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Regular paper

An improved TK-NOG mouse as a novel platform for humanized liver that overcomes limitations in both male and female animals

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ABSTRACT

We developed a novel immunodeficient NOG mouse expressing HSVtk mutant clone 30 cDNA under the control of mouse transthyretin gene enhancer/promoter (NOG-TKm30) to acquire fertility in males and high inducibility of liver injury in females. Maximum human albumin levels (approx. 15 mg/mL plasma) in both male and female NOG-TKm30 mice engrafted with human hepatocytes (humanized liver mice) were observed 8–12 weeks after transplantation. Immunohistochemical analyses revealed abundant expression of major human cytochrome P450 (CYP) enzymes (CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4) in reconstituted liver with original zonal distribution. *In vivo* drug–drug interactions were observed in humanized liver mice as decreased area under the curve of midazolam (CYP3A4/5 substrate) and omeprazole (CYP3A4/5 and CYP2C19 substrate) after oral administration of rifampicin. Furthermore, we developed a pregnant model for evaluating prenatal exposure to drugs. The detection of thalidomide metabolites in the fetuses of pregnant humanized liver mice indicates that the novel TK model can be used for developmental toxicity studies requiring the assessment of human drug metabolism. These results suggest that the limitations of traditional TK-NOG mice can be addressed using NOG-TKm30 mice, which constitute a novel platform for humanized liver for both *in vivo* and *in vitro* studies.

1. Introduction

Humanized liver chimeric mice were developed by transplanting human hepatocytes into immunodeficient mice with injured livers to limit species differences in drug metabolism and hepatotropism of hepatitis B virus (HBV) between mice and humans [1]. We established an inducible liver injury model, i.e., a TK-NOG transgenic mouse, which expresses the herpes simplex virus type 1 thymidine kinase gene (HSVtk) in liver with regulation by mouse albumin enhancer/promoter. Brief exposure to non-toxic doses of ganciclovir (GCV) ablated liver cells that express the HSVtk transgene, and host liver was then infiltrated with transplanted human hepatocytes [2]. HSVtk suicide gene technique allows selective depletion of target cells and is a widely accepted

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approach to examine functional aspects of cells, e.g., pituitary somatotropes [3] and bone marrow osteoblasts [4] *in vivo*. However, transgenic male mice that successfully express HSVtk have been determined to become infertile eventually, regardless of the promoter used [5,6]. Male infertility makes maintaining transgenic lines and developing animal models difficult. First, *in vitro* fertilization using sperm of HSVtk transgenic animals is not available for large-scale production. Inefficient natural mating with transgenic females is unavoidable even for large-scale production. Second, the inability to obtain HSVtk transgenic homozygotes means that PCR genotyping is always required. Two approaches have been made available to obtain a suicide gene that does not cause male infertility. Salomon et al. showed that male infertility is due to testis-specific high-level expression of short TK transcripts initiated mainly upstream of the second internal ATG of the TK gene [7]. Male transgenic ΔTK mice with a truncated TK gene starting at the second ATG and with its major internal transcription initiation sites deleted are fertile and thus transmit the transgene to progeny. The other approach is using an HSVtk mutant protein with a modified sequence by random sequence mutagenesis at the substrate binding motif [8]. Myeloid-specific CD11b promoter-driven transgenic mice (CD11b-TK^{mt-30}) were successfully generated using an HSVtk mutant clone 30 and reproduced normally, thus transmitting the transgene in expected Mendelian ratios [9]. TK-NOG mice also have another difficult issue of sexual dimorphism in transgene expression when used as an animal experimental model. In TK-NOG mice, liver-specific expression was achieved using mouse albumin enhancer/promoter to drive HSVtk gene expression; however, male-specific gene expression in transgenic rats using the 5′-flanking region of the mouse albumin gene has already been reported [10]. In fact, female TK-NOG mice have much lower expression of the transgene than male TK-NOG mice, hence only half of the transgenic mice can be used as transplant recipients.

