Received: 27 January 2021 | Revised: 2 April 2021 | Accepted: 28 April 2021

DOI: 10.1111/cts.13070

ARTICLE

Prediction of human pharmacokinetics for low-clearance compounds using pharmacokinetic data from chimeric mice with humanized livers

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Funding information
No funding was received for this work.

Abstract

Development of low-clearance (CL) compounds that are slowly metabolized is a major goal in the pharmaceutical industry. However, the pursuit of low intrinsic CL (CL_{int}) often leads to significant challenges in evaluating the pharmacokinetics of such compounds. Although in vitro–in vivo extrapolation is widely used to predict human CL, its application has been limited for low-CL_{int} compounds because of the low turnover of parent compounds in metabolic stability assays. To address this issue, we focused on chimeric mice with humanized livers (PXB-mice), which have been increasingly reported to accurately predict human CL in recent years. The predictive accuracy for nine low-CL_{int} compounds with no significant turnover in a human hepatocyte assay was investigated using PXB-mouse methods, such as single-species allometric scaling (PXB-SSS) approach and a novel physiologically based scaling (PXB-PBS) approach that assumes that the CL_{int} per hepatocyte is equal between humans and PXB-mice. The percentages of compounds with predicted CL within 2- and 3-fold ranges of the observed CL for low-CL_{int} compounds were 89% and 100%, respectively, for both PXB-SSS and PXB-PBS approaches. Moreover, the predicted CL was mostly consistent among the methods. Conversely, the percentages of compounds with predicted CL within 2- and 3-fold ranges of the observed CL for low-CL_{int} compounds were 50% and 63%, respectively, for multispecies allometric (MA) scaling. Overall, these PXB-mouse methods were much more accurate than conventional MA scaling approaches, suggesting that PXB-mice are useful tools for predicting the human CL of low-CL_{int} compounds that are slowly metabolized.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

It is important to identify low-clearance (CL) compounds that are slowly metabolized during the drug discovery process, but the ability of in vitro–in vivo extrapolation to

predict human CL decreases as the value of intrinsic CL (CL_{int}) decreases. Although chimeric mice with humanized livers (PXB-mice) have been reported to be useful for predicting human CL, their applicability to low-CL_{int} compounds with no significant turnover in human hepatocyte assays has not been clarified.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study examined the predictive accuracy of PXB-mouse methods for low-CL_{int} compounds.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

In addition to the previously reported single-species allometric scaling approach, we proposed a novel physiologically based scaling approach. Both methods displayed much greater predictive accuracy for low-CL_{int} compounds than conventional multi-species allometric scaling approaches.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The findings should improve the accuracy of human pharmacokinetic prediction and enable efficient and safe first-in-human studies.

INTRODUCTION

The accurate prediction of human pharmacokinetics using preclinical data is essential at the drug discovery stage because it provides important insights into drug candidate selection and first-in-human study design. Clearance (CL) is a key determinant of human pharmacokinetics, and several approaches, such as in vitro–in vivo extrapolation (IVIVE) and multispecies allometric (MA) scaling, have been widely used to predict human CL. Furthermore, the proportion of low-CL compounds in drug discovery portfolios has increased in the pharmaceutical industry to approximately 30%.[1,2] This is largely attributable to the tendency to extract metabolically stable compounds during high-throughput absorption, distribution, metabolism, and excretion (ADME) screening to achieve adequate exposure at a lower dosage in humans.[3] In other words, as a result of the selection of low intrinsic CL (CL_{int}) compounds, it has become increasingly common that IVIVE cannot be applied because of the low turnover of the parent compound in microsomal or hepatocyte stability assays. In these in vitro assays, it is difficult to estimate the in vitro CL_{int} of low-CL_{int} compounds because of the limited incubation time for which enzymatic activity can be maintained.

Chimeric mice with humanized livers (PXB-mice) were generated from urokinase-type plasminogen activator-cDNA/severe combined immunodeficiency mice injected with human hepatocytes.[4] A wide range of drug-metabolizing enzymes and transporters were expressed in the PXB-mice liver, this is considering the fact that greater than 80% of mouse hepatocytes are replaced by human hepatocytes.[5,6] Therefore, PXB-mice represent a useful animal model for predicting human CL.[7] Furthermore, the single-species allometric scaling (PXB-SSS) approach has been reported to provide high predictive accuracy[8,9] and to be applicable to compounds that undergo hepatic organic anion-transporting polypeptide-mediated transport[10] and compounds with long half-lives.[11] However, the predictive accuracy has not been investigated for low-CL_{int} compounds with no significant turnover of the parent compound in human hepatocyte assays.

This study evaluated the usefulness of PXB-mouse methods for predicting the human CL of low-CL_{int} compounds compared with the conventional MA scaling approach. Furthermore, we proposed a novel physiologically based scaling (PXB-PBS) approach that assumes that in vivo CL_{int} per hepatocyte is equal between humans and PXB-mice based on the concept that mouse-derived hepatocytes in PXB-mice are mostly replaced by human hepatocytes. Whereas the PXB-SSS approach is an empirical, simple equation using body weight and exponents, the PXB-PBS approach has physiological significance and aids in understanding the prediction of CL using PXB-mice, which can lead to improved confidence in its application in drug discovery. In addition, the PXB-PBS approach can potentially provide more accurate CL predictions than the PXB-SSS approach because it can incorporate more detailed information, such as physiological parameters and compound-dependent pharmacokinetic parameters.[12]

METHODS

Definition of low-CL_{int} compounds

A total of 16 commercially available compounds were selected, including those expected to have low CL_{int} based on clinical data (<5 ml/min/kg[13]) and those metabolized by cytochrome P450 (CYP) and non-CYP enzymes. Because most of these compounds were metabolized in the liver, this study

assumed that total CL (CL_t) is equal to hepatic CL. An in vitro metabolic stability assay using cryopreserved human hepatocytes purchased from BioIVT (ZOL, 10-donor mixed gender pool, Baltimore, MD, USA) was conducted to confirm whether the compounds exhibited low turnover. Tested compounds at a final concentration of 0.1 or 1 μmol/l were incubated at a cell density of 0.5 million cells/ml for 4 h with human hepatocytes suspended in Williams' E medium containing 0.125% bovine serum albumin, 15 mmol/l HEPES, and 2 mmol/l GlutaMAX-1. Low-CL_{int} compounds in this study were defined as those that did not display significant turnover (<20%[14]; Table 1). By contrast, compounds that exhibited significant turnover in human hepatocyte assays were classified as moderate- to high-CL_{int} compounds in this study. The details of chemicals and reagents used in this study and the detailed procedures of the human hepatocyte assay are provided in the Supporting information.

