Short Communication

Use of Sandwich-Cultured Human Hepatocytes to Predict Biliary Clearance of Angiotensin II Receptor Blockers and HMG-CoA Reductase Inhibitors

Received July 18, 2008; accepted December 12, 2008

ABSTRACT:

Previous reports have indicated that *in vitro* biliary clearance (Cl_{biliary}) determined in sandwich-cultured hepatocytes correlates well with *in vivo* Cl_{biliary} for limited sets of compounds. The purpose of this study was 1) to determine the *in vitro* Cl_{biliary} in sandwich-cultured human hepatocytes of angiotensin II receptor blockers and HMG-CoA reductase inhibitors that undergo limited metabolism and 2) to compare the predicted Cl_{biliary} values with estimated *in vivo* hepatic clearance data in humans. The average biliary excretion index and *in vitro* intrinsic Cl_{biliary} values of olmesartan, valsartan,

pravastatin, rosuvastatin, and pitavastatin in sandwich-cultured human hepatocytes were 35, 23, 31, 25, and 16%, respectively, and 0.943, 1.20, 0.484, 3.39, and 5.48 ml/min/kg, respectively. Cl_{biliary} values predicted from sandwich-cultured human hepatocytes correlated with estimated *in vivo* hepatic clearance values based on published data (no *in vivo* data in humans was available for pitavastatin), and the rank order was also consistent. In conclusion, *in vitro* Cl_{biliary} determined in sandwich-cultured human hepatocytes can be used to predict *in vivo* Cl_{biliary} of compounds in humans.

Many drugs, including cardiovascular, anticancer, and anti-infective agents (Bi et al., 2006; Shitara et al., 2006), undergo extensive biliary excretion in humans. Biliary excretion often plays an important role in the pharmacologic and pharmacokinetic behavior of these compounds. For example, enterohepatic recycling may prolong the pharmacologic effect of a compound by maintaining therapeutic concentrations for an extended period of time. In patients with renal impairment, biliary excretion may provide an alternative route of elimination, potentially avoiding elevated blood concentrations of drugs that might otherwise be excreted in the urine (Ishizuka et al., 1997; Nakagomi-Hagihara et al., 2006). Accordingly, knowledge regarding the extent of biliary excretion of compounds in humans during the early stages of drug development would be important in the drug discovery process. Elucidation of the biliary excretion properties of a drug candidate is also critical, considering the potential for drug-drug interactions and disease state alterations in hepatobiliary drug disposition.

Sandwich-cultured rat and human hepatocytes form intact bile canalicular networks, and they maintain functional expression levels of uptake and efflux transport proteins for several days (Hoffmaster et al., 2004; Zhang et al., 2005). *In vitro* biliary clearance (Cl_{biliary}) determined in sandwich-cultured rat and human hepatocytes correlates well with *in vivo* Cl_{biliary} (Liu et al., 1999a; Ghibellini et al., 2007), suggesting that this system can be used to predict the *in vivo* biliary excretion of drug candidates.

This work was supported by the National Institutes of Health National Institute of General Medical Sciences [Grant GM 41935].

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.

doi:10.1124/dmd.108.023465.

In a recent study, we demonstrated that the *in vitro* Cl_{biliary} of angiotensin II receptor blockers (ARBs; olmesartan and valsartan) and HMG-CoA reductase inhibitors (statins; pravastatin, rosuvastatin, and pitavastatin) determined in sandwich-cultured rat hepatocytes can be used to estimate *in vivo* Cl_{biliary} and to ascertain the involvement of transport proteins in the basolateral uptake and canalicular excretion of these drugs (Abe et al., 2008). All of these compounds undergo limited metabolism and are organic anions with a carboxylic acid moiety, and they are excreted extensively into bile by canalicular transport proteins (Hirano et al., 2005; Nakagomi-Hagihara et al., 2006; Shitara and Sugiyama, 2006; Yamashiro et al., 2006). These compounds exhibited a wide range of biliary clearance values (low, olmesartan and valsartan; medium, pitavastatin; and high, rosuvastatin and pravastatin). The biliary clearance of the selected compounds should be governed by the unbound fraction and the hepatic uptake ability because these compounds were subject to minimal metabolism and exhibited uptake-limited hepatic clearance. Similar investigations in sandwich-cultured human hepatocytes would be useful in the preliminary evaluation of the hepatobiliary disposition of candidate compounds; data generated could provide the necessary information to 1) predict the rank order of *in vivo* Cl_{biliary} in humans, 2) assess the potential for drug-drug interactions in hepatobiliary transport, and 3) understand species differences in hepatobiliary disposition. Limited data are available for *in vitro*-*in vivo* correlations of the Cl_{biliary} of compounds due to the complexity of the procedures required to measure biliary excretion in humans (Ghibellini et al., 2007).

