UTILITY OF HEPATOCYTES IN PREDICTING DRUG METABOLISM: COMPARISON OF HEPATIC INTRINSIC CLEARANCE IN RATS AND HUMANS IN VIVO AND IN VITRO

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ABSTRACT:

We investigated hepatic in vitro intrinsic clearance (CL_{int,in vitro}) in freshly isolated or cryopreserved hepatocytes and compared with CL_{int,in vivo} by using nine model compounds, FK1052, FK480, diazepam, diltiazem, troglitazone, quinotolact, FK079, zidovudine, and acetaminophen, in rats and humans. The compounds showed a broad range of in vivo hepatic extraction ratios (rat, 0.05–0.93; humans, 0.03–0.76) and were metabolized by hepatic P450, UDP-glucuronosyltransferase, sulfotransferase, and/or esterase. CL_{int,in vitro} was determined from substrate disappearance rate at 1 μM in hepatocytes. CL_{int,in vivo} was calculated from in vivo pharmacokinetic data using two frequently used mathematical models (the well stirred and dispersion models). When estimating rat CL_{int,in vitro} in freshly isolated hepatocytes, the rat scaling factor values (CL_{int,in vivo}/CL_{int,in vitro}) showed marked difference among the model compounds (0.2–73.1-fold). The rat CL_{int,in vitro} values in freshly isolated hepatocytes were in good agreement with these in cryopreserved hepatocytes. Human CL_{int,in vitro} were determined by use of cryopreserved hepatocytes. When human CL_{int,in vitro} was regarded as the predicted CL_{int,in vivo}, the observed and predicted CL_{int,in vivo} for FK1052, FK480, troglitazone, and FK079 differed markedly (12.4–199.0-fold). In contrast, using human CL_{int,in vitro} corrected with the rat scaling factors yielded better predictions of CL_{int,in vivo} that were mostly within 5-fold of the actual values. These results make the evaluation using hepatocytes more useful and provide a basis for predicting hepatic clearance using hepatocytes.

Recently, pharmacokinetic investigation has played an increasingly important role in drug discovery. In particular, it is very important to predict human hepatic metabolic clearance because most drugs are eliminated from the body predominantly by hepatic metabolism. For predicting hepatic clearance, theoretical aspects of in vitro/in vivo scaling based on a physiological model and clearance concepts have been developed (Roberts and Rowland, 1986). Application of this method has been successful in predicting in vivo hepatic clearance in rats for many drugs metabolized by P450 from in vitro metabolism studies and is based on the assumption that any in vitro-in vivo difference seen in humans is also apparent in animals to approximately the same degree.

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1 Abbreviations used are: P450, cytochrome P450; HPLC, high performance liquid chromatography; CL, plasma clearance; WME, Williams’ medium E; CLH1, hepatic clearance; CLint, intrinsic metabolic or hepatic clearance; CLR, renal clearance; DV, dispersion number; EX, hepatic extraction ratio; F, the fraction absorbed from the intestinal tract; fH, hepatic availability; fP, unbound fraction in plasma (or serum); fU, hepatic fraction in hepatocytes; QH, hepatic blood flow rate; RO, blood-to-plasma concentration ratio.
ulate the involvement of active transporters. Accordingly, isolated hepatocytes provide an in vitro system for studying the integrated metabolism and distribution of compounds. Some studies have reported the prediction of hepatic clearance from in vitro experiments using freshly isolated hepatocytes, especially in rats (Ashforth et al., 1995; Carlile et al., 1998). However, there has been limited application of the prediction using human freshly isolated hepatocytes (Bayliss et al., 1999). In general, there is limited availability of fresh human livers for research. Cryopreservation would greatly enhance the utility of human hepatocytes because cryopreserved hepatocytes could be used at any time for experiments. Successful cryopreservation of human hepatocytes has been reported by several researchers (Chesne et al., 1993; Coupondis et al., 1993). However, there have been few reports on the prediction of hepatic clearance using human cryopreserved hepatocytes.

