Prediction of in vivo hepatic clearance and half-life of drug candidates in human using chimeric mice with humanized liver

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d) Abbreviations

CYP, cytochrome P450; PXB mice, chimric mice with humanized liver; CL_{int}, in vitro, in vitro intrinsic clearance; CL_{int, in vivo}, in vitro intrinsic clearance; CL_{t}, total clearance; t_{1/2}, half life; UGT, UDP-glucuronosyltransferase; SULT, sulfotransferase; NAT, N-acetyltransferase; uPA^{+/+}, urokinase-type plasminogen activator; SCID, severe combined immunodeficiency; RI, replacement index; i.v., intravenously administration; DMSO, dimethylsulfoxide; PEG400, polyethylene glycol 400; h-hepatocytes, PXB mice hepatocytes; fu, plasma unbound fraction; Q, hepatic blood flow; Rb, blood/plasma concentration ratio; CL_{oral}, oral clearance; AO, aldehyde oxidase.
Abstract

Accurate prediction of pharmacokinetic (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 CYP and non-CYP enzymes in liver 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 the selected drugs involves multiple metabolic pathways mediated by not only CYP, but also non-CYP enzymes such as UGT, SULT, and aldehyde oxidase (AO) 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 ($r^2=0.421$, $p=0.016$). On other hand, when CL_{int,in vivo} values in humans and PXB mice were similarly compared, there was a good correlation ($r^2=0.785$, $p=5.556 \times 10^{-5}$). Elimination half-life ($t_{1/2}$) after intravenous administration also showed a good correlation ($r^2=0.886$, $p=1.506 \times 10^{-4}$) between human 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 semi-quantitative 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 pre-clinical stage of pharmaceuticals 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 alters the normal function of metabolic enzymes (Wang et al., 2005). Although this might be ameliorated by using fresh hepatocytes immediately after isolation from liver, these are not readily available, and in any case show considerable inter-individual differences.

Recently, it has become possible to predict CL and half life ($t_{1/2}$) by means of computational approach and physiologically based modeling (Ekins et al., 2000, De Buck et al., 2007, Tabata et al., 2009, Paixão et al., 2010). Accurate prediction of human PK is a key issue for development of new drugs, since many new drug candidates with diverse chemical structures are metabolized not only by CYP, but also by non-CYP enzymes, such as UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT). It is also necessary to take account of 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.), have been generated from urokinase-type plasminogen activator (uPA+/−)/severe combined immunodeficiency (SCID) 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 CYP and non-CYP enzymes in liver of PXB mice with a high replacement index (RI) are similar to those of humans (Kato et al., 2004, 2005), and human-specific metabolites are formed in PXB mice (Inoue et al., 2009, Yamazaki et al., 2010, Kamimura et al., 2010, Serres et al., 2011). Thus, PXB mice could be a good in vivo model for predicting drug metabolism in humans.

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

Chemicals

6-Deoxypenciclovir and mirtazapine were obtained from Toronto Research Chemicals Inc. (Ontario, Canada). Dapsone, lamotrigine, salbutamol, and sulindac were purchased from Sigma-Aldrich (Poole, UK). 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 Co. (Michigan, USA). Ibuprpfen and ketoprofen and (S)-warfarin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Zaleplon was kindly provided by King Pharm. Inc. (Bristol, UK).

All 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 in 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 B.D. Biosciences (San Jose, CA, USA). PXB mice were housed in a temperature- and humidity-controlled environment under a 12 hr/12 hr light/dark
cycle.

The replacement index (RI) was determined by 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). The RI values of PXB mice used in this study ranged from 73.4% to 93.4%.

**Administration**

Drug solution (5 mL/kg) was administered intravenously (i.v.) to PXB mice at 0.3-5 mg/kg body weight. Solutions of dapsone, diclofenac, 6-deoxypenciclovir, fasudil, ketoprofen, ibuprofen, mirtazapine, naproxen, salbutamol, sulindac were prepared in saline. In the cases of ketoprofen, ibuprofen, naproxen, and sulindac, equivalent amounts of alkali were added. Dapsone solutions contained 10% dimethylsulfoxide (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 (PEG400) in saline. Equivalent amounts of hydrochloric acid were also added to the solutions of lamotrigine and zaleplon. Warfarin was formulated in 3% DMSO, 97% saline with an equivalent amount of sodium hydroxide.

