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Non-invasive method to detect induction of CYP3A4 in chimeric mice with a humanized liver

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Abstract

Chimeric mice with a humanized liver have been previously established by the transplantation of human hepatocytes to urokinase-type plasminogen activator/severe combined immunodeficiency mice. A non-invasive method to detect the induction of cytochrome P450 (CYP) 3A4 was evaluated in chimeric mice with a humanized liver. Dexamethasone (DEX) was used as a probe drug to detect induction; and rifampicin was used as a model drug to induce CYP3A4. Before and after rifampicin treatment (50 mg kg^{-1}, intraperitoneal injection once a day for 4 days) in the chimeric mice, DEX was subcutaneously injected and the urinary excretion of 6β-hydroxydexamethason (6βOHD) and DEX was determined. The metabolic ratio (6βOHD/DEX) significantly increased after rifampicin treatment. Livers from the control and rifampicin-treated chimeric mice were stained immunohistolochemically with antibodies against CYP3A4 and CYP3A5. CYP3A4 and CYP3A5 were detected in the area of humanized liver, but staining was intense for CYP3A4 and very weak for CYP3A5. Only the staining of CYP3A4 was increased after rifampicin treatment. Formation of 6βOHD by human liver microsomes was higher than that formed by mouse liver microsomes. Metabolite formation was catalysed by both CYP3A4 and CYP3A5 and the intrinsic clearance (V_{max}/K_m) by CYP3A4 was found to be 50-fold higher than that of CYP3A5. The results of the present study indicate that estimation of the changes of the urinary metabolic ratio (6βOHD/DEX) in the chimeric mice with a humanized liver is a very useful tool for detecting the induction of CYP3A4 by a non-invasive method.

Keywords: *CYP3A4, CYP3A5, induction, chimeric mice with a humanized liver, non-invasive method, humanized liver, dexamethasone, rifampicin*

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Introduction

Cytochrome P450s (CYPs) consist of a gene super family of haemoproteins and play a critical role in the metabolic clearance of lipophilic compounds including many drugs (Nelson et al. 1996). CYP3A4 is one of the abundant CYPs in human liver (McGinnity et al. 2000) and participates in metabolizing more than half of drugs currently in use (Rendic 2002). Inhibition and induction of CYP-dependent metabolism, especially in the case of CYP3A4, are the causes in the serious problems that can arise in clinical practice and in the attrition of drug candidates through the drug-discovery and development stages (Dresser et al. 2000, Lin 2006). In order to develop a safe drug without induction potency, it is very important to develop a non-clinical system for detecting this characteristic.

Induction of CYP is regulated by a number of nuclear receptors such as pregnane X receptor (PXR) and constitutive androstane receptor (Cheng & Waxman 2006). The induction of CYP3A4 is mainly mediated through activation of PXR, and rifampicin activates human PXR and induces CYP3A4.

Chimeric mice with a humanized liver have been previously established by the transplantation of human hepatocytes to urokinase-type plasminogen activator ($uPA^{+/+}$)/severe combined immunodeficiency (SCID) mice (Tateno et al. 2004). These mice could be a very promising model for examining the absorption, distribution, metabolism and excretion of drugs and drug candidates for predicting problems in clinical practice (Katoh & Yokoi 2007). Induction of CYP3A4 in chimeric mice with a humanized liver has been previously characterized by measuring enzyme activities, protein and mRNA expression levels after treatment with rifampicin (Katoh et al. 2005a, b).

Although the majority of the mouse liver was replaced by the human liver in $uPA^{+/+}$/SCID chimeric mice, 20–30% of the mouse hepatocytes still existed in the mouse liver used in this study. To delete or minimize noise/false information for the sensitive detection of CYP induction in mice, a sensitive probe metabolized more rapidly by human liver microsomes than by mouse liver microsomes should be established. The rate of 6β-hydroxydexamethasone (6βOHD) formation by human liver microsomes is much higher than in mouse liver microsomes (Tomlinson et al. 1997), the reaction being catalysed by CYP3A4 (Gentile et al. 1996). After dexamethasone (DEX) administration to man, 6βOHD is excreted as a major urinary metabolite, and unchanged DEX and DEX glucuronide were detected as a small fraction of the excreted dose (Minagawa et al. 1986).

It is postulated that DEX is a suitable probe to detect induction of CYP3A4 mediated metabolism and in the current study, we have evaluated a non-invasive method in chimeric mice with a humanized liver using DEX as a probe drug and rifampicin as a model drug to induce CYP3A4. Specifically, we have compared the change in the urinary metabolic ratio of 6βOHD to DEX, both before and after rifampicin treatment in the same animal.

Materials and methods

Reagents

DEX was obtained from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan) (chemical purity, 99.9%). 6βOHD was synthesized at KNC Laboratories Co. Ltd (Kobe, Japan) (chemical purity, 97.4%). Rifampicin was purchased from Wako Pure Chemical Industries (Osaka, Japan). Beclomethasone was obtained from Sigma-Aldrich Japan (Tokyo, Japan). Tolubutamide was purchased from Ultrafine (Manchester, UK). β-Nicotinamide adenine

dinucleotide phosphate (reduced form, NADPH) was obtained from Oriental Yeast (Tokyo, Japan). Other reagents were high-performance liquid chromatography (HPLC) grade or better.

Polyclonal rabbit anti-human CYP3A4 and CYP3A5 antibodies were purchased from BD Gentest (Worburn, MA, USA). The CYP3A4 antibodies reacted with CYP3A4 and CYP3A7, and the CYP3A5 antibodies only reacted with CYP3A5 (manufacturer's information).

Recombinant CYP enzymes expressed in insect cells infected with baculovirus containing human CYP enzymes and human NADPH-CYP reductase cDNA inserts with human cytochrome b_5 and pooled liver microsomes from human and mouse (CD-1) liver were obtained from BD Gentest.

Generation of chimeric mice with a humanized liver

The generation of chimeric mice with a humanized liver was conducted as described previously (Tateno et al. 2004; Katoh et al. 2005a, b). All experiments were performed in accordance with Hiroshima Prefectural Institute of Industrial Science and Technology Ethics Board, the Ethics Committees of Kanazawa University and the Animal Ethics Committee of Pfizer Global Research and Development Nagoya Laboratories. The extent of the replacement from mouse liver to human liver for the metabolic ratio studies was 70–80% in the chimeric mice as assessed by the method described below. The extent of replacement was 60–90% for the immunohistochemistry study, as assessed by the method described below. The concentration of human albumin in the blood of the mice was determined using an enzyme-linked immunosorbent assay and the extent of the replacement was estimated with a correlation between the extent of the replacement and the human albumin concentration (Tateno et al. 2004).

Animal treatment

Chimeric mice with a humanized liver (two females and seven males: body weight, 16–20 g) received an intraperitoneal (i.p.) injection of rifampicin (dissolved in corn oil, $3.3\,\mu l\,g^{-1}$ body weight) once a day for 4 days at a dose of $50\,mg\,kg^{-1}$. Control chimeric mice with a humanized liver (one female and five males: body weight, 15–20 g) group received an i.p. injection of the same volume of corn oil. On 3 days before and 1 day after the treatment of rifampicin, animals received a subcutaneous (s.c.) injection of DEX (dissolved in polyethylene glycol 400, $5.0\,\mu l\,g^{-1}$ body weight) at a dose of $10\,mg\,kg^{-1}$. Urine samples were collected every 24 h for 48 or 72 h. Liver samples were obtained from corn oil-treated and rifampicin-treated mice on 1 day after the last refampicin injection. After animals were killed by exsanguination under diethyl ether anaesthesia, livers were dissected and fixed with 4% paraformaldehyde and processed for immunohistochemical analysis.