The TK-NOG chimeric mice with humanized livers have been widely used for drug metabolism and pharmacokinetic studies [11–13]. However, their usefulness in studies of drug-drug interactions and chemical toxicity has not been sufficiently evaluated. Cytochrome P450 (P450) induction mediated drug–drug interactions are a major concern in clinical practice because they result in reduced therapeutic efficacy in polypharmacy [14]. There exist species differences in PXR-mediated P450 induction between humans and rodents. Tg hPXR/human P450 mice models are useful for investigating the induction of some human P450 genes with human PXR ligands. However, the effect of the human PXR ligand on multiple human P450 genes cannot be examined using these mouse models [15]. Drug–drug interaction studies using TK-NOG chimeric mice with humanized livers are expected to provide critical insights on the effect of human PXR ligands on multiple human P450 genes (CYP3A4/5, CYP2C19, and others). Furthermore, safety decisions on reproductive and developmental toxicity are generally based on pharmacokinetic, metabolic, and toxicological properties of the candidates. Thalidomide has been previously used as a sedative and a treatment for morning sickness during pregnancy, but this drug was withdrawn owing to its teratogenic effects in humans [16]. Species differences have also been reported in teratogenic effects (e.g. humans and rabbits are sensitive to thalidomide, whereas rodents are resistant) [17]. The development of pregnant model using novel humanized liver mice is expected to provide novel insights for the evaluation of prenatal developmental toxicity via humanized maternal drug metabolism.

We generated a novel HSVtk transgenic strain that addresses both infertility in male and low transgene expression in female animals by replacing mouse albumin enhance/promoter with liver-specific mouse transthyretin promoter [18] and wildtype HSVtk gene to mutant HSVtk clone30 gene, which does not cause male sterility [9]. The HSVtk mutant30 transgenic (NOG-TKm30) homozygote has been determined to enable easy breeding to maintain transgenic mice without PCR genotyping and without the appearance of unusable non-transgenic mice. Additionally, female transgenic mice express the transgene at the same level as males. We then investigated the utility of NOG-TKm30 humanized liver mice for drug metabolism studies.

2. Materials and methods

2.1. Generation of NOG-TKm30 transgenic mice and liver humanization by human hepatocyte transplantation

All the experimental procedures and protocols used for this animal study were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals of the Central Institute for Experimental Animals (Permit Number: 17017A). All efforts were made to minimize animal suffering, reduce the number of animals used, and utilize alternatives to *in vivo* techniques. A herpes simplex virus type 1 thymidine kinase mutant clone 30 (UL23 mutant30 or HSVtk mutant30) gene expression unit was constructed (Supplementary Fig. 1A). The linearized construct was microinjected into fertilized NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic (NOG) strain mouse using standard methods. HSVtk mutant30 transgenic NOG mice (NOG-TKm30) were obtained and used as recipients for human hepatocyte transplantation. For detailed information about the preparation and characterization of NOG-TKm30 transgenic mice and humanized liver NOG-TKm30 mice, see Supplementary Materials and Methods.

2.2. In vivo drug–drug interaction studies with NOG-TKm30 humanized liver mice

NOG-TKm30 humanized liver mice were given oral administration of rifampicin (0, 10, 50, and 100 mg/kg once daily) for 3 days followed by oral administration of a CYP cocktail containing five human CYP probe substrates (caffeine, warfarin, omeprazole, metoprolol, and midazolam). The blood samples of the mice were collected at 0.5, 1, 2, 4, 7, and 24 h and centrifuged for plasma isolation. The plasma concentrations of CYP probe substrates were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Supplementary Fig. 2; see Supplementary Materials and Methods).

2.3. Drug metabolism study in pregnant humanized liver mice and fetuses

F1 hybrid TKm30 transgenic mice were obtained by cross-breeding the NOG-TKm30 with the immunodeficient inbred IQI strain (formally, IQI.Cg-*Prkdc*^{scid} *Sirpα*^{NOD} *Il2rg*^{tm1Sug}, abridged name: ISGS) established by backcross breeding NOG mouse to IQI strain to introduce *scid* mutation [19] and *Sirpα* polymorphic genotype of the NOD strain [20]. Female F1-TKm30 mice were used as a platform for pregnant humanized liver mice due to their high reproductive ability caused by heterosis. Pregnant humanized liver mice were prepared by mating humanized liver F1-TKm30 female mice with NOG male mice. On the 19th day of pregnancy, 100 mg/kg thalidomide was administered orally to humanized liver F1-TKm30 mice. Maternal and fetal blood samples were then collected 2 h after administration and centrifuged for plasma isolation. Plasma concentrations of thalidomide, 5-hydroxythalidomide, 5′-hydroxythalidomide, and 5,6-dihydroxythalidomide were measured by LC-MS/MS (see Supplementary Materials and Methods).

2.4. Statistical analyses

Data were analyzed using Prism 8 for macOS (GraphPad Software, San Diego, CA, USA). Differences in reproductive ability between homozygote and heterozygote and in GCV sensitivity between male and female were assessed using unpaired *t*-tests with Welch's correction, and *p*-values <0.05 were considered statistically significant. One-way analysis of variance with Dunnett post-hoc test was performed to compare drug-metabolizing activities, protein levels, and mRNA levels between vehicle- and rifampicin-treated groups.

