CYP3A4 Induction in the Liver and Intestine of Pregnan X
Receptor/CYP3A-Humanized Mice: Approaches by Mass Spectrometry Imaging and Portal Blood Analysis

Kaorui Kobayashi, Jiro Kuze, Satoshi Abe, Shoko Takehara, Genki Minegishi, Katsuhide Igarashi, Satoshi Kitajima, Jun Kanno, Takushi Yamamoto, Mitsuo Oshimura, and Yasuhiro Kazuki

ABSTRACT

Induction of cytochrome P450 enzyme 3A (CYP3A) in response to pregnane X receptor (PXR) activators shows species-specific differences. To study the induction of human CYP3A in response to human PXR activators, we generated a double-humanized mouse model of PXR and CYP3A. CYP3A-humanized mice generated by using a mouse artificial chromosome (MAC) vector containing the entire genomic human CYP3A locus (hCYP3A-MAC mouse line) were bred with PXR-humanized mice in which the ligand-binding domain of mouse PXR was replaced with that of human PXR, resulting in double-humanized mice (hCYP3A-MAC/hPXR mouse line). Oral administration of the human PXR activator rifampicin increased hepatic expression of CYP3A4 and triazolam metabolism in the intestine of CYP3A/PXR-humanized mice. These results suggest that the hCYP3A-MAC/hPXR mouse line may be a useful model to predict human PXR-dependent induction of metabolism of CYP3A4 substrates in the liver and intestine.

SIGNIFICANCE STATEMENT

We generated a double-humanized mouse line for CYP3A and PXR. Briefly, CYP3A-humanized mice generated by using a mouse artificial chromosome vector containing the entire genomic human CYP3A locus were bred with PXR-humanized mice in which the ligand-binding domain of mouse PXR was replaced with that of human PXR. Expression of CYP3A4 and metabolism of triazolam, a typical CYP3A substrate, in the liver of CYP3A/PXR-humanized mice were enhanced in response to rifampicin, a typical human PXR activator. Enhancement of triazolam metabolism in the intestine of CYP3A/PXR-humanized mice was firstly shown by combination of mass spectrometry imaging of sliced intestine and liquid chromatography with tandem mass spectrometry analysis of metabolite concentration in portal blood after oral dosing of triazolam.

Introduction

Pregnane X receptor (PXR, NR1I2), a member of the nuclear receptor superfamily, regulates the induction process of CYP3A4 (Willson and Kliewer, 2002). PXR is activated by a broad range of chemicals such as steroid hormones, herbs, and antibiotics (Mackowiak and Wang, 2016). Activated PXR translocates into the nucleus and forms a heterodimer with the retinoid X receptor α (RXRα). The PXR-RXRα heterodimer binds to DNA response elements and activates the transcription of target genes including CYP3A4 (Goodwin et al., 1999; Toriyabe et al., 2009).

Interestingly, there are significant species differences in response to PXR activators between humans and rodents (Blumberg et al., 1998; Kliewer et al., 1998; Lehmann et al., 1998; Jones et al., 2000; Moore et al., 2000; Quattrochi and Guzelian, 2001). The antibiotic rifampicin (rifampin) (rif) activates human PXR but is a weak activator of rodent PXR. In contrast, pregnenolone 16α-carbonitrile (PCN) potently activates rodent PXR but is a weak activator of human PXR. Because such differences lead to a problem for studies using rodents to predict DDIs in humans, some humanized mouse lines in which there are responses to human PXR activators have been developed (Xie et al., 2000; Cheung et al., 2006; Ma et al., 2008; Igarashi et al., 2012).

Hasegawa et al. (2011) generated a multiple humanized mouse line that combines humanization of PXR and constitutive androstane receptor (CAR) and replacement of the mouse Cyp3a cluster with a human genomic region carrying CYP3A4 and CYP3A7 (huPXR/huCAR/huCYP3A4/3A7). The huPXR/huCAR/huCYP3A4/3A7 mice showed a CYP3A4 induction response to human PXR activators. In the huPXR/huCAR/huCYP3A4/3A7 mice, plasma concentrations of triazolam (TRZ), a typical CYP3A4 substrate, were decreased by oral administration of RIF. However, the induction of intestinal CYP3A4 mRNA expression was negligible when RIF was orally administered. In addition, the area under the plasma concentration–time curves (AUCs) of TRZ metabolites were decreased by RIF treatment. Therefore, there is no direct evidence for enhancement of CYP3A4-mediated metabolism in the intestine of huPXR/huCAR/huCYP3A4/3A7 mice treated orally with human PXR activators.

In this study, we generated a double-humanized mouse line for CYP3A4 and PXR and examined CYP3A4 induction in the liver and intestine of the double-humanized mice in response to RIF. To demonstrate the functional induction of intestinal CYP3A4 by RIF, we determined the metabolite concentrations in portal blood after oral administration of TRZ and visualized the metabolite formation in the sliced small intestine by mass spectrometry imaging.

### Materials and Methods

#### Materials

TRZ and RIF were purchased from Wako Pure Chemicals (Osaka, Japan). Pregnenolone 16α-carbonitrile (PCN) was purchased from Toronto Research Chemicals (North York, ON, Canada). Both 1'-hydroxy TRZ and 4-hydroxy TRZ were supplied by Nihon Upjohn (Tokyo, Japan). All other reagents were purchased from commercial sources.