In the present study, we examined CL_{int,in vitro} obtained from in vitro experiments using hepatocytes and CL_{int,in vivo} calculated from in vivo pharmacokinetic data with nine model compounds (FK1052, FK480, diazepam, diltiazem, troglitazone, quinotolast, FK079, zidovudine, and acetaminophen) in rats and humans. In particular, we evaluated CL_{int,in vitro} using cryopreserved hepatocytes in humans. At the same time, the measurement method of CL_{int,in vitro}, which is determined from substrate disappearance rate at 1 μM in hepatocytes, was used because it is a simple and useful method.

**Materials and Methods**

**Chemicals.** FK1052, FK480, troglitazone, quinotolast, and FK079 were synthesized by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). Diltiazem hydrochloride, zidovudine, and acetaminophen were purchased from Sigma-Aldrich (St. Louis, MO). Diazepam was purchased from Wako Pure Chemicals (Osaka, Japan). The other reagents and solvents used were of analytical and HPLC grade.

**Selection of Model Compounds.** FK1052, FK480, diazepam, diltiazem, troglitazone, quinotolast, FK079, zidovudine, and acetaminophen (Fig. 1) were selected as the model compounds based on the following criteria: clearance of the unchanged model compounds in hepatocytes were obtained. Each compound was incubated with a reaction mixture (500 μl) of hepatocyte suspension. After preincubation at 37°C for 5 min, the reactions were initiated by adding 5 μl of model compound solution in methanol. The final concentration of each model compound used was 1 μM. After incubation at 37°C for various time periods, the reactions of FK1052, FK480, diltiazem, diazepam, quinotolast, and troglitazone were centrifuged at 10,000g for 5 min, and an aliquot of the supernatant was injected into an HPLC for measuring the unchanged compound concentration. The reactions of FK079, zidovudine, and acetaminophen were processed by extraction. After adding each internal standard (FK117460 (FK079 derivative), 4-nitrophenol, and 3-acetaminophen, respectively), their organic fractions were evaporated under N₂ and the residues were reconstituted in the mobile phase (see below) for HPLC analysis.

**Determination of unchanged model compound concentrations.** An LC module 1 plus (Millipore Corporation, Bedford, MA) was used. The column for analysis was an Inertil ODS-3 (5 μm, 150 × 4.6 mm) (GL Science Inc., Tokyo, Japan). The flow rate was 1.0 ml/min. The mobile phase and detection wavelength for the analysis of each model compound were as follows: FK1052, mobile phase: buffer/CH₃CN (40:60), detection UV 242 nm; FK480, mobile phase: buffer/CH₃CN (40:60), detection UV 295 nm; diltiazem, mobile phase: buffer/CH₃CN (40:60), detection UV 240 nm; diazepam, mobile phase: buffer/CH₃CN (40:60), detection UV 254 nm; quinotolast; mobile phase: buffer/CH₃CN (70:30), detection, fluorescence, excitation 265 nm, emission 280 nm; acetaminophen, mobile phase: buffer/CH₃CN (50:50), detection UV 230 nm; FK079, mobile phase: buffer/CH₃CN (70:30), detection UV 280 nm; zidovudine, mobile phase: buffer/CH₃OH (80:20), detection UV 267 nm; acetaminophen, mobile phase: 0.5% acetic acid/CH₃CN (90:10), detection UV 254 nm; and buffer*: 5 mM phosphate buffer (pH 7.2).

All assay methods were in the concentration range of 0.1 to 2 μM. Reproducibility was evaluated by performing five replicate analyses of hepatocyte samples containing 0.1, 0.5, and 1 μM compound, respectively. The coefficient of variation was less than 10%, and the actual concentration of the compounds ranged from 97.5 to 106%. All assay methods thus provide good accuracy and precision.

