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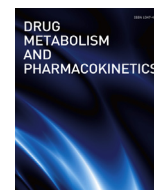

Review

Chimeric mice with humanized liver: Application in drug metabolism and pharmacokinetics studies for drug discovery

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A B S T R A C T

Predicting human drug metabolism and pharmacokinetics (PK) is key to drug discovery. In particular, it is important to predict human PK, metabolite profiles and drug-drug interactions (DDIs). Various methods have been used for such predictions, including *in vitro* metabolic studies using human biological samples, such as hepatic microsomes and hepatocytes, and *in vivo* studies using experimental animals. However, prediction studies using these methods are often inconclusive due to discrepancies between *in vitro* and *in vivo* results, and interspecies differences in drug metabolism. Further, the prediction methods have changed from qualitative to quantitative to solve these issues. Chimeric mice with humanized liver have been developed, in which mouse liver cells are mostly replaced with human hepatocytes. Since human drug metabolizing enzymes are expressed in the liver of these mice, they are regarded as suitable models for mimicking the drug metabolism and PK observed in humans; therefore, these mice are useful for predicting human drug metabolism and PK. In this review, we discuss the current state, issues, and future directions of predicting human drug metabolism and PK using chimeric mice with humanized liver in drug discovery.

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1. Introduction

Predicting human drug metabolism and pharmacokinetics (PK) in drug discovery is important for identifying safe and efficacious drug candidates for the treatment of human diseases. Conventional prediction research has been used to select drug candidates that show excellent drug metabolism and PK characteristics in humans. The approaches used have typically included qualitative screening methods to evaluate such parameters as metabolic stability, cytochrome P450 (P450) inhibition and induction, and metabolite profiles. In recent years, however, this type of study has been largely accomplished. Poor efficacy and safety of candidate drugs have become the major cause of attrition in clinical studies. To resolve these issues, prediction of human PK in drug discovery has changed from qualitative screening to quantitative and detailed research methods. Similarly, prediction of drug-drug interactions (DDIs) has changed from estimations of *in vitro* P450 inhibition and induction to accurate predictions of *in vivo* DDIs in humans. Therefore, it has become necessary to predict not only PK parameters, such as clearance, but also the plasma time course of drug concentrations in humans [1]. Previously, drug metabolism research was mainly focused on factors associated with P450 metabolism. Recently, non-P450-based drug metabolism, such as those associated with UDP-glucuronosyltransferase (UGT) and aldehyde oxidase (AO), has received increased attention [1,2]. The prediction of human-specific or disproportionate levels of metabolites has assumed greater importance in estimating toxicity in drug discovery.

Predicting human drug metabolism and PK has commonly been examined by *in vitro* metabolic studies using human biological samples, such as hepatic microsomes and hepatocytes, and *in vivo* studies using experimental animals. However, prediction studies using these methods are often inconclusive due to discrepancies between *in vitro* and *in vivo* data, and interspecies differences in drug metabolism. Although various correction methods have been proposed [3–6], the accuracy of these predictions remains inadequate. Therefore, more accurate prediction methods are required.

Novel tools have been developed to solve this issue. One of the more effective approaches is the development of humanized animals, such as chimeric mice with humanized liver. These mice are

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constructed by transplanting human hepatocytes into mice that are genetically modified to have immunodeficiencies, which facilitates the engraftment of transplanted human cells into the mouse liver by preventing rejection of the transplanted human cells and damage to endogenous mouse cells [7–9]. The mouse liver cells are mostly replaced with human hepatocytes, which express human drug metabolizing enzymes [10]. Therefore, drug metabolism and PK studies in these mice should mimic those in humans, making them useful for improving such predictions.

Here, we review current research on predicting human drug metabolism and PK in drug discovery using chimeric mice with humanized liver. In particular, we discuss the prediction of human PK, metabolite profiles and DDIs.

2. Characteristics of chimeric mice with humanized liver

There are three frequently used types of chimeric mice with humanized liver models [7–9]. The first is urokinase-type plasminogen activator (uPA)/severe combined immunodeficiency (SCID) mice transplanted with human hepatocytes (humanized liver uPA/SCID mice). Mercer et al. [11] developed humanized liver uPA/SCID mice for studying the human hepatitis C virus *in vivo*. Tateno et al. [12] reported humanized liver uPA/SCID mice with a replacement index (RI), which is the ratio of human hepatocytes in the liver, of more than 70%. The ratio of liver weight to body weight of humanized liver uPA/SCID mice is approximately two-fold compared to that of SCID mice [13]. Tateno et al. [14] have reported physiological parameters such as the levels of total and human albumin and total bilirubin in the blood of humanized uPA/SCID mice. They also reported that liver blood flow, which was determined by measuring blood indocyanine green concentrations after intravenous administration, is similar between humanized uPA/SCID and SCID mice. Tateno et al. [12] have used homozygous albumin enhancer/promoter-driven uPA/SCID mice. However, RI in these mice is decreased due to the deletion of the uPA transgene by homologous recombination. To solve this problem, Tateno et al. [15] generated hemizygous cDNA-uPA/SCID mice.