Data collection

In vitro and in vivo data for all compounds to predict human CL are summarized in Table 1. CL_t in humans, PXB-mice, rats, monkeys, and dogs and fraction unbound in plasma ($f_{u,p}$) in humans and rats were obtained from experiments or the literature. References are provided in Table S1. In vitro $CL_{int,human}$ per hepatocyte was calculated by correcting the compound disappearance rate in human hepatocyte assays with fraction unbound in hepatocytes and medium. Further, $f_{u,p}$ in humans and rats was measured by the equilibrium dialysis method using a Rapid Equilibrium Dialysis Device purchased from Thermo Fisher Scientific. The detailed methods for the determination of in vitro CL_{int} and $f_{u,p}$ are provided in the Supporting information. At various sampling points, the plasma samples after intravenous administration to PXB-mice ($N = 3$) at a cassette dose of 0.1 mg/kg were collected. The plasma samples were extracted with acetonitrile containing the internal standard verapamil, and then the plasma concentration was determined using a liquid chromatography/tandem mass spectrometry method. The details of tandem mass spectrometry are provided in Table S2. Moreover, cassette doses of 0.1 mg/kg were administered intravenously to rats and monkeys ($N = 3$ each), and the plasma concentration was determined in a similar manner. CL_t was calculated via noncompartmental analysis using Phoenix WinNonlin version 6.3 (Certara) based on the plasma concentration–time profiles following intravenous dosing. The sources of animals were as follows: 3 male PXB-mice weighing 18.5–21.5 g and aged 17–19 weeks at dosing were supplied by PhoenixBio Co., Ltd., and the replacement rate of mouse hepatocytes with human hepatocytes was 89%–91%; 3 male Sprague–Dawley rats weighing 242–261 g and aged 6 weeks at dosing were supplied by Charles River Laboratories Japan, Inc.; and 3 male cynomolgus monkeys weighing 2.9–3.6 kg and aged approximately 3.6–3.8 years at dosing were supplied by Hamri Co., Ltd. All in vivo experiments were approved by the Institutional Animal Care and Use Committee of Mitsubishi Tanabe Pharma Corporation (Kanagawa, Japan).

In vitro–in vivo extrapolation

In vitro $CL_{int,human}$ per hepatocyte was converted to scaled $CL_{int,human}$ using human scaling factors, such as liver weight (LW), body weight (BW), and hepatocellularity,[15] which are presented in Table 2, according to Equation 1. $CL_{t,human}$ was calculated from scaled $CL_{int,human}$ via a dispersion model (Equation 2) using hepatic blood flow (Q_h),[15] which is presented in Table 2, and fraction unbound in blood ($f_{u,b}$) was calculated using the human blood-to-plasma concentration ratio (R_b) obtained from the literature.

$$\text{Scaled CL}_{\text{int}}\,(\text{ml/min/kg}) = \text{CL}_{\text{int}}\,\text{per hepatocyte}\,\left(\text{ml/min/1}\times10^{6}\text{cells}\right) \times \frac{\text{LW (g)}}{\text{BW (kg)}} \times \text{hepatocellularity}\,\left(1\times10^{6}\text{cells/g liver}\right), \tag{1}$$

$$\text{CL}_h = Q_h\left[1 - \frac{4a}{(1+a)^2\exp\left[\frac{a-1}{2Dn}\right] - (1-a)^2\exp\left[-\frac{a+1}{2Dn}\right]}\right]. \tag{2}$$

In Equation 2, $Dn = 0.17$,[16] and $\text{Scaled CL}_{\text{int}} = \frac{Q_h(a^2-1)}{4Dn\times f_{u,b}}$.

MA scaling from nonclinical animal species

$CL_{t,human}$ was predicted using the rule of exponent (ROE)[17] and f_u corrected intercept method (FCIM).[18] According to ROE, CL_t values in rats, monkeys, and dogs were plotted against BW on a log–log scale as simple allometry (SA), and then $CL_{t,human}$ was predicted by an allometric equation (Equation 3, 4, or 5) that was based on the exponent values obtained from SA (Equation 3). SA was used when the exponents of SA ranged from 0.55 to 0.70. Maximum life-span potential (MLP) correction was applied when SA was 0.71–1.0; that is, the product of MLP and CL_t for each animal was plotted as a function of BW on a log–log scale using Equation 4. When SA greater than or equal to 1.0, brain weight (BrW) correction was applied; that is, the product of BrW and CL_t for each animal was plotted in a similar manner using Equation 5. In these equations, a, b, and c were the coefficients of the allometric equations, and x, y, and z were the exponents. BW, BrW, and MLP were set at 0.25 kg, 0.00174 kg, and 4.4 years, respectively, for rats; 3.75 kg,

TABLE 1 Summary of drug disposition and in vitro and in vivo parameters to predict human CL_t for all compounds

		In vitro parameters					In vivo parameters			
			CL_{int} per hepatocyte (μl/min/1 × 10^6 cells)	R_b[a]	$f_{u,p}$		CL_t (ml/min/kg)			
Compounds	**Disposition**	**% remaining after a 4-h incubation**	**Humans**	**Humans**	**Humans**	**Rats**	**PXB-mice**	**Rats**	**Monkeys**	**Dogs**
Antipyrine	P450	96	ND	1	0.990	0.690	4.60	7.86	11.5	7.22
Bosentan	P450, OATP	69	12.3	0.48	0.023	0.012	8.78	16.40	20.6	1.72
Carbazeran	AO	0	96.9	1	0.148	0.169	99.67	42.22	87.87	11.5
Dapsone	P450, NAT	94	ND	1.04	0.381	0.280	4.40	6.87	5.08	1.21
Diazepam	P450	62	3.9	0.71	0.036	0.171	42.147	61.51	17.90	46.71
Disopyramide	P450	71	3.2	1.2	0.161	0.610	28.86	179.46	19	29
Doxazosin	P450	11	41.5	1	0.066	0.050	34.245	30.00	15.47	11.21
Ranitidine	P450, FMO	83	ND	1	1.000	0.900	131.02	99.88	40.23	10.4
Reboxetine	P450	35	13.9	1	0.041	0.253	17.658	61.51	14.94	22.39
(S)-Naproxen	P450, UGT	102	ND	0.55	0.007	0.008	0.556	0.41	0.83	0.04
(S)-Warfarin	P450	80	ND	0.55	0.013	0.005	0.484	0.21	0.102	1.49
Tenoxicam	P450	96	ND	0.67	0.015	0.030	0.2878	0.49	0.061	0.104
Theophylline	P450	96	ND	0.85	0.580	0.400	4.70	1.91	1.08	1.73
Timolol	P450	85	ND	0.84	0.715	0.760	130.05	137.02	13.6	–[b]
Tolbutamide	P450	89	ND	0.55	0.039	0.049	0.58	0.39	0.0456	0.142
UCN−01	–[b]	4	83.5	1	0.003	0.0175	0.0369	77.37	3.36	10.27

Note: References are provided in Table S1.

