Prediction of In Vivo Hepatic Clearance and Half-Life of Drug Candidates in Human Using Chimeric Mice with Humanized Liver

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Abstract:

Accurate prediction of pharmacokinetics (PK) parameters in humans from animal data is difficult for various reasons, including species differences. However, chimeric mice with humanized liver (PXB mice; urokinase-type plasminogen activator/severe combined immunodeficiency mice repopulated with approximately 80% human hepatocytes) have been developed. The expression levels and metabolic activities of cytochrome P450 (P450) and non-P450 enzymes in the livers of PXB mice are similar to those in humans. In this study, we examined the predictability for human PK parameters from data obtained in PXB mice. Elimination of selected drugs involves multiple metabolic pathways mediated not only by P450 but also by non-P450 enzymes, such as UDP-glucuronosyltransferase, sulfotransferase, and aldehyde oxidase in liver. Direct comparison between in vitro intrinsic clearance (CL_{int,in vitro}) in PXB mice hepatocytes and in vivo intrinsic clearance (CL_{int,in vivo}) in humans, calculated based on a well stirred model, showed a moderate correlation (r^2 = 0.475, p = 0.009). However, when CL_{int,in vivo} values in humans and PXB mice were compared similarly, there was a good correlation (r^2 = 0.754, p = 1.174 \times 10^{-4}) between humans and PXB mice. The rank order of CL and t_{1/2} in human could be predicted at least, although it may not be possible to predict absolute values due to rather large prediction errors. Our results indicate that in vitro and in vivo experiments with PXB mice should be useful at least for semiquantitative prediction of the PK characteristics of candidate drugs in humans.

Introduction

It is important to predict human pharmacokinetics (PK) and metabolism of drug candidates in the preclinical stage of pharmaceutical development. Various approaches to predict human clearance (CL) with in vitro metabolic systems, such as human liver microsomes and hepatocytes, have been reported (Nagilla et al., 2006; Brown et al., 2007; Fagerholm, 2007; Stringer et al., 2008; Chiba et al., 2009; Hallifax et al., 2010) but with limited success. One of the reasons for the discrepancy between predicted and observed CL may be that the preparation, storage, and experimental treatment of hepatocytes alter the normal function of metabolic enzymes (Wang et al., 2005). Although this might be ameliorated by using fresh hepatocytes immediately after isolation from the liver, these are not readily available and in any case show considerable interindividual differences.

It has become possible recently to predict CL and half-life (t_{1/2}) by means of computational approaches and physiologically based modeling (Ekins and Obach, 2000; De Buck et al., 2007; Tabata et al., 2009; Paixão et al., 2010). Accurate prediction of human PK is a key issue for the development of new drugs, because many new drug candidates with diverse chemical structures are metabolized not only by cytochrome P450 (P450) but also by non-P450 enzymes, such as UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT). It is also necessary to take into account the effects of cell permeability, transporter-mediated uptake, and excretion (Chiba et al., 2009; Huang et al., 2010).

Chimeric mice with humanized liver (PXB mice; PhoenixBio Co., Ltd., Hiroshima, Japan) have been generated from urokinase-type plasminogen activator/severe combined immunodeficiency mice transplanted with human hepatocytes (Tateno et al., 2004). In these mice, approximately 80% of the hepatocytes are human. The expression levels and metabolic activities of P450 and non-P450 enzymes in
livers of PXB mice with a high replacement index (RI) are similar to those of humans (Katoh et al., 2004, 2005), and human-specific metabolites are formed in PXB mice (Inoue et al., 2009; Kamimura et al., 2010; Yamazaki et al., 2010; De Serres et al., 2011). Thus, PXB mice could be a good in vivo model for predicting drug metabolism in humans.

However, quantitative methods for predicting PK parameters of humans from data in PXB mice have not been established yet. Therefore, we selected 13 model compounds that are metabolized by P450 and/or non-P450 enzymes in liver and compared the PK parameters in humans and PXB mice, using both in vitro and in vivo approaches, to evaluate the utility of this animal model for the prediction of human PK.

