes (SCH) was used to show that basolateral efflux represents a significant elimination route from rat and human hepatocytes (Pfeifer et al. 2013). Of the candidate transport proteins known to mediate hepatic basolateral efflux of drugs and metabolites, RSV was shown to be a substrate of human MRP4 (Pfeifer et al. 2013), which likely contributes to the basolateral efflux of RSV in human liver.

ABBREVIATIONS: APAP-GSH, acetaminophen-glutathione; BCRP, breast cancer resistance protein; CL, clearance; CL_{BL} , basolateral efflux clearance; CL_{Bile} , biliary clearance; CL_{Met} , metabolic clearance of RSV to RSV-PA; CL_{Other} , clearance of RSV to entities other than RSV-PA; DDI, drug-drug interaction; EHBR, Eisai hyperbilirubinemic rats; f_e , fraction excreted; IPL, isolated perfused liver; LC-MS/MS, liquid chromatography-tandem mass spectroscopy; MRP, multidrug resistance-associated protein; OATP, organic anion transporting polypeptide; RSV, rosuvastatin; RSV-PA, pentanoic acid metabolite of RSV; d_6 -RSV, deuterated RSV; SCH, sandwich-cultured hepatocytes; TOF, time-of-flight; TR^{-} , Mrp2-deficient; WT, wild type.

The importance of hepatic basolateral efflux in drug disposition remains largely unrecognized except in the case of hepatically derived drug conjugates (Zamek-Gliszczynski et al., 2006; Hardwick et al., 2012). Other notable exceptions include fexofenadine (Tian et al., 2008), enalaprilat (de Lannoy et al., 1993), and methotrexate (Vlaming et al., 2009). As such, availability of tools and information for prediction and in vitro–in vivo extrapolation of hepatic basolateral efflux lags behind the development of these tools for more-recognized pathways, such as hepatic uptake and biliary excretion. Although isolated expression systems have been used for some proteins known to facilitate hepatic basolateral efflux, such as Mrp3/MRP3 and Mrp4/MRP4 (Hirohashi et al., 1999; Akita et al., 2002; Chen et al., 2002), other translational tools, such as quantitative proteomics data and identification of specific substrates/inhibitors, are extremely limited. Therefore, it is important to assess the basolateral efflux of RSV and other drugs in whole liver to ascertain the potential role of this pathway in vivo, and the predictive capability of the aforementioned SCH method and other in vitro systems that may be developed.

Following oral administration of RSV to wild-type (WT) Sprague-Dawley and Mrp2-deficient Eisai hyperbilirubinemic rats (EHBR), biliary clearance (liver-to-bile) was decreased without a significant increase in RSV liver concentrations, whereas systemic exposure was increased more than 3-fold (Kitamura et al., 2008). Although this may suggest efficient hepatic basolateral efflux of RSV in the setting of impaired biliary excretion, decreased hepatic uptake and/or impaired renal elimination in EHBR rats may contribute to the increased systemic exposure. An important advantage of the isolated perfused liver (IPL) model is that the role of hepatic processes can be evaluated in isolation from other organ systems (Brouwer and Thurman, 1996). RSV disposition was reported recently in recirculating isolated perfused livers from WT and Mrp2-deficient (TR^{-}) rats (Hobbs et al., 2012). Increased perfusate concentrations in TR^{-} compared with WT livers were attributed to decreased hepatic uptake; however, the role of basolateral excretion was not considered. The single-pass IPL system used in the present study allowed for direct evaluation of basolateral excretion from liver to perfusate.

RSV is not metabolized extensively in humans; identified metabolites include the inactive 5*S*-lactone, as well as *N*-desmethyl-RSV, which is formed by cytochrome 2C9 (CYP2C9) and retains up to 50% of the 3-hydroxy-3-methylglutaryl-CoA-reductase inhibitor activity of RSV (Martin et al., 2003b). Although metabolism plays a similarly minor role in rats in terms of overall mass balance, metabolites have been reported to account for a significant portion (~50%) of plasma and liver content in whole animal studies (Nezasa et al., 2002; Kitamura et al., 2008). Biotransformation of RSV in rats is mediated primarily by β -oxidation of the fatty acid chain, with no evidence of cytochrome P450 involvement. The pentanoic acid derivative of RSV (RSV-PA) has been suggested as the primary metabolite based on thin-layer chromatography of extracted plasma and liver samples from rats administered [^{14}C]RSV, cospotted with an authentic standard of RSV-PA (Nezasa et al., 2002); structural identification and confirmation of the RSV-PA metabolite by mass-spectrometric analysis has not been reported.

Interplay between transporters and metabolizing enzymes has been recognized. This presents a challenge in predicting the impact of altered function on hepatic and/or systemic exposure of drugs when multiple elimination pathways are involved (Benet et al., 2004; Zamek-Gliszczynski et al., 2006; Parker and Houston, 2008). This is clinically relevant for RSV because impaired hepatic transport due to drug-drug interactions (DDIs) and genetic polymorphisms has been shown to alter the pharmacokinetics of RSV (Schneck et al., 2004; Simonson et al., 2004; Zhang et al., 2006; Kiser et al., 2008; Keskitalo et al., 2009). Some of these changes have been associated with altered efficacy (low-density lipoprotein lowering) of RSV (Simonson et al., 2003; Tomlinson et al., 2010), whereas increased systemic exposure has been associated with life-threatening rhabdomyolysis related to statin use in general (Hamilton-Craig, 2001; Thompson et al., 2003).

The present experiments were designed to quantify the contribution of the basolateral efflux pathway to the hepatocellular elimination of RSV in single-pass rat IPLs. In addition, clearance values generated using a novel uptake/efflux protocol that was developed in the SCH model were compared with this dataset generated in whole liver. IPL data, combined with pharmacokinetic modeling, revealed that basolateral efflux represents a significant route of RSV hepatocellular excretion from rat liver, similar to findings in SCH. In addition, reduced CL_{BL} and CL_{Bile} of RSV in TR^{-} livers highlighted the contribution of biotransformation as an alternative elimination pathway in the setting of impaired hepatic efflux in rat liver, with RSV-PA identified as the primary metabolite.

Materials and Methods

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. RSV and the deuterated RSV (d_6 -RSV) internal standard were purchased from Moravek Biochemicals (Brea, CA). GF120918 (elacridar) was a generous gift from GlaxoSmithKline (Research Triangle Park, NC).