**Calculation of CL_{int,in vitro}, CL_{int,in vivo}** values were calculated from substrate disappearance rate in hepatocytes as follows. If substrate disappearance can be assumed to follow first-order reaction, the unchanged drug profile as a function of time [C(t)] is described as follows.

\[
C(t) = C_i \times e^{-k_i \times t}
\]

where \(C_i\) is the initial concentration of the compound, \(k_i\) is the disappearance rate constant of unchanged drug (min⁻¹).

Furthermore, initial metabolic rate (V₀) (micromoles per minute per cell) is described by eq. 2:

\[
V_0 = k_i \times C_{i,cell}
\]

where \(C_{i,cell}\) is the cell density (cells per milliliter).

On the other hand, from the Michaelis-Menten equation, \(V_0\) is described by the eq. 3:

\[
V_0 = V_{max} \times C_i / (K_m + C_i)
\]

If the substrate concentration used in the experiments (1 μM) is below the \(K_m\) for the drug-metabolizing enzyme reactions, the drug concentration may be assumed to be much smaller than \(K_m\) (\(K_m \gg C_i\)). Thus, \(V_0\) can be expressed by eq. 4:

\[
V_0 = V_{max} \times K_m / C_i
\]

Consequently,

\[
CL_{int,in vitro} = V_{max} / (K_m \times C_i)
\]

\(CL_{int,in vitro}\) was thus calculated by eq. 5 based on the time course of unchanged drug concentrations by a least-square linear regression. The \(CL_{int,in vitro}\) values...
expressed per cell calculated from the in vitro metabolism experiments were expressed per kilogram of body weight by taking the number of hepatocytes per gram liver and the liver weight per kilogram of body weight shown in Table 1 into consideration.

**In Vivo Data. Sources of pharmacokinetic data.** In vivo clearance under linear conditions, \( f_a \) and \( R_B \) data, were obtained from in-house and published literature. The in vivo pharmacokinetic data were considered to be trustworthy because the in vivo pharmacokinetic experiments were performed based on good accuracy methods and the appropriate protocols. In vivo clearance value was calculated by dividing the dose by area under the curve. In the case of high-clearance compounds, \( CL_{oral} \) was used, because \( CL_{int,in\,vivo} \) calculated from \( CL_{tot} \) is affected by changes of \( Q_H \) (Iwatsubo et al., 1997). For FK480 and diazepam, which are intermediate-clearance drugs in rats, \( CL_{oral} \) was also used in the same way as the in vitro-in vivo scaling using hepatic microsomes (Naritomi et al., 2001). When the in vivo clearance value was not expressed per kilogram of body weight, this value was converted so that it was expressed per kilogram of body weight by taking the mean value of body weight in published literature or body weight of 250 g, 10 kg, and 70 kg for rats, dogs, and humans, respectively. \( F_a \) of FK1052, FK480, quinotolast, and FK079 were estimated by summing the recoveries of radioactivity in bile and urine after oral administration of \(^{14}\)C model compounds to rats from our own data. \( F_a \) for diazepam, diltiazem, troglitazone, zidovudine, and acetaminophen were estimated from published data. FK1052, FK480, diazepam, and diltiazem were evaluated as the compounds metabolized mainly by P450. Troglitazone, quinotolast, FK079, zidovudine, and acetaminophen were evaluated as the compounds metabolized by different drug-metabolizing enzymes (Table 2).