**Materials and Methods**

**Chemicals.** 6-Deoxypenciclovir and mirtazapine were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). Dapsone, lamotrigine, salbutamol, and sulindac were purchased from Sigma-Aldrich (St. Louis, MO). Diclofenac was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Fasudil was obtained from Tocris Bioscience (Bristol, UK). (S)-Naproxen was purchased from Cayman Chemical (Ann Arbor, MI). Ibuprofen, ketoprofen, and (S)-warfarin were purchased from Wako Pure Chemicals (Osaka, Japan). Zaleplon was kindly provided by King Pharm. Inc. (Bristol, UK). All of the other regents and solvents were commercial products of the highest available grade or analytical grade.

**Animals.** The present study was approved by the animal ethics committee and was conducted in accordance with the regulations on the use of living modified organisms of Hiroshima University. PXB mice (10–14 weeks of age) with human hepatocytes were prepared by the reported method (Tateno et al., 2004). Human hepatocytes of a donor (African-American boy, 5 years old) were obtained from BD Biosciences (San Jose, CA). PXB mice were housed in a temperature- and humidity-controlled environment under a 12 h light/dark cycle. The RI was determined by the measurement of human albumin in blood collected from the tail vein. The RI was estimated by the correlation curve between the human albumin levels in mouse blood and determined by using human-specific cytokeratin 8/18-immunostained liver sections (Tateno et al., 2004). Human hepatocytes of a donor (African-American boy, 5 years old) were obtained from BD Biosciences (San Jose, CA). PXB mice were housed in a temperature- and humidity-controlled environment under a 12 h light/dark cycle.

**Administration.** Drug solution (5 ml/kg) was administered intravenously to PXB mice at 0.3 to 5 mg/kg body weight. Solutions of dapsone, diclofenac, 6-deoxypenciclovir, fasudil, ketoprofen, ibuprofen, mirtazapine, naproxen, salbutamol, and sulindac were prepared in saline. In the cases of ketoprofen, ibuprofen, naproxen, and sulindac, equivalent amounts of alkali were added. Dapsone solutions contained 10% dimethyl sulfoxide (DMSO), and mirtazapine solutions were prepared with 10% DMSO and equivalent amounts of hydrochloric acid. Lamotrigine, and zaleplon solutions were prepared with 10% DMSO and 10% polyethylene glycol 400 in saline. Equivalent amounts of hydrochloric acid also were added to the solutions of lamotrigine and zaleplon. Warfarin was formulated in 3% DMSO and 97% saline with an equivalent amount of sodium hydroxide.

Blood samples after dosing were collected from orbital veins of PXB mice at predetermined times using heparinized glass. These samples were centrifuged, and the plasma was stored at −30°C.

**Determination of Drug Concentrations in Plasma.** A 10 μl aliquot of plasma was added to 40 μl of acetonitrile or methanol containing an internal standard (carbamazepine, ketoprofen, or ibuprofen). The mixtures were centrifuged at 14,000 g for 5 min, and the supernatant was subjected to liquid chromatography tandem mass spectrometry (LC/MS/MS).

**Isolation and Purification of Hepatocytes from PXB Mice.** Fresh hepatocytes were isolated from PXB mice (13–15 weeks of age) by means of the in situ collagenase perfusion method and purified as described previously (Yamasaki et al., 2010). PXB mouse hepatocytes (h-hepatocytes) contained approximately 7% mouse hepatocytes. We used h-hepatocytes purified by the use of 66Z rat IgG and magnetic beads bearing anti-rat IgG antibodies. The magnetic removal of mouse hepatocytes reduced the level of mouse hepatocytes to approximately 2% (in this study, the purity of human hepatocytes from PXB mouse hepatocytes ranged from 96.6 to 99.7% after purification). Cell viability of the hepatocytes used in the experiments ranged from 79 to 91%, as determined by means of the trypan blue exclusion test.

**In Vitro Metabolic Studies Using h-Hepatocytes.** The h-hepatocyte suspension (1 × 10^6 cells/ml) was incubated in Krebs-Henseleit buffer without serum in the presence of 10 μM of the test drug at 37°C under an atmosphere of 5% CO2, 95% O2. The final concentration of acetonitrile was 0.5% (v/v) in the reaction mixture. The plates (24 wells) were shaken gently with an orbital shaker. The incubation mixture was sampled at 0, 0.25, 0.5, 1, and 2 h after treatment, and reactions were stopped by freezing the mixture in liquid nitrogen. When required, the samples were thawed, spiked with two volumes of acetonitrile or methanol containing an internal standard, and centrifuged. Aliquots of the supernatants were subjected to LC/MS/MS.