3. Results

3.1. Generation of NOG-TKm30 transgenic mice

We obtained three male transgenic mice carrying the HSVtk mutant30 transgene, which were screened using TaqMan real-time PCR assay (Supplementary Fig. 1B). Four transgenic lines were established from male founders by mating with female NOG mice, indicating that NOG-TKm30 transgenic mice successfully evaded male infertility. Details of the characterization of the NOG-TKm30 mice are described in the Supplementary Information file.

3.2. Characterization of humanized liver mice

Human serum albumin levels of four different donor hepatocytes transplanted into NOG-TKm30 mice are provided in Fig. 1A and Supplementary Fig. 3A. NOG-TKm30 allowed engrafting and proliferation of hepatocytes within the liver without gender differences. Cholinesterase activity in plasma, an alternative marker for evaluating human hepatocyte engraftment, was observed to increase in both male and female NOG-TKm30 mice after human hepatocyte transplantation (Supplementary Fig. 3B). Hematoxylin and eosin (H&E)-stained sections showed a repopulated humanized liver (exceeding 90%) that is occupied with human hepatocytes showing clear cytoplasm and round-shaped and red-colored colonies, which are hyperplastic nodules derived from residual mouse hepatocytes (Fig. 1B). The enlarged image (Fig. 1B) shows human hepatocytes with pale cytoplasm and lipid droplets, consistent with previous descriptions of the TK-NOG model [2]. We further examined histology using consecutive thin sections prepared from NOG-TKm30 humanized liver with specific antibodies for drug metabolism-related enzymes. Almost all hepatocytes were replaced by human hepatocytes that were stained with anti-HLA antibody, and typical lobular structure, a portal triad consisting of portal vein, bile duct, and hepatic artery, was conserved within the humanized liver (Fig. 1C). Immunohistochemical staining indicated that CYP1A2 and CYP3A4 were clearly localized in the perivenous regions. Conversely, CYP2C9, CYP2D6, and CYP2E1 were noted to be distributed homogeneously across hepatic lobules or weakly around the perivenous region. The distribution pattern of CYP proteins in the humanized liver was similar to that of humans (Fig. 1D).

3.3. In vivo drug–drug interaction studies with NOG-TKm30 humanized liver mice

To assess the function of human CYPs expressed in the humanized liver, *in vivo* pharmacokinetics of five CYP substrates