Abbreviations: CL_{int}, intrinsic clearance; R_b, blood-to-plasma concentration ratio; $f_{u,p}$, fraction unbound in plasma; CL_t, total clearance; PXB-mice, chimeric mice with humanized livers; ND, not determined because of the absence of significant turnover (<20%) during a 4-h incubation in the human hepatocyte assay; OATP, organic anion-transporting polypeptide; AO, aldehyde oxidase; NAT, N-acetyltransferase; FMO, flavin-containing monooxygenase; UGT, UDP-glucuronosyltransferase.

[a]R_b was assumed to be 1 for carbazeran, doxazosin, ranitidine, reboxetine, and UCN-01 because of a lack of data in the literature.

[b]Not available in the literature.

TABLE 2 Scaling factors for the IVIVE and PXB-PBS approaches

Species	Q_h (ml/min/kg)	Liver weight (g)	Body weight (kg)	Hepatocellularity (1×10^6 cells/g liver)
Humans	20	1470	70	120
PXB-mice	91.3	1.977	0.02	168

Abbreviations: IVIVE, in vitro–in vivo extrapolation; PXB-mice, chimeric mice with humanized livers; PXB-PBS, physiologically based scaling using PXB-mice; Q_h, hepatic blood flow.

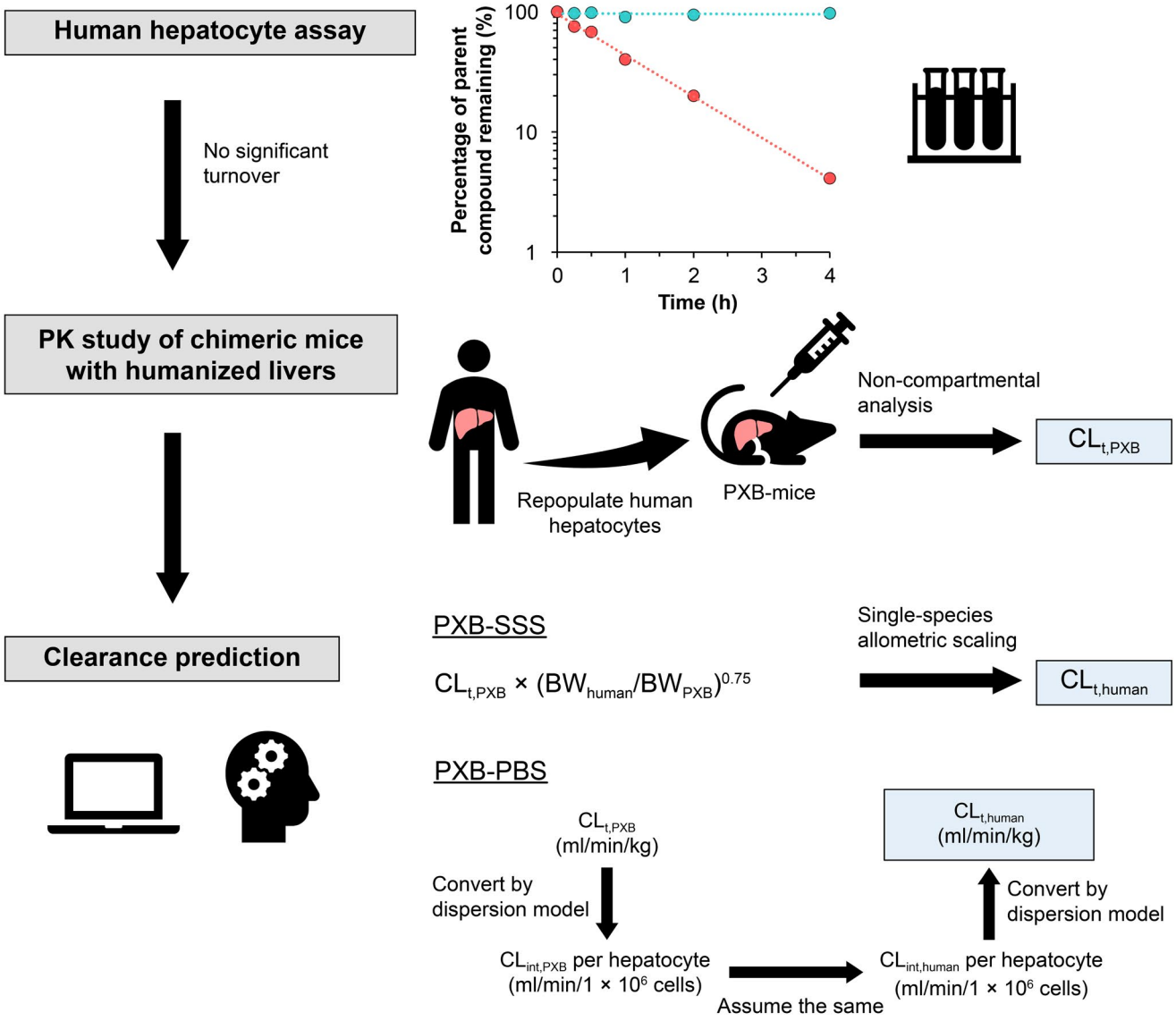

FIGURE 1 Scheme for predicting human clearance (CL) for low intrinsic CL compounds with no significant turnover in human hepatocyte assays. PXB-mice, chimeric mice with humanized livers that were repopulated with human hepatocytes. PXB-SSS, prediction method based on single-species allometric scaling using PXB-mice. PXB-PBS, prediction method based on physiologically based scaling using PXB-mice. CL_t, CL_{int}, and BW, total clearance, intrinsic clearance, and body weight, respectively

0.0424 kg, and 18.5 years, respectively, for monkeys; and 12 kg, 0.0754 kg, and 20.5 years, respectively, for dogs.[19] MLP in years was calculated as a function of BW and BrW for each animal according to Equation 6.

$$CL_t = a(BW)^x, \quad (3)$$

$$CL_t = \frac{b(MLP \times CL_t)^y}{8.18 \times 10^5}, \quad (4)$$

$$CL_t = \frac{c(BrW \times CL_t)^z}{1.53}, \quad (5)$$

$$\text{MLP (years)} = 185.4\text{BrW}^{0.636}\text{BW}^{-0.225}. \quad (6)$$

According to FCIM, $CL_{t,human}$ was predicted using Equation 7. In this equation, a was the intercept obtained from the log–log plot of CL_t versus BW, and $Rf_{u,p}$ was the ratio of $f_{u,p}$ for rats and humans.