Animals. Male Wistar wild-type (WT) rats (250–350 g) from Charles River Laboratories (Wilmington, MA) or male Mrp2-deficient (TR^{-}) rats bred at the University of North Carolina (250–350 g; breeding stock obtained from Dr. Mary Vore, University of Kentucky, Lexington, KY) were used as donors for isolated perfused liver studies. Rats were allowed water and food ad libitum and acclimated for a minimum of 1 week prior to experimentation. All animal procedures complied with the guidelines of the Institutional Animal Care and Use Committee (University of North Carolina, Chapel Hill, NC). All procedures were performed under full anesthesia with ketamine/xylazine (140/8 mg/kg i.p.).

Isolated Perfused Livers. WT and TR^{-} rat livers were perfused in a single-pass manner as described previously (Brouwer and Thurman, 1996; Chandra et al., 2005). In brief, following cannulation of the portal vein and bile duct, livers were perfused in situ with continuously oxygenated Krebs-Ringer bicarbonate buffer (35 ml/min) containing 5 μ M taurocholate to maintain bile flow. Livers were removed from the body cavity and placed in a humidified perfusion chamber heated to maintain liver temperature at 37°C. Perfusion was continued for a 15-minute equilibration period and then switched to a RSV-containing perfusate (0.5 μ M) for the 60-minute loading phase. At 60 minutes, the buffer was switched to RSV-free perfusate, and perfusion was continued for an additional 30 minutes. For conditions in the presence of inhibitor, the perfusate also contained 0.5 μ M GF120918 for the duration of the experiment (15-minute equilibration period followed by the 90-minute perfusion; Fig. 1). This

Equilibration Phase 15 min	Loading Phase + 0.5 μ M RSV, 60 min	Efflux Phase 30 min
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Fig. 1. Scheme depicting the experimental protocol in isolated perfused livers. Gray shading represents inclusion of rosuvastatin (RSV) in the Krebs-Ringer bicarbonate buffer during the uptake phase. For conditions in the presence of inhibitor, the perfusate also contained 0.5 μ M GF120918 for the duration of the experiment (15-minute equilibration phase followed by loading and efflux phases).

concentration was sufficient to inhibit RSV biliary excretion in TR⁻ SCH with minimal impact on uptake (Pfeifer et al. 2013). Liver viability was assessed by monitoring inflow perfusion pressure (<15 mm H₂O), gross morphology, and maintenance of bile flow

(within 30% of the baseline rate during the equilibration period). Bile and perfusate were collected over 5-minute intervals, and bile volume was determined gravimetrically in preweighed tubes. After perfusion, livers were blotted dry, weighed, and stored at -80°C until analysis.

Fig. 2. (A) Extracted ion current (XIC) chromatograph of rosuvastatin (RSV; red), rosuvastatin pentanoic acid (RSV-PA; purple), and d₆-RSV (internal standard; blue) in TR⁻ control liver tissue. (B) High-resolution product ion spectra of RSV (top, 482.20 ± 0.05 amu), d₆-RSV (middle, 488.20 ± 0.05 amu), and RSV-PA (bottom, 422.20 ± 0.05 amu). Note the expected $\Delta 6$ amu between the products of RSV and its internal standard (258.14/264.17, 300.15/306.19, 314.14/320.20, 378.12/384.16, 404.19/410.23, 422.15/428.19, and 446.15/452.19), as well as common product ions formed from RSV and RSV-PA (242.10, 256.12, and 270.17 amu), which are independent of the changes within the carboxylic acid side chain.

Fig. 3. Model scheme depicting the disposition of rosuvastatin (RSV) in rat IPL studies. Q denotes the flow rate of perfusate, X denotes mass of RSV, V denotes compartmental volume, and C denotes compartmental concentration. Subscripts with mass, flow, and concentration terms denote the corresponding compartments in the model scheme as follows: inflow perfusate (in), outflow perfusate (out), extracellular compartment (EC, assumed to be in rapid equilibration with the perfusate), intracellular liver compartment (L), and bile (Bile). Clearance values are designated as CL_{Uptake} for RSV uptake from the extracellular to intracellular liver compartment, CL_{BL} for RSV efflux from the intracellular to extracellular liver compartment, CL_{Met} for conversion from RSV to the pentanoic acid metabolite, and CL_{Other} for RSV conversion to metabolites other than the pentanoic acid derivative. V_{max} is the maximum velocity of RSV biliary excretion, K_m is the RSV concentration resulting in half-maximal biliary excretion, and f_u denotes unbound fraction.

Bioanalysis. RSV was quantified by liquid chromatography–tandem mass spectroscopy (MS/MS) as described previously (Abe et al., 2008). RSV-PA was identified by high resolution MS/MS (TOF/TOF) using an ABSciex 5600 TripleTOF mass spectrometer (Fig. 2). Direct absolute quantification of RSV-PA was not possible due to the lack of an analytical standard. However, estimating sample-to-sample differences in the relative concentrations of RSV-PA was possible by comparing normalized peak area ratios (RSV-PA: d_6 -RSV internal standard). The absolute concentration of RSV-PA was estimated by comparing normalized peak area ratios (RSV-PA: d_6 -RSV internal standard) to the calibration curve generated using the peak area ratios (RSV: d_6 -RSV internal standard) of samples with known concentrations of RSV, with the assumption that RSV and RSV-PA have similar ionization efficiencies.

Pharmacokinetic Modeling. Pharmacokinetic modeling and simulation were used to evaluate RSV disposition in rat IPLs and to determine the effects of GF120918 and loss of Mrp2 function on RSV hepatobiliary disposition. A model incorporating linear and nonlinear parameters governing RSV flux (Fig. 3) was fit to rate-versus-time data from individual experiments. The model fitting was performed with WinNonlin Phoenix, v6.1 (St. Louis, MO) using the stiff estimation method and a proportional model for residual error. The model scheme depicting the single-pass IPL system (Fig. 3) consisted of an extracellular (sinusoidal/perfusate) compartment, liver tissue, and a bile compartment, each divided into five subcompartments in the semi-physiologically based approximation of the dispersion model, as reported by Watanabe et al. (2009). The model was fit simultaneously to biliary excretion rate and appearance rate in outflow perfusate data, as well as terminal recovery of RSV and RSV-PA in liver tissue. Differential equations describing the model scheme in Fig. 3 are as follows:

Extracellular liver 1:

$$\frac{dX_{EC,1}}{dt} = Q \times C_{in} + \frac{CL_{BL}}{5} \times C_{u,L,1} - \frac{CL_{Uptake}}{5} \times C_{EC,1} - Q \times C_{EC,1}$$

Extracellular liver 2–5:

$$\frac{dX_{EC,n}}{dt} = Q \times C_{EC,n-1} + \frac{CL_{BL}}{5} \times C_{u,L,n} - \frac{CL_{Uptake}}{5} \times C_{EC,n} - Q \times C_{EC,n}$$