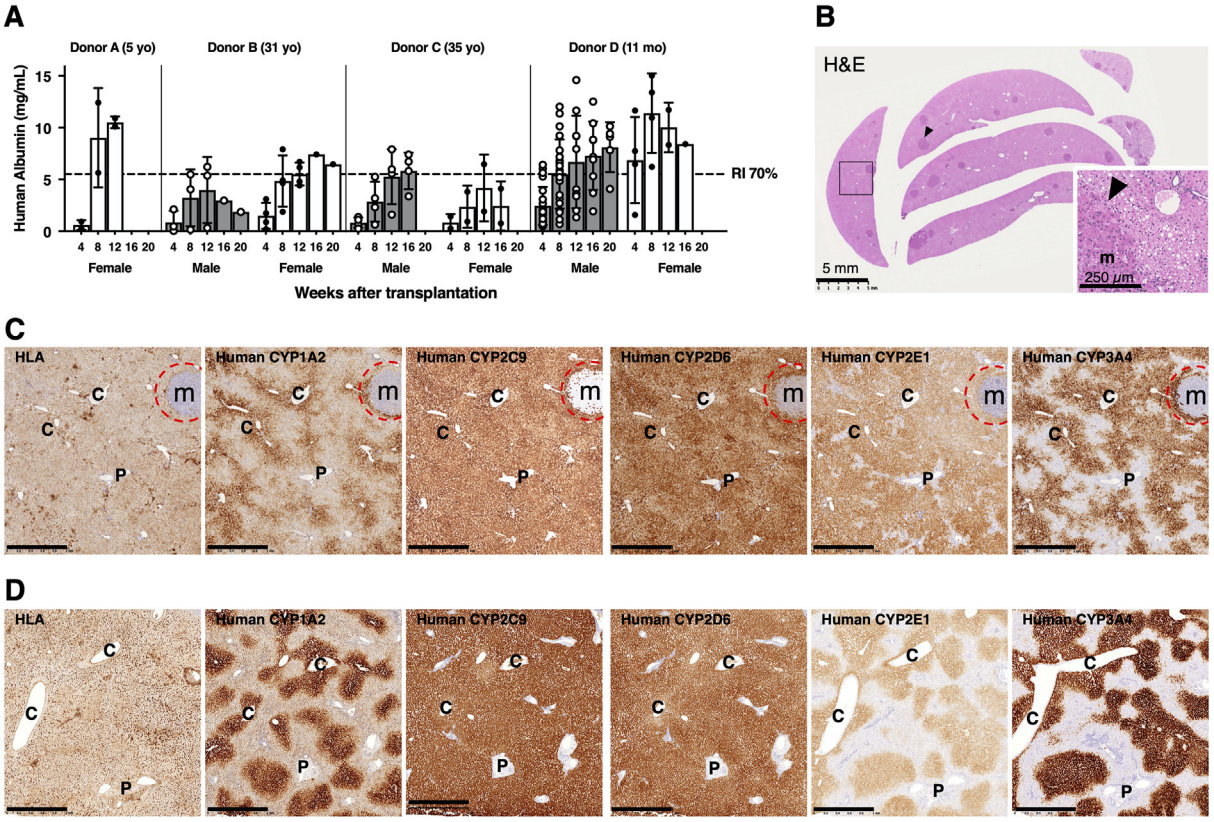

Fig. 1. Characterization of humanized liver mice. (A) The human albumin levels in the plasma from humanized liver mice were determined (donor A, 5-year-old, 4–12 weeks; donor B, 31-year-old, 4–20 weeks; donor C, 35-year-old, 4–16 weeks; donor D, 11-month-old, 4–16 or 4–20 weeks). The dotted line indicates the plasma levels of human albumin equivalent to 70% replacement. (B) The liver tissues of humanized liver from mice aged 16 weeks were stained with H&E and (C) the following antibodies anti-HLA, antihuman CYP1A2, antihuman CYP2C19, antihuman CYP2D6, antihuman CYP2E1, and antihuman CYP3A4. The area within the squares in (B) is magnified in (C). Dotted circles indicate the region comprising mouse hepatocytes (m). (D) Human liver tissues were used as staining control. P, portal tract, C, central vein. Scale bar, 1 mm.

(caffeine, warfarin, omeprazole, metoprolol, and midazolam, 5.0 mg/kg each) were analyzed in NOG-TKm30 humanized liver mice after repeated oral administration of rifampicin (10, 50, and 100 mg/kg daily for 3 days). $AUC_{0\text{-}\infty}$ of midazolam in humanized liver mice were significantly decreased in a dose-dependent manner by administering rifampicin (53%, 80%, and 84% compared with controls) (Fig. 2 and Table 1). Rifampicin treatment also decreased $AUC_{0\text{-}\infty}$ of omeprazole in humanized liver mice, though only at doses 50 and 100 mg/kg (41% and 50% compared with controls) (Fig. 2 and Table 1). In contrast, prior treatment with rifampicin did not affect $AUC_{0\text{-}\infty}$ or C_{max} of caffeine, warfarin, and metoprolol (Table 1). Midazolam 1′-hydroxylation and omeprazole sulfoxidation activities via CYP3A4/5 in the liver microsomes were significantly higher in rifampicin-treated mice (50 and 100 mg/kg) than in vehicle-treated mice (Fig. 3). Furthermore, *S*-warfarin 7-hydroxylation by CYP2C9 and omeprazole 5-hydroxylation activities by CYP2C19 in liver microsomes were significantly higher in rifampicin-treated mice (100 mg/kg) than in vehicle-treated mice. The protein/mRNA expression levels of CYP3A4, CYP3A5, CYP2C9, and CYP2C19 in the liver were upregulated by rifampicin treatment (Fig. 3). In contrast, significant differences between rifampicin- and vehicle-treated mice were not observed for ethoxyresorufin *O*-deethylation activity by CYP1A2 (Fig. 3) or metoprolol *O*-deethylation activity by CYP2D6 (data not shown) in liver microsomes. Further, the expression of hepatic CYP1A2 protein/mRNA (Fig. 3) and CYP2C8 mRNA (data not shown) was not upregulated.