Analysis of urine samples

Urine samples (0.1 ml) were mixed with an internal standard (beclomethasone, 1 μM) and treated with OASIS HLB μElution Plate (Waters Corporation, Milford, MA, USA). After washing with water, DEX, 6βOHD and beclomethasone were eluted with acetonitrile (0.1 ml). Eluates were diluted with water (0.3 ml) and analysed by HPLC with tandem mass spectrometric detection (LC/MS/MS). The detection of DEX and 6βOHD was performed

using an API-4000 mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Canada), a 1100 series HPLC system (Agilent Technologies, Taufkirchen, Germany), and a CTC-PAL auto injector (CTC Analytics AG, Zwingen, Switzerland). The eluates were injected into a YMC-Pack Pro C8 column (2.0 × 75 mm, YMC, Kyoto, Japan) and eluted by a linear gradient with the mobile phase, which consisted of a mixture of A (10% acetonitrile containing 10 mM ammonium acetate) and B (80% acetonitrile containing 10 mM ammonium acetate). The column temperature and the flow rate were 40°C and 0.5 ml min^{-1}, respectively. The gradient conditions for elution were as follows: 20% B (0.0–0.5 min); 20–100% B (0.5–2.5 min); 100% B (2.5–3.0 min); 100 to 20% B (3.0–3.05 min); and 20% B (3.05–5.5 min).

Immunohistochemistry

Polyclonal rabbit anti-human CYP3A4 and CYP3A5 were used as markers of enzyme induction in immunohistochemical staining. Paraformaldehyde-fixed livers were trimmed, embedded in paraffin, sectioned to a thickness of 4 μm, and mounted on glass slides. The avidin–biotin affinity system (Vectastain Elite ABC, Vector, CA, USA) was used for the immunohistochemistry studies. The dilutions of the CYP3A4 and CYP3A5 antibodies were 1:100 and 1:50, respectively. The liver sections were visualized with 3,3′-diaminobenzidine tetrachloride as the chromogen and counterstained with haematoxylin. Immuno-expression of CYP3A4 and CYP3A5 was analysed quantitatively using two-dimensional evaluation with the Image Processor for Analytical Pathology (IPAP-WIN, Sumika Technoservice, Osaka, Japan), and the positive area in the human hepatocyte area was calculated as a percentage.

Kinetic assay for 6βOHD formation

The reaction mixture consisted of 1.3 mM β-NADPH, 3.3 mM $MgCl_2$, 100 mM phosphate buffer (pH 7.4), microsomes, and a substrate in a final volume of 200 μl. The reaction was started by adding NADPH after pre-incubation for 5 min in a water bath at 37°C. The final human and mouse microsomal concentrations were 0.1 and 0.5 mg ml^{-1}, respectively. The final concentrations of CYP3A4 and CYP3A5 were 2 and 30 pmol ml^{-1}, respectively. Considering microsomal protein binding, the protein concentration in the reaction mixture containing recombinant CYP microsomes was adjusted to 0.23 mg ml^{-1} by the addition of control microsomes. Final substrate concentrations were from 4.4 to 220 μM. The incubation time was 10 min. The reaction was terminated by the addition of 400 μl of methanol containing tolbutamide (25 ng ml^{-1}) as an internal standard. After the centrifugation at 2000 rpm for 15 min at 4°C, the supernatants were subjected to LC/MS/MS analysis.

Analysis of in vitro samples

The detection of 6βOHD was performed using an API-4000 mass spectrometer, a 1100 series HPLC system, and a CTC-PAL auto injector. The supernatants of the reaction mixture were injected into an AtlanticTM dC18 column (4.6 × 150 mm, 5 μm, Waters Corporation) and eluted by a linear gradient with the mobile phase, which consisted of a mixture of A (90% 5 mM ammonium formate/10% acetonitrile containing 0.05% formic acid) and B (15% 5 mM ammonium formate/80% acetonitrile/5% methanol containing

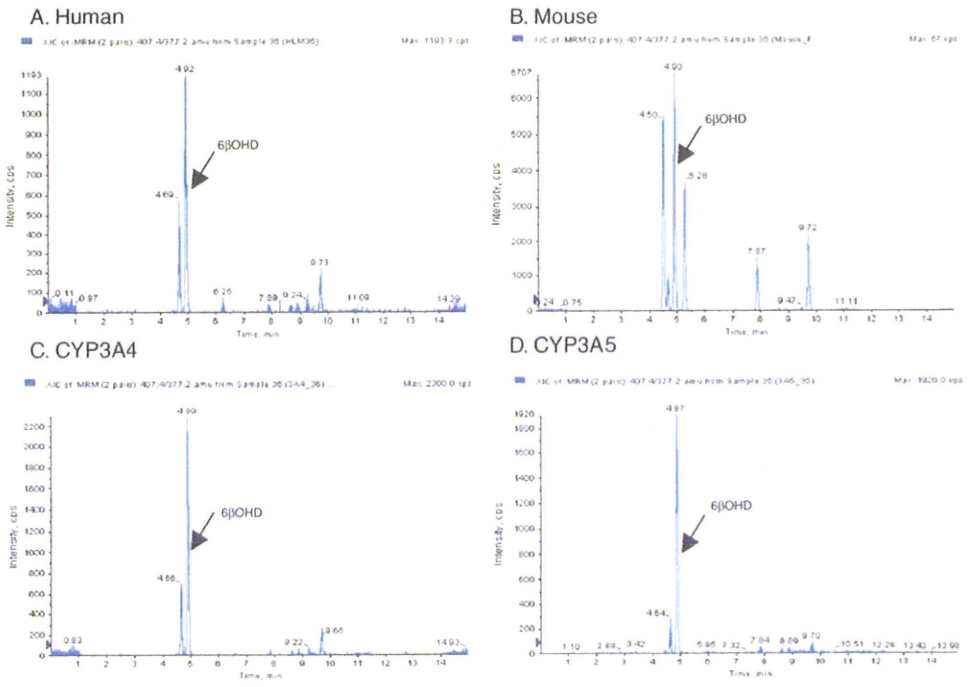

Figure 1. Typical chromatograms of 6βOHD elution formed by liver microsomes and recombinant CYPs. Human liver microsomes (A), mouse liver microsomes (B, male), recombinant 3A4 (C), and recombinant CYP3A5 (D).

0.05% formic acid). The column temperature and the flow rate were 40°C and 0.9 ml min^{-1}, respectively. The gradient conditions for elution were as follows: 100 to 5% B (0.0–9.0 min); 55 to 100% B (9.0–9.1 min); 100% B (9.1–10.5 min); 100 to 5% B (10.5–10.6 min); and 5% B (10.6–15.0 min). The inaccuracy value of each concentrations was <20%.

Kinetic analysis

The kinetic parameters for metabolite formation were calculated using GraphPad Prism 4.02 (GraphPad Software, San Diego, CA, USA).

Statistical analysis

Statistical differences were analysed by a Student's *t*-test.

Results

Kinetics of 6βOHD formation

The kinetic analysis for the DEX 6β-hydroxylation was conducted using liver microsomes from human and mouse, in addition to recombinant CYP3A4 and CYP3A5. As shown in Figure 1, 6βOHD was a major hydroxylated metabolite from DEX in human and mouse

