Drug Discovery: Recent Progress and the Future

Review

Utility of Chimeric Mice with Humanized Liver for Predicting Human Pharmacokinetics in Drug Discovery: Comparison with *in Vitro*–*in Vivo* Extrapolation and Allometric ScalingYoichi Naritomi,^{*,a} Seigo Sanoh,^b and Shigeru Ohta^{b,†}

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Predicting human pharmacokinetics (PK) such as clearance (CL) and volume of distribution (Vd) is a critical component of drug discovery. These predictions are mainly performed by *in vitro*–*in vivo* extrapolation (IVIVE) using human biological samples, such as hepatic microsomes and hepatocytes. However, some issues with this process have arisen, such as inconsistencies between *in vitro* and *in vivo* findings; the integration of predicted CYP, non-CYP and transporter-mediated human PK; and the difficulty of evaluating very metabolically stable compounds. Various approaches to solving these issues have been reported. Allometric scaling using experimental animals has also often been used. However, this method has also shown many problems due to interspecies differences, albeit that various correction methods have been proposed. Another approach involves the production of chimeric mice with humanized liver *via* the transplantation of human hepatocytes into mice. The livers of these mice are repopulated mostly with human hepatocytes and express human drug-metabolizing enzymes and drug transporters, suggesting that these mice are useful for solving the issues of IVIVE and allometric scaling, and more reliably predicting human PK. In this review, we summarize human PK prediction methods using IVIVE, allometric scaling and chimeric mice with humanized liver, and discuss the utility of predicting human PK in drug discovery by comparing these chimeric mice with IVIVE and allometric scaling.

Key words humanized mouse; prediction; pharmacokinetics; *in vitro*–*in vivo* extrapolation; allometric scaling

1. INTRODUCTION

Predicting human efficacy, toxicity, and drug metabolism and pharmacokinetics (PK) is critical to drug discovery. Historically, poor drug metabolism and PK profiles have been the most significant cause of attrition of drug candidates.¹⁾ Pharmaceutical companies have therefore tried to predict human drug metabolism and PK based on *in vitro* studies using human biological samples and *in vivo* studies using experimental animals, and selected drug candidates which were considered to show reliable drug metabolism and PK profiles in humans. Although these strategies produced a dramatic reduction in attrition, drug metabolism and PK still remain as non-negligible cause of attrition.²⁾

To select drug candidates, it is necessary to predict human quantitative PK, metabolite profiles and drug–drug interactions (DDIs) caused by drug enzyme inductions and inhibitions. In particular, predicting human PK is important for drug discovery. Conventionally, this has been done by evaluating PK parameters such as clearance (CL) and volume of distribution (Vd). In addition, more detailed human PK predictions such as the time course of plasma and tissue drug concentrations have become to be used because these predictions

are necessary to estimate quantitative efficacy and toxicity.³⁾

Prediction of human PK has mainly been conducted using compounds metabolized by CYP. However, recently, it has also become necessary to predict human PK for compounds metabolized by non-CYP, such as uridine 5'-diphosphate (UDP)-glucuronosyltransferase (UGT) and aldehyde oxidase (AO).^{3,4)} Further, attention has also focused on predicting human PK involving drug transporters.³⁾ It is also important to evaluate the integration of these prediction methods using CYP, non-CYP and drug transporters-mediated human PK.

Prediction of human PK is most commonly conducted using *in vitro*–*in vivo* extrapolation (IVIVE) and allometric scaling. IVIVE predicts *in vivo* CL using *in vitro* data for biological materials such as hepatic microsomes and hepatocytes, based on the CL concepts. This method is very useful for predicting human PK using human biological samples. However, differences between *in vitro* and *in vivo* findings have produced many difficulties with this prediction, for which various correction methods have been proposed.⁵⁾ As mentioned above, IVIVE has been mainly established for compounds metabolized by CYP. Presently, it is also necessary to establish prediction methods for compounds metabolized by non-CYP. Allometric scaling predicts CL and Vd based on the relationship between *in vivo* PK parameters and body weights in experimental animals such as mice, rats, dogs and monkeys. Prediction with allometric scaling has also often failed due

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to interspecies differences in drug metabolism and pharmacokinetics. However, this methodology has been reconsidered following proposals for various modified methods and its predictability has been revised.^{5,6)}

Despite these methods for improving IVIVE and allometric scaling, various issues concerning their accuracy and application remain unsolved. Accordingly, more accurate and useful prediction methods are required. Recently, human PK prediction methods using chimeric mice with humanized liver have been reported.⁷⁾ These mice are constructed by transplanting them with human hepatocytes.⁸⁾ The livers of these mice are then mostly repopulated with human hepatocytes and express human drug-metabolizing enzymes and drug transporters.⁹⁾ These chimeric mice with humanized liver are therefore considered useful animals for solving the issues of PK prediction using IVIVE and allometric scaling.