Intracellular liver 1–5:

$$\frac{dX_{L,n}}{dt} = \frac{CL_{Uptake}}{5} \times C_{EC,n} - \left[\left(\frac{CL_{BL}}{5} \right) + \left(\frac{CL_{Met}}{5} \right) + \left(\frac{CL_{Other}}{5} \right) \right] \times C_{u,L,n} - \frac{C_{u,L,n} \times (V_{max}/5)}{(K_m + C_{u,L,n})}$$

Bile 1–5:

$$\frac{dX_{Bile,n}}{dt} = \frac{C_{u,L,n} \times (V_{max}/5)}{(K_m + C_{u,L,n})}$$

where variables and parameters are defined as in Fig. 3, with further explanation as follows. $C_{EC,n}$ is the extracellular concentration, calculated as $X_{EC,n}/(V_{EC}/5)$, and V_{EC} is the extracellular volume of the liver, which was assumed to be in equilibration with the sinusoidal space and estimated at 20% of the total liver mass, as reported previously (Watanabe et al., 2009; Hobbs et al., 2012). Q is the perfusate flow rate of 35 ml/min, and C_{in} is the concentration of RSV in the inflow perfusate, measured for each preparation; binding of RSV to the perfusion tubing and apparatus was <10% and not considered further. $C_{u,L,n}$ is the unbound intracellular concentration of RSV in the liver based on binding of RSV to rat liver tissue, which was determined by equilibrium dialysis with an unbound fraction of 0.25, corrected for dilution. The total intracellular liver concentration was calculated as the sum of the mass in liver subcompartments 1–5, divided by the intracellular volume (V_L), calculated as total liver mass minus the V_{EC} . The RSV concentration resulting in half-maximal biliary excretion (K_m) was set to 10 μ M based on the reported affinity of RSV for Mrp2 and Bcrp in isolated expression systems (Huang et al., 2006; Deng et al., 2008). The $C_{EC,5} \times Q$ was fit to the observed appearance rate in outflow perfusate, while the sum of the excretion rate in bile ($dX_{Bile,n}/dt$) for liver subcompartments 1–5 was fit to the observed biliary excretion rate. The RSV and RSV-PA mass in liver subcompartments 1–5 was fit to observed recovery of RSV and RSV-PA in liver tissue at the end of the study. Initial parameter estimates were obtained from a combination of direct extrapolation of IPL data and simulations in Berkeley-Madonna. V_{max} was estimated initially as the steady-state excretion rate in bile, since the unbound liver concentrations were estimated from mass balance to be ~3–5 times the K_m for the biliary excretion process. Rapid attainment of steady-state in the outflow perfusate of WT IPLs precluded accurate estimation of the CL_{Uptake} . Therefore, CL_{Uptake} was fixed at 40 ml/min/g liver based on two independent reports of RSV initial uptake (determined at time points < 1 minute) in freshly isolated, suspended WT rat hepatocytes (Nezasa et al., 2003; Yabe et al., 2011), using standard conversion factors of 200 mg of protein/g of liver and 100 million cells/g liver (Swift et al., 2010). Metabolic clearance of RSV to the pentanoic acid derivative (CL_{Met}) and other potential metabolites (CL_{Other}) were estimated initially from simulations in Berkeley-Madonna.

Transporter-mediated clearance values [CL_{Uptake} , CL_{BL} , and CL_{Bile} (V_{max}/K_m in the linear range)] estimated from pharmacokinetic modeling of RSV IPL data were compared with analogous parameter values reported previously in rat SCH (Pfeifer et al. 2013); scaling factors were reported for the WT control conditions. Scaling factors represent the respective clearance value in the IPL divided by the corresponding value in SCH.

Data Analysis. All data are presented as mean $\pm$ S.D. of $n = 3$ livers in each treatment group. The effects of Mrp2 status (WT or TR⁻) and GF120918 on RSV disposition were determined independently by one-way ANOVA with Tukey's post-hoc test. The effect of

Mrp2 status was evaluated at each level of inhibitor (absent or present), and the effect of GF120918 was evaluated at each level of Mrp2 status (WT or TR⁻).

Results

Baseline bile flow in WT IPLs was $0.49 \pm 0.05 \mu\text{l/min/g}$ liver and $0.30 \pm 0.11 \mu\text{l/min/g}$ liver in the absence and presence of GF120918, respectively, with corresponding values in TR⁻ IPLs of 0.33 ± 0.04 and $0.25 \pm 0.07 \mu\text{l/min/g}$ liver, respectively. Outflow perfusate concentrations of RSV ranged from 0.003 to 0.23 μM in WT and TR⁻ IPLs. The rates of RSV appearance in bile and outflow perfusate are plotted in Fig. 4; recovery of RSV and RSV-PA at the end of the perfusion is summarized in Fig. 5. The biliary excretion rate of RSV was similar in WT livers in the absence and presence of GF120918 (Fig. 4A), whereas the addition of GF120918 in TR⁻ livers markedly reduced the biliary excretion rate of RSV (Fig. 4B). Interestingly, the initial appearance rate of RSV in the outflow perfusate was reduced in TR⁻ compared with WT livers (Fig. 4, C and D). Addition of GF120918 slightly reduced the rate of RSV appearance in outflow perfusate of WT livers (Fig. 4C) but had no effect on the rate of RSV appearance in outflow perfusate of TR⁻ livers (Fig. 4D). The cumulative recovery of RSV in perfusate over the 90-minute study tended to be reduced in TR⁻ compared with WT livers in the absence of GF120918 and also by GF120918 in WT livers (Fig. 5). However, the individual effects of Mrp2 status (WT

versus TR⁻) and GF120918 failed to reach significance after correcting for multiple comparisons. Biliary recovery of RSV was not affected significantly by GF120918 in WT IPLs, but the effect of GF120918 on the reduced biliary recovery in TR⁻ IPLs was statistically significant (Fig. 5). The effect of Mrp2 status on RSV biliary recovery was statistically significant in the presence of GF120918 but not in the absence of GF120918 (Fig. 5). Total recovery of the dosed RSV in perfusate, bile, and liver tissue at the end of the 90-minute studies was nearly complete following perfusion of WT IPLs ($96 \pm 9\%$ and $94 \pm 13\%$ in the absence and presence of GF120918, respectively, Fig. 5); however, it was reduced significantly in TR⁻ IPLs ($71 \pm 4\%$ and $56 \pm 7\%$ in the absence and presence of GF120918, respectively, Fig. 5). This reduction in total recovery of parent RSV in TR⁻ IPLs was offset, in part, by the presence of the pentanoic acid metabolite in TR⁻ liver tissue.