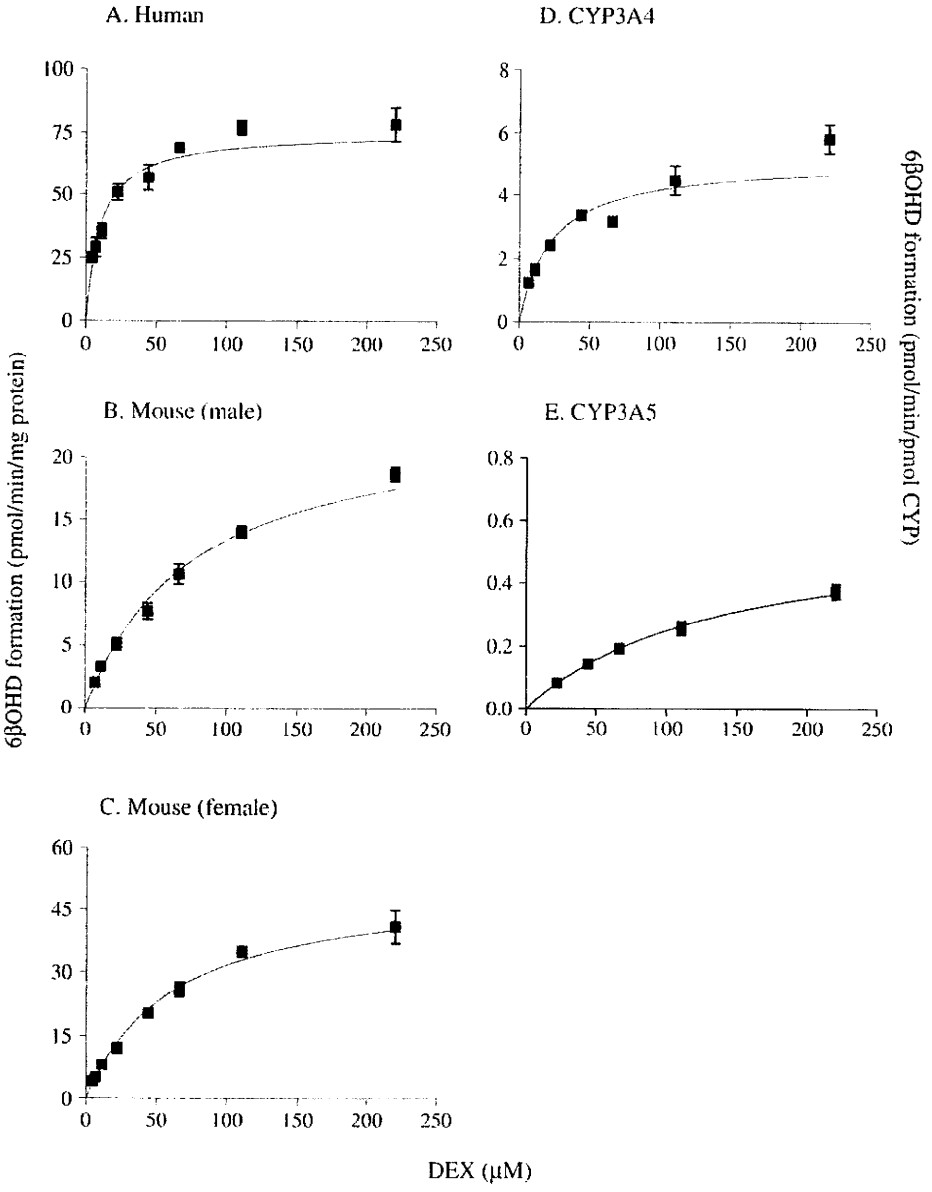

Figure 2. Activity versus substrate concentration profiles for DEX 6β-hydroxylation by liver microsomes and recombinant CYPs. Human liver microsomes (A), mouse liver microsomes (B, male), mouse liver microsomes (C, female), recombinant CYP3A4 (D), and recombinant CYP3A5 (E).

liver microsomes, and recombinant CYP3A4 and CYP3A5. 6βOHD formation was linear as a function of reaction time and microsomal protein concentrations used in the current study (data not shown). The activity versus substrate concentration profiles for 6βOHD formation are depicted in Figure 2 and the kinetic parameters are summarized in Table I. The intrinsic clearances (CL_{int}) with human liver microsomes were 23- and 8.9-fold higher than those of male and female mouse liver microsomes, respectively. Both CYP3A4

Table I. Kinetic parameters for 6βOHD formation in pooled liver microsomes and recombinant CYP microsomes.

Enzyme sources	Kinetic parameters		
	K_m (μM)	V_{max}	CL_{int}[a]
Liver microsomes[b]		(pmol min^{-1} mg^{-1} protein)	(μl min^{-1} mg^{-1} protein)
Human	10 ± 1	75 ± 4	7.5
Mouse (male)	76 ± 10	24 ± 2	0.32
Mouse (female)	61 ± 7	51 ± 4	0.84
Recombinant microsomes[c]		(pmol min^{-1} $pmol^{-1}$ CYP)	(μl min^{-1} $pmol^{-1}$ CYP)
CYP3A4	23 ± 3	5.1 ± 0.4	0.22
CYP3A5	140 ± 23	0.60 ± 0.06	0.0043

Kinetic parameters were calculated from the fit curves by non-linear regression using GraphPad Prism 4.02. Kinetic parameters are the mean ± standard error (SE) derived from triplicate determinations.
[a] *In vitro* intrinsic clearance (CL_{int}) = V_{max}/K_m.
[b] Microsomal concentrations of human and mouse liver were 0.1 and 0.5 mg ml^{-1}, respectively.
[c] CYP concentrations of CYP3A4 and CYP3A5 were 2 and 30 pmol ml^{-1}, respectively. Considering the microsomal binding, the protein concentration was adjusted to 0.23 mg ml^{-1} by the addition of control microsomes.

and CYP3A5 catalysed 6βOHD formation and the CL_{int} for CYP3A4 was 51-fold higher than that for CYP3A5.

Excretion of 6βOHD and DEX in chimeric mouse urine after DEX administration

Urinary excretion of 6βOHD was determined after the s.c. injection of DEX to chimeric mice at a dose of 10 mg kg^{-1} (Table II). There were no clear gender-dependent differences and no changes in excretion of 6βOHD and DEX after treatment of urine samples with β-glucuronidase/sulfatase (data not shown). Almost equal amounts (4–5% of dose) of 6βOHD and DEX were excreted in the urine collected for 72 h and the metabolic ratio of 6βOHD to DEX was almost constant (1.1–1.5) throughout collection the periods. Excretion of 6βOHD and DEX was almost complete after the first 48 h following the DEX injection.

Effect of rifampicin treatment on 6βOHD and DEX excretion

Before and after rifampicin or vehicle treatment, urinary samples were collected for 48 h after DEX administration and analysed to determine excretion of 6βOHD and DEX (Table III). When comparing the metabolic ratio of 6βOHD with DEX in the groups that did not receive the i.p. injection of vehicle or rifampicin (in the column titled Pre-rifampicin), the ratio was almost the same between the two groups. The ratio of 6βOHD to DEX was not changed before and after vehicle treatment. This ratio was increased twofold by treatment of rifampicin as shown in the column titled Change (post-/pre-).

Immunohistochemical analysis

Livers from the control and rifampicin-treated chimeric mice were stained immunohistochemically with antibodies against CYP3A4 and CYP3A5 (Figure 3). CYP3A4 and CYP3A5 were detected in the area of humanized liver as described previously (Tateno et al. 2004).

Table II. Excretion of 6βOHD and DEX after treatment of DEX in chimeric mice with a humanized liver.

Time (h)	Excretion (% of dose)		Metabolic ratio (6βOHD/DEX)
	6βOHD	DEX	
0–24	4.23 ± 0.97	3.83 ± 0.43	1.10 ± 0.17
24–48	0.45 ± 0.17	0.34 ± 0.16	1.39 ± 0.37
48–72	0.16 ± 0.12	0.11 ± 0.09	1.54 ± 0.15
Total	4.83 ± 1.22	4.28 ± 0.67	1.12 ± 0.17

Animals received the subcutaneous injection of DEX at a dose of 10 mg kg^{-1}. Urine samples were collected every 24 h for 72 h.
Values are the mean or mean ± standard deviation (SD) from three animals.

Table III. Effect of rifampicin treatment on urinary 6βOHD and DEX excretion in chimeric mice with a humanized liver.

Treatment	Metabolic ratio (6βOHD/DEX)		
	Pre-rifampicin	Post-rifampicin	Change (post-/pre-)
Control	1.00 ± 0.49	1.64 ± 0.72	1.75 ± 0.73
	(0.57–1.89)	(0.64–2.42)	(0.94–2.63)
Rifampicin	0.84 ± 0.35	2.56 ± 1.03	3.50 ± 1.96*
	(0.25–1.35)	(1.15–3.73)	(1.70–6.96)

Animals received the i.p. injection of vehicle or rifampicin once a day for 4 days at a dose of 50 mg kg^{-1}. On 3 days before and 1 day after vehicle or rifampicin treatment, animals received the s.c. injection of DEX at a dose of 10 mg kg^{-1}. Urine samples were collected for 48 h.
Values represent mean ± standard deviation (SD) from six to nine animals. Values in the parentheses represent the range of minimal and maximal values.
*Significantly different from control values ($p < 0.05$).