Chimeric mice with humanized liver have been used for PK, metabolite profiling and DDIs studies. There have been several reviews with respect to the studies, including our recent review.^{7,10–13)} In the present review, we particularly focus on human PK prediction using chimeric mice with humanized liver. First, we explain an overview of IVIVE and allometric scaling and problems with their use. Next, we discuss the utility of chimeric mice with humanized liver for predicting human PK in drug discovery by comparing this method with IVIVE and allometric scaling.

2. PREDICTION OF HUMAN PK USING IVIVE

In drug discovery, predicting human CL is essential for PK studies. IVIVE has been widely used for this purpose. Namely, mathematical models based on CL concepts were proposed for predicting quantitative hepatic CL from the 1970s to 1980s.^{14–16)} The reliability of the mathematical models was initially studied for compounds mainly metabolized by CYP using experimental animal biological samples such as hepatic microsomes and hepatocytes. Since the 1990s, prediction of human CL has progressed by the application of human biological samples. Hepatic CL (CL_h) is predicted using mathematical models. The equations of these models consist of intrinsic CL (CL_{int}), blood unbound fraction (f_u) for each compound, and hepatic blood flow (Q_h). Typical models include the well-stirred model¹⁴⁾ (Eq. 1), the parallel-tube model¹⁵⁾ (Eq. 2) and the dispersion model¹⁶⁾ (Eq. 3).

$$CL_h = \frac{Q_h \cdot f_u \cdot CL_{int}}{Q_h + f_u \cdot CL_{int}} \quad (1)$$

$$CL_h = Q_h \left[1 - \exp \left(- \frac{f_u \cdot CL_{int}}{Q_h} \right) \right] \quad (2)$$

$$CL_h = Q_h \left[1 - \frac{4a}{(1+a)^2 \exp \frac{a-1}{2D_n} - (1-a)^2 \exp \left(- \frac{a+1}{2D_n} \right)} \right] \quad (3)$$

where D_n (the dispersion number) = 0.17, $a = \sqrt{1 + 4R_n D_n}$ and $R_n = f_u \cdot CL_{int} / Q_h$

CL_h is predicted by substituting *in vitro* CL_{int} ($CL_{int, in vitro}$) estimated from *in vitro* metabolic experiments using hepatic microsomes or hepatocytes into CL_{int} in the equations (Fig. 1). However, difficulties in predicting human CL have been often

Fig. 1. Procedure for Predicting CL_h Using IVIVE

observed due to the inconsistency between *in vitro* and *in vivo* findings. Various correction methods have therefore been proposed.⁵⁾ Naritomi *et al.*^{17,18)} evaluated a prediction method using a compound-specific scaling factor, the ratio of animal $CL_{int, in vitro}$ and *in vivo* CL_{int} ($CL_{int, in vitro} / CL_{int, in vivo}$). Animal and human $CL_{int, in vitro}$ are first estimated using hepatic microsomes or hepatocytes. The animal $CL_{int, in vivo}$ is then calculated from *in vivo* animal PK experiments using the mathematical models. Finally, human CL_h is predicted using human $CL_{int, in vitro}$ corrected by the animal scaling factor and the mathematical models. Obach¹⁹⁾ evaluated the predictability of human CL using the mathematical models with or without plasma and microsomal protein binding. Poulin *et al.*²⁰⁾ proposed a novel method which considered the fraction unbound to hepatocytes in incubation and the unbound fraction in the liver. Despite these efforts, however, the discrepancy between *in vitro* and *in vivo* methods has still not necessarily been resolved. Resolution of this issue would require further improvement in IVIVE methods or completely different approaches.

As mentioned above, IVIVE has been primarily evaluated for compounds mainly metabolized by CYP. In contrast, non-CYP such as UGT and AO are involved in the metabolism of many compounds. Testa *et al.*²¹⁾ reported that although CYP is involved in 40% of drug metabolism, non-CYP is involved in the remaining part. Cerny²²⁾ reported the contributions of CYP and non-CYP in the formation of major metabolites for U.S. Food and Drug Administration (FDA)-approved intravenous and orally administered small-molecule drugs (2006–2015). He showed that although CYP contributes the largest percentage of metabolism, non-CYP or a mix of both CYP and non-CYP represent a considerable portion. Thus, it is also necessary to consider prediction methods for non-CYP metabolism.