The presence of the pentanoic acid metabolite of RSV was confirmed by both targeted and untargeted high-resolution MS/MS. The targeted approach was based on a previous report suggesting that RSV-PA was the primary metabolite in rat (Nezasa et al., 2002). First, targeted extracted ion chromatograms (422.20 ± 0.05 amu) from full-scan TOF revealed this parent ion to be present only in samples from RSV-perfused livers (Fig. 2A). Second, comparisons between the high-resolution product ion spectra of RSV (482.20 amu) and the 422.20 amu parent ion further substantiated this unique analyte to be RSV-PA (Fig. 2B). By use of the proposed structure for the metabolite, there were common product ions

Fig. 4. Rosuvastatin (RSV) biliary excretion (A and B) and outflow perfusate (C and D) rate-versus-time data in isolated perfused livers from wild-type (WT; A and C) and Mrp2-deficient (TR⁻; B and D) rats in the absence (open symbols; dashed line) or presence (closed symbols; solid line) of GF120918. The simulated excretion rate-time profiles were generated from the relevant equations based on the model scheme in Fig. 3 and the final parameter estimates reported in Table 1. Gray arrow indicates the switch from RSV-containing to RSV-free perfusate at 60 minutes. Data are presented as mean $\pm$ S.D. ($n = 3$ livers).

Fig. 5. Recovery of rosvastatin (RSV; solid bars) and rosvastatin pentanoic acid (RSV-PA; open bars) in perfusate, liver, and bile, as well as total recovery from WT and TR⁻ isolated perfused livers in the absence and presence of GF120918. Data are reported as the mean percentage $\pm$ S.D. based on the total dose administered to each liver ($n = 3$ per group) recovered at the end of the 90-minute perfusion. For recovery of parent drug: * $P < 0.05$, adjusted; effect of Mrp2 status (WT versus TR⁻) is statistically significant within the same level of inhibitor (+ or -GF120918). † $P < 0.05$, adjusted; effect of GF120918 (absence versus presence) is statistically significant within the same level of Mrp2 status (WT or TR⁻). For recovery of the metabolite: ‡ $P < 0.05$, adjusted; effect of Mrp2 status (WT versus TR⁻) is statistically significant within the same level of inhibitor (+ or -GF120918).

formed (242.10, 256.12, and 270.17 amu; Fig. 2B), which were independent of the changes within the carboxylic acid side chain. Additionally, the paired product ions, 402.19/404.19 for RSV and 342.17/344.17 for RSV-PA, represent loss of $\text{CH}_4\text{O}_2\text{S}$ while maintaining the $\Delta 60$ amu ($\text{C}_2\text{H}_4\text{O}_2$) between RSV and RSV-PA. RSV-PA also was identified independently by analyzing the extracted homogenates from nontreated (blank) and RSV-treated TR⁻ IPLs in an untargeted, or unbiased, manner using the ABSciex PeakView and MetabolitePilot software packages programmed to detect and to suggest likely chemical structures of potential metabolic products formed from RSV. No other specific RSV metabolites were identified by MS/MS. Estimated concentrations of RSV-PA were low in perfusate and bile samples, representing $<2\%$ of the total dose over the course of the study. As such, RSV-PA was reported only in the liver tissue (Fig. 5). RSV-PA comprised approximately 40% of total RSV content (metabolite/parent ratio of 0.70 ± 0.24 and 0.70 ± 0.30 in the absence and presence of GF120918, respectively) in TR⁻ liver tissue, or 16% of the total administered dose of RSV at the end of the perfusion (Fig. 5). In contrast, while RSV-PA accounted for a similar proportion of total RSV content in WT liver tissue at the end of the perfusion ($\sim 30\%$; metabolite/parent ratio of 0.58 ± 0.28 and 0.50 ± 0.10 in the absence and presence of GF120918, respectively), this comprised only $\sim 3\%$ of the total administered dose of RSV (Fig. 5).

Parameter estimates recovered from fitting the differential equations based on the model scheme depicted in Fig. 3 to the data are listed in Table 1. The estimated maximum velocity ($V_{\max}$) values of the biliary excretion process and resulting biliary clearance of RSV ($\text{CL}_{\text{Bile}} = V_{\max}/[K_m + C]$) were reduced significantly in TR⁻ compared with WT livers in the absence and presence of GF120918. GF120918 reduced $V_{\max}$ significantly in TR⁻ but not WT livers. CL_{BL} was significantly decreased in TR⁻ compared with WT livers in the absence of

GF120918. GF120918 tended to reduce CL_{BL} in WT IPLs, with a minimal effect in TR⁻ livers, but these differences were not statistically significant. The CL_{Met} and CL_{Other} tended to be increased in TR⁻ compared with WT IPLs, and the presence of GF120918 also tended to increase the metabolic clearance, but these differences were not statistically significant.

Transporter-mediated clearance values recovered from the current IPL studies were compared with analogous values obtained in SCH studies reported previously (Pfeifer et al. 2013). Comparison of WT (control) IPL and SCH data resulted in empirical scaling factors of 4.2 for $\text{CL}_{\text{Uptake}}$ (40 ml/min/g liver in IPLs versus 9.5 ml/min/g liver in SCH), 6.4 for CL_{BL} (1.4 versus 0.21 ml/min/g liver in IPLs compared with SCH), and 1.1 for CL_{Bile} [0.26 ml/min/g liver (calculated as $V_{\max}/K_m$) in IPLs versus 0.23 ml/min/g liver in SCH].

Discussion

The present perfused liver studies confirm a significant role for basolateral efflux in the hepatobiliary disposition of RSV in rat hepatocytes, as recently demonstrated in rat SCH (Pfeifer et al. 2013). The effects of modulating Mrp2 and Bcrp function using TR⁻ rat livers and GF120918, respectively, were consistent with SCH data and previous reports suggesting that each transporter contributes to a similar degree and together comprise approximately 90 to 95% of RSV biliary excretion in rats (Table 1) (Kitamura et al., 2008; Hobbs et al., 2012; Pfeifer et al. 2013).