The immunohistochemical staining was intense for CYP3A4 (CYP% = 50.0%) and faint for CYP3A5 (CYP% = 15.2%). The positive reaction for CYP3A4 was increased (CYP% = 73.7%) after rifampicin treatment. Only CYP3A4 staining significantly increased after rifampicin treatment ($p < 0.01$) and CYP3A5 staining did not changed.

Discussion

6βOHD formation has been previously reported to be catalysed by CYP3A4 (Gentile et al. 1996), but participation of CYP3A5, another member of the CYP3A subfamily, has not been previously determined. As shown in the current study, 6βOHD formation is catalysed by both CYP3A5 and CYP3A4. The intrinsic clearance by CYP3A4 is more than 50-fold higher than that of CYP3A5. These results indicate that DEX 6β-hydroxylation is a suitable substrate that partially differentiates CYP3A4 and CYP3A5 activities.

Although mouse liver was highly replaced by human liver in $uPA^{+/+}$/SCID chimeric mice, 20–30% of mouse liver was still present. For sensitive detection of CYP induction in the mouse, a probe should be metabolized more rapidly in human liver microsomes than in mouse liver microsomes. Activities of DEX 6β-hydroxylation by human liver microsomes are higher than those in mouse liver microsomes (Tomlinson et al. 1997) and the reaction is catalysed by CYP3A4 (Gentile et al. 1996). As shown in the current study,

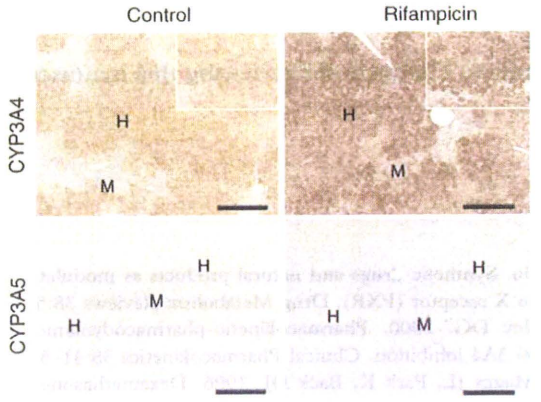

Figure 3. Immunohistochemical analyses of CYP3A4 and CYP3A5 in chimeric mice with a humanized liver. Immunohistochemical staining of CYP3A4 and CYP3A5 revealed cytoplasmic positive reactions in large clear human hepatocytes. The immuno-expression (CYP%, mean± SD from five animals) of CYP3A4 and CYP3A5 were 50.0% ± 5.2% and 15.2% ± 21.9%, respectively, in control mice. The expression of CYP3A4 and CYP3A5 were 73.7% ± 9.8% and 11.2% ± 11.8%, respectively, in rifampicin-treated animals. H, human hepatocyte area; M, mouse hepatocyte area; bar, 100 μm; and insert, higher magnification (× 2.1) for each figure.

6βOHD formation by CYP3A5 was much lower that that by CYP3A4. After DEX administration to man, 6βOHD is excreted as a major urinary metabolite, and only a small fraction of unchanged DEX and DEX glucuronide were detected (Minagawa et al. 1986). Accordingly, it is concluded that DEX is a suitable probe to detect induction of CYP3A4-mediated metabolism in chimeric mice with a humanized liver by a non-invasive method.

In the current study, the ratio of 6βOHD to DEX after DEX treatment was 1.1 in the urine of the chimeric mice with a humanized liver. Although the first study reported that after DEX administration to man, 6βOHD was detected as a major urinary metabolite (about 30% of the dose) and DEX as only a small portion (about 2% of the dose) (Minagawa et al. 1986), a recent study reported a ratio of 1.4 by an LC/MS method (Zurbonsen et al. 2004). The cause of the differences in these latter two studies is not clear but may arise to different assay methods, individual differences, different population of subjects administered, etc.

Although DEX is also reported as an inducer of CYP3A4, a metabolic ratio of 6βOHD to DEX is not changed before and after vehicle treatment. The result indicates that CYP3A4 is not induced by a single s.c. treatment of DEX at a dose of 10 mg kg^{-1}.

The present paper has reported a non-invasive method to detect induction of CYP3A4 in chimeric mice with a humanized liver. A change of the metabolic ratio (6βOHD/DEX) before and after inducer treatment in the same animal minimizes inter-individual differences of any factors which could affect the ratio. It is concluded that the chimeric mice with a humanized liver coupled with determining the changes of the urinary metabolic ratio (6βOHD/DEX) before and after inducer treatment are a useful animal model for detecting the induction of CYP3A4 by a non-invasive method.

Further studies such as induction study using another CYP3A4 inducers and co-administration study of CYP3A4 substrates with inhibitors will provide further information on usefulness of the chimeric mice with a humanized liver for detecting CYP3A4 induction by a non-invasive method.

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Review

Chimeric mice with humanized liver

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Abstract

Recently, chimeric mice with humanized liver were established by transplanting human hepatocytes into an urokinase-type plasminogen activator$^{+/+}$/severe combined immunodeficient transgenic mouse line. The replacement with human hepatocytes is more than 80–90% and is higher than any other chimeric mouse reported previously. In drug development, the liver is one of the most important organs because it is mainly involved in the pharmacokinetics of drugs and is frequently damaged by many drugs due to the accumulation of drugs and/or metabolites. The pharmacokinetics could affect the efficacy and toxicity of a drug, and thus prediction of the human pharmacokinetics is important for developing new drugs without adverse reactions and toxicity. Extrapolation from experimental animals or in vitro studies to the human in vivo pharmacokinetics is still difficult. To date, human hepatocytes and liver microsomes are recognized as better tools and are frequently used to estimate the human pharmacokinetics. We thought that chimeric mice with humanized liver could become a new tool for estimating the human toxicity and pharmacokinetics. At first, metabolism, which plays an essential role in pharmacokinetics, was investigated in the chimeric mice. In the liver of the chimeric mice, human drug metabolizing enzymes were found to be expressed and to reflect the capacities and genetic polymorphism of the donor. In an in vivo study on metabolism, human specific metabolites could be detected in the serum of the chimeric mice indicating that the chimeric mice could be used as an in vivo model to address human metabolism. These results suggested that the chimeric mice could overcome the species differences in drug metabolism and be used to evaluate drug toxicity due to genetic polymorphism. The reasons for drug interaction are often enzyme induction and inhibition. By the treatment with a typical inducer of cytochrome P450 (P450), which is the central drug-metabolizing enzyme, P450s expressed in the liver of the chimeric mice were found to possess induction potencies. After the treatment with a specific inhibitor of human P450, the area under the curve of the P450 metabolite was significantly decreased in the chimeric mice but not in the control mice. Therefore, it was indicated that the chimeric mice could be useful for assessing drug interactions in vivo. Moreover, drug excretion was determined to be humanized because cefmetazole was mainly excreted in urine both in the chimeric mice and humans but in the feces in control uPA$^{-/-}$/SCID mice. Drug transporters expressed in the liver of the chimeric mice were also humanized.

In this review, studies of the chimeric mice with humanized liver, particularly on metabolism and excretion, are summarized and the possibility of using the chimeric mice is proposed for the advanced prediction of human pharmacokinetics and toxicity.