When predicting CL using IVIVE for a compound, it is necessary to select *in vitro* reaction conditions which accord with the characteristics of the drug metabolizing enzymes involved in the metabolism. So which enzymes metabolize the compounds should be clarified. Drug metabolizing enzymes involved in the metabolism of test compounds are usually predicted by detecting comprehensive metabolites, the quantitative relative amount of the metabolites, and structural elucidation of the metabolites in hepatocytes. The metabolites produced are generally identified using LC-MS. Radio-labelled test compounds are useful for detecting comprehensive metabolite. However, the use of radio-labelled test compounds is limited in drug discovery. One method for identifying enzymes involved in the metabolism of a compound is to use a specific inhibitor for each drug-metabolizing enzyme in *in*

in vitro metabolic studies using hepatocytes. Jinno *et al.*²³⁾ evaluated the contribution of CYP and UGT to the metabolism of various carboxylic acid compounds by adding 1-aminobenzotriazole, a CYP inhibitor, or (–)-borneol, a UGT inhibitor, in *in vitro* human hepatocytes reaction mixtures. As an alternative method, Di *et al.*²⁴⁾ reported an approach for providing mechanistic insights into metabolic pathways by comparing $CL_{\text{int, in vitro}}$ in hepatic microsomes (CYP-mediated reaction) and hepatocytes: for compounds mainly metabolized by CYP, the two $CL_{\text{int, in vitro}}$ values are comparable. On the other hand, for compounds mainly metabolized by non-CYP, $CL_{\text{int, in vitro}}$ in hepatocytes is larger than in hepatic microsomes. When uptake is rate-limiting in hepatocytes, $CL_{\text{int, in vitro}}$ in hepatocytes is smaller than in hepatic microsomes.

Unlike the case with CYP, it is necessary to establish an IVIVE method for each non-CYP enzyme. It has been reported that $CL_{\text{int, in vitro}}$ in hepatic microsomes underpredicts $CL_{\text{int, in vivo}}$ for UGT metabolism.^{25,26)} One reason is that unsaturated fatty acids released from microsomal membranes inhibit the metabolic activities of UGT isoforms such as UGT1A9 and UGT2B7.²⁷⁾ The predictability of human CL_h was improved by the addition of bovine serum albumin (BSA), which can sequester unsaturated fatty acids in *in vitro* reaction mixtures. For AO metabolism, $CL_{\text{int, in vitro}}$ in hepatic cytosol and S9 and hepatocytes underpredicted human $CL_{\text{int, in vivo}}$.²⁸⁾ Akabane *et al.*²⁹⁾ compared human $CL_{\text{int, in vivo}}$ and $CL_{\text{int, in vitro}}$ in pooled hepatocytes for eight AO substrates. $CL_{\text{int, in vitro}}$ showed an approximately 10-fold underestimation compared to $CL_{\text{int, in vivo}}$. By taking account of this result, they were able to predict CL_h quantitatively. Sulfotransferase (SULT), carboxylesterase (CES), flavin-containing monooxygenase (FMO) and monoamine oxidase (MAO) are also important non-CYP enzymes. However, reports on predicting human CL with these drug-metabolizing enzymes are limited. Cubit *et al.*³⁰⁾ tried to predict human $CL_{\text{int, in vivo}}$ based on $CL_{\text{int, in vitro}}$ of hepatic and intestinal CYP, UGT and SULT for four compounds. They used hepatic and intestinal cytosol for SULT metabolism. Nishimuta *et al.*³¹⁾ evaluated the prediction of human CL for eight CES 1 substrates using hepatocytes and S9. The mean value of predicted $CL_{\text{int, in vivo}}$ based on hepatocyte $CL_{\text{int, in vitro}}$ showed only 20% of the observed value. On the other hand, prediction bias was reduced with hepatic S9. Jones *et al.*³²⁾ studied the correlation between $CL_{\text{int, in vivo}}$ and $CL_{\text{int, in vitro}}$ in humans for 10 FMO substrates. They found that $CL_{\text{int, in vitro}}$ in hepatocytes can predict $CL_{\text{int, in vivo}}$ and CL_h well. For MAO metabolism, PK prediction methods using human hepatic mitochondria and microsomes have been reported.^{33,34)} However, further improvement in predictability appears to require additional evaluation.

Recently, predicting drug transporter-mediated human PK using IVIVE has attracted attention.³⁵⁾ Watanabe *et al.*³⁶⁾ predicted human PK for pravastatin, which is a substrate of organic anion-transporting polypeptide (OATP) 1B1 and multidrug resistance-associated protein (MRP) 2, using a physiologically based pharmacokinetic (PBPK) model with human cryopreserved hepatocytes. They showed the excellent predictability of plasma concentration profiles and distribution of pravastatin by incorporating empirical scaling factors which were calculated from *in vivo* PK data and *in vitro* data determined in hepatocytes in rats. De Bruyn *et al.*³⁷⁾ reported that the use of the empirical scaling factors determined from

monkey data improved the prediction of human PK for nine OATP substrates. The empirical value of the scaling factors is thought to differ among compounds, donors of human hepatocytes, *in vitro* conditions and animal species.^{38–41)}

As described above, various IVIVE methods for predicting human PK participants for each drug-metabolizing enzyme or drug transporter have been evaluated. However, it is difficult to clarify which of the drug-metabolizing enzymes and transporters contributes to the PK of test compounds in early drug discovery. It should be also considered that multiple drug-metabolizing enzymes and transporters are involved in PK. Therefore, it is necessary to establish unified PK prediction methods for various drug-metabolizing enzymes and drug transporters.