**Calculation of \( CL_{int,in\,vivo} \) values.** \( CL_H \) values were determined from eqs. 6 and 7 by use of the \( CL_{oral} \) and \( CL_{int,in\,vivo} \) values, respectively. For quinotolast, \( CL_H \) in rats were calculated from pharmacokinetic parameters after administration into femoral vein and hepatic portal vein (Katashima et al., 1993a). \( CL_H \) was considered to be negligible for FK1052, FK480, diazepam, diltiazem, quinotolast, troglitazone, and FK079.

\[
CL_H = (CL_{oral}/R_B) - CL_R
\]

**Fig. 1. Chemical structures of model compounds.**
In Vivo Pharmacokinetic Data for Model Compounds. In vivo pharmacokinetic data for the model compounds are summarized in Table 2. The fractions absorbed from the intestinal tract \((F_a)\) were high, in the range of 0.7 to 1.0. The values of unbound fraction in plasma (or serum) \((f_u)\) ranged widely from the highest values for zidovudine \((f_u, \text{rat}: 0.786; \text{human}, 0.8)\) and acetaminophen \((f_u, \text{rat}: 0.82; \text{human}, 0.79)\) to the lowest value for troglitazone \((f_u, \text{rat}: 0.000921; \text{human}, 0.000941)\). In vivo clearance, \(C_{\text{L,int,in vivo}}\), and \(E_H\) values differed markedly between rats and humans for each compound. For example, FK480 and diazepam are characterized by intermediate to high \(E_H\) (0.62 and 0.64) in rats. On the contrary, low \(E_H\) values (0.12 and 0.03) were observed in humans. In vivo rat and human \(E_H\) ranged widely among the model compounds (rat: 0.05 for FK079 and \(-0.93\) for FK1052; human: 0.03 for diazepam and \(-0.76\) for diltiazem). The model compounds represented a variety of metabolic pathways. FK1052, FK480, diltiazem, and diazepam are metabolized mainly by P450. In contrast, quinolost, FK079, and zidovudine were metabolized by UDP-glucuronosyltransferase or esterase besides P450. Troglitazone and acetaminophen were metabolized mainly by UDP-glucuronosyltransferase and sulfotransferase. Of the compounds metabolized by different drug-metabolizing enzymes, some compounds showed species differences in the elimination routes and metabolite profiles. For example, FK079 is metabolized mainly by P450 in rats and by esterase in humans (respectively (Tokuda et al., 1997). For zidovudine, approximately 75 and 10% of the dose were excreted as its unchanged and glucuronide in rats, respectively (Mays et al., 1991). On the other hand, approximately 14 and 75% of the dose were excreted as its unchanged and glucuronide in humans, respectively (Blum et al., 1996). For acetaminophen, the primary route of metabolism is glucuronidation in rats and sulfation in humans (Hjelle and Klaassen, 1984; Sonne et al., 1988).

In Vitro Metabolism in Rat Hepatocytes and Estimation of Scaling Factor. In Fig. 2 illustrates the time courses of the unchanged model compounds in freshly isolated rat hepatocytes. The time courses were estimated by use of Sprague-Dawley rat hepatocytes, except for troglitazone. For troglitazone, the time course was estimated by use of Wistar-Imamichi rat hepatocytes, because the in vivo pharmacokinetic data in Wistar-Imamichi rats were used for evaluation. The unchanged drug profiles at 1 \(\mu M\) showed linear log concentration declines so that the metabolism follows a first-order reaction under these conditions. Table 3 shows the \(C_{\text{L,int,in vivo}}\) calculated from the time courses of the model compounds in freshly isolated rat hepatocytes and the values for rat scaling factor (the ratios of \(C_{\text{L,int,in vivo}}\) obtained from in vivo pharmacokinetic data to \(C_{\text{L,int,in vivo}}\)) the model compounds. In the same way, the rat scaling factor values calculated using the dispersion models were 0.2- to 47.1-fold for diazepam, 43.6-fold for FK1052, 25.1-fold for FK480, 10.5-fold for quinolost, 6.1-fold for FK079, and 0.5- to 2.2-fold for diltiazem, diltiazem, zidovudine, and acetaminophen, showing a marked difference among the model compounds. In the same way, the rat scaling factor values calculated using the dispersion models were 0.2- to 47.1-fold, showing marked differences among the model compounds.

