-Review-

# Drug Metabolism and Toxicity in Chimeric Mice with Humanized Liver

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The cytochrome P450 (P450, CYP) enzyme superfamily is associated with the metabolism of drugs, environmental pollutants and endogenous substrates with broad overlapping substrate specificities. In addition, species differences between experimental animals and humans in the roles of P450 enzymes in drug metabolism are determinant factors in the drug discovery process. The induction and inhibition of P450-dependent metabolism, especially in the case of P450 3A enzyems, can cause serious problems in clinical practice and in the attrition of drug candidates because of adverse toxicology or pharmacokinetics. Recently, chimeric mice whose livers have been replaced to a level of 80% or more with human hepatocytes were established using urokinase-type plasminogen activator transgenic/severe combined immunodeficiency (uPA/SCID) mice. In the livers of these chimeric mice, hepatic cords and sinusoid-like structures were observed. Moreover, bile canaliculi associated with human hepatocytes were also detected. The mRNA expressions derived from humans of 52 phase I and 26 phase II enzymes (including 20 P450 s) and 21 transporters were ascertained in the chimeric mice liver. Taken together, these chimeric mice exhibited the glutathione conjugate form of a possible quinine-imine metabolite and responded to treatment with rifampicin or rifabutin by induction of P450 3A enzyems. This animal model should prove to be a good *in vivo* tool to assess the safety of drug candidates in terms of toxicity and drug-drug interactions caused by P450 induction.

Key words ----- metabolism, cytochrome P450, toxicity, chimeric mice, human

#### INTRODUCTION

distribution, Acceptable absorption, metabolism, and elimination (ADME) properties are essential to the success of any drug candidate.<sup>1)</sup> From analysis of the causes of drug candidate attrition from 1991 to 2000, it was found that adverse pharmacokinetics and bioavailability were dramatically reduced in this period.<sup>2)</sup> These data provide evidence that the industry can identify and ameliorate the causes of attrition; however, unfortunately, issues regarding the efficacy and toxicology of drug candidates remain. It is important that the mindset of reducing attrition in drug development should be in place from the earliest stages of discovery.

Drug-induced liver injury is one of the most frequent causes of safety-related withdrawal of drugs from the market.<sup>3)</sup> It has been reported that druginduced hepatotoxicity may be caused by active intermediates formed from acetaminophen or troglitazone by animal and/or human drug-metabolizing enzymes; indeed, idiosyncratic hepatotoxicity by troglitazone was the cause of the withdrawal of this drug from the market.<sup>4,5)</sup> Idiosyncratic drug toxicity is a major complication and its prediction is very difficult. Circumstantial evidence suggests the involvement of reactive metabolites in this reaction.<sup>6)</sup> Small organic molecules may be bioactivated in vivo to electrophiles that can adduct to biological macromolecules and subsequently elicit organ toxicitv.<sup>7)</sup>

Cytochrome P450 (P450, CYP) enzymes make up a superfamily of heme-containing monooxygenases, and multiple forms of P450 enzymes exist

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in mammals.<sup>8)</sup> These P450 enzymes are responsible for the oxidation of many drugs, environmental chemicals, and endogenous substrates. P450 enzymes exist in liver and in extrahepatic tissues such as intestine, lung, and kidney.<sup>9)</sup> CYP3A4 (an individual form of P450) is the most abundant CYP3A form in adult human liver and intestine.<sup>10)</sup> Induction and inhibition of P450-dependent metabolism, especially in the case of CYP3A4, can cause serious problems in clinical practice and in the attrition of drug candidates during the drug discovery and development stages.<sup>11,12</sup>

Species differences between experimental animals and humans in the roles of P450 enzymes in drug metabolism and the induction of P450 enzymes are considerable factors in drug discovery.<sup>13)</sup> Because of these differences, human P450 enzyme sources such as liver microsomes, hepatocytes, and recombinant microsomes are used as in vitro tools. Human hepatocytes in primary culture are the most suitable in vitro tool for induction studies, but this approach has some problems such as the inability of cells to proliferate, the quick degradation of P450 activities during culture, and the requirement for specific culturing or technical conditions.<sup>14)</sup> The main functions of the liver include not only drug metabolism (including detoxification), but also the synthesis and secretion of bile, the destruction of spent blood cells, and the synthesis of plasma proteins.<sup>15)</sup> Because these additional liver functions are hard to replicate in the laboratory, they represent an inherent limitation in risk assessment using in vitro and ex vivo assays. Therefore, in this review, we introduce the utility of chimeric mice with humanized liver as a new experimental animal model.

