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1. Title

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

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2. Running title

a) Biliary clearance prediction of ARBs and statins in human

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d) Nonstandard abbreviations: ARB, angiotensin II receptor blocker; Bcrp, breast cancer resistance protein; BEI, biliary excretion index; $Cl_{biliary}$, biliary clearance; Mrp2, multidrug resistance-associated protein 2; Oatp, organic anion transporting polypeptide; Statin, HMG-CoA reductase inhibitor.

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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 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 to compare the predicted $Cl_{biliary}$ values with estimated *in vivo* hepatic clearance data in humans. The average biliary excretion index (BEI) 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 also was 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.

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Introduction

Many drugs including cardiovascular, anti-cancer, 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 provides an alternative route of elimination potentially avoiding elevated blood concentrations of drugs that might otherwise be excreted in 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 also is 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 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 is useful for predicting the *in vivo* biliary excretion of drug candidates.

Recently, 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, are organic anions with a carboxylic acid moiety, and are excreted extensively into bile by canalicular transport proteins (Nakagomi-Hagihara et al., 2006; Yamashiro et al., 2006; Shitara and Sugiyama, 2006; Hirano et al., 2005). These compounds exhibited a wide range of biliary clearance values (*low*: olmesartan, valsartan; *medium*: pitavastatin; *high*: rosuvastatin and pitavastatin). 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, predicting the rank order of *in vivo* $Cl_{biliary}$ in humans, assessing the potential for drug-drug interactions in hepatobiliary transport, and understanding 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 to determine in sandwich-cultured human hepatocytes the *in vitro* $Cl_{biliary}$ of ARBs and statins that undergo limited metabolism, and to compare the predicted *in vivo* $Cl_{biliary}$ values based on the *in vitro* data with estimated published *in 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.

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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). [^{3}H]Taurocholate (5 Ci/mmol; purity >97%) was purchased from PerkinElmer Inc. (Waltham, MA). Dulbecco's modified Eagle's medium (DMEM), MEM non-essential amino acids, and 5(and 6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) were purchased from Invitrogen (Carlsbad, CA). Insulin/transferrin/selenium culture supplement (ITS^{TM}) and Matrigel^{TM} were purchased from BD Biosciences Discovery Labware (Bedford, MA). Penicillin-streptomycin solution, taurocholic acid, dexamethasone and Triton X-100 were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). All other chemicals and reagents were of analytical grade and readily available from commercial sources.

Hepatocyte Culture

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. Media consisted of DMEM containing 1% ITS^{TM}, 0.1 µM dexamethasone, 2 mM L-glutamine, 1% MEM non-essential amino acids, 100 units penicillin G sodium and 100 µg streptomycin sulfate. Hepatocytes were cultured at 37°C in a humidified incubator with 95% O_2/5% CO_2 for 7-10 days; media was changed daily.

Fluorescence Microscopy

Retention of 5 (and 6)-carboxy-2',7'-dichlorofluorescein (CDF) in bile canalicular 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 pre-incubated 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), in order to maintain or disrupt the tight junctions sealing bile canalicular networks, respectively. Subsequently, hepatocytes were incubated with test compound (5 μM for ARBs or statins; 1 μM for [^{3}H]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 [^{3}H]taurocholate, cells were lysed with 1 ml of 0.5% Triton X-100 in phosphate-buffered saline. For ARBs and statins, cells were lysed with 1 ml of 70% (v/v) methanol, and sonicated for 20 sec with a sonic dismembrator (model 100, Fisher Scientific, Pittsburgh, PA) and 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^{TM}-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 standard HBSS or Ca^{2+}-free HBSS 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 $Cl_{biliary}$ (ml/min/kg) were calculated in hepatocytes using B-CLEAR® technology (Qualyst, Inc., Raleigh, NC) based on the following equations (Liu et al., 1999a).