Table 4 shows the unbound fraction in freshly isolated rat hepatocytes, rat \(C_{\text{L,int,in vitro}}\) corrected with \(f_{\text{u,hepatocytes}}\), and the scaling factor values. Rat \(f_{\text{u,hepatocytes}}\) values were determined by use of Sprague-Dawley rat hepatocytes, except for the \(f_{\text{u,hepatocytes}}\) value for troglitazone, which was determined by use of Wistar-Imamichi rat hepatocytes. Rat \(f_{\text{u,hepatocytes}}\) values were dependent on the model compounds. For example, although FK1052, FK480, and troglitazone were highly bound to hepatocytes with free-fraction values ranging...
TABLE 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Species</th>
<th>CL int,in vitro</th>
<th>f u</th>
<th>f hepaticocytes</th>
<th>f hepaticocytes corrected with rat scaling factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FK1052, FK480</td>
<td>Human</td>
<td>0.95 0.03 1.21</td>
<td>0.78 0.02 1.51</td>
<td>0.22 0.01 1.00</td>
<td>0.22 0.01 1.00</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
</tr>
<tr>
<td>FK480, FK1052</td>
<td>Rat</td>
<td>0.70 0.70 1.40</td>
<td>0.39 0.39 0.78</td>
<td>0.24 0.24 0.51</td>
<td>0.24 0.24 0.51</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
</tr>
<tr>
<td>Diazepam, FK480</td>
<td>Rat</td>
<td>1.00 1.00 1.00</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>Rat</td>
<td>1.00 1.00 1.00</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
</tr>
<tr>
<td>Quinotolast, FK079</td>
<td>Rat</td>
<td>1.25 1.25 1.25</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
</tr>
<tr>
<td>Diltiazem, FK079,</td>
<td>Human</td>
<td>0.78 0.78 0.78</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
</tr>
<tr>
<td>Zidovudine, FK079,</td>
<td>Human</td>
<td>0.78 0.78 0.78</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Human</td>
<td>0.78 0.78 0.78</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>0.93 0.93 0.93</td>
<td>Diaz-Garcia et al. (1992), Luirila et al. (1998), Sawada et al. (1995), Kiy et al. (1970)</td>
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</tbody>
</table>

**Discussion**

In the present study, we investigated CL int,in vitro obtained from in vitro experiments using hepatocytes and CL int,in vivo calculated from in vivo pharmacokinetic data with nine model compounds (FK1052, FK480, diltiazem, troglitazone, quinotolast, FK079, zidovudine, and acetaminophen) in rats and humans. As a result, 1) rat scaling factor values (CL int,in vivo/CL int,in vitro) were different among the compounds; 2) when human CL int,in vitro was regarded as the predicted CL int,in vivo, the observed and predicted CL int,in vivo differed markedly for some compounds; and 3) use of human CL int,in vitro corrected with rat scaling factor improved the predictability of human CL int,in vivo.

In this study, CL int,in vitro values were estimated using hepatocytes. In drug discovery, enzymes involved in the metabolism of compounds are not sufficiently characterized. Compared with subcellular fractions such as hepatic microsomes, hepatocytes seem to be suitable for measuring CL int,in vitro because they possess a larger complement of
drug-metabolizing enzymes. In addition, the hepatocyte system is a useful tool to evaluate compounds that are metabolized by multiple drug-metabolizing enzymes. Therefore, we selected model compounds metabolized by various enzymes, such as P450, esterase, UDP-glucuronosyltransferase, and sulfotransferase, to confirm the advantage of hepatocytes (Table 2). Of the model compounds, troglitazone (FK1052, FK480, diazepam, diltiazem, quinotolast, and troglitazone), 1 × 10⁶ cells/ml (acetaminophen), 2 × 10⁶ cells/ml (zidovudine), and 4 × 10⁶ cells/ml (FK079). Each point and bar represents the mean ± S.D. of three experiments. The solid lines represent the linear regression lines by the least-squares method.