When test compounds are metabolically very stable, it is difficult to estimate $CL_{\text{int, in vitro}}$ accurately since incubation times are limited in *in vitro* experiments with microsomes and hepatocytes.⁴²⁾ Various approaches to this issue have been reported. Di *et al.*⁴³⁾ proposed the relay method, in which human cryopreserved hepatocytes are transferred in the supernatant of an *in vitro* incubation mixture to another *in vitro* incubation mixture with freshly thawed hepatocytes to prolong the reaction time. They reported that $CL_{\text{int, in vitro}}$ estimated using the relay method showed excellent predictions of human $CL_{\text{int, in vivo}}$. Further, novel *in vitro* systems such as HepatoPac^{44,45)} and HμREL,^{46,47)} which are micropatterned hepatocyte coculture systems, and cell systems such as upcyte,⁴⁸⁾ which is derived from human hepatocytes, have been used to estimate low $CL_{\text{int, in vitro}}$ and predict *in vivo* CL in humans.

Prediction of human CL_h has been mainly investigated using IVIVE methods. However, predicting extrahepatic CL is also important. To predict intestinal clearance or intestinal availability, various mathematical models^{4,49)} such as the Q_{gut} model⁵⁰⁾ and the simplified intestinal availability model⁵¹⁾ have been proposed. To predict renal clearance, IVIVE methods have been also evaluated.^{52–55)} Further, future studies will require human PK prediction methods which unify hepatic and extrahepatic clearances.

3. PREDICTION OF HUMAN PK USING ALLOMETRIC SCALING

Allometric scaling is a PK prediction method based on the relationship between *in vivo* PK parameters such as total CL (CL_t) and V_d , and body weights in experimental animals such as

Fig. 2. Illustration of Simple Allometry for Predicting Human CL_t

mice, rats, dogs and monkeys. Simple allometry is a basic method. To predict human CL_r , the following equation (Eq. 4) obtained from *in vivo* PK studies in three or more species is used.

$$CL_r = a \times BW^b \quad (4)$$

where a and b are the coefficient and scaling exponent, respectively, and BW is the body weight.

Figure 2 shows an illustration of simple allometry for predicting human CL_r . However, this method has often failed at predictions. Therefore, various modified allometric scaling methods aimed at improving the prediction of human pharmacokinetics have been proposed.

Mahmood and Balian⁵⁶⁾ proposed the rule of exponents (ROE). This method predicts human CL_r based on the scaling exponents of simple allometry as follows:

$$\text{When } 0.55 \leq b \leq 0.70, CL = a \times BW^b \quad (5)$$

When $0.71 \leq b \leq 1.0$,

$$CL_{\text{human}} = a \times (MLP_{\text{animal}} \times CL_{\text{animal}})^b / MLP_{\text{human}} \quad (6)$$

$$\text{When } b > 1.0, CL_{\text{human}} = a \times (BrW_{\text{animal}} \times CL_{\text{animal}})^b / BrW_{\text{human}} \quad (7)$$

$$\text{When } b > 1.3, CL \text{ may be overpredicted} \quad (8)$$

$$\text{When } b < 0.55, CL \text{ may be underpredicted} \quad (9)$$

where MLP represents the maximum lifespan potential and BrW indicates brain weight.

Ward and Smith⁵⁷⁾ proposed the liver blood flow (LBF) method. This method predicts human CL_r using animal CL_r and the ratio of human to animal LBF rates. Predicted human CL_r can be calculated using the following equation (Eq. 10):

$$CL_{r \text{ human}} = CL_{r \text{ animal}} \times (LBF_{\text{human}} / LBF_{\text{animal}}) \quad (10)$$

The LBF values of 85, 30, 45 and 21 mL/min/kg for rats, dogs, monkeys and humans, respectively, were used.^{58,59)} Prediction accuracy of the LBF method was better with monkey clearance data than with rat and dog data. Nagilla and Ward,⁶⁰⁾ Ward *et al.*,⁶¹⁾ and Tang *et al.*⁶²⁾ reported similar results. Tang and Mayersohn⁶³⁾ developed the unbound fraction in plasma (f_u) corrected intercept method (FCIM), which incorporates rat and human plasma protein binding data with allometric scaling. The equation (Eq. 11) for predicting human CL_r is as follows:

$$CL_{r \text{ human}} = 33.35 \text{ mL/min} \times (a / Rf_u)^{0.77} \quad (11)$$

where a is the coefficient of simple allometric scaling, and Rf_u is the ratio of f_u in rats to humans. This method showed better predictability than the ROE.