Keywords: Humanized model; Drug-metabolizing enzyme; Transporter; Drug interactions; Genetic polymorphism

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1. Introduction

There is large interindividual variability in the efficacy and toxicity of drugs, which may be mainly caused by differences in the drug pharmacokinetics. The pharmacokinetics can be determined by ADME (absorption, distribution, metabolism, and excretion), especially drug metabolism. Drug metabolism consists of the phase I reactions (oxidation, reduction, and hydrolysis) and phase II reaction (conjugation), which occur predominantly in the liver. Therefore, the liver is the essential organ to determine the drug pharmacokinetics. One of the phase I enzymes, cytochrome P450 (P450, CYP), plays a central role in drug metabolism. P450 can metabolize various compounds including xenobiotic and endogenous compounds (Nelson et al., 1996). In addition, bioactivation leading to toxicity can sometimes be initiated by some P450s. Recently, the phase II reaction has been well studied since a parent drug and/or phase I metabolites are frequently excreted after conjugation. Furthermore, several drug-metabolizing enzymes have been shown to be polymorphic (Ingelman-Sundberg, 2002; Miners et al., 2002) indicating that genetic polymorphism can affect differences in the drug pharmacokinetics.

The mechanism of drug interactions can be often explained by the induction and inhibition of drug metabolizing enzymes. Serious drug interactions involving P450 have been reported (Dresser et al., 2000; Niemi et al., 2003). The QT prolongation caused by the inhibition of CYP3A4 by a coadministered drug resulted in the withdrawal of terfenadine and cisapride from the market. The prediction of adverse drug reactions is essential in drug development.

There are many reports regarding drug interactions and toxicity of various drugs, although the drugs were approved only after their safety was thought to have been confirmed in preclinical and clinical studies. In the preclinical stage, the pharmacokinetics of a drug candidate are investigated using human-derived sources or experimental animals. The results from experimental animals and in vitro studies sometimes wrongly predict the human pharmacokinetics and toxicity. Many researchers have made much effort to overcome such difficulties including those caused by species differences. Nowadays, human liver microsomes and human hepatocytes in primary culture are recognized as better tools and are frequently used during drug development. Human liver microsomes can be stored for a few years without the loss of enzyme activities but cannot be used to evaluate the induction potencies. Human hepatocytes express all the drug metabolizing enzymes, but a novel technique for culture is needed to avoid decreasing the enzyme activities. Such in vitro models have various advantages and limitations, as have been described previously (Gomez-Lechon et al., 2003; Rodrigues and Rushmore, 2002).

To develop an artificial human liver is one of the best approaches for predicting human pharmacokinetics and safety. An urokinase-type plasminogen activator (uPA)$^{+/+}$/severe combined immunodeficient transgenic mouse line, in which the liver could be replaced by 80–90% with human hepatocytes, was established in Japan (Tateno et al., 2004). In this review, basic researches concerning drug metabolism and drug interactions are summarized for the application of chimeric mice in drug development and toxicology.

2. Generation of chimeric mice with humanized liver

In 2001, chimeric mice with partially humanized liver were described by Dandri et al. (2001) and Mercer et al. (2001). In the former report, the livers in uPA/recombinant activation gene-2 mice could be repopulated with approximately 15% human hepatocytes (Dandri et al., 2001). In the latter, a portion the liver in uPA$^{+/+}$/SCID mice was replaced with human hepatocytes (Mercer et al., 2001). Since they were investigating on hepatitis virus, their chimeric mice with low replacement might be sufficient to achieve their purposes. However, the livers of such chimeric mice are not suitable to investigate human ADME in mice in vivo. In 2004, Tateno et al. (2004) succeeded in generating chimeric uPA$^{+/+}$/SCID mice, in which the livers could be replaced by more than 90% with human hepatocytes. The uPA$^{+/+}$/SCID mice at 20–30 days after birth were injected with human hepatocytes through a small left-flank incision into the inferior splenic pole (Tateno et al., 2004). We transplanted 7.5×10^5 hepatocytes into the livers of the uPA$^{+/+}$/SCID mice, and monitored their growth by determining the concentration of hAlb in the host serum. After transplantation using hepatocytes from 9-month-old Caucasian male into 19 uPA$^{+/+}$/SCID mice, serum human albumin (hAlb) concentrations in 14 mice reached 5 mg/ml around 60 days post-transplantation (Fig. 1). All mice survived for up to 60 days post-transplantation. To identify human hepatocytes in the chimeric livers, liver sections of mice were subjected to human specific cytokeratin 8/18 (hCK8/18) immunostaining (Fig. 2). The anti-hCK8/18 antibodies reacted specifically with hepatocytes and bile duct cells in the human livers, but did not react with the livers of the host mice. The replacement index (RI) of the mouse liver that received the human hepa-

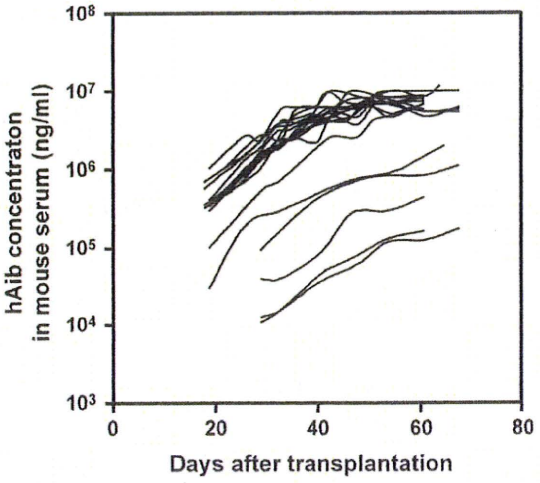

Fig. 1. Changes in the hAlb concentration in mouse serum. Nineteen uPA$^{+/+}$SCID mice were transplanted with 7.5×10^5 hepatocytes from 9-month-old Caucasian male. The hAlb concentrations increased in all the transplanted mice, and were >5 mg/ml in 14 mice among them.

tocytes was determined as the ratio of the area occupied by hCK8/18-positive hepatocytes to the entire area examined in immunostained sections using all lobules. The RI values correlated well with the serum hAlb concentrations of chimeric mice ($y = 0.0413e^{0.0676x}$, where y = hAlb concentration (mg/ml) and x = RI (%), $r = 0.92$). The correlation formula suggests that mice with >5 mg/ml hAlb concentration should have RI > 70%. Chimeric mice more than 11 weeks old would be used for experiments because the hAlb concentration will have reached a plateau. This chimeric mouse line has many advantages. By measurement of the hAlb concentration, it is easy to estimate the degree of replacement by human hepatocytes. Hepatocytes from various donors and cryopreserved hepatocytes could be used to generate such chimeric mice. Therefore, interindividual variabilities of pharmacokinetics and toxicity can be evaluated by the chimeric mice with hepatocytes from different donors.

Table 1
Correlation between enzyme activities and hAlb concentrations in chimeric mice with humanized liver

Isoform	Enzyme activity	Correlation (r)	
		Donor A	Donor B
CYP2C8	PTXOH	0.93*	0.69
CYP2C9	DICOH	0.98**	0.93**
CYP2D6	DBOH	0.97**	0.72
CYP3A4	DEXOH	0.97**	0.87*
UGT2B7	Morphine 6-glucuronosyltransferase activity	0.93*	0.93***
SULT1A1	Troglitazone sulfotransferase activity	0.82	0.71*
SULT1E1	Estrone 3-sulfotransferase activity	0.90*	0.89**

Donor A chimeric mice ($n = 5$); donor B chimeric mice ($n = 7$). Donor A, 12-year-old Japanese male; donor B, 9-month-old Caucasian male. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. PTXOH: paclitaxel 6α-hydroxylase activity; DICOH: diclofenac 4′-hydroxylase activity; DBOH: debrisoquine 4′-hydroxylase activity; DEXOH: dexamethasone 6-hydroxylase activity.

