Effect of Plasma Protein Binding on in Vitro-in Vivo Correlation of Biliary Excretion of Drugs Evaluated by Sandwich-Cultured Rat Hepatocytes

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Received September 26, 2007; accepted April 2, 2008

ABSTRACT:

In the present study, we examined in vitro biliary clearance of several compounds in sandwich-cultured rat hepatocytes (SCRH) and compared it with that observed in vivo in rats; the effect of plasma protein binding on in vitro-in vivo correlation of biliary excretion was also assessed. The in vitro biliary excretion was determined by differential cumulative uptake of compounds in SCRH preincubated in the presence and absence of Ca\(^{2+}\)/Mg\(^{2+}\). The cumulative uptake study of radiolabeled substrates revealed that the function of canalicular efflux transporters such as bile salt export pump, multidrug resistance-associated protein 2, breast cancer resistance protein, and multidrug resistance 1 was adequately maintained in SCRH. Unlabeled test compounds, pravastatin, rosuvastatin, valsartan, cefmetazole, and cepofamexone exhibited varying degrees of in vitro biliary excretion in the cumulative uptake study using SCRH. In vivo biliary excretions of these compounds were measured in common bile duct-cannulated rats. Whereas their biliary excretion ratios were all more than 60% of the dose, the in vivo intrinsic biliary clearances varied from 10.5 to 1787.2 ml/min/kg. The in vitro intrinsic biliary clearances of test compounds were well correlated with their corresponding in vivo intrinsic clearances calculated on the basis of the plasma unbound concentration ($r^2 = 0.984$), whereas less correlation was observed when they were calculated on the basis of plasma total concentration ($r^2 = 0.217$). These results indicate that SCRH is a useful in vitro model for predicting in vivo intrinsic biliary clearance in rats. In addition, for an accurate prediction, it is necessary to evaluate the in vivo intrinsic biliary clearance based on plasma unbound concentration but not total concentration.

Biliary excretion is one of the important routes for drug elimination (Levine, 1978; Rollins and Klaassen, 1979). Therefore, elucidating the biliary excretion properties of new chemical entities (NCEs) is a critical issue in the drug discovery and development stage. Several experimental methods have been used to investigate the mechanism of biliary excretion, including liver canalicular membrane vesicles, isolated and cultured hepatocytes, isolated perfused liver, and bile duct-cannulated animals (Kukongviriyapan and Stacey, 1990; Tamai et al., 1999a; Handler et al., 1994; Chandra et al., 2005; Tsuda-Tsukimoto et al., 2006). However, because of the lack of acceptable throughput, development of more suitable methods for evaluating the hepatobiliary disposition of NCEs in the drug discovery and development stage is desired.

In view of convenience and an advantage of accessibility, primary cultured rat hepatocytes have been expected to be a potential in vitro tool for evaluating hepatobiliary disposition; however, primary cultured rat hepatocytes are well known for rapidly losing cell polarity and liver-specific functions such as albumin secretion, hepatic uptake, and enzyme activity under conventional monolayer culture conditions (Foliot et al., 1985; Dunn et al., 1989). In contrast with conventional conditions, hepatocytes cultured in a collagen-sandwich configuration were reported to maintain the liver-specific functions for several days and exhibit a three-dimensional configuration, formation of bile canaliculi, and proper localization of drug efflux transporters on the canalicular membrane (LeCluyse et al., 1994; Talamini et al., 1997).

Brouwer and colleagues reported an application of sandwich-cultured rat hepatocytes (SCRH) to evaluate biliary excretion (Liu et al., 1999a). In their technique, biliary excretion in SCRH was assessed by cumulative uptake of a compound in the presence (a standard condition) and absence of Ca\(^{2+}\)/Mg\(^{2+}\) in the incubation medium. The cumulative uptake in the standard condition represented the amount of compound in both cytosol and the bile canalicular lumen, whereas that in Ca\(^{2+}\)/Mg\(^{2+}\)-free condition represented the amount only in cytosol because the tight junction of bile canaliculi was disrupted under this condition. Therefore, the amount of compound excreted into the bile canalicular lumen was estimated from the differential uptake of compound between standard and Ca\(^{2+}\)/Mg\(^{2+}\)-free conditions.