Tang *et al.*⁶²⁾ proposed alternative approaches based on CL_r data sets from one or two animal species. The one animal species method uses rat, dog, or monkey CL_r data with a fixed coefficient as follows:

$$CL_{r \text{ human}} / \text{kg} = 0.152 \times CL_{r \text{ rat}} / \text{kg} \quad (12)$$

$$CL_{r \text{ human}} / \text{kg} = 0.410 \times CL_{r \text{ dog}} / \text{kg} \quad (13)$$

$$CL_{r \text{ human}} / \text{kg} = 0.407 \times CL_{r \text{ monkey}} / \text{kg} \quad (14)$$

The two animal species method uses rat and dog, or rat and monkey CL data with a fixed coefficient and exponent as fol-

lows:

$$CL_{r \text{ human}} = a_{\text{rat-dog}} \times (BW_{\text{human}})^{0.628} \quad (15)$$

$$CL_{r \text{ human}} = a_{\text{rat-monkey}} \times (BW_{\text{human}})^{0.650} \quad (16)$$

where a is the coefficient calculated from allometric scaling using the two species. They reported that the one species method using monkey data shows better predictability than that using rat or dog data, and that two species methods show comparable predictability with that of the ROE.

Hosea *et al.*⁶⁴⁾ reported that, from retrospective analysis, single species scaling (SSS) with rat, dog or monkey data shows similar or improved accuracy compared to multiple species allometric scaling methods such as simple allometric scaling and ROE. This method for predicting human CL uses a fixed scaling exponent of 0.75 as follows:

$$CL_{r \text{ human}} = CL_{r \text{ animal}} \times (BW_{\text{human}} / BW_{\text{animal}})^{0.75} \quad (17)$$

Deguchi *et al.*⁶⁵⁾ evaluated the accuracy of predicting human CL_r using the ROE and SSS with mouse, rat, monkey and dog PK data for UGT substrates. They reported that SSS using monkeys showed the most accurate prediction of human CL_r . Recently, Yoshimatsu *et al.*⁶⁶⁾ evaluated the prediction of human CL_r using SSS with minipigs. SSS with minipigs showed higher predictability compared with mice and rats.

In their comprehensive assessment of human CL_r prediction methods, Lombardo *et al.*⁶⁷⁾ compared the accuracy of 37 different human CL_r prediction methods by analyzing intravenous PK data from rats, dogs and monkeys for approximately 400 compounds. They estimated human CL_r and calculated the geometric mean-fold error, % of <2-fold error and median bias as the predictability indices for each method. They evaluated allometric scaling methods, including the one animal species method and LBF method using rat, dog and monkey data, and FCIM using rat, dog and monkey data. The one animal species method and LBF method using monkey data, and FCIM showed good predictability. Ring *et al.*⁶⁸⁾ evaluated the accuracy of 29 different human CL_r prediction methods for 108 compounds. They reported that several allometric scaling methods, including ROE, FCIM, the one animal species method using dog data, and the two animal species method using rat and dog data performed relatively well. Liu *et al.*⁶⁹⁾ collected rat, dog and monkey PK data for 446 compounds and evaluated the predictabilities of human CL_r using various allometric scaling methods including SSS, the two animal species method, ROE, FCIM and LBF methods. They classified reliable CL_r prediction methods based on their disposition pathway (renal excretion or non-renal excretion (metabolism, biliary excretion or minor renal excretion)), organ extraction ratio (hepatic extraction ratio or ratio of unbound CL to renal glomerular filtration rate) and $\log P$ of the compounds.

Volume of distribution at steady state (V_{dss}) is an important PK parameter. In general, V_{dss} can be predicted using allometric scaling. It has been also reported that correction with unbound fraction in plasma improves human V_{dss} prediction.^{6,70)} Lombardo *et al.*⁷¹⁾ comprehensively estimated the predictabilities of prediction methods of V_{dss} in humans using rat, dog, and monkey PK data for approximately 400 compounds. They suggested that SSS using dogs may be a reasonable method for allometric scaling.

As described above, various modified methods have been proposed to improve the predictability of human PK param-

eters, especially CL . However, as long as allometric scaling methods use typical experimental animals such as mice, rats, dogs and monkeys, failures in predicting human PK will occur due to interspecies differences.