## CHIMERIC MICE WITH HUMANIZED LIVER

Tateno *et al.*<sup>16)</sup> succeeded in establishing chimeric mice whose livers could be more than 80% replaced with human hepatocytes using urokinasetype plasminogen activator transgenic/severe combined immunodeficiency (uPA/SCID) mice. Heterozygous uPA-transgenic mice (albumin promoter) were crossed with SCID mice to produce uPA<sup>+/-</sup>/SCID mice that were then crossed to produce uPA<sup>+/+</sup>/SCID mice. uPA<sup>+/+</sup>/SCID mice undergo continuous hepatocellular damage due to expression of the albumin-uPA transgene, and also have immunologic tolerance to human hepatocytes as a result of the SCID mutation. It follows that human hepatocytes can be transplanted into these mice, and establishment of human hepatocytes can compensate for damaged endogenous murine hepatocyte functions.

For true human liver function. threedimensional architectures of hepatocytes, vasculature cells, and associated nonparenchymal cells are necessary. Chimeric mice with humanized liver have various amounts of human hepatocytes that proliferate and replace mouse hepatocytes. In liver from chimeric mice, hepatic cords and sinusoid-like structures have been observed.<sup>15)</sup> Moreover, bile canaliculi associated with human hepatocytes have also been observed. The mRNAs of 58 human phase I enzymes, 26 human phase II enzymes, 23 human transporters, and 5 mouse P450s were measured in chimeric mice with almost completely humanized liver (replacement index: 71-89%) generated using hepatocytes from a Japanese donor.<sup>17)</sup> The mRNA expression of 20 human P450s and the other 32 phase I and 26 phase II enzymes and 21 human transporters was ascertained in the chimeric mice liver.

#### IN VIVO TOXICITY IN CHIMERIC MICE

Acetaminophen at high doses is known to be a common toxicant and to produce centrilobular hepatic necrosis; its metabolism is mediated by P450 enzymes to yield the metabolite N-acetyl-pbenzoquinone imine (NAPOI).<sup>18)</sup> The metabolism of acetaminophen to NAPQI is catalyzed by CYP2E1, CYP3A4, and CYP1A2. Chimeric mice survived the oral administration of acetaminophen at 1400 mg/kg at 24 hr postdose, whereas ICR mice, a standard toxicology strain in Japan, died.<sup>15)</sup> In these chimeric mice, a reduction in immunoexpression level of CYP2E1 was observed in the human hepatocyte liver regions after the administration of acetaminophen. CYP2E1 knockout mice have been shown to be more resistant to acetaminophen toxicity.<sup>19)</sup> In patients undergoing treatment with acetaminophen, liver injury was intensified in those suffering chronic alcohol abuse, which stabilizes CYP2E1 protein and depletes the amount of glutathione.<sup>20)</sup> The mechanism of acetaminophen toxicity is incompletely understood, and further investigation of the species differences between human and rodents are needed.

As mentioned above, there are species differ-



Fig. 1. Possible Mechanism of Rat and Human Metabolite Formation from M-5 Catalyzed by Rat and Human P450 1A2

Metabolic scheme of human hepatotoxic pyrazolopyrimidine derivative OT-7100 and its primary metabolite M-5 after biotransformation by human and rat P450 1A2 enzymes. Only M-23OH was detected in human liver microsomal incubation, but M-23OH and M-22OH were seen in rat liver microsomal incubation.<sup>21)</sup> Prior to docking simulation, the energy of rat P450 1A2 was minimized using the CHARM22 force field in the MOE software package (ver. 2008.10, Chemical Computing Group, Montreal, Canada) based on the structure of human P450 1A2 (Protein Data Bank code, 2HI4) to model the three-dimensional structure. Docking simulation was carried out for M-5 binding to the homology model of rat and human P450 1A2 using the MMFF94x force field distributed in the MOE Dock software.