3.4. Drug metabolism study in pregnant humanized liver mice and fetuses

The availability of female humanized liver mice may allow the assessment of developmental toxicity and teratogenicity of drugs and metabolites produced in the human liver. Thalidomide, a sedative and an antiemetic drug, is metabolized to 5-hydroxy and dihydroxy metabolites, resulting in the formation of an arene oxide [21], whose toxicity may be relevant. Circulating thalidomide and metabolite levels at 2 h after the oral administration of thalidomide (100 mg/kg) were compared between pregnant humanized liver mice and pregnant non-humanized liver mice. The plasma concentrations of 5-hydroxythalidomide and 5′-hydroxythalidomide were higher in the pregnant non-humanized liver mice (Fig. 4B and C). On contrary, the plasma concentrations of thalidomide and 5,6-dihydroxythalidomide were higher in the pregnant humanized liver mice (Fig. 4A and D). Similarly, thalidomide and 5,6-dihydroxythalidomide in the plasma were abundant in the fetuses of pregnant humanized liver mice (Fig. 4A and D). Unidentified dihydroxythalidomide was abundantly detected in the plasma of pregnant humanized liver mice and their fetuses (Fig. 4E). Thus,

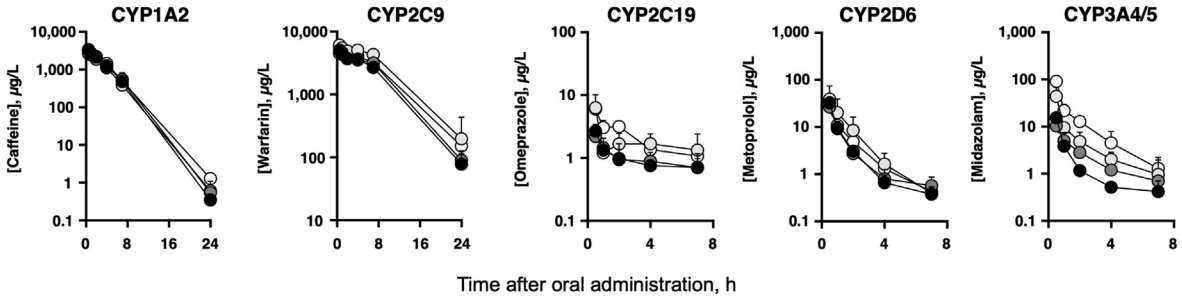

Fig. 2. Plasma concentrations of caffeine, warfarin, omeprazole, metoprolol, and midazolam after repeated oral administration of rifampicin or vehicle to humanized liver mice. Humanized liver mice were administered 5.0 mg/kg caffeine, warfarin, omeprazole, metoprolol and midazolam after repeated oral administration of rifampicin [10 (light gray circles), 50 (dark gray circles), or 100 (closed circles) mg/kg] or vehicle (open circles) for 3 days. Plots and bars are mean ± SD values of three animals.

Table 1
Pharmacokinetic parameters for caffeine, warfarin, omeprazole, metoprolol, and midazolam in humanized liver mice after single simultaneous administrations orally (5.0 mg/kg each).

Drug	Dose of rifampicin	C_{max}	$AUC_{0\text{-}\infty}$	AUC decrease[a]
	mg/kg	μg/mL	μg·h/mL	%
Caffeine	Vehicle	3.4 ± 0.6	13 ± 3	–
	10	3.2 ± 0.7	11 ± 2	15
	50	2.7 ± 0.5	12 ± 5	8
	100	3.4 ± 0.3	13 ± 3	0
Warfarin	Vehicle	5.9 ± 0.3	58 ± 11	–
	10	6.2 ± 1.1	74 ± 13	0
	50	4.7 ± 0.6	53 ± 8	9
	100	5.0 ± 0.6	51 ± 2	12
Omeprazole	Vehicle	0.0061 ± 0.0012	0.022 ± 0.010	–
	10	0.0067 ± 0.0036	0.014 ± 0.003	36
	50	0.0022 ± 0.0006	0.013 ± 0.009*	41
	100	0.0025 ± 0.0008	0.011 ± 0.004*	50
Metoprolol	Vehicle	0.035 ± 0.003	0.038 ± 0.002	–
	10	0.039 ± 0.035	0.053 ± 0.046	0
	50	0.027 ± 0.006	0.029 ± 0.006	24
	100	0.034 ± 0.012	0.032 ± 0.012	16
Midazolam	Vehicle	0.091 ± 0.016	0.098 ± 0.017	–
	10	0.044 ± 0.024*	0.046 ± 0.017**	53
	50	0.010 ± 0.002***	0.020 ± 0.003***	80
	100	0.014 ± 0.003***	0.016 ± 0.002***	84

[a] $AUC_{0\text{-}\infty}$ decrease in rifampicin-treated mice to $AUC_{0\text{-}\infty}$ in vehicle-treated mice. The $AUC_{0\text{-}\infty}$ represent the mean ± SD, N = 3. *p<0.05, **p<0.01, and ***p<0.001 vs. the vehicle-treated mice.