4. PREDICTION OF HUMAN PK USING CHIMERIC MICE WITH HUMANIZED LIVER

As described in Sections 2 and 3, IVIVE and allometric scaling have been widely used to predict human PK. However, the various issues mentioned above remain. One reason for these issues is that *in vivo* situations are not reflected in IVIVE methods. In addition, predictions with allometric scaling fail due to interspecies differences in PK. To solve these issues, the application of humanized mouse models has been considered. For human PK prediction methods, genetically humanized mice and chimeric mice with humanized liver are often investigated.⁸⁾ Genetically humanized mice result from the introduction of specific human genes into the mouse genome. The mice then express only the selected human drug metabolizing enzymes and transporters. Accordingly, the use of these mice in predicting human PK is limited to those compounds whose metabolism and disposition involve human drug metabolizing enzymes and transporters expressed in the mice. It is also necessary to consider the effects of drug metabolizing enzymes and transporters originally expressed in the host mice. Mitsui *et al.*⁷²⁾ predicted human CL for compounds mainly metabolized by CYP3A4 using transgenic mice carrying the human CYP3A4 gene and lacking their mouse *Cyp3a* gene. They reported an excellent correlation of $CL_{int, in vivo}$ between the mice and humans for six CYP3A4 substrates. Further, they showed that the correlation could be improved by considering the relative contribution of CYP3A4 to overall metabolism in the mice.

In contrast, chimeric mice with humanized liver are generated by transplanting human cells into host mice which have immunodeficiencies and impaired hepatic function. The mice express human drug metabolizing enzymes and transporters in the liver since the mouse hepatocytes are mostly replaced with human hepatocytes.⁷⁻¹²⁾ Thus, the mice are useful for predicting human PK which is mainly determined by the liver. To produce such chimeric mice with humanized liver, various host mice have been developed, such as uPA/SCID, FRG, TK-NOG, AFC8, Alb-TRECK/SCID and FDG/NOD mice.⁸⁾ Among the models of chimeric mice with humanized liver, the most frequently used are humanized liver uPA/SCID mice, humanized liver TK-NOG mice and humanized liver FRG mice.^{7,8,12)} A number of researchers reported that levels of mRNA and protein expression and activities of drug metabolizing enzymes and transporters in the livers of chimeric mice with humanized liver were similar to those in human liver,⁷³⁻⁷⁸⁾ suggesting that chimeric mice with humanized liver would be useful in quantitatively predicting human PK.

However, it is only recently that chimeric mice with humanized liver have been applied to the prediction of human PK. In a previous review, we summarized human PK studies using chimeric mice with humanized liver.⁷⁾ In the present review, we add a number of novel research reports and discuss points to consider when applying chimeric mice with humanized liver to predict human PK.

As basic research into predicting human CL , Sanoh *et al.*⁷⁹⁾

Fig. 3. Comparison of $CL_{int, in vitro}$ in Human Hepatocytes Isolated from the Liver of Humanized Liver uPA/SCID Mice and $CL_{int, in vivo}$ in Humans

Solid line represents unity. Dotted lines represent 3-fold differences. Adapted from Sanoh *et al.*⁷⁹⁾ with permission from the American Society for Pharmacology and Experimental Therapeutics.

Fig. 4. Comparison of $CL_{int, in vivo}$ in PXB Mice, a Humanized Liver uPA/SCID Mouse Model and Humans

Solid line represents unity. Dotted lines represent 3-fold differences. Adapted from Sanoh *et al.*⁷⁹⁾ with permission from the American Society for Pharmacology and Experimental Therapeutics.

evaluated the relationship of CL_{int} in humanized liver uPA/SCID mice and humans for 13 compounds metabolized by CYP and/or non-CYP. First, they compared $CL_{int, in vitro}$ estimated from human hepatocytes isolated from the liver of the mice and $CL_{int, in vivo}$ in humans. Human $CL_{int, in vivo}$ was calculated from *in vivo* human CL_i using the well-stirred model. These CL_{int} data showed a poor correlation (Fig. 3), suggesting the need for a specific scaling factor for each compound, the ratio of $CL_{int, in vitro}$ and $CL_{int, in vivo}$, as was also reported by Naritomi *et al.*^{17,18)} Next, they compared $CL_{int, in vivo}$ in humanized liver uPA/SCID mice and humans. These $CL_{int, in vivo}$ showed a good correlation except for a few compounds (Fig. 4), suggesting that it would be possible to quantitatively predict human CL based on *in vivo* PK studies using chimeric mice with humanized liver. However, absolute values of $CL_{int, in vivo}$ in the mice were not consistent with those in humans, and the same scaling factor, the ratio of $CL_{int, in vivo}$ between the mice and humans, were observed among the compounds. To predict human $CL_{int, in vivo}$ using chimeric mice with humanized liver, it is necessary to consider the compound common scaling factor.