$$CL_t = 33.35 \times \left(\frac{a}{Rf_{u,p}}\right)^{0.77}. \quad (7)$$

PXB-SSS

$CL_{t,human}$ was predicted by the PXB-SSS approach using Equation 8.[9] BW was set at 70 and 0.02 kg for humans and PXB-mice, respectively.

$$CL_{t,\,human} = CL_{t,PXB} \times (BW_{human}/BW_{PXB})^{0.75}. \quad (8)$$

PXB-PBS

$CL_{t,human}$ was predicted using the PXB-PBS approach following several steps (Figure 1). Based on the scaling factors of PXB-mice presented in Table 2, scaled $CL_{int,PXB}$ was calculated from $CL_{t,PXB}$ via a dispersion model (Equation 2) using Q_h and $f_{u,b}$, and then in vivo $CL_{int,PXB}$ per hepatocyte was calculated (Equation 1). The estimation or measurement methods of LW and hepatocellularity in PXB-mice, which were used as scaling factors, are provided in the Supporting information. Based on the concept that mouse-derived hepatocytes in PXB-mice are largely replaced by human hepatocytes, in vivo CL_{int} per hepatocyte was assumed to be equal between humans and PXB-mice (Equation 9). The same assumption was applied for $f_{u,p}$ and R_b.[7] Based on the assumption that Q_h is equal in normal and PXB-mice,[7] the Q_h values presented in Table 2 were used.[20] The inverse conversion was performed by a dispersion model using human scaling factors, and then $CL_{t,human}$ was estimated. For compounds for which $CL_{t,PXB}$ exceeds Q_h, CL_t was set at 90% of Q_h.[7]

$$CL_{int,PXB}\ \text{per hepatocyte}\ \left(\text{ml/min/1}\times 10^6\text{cells}\right) = CL_{int,human}\ \text{per hepatocyte}\ \left(\text{ml/min/1}\times 10^6\text{cells}\right). \quad (9)$$

Evaluation of predictive accuracy

Observed $CL_{t,human}$ was compared with the predicted value for the IVIVE, PXB-SSS, PXB-PBS, ROE, and FCIM approaches to calculate the fold error. For each low-CL_{int} compound, moderate- to high-CL_{int} compounds, and all compounds, the percentages predicted within 2- and 3-fold ranges of the observed $CL_{t,human}$ were calculated. Moreover, the geometric mean of the ratio between the predicted and observed values, which was frequently used as the absolute average fold error (AAFE), was calculated according to Equation 10,[21] and subsequently the predictability of each method was compared. For low-CL_{int} compounds, the PXB-SSS, PXB-PBS, ROE, and FCIM approaches were compared, and IVIVE was additionally included in the comparison for moderate- to high-CL_{int} compounds and all compounds.

$$\text{AAFE} = 10^{\frac{\sum\left|\log\left(\frac{\text{Observed}}{\text{Predicted}}\right)\right|}{n}}. \quad (10)$$

Sensitivity analysis

To fully understand the PXB-PBS approach, sensitivity analyses were conducted using a dataset of (S)-naproxen as a representative of low-CL_{int} compounds and diazepam as a representative of moderate- to high-CL_{int} compounds. The impact of parameters such as $f_{u,p}$, R_b, and Q_h, which included assumptions in the PXB-PBS approach in this study, on predicted $CL_{t,human}$ was investigated. Simulated $CL_{t,human}$ was defined as the CL generated by changing each parameter, and the magnitude of change from the predicted $CL_{t,human}$ was evaluated according to Equation 11 for an increased predicted value of $CL_{t,human}$ and Equation 12 for a decreased predicted value of $CL_{t,human}$.[22] These equations were primarily used to express the changes in predicted $CL_{t,human}$ on a similar magnitude for both positive and negative value and to evaluate the compounds on the same scale on a 3D plot, regardless of the observed $CL_{t,human}$. Sensitivity analysis was conducted for $f_{u,p}$ in humans and PXB-mice within a 3-fold range of human $f_{u,p}$, for R_b in humans and PXB-mice within the range of 0.5–2, for Q_h in PXB-mice within the range of 90–180 ml/min/kg, and for Q_h in humans within the range of 18–23 ml/min/kg, which were frequently used as physiological parameters in literature.

$$\text{Percent change (\%)} = \frac{\text{Simulated}\,CL_{t,human} - \text{Predicted}\,CL_{t,human}}{\text{Predicted}\,CL_{t,human}} \times 100, \quad (11)$$

$$\text{Percent change (\%)} = \frac{\text{Simulated}\,CL_{t,human} - \text{Predicted}\,CL_{t,human}}{\text{Simulated}\,CL_{t,human}} \times 100. \quad (12)$$

RESULTS

Determination of low-CL_{int} compounds based on human hepatocyte assays

As the purpose of this study was to verify whether the prediction method using PXB-mice was useful for low-CL_{int} compounds, we first conducted an in vitro metabolic stability assay using cryopreserved human hepatocytes to identify low-CL_{int} compounds.

The proportion of the parent compound remaining after 4 h of incubation is presented in Table 1. Antipyrine, dapsone, ranitidine, (S)-naproxen, (S)-warfarin, tenoxicam, theophylline, timolol, and tolbutamide met the criteria for low-CL_{int} compounds; that is, they did not display significant turnover (<20%) during a 4-h incubation. Conversely, bosentan, carbazeran, diazepam, disopyramide, doxazosin, reboxetine, and UCN-01 (7-hydroxystaurosporine) were classified as moderate- to high-CL_{int} compounds because they exhibited significant turnover. The plots of the proportion of each parent compound remaining after 4 h of incubation are summarized in Figure S1.

Prediction of human CL by IVIVE, MA, and PXB-mouse methods

For low-CL_{int} compounds, CL was predicted using conventional MA scaling approaches and PXB-mouse methods, whereas for moderate- to high-CL_{int} compounds, CL was predicted by all methods including IVIVE. The relationships between observed and predicted $CL_{t,human}$ are presented in Figure 2. The observed and predicted $CL_{t,human}$ and the fold errors are summarized in Table 3. To compare the predictability of each method, both the percentages predicted within 2- and 3-fold ranges of the observed value and AAFE are summarized in Table 4.