The purpose of this study was 1) to determine in sandwich-cultured human hepatocytes the *in vitro* Cl_{biliary} of ARBs and statins that undergo limited metabolism and 2) to compare the predicted *in vivo* Cl_{biliary} values based on the *in vitro* data with estimated published in

ABBREVIATIONS: Cl_{biliary} , biliary clearance; ARB, angiotensin II receptor blocker; CDFDA, 5 (and 6)-carboxy-2',7'-dichlorofluorescein diacetate; CDF, 5 (and 6)-carboxy-2',7'-dichlorofluorescein; HBSS, Hank's balanced salt solution; BEI, biliary excretion index; AUC, area under the medium concentration-time curve; MRP, multidrug resistance-associated protein; OATP, organic anion-transporting polypeptide.

TABLE 1
Demographics of human liver donors

Donors had no history of tobacco and alcohol use within the last 2 years.

Liver Identification	Age (years)	Gender	Race	Comedications
1	57	Female	African American	Metoclopramide, docusate, acetaminophen, hydrocodone
2	64	Female	White	Atorvastatin, alendronate, amlodipine, benazepril
3	51	Male	White	Glyburide, metformin, pravastatin, bevacizumab, pioglitazone

vivo hepatic clearance data. In this study, hepatic clearance values for these drugs were estimated based on the difference between total clearance and renal clearance in humans.

Materials and Methods

Chemicals. Olmesartan was kindly provided by Daiichi-Sankyo Co., Ltd. (Tokyo, Japan). Valsartan, pravastatin, rosuvastatin, and pitavastatin were obtained from Toronto Research Chemicals Inc. (Ontario, Canada). [^3H]Taurocholate (5 Ci/mmol; purity >97%) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). Dulbecco's modified Eagle's medium, minimum essential medium nonessential amino acids, and 5 (and 6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) were purchased from Invitrogen (Carlsbad, CA). Insulin/transferrin/selenium culture supplement and Matrigel were purchased from BD Biosciences Discovery Labware (Bedford, MA). Penicillin-streptomycin solution, taurocholic acid, dexamethasone, and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of analytical grade and were readily available from commercial sources.

Hepatocyte Culture. Freshly isolated human hepatocytes (seeding density: 1.5 million cells per well) overlaid with Matrigel in 6-well plates were provided by CellZDirect (Durham, NC). The demographics of the human liver donors are shown in Table 1. Hepatocytes were cultured in Dulbecco's modified Eagle's medium containing 1% insulin/transferrin/selenium, 0.1 μM dexamethasone, 2 mM L-glutamine, 1% minimum essential medium nonessential amino acids, 100 units of penicillin G sodium, and 100 μg of streptomycin sulfate at 37°C in a humidified incubator with 95% O_2 /5% CO_2 for 7 to 10 days; medium was changed daily.

Fluorescence Microscopy. Retention of 5 (and 6)-carboxy-2',7'-dichlorofluorescein (CDF) in bile canaliculi networks was examined by fluorescence microscopy. Hepatocytes were rinsed with 2 ml of standard buffer and then 1.5 ml of CDFDA (10 μM) in standard buffer was added. After a 10-min incubation at 37°C, the buffer was removed and 1.5 ml of standard buffer was added. The cells and bile canaliculi were imaged with a Zeiss Axiovert 100TV inverted fluorescence microscope (Carl Zeiss Inc., Thornwood, NY).