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

**Determination of drug concentration in plasma**

A 10 μL aliquot of plasma was added to 40 μL of acetonitrile or methanol containing an internal standard (carbanazepine, ketoprofen, ibuprofen). The mixtures were centrifuged at 14000 g for 5 min, and the supernatant was subjected to 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 (Yamasaki et al, 2010). PXB mouse hepatocytes (h-hepatocytes) contained about 7% of mouse hepatocytes. We employed 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 the mouse hepatocyte to approximately 2% (in this study, purity of human hepatocytes values of PXB mouse hepatocytes ranged from 96.6% to 99.7% after purification). Cell viability of hepatocytes used in experiments was ranged from 79% to 91%, as determined by means of the trypan blue exclusion test.
In vitro metabolic studies using h-hepatocytes

h-Hepatocyte suspension (1x10⁶ cells /mL) was incubated in Krebs-Henseleit buffer without serum in the presence of 10 μM test drug at 37 ºC 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 hrs after treatment and reactions were stopped by freezing the mixture in liquid nitrogen. When required, the samples were thawed, spiked with 2 volumes of acetonitrile or methanol containing 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 HPLC system with an autosampler (Agilent Technologies, Palo Alto, CA). Several mobile phase conditions were used. Mobile phase condition 1 consisted of 10 mM ammonium acetate (A) and acetonitrile (B) on a column of Inertsil ODS-3 (3 μm, 50 x 2.1 mm) at 40 ºC for 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 HPLC gradient was 90:10 (A:B). From 0 min to 5 min, the mobile phase composition was changed linearly to 10:90 (A:B), and this was held until 8 min. The gradient was then returned to 90:10 (A:B) linearly.
from 8 min to 8.1 min and the column was re-equilibrated to the initial condition.

Mobile phase condition 2 consisted of 0.1% formic acid (A) and methanol (B) on a column of YMC-Triart C18 (3 μm, 50 x 2.1 mm) for analysis of dapsone, 6-deoxypenciclovir, fasudil, lamotrigine, salbutamol, and zaleplon. The starting condition was 90:10 (A:B). From 0 min to 5 min, the mobile phase composition was changed linearly to 10:90 (A:B), and this was maintained until 8 min, then the column was re-equilibrated to the initial condition.

The MS/MS experiments were conducted by using API2000 LC/MS/MS systems (Applied Biosystems, Foster, CA). Mass number of the ionization mode, molecular ion and product ion for the model compounds were as follows:

- **dapsone** $m/z=248.99 \ [M+H]^+$ to 92.18,
- **6-deoxypenciclovir** $m/z=238.05 \ [M+H]^+$ to 210.95,
- **diclofenac** $m/z=294.14 \ [M+H]^-$ to 249.53,
- **fasudil** $m/z=292.07 \ [M+H]^+$ to 99.09,
- **ibuprofen** $m/z=204.88 \ [M+H]^-$ to 158.52,
- **ketoprofen** $m/z=253.16 \ [M+H]^-$ to 208.73,
- **lamotrigine** $m/z=256.03 \ [M+H]^+$ to 210.96,
- **mirtazapine** $m/z=266.14 \ [M+H]^+$ to 194.97,
- **(S)-naproxen** $m/z=228.68 \ [M+H]^-$ to 168.55,
- **salbutamol** $m/z=240.18 \ [M+H]^+$ to 148.03,
- **sulindac** $m/z=357.07 \ [M+H]^+$ to 232.96,
- **(S)-warfarin** $m/z=309.06 \ [M+H]^+$ to 162.97,
- **zaleplon** $m/z=306.08 \ [M+H]^+$ to 236.12.

**Determination of PK parameters**

Pharmacokinetic parameters were determined by non-compartmental methods using the
concentration-time curve profile. The total clearances (CLt) after i.v. administration were calculated as Dose/AUCiv. AUCiv were estimated from the time course using the trapezoidal method with extrapolation from the last quantifiable point to infinity. The terminal elimination half-life t1/2 was estimated as ln2/ke, where ke is that of the plot of the terminal elimination phase on a logarithmic scale.