The percentage predicted within 2-fold ranges of the observed $CL_{t,human}$ for low-CL_{int} compounds was 89% for both the PXB-SSS and PXB-PBS approaches, and the AAFEs were 1.51 and 1.46, respectively. Furthermore, the percentage predicted within 3-fold ranges of the observed $CL_{t,human}$ was 100% for both methods. Conversely, according to ROE and FCIM approaches, some compounds displayed large discrepancies exceeding 3-fold between the observed and predicted values, leading to higher AAFEs, such as 2.58 and 2.50. When the predictability of each method was compared among moderate- to high-CL_{int}

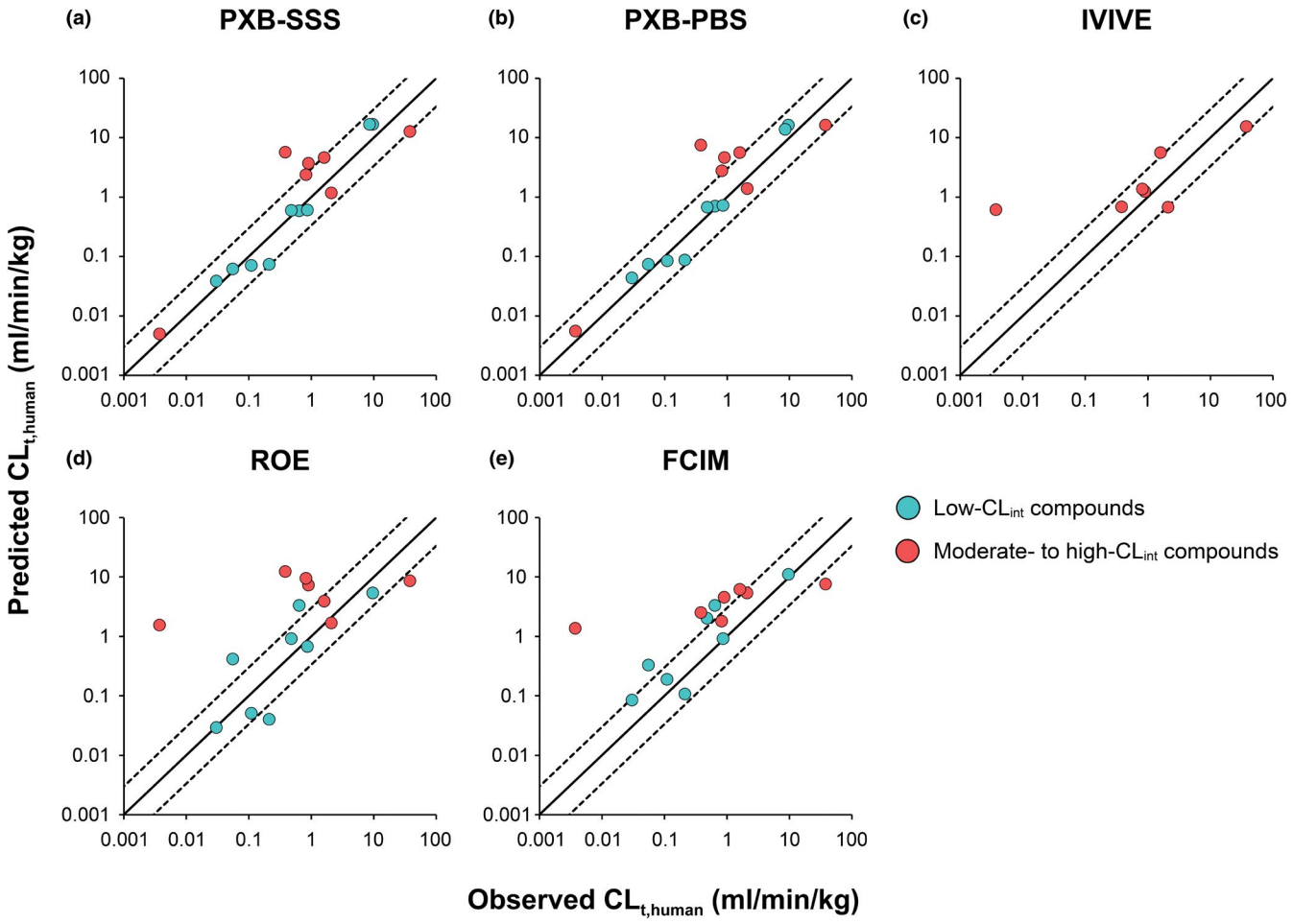

FIGURE 2 Relationships between observed and predicted total human clearance ($CL_{t,human}$) for low intrinsic clearance (CL_{int}) compounds and moderate- to high-CL_{int} compounds. Panels (a), (b), (c), (d), and (e) presents the results of single-species allometric scaling from chimeric mice with humanized livers (PXB-mice), a physiologically based scaling using PXB-mice, in vitro–in vivo extrapolation (IVIVE), rule of exponent, and the f_u corrected intercept method, respectively. Solid and dotted lines represent the unity and 3-fold error, respectively. PXB-SSS, prediction method based on single-species allometric scaling using PXB-mice; PXB-PBS, prediction method based on physiologically based scaling using PXB-mice; ROE, rule of exponent; FCIM, f_u corrected intercept method

TABLE 3 Comparisons of observed and predicted $CL_{t,human}$ for all compounds

Category of compounds	Compounds	Observed $CL_{t,human}$ (ml/min/kg)	Predicted $CL_{t,human}$ (ml/min/kg)					Fold error (predicted/observed)				
			PXB-SSS	PXB-PBS	IVIVE	ROE	FCIM	PXB-SSS	PXB-PBS	IVIVE	ROE	FCIM
Low-CL_{int} compounds	Antipyrine	0.64	0.59	0.71	ND	3.32	3.31	0.92	1.10	ND	5.19	5.17
	Dapsone	0.48	0.60	0.67	ND	0.91	2.01	1.24	1.40	ND	1.90	4.19
	Ranitidine	9.6	16.9	16.4	ND	5.4	11.1	1.76	1.70	ND	0.56	1.16
	(S)-Naproxen	0.11	0.072	0.085	ND	0.051	0.189	0.65	0.77	ND	0.46	1.71
	(S)-Warfarin	0.055	0.062	0.074	ND	0.417	0.330	1.13	1.34	ND	7.58	5.99
	Tenoxicam	0.03	0.039	0.044	ND	0.029	0.085	1.29	1.46	ND	0.98	2.83
	Theophylline	0.86	0.60	0.72	ND	0.68	0.91	0.70	0.84	ND	0.79	1.06
	Timolol	8.5	16.8	13.7	ND	NC	NC	1.98	1.62	ND	NC	NC
	Tolbutamide	0.21	0.074	0.088	ND	0.040	0.107	0.35	0.42	ND	0.19	0.51
Moderate- to high-CL_{int} compounds	Bosentan	2.1	1.18	1.39	0.68	1.68	5.37	0.56	0.66	0.32	0.80	2.56
	Carbazeran	37.6	12.8	16.4	15.5	8.6	7.6	0.34	0.44	0.41	0.23	0.20
	Diazepam	0.38	5.68	7.52	0.69	12.46	2.53	14.94	19.80	1.82	32.79	6.67
	Disopyramide	0.9	3.72	4.63	1.24	7.36	4.56	4.14	5.15	1.38	8.17	5.06
	Doxazosin	1.6	4.61	5.65	5.62	3.93	6.20	2.88	3.53	3.51	2.46	3.88
	Reboxetine	0.82	2.39	2.79	1.37	9.52	1.82	2.91	3.40	1.67	11.61	2.22
	UCN−01	0.0037	0.0050	0.0056	0.6186	1.5640	1.3765	1.35	1.52	167.20	422.70	372.03

Note: References are provided in Table S1.