Accumulation Studies and Analysis. Hepatocytes were rinsed twice and then preincubated for 10 min at 37°C with 2 ml of warmed Hank's balanced salt solution (HBSS) containing Ca^{2+} (standard; cells + bile) or Ca^{2+} -free HBSS (cells), to maintain or disrupt the tight junctions sealing bile canaliculi networks, respectively. Subsequently, hepatocytes were incubated with test compound (5 μM for ARBs or statins; 1 μM for [^3H]taurocholate) in standard HBSS for 10 min at 37°C. After incubation, the dosing solution was aspirated from the cells and uptake was stopped by washing the cells 3 times with ice-cold standard HBSS. For [^3H]taurocholate, cells were lysed with 1 ml of Triton X-100 (0.5%) in phosphate-buffered saline. For ARBs and statins, cells were lysed with 1 ml of methanol (70%; v/v) and sonicated for 20 s with a sonic dismembrator (model 100; Thermo Fisher Scientific, Waltham, MA) then stored at -70°C until analysis. The samples were analyzed for drug concentrations by liquid scintillation counting or by liquid chromatography with tandem mass spectrometry, as described previously (Abe et al., 2008). Substrate accumulation was corrected for nonspecific binding by using Matrigel-precoated dishes without cells. The total protein concentration in cell lysates was quantified by the BCA protein assay (Pierce Biotechnology, Inc., Rockford, IL) using bovine serum albumin as the reference standard, and accumulation was normalized to protein concentration. Due to incompatibility of the protein assay with methanol, the average protein concentration for samples lysed with Triton X-100 incubations in the same liver preparation was used to normalize accumulation.

Data Analysis. The accumulation (pmol/mg protein), biliary excretion

index (BEI; %), and in vitro intrinsic $\text{Cl}_{\text{biliary}}$ (ml/min/kg) were calculated in hepatocytes using B-CLEAR technology (Qualyst, Inc., Raleigh, NC) based on eqs. 1 and 2 (Liu et al., 1999a).

$$\text{BEI} = \frac{\text{Accumulation}_{\text{cells} + \text{bile}} - \text{Accumulation}_{\text{cells}}}{\text{Accumulation}_{\text{cells} + \text{bile}}} \times 100 \quad (1)$$

$$\text{Intrinsic } \text{Cl}_{\text{biliary}} = \frac{\text{Accumulation}_{\text{cells} + \text{bile}} - \text{Accumulation}_{\text{cells}}}{\text{AUC}_{\text{medium}}} \quad (2)$$

where $\text{AUC}_{\text{medium}}$ was determined as the product of the incubation time and the medium concentration. The concentration of drug in the medium was defined as the initial substrate concentration in the incubation medium, because the medium concentration at the end of incubation did not differ by more than 10% from the medium concentration at the beginning of incubation. In the absence of exogenous protein in the incubation medium, unbound intrinsic $\text{Cl}_{\text{biliary}}$ (intrinsic $\text{Cl}'_{\text{biliary}}$) was assumed to be equivalent to in vitro intrinsic $\text{Cl}_{\text{biliary}}$ (eq. 2). The in vitro intrinsic $\text{Cl}_{\text{biliary}}$ (ml/min/mg protein) was scaled to kilogram of body weight assuming the following: 1 mg protein/1.5 $\times 10^6$ cells, 107 $\times 10^6$ hepatocytes/g human liver tissue (Wilson et al., 2003), and 25.7 g of liver tissue per kilogram of body weight (Davies and Morris, 1993). The predicted in vivo $\text{Cl}_{\text{biliary}}$ values were estimated according to the equations below based on the well-stirred model of hepatic disposition, assuming that red blood cell partitioning of test compounds was minimal.

$$\text{Predicted } \text{Cl}_{\text{biliary}} = \frac{Q_p \times f_{u,p} \times \text{intrinsic } \text{Cl}'_{\text{biliary}}}{Q_p + f_{u,p} \times \text{intrinsic } \text{Cl}'_{\text{biliary}}} \quad (3)$$

where Q_p and $f_{u,p}$ represent the hepatic plasma flow rate and plasma unbound fraction, respectively. Hepatic plasma flow in humans (750 ml/min) (Sandker et al., 1994) was normalized by assuming a body weight of 70 kg.