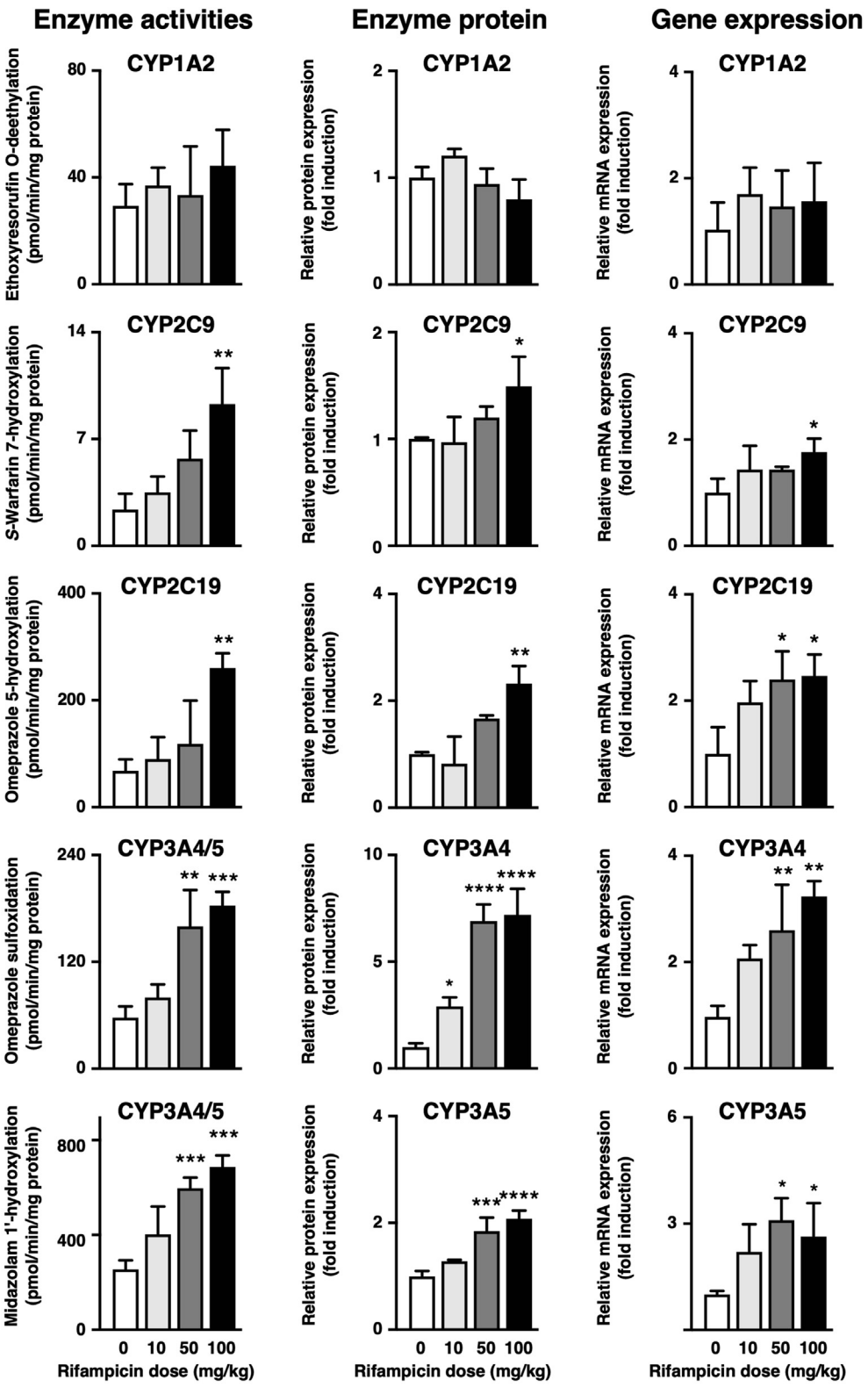

Fig. 3. Microsomal drug oxidation activities, relative P450 protein levels, and relative P450 mRNA levels in the livers of rifampicin or vehicle-treated humanized liver mice. Data represent the mean ± SD values of liver microsomal P450 activities of three animals. The fold induction of human P450 mRNAs and proteins in the rifampicin-treated group when compared with those in the vehicle-treated group. Statistically significant difference when compared with that in the vehicle-treated group; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.005$, and ****, $p < 0.001$.