Recently, sandwich-cultured human hepatocytes have been used to evaluate human biliary excretion (Bi et al., 2006). Establishing an in

ABBREVIATIONS: NCEs, new chemical entities; SCRH, sandwich-cultured rat hepatocytes; Bsep, bile salt export pump; Mrp, multidrug resistance-associated protein; Bcrp, breast cancer resistance protein; DMEM, Dulbecco’s modified Eagle’s medium; HBSS, Hank’s balanced salt solution; CDF, 5 (and 6)-carboxy-2’,7’-dichlorofluorescein; BEI, biliary excretion index; Mdr, multidrug resistance.
vitro-in vivo correlation of biliary excretion is required for the prediction of human hepatobiliary excretion. Previously, the in vitro-in vivo correlation of biliary excretion for Bsep and Mrp2 substrates was evaluated using radiolabeled substrates in SCRH (Liu et al., 1999b). However, the in vivo intrinsic biliary clearance was calculated on the basis of the plasma total concentration without consideration of the plasma unbound fraction in the correlation study. In addition, although Bcrp is also an important transporter for the biliary excretion of drugs (Hirano et al., 2005; Zamek-Gliszczynski et al., 2005; Ando et al., 2007), the correlation of Bcrp substrates has not been examined. Furthermore, when the SCRH system is applied to the drug discovery process, it is a great advantage if the assay system is available for unlabeled compounds.

In the present study, we investigated the in vitro biliary clearance of several unlabeled compounds including rosuvastatin as a rat Bcrp substrate (unpublished observation) in SCRH and compared it with their corresponding in vivo intrinsic clearance. Furthermore, the influence of plasma protein binding on the in vitro-in vivo correlation of biliary clearance was assessed. Test compounds such as pravastatin, rosuvastatin, valsartan, cefmetazole, and cefoperazone that were known to be mainly excreted into bile in rats were chosen for the correlation study (Pattinson et al., 1987; García-Agundez et al., 1992; Komai et al., 1992; Nezasa et al., 2002; Yamashiro et al., 2006).

**Materials and Methods**

**Chemicals.** [3H]Taurocholic acid (50 Ci/nmol; purity 99%) and [3H]Ala-tyrosine [50 Ci/nmol; purity 99%] were obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). [3H]Estradiol 17β-d-glucuronide (53 Ci/nmol; purity 97%), [3H]Estrone 3-sulfate (57.3 Ci/nmol; purity 97%), and [3H]Dexamethasone (23.5 Ci/nmol; purity 97%) were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). Dulbecco’s modified Eagle’s medium (DMEM), William’s medium E, standard Hanks’ balanced salt solution (GIBCO, Life Technologies), bovine serum albumin (BSA; Sigma-Aldrich, Inc., Tokyo, Japan). Fetal bovine serum was purchased from Nissui Bioscience (Tokyo, Japan). Sodium selenite, dexamethasone, pravastatin, and cefmetazole, and cefoperazone were purchased from Sigma-Aldrich, Inc. (Tokyo, Japan). Betaine was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Rosuvastatin and valsartan were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). BCA protein assay kit was used to determine the protein concentration in the cell lysate. For unlabeled compounds, cells were lysed with 0.5 ml of 1% Triton X-100 in phosphate-buffered saline by shaking for 20 min at room temperature. An aliquot of lysate was analyzed by liquid scintillation spectrometry. A BCA protein assay kit was used to determine the protein concentration in the cell lysate. For unlabeled compounds, cells were lysed with 0.5 ml of 1% Triton X-100 in phosphate-buffered saline by shaking for 20 min at room temperature. An aliquot of lysate was analyzed by liquid scintillation spectrometry. A BCA protein assay kit was used to determine the protein concentration in the cell lysate. For unlabeled compounds, cells were lysed with 0.5 ml of 1% Triton X-100 in phosphate-buffered saline by shaking for 20 min at room temperature. An aliquot of lysate was analyzed by liquid scintillation spectrometry. A BCA protein assay kit was used to determine the protein concentration in the cell lysate. For unlabeled compounds, cells were lysed with 0.5 ml of 1% Triton X-100 in phosphate-buffered saline by shaking for 20 min at room temperature. An aliquot of lysate was analyzed by liquid scintillation spectrometry. A BCA protein assay kit was used to determine the protein concentration in the cell lysate. For unlabeled compounds, cells were lysed with 0.5 ml of 1% Triton X-100 in phosphate-buffered saline by shaking for 20 min at room temperature. An aliquot of lysate was analyzed by liquid scintillation spectrometry. A BCA protein assay kit was used to determine the protein concentration in the cell lysate. For unlabeled compounds, cells were lysed with 0.5 ml of 1% Triton X-100 in phosphate-buffered saline by shaking for 20 min at room temperature. An aliquot of lysate was analyzed by liquid scintillation spectrometry. A BCA protein assay kit was used to determine the protein concentration in the cell lysate.
column (50 mm × 2.1 mm, i.d., 1.7-μm particle size; Waters) maintained at 40°C. The initial mobile phase was 95% 20 mM ammonium acetate and 5% acetonitrile and the proportion of acetonitrile was linearly increased to 90% over 3 min with the flow rate of 0.3 ml/min. The autosampler was conditioned at 8°C with the injection volume of 3 μl.