The hepatic clearance of olmesartan in humans was reported to be 0.86 l/h based on total and renal clearance after intravenous administration (Nakagomi-Hagihara et al., 2006). The renal clearance of valsartan was estimated as the product of the total clearance (2.19 l/h) and the fraction excreted unchanged in urine after an intravenous dose (0.29) (Flesch et al., 1997). Likewise, the renal clearance of rosuvastatin was estimated from the total clearance (48.9 l/h) and the fraction excreted unchanged in urine (0.28) (regulatory documentation). Total clearance and renal clearance of pravastatin after intravenous administration in humans were reported to be 13.5 and 6.3 ml/min/kg, respectively (Singhvi et al., 1990). Hepatic clearance values of these compounds in humans were estimated based on the difference between total clearance and renal clearance using the above information, assuming a body weight of 70 kg. No information regarding the total and renal clearance of pitavastatin after intravenous administration was available.

Results and Discussion

CDF retention in bile canaliculi of sandwich-cultured human hepatocytes was evaluated to assess canaliculi excretory function of the hepatocytes. CDFDA readily diffuses into hepatocytes, where it is hydrolyzed rapidly to CDF, a fluorescent compound that is extensively excreted into the bile canaliculi by multidrug resistance-associated protein (MRP)2 (Hoffmaster et al., 2004). CDF fluorescence accumulated in bile canaliculi networks of sandwich-cultured human hepatocytes after a 10-min incubation with CDFDA (Fig. 1). [^3H]Taurocholate accumulation in standard buffer (101–205 pmol/mg protein) and BEI (52.4–70.8%), equivalent to the percentage of retained substrate in the canaliculi networks, were in good agreement with

FIG. 1. Retention of CDF in bile canaliculi of sandwich-cultured human hepatocytes. Sandwich-cultured human hepatocytes were incubated with CDFDA ($10 \mu\text{M}$) in standard buffer for 10 min. Representative images of CDF fluorescence in hepatocyte cultures from liver 2 (A) and liver 3 (B).

reported values (Ghibellini et al., 2007) (Fig. 2). These findings demonstrated functional excretion of substrates into the canalicular networks of sandwich-cultured human hepatocytes.

K_m values for the hepatic uptake of olmesartan (Nakagomi-Hagihara et al., 2006), pravastatin (Nakai et al., 2001), and pitavastatin (Fujino et al., 2004) were reported to be 29.3, 11.5, and $3.0 \mu\text{M}$ in human hepatocytes, respectively. K_m values for valsartan uptake in HEK293 cells expressing OATP1B1 and OATP1B3 were 1.4 and $18 \mu\text{M}$, respectively (Yamashiro et al., 2006). K_m values for rosuvastatin uptake in HeLa cells expressing OATP1A2, OATP1B1, OATP1B3, and OATP2B1 were 2.6, 4 to 7.3, 9.8, and $2.4 \mu\text{M}$, respectively (Ho et al., 2006). In the present study, $5 \mu\text{M}$ was selected as the substrate concentration based on the similarity to K_m values for uptake in hepatocytes and comparison with data generated in rats.