**LC/MS/MS Conditions.** Aliquots (10 μl) of plasma and h-hepatocyte suspension were introduced into the high-performance liquid chromatography system with an autosampler (Agilent Technologies, Santa Clara, CA). Several mobile phase conditions were used. Mobile phase condition 1 consisted of 10 mM ammonium acetate (A) and acetonitrile (B) on an Inertsil ODS-3 column (3 μm, 50 × 2.1 mm; GL Sciences Inc., Tokyo, Japan) at 40°C for the analysis of diclofenac, ibuprofen, ketoprofen, mirtazapine, (S)-naproxen, sulindac, and (S)-warfarin. The flow rate was set at 0.2 ml/min. The starting condition for the high-performance liquid chromatography gradient was 90:10 (A/B). From 0 to 5 min, the mobile phase composition was changed linearly to 10:90 (A/B), and this was held until 8 min. The gradient then was returned to 90:10 (A/B).

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**FIG. 1. Chemical structures of the model compounds used in this study.**
Literature values of plasma clearance, half-life, unbound fraction in plasma, blood/plasma concentration ratio, and metabolic enzymes in humans for the model compounds examined in this analysis

Rb values of fasudil, lamotrigine, and sulindac were assumed to be 1 due to unavailable data from the literature. References are in Supplemental Tables 1 and 2.

In vivo intrinsic clearance (CL\textsubscript{int,in vivo}) was calculated from the in vivo CL\textsubscript{v}, fu, Rb, and average hepatic blood flow (Q) based on a well stirred model as

\[
CL\textsubscript{int,in vivo} = CL\textsubscript{v}/((fu/Rb) \times (1 - CL\textsubscript{v}/Q))
\]

(Pang and Rowland, 1977). These CL\textsubscript{v} values were converted to blood clearance using Rb values.

The Q values of humans and PXB mice were set at 21 and 90 ml \textminuss1 \times 10\textsuperscript{-6} kg\textsuperscript{-1} (same as in normal mice), respectively (Davies and Morris, 1993). In addition, Rb and fu of human were assumed to be equivalent to those of PXB mice. If CL\textsubscript{v} of drugs exceeded liver blood flow, then the hepatic clearance was taken as 90% of liver blood flow. CL\textsubscript{int,in vivo} of 6-deoxypenciclovir, lamotrigine, and sulindac were evaluated from oral clearance (CL\textsubscript{oral}) as

\[
CL\textsubscript{oral} = CL\textsubscript{int,in vivo}/fu/Rb.
\]

### Results

#### Selection of the Model Compounds for Analysis

In this study, we selected 13 compounds with diverse chemical structures (Fig. 1). Elimination of these selected drugs involves multiple metabolic pathways mediated not only by P450 but also by non-P450 enzymes, such as UGT, SULT, and aldehyde oxidase (AO) in liver. Mirtazapine and warfarin were known to be mainly metabolized by P450. Diclofenac, ibuprofen, and naproxen were metabolized by both UGT and P450. Furthermore, the model compounds metabolized by AO, such as...