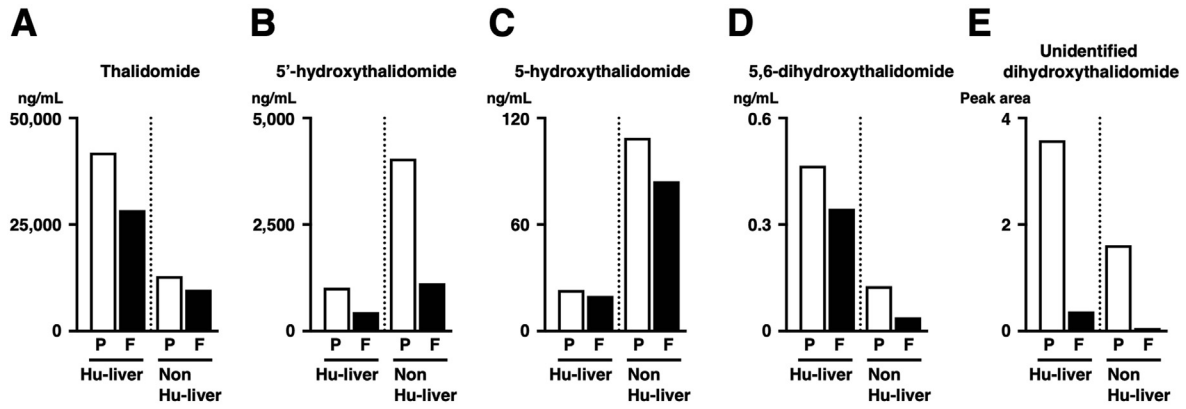

Fig. 4. Plasma concentration of thalidomide and its metabolites in pregnant humanized liver mice and fetuses. The plasma concentrations of thalidomide (A), 5′-hydroxythalidomide (B), 5-dihyrdoxythalidomide (C), and 5,6-dihydroxythalidomide (D), and unidentified dihydroxythalidomide (E) in non-humanized liver and humanized liver mice were measured at 2 h after the oral administration of thalidomide (100 mg/kg). The results are expressed as the mean values of duplicate determinations. P, pregnant; F, fetuses, Hu-liver, pregnant F1-TKm30 mouse with humanized liver; non-Hu-liver, pregnant F1-TKm30 mouse.

fetal circulating drug levels based on humanized maternal drug metabolism can be assessed using pregnant humanized liver mice.

4. Discussion

A novel HSVtk transgenic mouse (NOG-TKm30) was constructed that successfully surmounts issues of both infertility in males and low transgene expression in females. This homozygous mouse model is easily bred, and transgenic mice can be obtained without genotyping. In NOG-TKm30 humanized mice, highly repopulated humanized liver stably maintains human hepatocytes for long periods after transplantation and substantially expresses important human P450 enzymes, including CYP3A4 (most abundant hepatic CYP), CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP2E1.

In *in vivo* human P450 induction by a human PXR agonist rifampicin, decreased AUC values of midazolam and omeprazole in humanized liver mice were found to be consistent with the inductive effect of rifampicin on hepatic CYP3A4 and CYP2C19 in terms of enzyme activity levels and protein and mRNA expression. In a clinical study, the concomitant effects of rifampicin administration on CYP3A4 and CYP2C substrates were reported [22]. The AUC of orally administered midazolam was decreased to 2%—4% of control by treatment with 600 mg/day rifampicin for 5 days [23,24]. The AUC of orally administered omeprazole was decreased in all subjects irrespective of CYP2C19 genotypes by treatment with rifampicin 450 mg/day for 7 days [25]. The AUC of orally administered warfarin was decreased to 43% of control by treatment with rifampicin 600 mg/day for 4 days [26]. In humanized liver mice, the AUC values of orally administered midazolam and omeprazole were decreased to approximately 84% and 50%, respectively, by treatment with rifampicin 10—100 mg/kg for 3 days (Table 1). However, the AUC of warfarin in humanized liver mice was not significantly decreased by rifampicin treatment, although CYP2C9 induction was observed in *S*-warfarin 7-hydroxylation activity levels, protein expression, and mRNA expression (Fig. 3). *S*-warfarin is metabolized primarily by CYP2C9 to 7-hydroxywarfarin, whereas *R*-warfarin is metabolized primarily by CYP1A2 to 6- and 8-hydroxywarfarin and by CYP3A4 to 10-hydroxywarfarin. Therefore, warfarin potentially leads to drug—drug interactions with a wide range of drugs that are metabolized by these P450 enzymes. The unexpected small contribution of CYP2C9 in warfarin metabolism in humanized liver mice may lead to no apparent differences in warfarin AUC between the rifampicin- and vehicle-treated groups under the conditions used in this study (Table 1). Further, no change in warfarin exposure after rifampicin treatment (50 mg/kg) in other humanized liver mice was observed [27]. The effects of rifampicin on CYP2D6 mRNA expression and its activity were minute and negligible in the *in vitro* assay using human hepatocytes [28,29]. Therefore, rifampicin is known to clinically produce no significant induction of CYP2D6 [30]. Similarly, CYP2D6 mRNA expression/activity in humanized livers was not induced by rifampicin (data not shown) and the AUC of metoprolol in humanized liver mice was not significantly altered by rifampicin treatment. These results suggest that the *in vivo* induction of hepatic CYP3A4/5 and CYP2C19 in humanized liver mice mostly reflects that in humans, with the exceptions observed in the pharmacokinetics of warfarin.