The second type, known as humanized liver TK-NOG mice, was developed by Hasegawa et al. [16]. They produced TK-NOG mice by expressing a herpes simplex virus type 1 thymidine kinase transgene in the liver of highly immunodeficient NOG mice. Mouse hepatocytes were ablated by exposure to ganciclovir and human hepatocytes were subsequently transplanted and maintained in the liver of TK-NOG mouse.

The third type was developed by Azuma et al. [17] and generated by transplanting human hepatocytes into *Fah*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ (FRG) mice (humanized liver FRG mice). Fumarylacetoacetate hydrolase (Fah) is involved in the tyrosine catabolic pathway, and genetic knockout of Fah causes damage to endogenous mouse hepatocytes.

The mRNA and protein expression levels and activities of human drug metabolizing enzymes and transporters have been examined in the livers of chimeric mice with humanized liver. Tateno et al. [12] showed that the mRNA expression profiles of human CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 in the liver of humanized liver uPA/SCID mice were similar to those in the donor liver. Katoh et al. [18] reported that the protein and enzyme activity levels of major human P450s in the liver of humanized liver uPA/SCID mice, which showed RIs of close to 90%, were similar to those in the donor liver. Yamasaki et al. [19] observed that the activity levels of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A in human hepatocytes isolated from humanized liver uPA/SCID mice were similar to or greater than those in cryopreserved human hepatocytes. Katoh et al. [20] reported that the protein and enzyme activity levels of human UGT, sulfotransferase (SULT), *N*-acetyltransferase and glutathione-*S*-transferase in the liver of humanized liver uPA/SCID mice showed RIs of close to 90%, indicating that they were similar to those in the donor liver. Hasegawa et al. [16] showed that the mRNA expression levels of 12 P450s, two UGTs, five SLC, four ABC transporters, and three nuclear receptors in the liver of humanized liver TK-NOG mice were comparable to those in donor livers. Ohtsuki et al. [21] quantitatively measured the protein levels of 12 human P450s, two UGTs, and various transporters (eight ABC and eight solute carrier transporters) in microsomes and the plasma membrane of the liver of humanized liver uPA/SCID mice using LC/MS/MS. They showed that the levels of most of the proteins, except for CYP2A6, CYP4A11, BSEP and MDR3, in the liver of humanized liver uPA/SCID mice were within 4-fold of that found in human liver, suggesting that protein expression levels are comparable between the livers of humanized liver uPA/SCID mice and humans. Therefore, chimeric mice with humanized liver may retain the functions of human drug metabolizing enzymes and transporters in the liver.

3. Prediction of human PK

The prediction of human PK parameters, especially clearance (CL) and volume of distribution at steady state (Vd_{ss}), is important in drug discovery. *In vitro-in vivo* extrapolation (IVIVE) and allometric scaling methods have predominantly been used to predict human CL. Predicting plasma concentration-time profiles is also important for estimating drug efficacy and toxicity. To predict clearance using IVIVE, *in vitro* intrinsic clearance ($CL_{int,\ in\ vitro}$) is estimated from *in vitro* metabolic experiments using human biological samples, such as hepatic microsomes and hepatocytes. CL can subsequently be predicted from $CL_{int,\ in\ vitro}$ using mathematical models, such as the well-stirred and dispersion models [2,3]. Allometric scaling can be used to predict CL and Vd_{ss} in experimental animals based on the relationship between body weight and PK parameters [4–6]. However, many IVIVE studies have demonstrated discrepancies between *in vitro* and *in vivo* results. Various correction methods have been proposed to improve the predictability of IVIVE [3,4] (Fig. 1A). Similarly, allometric scaling can result in low predictability due to interspecies differences in drug metabolism. However, despite the proposal of various modified methods for improving the predictability of allometric scaling [4–6] (Fig. 1A), these predictions continue to have associated problems.

Chimeric mice with humanized liver contain repopulated human hepatocytes in the majority of the liver, which express human drug-metabolizing enzymes. These mice are therefore model animals that can potentially mimic human drug metabolism and PK (Fig. 1B).

Sanoh et al. [22] compared the *in vitro* intrinsic clearance ($CL_{int,\ in\ vitro}$) in fresh human hepatocytes isolated from humanized liver uPA/SCID mice, and the *in vivo* intrinsic clearance ($CL_{int,\ in\ vivo}$) in these mice and humans. They assessed 13 model compounds that were metabolized by P450 and non-P450 enzymes, such as UGT and AO, and calculated the $CL_{int,\ in\ vivo}$ from the mouse and human CL using the well-stirred model. While $CL_{int,\ in\ vitro}$ in human hepatocytes isolated from humanized liver uPA/SCID mice and $CL_{int,\ in\ vivo}$ in humans were only moderately correlated ($r^2 = 0.475$), $CL_{int,\ in\ vivo}$ in humanized liver uPA/SCID mice was well correlated with $CL_{int,\ in\ vivo}$ in humans ($r^2 = 0.754$). However, some compounds showed large differences in $CL_{int,\ in\ vivo}$ in humanized liver uPA/SCID mice and humans. In these cases, the study was at least useful for predicting the rank order of clearance of these compounds in humans.

Sanoh et al. [23] predicted total clearance (CL_t) and Vd_{ss} in humans for 17 compounds metabolized by P450 and/or non-P450

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