**TABLE 3**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CLₜₐₓₘᵢₓ,ᵢ₉ᵦᵲᵢⱼ</th>
<th>Scaling Factor</th>
<th>Well Stirred</th>
<th>Dispersion</th>
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<tr>
<td></td>
<td>ml/min/kg body weight</td>
<td>(CLₜₐₓₘᵢₓ,ᵢ₉ᵦᵲᵢⱼ / CLₜₐₓₘᵢₓ,ᵢ₉ᵦᵲᵢⱼ)</td>
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<tr>
<td>FK1052</td>
<td>635 ± 24</td>
<td>43.6</td>
<td>12.5</td>
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<tr>
<td>FK480</td>
<td>226 ± 13</td>
<td>25.1</td>
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<td>Diazepam</td>
<td>345 ± 39</td>
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<td>Diltiazem</td>
<td>3300 ± 1330</td>
<td>0.5</td>
<td>0.2</td>
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<td>Quinotolast</td>
<td>54.6 ± 3.4</td>
<td>10.5</td>
<td>9.0</td>
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<tr>
<td>Troglitazone</td>
<td>928 ± 9</td>
<td>73.1</td>
<td>47.1</td>
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<tr>
<td>FK079</td>
<td>4.75 ± 0.14</td>
<td>6.1</td>
<td>6.0</td>
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<tr>
<td>Zidovudine</td>
<td>14.3 ± 1.0</td>
<td>1.4</td>
<td>1.3</td>
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<tr>
<td>Acetaminophen</td>
<td>71.3 ± 10.9</td>
<td>0.7</td>
<td>0.6</td>
<td></td>
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</table>

*Data represent the mean ± S.D. of three experiments.

**TABLE 4**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>fᵢₜₐₓₘᵢₓ,ᵢ₉ᵦᵲᵢⱼ</th>
<th>Rat CLₜₐₓₘᵢₓ,ᵢ₉ᵦᵲᵢⱼ</th>
<th>Rat Scaling Factor</th>
<th>Well Stirred</th>
<th>Dispersion</th>
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<tr>
<td></td>
<td>µL/min/10⁶ cells</td>
<td>(µL/min/10⁶ cells)</td>
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<tr>
<td>FK1052</td>
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<td>2600</td>
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<td>FK480</td>
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<td>Diazepam</td>
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<tr>
<td>Diltiazem</td>
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<td>6240</td>
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<td>Quinotolast</td>
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<td>FK079</td>
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<tr>
<td>Acetaminophen</td>
<td>0.933</td>
<td>76.4</td>
<td>0.7</td>
<td>0.5</td>
<td></td>
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</table>

The dotted lines represent the lines of unity.

**FIG. 2.** Time courses of unchanged compounds in freshly isolated rat hepatocytes.

Each compound (at a concentration 1 µM) was incubated for various time periods at 37°C in freshly isolated rat hepatocytes. The values for cell density in the reaction mixtures were 0.5 × 10⁶ cells/ml (FK1052, FK480, diazepam, diltiazem, quinotolast, and troglitazone), 1 × 10⁶ cells/ml (acetaminophen), 2 × 10⁶ cells/ml (zidovudine), and 4 × 10⁶ cells/ml (FK079). Each point and bar represents the mean ± S.D. of three experiments. The solid lines represent the linear regression lines by the least-squares method.

**FIG. 3.** Correlation of CLₜₐₓₘᵢₓ,ᵢ₉ᵦᵲᵢⱼ between freshly isolated and cryopreserved rat hepatocytes.

The dotted lines represent the lines of unity.
tazone, quinotolast, FK079, zidovudine, and acetaminophen are metabolized by different drug-metabolizing enzymes.