Mass spectrometry analysis was performed on a Quattro Premier XE tandem quadrupole mass spectrometer (Waters) using an electrospray source in positive or negative ion mode. The ionization source parameters were capillary voltage 1.0 kV; source temperature 120°C; desolvation gas temperature 400°C at a flow rate of 1000 liters/h (nitrogen) and a cone gas flow rate of 50 liters/h. Nitrogen (99.9% purity) and argon (99.9999% purity) were used as cone and collision gases, respectively. The acid form was monitored in the measurement of pravastatin concentration-time curve), Vdss (distribution volume), CLtot (total body clearance), CLbile (intrinsic biliary clearance), CLuptake (intrinsic uptake clearance) at 18°C with the injection volume of 3 μl.

Data Analysis. Apparent intrinsic biliary clearance is defined as intrinsic biliary clearance in the present study. In vitro intrinsic uptake clearance (CLuptake, int) and intrinsic biliary clearance (CLbile, int) were estimated according to eq.1 and eq.2, respectively. Rat liver weight and protein content in liver tissue were assumed to be 40 g/kg b.wt. and 200 mg of protein/g liver weight, respectively, in each calculation (Seglen, 1976; Davies and Morris, 1993).

\[ CL_{\text{uptake, int}} = \frac{\Delta \text{uptake} (+ \text{Ca}^{2+}/\text{Mg}^{2+} \text{ at 2-0 min})}{\text{incubation time} \times \text{concentration} \text{medium}} \]  

where \( \Delta \text{uptake} \) represents the differences of uptake amount between 0 and 2 min and (medium) represents the concentration in the incubation medium at 0 min.

\[ CL_{\text{bile, int}} = \frac{\text{uptake} (+ \text{Ca}^{2+}/\text{Mg}^{2+}) - \text{uptake} (- \text{Ca}^{2+}/\text{Mg}^{2+})}{\text{incubation time} \times \text{concentration} \text{medium}} \]  

where uptake \((+ \text{Ca}^{2+}/\text{Mg}^{2+})\) and uptake \((- \text{Ca}^{2+}/\text{Mg}^{2+})\) represent the cumulative uptake of compound in SCRH preincubated in the presence and absence of \(\text{Ca}^{2+}/\text{Mg}^{2+}\), respectively. The intrinsic biliary clearance was determined at 10 min. Biliary excretion was quantitatively assessed by the biliary excretion index (BEI) at the time point of 10 min based on eq. 3 (Liu et al., 1999c). BEI was determined using B-CLEAR technology (Qualyst, Inc., Raleigh, NC).