Abbreviations: CL_{int}, intrinsic clearance; $CL_{t,human}$, total human clearance; FCIM, f_u corrected intercept method; IVIVE, in vitro–in vivo extrapolation; NC, not calculated because of a lack of data; ND, not determined because of the absence of significant turnover (<20%) during a 4-h incubation in the human hepatocyte assay; PXB-PBS, physiologically based scaling using chimeric mice with humanized livers; PXB-SSS, single-species allometric scaling using chimeric mice with humanized livers; ROE, rule of exponents.

TABLE 4 Comparative evaluation of various prediction methods

	PXB-SSS	PXB-PBS	IVIVE	ROE	FCIM
Low-CL_{int} compounds					
Number of compounds	9	9	ND	8	8
Within 2-fold error (%)	89	89	ND	50	50
Within 3-fold error (%)	100	100	ND	63	63
AAFE	1.51	1.46	ND	2.58	2.50
Moderate- to high-CL_{int} compounds					
Number of compounds	7	7	7	7	7
Within 2-fold error (%)	29	29	43	14	0
Within 3-fold error (%)	71	43	57	29	29
AAFE	3.23	3.50	4.07	10.84	7.52
All compounds					
Number of compounds	16	16	7	15	15
Within 2-fold error (%)	63	63	43	33	27
Within 3-fold error (%)	88	75	57	47	47
AAFE	2.11	2.14	4.07	5.04	4.18

Abbreviations: CL_{int}, intrinsic clearance; PXB-SSS, single-species allometric scaling using chimeric mice with humanized livers; PXB-PBS, physiologically based scaling using chimeric mice with humanized livers; IVIVE, in vitro–in vivo extrapolation; ROE, rule of exponents; FCIM, f_u corrected intercept method; ND, not determined because of the absence of significant turnover (<20%) during a 4-h incubation in the human hepatocyte assay; AAFE, absolute average fold error.

compounds, the percentages predicted within 3-fold ranges of observed $CL_{t,human}$ values and AAFEs were 71% and 3.23, respectively, for the PXB-SSS approach, 43% and 3.50, respectively, for the PXB-PBS approach, 57% and 4.07, respectively, for IVIVE, 29% and 10.84, respectively, for the ROE approach, and 29% and 7.52, respectively, for the FCIM approach. It was suggested that both PXB-SSS and PXB-PBS approaches were more accurate than the MA scaling approach, but they had comparable accuracy as IVIVE. Thus, even for moderate- to high-CL_{int} compounds, PXB-mouse methods displayed relatively high predictive accuracy, but large discrepancies were noted between the observed and predicted values for diazepam in PXB-mouse methods, leading to large AAFEs. IVIVE also provided highly accurate predictions overall, but AAFE was large because of the considerably greater overprediction in UCN-01. For all compounds, the percentages predicted within 3-fold ranges of observed $CL_{t,human}$ and AAFE were 88% and 2.11, respectively, for the PXB-SSS approach, 75% and 2.14, respectively, for the PXB-PBS approach, 47% and 5.04, respectively, for the ROE approach, and 47% and 4.18, respectively, for the FCIM approach, suggesting that PXB-mouse methods exhibited the best accuracy.

Predictive performance of the PXB-PBS approach and investigation for critical parameters by sensitivity analysis

As illustrated in Table 3 and Figure S2, $CL_{t,human}$ predicted using the PXB-PBS approach for all compounds was mostly consistent with that predicted using the PXB-SSS approach, revealing the high predictive accuracy as described previously despite having several assumptions that in vivo CL_{int} per hepatocyte, $f_{u,p}$, and R_b were equal between humans and PXB-mice and that Q_h was equal between normal and PXB-mice. Sensitivity analysis was conducted using the (S)-naproxen and diazepam dataset to examine the impact of $f_{u,p}$, R_b, and Q_h on predicted $CL_{t,human}$, and the results are summarized in Figure 3. When $f_{u,p}$ was larger in humans than in PXB-mice, predicted $CL_{t,human}$ increased, but in the opposite case, predicted $CL_{t,human}$ decreased. The magnitude of this change was greater for (S)-naproxen than for diazepam. Conversely, when there was no significant difference in $f_{u,p}$ between humans and PXB-mice, predicted $CL_{t,human}$ was constant regardless of the value of $f_{u,p}$ for both compounds. R_b in humans and PXB-mice and Q_h in humans and PXB-mice did not significantly affect predicted $CL_{t,human}$ within the range examined for both compounds.

DISCUSSION

The discovery and development of low-CL_{int} compounds is one of the most important tasks common to all pharmaceutical companies. It is an important mission of drug metabolism and pharmacokinetics scientists to efficiently assess drug metabolism and accurately predict $CL_{t,human}$ during the drug discovery process. In suspended hepatocyte assays, low-CL_{int} compounds frequently display no significant turnover