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Scaled CL\textsubscript{int,in vivo} (µl \textminuss1 \times 10\textsuperscript{6} cells\textsuperscript{-1})</th>
<th>Scaled CL\textsubscript{int,in vivo} (Human)</th>
<th>Scaled CL\textsubscript{int,in vivo} (PXB Mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl \textminuss1 \times 10\textsuperscript{6} cells\textsuperscript{-1}</td>
<td>µl \textminuss1 \times 10\textsuperscript{6} cells\textsuperscript{-1}</td>
<td>µl \textminuss1 \times 10\textsuperscript{6} cells\textsuperscript{-1}</td>
</tr>
<tr>
<td>Dapsone</td>
<td>2.3 \pm 1.2</td>
<td>8.0 \pm 4.0</td>
<td>43.1 \pm 21.3</td>
</tr>
<tr>
<td>6-Deoxyxenciclovir</td>
<td>5.3 \pm 1.2</td>
<td>18.3 \pm 4.2</td>
<td>98.6 \pm 22.4</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>24.7 \pm 1.2</td>
<td>84.7 \pm 4.0</td>
<td>455.8 \pm 21.3</td>
</tr>
<tr>
<td>Fasudil</td>
<td>35.7 \pm 1.3</td>
<td>122.4 \pm 4.5</td>
<td>659.1 \pm 24.5</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>13.3 \pm 2.1</td>
<td>45.8 \pm 7.1</td>
<td>246.4 \pm 35.8</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>6.0 \pm 1.0</td>
<td>20.6 \pm 3.4</td>
<td>110.9 \pm 18.5</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>1.4 \pm 0.7</td>
<td>4.8 \pm 3.6</td>
<td>25.9 \pm 19.2</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>6.3 \pm 1.2</td>
<td>21.7 \pm 4.0</td>
<td>117.0 \pm 21.3</td>
</tr>
<tr>
<td>(S)-Naproxen</td>
<td>12.7 \pm 2.5</td>
<td>43.5 \pm 8.6</td>
<td>234.1 \pm 46.5</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>1.0 \pm 1.0</td>
<td>3.3 \pm 3.3</td>
<td>17.9 \pm 17.8</td>
</tr>
<tr>
<td>Sulindac</td>
<td>2.0 \pm 2.0</td>
<td>7.0 \pm 6.7</td>
<td>37.6 \pm 36.0</td>
</tr>
<tr>
<td>(S)-Warfarin</td>
<td>1.2 \pm 0.7</td>
<td>4.1 \pm 2.5</td>
<td>22.2 \pm 13.3</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>2.3 \pm 1.2</td>
<td>8.0 \pm 4.0</td>
<td>43.1 \pm 21.3</td>
</tr>
</tbody>
</table>
administration of drugs to humans were obtained from the literature. If CLt after intravenous administration was not available, the values of CL/F after oral administration of selected model drugs to humans were obtained from the literature, we used the value of CL/F after oral administration. The PK parameters and the major enzymes responsible for drug metabolism in humans are shown in Table 1. The spreadsheet containing these values with the literature references is included as an attachment in the supplemental data (Supplemental Tables 1 and 2).

Disappearance of Parent Drugs after Incubation. Remaining amounts of all of the compounds decreased linearly for 2 h on incubation with h-hepatocytes. The values of CLint,in vitro in hepatocytes, calculated using scaling factors to humans and PXB mice whole body as described under Materials and Methods, are listed in Table 2. These CLint,in vitro values covered a wide range. Fasudil showed high clearance, whereas warfarin was very stable.

PK Study of the Model Compounds in PXB Mice. Plasma concentrations and PK parameters in PXB mice after intravenous administration of drug solutions at 0.3 to 5 mg/kg are shown in Fig. 2 and Tables 3 and 4. Each RI value in PXB mice was 73.4 to 93.4%.

CLt values of warfarin and lamotrigine were relatively low, whereas those of fasudil and salbutamol were much higher; the range of CLt was 0.2 to 198 ml · min⁻¹ · kg⁻¹. The t½2 value of lamotrigine was the longest, and those of 6-deoxypenciclovir and fasudil were short, as shown in Table 3.

Comparison of Intrinsic CL between h-Hepatocytes and Humans. Direct comparison between CLint,in vitro from h-hepatocytes and CLint,in vivo calculated for a well stirred model in humans showed a moderate correlation (r² = 0.475, p = 0.009) (Fig. 3). For 2 of 13 (15%) compounds, observed CLint,in vivo was predicted within a 3-fold error from hepatocyte CLint,in vitro. However, for 8 of 13 (62%) compounds, observed CLint,in vivo was predicted with a 3- to 10-fold error. Figure 4 shows the relationship between CLint,in vivo and CLint,in vitro for PXB mice; again, the correlation was moderate (r² = 0.435, p =

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human CLint,in vivo</th>
<th>PXB Mice CLint,in vivo</th>
</tr>
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<tbody>
<tr>
<td>Dapsone</td>
<td>2.0</td>
<td>8.6</td>
</tr>
<tr>
<td>6-Deoxypenciclovir</td>
<td>118.0</td>
<td>209.0</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1004.3</td>
<td>4905.1</td>
</tr>
<tr>
<td>Fasudil</td>
<td>370.6</td>
<td>1588.2</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>147.1</td>
<td>686.0</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>232.2</td>
<td>442.0</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>0.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>123.6</td>
<td>408.7</td>
</tr>
<tr>
<td>(S)-Naproxen</td>
<td>10.1</td>
<td>230.2</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>13.5</td>
<td>1148.2</td>
</tr>
<tr>
<td>Sulindac</td>
<td>47.8</td>
<td>86.5</td>
</tr>
<tr>
<td>(S)-Warfarin</td>
<td>3.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>173.6</td>
<td>261.3</td>
</tr>
</tbody>
</table>