\[ \text{BEI} = \frac{\text{uptake} (+ \text{Ca}^{2+}/\text{Mg}^{2+}) - \text{uptake} (- \text{Ca}^{2+}/\text{Mg}^{2+})}{\text{uptake} (+ \text{Ca}^{2+}/\text{Mg}^{2+})} \]  

The in vivo kinetic parameters such as AUCinf (area under the plasma concentration-time curve), Vdss (distribution volume), CLtot (total body clearance), \(C_{f}\) (initial concentration) and \(t_{1/2g}\) (plasma half-life) were calculated by a model-independent method using the MULTI computer program (Yamaoka and Nakagawa, 1983). The in vivo biliary clearance (CLbile) was calculated according to eq. 4

\[ CL_{\text{bile}} = \frac{\text{accumulation in bile (180 min)}}{\text{AUC (180 min)}} \]  

where accumulation in bile (180 min) represents the amount of compounds excreted into bile for 180 min and AUC (180 min) represents the area under the plasma concentration-time curve from 0 to 180 min.

The in vivo intrinsic biliary clearance was calculated by eq. 5a and 5b using the well-stirred model, where \( Q_h \) represents rat hepatic blood flow, 67 ml/min/kg (Nies et al., 1976), and \( f_p \) and \( R_b \) represent plasma unbound fraction and blood/plasma ratio of test compounds, respectively. In these equations, \( CL_{\text{bile, int}} \) and \( CL_{\text{bile, int}}^* \) represent the intrinsic biliary clearance calculated on the basis of plasma unbound concentration and plasma total concentration, respectively.

\[ CL_{\text{bile, int}} = \frac{Q_h \times CL_{\text{bile, int}}^*}{Q_h - CL_{\text{bile, int}}^*} \times \frac{1}{f_p} \]  

\[ CL_{\text{bile, int}}^* = \frac{Q_h \times CL_{\text{bile}}}{Q_h - CL_{\text{bile}}} \]  

Results

Cell Morphology and Biliary Excretion of CDF in SCRH. Figure 1 shows a phase-contrast image of SCRH cultured for 4 days (left) and fluorescence images after a 20-min preincubation with 10 μM CDF diacetate in standard buffer (middle) and Ca2+/Mg2+-free buffer (right). The formation of bile canaliculi lumen was clearly observed in SCRH. The fluorescent Mrp2 substrate, CDF (Kitamura et al., 1990), was localized in hepatocytes and the bile canalicular lumen when it was incubated in the standard condition. In contrast, CDF localization in the bile canalicules lumen but not in hepatocytes disappeared in Ca2+/Mg2+-free condition. These results suggest that the bile canaliculi and intact tight junctions were reestablished and that Mrp2 functioned to accumulate the substrate into the canalicular lumen in SCRH used in this study.

Cumulative Uptake of the Radiolabeled Substrates in SCRH. Several radiolabeled compounds were chosen to assess the function of efflux transporters in SCRH. [3H]Taurocholic acid, [3H]estradiol-17β-d-glucuronide, [3H]estrone 3-sulfate, and [3H]digoxin were used as a substrate of Bsep, Mrp2, Bcrp, and Mdr1, respectively. Time courses of cumulative uptake of these compounds are shown in Fig. 2. The cumulative uptake in the standard condition was significantly higher than that in the Ca2+/Mg2+-free condition. The BEI is summarized in Table 1. The BEI of [3H]salicylic acid showed the lowest
value of 4.4%, which is consistent with this compound not being excreted into bile in vivo.