When rat CL\textsubscript{int,in vivo} were evaluated using freshly isolated rat hepatocytes, rat scaling factor values for a few drugs (diazepam, diltiazem, zidovudine, and acetaminophen) were close to unity (Table 3). However, the values for some drugs (FK1052, FK480, quinotolast, troglitazone, and FK079) were from 6.0 to 73.1, resulting in CL\textsubscript{int,in vivo} values larger than the CL\textsubscript{int,in vitro} values (Table 3). These findings suggest that CL\textsubscript{int,in vivo} values are not always in agreement with the CL\textsubscript{int,in vitro} values. In the same way, when using cryopreserved human hepatocytes, the differences between observed and predicted human CL\textsubscript{int,in vivo} for FK1052, FK480, troglitazone, and FK079 were especially large (12.4–199.0-fold) (Table 5), resulting in discrepancies between CL\textsubscript{int,in vitro} and CL\textsubscript{int,in vivo}.

Several studies have reported the use of freshly isolated rat hepatocytes to predict quantitative hepatic clearance (Ashforth et al., 1995; Carlile et al., 1998). However, there have been few studies of prediction using freshly isolated human hepatocytes (Bayliss et al., 1999).
One reason is that there is a limited availability of fresh human livers for research. Thus, human hepatocytes are not yet widely used as an experimental system. In this respect, cryopreservation is especially important for hepatocytes from species such as humans, where tissue supply is limited. There are several reports of the cryopreservation of human hepatocytes (Chesne et al., 1993; Coundouris et al., 1993). Furthermore, a few groups have reported the evaluation of metabolic pathways, enzyme induction, and inhibition using cryopreserved human hepatocytes applied to drug metabolism research (Ruegg et al., 1997; Li et al., 1999; Hewitt et al., 2001).

For predicting hepatic metabolic clearance using cryopreserved hepatocytes, there is a requirement that \( CL_{\text{int,in vitro}} \) (or drug-metabolizing enzyme activity) in cryopreserved hepatocytes is in agreement with that in the freshly isolated hepatocytes. In this study, we compared \( CL_{\text{int,in vitro}} \) in freshly isolated and cryopreserved rat hepatocytes for the model compounds. As a result, for most of the compounds, the \( CL_{\text{int,in vitro}} \) in the freshly isolated hepatocytes were in good agreement with those in the cryopreserved hepatocytes (Fig. 3). In addition, Li et al. (1999) have reported that cryopreserved human hepatocytes had equivalent enzyme activities for P450 (CYP1A2, 2A6, 2C9, 2C19, 2D6, and 3A4), UDP-glucuronosyltransferase, and sulfotransferase to their corresponding freshly isolated human hepatocytes. Judging from these results, it is reasonable to use cryopreserved hepatocytes for predicting hepatic metabolic clearance.

\( CL_{\text{int,in vitro}} \) was determined from substrate disappearance rate at a single drug concentration (1 \( \mu M \)) in hepatocytes. The measurement method can be thought of as a simple and useful method with advantages of the method as follows: 1) simple to conduct, 2) can be done for many compounds, 3) metabolites do not need to be known, 4) can be easily done without radiolabeling, and 5) can yield enzyme kinetic data based on the disappearance of parent compounds. On the other hand, disadvantages of the method are as follows: 1) it is difficult to measure very low \( CL_{\text{int,in vitro}} \) values; 2) do not get individual metabolite information; and 3) do not obtain \( K_m \) and \( V_{\text{max}} \) parameters (Naritomi et al., 2001). We could not detect \( CL_{\text{int,in vitro}} \) for quinotolast in human hepatocytes because the \( CL_{\text{int,in vitro}} \) values were very low (Fig. 4). In this case, it is difficult to predict \( CL_{\text{int,in vivo}} \) quantitatively. However, it seems reasonable to assume that the predicted \( CL_{\text{int,in vivo}} \) in humans was very low in comparison with that in rats, from a qualitative level.