**Calculation of in vitro intrinsic clearance**

In vitro intrinsic clearance (CLint,in vitro) was calculated from the time course of disappearance of the test drug during incubation with h-hepatocytes. Each plot was fitted to the first-order elimination rate constant as follows:

\[ C(t) = C0 \times e^{-ke \times t} \]

where C(t) and C0 are the concentration of unchanged test drug at incubation time t and that at pre-incubation; ke is the disappearance rate constant of unchanged drug.

Subsequently, CLint, in vitro (μL/min/10⁶ cells) values were converted to CLint, in vitro (mL/min/kg) for the whole body. CLint, in vitro data were scaled up using physiological parameters; human liver weight 26 g/kg (Davies et al., 1993) and PXB mouse liver weight 140 g/kg, and the hepatocellularity (132 x 10⁶ cells/g liver) of PXB mice. These parameters were taken from the average of observed data in PXB mice (RI=80%).
Calculation of in vivo intrinsic clearance

CL\textsubscript{t} of PXB mice was calculated from the plasma concentrations after dosing using non-compartmental methods as described. CL\textsubscript{t} was assumed to be equal to the hepatic clearance.

Values of CL\textsubscript{t}, plasma unbound fraction (fu), blood/plasma concentration ratio (R\textsubscript{b}) in humans were taken from the literature.

In vivo intrinsic clearance (CL\textsubscript{int, in vivo}) was calculated from the in vivo CL\textsubscript{t}, fu, R\textsubscript{b}, and average hepatic blood flow (Q) based on a well-stirred model as follows (Pang et al., 1977):

\[
CL\textsubscript{int, in vivo} = CL\textsubscript{t} / \left( \frac{fu}{R\textsubscript{b}} \times \left( 1 - \frac{CL\textsubscript{t}}{Q} \right) \right).
\]

Average hepatic flow (Q) of humans and PXB mice were set at 21 and 90 mL/min/kg (same as in normal mice), respectively (Davies et al., 1993). In addition, R\textsubscript{b} and fu of human were assumed to be equivalent to those of PXB mice. If total CL of drugs exceeded liver blood flow, 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}/fu. (Table 4)
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 by not only CYP, but also non-CYP enzymes such as UGT, SULT, and aldehyde oxidase (AO) in liver. Mirtazapine and warfarin were known to be mainly metabolized by CYP. Diclofenac, ibuprofen, naproxen were metabolized by both UGT and CYP. Furthermore, the model compounds metabolized by AO such as 6-deoxypenciclovir, fasudil, sulindac, and zaleplon were added in this study. These reflected in data set spanned a wide range of PK parameters characteristics. Total clearance (CL_t) and t_{1/2} after i.v. administration of selected model drugs to humans were obtained from the literature. If CL_t after i.v. administration was not available from the literature, we used the value of CL_t/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 Table 1 and Supplemental Table 2).

Disappearance of parent drugs after incubation

Remaining amounts of all compounds decreased linearly for 2 hrs on incubation with h-hepatocytes. The values of CL_{int,in vitro} in hepatocytes, calculated using scaling factors to
humans and PXB mice whole body as described in methods, are listed in Table 2. These
CL_{int,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 i.v. administration of drug
solutions at 0.3 to 5 mg/kg are shown in Fig. 2 and Table 3. Each RI value in PXB mice was
73.4 to 93.4%.

CL_t values of warfarin and lamotrigine were relatively low, whereas those of fasudil and
salbutamol were much higher; the range of CL_t was 0.2 to 198 mL/min/kg. The t_{1/2} value of
lamotrigine was the longest, and those of 6-deoxypenciclovir and fasudil were short, as shown
as Table 3.

**Comparison of intrinsic CL between h-hepatocytes and humans**

Direct comparison between CL_{int, in vitro} from h-hepatocytes and CL_{int,in vivo} calculated for a
well-stirred model in human showed a moderate correlation (r^2=0.421, p=0.016) (Fig. 3) For
3 of 13 (23%) compounds, observed CL_{int,in vivo} was predicted within a 3-fold error from
hepatocyte CL_{int, in vitro}. However, for 9 of 13 (69%) compounds, observed CL_{int,in vivo} was
predicted with a 3- to 10-fold error.
Fig. 4 shows the relationship between \( \text{CL}_{\text{int, in vivo}} \) and \( \text{CL}_{\text{int, in vitro}} \) for PXB mice; again the correlation was moderate \((r^2=0.419, p=0.017)\). For 7 of 13 (54%) compounds, \( \text{CL}_{\text{int, in vivo}} \) of PXB mice was predicted within 3-fold error from h-hepatocyte \( \text{CL}_{\text{int, in vitro}} \). For 5 of 13 (38%) compounds, \( \text{CL}_{\text{int, in vivo}} \) was predicted with a 3- to 10-fold error.