Both rosuvastatin and pitavastatin exhibited strikingly higher 10-min accumulation in sandwich-cultured human hepatocytes compared with the other drugs, suggesting higher uptake activity (Fig. 2). In sandwich-cultured human hepatocytes, the average accumulation in standard buffer (cells + bile) of olmesartan, pravastatin, and pitavastatin were 72, 45, and $980 \text{ pmol/mg protein}$, respectively. The rank order of these values was consistent with the intrinsic clearance values (V_{max}/K_m) for the hepatic uptake of these drugs in suspended human hepatocytes [olmesartan: $2.5 \mu\text{l/min}/10^6\text{cells}$ (Nakagomi-Hagihara et al., 2006); pravastatin: $0.89 \mu\text{l/min}/10^6\text{cells}$ (Nakai et al., 2001); pitavastatin: $27 \mu\text{l/min}/10^6\text{cells}$ (Fujino et al., 2004); no data were available for valsartan and rosuvastatin].

The *in vitro* intrinsic $\text{Cl}_{\text{biliary}}$ values were higher for both rosuvastatin and pitavastatin compared with the other drugs (Table 2). Sandwich-cultured rat hepatocytes also exhibited higher accumulation and intrinsic $\text{Cl}_{\text{biliary}}$ values of rosuvastatin and pitavastatin compared with the other drugs (Abe et al., 2008).

For a given ARB or statin, accumulation and intrinsic $\text{Cl}_{\text{biliary}}$ values from the three different human livers varied by <5- and <4-fold, respectively. Approximately 5-fold variability was also observed for the uptake of estradiol-17 β -D-glucuronide in suspended human hepatocytes and sandwich-cultured human hepatocytes prepared from cryopreserved human hepatocytes (Shitara et al., 2003; Bi et al., 2006). Multiple OATPs are involved in the hepatic uptake of

these ARBs and statins (Nakagomi-Hagihara et al., 2006; Shitara et al., 2006; Yamashiro et al., 2006). MRP2 is involved in the canalicular excretion of these ARBs and statins; multidrug resistance protein 1 and breast cancer resistance protein are also involved in the canalicular excretion of these statins (Nakagomi-Hagihara et al., 2006; Shitara et al., 2006; Yamashiro et al., 2006). Differences in the contribution of these transport proteins between donors may be the reason for some interliver variability.

The relationship between predicted $\text{Cl}_{\text{biliary}}$ values from sandwich-cultured human hepatocytes and estimated hepatic clearance values for olmesartan, valsartan, pravastatin, and rosuvastatin was well described (Fig. 3). Estimated *in vivo* hepatic clearance values were used in this comparison, because $\text{Cl}_{\text{biliary}}$ for these compounds has not been measured directly in humans. The assumption in this study that *in vivo* hepatic clearance is equivalent to $\text{Cl}_{\text{biliary}}$ is reasonable due to the limited metabolism of these ARBs and statins in humans. Pitavastatin was not included in this relationship because no information was available to estimate the *in vivo* hepatic clearance in humans. In contrast, there was no discernable relationship between predicted $\text{Cl}_{\text{biliary}}$ values and estimated *in vivo* hepatic clearance values for these drugs based on eq. 3 assuming $f_{u,p}$ was unity (data not shown). According to the present data, when *in vivo* $\text{Cl}_{\text{biliary}}$ values are predicted based on sandwich-cultured human hepatocyte data, the unbound fraction should be included as shown in eq. 3. Abe et al. (2008) reported that the predicted $\text{Cl}_{\text{biliary}}$ values, corrected for plasma unbound fraction, from sandwich-cultured rat hepatocytes based on 10-min incubations at $5 \mu\text{M}$ substrate concentration correlated well with the *in vivo* $\text{Cl}_{\text{biliary}}$ values for these drugs in rats.

The rank order of the predicted $\text{Cl}_{\text{biliary}}$ values was consistent with the estimated *in vivo* hepatic clearance values for these drugs, although both ARBs have low *in vivo* hepatic clearance values. Both the predicted and the estimated *in vivo* hepatic clearance values of rosuvastatin were the highest, followed by pravastatin, valsartan, and olmesartan (Fig. 3; Table 2). Based on this study, the *in vivo* $\text{Cl}_{\text{biliary}}$ of pitavastatin was predicted to be much lower than pravastatin and rosuvastatin. Although the bioavailability of pitavastatin is unknown in humans because no intravenous formulation is available, the oral clearance of pitavastatin in humans was reported to be lower than pravastatin and rosuvastatin (Ieiri et al., 2007).