Each compound was administered intravenously to PXB mice at 0.3 to 5 mg/kg body weight. The values of RI of PXB mice ranged from 73.4 to 93.4%. Each value represents the mean ± S.D. (n = 3–5).
For 6 of 13 (46%) compounds, $CL_{\text{int,in vivo}}$ of PXB mice was predicted within a 3-fold error from h-hepatocyte $CL_{\text{int,in vitro}}$. For 5 of 13 (38%) compounds, $CL_{\text{int,in vivo}}$ was predicted within a 3- to 10-fold error.

**Relationship between Intrinsic Clearance in Humans and PXB Mice In Vivo.** We directly compared $CL_{\text{int,in vivo}}$ calculated based on a well stirred model in humans and PXB mice. As shown in Fig. 5, there was a good correlation ($r^2 = 0.754, p = 1.174 \times 10^{-5}$) between literature $CL_{\text{int,in vivo}}$ in human and measured $CL_{\text{int,in vivo}}$ of PXB mice for these compounds. For 4 of 13 (31%) compounds, observed $CL_{\text{int,in vivo}}$ in humans was predicted within a 3-fold error from PXB mouse $CL_{\text{int,in vivo}}$. For 7 of 13 (54%) compounds, human $CL_{\text{int,in vivo}}$ was predicted within a 3- to 10-fold error.

**Relationship of Elimination $t_{1/2}$ between Humans and PXB mice.** Figure 6 shows the relationship of $t_{1/2}$ after intravenous administration between humans and PXB mice. Compounds for which literature data were not available were excluded from this figure. A good correlation ($r^2 = 0.886, p = 1.506 \times 10^{-5}$) was found. For 6 of 9 (67%) compounds, human observed $t_{1/2}$ was predicted within a 3-fold error from PXB mouse $t_{1/2}$. For 3 of 9 (33%) compounds, the error was in the range of 3- to 10-fold.

**Discussion**

The prediction of human PK parameters is an important step during the preclinical development of pharmaceuticals to reduce costs by enabling the early elimination of candidates with unsuitable properties. However, species differences make it difficult to predict human PK from animal data; monkey data may lead to underprediction (Chiou and Buehler, 2002; Akabane et al., 2010), whereas dog data may cause overestimation (Chiou et al., 2000). In vitro-in vivo scaling from data obtained with human hepatic microsomes and hepatocytes is a widely used approach but often results in the underprediction of in vivo CL (Obach, 1999). We considered the possibility that PXB mice, in which hepatocytes are replaced with human hepatocytes to the extent of approximately 80% (Tateno et al., 2004), may have superior predictive utility, because the expression levels and activities of both P450 and non-P450 enzymes well reflect those of the donor hepatocytes (Yoshitsugu et al., 2006; Yamasaki et al., 2010). In this study, we checked metabolic activities (CYP2C9, CYP2D6, UGT, SULT, and AO) using probe substrates between donor hepatocytes and h-hepatocytes purified from PXB mice (Supplemental Table 3) as well as the expression of drug transporters and blood albumin (Tateno et al., 2004; Nishimura et al., 2005).

For the present study, we selected 13 model compounds with diverse chemical structures (Fig. 1), which are metabolized through multiple pathways by P450 and non-P450 enzymes, such as UGT, SULT, and AO. Their values of CL cover a wide range from 0.055 to 118 ml/min/kg (Table 1).
First, we performed an in vitro metabolic study using fresh h-hepatocytes isolated from PXB mice. We calculated CL_{int,in vivo} using fresh h-hepatocytes and compared the results with human CL_{int,in vivo} estimated by use of a well stirred model (Pang and Rowland, 1977). These results using a parallel tube model and a dispersion model were also similar to those of a well stirred model (S. Sanoh, unpublished observations). A moderate correlation ($r^2 = 0.475, p = 0.009$) was found, but this approach was not superior to prediction using other methods. 