ences between experimental animals and humans in the roles of P450 enzymes. Development of potential analgesic agent 5-n-butyl-7-(3,4,5trimethoxybenzoylamino)pyrazolo[1,5-a]pyrimidine (OT-7100) was cancelled because of limited hepatotoxic effects in humans that had not been predicted from studies in regulatory animals such as rats or in vitro studies.<sup>21)</sup> Human liver microsomal CYP1A2 was able to effectively mediate the transformation of a primary metabolite 5-n-butyl-pyrazolo[1,5-a]pyrimidine (M-5) to 3-hydroxy-5-n-butyl-pyrazolo[1,5-a]pyrimidine (M-23OH), yielding a possible quinine-imine metabolite that could bind to glutathione or biomolecules such as CYP1A2 and other proteins in livers (Fig. 1). However, on the metabolism of M-5 by rat CYP1A2, M-5 was metabolized maily to 6-hydroxy-5-*n*-butyl-pyrazolo[1,5-*a*]pyrimidine (M-22OH) and unknown metabolites in addition to M-23OH. Chimeric mice with humanized liver preferentially yielded M-23OH and its glutathione conjugate in the plasma and liver.<sup>21)</sup> In contrast,

liver microsomes from chimeric mice with rat liver yielded some rat-specific metabolites *in vivo*.

Regarding the chimeric mice with humanized liver used in this study, the replacement index of mouse liver by human hepatocytes ranged from 78% to 90%. The chimeric mice have much lower activities of mouse P450 enzymes and high activities of human P450 enzymes because uPA<sup>+/+</sup>/SCID mice experience severe liver failure due to the death of their original mouse hepatocytes, and the proliferation of human hepatocytes is needed for the survival of these chimeric mice.<sup>22)</sup> The barely detectable mouse P450 activities might be due to the partial repopulation of murine hepatocytes rather than human hepatocytes.<sup>23)</sup> The involvement of these remaining mouse P450s in chimeric mice in the metabolism of M-5 is uncertain. From the docking simulation of M-5 into human or rat P450 1A2, the top-rank docking model is illustrated in Fig. 1. In docking simulation using the three-dimensional structure of human and rat P450 1A2, the 3- and 6-positions of the pyrazolo[1,5-a]pyrimidine ring

were closely oriented to the center of the heme of human and rat P450 1A2, respectively. These findings support the phenomenon of M-5 metabolism in chimeric mice with human and rat liver microsomes.

## *IN VIVO* INDUCTION OF P450 ENZYMES IN CHIMERIC MICE

Induction of P450 enzymes is regulated by a number of nuclear receptors such as the pregnane X receptor (PXR) and the constitutive androstane receptor.<sup>24)</sup> The induction of CYP3A4 is mainly mediated through activation of PXR, and rifampicin activates human PXR and induces CYP3A4. Human CYP3A was induced by rifampicin, but rat CYP3A was not.<sup>25)</sup> The amino acid sequence for the ligand-binding domain is different in humans and rodents. Therefore, chimeric mice with humanized liver would be an appropriate model to evaluate the in vivo induction of candidates at the drug discovery and development stage. Although the majority of the mouse liver was replaced by human liver in uPA<sup>+/+</sup>/SCID chimeric mice, mouse hepatocytes still existed in the mouse liver used. In order to delete or minimize noise/false information during the sensitive detection of P450 induction in mice, dexamethasone (DEX) is suggested as a human selective CYP3A substrate. The formation rate of  $6\beta$ -hydroxydexamethasone ( $6\beta$ OHD) in human liver microsomes is much higher than that in mouse liver microsomes.<sup>26)</sup> In our previous study, the intrinsic clearance of 6BOHD formation from dexamethazone in human liver microsomes was 23-fold and 8.9-fold higher than that of male and female mouse liver microsomes, respectively (Table 1).<sup>27)</sup> In addition, 6BOHD was excreted as a major urinary metabolite after DEX administration in humans.<sup>28)</sup> Unchanged DEX and DEX glucuronide were detected as a small fraction of the excreted dose. The metabolic ratio of 6BOHD to DEX in chimeric mice increased two-fold on treatment with concomitant rifampicin, as shown in Table 2.27) In addition, in liver microsomes from chimeric mice with humanized liver, the expression levels of human CYP3A4 mRNA and CYP3A4 protein and the metabolic activity of dexamethasone 6\beta-hydroxylation increased 8- to 22-fold, 3to 10-fold, and 5- to 12-fold, respectively, on treatment with rifampicin. In contrast, treatment with rifabutin, which is an analogue of rifampicin, yielded 7.4-, 3.0-, 2.4-, and 1.9-fold changes in human CYP3A4 mRNA content, CYP3A4 protein content, testosterone  $6\beta$ -hydroxylase activity, and dexamethasone 6-hydroxylase activity, respectively.<sup>29)</sup> The chimeric mice with humanized liver demonstrated the CYP3A-related induction by rifampicin as well as rifabutin.