Previously, we reported the quantitative prediction of human hepatic clearance using hepatic microsomes for compounds metabolized by P450 (Naritomi et al., 2001). As a result, we found that use of human \( CL_{\text{int,in vitro}} \) corrected with rat and/or dog scaling factors yielded better predictions of human \( CL_{\text{int,in vivo}} \). In this study, we have examined the effect of rat scaling factor in the case of using hepatocytes. Without consideration of rat scaling factor, some compounds showed marked differences between observed and predicted human \( CL_{\text{int,in vivo}} \), as mentioned above. In contrast, with rat scaling factor, most of the differences were within 5-fold (Table 5). These results indicate that it is likely that the prediction method using human hepatic materials, which includes animal scaling factors, improves predictability. However, the differences were more than 2-fold except for FK480 (1.3–1.9-fold) (Table 5). Increasing the accuracy of the predictability requires the improvement of the prediction method. In addition, the results were obtained by use of the limited compounds. In the future, we would improve the prediction method for many more compounds and confirm its validity.

When using hepatic microsomes, inclusion of dog scaling factor also improved the predictability of human \( CL_{\text{int,in vivo}} \) (Naritomi et al., 2001). In the present study, we evaluated the prediction by use of hepatocytes, focusing on the application of rat scaling factor. To give a better human prediction, future studies should also examine the prediction applying dog scaling factor. A major problem facing researchers using hepatocytes from the livers of larger species such as dogs and humans is the scarcity of tissue available. Recently, cryopreservation of dog hepatocytes has been reported (Swales and Utech, 1998). Use of cryopreserved dog hepatocytes would be useful in prediction studies.

At the present time, it is not clear why each compound has an intrinsic scaling factor. However, there are a few possible reasons. To begin with, if \( f_a, \text{hepatocytes} \) in the reaction mixture may have an influence on the \( CL_{\text{int,in vitro}} \) values. For in vitro-in vivo scaling, correction with \( f_a \) in the reaction mixture has been reported to be important (Lin et al., 1980; Obach, 1999). Therefore, we have evaluated \( f_a, \text{hepatocytes} \) of the model compounds in rats and the changes in scaling factor values when correcting with \( f_a, \text{hepatocytes} \). However, \( CL_{\text{int,in vitro}} \) corrected with \( f_a, \text{hepatocytes} \) were not in agreement with the \( CL_{\text{int,in vivo}} \) for some compounds. Namely, the scaling factor values for FK1052, FK480, troglitazone, quinotolast, and FK079 still showed high values, 3.1- to 15.8-fold (Table 4). This suggests that a scaling factor different from unity might not be due only to the drug binding to hepatocytes. The following assumptions in the mathematical models may relate to the result: 1) a rapid equilibrium between blood and hepatocytes, 2) availability of only unbound drug for elimination and transport across membranes, and 3) homogeneous distribution of metabolic enzymes and transport carriers along the blood flow pathways in the liver (Iwatsubo et al., 1996). If any of these assumptions are incorrect, discrepancies between \( CL_{\text{int,in vivo}} \) and \( CL_{\text{int,in vitro}} \) will be observed.

In conclusion, we investigated \( CL_{\text{int,in vitro}} \) obtained from in vitro experiments using freshly isolated or cryopreserved hepatocytes and compared with \( CL_{\text{int,in vitro}} \) calculated from in vivo pharmacokinetic data with nine model compounds in rats and humans. When using freshly isolated rat hepatocytes, rat scaling factor values (\( CL_{\text{int,in vivo}}/CL_{\text{int,in vitro}} \)) showed marked difference among the model compounds. Human \( CL_{\text{int,in vitro}} \) were determined by use of cryopreserved hepatocytes. When human \( CL_{\text{int,in vitro}} \) was regarded as the predicted \( CL_{\text{int,in vivo}} \), the observed and predicted \( CL_{\text{int,in vivo}} \) for some drugs differed markedly. In contrast, use of human \( CL_{\text{int,in vitro}} \) corrected with rat scaling factors improved the predictability of human \( CL_{\text{int,in vivo}} \). These results make the evaluation using hepatocytes more useful and provide a basis for predicting hepatic clearance using hepatocytes.

References