CL_{int,in vivo} values of diclofenac, ibuprofen, warfarin, and zaleplon were approximately similar to reported values using cryopreserved hepatocytes (Ekins and Obach, 2000; Nagilla et al., 2006; Stringer et al., 2008), supporting the idea that CL_{int,in vivo} values are similar in fresh hepatocytes and cryopreserved hepatocytes (Naritomi et al., 2003; McGin尼ty et al., 2004).

A similar correlation ($r^2 = 0.435, p = 0.014$) was observed between CL_{int,in vivo} and CL_{int,in vivo} in PXB mice (Fig. 4). In both cases, the numbers of compounds for which absolute values of CL_{int} were predicted within a 3-fold error were insufficient. Therefore, we next evaluated the predictability of hepatic clearance and $t_{1/2}$ from in vivo data in PXB mice. The values of CL_{int,in vivo} estimated by intravenous administration in PXB mice were well correlated ($r^2 = 0.754, p = 1.174 \times 10^{-4}$) with observed CL_{int,in vivo} in human. Surprisingly, we also found a good correlation ($r^2 = 0.886, p = 1.506 \times 10^{-4}$) between $t_{1/2}$ values in PXB mice and humans. However, although the rank order was the same, there were rather large prediction errors, so it may not be possible to predict absolute values. This is consistent with the findings of Xiao et al. (2010) in PXB mice.

We used PXB mice that showed that the average RI values were approximately 80%. It was a concern that the contribution of the remaining approximately 20% mice hepatocytes may be reflected on clearance. CL_{int,in vivo} values of these model compounds in host mice hepatocytes (several combined immunodeficiency mice) were almost higher than those of h-hepatocytes within a 10-fold range (Supplementary Fig. 1). The extent of the difference may not influence the predictability of CL_{int,in vivo}.

For the estimation of CL_{int,in vivo} in PXB mice, the fu values of those model compounds is the same as those in humans because human albumin is expressed in the blood of PXB mice. Inoue et al. (2009) reported fu value of warfarin in PXB mice was similar to that in humans. Furthermore, fu values of some compounds (dapsone, diclofenac, ketoprofen, salbutamol, and zaleplon) in this study were also approximately similar to those in humans (S. Sanoh, unpublished observations).

We assumed that the RB values of those model compounds is also the same as those in humans, because RB values of some compounds (dapsone, diclofenac, ketoprofen, salbutamol, and zaleplon) in this study were also approximately similar to those in humans (S. Sanoh, unpublished observations).

$Q$ values were assumed to be 90 ml · min$^{-1}$ · kg$^{-1}$, respectively, corresponding to the values of normal mice (Davies and Morris, 1993). In further work, it would be desirable to examine whether these values are appropriate.

In this study, we selected model compounds metabolized not only by P450, but also by non-P450 enzymes, including AO. 6-Deoxypenecillinocovir, fusudil, sulindac, and zaleplon are metabolized mainly by AO in humans. It has been reported that human CL for drugs metabolized by AO may be underpredicted from data obtained with human liver cytosol and S9 due to the loss or deactivation of AO during preparation, homogenization, storage, and experimental procedures (Zientek et al., 2010). PXB mice have high AO activity, being similar to humans (Kitamura et al., 2008), and may be a useful source of fresh h-hepatocytes.

Our results indicate that PXB mice can be used at least for semi-quantitative prediction of not only CL, but also $t_{1/2}$ in humans. PXB mice also would be useful for in vitro estimation and comparison of PK of various candidate compounds, because large amounts of fresh, identical hepatocytes ($1.1 \times 10^6$ cells/mouse) are available by transplantation of donor hepatocytes ($2.5 \times 10^6$ cells/mouse). The combination of in vitro study in PXB mice and in vitro study using PXB hepatocytes may prove to be particularly effective.

Acknowledgments

We thank members in PhoenixBio Co., Ltd. for the isolation of hepatocytes from PXB mice.

Authorship Contributions

Participated in research design: Sanoh, Sugihara, Kotake, Tayama, Horie, Kitamura, and Ohta.

Conducted experiments: Sanoh and Horiguchi.

Contributed new reagents or analytic tools: Sugihara, Ohshita, and Tateno.

Performed data analysis: Sanoh and Horiguchi.

Wrote or contributed to the writing of the manuscript: Sanoh, Kotake, Tateno, and Ohta.

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PREDICTION OF PK PARAMETERS USING HUMAN LIVER CHIMERIC MICE


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