**Relationship between intrinsic clearance in humans and PXB mice in vivo**

We directly compared \( \text{CL}_{\text{int, in vivo}} \) calculated based on a well-stirred model in humans and PXB mice. As shown in Fig. 5, there was a good correlation \((r^2=0.785, p=5.556 \times 10^{-5})\) between literature \( \text{CL}_{\text{int, in vivo}} \) in human and measured \( \text{CL}_{\text{int, in vivo}} \) of PXB mice for these compounds. For 4 of 13 (31%) compounds, observed \( \text{CL}_{\text{int, in vivo}} \) in humans was predicted within 3-fold error from PXB mouse \( \text{CL}_{\text{int, in vivo}} \). For 7 of 13 (54%) compounds, human \( \text{CL}_{\text{int, in vivo}} \) was predicted with a 3- to 10-fold error.

**Relationship of elimination half-life \((t_{1/2})\) between humans and PXB mice**

Fig. 6 shows the relationship of \( t_{1/2} \) after i.v. administration between humans and PXB mice. Compounds for which literature data were not available were excluded from this figure. A good correlation \((r^2=0.886, p=1.506 \times 10^{-4})\) was found. For 6 of 9 (67%) compounds, human observed \( t_{1/2} \) was predicted within 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 pre-clinical development of pharmaceuticals, in order to reduce costs by enabling 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 under-prediction (Chiou et al., 2002, Akabane et al., 2010), while dog data may cause over-estimation (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 under-prediction of in vivo CL (Obach, 1999). We considered 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 CYP and non-CYP 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, AO) using probe substrates between the donor hepatocytes and h-hepatocytes purified from PXB mice (Supplemental Table 3). As do 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 CYP and non-CYP enzymes, such as UGT, SULT, and aldehyde oxidase (AO). Their values of CL cover a wide range from 0.055 to 118 mL/min/kg (Table 1).
First, we performed in vitro metabolic study using fresh h-hepatocytes isolated from PXB mice. We calculated in vitro intrinsic clearance (CL_{int, in vitro}) using fresh h-hepatocytes and compared the results with human CL_{int, in vivo} estimated by use of the well-stirred model (Pang et al., 1977). These results using parallel tube model and dispersion model were also similar to well-stirred model. (data not shown). A moderate correlation (r^2=0.421, p=0.016) was found, but this approach was not superior to prediction using other methods.

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

A similar correlation (r^2=0.418, p=0.017) was observed between CL_{int, in vitro} 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 3-fold error were insufficient.

Therefore, we next evaluated the predictability of hepatic clearance and half life (t_{1/2}) from in vivo data in PXB mice. The values of CL_{int, in vivo} estimated by i.v. administration in PXB mice were well correlated (r^2=0.785, p=5.556 x 10^{-5}) with observed CL_{int, in vivo} in human. Surprisingly, we also found a good correlation (r^2=0.886, p=1.506 x 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 which showed that the average RI values were about 80%. It was a concern that the contribution of remaining about 20% mice hepatocytes which may be reflected on clearance. \( \text{CL}_{\text{int, in vitro}} \) of these model compounds in host mice hepatocytes (SCID mice) almost showed higher than those of h-hepatocytes within 10-fold range (Supplemental Fig.1). The extent of difference may not influence on predictability of \( \text{CL}_{\text{int, in vivo}} \).

For the estimation of \( \text{CL}_{\text{int, in vivo}} \) in PXB mice, the plasma unbound fraction (fu) of those model compounds is the same as in humans because human albumin are expressed in blood in PXB mice. Inoue et al, 2009 reported fu values of warfarin of PXB mice was similar to that of human. Furthermore, fu values of some compounds (dapsone, diclofenac, ketoprofen, salbutamol, zaleplon) in this study were also approximately similar to those of human (data not shown).