It is clear that differences exist in the handling of RSV by WT and TR⁻ livers beyond the expected decrease in biliary excretion rate resulting from loss of Mrp2 function (Figs. 4 and 5; Table 1). The biliary excretion rate appeared to reach steady state by ~ 30 minutes in both WT and TR⁻ IPLs; however, there was a delay in the attainment of steady-state appearance rate of RSV in the outflow perfusate during the loading phase of TR⁻ compared with WT IPLs (Fig. 4, C and D). Qualitatively, this supports saturation of the biliary excretion capacity in TR⁻ IPLs, which was represented by parameterizing CL_{Bile} as ($V_{\max}/[K_m + C]$) in the model structure (Figs. 3 and 4, B and D). Quantitatively, it is curious that the excretion rate in the outflow perfusate remains lower in TR⁻ compared with WT throughout the loading phase (Fig. 4, C and D), suggesting that the basolateral efflux of RSV may be impaired in TR⁻ livers. Rather, it would be expected that RSV excretion in the outflow perfusate would be greater in TR⁻ than WT livers due to increased hepatocellular concentrations driving the efflux process(es). Basolateral efflux is regarded commonly as a compensatory route of elimination to protect hepatocytes in the setting of cholestasis or otherwise impaired biliary excretion (Ogawa et al., 2000; Scheffer et al., 2002; Denk et al., 2004; Gradhand et al., 2008). Mrp3 exhibits increased expression in TR⁻ rat livers, and Mrp4 expression is increased following bile duct ligation, but remains unchanged in TR⁻ livers (Akita et al., 2001; Donner and Keppler, 2001; Chen et al., 2005; Johnson et al., 2006). Therefore, it would be expected that RSV CL_{BL} might be greater in TR⁻ livers. Instead, the recovered CL_{BL} value was reduced significantly in TR⁻ compared with WT livers in the absence of GF120918 (Table 1). This is entirely consistent with acetaminophen-glutathione (APAP-GSH) data in WT and TR⁻ rats, in

TABLE 1

Summary of recovered parameter estimates based on the model scheme depicted in Fig. 3 describing rosuvastatin disposition in wild-type (WT) and Mrp2-deficient (TR⁻) isolated perfused livers in the absence (control) or presence of GF120918.

Values are presented as mean $\pm$ S.D. ($n = 3$ livers). CL_{Uptake} was fixed at 40 ml/min/g liver (Nezasa et al., 2003; Yabe et al., 2011). K_m was fixed at 10 μ M based on the reported affinity of RSV for Mrp2 and Bcrp (Huang et al., 2006; Deng et al., 2008).

Conditions	CL_{BL}	V_{max}	CL_{Met}	CL_{Other}
	ml/min/g liver	nmol/min/g liver	μ l/min/g liver	
WT				
Control	1.4 $\pm$ 0.3	2.6 $\pm$ 1.2	0.83 $\pm$ 0.25	16 $\pm$ 22
+GF120918	0.83 $\pm$ 0.29	3.2 $\pm$ 0.7	1.3 $\pm$ 0.5	24 $\pm$ 34
TR ⁻				
Control	0.55 $\pm$ 0.19*	1.3 $\pm$ 0.1*	3.2 $\pm$ 1.5	46 $\pm$ 13
+GF120918	0.51 $\pm$ 0.13	0.26 $\pm$ 0.08*†	4.2 $\pm$ 1.7	85 $\pm$ 27

* $P < 0.05$, adjusted; effect of Mrp2 status (WT versus TR⁻) is statistically significant within the same level of inhibitor (+ or -GF120918).

† $P < 0.05$, adjusted; effect of GF120918 (absence versus presence) is statistically significant within the same level of Mrp2 status (WT or TR⁻).

which hepatic basolateral excretion of APAP-GSH was impaired in TR⁻ rats, along with biliary excretion, leading to pronounced retention of APAP-GSH in TR⁻ livers (Chen et al., 2003). APAP-GSH and RSV likely compete for basolateral excretion with GSH and other organic anions that accumulate in TR⁻ livers due to the absence of Mrp2 (Elferink et al., 1989). Similar to recent data reported by our group for RSV (Pfeifer et al. 2013), GSH and GSH conjugates are poor substrates for Mrp3, with basolateral excretion mediated primarily by Mrp4 (Hirohashi et al., 1999; Rius et al., 2008).

The absence of impaired CL_{BL} in TR⁻ compared with WT SCH (Pfeifer et al. 2013) may be due to decreased accumulation of endogenous anions in vitro. Although the SCH system retains much of the synthetic function of the liver (Swift et al., 2010), bile acids and bilirubin are recycled extensively from the intestine in vivo, which is absent in the SCH model (Chiang, 2009; Monte et al., 2009). Extrapolation of transporter-mediated RSV clearance values between SCH and IPLs was confined to the WT control condition because of the difference observed between SCH and IPLs regarding the effect of Mrp2 status (WT versus TR⁻) on CL_{BL} , as described above. The ~4-fold decrease in CL_{Uptake} in WT rat SCH compared with IPLs (9.5 versus 40 ml/min/g liver) corresponds with reduced Oatp expression over days in culture in rat SCH (Tchaparian et al., 2011). CL_{BL} was decreased ~6-fold (0.21 versus 1.4 ml/min/g liver) in WT SCH compared with IPLs. Interestingly, the CL_{Bile} translated almost directly [0.23 ml/min/g liver in SCH versus 0.26 ml/min/g liver (V_{max}/K_m) in IPLs]. Bcrp and Mrp2 expression have been reported to increase and decrease, respectively, in rat SCH compared with liver tissue (Li et al., 2009; Li et al., 2010). Therefore, the effects of these changes appear to have a minimal impact on RSV CL_{Bile} .

Following intravenous administration of RSV to healthy humans, approximately 72% of the dose was eliminated by the liver, with an estimated hepatic extraction of 0.63 (Martin et al., 2003a). Similarly, the mean hepatic extraction observed at steady state (30–60 minutes) in WT rat IPLs without GF120918 in the current studies was 0.66. However, perfusate outflow profiles from WT and TR⁻ rat IPLs clearly indicated that RSV is almost completely extracted by the liver in a single pass and that RSV appearance in outflow perfusate

is a result of basolateral efflux. This is evident from the low initial appearance rate of RSV in outflow perfusate, especially from the TR⁻ IPLs, and the absence of a “drop-off” in the outflow perfusate profile when the RSV-containing inflow perfusate was switched to blank buffer at 30 minutes, thereby initiating the efflux phase in single-pass IPL studies (Fig. 4, C and D) (Akita et al., 2001; Chandra et al., 2005). These studies demonstrate for the first time the important role of hepatic basolateral efflux in mediating systemic RSV exposure, including impairment of this efflux pathway in TR⁻ compared with WT IPLs.