$$BEI = \frac{Accumulation_{Standard} - Accumulation_{Ca^{2+}\,free}}{Accumulation_{Standard}} \times 100 \qquad (1)$$

$$Intrinsic\ Cl_{biliary} = \frac{Accumulation_{Standard} - Accumulation_{Ca^{2+}\,free}}{AUC_{medium}} \qquad (2)$$

where AUC_{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, since 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 $Cl_{biliary}$ (intrinsic $Cl'_{biliary}$) is assumed to be equivalent to *in vitro* intrinsic $Cl_{biliary}$ [Equation (2)]. The *in vitro* intrinsic $Cl_{biliary}$ (ml/min/mg protein) was scaled to kilogram of body weight assuming the following: 1 mg protein/1.5×10^6 cells, 107×10^6 hepatocytes/g of 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*

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vivo $Cl_{biliary}$ 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.

$$Predicted\ Cl_{biliary} = \frac{Qp \times fu,p \times intrinsic\ Cl'_{biliary}}{Qp + fu,p \times intrinsic\ Cl'_{biliary}} \quad (3)$$

where Qp and fu,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 following 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 canalicular 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 canalicular networks of sandwich-cultured human hepatocytes after a 10-min incubation with CDFDA (Fig. 1). [^{3}H]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 canalicular networks, were in good agreement with 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 μM in human hepatocytes, respectively. K_m values for valsartan uptake in HEK293 cells expressing OATP1B1 and OATP1B3 were 1.4 and 18 μM, respectively (Yamashiro et al., 2006). K_m values for rosuvastatin uptake in HeLa cells expressing OATP1A2, OATP1B1, OATP1B3 and OATP2B1 were 2.6, 4-7.3, 9.8 and 2.4 μM, respectively (Ho et al., 2006). In the present studies, 5 μM 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 to 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 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 μl/min/10^6cells (Nakagomi-Hagihara et al., 2006); pravastatin: 0.89 μl/min/10^6cells (Nakai et al., 2001); pitavastatin: 27 μl/min/10^6cells (Fujino et al., 2004); no data were available for valsartan and rosuvastatin].

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

For a given ARB or statin, accumulation and intrinsic $Cl_{biliary}$ values from the three different human livers varied by <5-fold and <4-fold, respectively. Approximately 5-fold variability also was observed for the uptake of estradiol-17β-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 organic anion transporting polypeptides (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 (MDR) 1 and breast cancer resistance protein (BCRP) also are 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 inter-liver variability.

The relationship between predicted $Cl_{biliary}$ 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, since $Cl_{biliary}$ for these compounds has not been measured directly in humans. The assumption in this study that *in vivo* hepatic clearance is equivalent to $Cl_{biliary}$ 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 $Cl_{biliary}$ values and estimated *in vivo* hepatic clearance values for these drugs based on Equation (3) assuming fu,p was unity (data not shown). According to the present data, when *in vivo* $Cl_{biliary}$ values are predicted based on sandwich-cultured human hepatocytes data, the unbound fraction should be included as shown in Equation (3). Abe et al. (2008) reported that the predicted $Cl_{biliary}$ values, corrected for plasma unbound fraction, from sandwich-cultured rat hepatocytes based on 10-min incubations at 5 μM substrate concentration correlated well with the *in vivo* $Cl_{biliary}$ values for these drugs in rats.

The rank order of the predicted $Cl_{biliary}$ values was consistent with the estimated *in vivo* hepatic clearance values for these drugs, even though 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* $Cl_{biliary}$ 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 $Cl_{biliary}$ values were 5-30-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 to 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 likely that a scaling factor might be necessary for the prediction of *in vivo* biliary clearance. Although unlikely, it is possible that the underestimation of $Cl_{biliary}$ is due to more extensive metabolism of these compounds than previously recognized. According to the information on the FDA 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 (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.

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%), even though the published *in vitro* intrinsic $Cl_{biliary}$ of taurocholate (5.8-10 µl/min/mg protein; Bi et al., 2006) was slightly lower than our results (12-25 µl/min/mg protein). However, the BEI (43-58%) and the *in vitro* intrinsic $Cl_{biliary}$ (4.0-12 µl/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 µl/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 vs Day 7-10). Previously, we confirmed that sandwich-cultured human hepatocytes cultured for 7-10 days maintain good morphology, form distinct bile canalicular networks, and express functional transport proteins on the proper membrane domain (Hoffmaster et al., 2004).