The predicted $\text{Cl}_{\text{biliary}}$ values were 5- to 30-fold lower than the estimated *in vivo* hepatic clearance values for ARBs and statins (Table 2). These findings may be due to less extensive canalicular network formation in culture compared with liver tissue *in vivo*. Consistent underestimation also may be due to several factors including, but not limited to, decreased activity of transport proteins in culture or leakage from the biliary compartment in sandwich-cultured hepatocytes, as discussed previously (Liu et al., 1999b; Hoffmaster et al., 2005). A recent study with hepatocytes demonstrated a significant underprediction of *in vivo* clearance for a distinct set of drugs, which was attributed to hepatic uptake (Riley et al., 2005). It is probable that a scaling factor might be necessary for the prediction of *in vivo* biliary clearance. Although unlikely, it is possible that the underestimation of $\text{Cl}_{\text{biliary}}$ is due to more extensive metabolism of these compounds than previously recognized. According to the information on the Food and Drug Administration website, the contribution of metabolic clearance to total clearance for olmesartan, valsartan, and rosuvastatin in humans is quite low; these drugs are recovered primarily as unchanged species, with at most ~20% of the dose recovered as metabolites. Pravastatin is highly hydrophilic [LogD at pH 7 = -0.47], undergoes minimal metabolism by cytochrome P450 (Shitara and Sugiyama, 2006), and the contribution of metabolism to pravastatin clearance should be quite low.

FIG. 2. Accumulation [cells + bile (solid bars) and cells (white bars)] and BEI of taurocholate (A), olmesartan (B), valsartan (C), pravastatin (D), rosuvastatin (E), and pitavastatin (F) in sandwich-cultured human hepatocytes. Dosing concentration: taurocholate (1 μ M), ARBs and statins (5 μ M); incubation time: 10 min. Data represent mean + S.D. (triplicate).

Bi et al. (2006) reported that sandwich-cultured human hepatocytes prepared from cryopreserved human hepatocytes form intact bile canalicular networks and exhibit functional uptake and transporter-mediated excretion. Use of cryopreserved human hepatocytes would be more convenient than isolating and preparing sandwich-cultured human hepatocytes from fresh human liver. The reported BEI of taurocholate (41–63%) (Bi et al., 2006) was consistent with our results (53–71%), although the published in vitro intrinsic $Cl_{biliary}$ of taurocholate (5.8–10 μ l/min/mg protein) (Bi et al., 2006) was slightly lower than our results (12–25 μ l/min/mg protein). However, the BEI

(43–58%) and the in vitro intrinsic $Cl_{biliary}$ (4.0–12 μ l/min/mg protein) of rosuvastatin (Bi et al., 2006) was higher than our results (BEI, 17–33%; in vitro intrinsic $Cl_{biliary}$, 1.2–2.8 μ l/min/mg protein). These differences may be attributed to differences in the type of medium, hepatocytes, or the day in culture when the experiments were performed (day 5 versus days 7–10). We previously confirmed that sandwich-cultured human hepatocytes cultured for 7 to 10 days maintain good morphology, form distinct bile canalicular networks, and express functional transport proteins on the proper membrane domain (Hoffmaster et al., 2004).

TABLE 2

BEI, in vitro intrinsic $Cl_{biliary}$, and predicted $Cl_{biliary}$ compared with estimated in vivo hepatic clearance for selected ARBs and HMG-CoA reductase inhibitors in humans

Data represent mean $\pm$ S.E.M. of three individual experiments in triplicate.