3. Drug metabolism in chimeric mice with humanized liver

3.1. Cytochrome P450

The most important drug metabolizing enzyme involving phase I reactions is P450. One of the major isoforms, CYP3A4, has been reported to be responsible for the metabolism of more than 50% of clinical drugs (Pelkonen et al., 1998). In chimeric mice, human CYP3A4 mRNA and human CYP3A4 protein could be detected in an hAlb concentration-dependent manner by real-time reverse-transcription polymerase chain reaction (RT-PCR) and Western blotting, respectively, which detected human CYP3A4 but not murine Cyp3a isoforms (Katoh et al., 2004). As shown in Table 1, dexamethasone 6-hydroxylase activity (DEXOH) catalyzed by human CYP3A4 was also significantly correlated with the hAlb concentrations. Dexamethasone is primarily metabolized to 6-hydroxydexamethasone

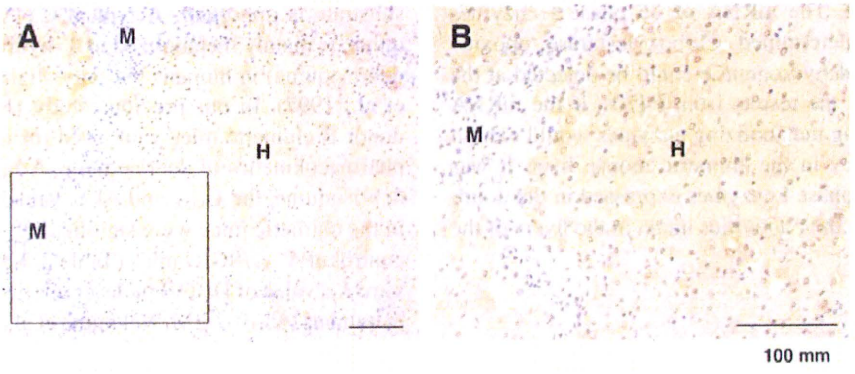

Fig. 2. Demonstration of mouse liver chimerism. (A) A uPA$^{+/+}$SCID mouse was transplanted with 7.5×10^5 hepatocytes from 9-month-old Caucasian male, and killed at 68 days post-transplantation. Frozen liver sections were prepared from the chimeric mouse, and stained with anti-hCK8/18 antibodies. Positive (brown colored) and negative (blue colored) regions showed human (H) and mouse (M) hepatocyte-occupied areas, respectively. RI determined as described in the text was 93%. (B) The region enclosed by the square in A is magnified and shown in B. Bar, 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

in humans but to 6-hydroxy-9α-fluoro-androsta-14-diene-11ß-hydroxy-16α-metyl-3,17-dione in mice (Tomlinson et al., 1997). DEXOH in liver microsomes from the donor A chimeric mouse with an RI of 90% showed almost the same activity as those from the donor A (Katoh et al., 2004) indicating that the liver of the chimeric mice possessed CYP3A4 capacity similar to that of the donor.

CYP2C9 is responsible for the hydroxylation of many non-steroidal anti-inflammatory drugs such as diclofenac. CYP2C8 and CYP2D6 are involved in the hydroxylation of the anticancer drug paclitaxel and many antipsychotropic drugs, respectively. In the chimeric mice, human specific activities such as diclofenac 4′-hydroxylase activity (DICOH) by CYP2C9, paclitaxel 6α-hydroxylase activity (PTXOH) by CYP2C8, and debrisoquine 4′-hydroxylase activity (DBOH) by CYP2D6 could be detected (Katoh et al., 2004). DICOH, PTXOH, and DBOH as well as DEXOH correlated with the hAlb concentrations (Table 1) and the chimeric mouse with the highest hAlb concentration exhibited similar enzyme activities to those of the donor. Therefore, the liver of the chimeric mice could reflect the phenotype of the donor. In our previous report (Katoh et al., 2004), the other four human P450 isoforms, CYP1A2, CYP2A6, CYP2C19, and CYP3A5, could be detected at the mRNA, protein and/or enzyme activity level. In the report by Nishimura et al. (2005b), the mRNA of 20 human P450 isoforms could be detected in the liver of the chimeric mice. It is surmised that all P450 isoforms expressed in the donor can be expressed in the liver of the chimeric mice. The chimeric mice have much fewer activities of mouse P450s and high activities of human P450s because uPA$^{+/+}$/SCID mice causes sever liver failure in mouse hepatocytes and the proliferation of human hepatocytes is needed for survival. That is one of the advantages as compared to transgenic mice with human P450s, which usually express only one human P450 isoform with many mouse P450s retaining normal activities.

3.2. Other phase I enzymes

Nishimura et al. (2005b) investigated the hepatic mRNA expression of human phase I enzymes in chimeric mice by TaqMan real-time RT-PCR. The mRNA of 35 phase I enzymes except for P450s was determined. Carboxylesterase, epoxide hydrolase, and alcohol dehydrogenase could be detected at the mRNA level. Based on the results from P450s, if the mRNA is expressed, human drug metabolizing enzymes would exhibit almost the same potency in the chimeric mouse liver. It was assumed that all of the phase I enzymes expressed in the donor could be expressed with their activities intact in the livers of the chimeric mice.

3.3. Phase II enzymes

The major hepatic phase II enzymes in humans are UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), *N*-acetyltransferase (NAT), and glutathione-*S*-transferase (GST). The contribution of phase II conjugation to the clearance of a drug was estimated to be approximately 30% or higher in humans (Bjornsson et al., 2003). In recent drug development, the relative involvement of phase II enzymes in the metabolic clearance of drugs is greater due to preselection concerning P450 metabolism in the early preclinical stage (Fisher et al., 2001). Taken together, attention needs to be paid to the interactions via UGT. Hyperbilirubinemia caused by the inhibition of UGT1A1 has been reported (Zucker et al., 2001), since bilirubin glucuronosyltransferase activity is catalyzed by UGT1A1.

The mRNA and protein of major UGT isoforms could be detected in the liver of chimeric mice (Katoh et al., 2005b). A specific human UGT2B7 activity, morphine 6-glucuronosyltransferase activity, correlated with the hAlb concentration as shown in Table 1. As in the case of phase I enzymes, human UGTs are also expressed in the chimeric mice and possess enzyme activities. Generally, GST plays an important role in the detoxification of various xenobiotics and reactive metabolites. For applying the chimeric mice to toxicological studies, the expression of human GST needed to be evaluated. The mRNA of human GSTs could be detected in the chimeric mice indicating that human GST may also have the potency of conjugation. In terms of SULT and NAT, since mRNA, protein, and/or enzyme activities could be detected (Katoh et al., 2005b), human phase II enzyme can also be expressed in the chimeric mice. That the livers of the chimeric mice expressed both human phase I and phase II enzymes is of great value because a series of human drug metabolisms in the liver can be investigated.

3.4. In vivo drug metabolism

In vivo experiments of chimeric mice would be more appropriate for the evaluation of human pharmacokinetics and toxicity. Therefore, we examined the in vivo drug metabolism catalyzed by human CYP2D6 in the chimeric mice. CYP2D6 function has been reported to exhibit large species differences, but is responsible for the metabolism of one quarter of the known clinical drugs (Ingelman-Sundberg, 2005). In addition, genetic polymorphism of human CYP2D6 is known to exist and many mutant alleles have been reported (http://www.cypalleles.ki.se/cyp2d6.htm). The prediction of the human pharmacokinetics of a CYP2D6 substrate is important. A typical CYP2D6 substrate, debrisoquine, is mainly metabolized to 4′-hydroxydebrisoquine (4-OH debrisoquine) in humans but only slightly in mice (Masubuchi et al., 1997). In our previous study (Katoh et al., 2007), the donor B chimeric mice were used for investigating the in vivo pharmacokinetics of debrisoquine. After oral administration of debrisoquine, the C_{max} and AUC values of 4-OH debrisoquine in the chimeric mice were significantly higher than those in the control uPA$^{-/-}$/SCID mice (Table 2, Katoh et al., 2007). The in vitro K_m value of DBOH in the chimeric mice was similar to that in humans (62–107 μM, Nakajima et al., 2002) but was different from that in uPA$^{-/-}$/SCID mice (213 μM) suggesting that the chimeric mice could reliably shown to exhibit a humanized profile of drug metabolism in both in vivo and in vitro studies. Thus the chimeric mice could become a useful model for evaluating the biotransformation of drugs by human drug metabolizing enzymes.