In the present studies, the addition of GF120918 nearly ablated RSV CL_{Bile} in TR⁻ livers, with minimal effects on accumulation and CL_{Bile} in WT livers. This finding was expected based on the fraction excreted (f_e) concept (Zamek-Gliszczynski et al., 2009), which states that loss-of-function of a transport pathway associated with $f_e < 0.5$ will have minor consequences on excretion and tissue exposure; in contrast, exposure will change exponentially in response to loss-of-function of transport pathways with $f_e > 0.5$. RSV is excreted into rat bile by Bcrp and Mrp2. In WT rat livers, GF120918 appeared to impair <50% of RSV CL_{Bile} , which resulted in minimal changes in accumulation and biliary recovery of RSV. In contrast, in TR⁻ livers lacking Mrp2, the addition of GF120918 resulted in $\geq 90\%$ impairment of RSV CL_{Bile} , which appeared to be greater than proportional based on the loss of Mrp2 (TR⁻ livers) and Bcrp (GF120918) function in isolation; however, this effect is well established (Zamek-Gliszczynski et al., 2009).

Interestingly, in a whole-animal study comparing WT Sprague-Dawley and Mrp2-deficient Eisai hyperbilirubinemic rats, liver concentrations of RSV were not increased significantly in EHBR animals, as measured by total radioactivity with separation of metabolites by thin-layer chromatography (Kitamura et al., 2008). RSV disposition also was reported recently in recirculating perfused WT and TR⁻ IPLs using LC-MS/MS detection (Hobbs et al., 2012). Increased liver accumulation was observed in TR⁻ compared with WT IPLs, similar to the present study; however, the roles of metabolism and/or basolateral excretion were not considered. The present studies suggest that impaired CL_{BL} as well as impaired CL_{Bile} contribute to the increased hepatic exposure of RSV in TR⁻ compared with WT rat livers.

Results of the present study confirmed the presence of RSV-PA as the primary metabolite, contributing ~30–40% of total RSV content in both WT and TR⁻ livers following the 30-minute efflux period, consistent with previous reports in whole animals (Nezasa et al., 2002; Kitamura et al., 2008). Delayed elimination of RSV metabolites, including RSV-PA, also is consistent with data in whole animals (Nezasa et al., 2002). Measurement of total radioactivity in previous SCH experiments precluded comparisons of RSV-PA formation between IPL and in vitro studies. However, the increase in hepatic accumulation of RSV and RSV-PA in TR⁻ compared with WT IPLs is consistent with impaired RSV excretion and “shunting” to the metabolic pathway(s).

Tools and information remain scarce to help predict the consequences of altered function of hepatic basolateral efflux mechanisms. Quantitative proteomics data and substrate/inhibitor specificity for transport proteins mediating hepatic basolateral efflux lag behind availability of such information for hepatic uptake and biliary excretion pathways. For example, quantitative proteomics data for MRP3 and MRP4 in whole liver and isolated hepatocytes has become available only recently and is exclusive to humans. Additionally, the data are based on a minimal number of samples compared with data for other transporters (Ohtsuki et al., 2012; Schaefer et al., 2012). Similarly, screening for substrates and inhibitors of MRP3 and MRP4 is extremely limited compared with other hepatic transporters (Köck et al., 2013; Sedykh et al., 2013). The number and role of precise proteins involved in basolateral efflux remains to be elucidated. OATPs have been reported to function in a bidirectional manner (Li et al., 2000; Mahagita et al., 2007). Emerging transporters include the organic solute transporter (OST) α/β , which is localized to the basolateral membrane of the entero- and hepatocytes, and serves as a bidirectional transporter of bile acids between cells and blood (Ballatori et al., 2005); OST α/β has been postulated to transport RSV in Caco-2 cells (Li et al., 2012). Given the limitations of current knowledge, it is important to assess the basolateral efflux of RSV and other drugs in whole liver to ascertain the potential role of this pathway in vivo.

Based on the present studies, impaired basolateral efflux clearly has the potential to impact hepatic and systemic exposure of RSV and shift routes of elimination through interplay between transport and metabolism. Although the importance of hepatic basolateral efflux has long been recognized for phase II conjugates, this work demonstrates the significance of this pathway in disposition of the parent drug, RSV. Increasing recognition of the contribution of hepatic basolateral efflux transporters to systemic and hepatic exposure of drugs/metabolites highlights the need to evaluate the consequences of altered function of these transport proteins due to DDIs, genetic variation, and/or disease on drug disposition, which may ultimately impact the efficacy and/or toxicity of medications that are substrates for these transporters.

Acknowledgments

The authors thank Certera, as a member of the Pharsight Academic Center of Excellence Program, for providing Phoenix WinNonlin software to the Division of Pharmacotherapy and Experimental

Therapeutics, UNC Eshelman School of Pharmacy, and Drs. Gary Pollack and Dhiren Thakker for insightful contributions to analysis of the data and manuscript preparation.

Authorship Contributions

Participated in research design: Pfeifer, Bridges, Ferslew, Brouwer.
Conducted experiments: Pfeifer, Hardwick, Bridges.
Performed data analysis: Pfeifer, Bridges, Ferslew, Brouwer.
Wrote or contributed to the writing of the manuscript: Pfeifer, Bridges, Hardwick, Brouwer.