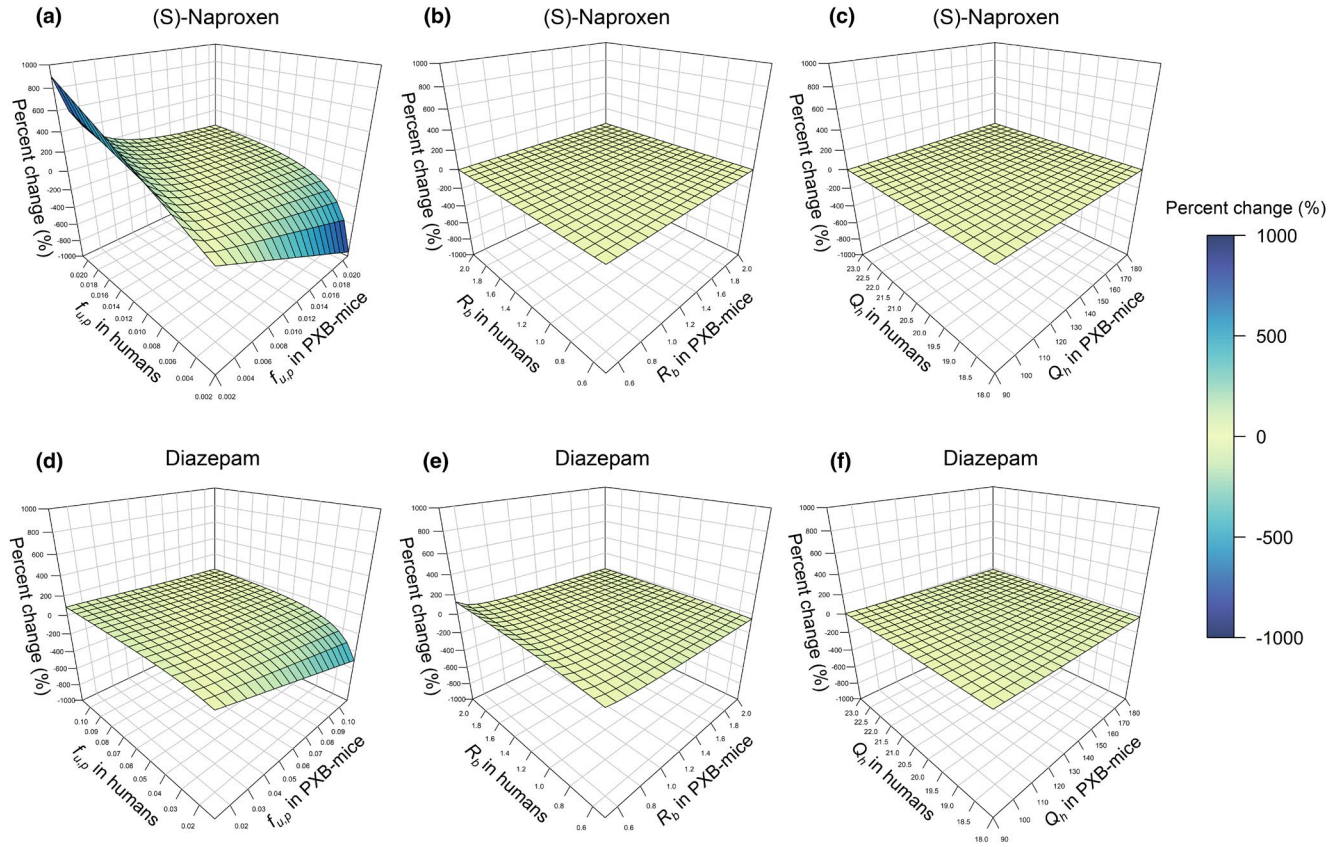

FIGURE 3 Sensitivity analysis of total human clearance ($CL_{t,human}$) predicted using the PXB-PBS approach. The impact of fraction unbound in plasma ($f_{u,p}$), blood-to-plasma concentration ratio (R_b), and hepatic blood flow (Q_h) on predicted $CL_{t,human}$ value was examined using a dataset of (S)-naproxen as a representative of low intrinsic clearance (CL_{int}) compounds and diazepam as a representative of moderate- to high-CL_{int} compounds. Sensitivity analysis was conducted for each $f_{u,p}$ value in humans and PXB-mice within a 3-fold range of human $f_{u,p}$, for each R_b value in humans and PXB-mice within a 0.5–2 range, and for each Q_h value within the ranges of 90–180 ml/min/kg in PXB-mice and 18–23 ml/min/kg in humans. The simulated $CL_{t,human}$ was defined as the clearance generated by changing each parameter, and the impact of these parameters on predicted $CL_{t,human}$ was evaluated as the percent change (%) calculated according to the following equations: ((simulated $CL_{t,human}$ −predicted $CL_{t,human}$)/predicted $CL_{t,human}$) ×100% for an increase in predicted $CL_{t,human}$ and ((simulated $CL_{t,human}$ −predicted $CL_{t,human}$)/simulated $CL_{t,human}$) ×100% for a decrease in predicted $CL_{t,human}$. Panels (a), (b), and (c) present the results obtained using $f_{u,p}$, R_b, and Q_h for (S)-naproxen as variables, respectively. Panels (d), (e), and (f) present the results obtained using $f_{u,p}$, R_b, and Q_h for diazepam as variables, respectively. PXB-mice, chimeric mice with humanized livers; PXB-PBS, physiologically based scaling using PXB-mice

within the incubation period in which enzymatic activity can be maintained. Therefore, an in vitro metabolic stability assay to detect slow turnover for a longer period, such as the Hepatopac,[13] HμREL,[2] and relay methods,[14] have been developed, whereas in vivo prediction methods specific for low-CL_{int} compounds have not been developed.

In this study, we used PXB-mice to construct an alternative approach to the suspended hepatocyte assay. In fact, although it is difficult to accurately compare the predictive accuracy of these in vitro assays with PXB-mouse methods because of the limited number of overlapping tested compounds, we propose two main advantages of PXB-mouse methods. First, in vitro assays successfully maintained not only CYP activity but also non-CYP activity for approximately 1 week.[23] However, how a longer incubation period affects activity remains unclear, particularly for non-CYP enzymes and transporters.[23] In addition, impact of longer incubations on IVIVE predictive accuracy requires further elucidation. Conversely, PXB-mice are not limited to the in vitro specific time-dependent loss of activity. Therefore, it is easy to estimate CL_t if appropriate blood sampling time points can be set. Second, it has been reported that CL_{int} may lead to underprediction if compound binding to the culture ware or fibroblasts is not considered,[13] whereas PXB-mouse methods do not have such limitations. Overall, PXB-mouse methods can provide high predictive accuracy, ease of use, and ease of interpretation, resulting in efficient drug discovery and development.

For the nine compounds defined in this study as low-CL_{int} compounds, the predictive accuracy of PXB-mouse methods was evaluated, and the higher predictive accuracy was confirmed. By contrast, antipyrine, (S)-warfarin, and