**Cumulative Uptake of the Unlabeled Compounds in SCRH.**

Figure 3 shows the cumulative uptake of unlabeled compounds that are known to be mainly excreted into bile in vivo, such as pravastatin, rosuvastatin, valsartan, cefmetazole, and cefoperazone. There were differences in cumulative uptake of compounds under standard and Ca<sup>2+</sup>/Mg<sup>2+</sup>-free conditions except for cefmetazole. Table 2 summarizes intrinsic uptake clearance, intrinsic biliary clearance, and BEI of test compounds in SCRH. The intrinsic uptake clearance, which represents the sinusoidal uptake in hepatocytes, was high for rosuvastatin, intermediate for pravastatin and valsartan, and low for cefmetazole and cefoperazone. BEI, which represents canalicular efflux, was high for rosuvastatin, cefoperazone, pravastatin, and valsartan and low for cefmetazole. The intrinsic biliary clearance, which represents the net biliary excretion, was high for rosuvastatin, intermediate for pravastatin and valsartan, and low for cefmetazole and cefoperazone.

**Plasma Concentration and Cumulative Biliary Excretion of Unlabeled Compounds in Rats.**

Pravastatin, rosuvastatin, valsartan, cefmetazole, and cefoperazone were administered i.v. to common bile duct-cannulated rats at the dose of 1 mg/kg. The plasma concentration-time curve and the cumulative biliary excretion-time curve of test compounds were shown in Fig. 4. Pharmacokinetic and biliary excretion parameters of test compounds are summarized in Table 3. All compounds were rapidly eliminated with a plasma half-life within 40 min. Biliary excretions of these compounds were all >60% of dose.

As shown in Table 3, the plasma protein binding ratio was extremely high for valsartan, modestly high for rosuvastatin, and low for others. Especially large differences were observed between CL<sub>bile, int</sub> and CL<sub>bile, un</sub> of valsartan and rosuvastatin because of their high protein binding. The blood/plasma ratios of pravastatin, rosuvastatin, valsartan, cefmetazole, cefoperazone, and taurocholic acid were 0.65, 0.67, 0.60, 0.66, 0.57, and 0.63, respectively. All test compounds were less distributed to the blood cells.

**Comparison of Biliary Excretion between in Vitro and in Vivo.**

The parameters of in vitro and in vivo biliary excretion of test compounds were compared by several approaches. The following in vivo parameters of taurocholic acid were obtained from the literature: biliary excretion ratio, biliary clearance, and plasma unbound fraction were 100%, 29.8 ml/min/kg, and 0.15, respectively (Inoue et al., 1985). Based on these parameters, the in vivo intrinsic biliary clearance calculated from eq. 5a and eq. 5b were 675.7 and 160.9 ml/min/kg, respectively. Figure 5A shows a comparison of the in vivo biliary excretion ratio and the BEI obtained from in vitro study. No clear correlation was observed between these parameters (\( r^2 = 0.638 \)). Furthermore, the in vitro intrinsic biliary clearances of test compounds were compared with their corresponding in vivo clearances calculated on the basis of plasma total or unbound concentration (Fig. 5, B and C). There was poor in vitro-in vivo correlation (\( r^2 = 0.217 \)) when the in vivo intrinsic biliary clearances were calculated on the basis of plasma total concentration (CL<sub>bile, int</sub>) (Fig. 5B). In contrast, the in vitro intrinsic biliary clearances were well correlated (\( r^2 = 0.984 \)) when the in vivo intrinsic biliary clearances were calculated on the basis of plasma unbound concentration (CL<sub>bile, un</sub>) (Fig. 5C).
Discussion