Prenatal exposure to chemicals, drugs, and environmental pollutants that pass through the placenta can affect fetal development [31]. Thalidomide, a well-known teratogen, is oxidized to 5-hydroxy and dihydroxy metabolites by human CYP3A enzymes [21,32]. Hydroxylation of 5-hydroxythalidomide was determined to be involved in the formation of a reactive immediate, possibly an arene oxide, that can be trapped to form glutathione (GSH) conjugates [32]. 5,6-Dihydroxythalidomide is further oxidized to a potentially toxic quinone intermediate that can also form GSH adduct of 5,6-dihydroxythalidomide [33], which has DNA damaging property via generation of reactive oxygen species [34]. These metabolites are likely crucial for toxicological activity. The non-humanized liver mice extensively converted thalidomide into a more polar metabolite, 5′-hydroxythalidomide (Fig. 4B). In particular, a higher level of circulating 5,6-dihydroxythalidomide in the fetus of pregnant humanized liver mice than in those of pregnant non-humanized liver mice was observed (Fig. 4D), which is an oxidative metabolite of the human disproportionate metabolite, 5-hydroxythalidomide. Circulating 5,6-dihydroxythalidomide in the fetus of pregnant humanized liver mice may be involved in reactive metabolite-mediated developmental toxicity. In addition, the abundant circulating thalidomide would preferentially convert thalidomide to 5-hydroxythalidomide in humanized liver mice when compared with that in non-humanized liver mice, according to our previous study [35]. However, the reason behind dihydroxythalidomide being much higher in concentration in the pregnant humanized liver mice than in the fetuses remains unclear. The differences in the plasma levels of thalidomide metabolites in the pregnant humanized liver and non-humanized liver mice may be attributed to the differences in thalidomide biotransformation by hepatic CYP3A enzymes and other P450 family enzymes between

humans and mice. 5-Hydroxythalidomide-GSH conjugate (m/z 580 → 451 transition) formed *in vivo* was below the detection limit under the current conditions. These results suggested that the prediction of exposure to human-specific or disproportionate metabolites in fetus was possible for preclinical drug metabolism studies using pregnant humanized liver mice. In this study, teratogenicity in the fetuses of humanized liver mice was not investigated because only single administration for a short period was performed; however, future investigations to optimize the gestational timing of dose initiation, the dose of the test substance, and the exposure period will enable to use new pregnant humanized liver mice in thalidomide teratogenicity studies.

In conclusion, we successfully established a humanized liver mouse model using the novel NOG-TKm30 mouse platform. The application of humanized liver mouse models to developmental toxicity studies has not previously been reported. Therefore, the ability to evaluate prenatal exposure using pregnant humanized liver mice will be a unique advantage of this novel model over the traditional TK model. Further analysis is needed to confirm the usefulness of this model for studying developmental toxicity related to teratogenicity. In addition, the abundant expression, and precise localization of human P450 enzymes within the lobule of NOG-TKm30 humanized liver may contribute to the superiority of this model compared with the traditional TK-NOG model for evaluating any drug interactions caused by PXR-mediated induction of CYP3A/2C enzymes. Overall, the NOG-TKm30 humanized mouse model is expected to become a preferred platform for studying human liver physiology, human drug metabolism and pharmacokinetics, toxicology, and hepatitis virus infection both *in vivo* and *in vitro*.

Author contributions

H.S. designed the experiments. H.K., Y.I., M.O., and Y.K. contributed to establish transgenic mice. N.Y., H.H., T.T., and H.S. performed *in vivo* experiments. S.U., Y.H., and M.Y. performed *in vitro* experiments. H.Y. and H.S. analyzed the *in vitro* data. K.K. performed histological analyses. H.S. and S.U. wrote the manuscript. All authors read and revised the manuscript.

Declarations of competing interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

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