**Table 1.** Kinetic Parameters for 6βOHD Formation from Dexamethasone in Pooled Human Liver Microsomes and in Pooled Mouse Liver Microsomes

Enzyme	K <sub>m</sub>	V <sub>max</sub>	CL <sub>int</sub>
	$(\mu M)$	(pmol/min per	(µl/min per
		mg protein)	mg protein)
Human	10 ± 1	$75 \pm 4$	7.5
Mouse (male)	$76 \pm 10$	$24 \pm 2$	0.32
Mouse (female)	61 ± 7	$51 \pm 4$	0.84

These data are from Emoto *et al.*<sup>27)</sup> Kinetic parameters were calculated from the curves fitted by non-linear regression using GraphPad Prism 4.02. Kinetic parameters are the mean  $\pm$  standard error derived from triplicate determinations. *In vitro* intrinsic clearance (CL<sub>int</sub>) was calculated from K<sub>m</sub> and V<sub>max</sub> values: CL<sub>int</sub> = V<sub>max</sub>/K<sub>m</sub>.

**Table 2.** Effect of Rifampicin Treatment on Urinary  $6\beta$ OHD and DEX Excretion in Chimeric Mice with a Humanized Liver

Treatment	Metabolic ratio (6βOHD/DEX)		
	Pre-treatment	Post-treatment	Change (post-/pre-)
Control	$1.00\pm0.49$	$1.64\pm0.72$	$1.75\pm0.73$
	(0.57 - 1.89)	(0.64 - 2.42)	(0.94 - 2.63)
Rifampicin	$0.84 \pm 0.35$	$2.56 \pm 1.03$	$3.50 \pm 1.96^{a}$
	(0.25 - 1.35)	(1.15 - 3.75)	(1.70 - 6.96)

These data are from Emoto *et al.*<sup>27)</sup> Animals received an intraperitoneal injection (i.p.) of vehicle or rifampicin once a day for 4 days at a dose of 50 mg/kg. Three days before and 1 day after vehicle or rifampicin injection, animals received a subcutaneous injection (s.c.) of DEX at a dose of 10 mg/kg. Urine samples were collected for 48 hr. Values represent mean  $\pm$  standard deviation from six to nine animals. Values in parentheses represent the range of minimal and maximal values. *a*) Significantly different from control values (p < 0.05).

### CONCLUSION

Chimeric mice with humanized liver contain functionally differentiated human hepatocytes.<sup>15)</sup> Moreover, the mRNAs of human phase I enzymes, human phase II enzymes, and human transport proteins were detected.<sup>17)</sup> OT-7100, which is known to be toxic in humans but not in mice, was activated to reactive intermediate(s) in the chimeric mice with humanized liver.<sup>21)</sup> This animal should provide a good model for investigating drug toxicity in vivo in preclinical studies. The chimeric mice also exhibited CYP3A-induction on treatment of rifampicin and rifabutin.<sup>27,29</sup> Because of the limited availability of high-quality human hepatocytes, in addition to chimeric mice, several hepatic cell lines, including HepG2, BC2, HepaRG, and the immortalized Fa2N-4 cell line, have recently been used for preliminary drug candidate evaluation as alternatives to primary human hepatocytes for CYP3A4 induction studies. HepaRG cells were developed as human hepatoma cells that express several P450s, PXR, aryl hydrocarbon receptor, and the constitutive androstane receptor at levels comparable with cultured primary human hepatocytes.<sup>30)</sup> After the selection of drug candidates by initial in vitro screening using HepaRG cells, the best way to study enzyme induction *in vitro* and *in vivo* is by using primary human hepatocytes and chimeric mice with humanized human liver, respectively. Chimeric mice with humanized liver should provide a good in vivo tool to assess candidate drugs in terms of their toxicity and drug-drug interaction caused by P450 induction.

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