In the present study, the in vitro biliary excretion was evaluated using SCRH to predict biliary excretion in vivo. First, we investigated whether the SCRH prepared in our study was capable of investigating in vitro biliary excretion or not. The phase-contrast microscopy study revealed that the SCRH exhibited liver-like cell morphology and the formation of bile canaliculi (Fig. 1, left). When the transporters are expressed and tight junctions are formed adequately, the accumulation of substrates of hepatic efflux transporters can be detected in the bile canalicular lumen; however, the accumulation of substrates in the bile canalicular lumen is not observed under the Ca\(^{2+}\)/Mg\(^{2+}\)-free condition, because the integrity of the tight junctions is Ca\(^{2+}\)/Mg\(^{2+}\)-dependent. As an example, the fluorescent Mrp2 substrate, CDF, was accumulated in bile canalicular lumen under the standard condition (Fig. 1, middle). However, the accumulation of fluorescence was not observed under the Ca\(^{2+}\)/Mg\(^{2+}\)-free condition (Fig. 1, right). These
results demonstrated that our SCRH reestablished bile canaliculi and intact tight junctions and that it maintained the functional activity of Mrp2.

Polarized excretion by canicular membrane transporters is essential for biliary excretion of compounds. Bsep, Mrp2, Bcrp, and Mdr1 are dominant transporters responsible for biliary excretion of endogenous and xenobiotic substances (Chanda and Brouwer, 2004). As shown in Fig. 2 and Table 1, taurocholic acid, estradiol-17ß-glucuronide, estrone 3-sulfate, and digoxin, which are substrates for Bsep, Mrp2, Bcrp, and Mdr1, respectively, were excreted into bile canicular lumen in SCRH. These observations indicated that our SCRH expressed the functional canicular transporters.

Next, SCRH were applied for the in vitro biliary excretions of unlabeled compounds. The biliary excretion of pravastatin, rosuvastatin, valsartan, and cefoperazone was clearly observed in SCRH, whereas it was negligible in case of cefmetazole (Fig. 3). The follow-

ing three parameters were determined in the present study using SCRH: 1) intrinsic uptake clearance for sinusoidal uptake from incubation medium into the cells, 2) BEI for translocation from hepatic intracellular space to bile canicular lumen, and 3) intrinsic biliary clearance for net biliary excretion from incubation medium to bile canicular lumen (Table 2). Rosuvastatin showed high biliary excretion, sinusoidal uptake, and canicular efflux. Pravastatin and valsartan exhibited intermediate biliary excretion, sinusoidal uptake, and canicular efflux. The higher uptake of rosuvastatin compared with pravastatin is consistent with the results of a previous report (Nezasa et al., 2003). Biliary excretions of cefmetazole and cefoperazone were low compared with those of rosuvastatin, pravastatin, and valsartan as were their sinusoidal uptake. Whereas the sinusoidal uptake of cef-

operazone into hepatocytes was lower than that of cefmetazole, the canicular efflux of cefoperazone was fairly higher than that of cefmetazole as reported previously (Nakakariya et al., 2008; Kato et al., 2008). Consequently, net biliary excretion of cefoperazone was higher than that of cefmetazole. These results suggest that the canicular membrane transport as well as sinusoidal uptake greatly affects the intrinsic biliary clearance of drugs.

It is important to note that the actual intrinsic biliary clearance should be determined by the transport across the canicular membrane based on the intracellular unbound concentration of compounds. However, it is practically impossible to determine experimentally the intracellular unbound concentration of compounds in both in vitro and in vivo studies, because many compounds could bind to various intracellular components such as proteins, lipids, DNAs, and others. Accordingly, in vitro biliary intrinsic clearance was calculated on the basis of the unbound concentration in incubation medium instead of the intracellular unbound concentration. Similarly, in vivo biliary intrinsic clearance was calculated on the basis of the plasma unbound concentration. Therefore, the in vitro and in vivo intrinsic biliary clearance in this study should be considered as an “apparent” intrinsic biliary clearance.
As shown in Fig. 4 and Table 3, pravastatin, rosuvastatin, valsartan, cefmetazole, and ceferozone were excreted mainly into bile with comparable values of their biliary excretion ratios that ranged from 62.7 to 86.3%. In contrast, their biliary intrinsic clearances showed more variability, ranging from 10.5 to 1787.2 ml/min/kg. Even though biliary intrinsic clearance was low, compounds could exhibit high biliary excretion ratios as observed for cefmetazole and ceferozone. Thus, the biliary excretion ratio was determined not only by biliary intrinsic clearance but also by other factors, such as plasma protein binding, hepatic blood flow, and metabolic and renal clearances.