Table 2
Pharmacokinetic and kinetic parameters of 4-OH debrisoquine in chimeric mice with humanized liver and uPA$^{-/-}$/SCID mice

	Pharmacokinetic parameter		Kinetic parameter
	C_{max} (nM)	AUC_{0-8} (nM·h)	K_m (μM)
Chimeric mouse	297.1 ± 37.7***	887 ± 90***	72 ± 8***
uPA$^{-/-}$/SCID mouse	61.4 ± 9.0	279 ± 32	213 ± 12

In vivo study for pharmacokinetic analysis, debrisoquine was orally administered at 2.0 mg/kg. Data represent the mean ± S.E. ($n = 8$). The donor B chimeric mice with high hAlb concentrations (>5.0 mg/ml and RI > 70) were used. ***$p < 0.001$, compared with uPA$^{-/-}$/SCID mice.

3.5. Genetic polymorphism

The interindividual variability of the pharmacokinetics and toxicity is partly related to the genetic polymorphism of drug metabolizing enzymes (Eichelbaum et al., 2006; Nagar and Remmel, 2006). Pharmacogenetics has become important in the developing concept of personalized medicine. FDA has approved genetic testing for mutations in the CYP2C19, CYP2D6, and UGT1A1 genes. In the case of CYP2D6, the CYP2D6 polymorphism might affect 3,4-methylenedioxymethamphetamine cytotoxicity (Carmo et al., 2006) and is associated with the occurrence of adverse effects of some antidepressants (Rau et al., 2004). The necessary dose adjustments of antidepressants according to the CYP2D6 genotype have been summarized (Kirchheiner et al., 2004) and will facilitate the selection of a drug and its dosage. However, the necessary dose adjustment and the effects on drug safety by many variants of other drug metabolizing enzymes remain unclear.

Genetic polymorphism of the CYP2A6 gene has been suggested to be related to the risk of lung cancer because CYP2A6 has a major impact on nicotine clearance (Nakajima and Yokoi, 2005). Bosch et al. (2007) hypothesized that the lethal toxicity of an anticancer drug, UFT (uracil/tegafur), is probably caused by a decrease in tegafur metabolism due to CYP2A6 polymorphism. The donor A was homozygous for *CYP2A6*4* alleles, in which the whole *CYP2A6* gene is deleted (Katoh et al., 2004). Human CYP2A6 protein and coumarin 7-hydroxylase activity catalyzed by CYP2A6 could not be detected in the liver of the donor A chimeric mouse with a high hAlb concentration (13.7 mg/ml; RI = 90), whereas they could be detected in that of the donor B chimeric mouse with a high hAlb concentration (7.7 mg/ml; RI = 90). The donor A chimeric mice were genotyped as *CYP2A6*4/CYP2A6*4* using genomic DNA extracted from the liver suggesting that the chimeric mice have the same genotype and phenotype as the donor. With regard to other P450 isoforms, CYP2C19 and CYP3A5, the genotype of the chimeric mouse was the same as the donor.

NAT2 carries out the acetylation of arylamines and arylhydrazines including environmental carcinogens. According to the isoniazid metabolism, individuals have been classified as slow and rapid acetylator phenotypes. NAT2 genetic polymorphism is known to be involved in such variations (Meyer and Zanger, 1997). The frequency of the main adverse reaction of isoniazid, peripheral neuropathy, in the slow acetylator phenotype was higher than that in the rapid acetylator phenotype (Hughes et al., 1954). The donor B and the donor B chimeric mice were genotyped as *NAT2*6/NAT2*13*, which was classified as slow acetylator (Cascorbi et al., 1995). Sulfamethazine *N*-acetyltransferase activity is a human specific activity catalyzed by human NAT2. Such activity in the donor B chimeric mice was lower than that in the donor A chimeric mice (Katoh et al., 2005b), which was consistent with the result of the genotyping.

Troglitazone is a thiazolidinedione antidiabetic agent that was withdrawn from the market in 2000 due to its association with idiosyncratic hepatotoxicity. A strong correlation between transaminase elevation and the combined *GSTM1* and *GSTT1* null genotype was observed (Watanabe et al., 2003). The susceptibility to tacrine hepatotoxicity was increased in individuals with these null genotypes (Simon et al., 2000). GSTM1 and GSTT1 are expressed in the liver in humans. Human GSTM1 mRNA in the liver of the donor B chimeric mice could not be detected by TaqMan real-time RT-PCR suggesting that the GSTM1 gene was deleted in donor B. Chimeric mice generated by a donor with *GSTM1* and *GSTT1* null genotypes may become a useful tool for predicting such hepatotoxicity.

If a donor with a poor metabolizer phenotype is used for generating the chimeric mice, the influence of genetic polymorphism on the pharmacokinetics and toxicity could be investigated. It is a great advantage that the chimeric mice can retain both the genotype and phenotype of the donor for personalized medicine and toxicological studies.

4. Induction of P450 in chimeric mice with humanized liver

Enzyme induction of a drug-metabolizing enzyme is a long-term consequence of chemical exposure and leads to an elevation of the enzyme activity (Lin and Lu, 2001). Drug interactions caused by the induction of P450, especially CYP3A4, are sometimes a serious problem because the induction may result in changes in the efficacy and toxicity of a drug (Pascussi et al., 2003; Niemi et al., 2003). Many CYP3A4 inducers are used as drugs in clinical practice and they can induce CYP3A4 at clinically used doses (Pascussi et al., 2003). By the coadministration of a typical CYP3A4 inducer, rifampicin, the pharmacokinetic of other drugs is sometimes changed significantly (Niemi et al., 2003). Moreover, P450 induction by herbal medicines including St John's wort could become a serious problem depending on the increased use of herbal medicines (Henderson et al., 2002). Therefore, the screening of drug candidates for induction potencies regarding drug-metabolizing enzymes is important in preclinical studies.

Using a primary culture of cryopreserved chimeric mouse hepatocytes, Nishimura et al. (2005a) investigated the induc-

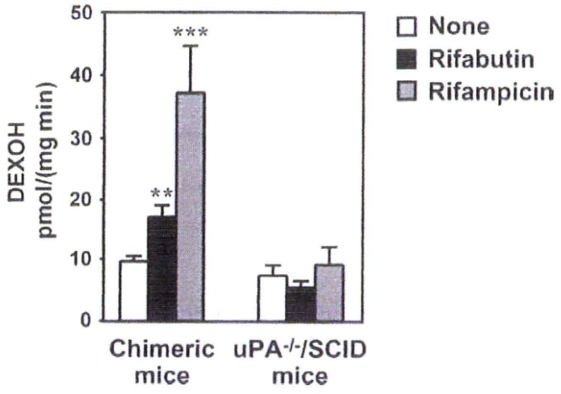

Fig. 3. Induction of DEXOH in rifabutin- or rifampicin-treated donor B chimeric mice with humanized liver. Rifabutin and rifampicin were intraperitoneally administered daily for 4 days at 50 mg/(kg day). Data represent the mean ± S.D. ($n \geq 3$). $^{**}p < 0.01$, $^{***}p < 0.001$ compared with none.

tion of human CYP3A4 mRNA by TaqMan real-time RT-PCR. They reported that CYP3A4 mRNA was significantly increased 2.2–8.4-fold by the treatment with 50 μM rifampicin for 24 h, which was a similar increase to that shown in fresh and cryopreserved human hepatocytes. After intraperitoneal treatment with rifampicin at 50 mg/(kg day) for 4 days, DEXOH had significantly increased by 4.1-fold on average in liver microsomes from the chimeric mice (Fig. 3, Katoh et al., 2005a). As mentioned above, since DEXOH is a human specific activity for CYP3A4, the increase of DEXOH would indicate that human CYP3A4 in the chimeric mice has in vivo induction potencies. Iwasaki et al. (2005) also determined the changed excretion of 6-hydroxydexamethasone after treatment with rifampicin in chimeric mice. After 4-day treatment with rifampicin (50 mg/kg, intraperitoneally), dexamethasone at 10 mg/kg was administered subcutaneously in chimeric mice and then the urinary excretion of 6-hydroxydexamethasone was measured. The excretion of 6-hydroxydexamethasone was increased 1.9–3.3-fold suggesting that the chimeric mice could be applied for in vivo and in vitro induction studies.