Sanoh *et al.*⁸⁰⁾ reported a human PK prediction method based on allometric scaling using humanized liver uPA/SCID

mice. First, they predicted human CL_t and Vd_{ss} for various compounds metabolized by CYP and/or non-CYP using SSS. 82.4% and 100% of predicted human CL_t and Vd_{ss} were within 3-fold of the observed values, respectively, indicating that SSS using chimeric mice with humanized liver is useful for quantitatively predicting human PK. However, this method failed the prediction of CL_t for diazepam. In general, the liver of chimeric mice with humanized liver is not perfectly replaced with human hepatocytes.^{9–11} The replacement index (RI), the replacement rate of mouse hepatocytes with human hepatocytes, of the mice used in the study was approximately 80%. The authors suggested that the reason for this failure to predict human CL_t for diazepam was metabolism by residual mouse hepatocytes. Next, they evaluated the prediction of intravenous plasma concentration–time curves in humans. They used the complex Dedrick plot,⁸¹ a prediction method for human plasma concentration–time profiles based on allometric scaling. Results also showed good predictability for most of the compounds evaluated. However, this method failed to predict the plasma concentration–time curve for diazepam, and also failed to predict CL_t .

Miyamoto *et al.*⁸² evaluated the utility of SSS using chimeric mice with humanized liver by comparing the predictability of human CL_t and Vd_{ss} using humanized liver uPA/SCID mice, rats and monkeys. When using the mice, 83.3% and 79.3% of the predicted values of CL_t and Vd_{ss} were within 3-fold of the observed values, respectively. They also showed the excellent predictability of human PK parameters using the mice compared with those with rats and monkeys. These results indicate that chimeric mice with humanized liver are useful for predicting human PK based on SSS. However, when using mice, human CL_t for diazepam was overpredicted, as was also found by Sanoh *et al.*⁸⁰ In addition, human CL_t was also overpredicted for benzydamine, which is metabolized by FMO, a non-CYP enzyme. It was considered that these overpredictions were due to metabolism of the compound by residual mouse hepatocytes in the liver of the mice. On the other hand, for mycophenolic acid and indomethacin, human CL_t were underpredicted using the mice, estimated due to differences in extra-hepatic metabolism between the mice and humans.

Physiologically based pharmacokinetic (PBPK) modeling is

a mathematical model which uses a pharmacokinetic model consisting of multiple compartments. This model mimics human physiology to quantitatively predict concentration–time profiles of compounds in the blood or other biological fluids, and tissues.⁸³ Human PK prediction methods based on PBPK models using chimeric mice with humanized liver have been also reported. Utoh *et al.*⁸⁴ reported the suitable predictability of human plasma concentration profiles of CYP probes based on a simplified PBPK model consisting of three compartments using humanized liver TK-NOG mice. However, Yamazaki-Nishioka *et al.*⁸⁵ reported the overprediction of human CL_h for benzydamine, a FMO substrate, based on a simplified PBPK model using humanized liver TK-NOG mice, as was also reported by Miyamoto *et al.*⁸² They suggested that the failure of prediction might be due to the effect of oxidation in the kidney, extra-hepatic metabolism. Nakayama *et al.*⁸⁶ predicted human CL_t and Vd_{ss} from *in vivo* PK data of humanized liver uPA/SCID mice using the well-stirred model and the Rodgers equation for 16 CYP and non-CYP substrates, and two OATP1B1 or OATP1B3 substrates. Results showed excellent predictability. Further, they estimated plasma concentration–time profiles in humans using a more complicated PBPK model composed of 11 tissue compartments for 17 compounds. They observed generally successful prediction of human plasma concentration–time profiles.

Here, we consider some points for the application of chimeric mice with humanized liver for predicting human PK. When generating chimeric mice with humanized liver, human hepatocytes transplanted into host mice are usually prepared from a specific donor. Therefore, it is necessary to evaluate the differences in PK among chimeric mice with humanized liver transplanted with human hepatocytes obtained from different donors. As described above, Sanoh *et al.*⁸⁰ and Miyamoto *et al.*⁸² reported the excellent predictability of human CL_t and Vd_{ss} using chimeric mice with humanized liver based on SSS. These reports used humanized liver uPA/SCID mice transplanted with commercially available human hepatocytes of different lots. Figure 5 compares CL_t and Vd_{ss} between humanized liver uPA/SCID mice transplanted with human hepatocytes of Lots. BD85 and BD195. PK parameters in the mice transplanted with Lot. BD85 were reported by Sanoh *et al.*⁸⁰ whereas those transplanted with human hepatocytes of Lot.

Fig. 5. Comparison of CL_t and Vd_{ss} between Humanized Liver uPA/SCID Mice Transplanted with Human Hepatocytes of Lot. BD85 and BD195. Solid line represents unity. Data from Sanoh *et al.*⁸⁰ and Miyamoto *et al.*⁸²

Fig. 6. Procedure of SSS (A) and Administration of Compounds (B) in *in Vivo* PK Studies Using Chimeric Mice with Humanized Liver
A–L: test compounds.