tenoxicam exhibited more than 3-fold discrepancies between the observed and predicted values in both the ROE and FCIM approaches. Although these MA scaling approaches using pharmacokinetic data from nonclinical animal species have been commonly used, their use in CL prediction for compounds with large interspecies difference in drug disposition has been limited.[24] In addition, there are some compounds for which CL is overpredicted for unknown reasons.[25] Antipyrine and (S)-warfarin are already known to follow vertical allometry as compounds for which CL is overpredicted,[25] and the present results again indicated the limitation of MA scaling approaches. Furthermore, the predictive accuracy of PXB-mouse methods for moderate- to high-CL_{int} compounds was also higher than those of MA scaling approaches, but AAFE was similar to that of IVIVE. There have been some reports of overprediction for diazepam, which leads to a high AAFE in PXB-SSS approach.[8,9] These reports suggest that the metabolic activity derived from mouse hepatocytes remaining in the livers of PXB-mice may contribute to the overprediction, but these findings have not been clearly elucidated. Concurrently, PXB-mouse methods were relatively less accurate for moderate- to high-CL_{int} compounds than for low-CL_{int} compounds. Sawada et al. noted a case of benzydamine, in which the flavin-containing monooxygenase (FMO) enzymes that were expressed in the mouse kidney were attributed for causing the overprediction of CL_t, and proposed that interspecies differences other than hepatic metabolism may lead to poor predictive accuracy.[26] They may also have caused poor predictive accuracy in moderate- to high-CL_{int} compounds in this study; however, there is no evidence that such a trend is observed more than low-CL_{int} compounds, thereby requiring further investigation. Furthermore, despite having renal CL mechanism, ranitidine, which is a low-CL_{int} compound, successfully predicted CL by PXB-SSS approach, but it was not successful for disopyramide, which is a moderate- to high-CL_{int} compound. Further analysis after measuring renal CL in PXB-mice is warranted for more accurate predictions. On the contrary, overprediction of UCN-01 also led to a high AAFE for the IVIVE method. Because UCN-01 is known to bind to α_1-acid glycoprotein more strongly than to albumin[27] and α_1-acid glycoprotein was not contained in this hepatocyte assay, such a difference between in vitro and in vivo conditions may have resulted in high CL_{int} and a large overprediction. It is noteworthy that PXB-mouse methods exhibited high predictive accuracy for UCN-01, and IVIVE displayed high predictive accuracy for diazepam. Therefore, the prediction strategy of selecting PXB-mouse methods or IVIVE according to the compound profile is most effective for moderate- to high-CL_{int} compounds. In IVIVE, a strategy for increasing confidence in the accuracy of the prediction by confirming the in vitro–in vivo correlation in at least two preclinical species has been proposed,[28] but there is currently no clear method for prospectively recognizing whether PXB-mouse methods are adaptive. Overall, it is expected that factors related to overprediction and the characteristics of the compound will be clarified and appropriate prediction strategies will be devised, or a prediction method exhibiting high predictive accuracy for all compounds will be found.

It has been reported that in vivo CL_{int} per LW is correlated between humans and PXB-mice,[7] and cases of prediction in which the values were assumed to be equal have been reported.[29] On the contrary, based on the concept that mouse-derived hepatocytes in PXB-mice are largely replaced by human hepatocytes, we proposed the PXB-PBS approach on the assumption that in vivo CL_{int} per hepatocyte was equal between humans and PXB-mice. It has been proven that hepatocellularity should be considered to improve the predictive accuracy for CL[30] ; thus, we also incorporated interspecies differences in hepatocellularity into the PXB-PBS approach. This approach and sensitivity analysis of certain parameters provided several new findings. First, $CL_{t,human}$ predicted using the PXB-PBS approach was mostly consistent with that predicted using the PXB-SSS approach for all compounds. The PXB-SSS approach is a simple allometric equation using the BW ratio and exponent. This exponent describes the relationship of physiological parameters between humans and PXB-mice, and it might feature the same concepts as the various assumptions in the PXB-PBS approach. Second, the results of sensitivity analysis demonstrated that a small interspecies difference in $f_{u,p}$ had little effect on predicted $CL_{t,human}$, whereas a large interspecies difference in $f_{u,p}$ greatly affected predicted $CL_{t,human}$. Because the PXB-PBS approach exhibited high predictive accuracy for many compounds, it was suggested that $f_{u,p}$ might be similar between humans and PXB-mice. In fact, human albumin and human α_1-acid glycoprotein involved in the plasma protein binding are secreted into the plasma of PXB-mice[4,31] and Miyamoto et al. have reported that interspecies differences in $f_{u,p}$ are within 3-fold for many compounds.[31] However, the differences exceeded 3-fold for diazepam, (S)-naproxen, and UCN-01, and $f_{u,p}$ was larger in PXB-mice than in humans for each compound.[31] As revealed in the sensitivity analysis, predicted $CL_{t,human}$ decreased when this interspecies difference was considered, which may have resulted in a relaxation of overprediction. Therefore, this interspecies difference in $f_{u,p}$ might be one of the causes of overprediction for diazepam. By contrast, for (S)-naproxen and UCN-01, considering these interspecies differences resulted in a less accurate CL prediction, and as Miyamoto et al. also stated, the factors responsible for these interspecies differences should be further investigated. Third, the result of sensitivity analysis illustrated that if $f_{u,p}$ was equal between humans and PXB-mice, predicted CL_t was constant regardless of the value of $f_{u,p}$. This finding appeared useful for compounds with high plasma protein binding, for which it is difficult to accurately measure $f_{u,p}$ in terms of the sensitivity and robustness of the

analytical method[32] or necessity of setting appropriate experimental conditions.[33] Finally, the results of sensitivity analysis indicated that R_b or Q_h did not significantly affect predicted $CL_{t,human}$. In fact, there were no cases in which R_b or Q_h was accurately estimated in PXB-mice, and this finding appeared meaningful.

In conclusion, PXB-mouse methods predicted $CL_{t,human}$ for 89% of low-CL_{int} compounds within a 2-fold range of the observed values and 100% of low-CL_{int} compounds within a 3-fold range of the observed values. These prediction methods displayed much greater predictive accuracy than the conventional MA scaling approaches, indicating the usefulness of PXB-mice as novel predictive tools for low-CL_{int} compounds with no significant turnover in human hepatocyte assays. Furthermore, $CL_{t,human}$ predicted using a novel PXB-PBS approach was mostly consistent with that predicted using the PXB-SSS approach for all 16 tested compounds. This finding and the results of sensitivity analysis suggested that the high predictive accuracy of the PXB-SSS approach may be attributable to the similarity of CL_{int} per hepatocyte and $f_{u,p}$ between humans and PXB-mice. Overall, a translational understanding from a physiological perspective has increased the confidence in the application of PXB-mice to drug discovery.

CONFLICT OF INTEREST

C.T. is an employee of PhoenixBio Co., Ltd. and S.S. received financial support from PhoenixBio Co., Ltd. in collaboration study. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

K.Y., T.N., T.W., C.T., S.S., and Y.K. wrote the manuscript. K.Y., N.I., H.S., and T.N. designed the research. K.Y., Y.D., N.I., H.Y., Y.I., and C.T. performed the research. K.Y. and Y.D. analyzed the data. K.Y. and Y.D. contributed new reagents/analytical tools.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Yoshida K, Doi Y, Iwazaki N, et al. Prediction of human pharmacokinetics for low-clearance compounds using pharmacokinetic data from chimeric mice with humanized livers. *Clin Transl Sci*. 2022;15:79–91. https://doi.org/10.1111/cts.13070