Rifabutin, which is a specific inducer of human CYP3A4 but not mouse Cyp3a, was used to evaluate the induction of human CYP3A4 in chimeric mice (Katoh et al., 2005c). DEXOH was increased in the liver microsomes from rifabutin-treated chimeric mice (Fig. 3, Katoh et al., 2005c). The induction potencies of rifampicin have been reported to be stronger than those of rifabutin (Perucca et al., 1988; Li et al., 1997), which is consistent with our result. The degree of induction can be evaluated using the chimeric mice.

Other inducible P450 isoforms, CYP1A1 and CYP1A2, were also examined for the induction potency in the chimeric mice. CYP1A1 and CYP1A2 play critical roles in the metabolic activation of many carcinogens. CYP1A induction has long been studied concerning its association with the toxicity of carcinogens and the risk of cancer (Ma and Lu, 2007). In the chimeric mice, the mRNA and protein of both CYP1A1 and CYP1A2 were induced by the treatment with typical inducers, ß-naphthoflavone or 3-methylcolanthrene, in in vitro and in vivo studies (Katoh et al., 2005a; Nishimura et al., 2005b; Yoshitsugu et al., 2006). Although the human specific CYP1A1/CYP1A2 activities could not be evaluated, these activities in the chimeric mice might be induced as in the case of human CYP3A4.

For induction studies, it is believed that human hepatocytes are better than any other models, although human hepatocytes loose the enzyme activity very quickly. Because this chimeric mouse line could overcome such difficulties, it could become a better tool for induction studies in humans.

5. Inhibition of P450 in chimeric mice with humanized liver

Enzyme inhibition is an acute decrease of metabolism by a coadministered drug or a time-dependent decrease in the amount of an enzyme by several factors (Pelkonen et al., 1998). Drug interactions are often caused by the inhibition of P450 activities (Dresser et al., 2000). The prediction of human pharmacokinetic parameters from in vitro technologies has progressed (Houston and Galetin, 2003) but quantitative extrapolations from in vitro to human in vivo and from experimental animals to humans are still difficult. It would be a great advantage if drug interactions in humans could be predicted using this chimeric mouse line.

The P450 inhibition by a coadministered drug is thought to be very critical when a patient requires long-term treatment with it. Some CYP2D6 inhibitors such as antidepressants are prescribed for long periods of time. The inhibition of CYP2D6 enzyme activity by quinidine and selective serotonin reuptake inhibitors is well known in clinical practice (Caporaso and Shaw, 1991; Hemeryck and Belpaire, 2002). Therefore, we focused on CYP2D6 inhibition and the drug interactions between debrisoquine and quinidine were investigated in chimeric mice in vivo (Katoh et al., 2007). Quinidine, a frequently prescribed antiarrhythmic agent, is a specific inhibitor of human CYP2D6 but not mouse Cyp2d indicating that the specific inhibition of human CYP2D6 could be determined in this experiment. After 3-day treatment with quinidine, the AUC and C_{max} values of 4-OH debrisoquine were significantly decreased in the chimeric mice, but such values did not change in the control uPA$^{-/-}$/SCID mice (Fig. 4, Katoh et al., 2007).

An in vitro inhibition study using liver microsomes also demonstrated the human specific inhibition. The K_i value of quinidine on DBOH in the chimeric mice (0.049 μM) was significantly lower than that in the control uPA$^{-/-}$/SCID mice (29 μM, $p < 0.001$ compared with the chimeric mice, Katoh et al., 2007). Therefore, the specific inhibition of human the CYP2D6 highly expressed in the chimeric mice suggested that human drug interactions could occur in the chimeric mice. Given the possibility of using chimeric mice for induction and inhibition studies, they could be a useful in vivo tool to predict drug interactions and toxicity.

6. In vivo excretion in chimeric mice with humanized liver

A drug is mostly eliminated by biliary and urinary excretion. To elucidate the excretion of a drug as well as the metabolism is essential for understanding the pharmacokinetics and toxic-

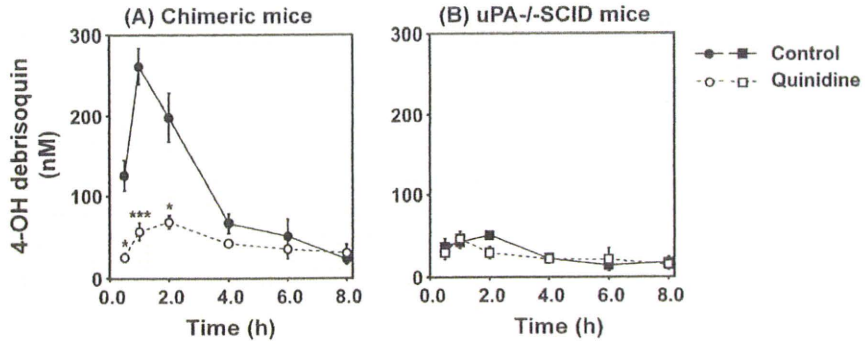

Fig. 4. Effect of quinidine treatment on serum concentration of 4-OH debrisoquine in donor B chimeric mice (A) and the control uPA$^{-/-}$/SCID mice (B). After treatment with quinidine (100 mg/(kg day) for 3 days), debrisoquine was orally administered at 2.0 mg/kg. Data represent the mean ± S.E. ($n = 4$). Open and closed symbols represent the values with or without quinidine treatment, respectively. *$p < 0.05$, ***$p < 0.001$ compared with control.

Table 3
Cefmetazole excretion in chimeric mice with humanized liver

	n	Excretion (% of the dose)	
		Urine	Feces
Chimeric mouse	5	81.0 ± 9.5***	5.9 ± 4.7***
uPA$^{-/-}$/SCID mouse	7	23.7 ± 8.8	59.4 ± 11.0

Cefmetazole was intraperitoneally administered at 25.0 mg/kg. Urine and feces samples were collected during 24 h after the cefmetazole administration. Data represent the mean ± S.D. ***$p < 0.001$, compared with uPA$^{-/-}$/SCID mice.

ity. Species differences in the excretory pathway may make the extrapolation from experimental animals to humans difficult. In the case of an antibiotic agent, cefmetazole was mainly excreted in urine in humans (Ko et al., 1989) but in feces in rats and mice in an unchanged form (Murakawa et al., 1980; Okumura et al., 2007). Thus, cefmetazole was a better probe to investigate the humanization of the excretion pathway in chimeric mice. Following intraperitoneal administration, the 24-h cumulative recovery of cefmetazole in urine and feces was quantified in the chimeric mice (Okumura et al., 2007). As shown in Table 3, the main pathway was urinary excretion in chimeric mice whereas it was fecal excretion in control uPA$^{-/-}$/SCID mice indicating that the excretory profile was humanized by the replacement with human hepatocytes.

Many drug transporters have been clarified to be responsible for drug excretion. Species differences of drug transporters have been clarified (Katoh et al., 2006; Suzuyama et al., 2007). As shown by Nishimura et al. (2005b) and us (Katoh et al., 2005c; Okumura et al., 2007), human drug transporters were expressed in the liver of the chimeric mice. The humanization of drug transporters may be also one of the determinants of the alteration of the cefmetazole excretory pathway. Although further study is needed, this chimeric mouse line might be a suitable model for predicting the humanized type of excretion. Since genetic polymorphism of drug transporters exists, the changed hepatic uptake and excretion can also be studied using the chimeric mice.

7. Conclusions

The chimeric mice have been shown to exhibit a humanized profile of drug metabolism, induction and inhibition of drug metabolizing enzymes, and excretion in in vivo studies. This chimeric mouse line should be a promising model for evaluating the in vivo pharmacokinetics in humans. In recent drug development, adverse pharmacokinetic and bioavailability have reduced as a cause of attrition although toxicology has increased (Kola and Landis, 2004). Drug-induced hepatotoxicity is one of the major problems in drug development and clinical practice. Recently, Yamamoto et al. (2007) demonstrated the usefulness of the chimeric mice in a toxicological study that evaluated acetaminophen. This chimeric mouse model could therefore contribute to estimate human specific toxicity.

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