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 µl/min/mg protein, respectively, based on data generated from 10-min incubations at 5 µM substrate concentration (Abe et al., 2008). Differences in the *in vitro* intrinsic $Cl_{biliary}$ in sandwich-cultured human hepatocytes (0.514, 0.654, 0.264, 1.85 and 2.99 µl/min/mg protein, respectively), might be due to differences in the contribution of transport proteins involved between rats and humans. For example, transport properties of human OATPs may be difficult to predict from rat data since 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.

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Footnote

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Dr. Kim Brouwer is a co-founder 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®).

Legends for Figures

Figure 1. Retention of carboxydichlorofluorescein (CDF) in bile canaliculi of sandwich-cultured human hepatocytes. Sandwich-cultured human hepatocytes were incubated with CDFDA (10 μM) in standard buffer for 10 min. Representative images of CDF fluorescence in hepatocyte cultures from Liver 2 (A) and Liver 3 (B).

Figure 2. Accumulation [cells + bile (solid bars) and cells (white bars)] and biliary excretion index of taurocholate (A), olmesartan (B), valsartan (C), pravastatin (D), rosuvastatin (E) and pitavastatin (F) in sandwich-cultured human hepatocytes. Dosing concentration: taurocholate (1 μM), ARBs and statins (5 μM); Incubation time: 10 min. Data represent mean + SD (triplicate).

Figure 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 ± SEM of three individual experiments in triplicate. When not visible, error bars are within the size of the symbol. Pitavastatin data was excluded from the plot because published information was not available to estimate *in vivo* hepatic clearance in humans.

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TABLE 1

Demographics of human liver donors[a]

Liver identification	Age (years)	Gender	Race	Co-medications
1	57	Female	African American	Metoclopramide, Docusate, Acetaminophen, Hydrocodone
2	64	Female	Caucasian	Atorvastatin, Alendronate, Amlodipine, Benazepril
3	51	Male	Caucasian	Glyburide, Metformin, Pravastatin, Bevacizumab, Pioglitazone

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

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TABLE 2

Biliary excretion index (BEI), *in vitro* intrinsic $Cl_{biliary}$ and predicted $Cl_{biliary}$ compared with estimated *in vivo* hepatic clearance for selected angiotensin II receptor blockers and HMG-CoA reductase inhibitors in humans. Data represent mean ± SEM of three individual experiments in triplicate.

Compound	BEI (%)	*In vitro* intrinsic $Cl_{biliary}$ (ml/min/kg)[a]	Plasma unbound fraction (fu,p)	Predicted $Cl_{biliary}$ (ml/min/kg)[b]	Estimated *in vivo* hepatic clearance (ml/min/kg)[c]
Olmesartan	34.6 ± 3.0	0.943 ± 0.330	0.01[d]	0.00942 ± 0.00329	0.205
Valsartan	23.0 ± 3.4	1.20 ± 0.33	0.059[e]	0.0702 ± 0.0193	0.370
Pravastatin	30.9 ± 3.1	0.484 ± 0.061	0.5[f]	0.237 ± 0.029	7.20
Rosuvastatin	24.6 ± 4.7	3.39 ± 0.89	0.12[g]	0.390 ± 0.098	8.38
Pitavastatin	15.6 ± 2.7	5.48 ± 1.21	0.004[h]	0.0219 ± 0.0048	No data

[a]*In vitro* intrinsic $Cl_{biliary}$ values were calculated according to Equation (2). [b]Predicted $Cl_{biliary}$ values were calculated according to Equation (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