References

- Abe K, Bridges AS, Yue W, and Brouwer KLR (2008) In vitro biliary clearance of angiotensin II receptor blockers and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in sandwich-cultured rat hepatocytes: comparison with in vivo biliary clearance. *J Pharmacol Exp Ther* **326**:983–990.
- Akita H, Suzuki H, Hirohashi T, Takikawa H, and Sugiyama Y (2002) Transport activity of human MRP3 expressed in Sf9 cells: comparative studies with rat MRP3. *Pharm Res* **19**:34–41.
- Akita H, Suzuki H, and Sugiyama Y (2001) Sinusoidal efflux of taurocholate is enhanced in MRP2-deficient rat liver. *Pharm Res* **18**:1119–1125.
- Ballatori N, Christian WV, Lee JY, Dawson PA, Soroka CJ, Boyer JL, Madejczyk MS, and Li N (2005) OSTalpha-OSTbeta: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. *Hepatology* **42**:1270–1279.
- Benet LZ, Cummins CL, and Wu CY (2004) Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharm* **277**:3–9.
- Brouwer KLR and Thurman RG (1996) Isolated perfused liver. *Pharm Biotechnol* **8**:161–192.
- Chandra P, Johnson BM, Zhang P, Pollack GM, and Brouwer KLR (2005) Modulation of hepatic canalicular or basolateral transport proteins alters hepatobiliary disposition of a model organic anion in the isolated perfused rat liver. *Drug Metab Dispos* **33**:1238–1243.
- Chen C, Hennig GE, and Manautou JE (2003) Hepatobiliary excretion of acetaminophen glutathione conjugate and its derivatives in transport-deficient (TR⁻) hyperbilirubinemic rats. *Drug Metab Dispos* **31**:798–804.
- Chen C, Slitt AL, Dieter MZ, Tanaka Y, Scheffer GL, and Klaassen CD (2005) Up-regulation of MRP4 expression in kidney of MRP2-deficient TR⁻ rats. *Biochem Pharmacol* **70**:1088–1095.
- Chen ZS, Lee K, Walther S, Raftogianis RB, Kuwano M, Zeng H, and Kruh GD (2002) Analysis of methotrexate and folate transport by multidrug resistance protein 4 (ABCC4): MRP4 is a component of the methotrexate efflux system. *Cancer Res* **62**:3144–3150.
- Chiang JY (2009) Bile acids: regulation of synthesis. *J Lipid Res* **50**:1955–1966.
- de Lannoy IA, Barker F, 3rd, and Pang KS (1993) Formed and preformed metabolite excretion clearances in liver, a metabolite formation organ: studies on enalapril and enalaprilat in the single-pass and recirculating perfused rat liver. *J Pharmacokin Biopharm* **21**:395–422.
- Deng JW, Shon JH, Shin HJ, Park SJ, Yeo CW, Zhou HH, Song IS, and Shin JG (2008) Effect of silymarin supplement on the pharmacokinetics of rosuvastatin. *Pharm Res* **25**:1807–1814.
- Denk GU, Soroka CJ, Takeyama Y, Chen WS, Schuetz JD, and Boyer JL (2004) Multidrug resistance-associated protein 4 is up-regulated in liver but down-regulated in kidney in obstructive cholestasis in the rat. *J Hepatol* **40**:585–591.
- Donner MG and Keppler D (2001) Up-regulation of basolateral multidrug resistance protein 3 (MRP3) in cholestatic rat liver. *Hepatology* **34**:351–359.
- Elferink RP, Ottenhoff R, Liefing W, de Haan J, and Jansen PL (1989) Hepatobiliary transport of glutathione and glutathione conjugate in rats with hereditary hyperbilirubinemia. *J Clin Invest* **84**:476–483.
- Gradhand U, Lang T, Schaeffeler E, Glaeser H, Tegude H, Klein K, Fritz P, Jedlitschky G, Kroemer HK, and Bachmakov I, et al. (2008) Variability in human hepatic MRP4 expression: influence of cholestasis and genotype. *Pharmacogenomics J* **8**:42–52.
- Hamilton-Craig I (2001) Statin-associated myopathy. *Med J Aust* **175**:486–489.
- Hardwick RN, Fisher CD, Street SM, Canet MJ, and Cherrington NJ (2012) Molecular mechanism of altered ezetimibe disposition in nonalcoholic steatohepatitis. *Drug Metab Dispos* **40**:450–460.
- Hirohashi T, Suzuki H, and Sugiyama Y (1999) Characterization of the transport properties of cloned rat multidrug resistance-associated protein 3 (MRP3). *J Biol Chem* **274**:15181–15185.
- Ho RH, Tirona RG, Leake BF, Glaeser H, Lee W, Lemke CJ, Wang Y, and Kim RB (2006) Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* **130**:1793–1806.
- Hobbs M, Parker C, Birch H, and Kenworthy K (2012) Understanding the interplay of drug transporters involved in the disposition of rosuvastatin in the isolated perfused rat liver using a physiologically-based pharmacokinetic model. *Xenobiotica* **42**:327–338.
- Huang L, Wang Y, and Grimm S (2006) ATP-dependent transport of rosuvastatin in membrane vesicles expressing breast cancer resistance protein. *Drug Metab Dispos* **34**:738–742.
- Johnson BM, Zhang P, Schuetz JD, and Brouwer KLR (2006) Characterization of transport protein expression in multidrug resistance-associated protein (MRP) 2-deficient rats. *Drug Metab Dispos* **34**:556–562.

- Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ, and Niemi M (2009) ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther* **86**:197–203.
- Kiser JJ, Gerber JG, Predhomme JA, Wolfe P, Flynn DM, and Hoody DW (2008) Drug/Drug interaction between lopinavir/ritonavir and rosuvastatin in healthy volunteers. *J Acquir Immune Defic Syndr* **47**:570–578.
- Kitamura S, Maeda K, Wang Y, and Sugiyama Y (2008) Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. *Drug Metab Dispos* **36**:2014–2023.
- Köck K, Ferslew BC, Netterberg I, Yang K, Urban TJ, Swaan PW, Stewart PW, and Brouwer KLR (2013) Risk factors for development of cholestatic drug-induced liver injury: Inhibition of hepatic basolateral bile acid transporters MRP3 and MRP4. *Drug Metab Dispos* DOI 10.1124/dmd.113.054304
- Li J, Wang Y, Zhang W, Huang Y, Hein K, and Hidalgo IJ (2012) The role of a basolateral transporter in rosuvastatin transport and its interplay with apical breast cancer resistance protein in polarized cell monolayer systems. *Drug Metab Dispos* **40**:2102–2108.
- Li L, Meier PJ, and Ballatori N (2000) Oatp2 mediates bidirectional organic solute transport: a role for intracellular glutathione. *Mol Pharmacol* **58**:335–340.
- Li N, Bi YA, Duignan DB, and Lai Y (2009) Quantitative expression profile of hepatobiliary transporters in sandwich cultured rat and human hepatocytes. *Mol Pharm* **6**:1180–1189.
- Li N, Singh P, Mandrell KM, and Lai Y (2010) Improved extrapolation of hepatobiliary clearance from in vitro sandwich cultured rat hepatocytes through absolute quantification of hepatobiliary transporters. *Mol Pharm* **7**:630–641.
- Mahagita C, Grassl SM, Piyachaturawat P, and Ballatori N (2007) Human organic anion transporter 1B1 and 1B3 function as bidirectional carriers and do not mediate GSH-bile acid cotransport. *Am J Physiol Gastrointest Liver Physiol* **293**:G271–G278.
- Martin PD, Warwick MJ, Dane AL, Brindley C, and Short T (2003a) Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers. *Clin Ther* **25**:2553–2563.
- Martin PD, Warwick MJ, Dane AL, Hill SJ, Giles PB, Phillips PJ, and Lenz E (2003b) Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. *Clin Ther* **25**:2822–2835.
- Monte MJ, Marin JJ, Antelo A, and Vazquez-Tato J (2009) Bile acids: chemistry, physiology, and pathophysiology. *World J Gastroenterol* **15**:804–816.
- Nezasa K, Higaki K, Takeuchi M, Nakano M, and Koike M (2003) Uptake of rosuvastatin by isolated rat hepatocytes: comparison with pravastatin. *Xenobiotica* **33**:379–388.
- Nezasa K, Takao A, Kimura K, Takaichi M, Inazawa K, and Koike M (2002) Pharmacokinetics and disposition of rosuvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, in rat. *Xenobiotica* **32**:715–727.
- Ogawa K, Suzuki H, Hirohashi T, Ishikawa T, Meier PJ, Hirose K, Akizawa T, Yoshioka M, and Sugiyama Y (2000) Characterization of inducible nature of MRP3 in rat liver. *Am J Physiol Gastrointest Liver Physiol* **278**:G438–G446.
- Ohtsuki S, Schaefer O, Kawakami H, Inoue T, Liehner S, Saito A, Ishiguro N, Kishimoto W, Ludwig-Schwelling E, and Ebner T, et al. (2012) Simultaneous absolute protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases as a novel approach for the characterization of individual human liver: comparison with mRNA levels and activities. *Drug Metab Dispos* **40**:83–92.
- Parker AJ and Houston JB (2008) Rate-limiting steps in hepatic drug clearance: comparison of hepatocellular uptake and metabolism with microsomal metabolism of saquinavir, nelfinavir, and ritonavir. *Drug Metab Dispos* **36**:1375–1384.
- Pfeifer ND, Yang K, and Brouwer KLR (2013) Hepatic basolateral efflux contributes significantly to rosuvastatin disposition I: Characterization of basolateral versus biliary clearance using a novel protocol in sandwich-cultured hepatocytes. *J Pharmacol Exp Ther* **347**:727–736.
- Rius M, Hummel-Eisenbeiss J, and Keppler D (2008) ATP-dependent transport of leukotrienes B4 and C4 by the multidrug resistance protein ABCB4 (MRP4). *J Pharmacol Exp Ther* **324**:86–94.
- Schaefer O, Ohtsuki S, Kawakami H, Inoue T, Liehner S, Saito A, Sakamoto A, Ishiguro N, Matsumaru T, and Terasaki T, et al. (2012) Absolute quantification and differential expression of drug transporters, cytochrome P450 enzymes, and UDP-glucuronosyltransferases in cultured primary human hepatocytes. *Drug Metab Dispos* **40**:93–103.
- Scheffer GL, Kool M, de Haas M, de Vree JM, Pijnenborg AC, Bosman DK, Elferink RP, van der Valk P, Borst P, and Scheper RJ (2002) Tissue distribution and induction of human multidrug resistant protein 3. *Lab Invest* **82**:193–201.
- Schneck DW, Birmingham BK, Zalikowski JA, Mitchell PD, Wang Y, Martin PD, Lasseter KC, Brown CD, Windass AS, and Raza A (2004) The effect of gemfibrozil on the pharmacokinetics of rosuvastatin. *Clin Pharmacol Ther* **75**:455–463.
- Sedykh A, Fourches D, Duan J, Hucke O, Garneau M, Zhu H, Bonneau P, and Tropsha A (2013) Human Intestinal Transporter Database: QSAR Modeling and Virtual Profiling of Drug Uptake, Efflux and Interactions. *Pharm Res* **30**:996–1007.
- Simonson SG, Martin PD, Mitchell P, Schneck DW, Lasseter KC, and Warwick MJ (2003) Pharmacokinetics and pharmacodynamics of rosuvastatin in subjects with hepatic impairment. *Eur J Clin Pharmacol* **58**:669–675.
- Simonson SG, Raza A, Martin PD, Mitchell PD, Jarcho JA, Brown CD, Windass AS, and Schneck DW (2004) Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. *Clin Pharmacol Ther* **76**:167–177.
- Swift B, Pfeifer ND, and Brouwer KLR (2010) Sandwich-cultured hepatocytes: an in vitro model to evaluate hepatobiliary transporter-based drug interactions and hepatotoxicity. *Drug Metab Rev* **42**:446–471.
- Tchaparian EH, Houghton JS, Uyeda C, Grillo MP, and Jin L (2011) Effect of culture time on the basal expression levels of drug transporters in sandwich-cultured primary rat hepatocytes. *Drug Metab Dispos* **39**:2387–2394.
- Thompson PD, Clarkson P, and Karas RH (2003) Statin-associated myopathy. *JAMA* **289**:1681–1690.
- Tian X, Swift B, Zamek-Gliszczynski MJ, Belinsky MG, Kruh GD, and Brouwer KLR (2008) Impact of basolateral multidrug resistance-associated protein (Mrp) 3 and Mrp4 on the hepatobiliary disposition of fexofenadine in perfused mouse livers. *Drug Metab Dispos* **36**:911–915.
- Tomlinson B, Hu M, Lee VW, Lui SS, Chu TT, Poon EW, Ko GT, Baum L, Tam LS, and Li EK (2010) ABCG2 polymorphism is associated with the low-density lipoprotein cholesterol response to rosuvastatin. *Clin Pharmacol Ther* **87**:558–562.
- Vlaming ML, van Esch A, Pala Z, Wagenaar E, van de Wetering K, van Tellingen O, and Schinkel AH (2009) Abcc2 (Mrp2), Abcc3 (Mrp3), and Abcg2 (Bcrp1) are the main determinants for rapid elimination of methotrexate and its toxic metabolite 7-hydroxymethotrexate in vivo. *Mol Cancer Ther* **8**:3350–3359.
- Watanabe T, Kusuhashi H, Maeda K, Shitara Y, and Sugiyama Y (2009) Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. *J Pharmacol Exp Ther* **328**:652–662.
- Yabe Y, Galetin A, and Houston JB (2011) Kinetic characterization of rat hepatic uptake of 16 actively transported drugs. *Drug Metab Dispos* **39**:1808–1814.
- Zamek-Gliszczynski MJ, Hoffmaster KA, Nezasa K, Tallman MN, and Brouwer KLR (2006) Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. *Eur J Pharm Sci* **27**:447–486.
- Zamek-Gliszczynski MJ, Kalvass JC, Pollack GM, and Brouwer KLR (2009) Relationship between drug/metabolite exposure and impairment of excretory transport function. *Drug Metab Dispos* **37**:386–390.
- Zhang W, Yu BN, He YJ, Fan L, Li Q, Liu ZQ, Wang A, Liu YL, Tan ZR, and Feng-Jiang, et al. (2006) Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. *Clin Chim Acta* **373**:99–103.

Address correspondence to: Kim L. R. Brouwer, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, CB #7569, Chapel Hill, NC 27599-7569. E-mail: kbrouwer@email.unc.edu