We assumed that blood/plasma concentration ratio (Rb) of those model compounds is also the same as in humans, since Rb values of some compounds (dapsone, diclofenac, ketoprofen, salbutamol, zaleplon) in this study were also approximately similar to those of human (data not shown).

\( Q_h \) were assumed to be 90 mL/min/kg, respectively, corresponding to the values of normal mice (Davies et al., 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 CYP, but also by non-CYP enzymes, including aldehyde oxidase (AO). 6-Deoxypenciclovir, fasudil, sulindac, and zaleplon are mainly metabolized 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 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 at least be used for semi-quantitative prediction of not only CL, but also t₁/₂ in humans. PXB mice would also be useful for in vitro estimation and comparison of PK of various candidate compounds, since large amounts of fresh, identical hepatocytes (1.1x10⁸ cells/mouse) are available by transplantation of donor hepatocytes (2.5x10⁵ 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

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Authorship Contributions

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

Conducted experiments: Sanoh, Horiguchi

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

Performed data analysis: Sanoh, Horiguchi

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

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Figure titles and legends

Fig. 1  Chemical structures of the model compounds used in this study.

Fig. 2  Plasma concentration after i.v. administration to human chimeric mice.
Each point represents the mean±S.D. (n=3~5)

Fig. 3  Correlation between observed human CL_{int, in vivo} and CL_{int, in vitro} of PXB mouse hepatocytes, calculated as described in the text.
The solid line represents unity. The dotted line represents the range within 3-fold of unity.

Fig. 4  Correlation between CL_{int, in vivo} of PXB mice and CL_{int, in vitro} of their hepatocytes, calculated as described in the text.
The solid line represents unity. The dotted line represents the range within 3-fold of unity.

Fig. 5  Correlation of CL_{int, in vivo} between human and PXB mice calculated as described in the text.
The solid line represents unity. The dotted line represents the range within 3-fold of unity.

Fig. 6  Correlation of t_{1/2} after i.v. administration between human and PXB mice.
Compounds for which literature data were not available were excluded from this figure. The solid line represents unity. The dotted line represents the range within 3-fold of unity.
Table 1  Literature values of systemic clearance, half life, and unbound fraction in plasma, Blood / plasma concentration ratio, and the metabolic enzymes in humans for the model compounds examined in this analysis.

<table>
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<th>Compounds</th>
<th>CL&lt;sub&gt;tot&lt;/sub&gt; or CL&lt;sub&gt;oral&lt;/sub&gt;</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt;</th>
<th>fu</th>
<th>Rb</th>
<th>Metabolic enzymes</th>
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<td>0.25</td>
<td>1.04</td>
<td>CYP2C9, CYP3A4, NAT</td>
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<td>6-Deoxypenciclovir&lt;sup&gt;(a)&lt;/sup&gt; &lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>118</td>
<td>-</td>
<td>1</td>
<td>1.2</td>
<td>AO</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>3.5</td>
<td>1.4</td>
<td>0.005</td>
<td>0.55</td>
<td>CYP2C9, UGT2B7, UGT1A9</td>
</tr>
<tr>
<td>Fasudil</td>
<td>73.2</td>
<td>0.26</td>
<td>0.51</td>
<td>1</td>
<td>AO</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.82</td>
<td>1.6</td>
<td>0.006</td>
<td>0.55</td>
<td>CYP2C9, UGT2B7</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>1.6</td>
<td>2.1</td>
<td>0.008</td>
<td>0.55</td>
<td>UGT2B7</td>
</tr>
<tr>
<td>Lamotrigine&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>0.3</td>
<td>-</td>
<td>0.45</td>
<td>1</td>
<td>UGT1A4, UGT2B7</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>8.0</td>
<td>15</td>
<td>0.15</td>
<td>0.67</td>
<td>CYP1A2, CYP2D6, CYP3A4</td>
</tr>
<tr>
<td>(S)-Naproxen</td>
<td>0.1</td>
<td>-</td>
<td>0.01</td>
<td>0.55</td>
<td>CYP2C9, CYP1A2, UGT2B7</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>7.7</td>
<td>3.9</td>
<td>0.925</td>
<td>0.96</td>
<td>SULT1A3</td>
</tr>
<tr>
<td>Sulindac&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>3.3</td>
<td>-</td>
<td>0.069</td>
<td>1</td>
<td>AO</td>
</tr>
<tr>
<td>(S)-Warfarin</td>
<td>0.055</td>
<td>29</td>
<td>0.015</td>
<td>0.55</td>
<td>CYP2C9</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>16</td>
<td>1.1</td>
<td>0.4</td>
<td>0.99</td>
<td>AO, CYP3A4</td>
</tr>
</tbody>
</table>