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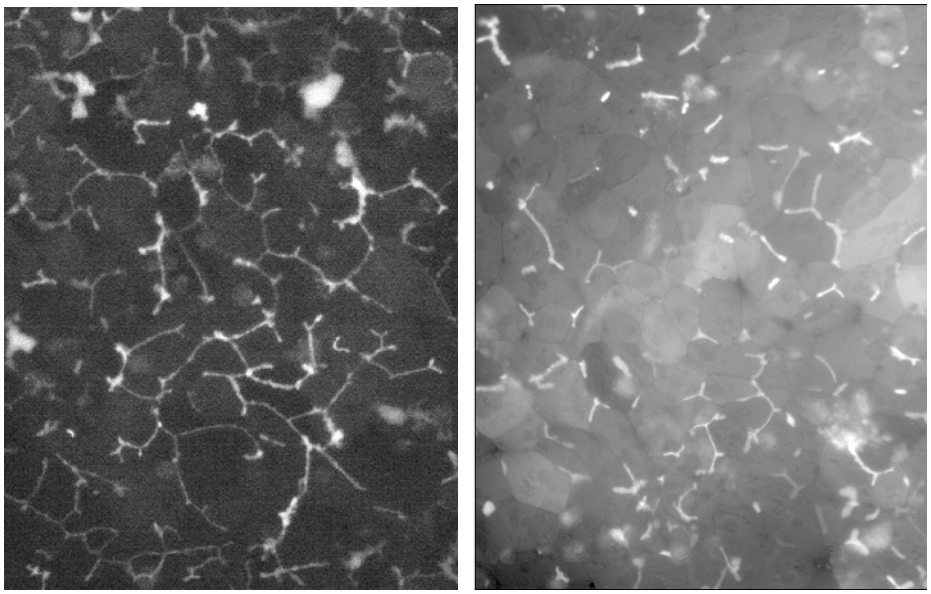

Figure 1

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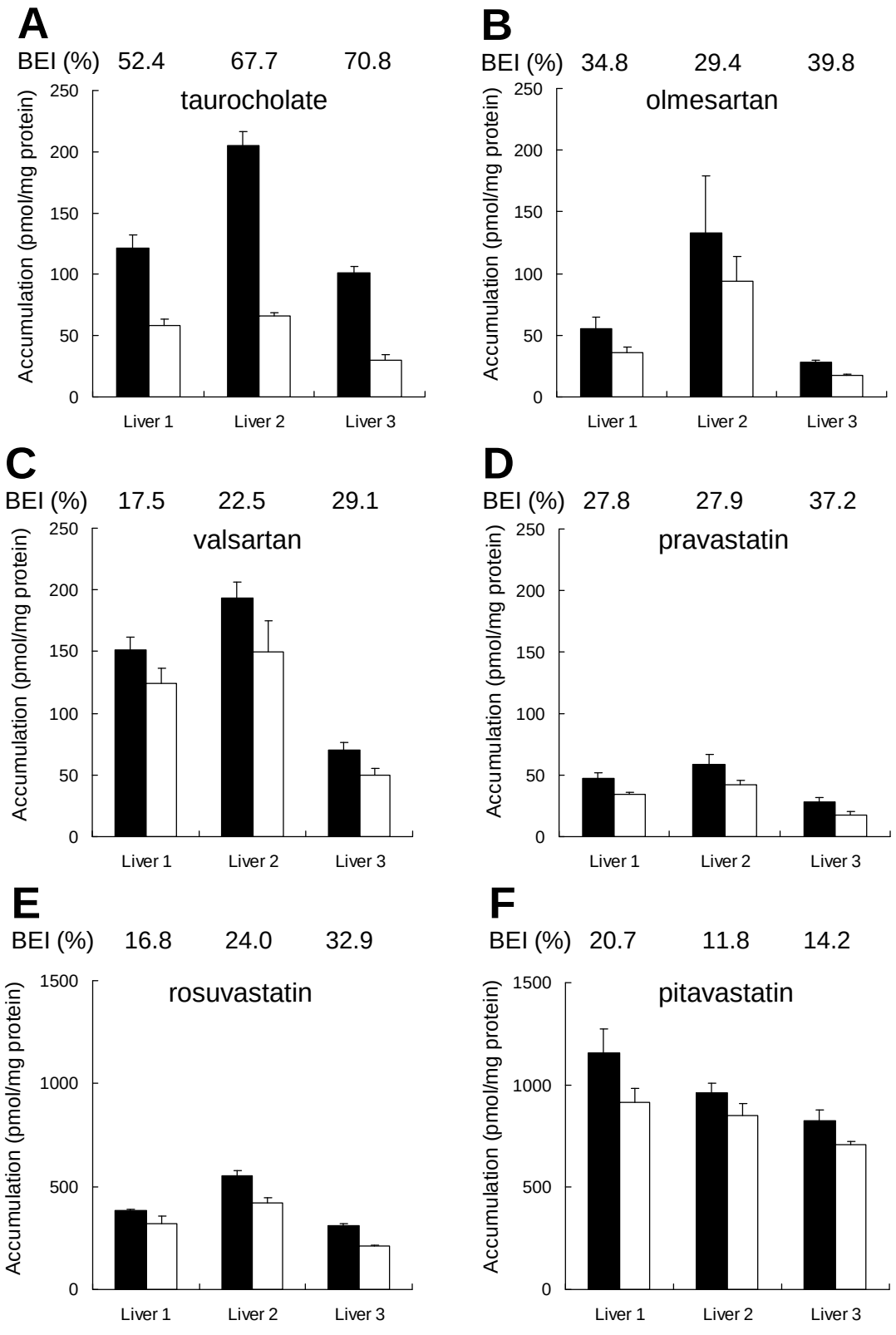