BD195 were reported by Miyamoto *et al.*⁸²⁾ Although the CL_t values obtained from Lot. BD195 tended to be slightly higher than those from Lot. BD85, an excellent correlation was observed. Moreover, V_{dss} were similar between mice obtained from Lot. BD85 and 195 for most of the compounds, showing an excellent correlation. In the future, comparison of chimeric mouse with humanized liver PK parameters should be evaluated for additional human hepatocyte lots.

By considering the relationship of mice and human PK, it is possible to reliably predict human PK. Therefore, estimation of PK in mice for test compounds should incorporate the evaluation of PK for a reference compound whose human PK has already been clarified. For example, prediction of human CL_t and V_{dss} using SSS should include application of the fixed values of a scaling exponent calculated from the relationship between chimeric mouse with humanized liver and human PK for a reference compound (Fig. 6(A)).

Since chimeric mice with humanized liver are expensive, cost effectiveness can be improved by performing *in vivo* PK studies by administering test compounds at a certain interval and sequentially estimating the PK, and using the cassette dosing method and simultaneously estimating the PK to reduce the number of mice used (Fig. 6(B)).^{82,84,86)}

As described above, drug metabolism by residual mouse hepatocytes in the liver of chimeric mice with humanized liver affects the predictability of human PK. To improve predictability, correction methods using $CL_{int, in vitro}$ in humans and animals have been reported.^{87,88)} To improve prediction accuracy, use of *in vitro* data using hepatic microsomes in chimeric mice with humanized liver and humans might be effective. Kamimura *et al.*⁸⁹⁾ estimated CL_t in humanized TK-NOG mice with 100% RI by extrapolating the values of CL_t in humanized TK-NOG mice which showed different RIs for PF-04937319, a glucokinase activator. Further, they predicted human PK based on the estimated CL_t in the mice. To suppress the metabolism of residual mouse hepatocytes, novel types of chimeric mice with humanized liver have been reported. Examples include Cyp3a-knockout chimeric mice with humanized liver,⁹⁰⁾ which avoid drug metabolism by mouse

Cyp3a, and mouse reduced nicotinamide adenine dinucleotide phosphate (NADPH)-CYP oxidoreductase-knockout chimeric mice with humanized liver,⁹¹⁾ which avoid mouse CYP metabolism.

It is also important to consider the influence of extra-hepatic metabolism of chimeric mice with humanized liver. Although a difficult issue to solve, incorporation of *in vitro* metabolic studies using chimeric mouse with humanized liver and human biological materials may be effective.

For IVIVE, it is difficult to estimate $CL_{int, in vitro}$ and predict human PK for very metabolically stable compounds. On the contrary, it is easy to predict human PK using chimeric mice with humanized liver because the *in vivo* PK data of mice are directly used in the predictions.

Human PK prediction methods using chimeric mice with humanized liver have mainly evaluated CYP and non-CYP substrates. Results have suggested that it is possible to simultaneously evaluated both types of substrate.^{80,82,86)} However, the prediction of drug transporter-mediated human PK remains limited; future studies may need to evaluate the prediction of human PK for various compounds involved in transport and integrate results for prediction of CYP-, non-CYP- and transporter-mediated human PK.

5. CONCLUSION

This review summarizes the prediction of human PK using IVIVE, allometric scaling and chimeric mice with humanized liver, and discusses the utility of predicting human PK using these mice in drug discovery by comparing results with those obtained with IVIVE and allometric scaling. IVIVE is a typical method for predicting human PK and has been applied to numerous compounds. However, inconsistency between predicted and actual CL has often been observed, and various correction methods have been reported. For IVIVE, it is necessary to establish separate methods for CYP, non-CYP enzyme and drug transporter metabolism and then integrate these methods in predicting human PK. It is difficult to evaluate very metabolically stable compounds, although various

approaches such as the relay method, HepatoPac, H μ REL and upcyte have been proposed. Allometric scaling using experimental animals has been also often used. Since there have been many failures in predicting human PK due to interspecies differences, various correction methods have been proposed. However, these difficulties in prediction still remain. In contrast, it is considered that PK in chimeric mice with humanized liver mimics that in humans, and that human PK prediction methods such as allometric scaling and PBPK modeling using *in vivo* PK data of these mice may solve the issues of IVIVE and allometric scaling. Nevertheless, a number of issues in predicting human PK remain, such as the influence of residual mouse hepatocytes and extra-hepatic metabolism. Solving these issues would make human PK prediction methods using chimeric mice with humanized liver more effective and useful.

Conflict of Interest S.S. and S.O. have performed a collaborative study with PhoenixBio Co., Ltd. The authors report no other conflict of interest.

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