Compound	BEI	In Vitro Intrinsic $Cl_{biliary}$	Plasma Unbound Fraction	Predicted $Cl_{biliary}$	Estimated In Vivo Hepatic Clearance
	%	ml/min/kg ^a	$f_{u,p}$	ml/min/kg ^b	ml/min/kg ^c
Olmesartan	34.6 $\pm$ 3.0	0.943 $\pm$ 0.330	0.01 ^d	0.00942 $\pm$ 0.00329	0.205
Valsartan	23.0 $\pm$ 3.4	1.20 $\pm$ 0.33	0.059 ^e	0.0702 $\pm$ 0.0193	0.370
Pravastatin	30.9 $\pm$ 3.1	0.484 $\pm$ 0.061	0.5 ^f	0.237 $\pm$ 0.029	7.20
Rosuvastatin	24.6 $\pm$ 4.7	3.39 $\pm$ 0.89	0.12 ^g	0.390 $\pm$ 0.098	8.38
Pitavastatin	15.6 $\pm$ 2.7	5.48 $\pm$ 1.21	0.004 ^h	0.0219 $\pm$ 0.0048	No data

^a In vitro intrinsic $Cl_{biliary}$ values were calculated according to eq. 2.

^b Predicted $Cl_{biliary}$ values were calculated according to eq. 3.

^c Estimated in vivo hepatic clearance values were calculated based on the difference between total clearance and renal clearance (see *Materials and Methods*).

^d Nakagomi-Hagihara et al., 2006.

^e Colussi et al., 1997.

^f Nakagomi-Hagihara et al., 2007.

^g Rosuvastatin regulatory documentation.

^h Pitavastatin regulatory documentation.

FIG. 3. Relationship between estimated in vivo hepatic clearance for ARBs and statins in humans based on published data and in vitro $Cl_{biliary}$ values predicted from sandwich-cultured human hepatocytes data. $Cl_{biliary}$ values predicted from in vitro data represent mean $\pm$ S.E.M. of three individual experiments in triplicate. When not visible, error bars are within the size of the symbol. Pitavastatin data were excluded from the plot because published information was not available to estimate in vivo hepatic clearance in humans.

In sandwich-cultured rat hepatocytes, the average in vitro intrinsic $Cl_{biliary}$ of olmesartan, valsartan, pravastatin, rosuvastatin, and pitavastatin was 0.215, 0.405, 0.553, 5.76, and 4.33 μ l/min/mg protein, respectively, based on data generated from 10-min incubations at 5 μ M substrate concentration (Abe et al., 2008). Species-specific differences in the contribution of individual transport proteins to hepatic disposition of olmesartan, valsartan, pravastatin, rosuvastatin, and pitavastatin may account for variations in the in vitro intrinsic $Cl_{biliary}$ in sandwich-cultured human hepatocytes (0.514, 0.654, 0.264, 1.85, and 2.99 μ l/min/mg protein, respectively), compared with values determined in sandwich-cultured rat hepatocytes. For example, transport properties of human OATPs may be difficult to predict from rat data because most rat *Slco* gene products are not orthologs of the human OATP proteins (Tamai et al., 2000). Furthermore, the intrinsic clearance of dinitrophenyl-S-glutathione, an Mrp2 substrate, is much higher in rats than in other species such as mouse, guinea pig, rabbit, dog, and human (Ishizuka et al., 1999). These observed differences between species clearly emphasize the importance of using an in vitro human tool to predict the hepatobiliary disposition of drug candidates in vivo in humans.

In summary, the in vitro $Cl_{biliary}$ determined in sandwich-cultured human hepatocytes is useful in predicting the in vivo $Cl_{biliary}$ of

selected ARBs and statins that undergo limited metabolism in humans. This model could provide important information regarding the $Cl_{biliary}$ of compounds in development, due to the difficulty of directly determining the in vivo $Cl_{biliary}$ of compounds in humans. Moreover, knowledge regarding the contribution of $Cl_{biliary}$ is essential to predict how disease states, genetic variability, and/or transporter-mediated drug-drug interactions may alter hepatobiliary drug disposition.

Acknowledgments. Dr. Kim Brouwer is a cofounder and Chair of the Scientific Advisory Board for Qualyst, Inc., which has exclusively licensed the sandwich-cultured hepatocyte technology for quantification of biliary excretion (B-CLEAR). K.A. was supported by a scholarship from Daiichi-Sankyo.

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