Figure 2

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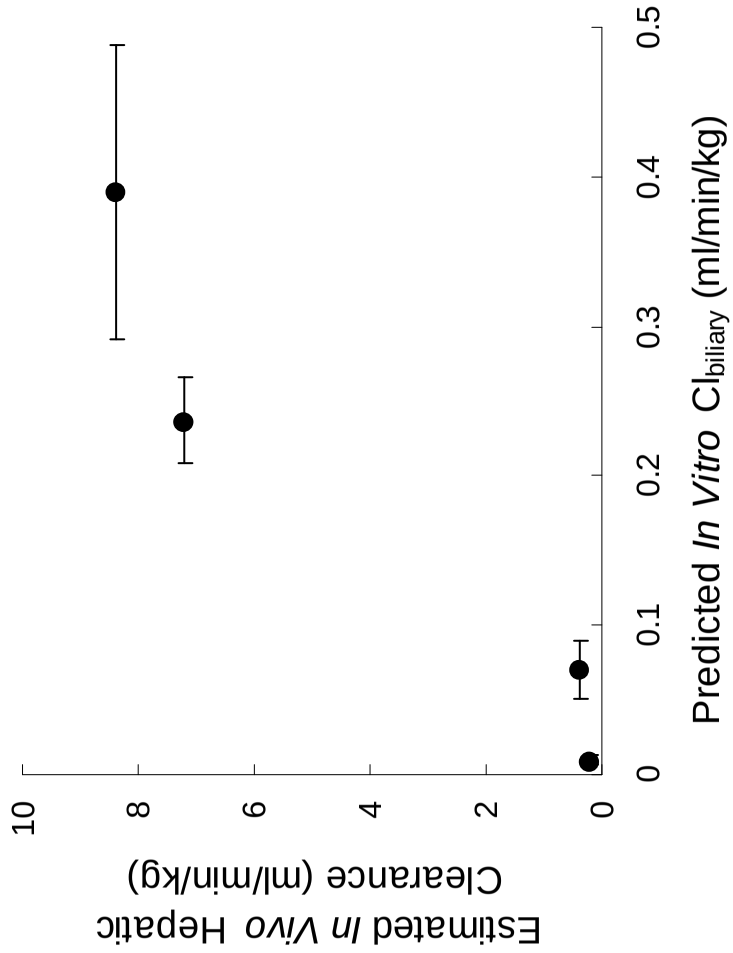

Figure 3