<sup>(a)</sup> From orally administration data  <sup>(b)</sup> Calculated as famciclovir which is prodrug of 6-deoxypenciclovir. −; Unavailable data from i.v. PK study  Rb values of fasudil, lamotrigine, and sulindac were assumed as 1 due to unavailable data from the literatures. References are in Supplemental Table1 and Supplemental Table2.
Table 2  *Estimation of CL\textsubscript{int, in vitro} (µL/min/kg) in PXB mice hepatocytes and scaling to humans and PXB mice.*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CL\textsubscript{int, in vitro} (µL/min/1x10\textsuperscript{6} cells)</th>
<th>Scaled CL\textsubscript{int, in vitro} (human) (mL/min/kg)</th>
<th>Scaled CL\textsubscript{int, in vitro} (PXB mice) (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapsone</td>
<td>2.3±1.2</td>
<td>8.0±4.0</td>
<td>43.1±21.3</td>
</tr>
<tr>
<td>6-Deoxypenciclovir</td>
<td>5.3±1.2</td>
<td>18.3±4.2</td>
<td>98.6±22.4</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>24.7±1.2</td>
<td>84.7±4.0</td>
<td>455.8±21.3</td>
</tr>
<tr>
<td>Fasudil</td>
<td>35.7±13.3</td>
<td>122.4±45.6</td>
<td>659.1±245.4</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>13.3±2.1</td>
<td>45.8±7.1</td>
<td>246.4±38.5</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>6.0±1.0</td>
<td>20.6±3.4</td>
<td>110.9±18.5</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>1.4±1.0</td>
<td>4.8±3.6</td>
<td>25.9±19.2</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>6.3±1.2</td>
<td>21.7±4.0</td>
<td>117.0±21.3</td>
</tr>
<tr>
<td>(S)-Naproxen</td>
<td>12.7±2.5</td>
<td>43.5±8.6</td>
<td>234.1±46.5</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>1.0±1.0</td>
<td>3.3±3.3</td>
<td>17.9±17.8</td>
</tr>
<tr>
<td>Sulindac</td>
<td>2.0±2.0</td>
<td>7.0±6.7</td>
<td>37.6±36.0</td>
</tr>
<tr>
<td>(S)-Warfarin</td>
<td>1.2±0.7</td>
<td>4.1±2.5</td>
<td>22.2±13.3</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>2.3±1.2</td>
<td>8.0±4.0</td>
<td>43.1±21.3</td>
</tr>
</tbody>
</table>