In the present study, the in vitro and in vivo biliary excretions were compared by several approaches. When the in vivo biliary excretion ratio was compared with BEI, there was no good relationship between them (Fig. 5A). As described previously, BEI reflected the translocation of compound from intracellular space to canalicular lumen across canalicular membrane. At present, it is difficult to predict the in vivo biliary excretion ratio directly from the parameters of SCRH. Subsequently, we also compared the in vitro intrinsic biliary clearance with that in vivo. The in vitro intrinsic biliary clearances of test compounds were well correlated with their corresponding in vivo clearances when the in vivo clearances were calculated on the basis of plasma unbound concentrations ($r^2 = 0.984$), whereas less correlation was observed when they were calculated on the basis of plasma total concentrations ($r^2 = 0.217$). It has been well recognized that only protein-unbound substrates but not protein-bound ones are transported and metabolized (Obach, 1999; Takahashi et al., 1999; Takeda et al., 2002). The in vitro biliary excretion was determined under protein-free conditions in the present SCRH experiments. Therefore, in vitro results should be compared with in vivo biliary excretion profiles based on the plasma unbound fraction. These results indicated that it was essential for a good in vitro-in vivo correlation to consider the plasma unbound fraction and that the in vitro intrinsic biliary clearance was the best parameter for the prediction of the in vivo biliary excretion profile.

In our preliminary study using rat Bcrp-expressing membrane vesicles, it was shown that rosuvastatin could be a substrate of rat Bcrp (unpublished observation). This is the first report to successfully demonstrate the good in vitro-in vivo correlation of intrinsic biliary clearance using a Bcrp substrate. Our SCRH system turned out to be applicable to a diverse range of compounds in drug discovery and development stages.

To apply the SCRH assay system to drug discovery, we made some improvement in the present study. Instead of 60-mm plastic culture dishes with a thick gelled collagen substratum and an overlaid thick gelled collagen, we used 24-well plates with a rigid collagen substratum (collagen-coated plate) and an overlaid thin collagen layer according to a previous report (Sudhu et al., 1993). The application of 24-well plates reduced the amount of hepatocytes and test compounds. In addition, it was easier to keep the configuration of the collagen layer in a thin form than in a thick one. In addition, LC-MS/MS enabled us to quantify the uptake amount of unlabeled compounds at low nanomolar concentrations and to perform the assay with acceptable throughput. The present modified experimental methods of SCRH help us apply the in vitro system to an early drug discovery stage, which requires high throughput and generality.

In a drug discovery and development stage, it is also important to elucidate whether the elimination pathway of NCEs is single or multiple. It is undesirable for NCEs to have a single elimination pathway from the viewpoint of the alteration of elimination clearance caused by drug-drug interactions and/or genetic polymorphisms on drug transporters and/or metabolizing enzymes. By comparing the present prediction of in vivo biliary clearance with in vivo metabolic clearance predicted from conventional experiments, the contribution of biliary excretion to total clearance can be estimated. Therefore, the sandwich culture system is a useful tool not only for the prediction of hepatobiliary disposition of NCEs but also for estimating the possible alteration of hepatic clearances.

In summary, the present study demonstrates that SCRH is a useful in vitro model to predict in vivo biliary excretion of a diverse range of compounds including Bcrp substrates. Plasma protein binding has a large impact on in vivo biliary intrinsic clearance, especially for highly bound compounds. In additional, to achieve an accurate prediction, it is necessary to calculate the in vivo intrinsic biliary clearance on the basis of plasma unbound concentration.

Acknowledgments. We gratefully acknowledge Dr. Hisako Fujimura and Naoko Murakami for supporting the isolation of rat hepatocytes and cell culture, Dr. Akira Saito for valuable advice, and Masaya Fukuda and Masao Yamanouchi for their expert technical assistance for the in vivo studies.

References


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