CL\textsubscript{int, in vitro} (µL/min/1x10\textsuperscript{6} cells) values were converted to CL\textsubscript{int, in vitro} (mL/min/kg) for the whole body. CL\textsubscript{int, in vitro} data were scaled up using physiological parameters; human liver weight 26 g/kg and PXB mouse liver weight 140 g/kg, and the hepatocellularity (132 x 10\textsuperscript{6} cells/g liver) of PXB mice. Each value represents the mean±S.D. (n=3)
Table 3  *Experimental conditions and RI values in PXB mice used for PK study.*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (mg/kg)</th>
<th>RI (mean±S.D.)</th>
<th>CLₜ (mL/min/kg)</th>
<th>t₁/₂ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapsone</td>
<td>3.0</td>
<td>77.4±5.5</td>
<td>2.1±0.5</td>
<td>4.5±1.1</td>
</tr>
<tr>
<td>6-Deoxypenciclovir</td>
<td>3.0</td>
<td>93.4±4.2</td>
<td>71.2±13.0</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>3.0</td>
<td>76.4±2.1</td>
<td>16.4±4.3</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>Fasudil</td>
<td>3.0</td>
<td>75.8±1.3</td>
<td>198.1±14.6</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>5.0</td>
<td>73.4±3.2</td>
<td>3.8±1.0</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>3.0</td>
<td>74.0±1.1</td>
<td>3.3±0.6</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>3.0</td>
<td>77.1±4.0</td>
<td>1.4±0.2</td>
<td>10.1±0.9</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>3.0</td>
<td>79.8±1.7</td>
<td>30.4±9.4</td>
<td>6.0±1.4</td>
</tr>
<tr>
<td>(S)-Naproxen</td>
<td>5.0</td>
<td>82.2±6.1</td>
<td>2.2±0.5</td>
<td>4.8±2.7</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>3.0</td>
<td>74.5±0.7</td>
<td>79.9±34.0</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>Sulindac</td>
<td>3.0</td>
<td>74.5±2.0</td>
<td>5.6±1.3</td>
<td>1.2±0.8</td>
</tr>
<tr>
<td>(S)-Warfarin</td>
<td>0.3</td>
<td>75.3±1.8</td>
<td>0.2±0.1</td>
<td>8.2±2.8</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>3.0</td>
<td>77.1±3.7</td>
<td>48.1±7.1</td>
<td>0.7±0.4</td>
</tr>
</tbody>
</table>

Each compound was administered intravenously (i.v.) to PXB mice at 0.3-5 mg/kg body weight. The values of replacement index (RI) of PXB mice were ranged from 73.4% to 93.4%. Each value represents the mean±S.D. (n=3~5)
Table 4  *In vivo intrinsic clearance (CL_{int, in vivo}) of humans and PXB mice, calculated by well-stirred model*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human CL_{int, in vivo} mL/min/kg</th>
<th>PXB mice CL_{int, in vivo} mL/min/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapsone</td>
<td>2.0</td>
<td>8.9</td>
</tr>
<tr>
<td>6-Deoxypenciclovir</td>
<td>141.6</td>
<td>409.0</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>462.0</td>
<td>2206.0</td>
</tr>
<tr>
<td>Fasudil</td>
<td>370.6</td>
<td>1588.2</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>78.2</td>
<td>363.7</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>119.1</td>
<td>235.5</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>0.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>57.7</td>
<td>205.0</td>
</tr>
<tr>
<td>(S)-Naproxen</td>
<td>5.5</td>
<td>124.0</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>12.6</td>
<td>738.9</td>
</tr>
<tr>
<td>Sulindac</td>
<td>47.8</td>
<td>86.5</td>
</tr>
<tr>
<td>(S)-Warfarin</td>
<td>2.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Zaleplon</td>
<td>166.3</td>
<td>255.7</td>
</tr>
</tbody>
</table>

CL_{int, in vivo} was calculated from the in vivo CL, fu, Rb, and average hepatic blood flow (Q) based on a well-stirred model. Average hepatic flow (Q) of humans and PXB mice were set at 21 and 90 mL/min/kg (same as in normal mice), respectively. In addition, Rb and fu of human were assumed to be equivalent to those of PXB mice. If total CL of drugs exceeded liver blood flow, the hepatic clearance was taken as 90% of liver blood flow. CL_{int, in vivo} of 6-deoxypenciclovir, lamotrigine, and sulindac were evaluated from oral clearance (CL_{oral}) as CL_{oral}/fu.
Fig. 1

- Dapsone
- 6-Deoxypenciclovir
- Diclofenac
- Fasudil
- Ibuprofen
- Ketoprofen
- Lamotrigine
- Mirtazapine
- (S)-Naproxen
- Salbutamol
- Sulindac
- (S)-Warfarin
- Zaleplon
Fig. 2

Plasma concentration (ng/mL) vs. time after administration (hr) for various drugs:

- Dapsone
- 6-Deoxypenciclovir
- Diclofenac
- Fasudil
- Ibuprofen
- Ketoprofen
- Lamotrigine
- Mirtazapine
- (S)-Naproxen
- Salbutamol
- Sulindac
- (S)-Warfarin
- Zaleplon
Fig. 5

![Graph showing the comparison of PXB mice CL_{int, in vivo} vs. Human CL_{int, in vivo} (mL/min/kg).]