#### Animals

The generation and characterization of CYP3A-humanized mice and PXR-humanized mice were described elsewhere (Igarashi et al., 2012; Kazuki et al., 2019). Briefly, a trans-chromosomic mouse line with a mouse artificial chromosome (MAC) vector containing the entire human CYP3A gene cluster (CYP3A4, CYP3A5, CYP3A7, and CYP3A43) and their regulatory regions (promoters and enhancers) was crossed with Cyp3a-knockout (Cyp3a-KO) mice to produce CYP3A-humanized mice with a Cyp3a-knockout background (hCYP3A-MAC mouse line).

The double-humanized mice of CYP3A and PXR (hCYP3A-MAC/hPXR mouse line) and PXR-humanized mice with a Cyp3a-knockout background (Cyp3a-KO/hPXR mouse line) were generated by mating the hCYP3A-MAC mouse line with a PXR-humanized mouse line in which the ligand-binding domain of mouse PXR was replaced with that of human PXR. Each mouse line was backcrossed to the C57BL/6 or ICR strain at least six generations.

The primer sequences for the genomic polymerase chain reaction (PCR) analyses are described in Supplemental Table 1. Primer sets for the genotyping of hCYP3A-MAC, Cyp3a KO (double KO of Cyp3a7-59 and Cyp3a13), and hPXR knock-in are described in Supplemental Table 2. All experiments were done using male mice between 10 and 11 weeks of age.

Animals were kept in a temperature-controlled environment with a 12-hour light/dark cycle. The light-cycle hours were between 7:00 AM and 7:00 PM. They received a standard diet (CE-2; CLEA, Tokyo, Japan). The present study was conducted in accordance with the guidelines for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research of Taiho Pharmaceutical Co., Tottori University and Chiba University.

*Analysis of PXR-Specific Induction in the Liver.* We treated hCYP3A-MAC mice and hCYP3A-MAC/hPXR mice intraperitoneally with RIF (10 mg/kg, an hPXR activator), PCN (100 mg/kg, an mPXR activator), or a vehicle (corn oil) for 4 consecutive days. Sixteen hours after the final treatment, liver pieces were collected.

*Analysis of CYP3A4 Induction by RIF in the Liver and Intestine.* We treated hCYP3A-MAC/MAC mice orally with RIF (30 mg/kg) or a vehicle (0.5% carboxymethyl cellulose) for 4 consecutive days. Sixteen hours after the final treatment, the liver and small intestine were collected.

#### mRNA Analysis

Total RNA was isolated from tissue pieces of livers and small intestines (1-cm segments collected at 10 cm from the pyloric region) by using the RNeasy Mini Kit (Qiagen, Germany). Reverse transcription was performed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA). The expression levels of CYP3A4 mRNA were determined by TaqMan reverse transcription PCR using TaqMan Gene Expression Assays (Hs00604506_m1; Thermo Fisher Scientific). The mRNA expression levels were normalized to endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression detected by 20− Pre-Developed TaqMan Assay Reagent mouse GAPDH (Thermo Fisher Scientific).

#### TRZ Hydroxylation Activity In Vitro

Microsomal fractions of the liver and intestine were prepared as described elsewhere (Kazuki et al., 2013). Incubations were performed in a total volume of 250 μl containing 0.1 mM EDTA, 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system (0.5 mM NADP+, 2 mM glucose-6-phosphate, 1 IU/ml glucose-6-phosphate dehydrogenase, 4 mM MgCl2), and a substrate (200 μM TRZ).

**ABBREVIATIONS:** AUC, area under the plasma concentration–time curve; CAR, constitutive androstane receptor; P450, cytochrome P450 enzyme; DDI, drug–drug interaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; h, human; KO, knockout; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MAC, mouse artificial chromosome; PCN, pregnenolone 16α-carbonitrile; PCR, polymerase chain reaction; PXR, pregnane X receptor; RIF, rifampicin; TRZ, triazolam.
The microsomal protein concentrations and incubation times used were selected from the linear range of formation of TRZ metabolites.

The mixtures were preincubated for 1 minute at 37°C, and reactions were initiated by the addition of 25 μl of NADPH-generation system. Incubation was stopped by the addition of 100 μl of acetonitrile followed by the addition of 50 μl of 1 μg/ml oxazepam in methanol as an internal control.

Determination of 1'-hydroxy TRZ and 4-hydroxy TRZ was performed using a Hitachi L-7000 model HPLC system (Tokyo, Japan) consisting of an L-7400 UV detector and a CAPCELL PAK C18 UG120 column (4.6 × 250, 5 mm; Shiseido, Tokyo, Japan). The mobile phase consisted of 10 mM potassium phosphate buffer (pH 7.4), acetonitrile, and methanol (60/30/10, v/v/v), with a flow rate of 1.0 ml/min. The eluent was monitored at a wavelength of 220 nm.

**Immunoblot Analysis.** Microsomal fractions (1 μg) of small intestines were separated by 10% SDS-PAGE, transferred to a polyvinylidene fluoride membrane, and probed using a mouse monoclonal anti-CYP3A4 antibody (1:1000 dilution, sc-53850; Santa Cruz Biotechnology, Dallas, TX) and a rabbit monoclonal anti-GAPDH antibody (1:10,000 dilution, ab181602; Abcam, Cambridge, United Kingdom). The secondary antibodies used were horseradish peroxidase-conjugated rabbit anti-mouse IgG (1:5000 dilution, ab6728; Abcam) and goat anti-rabbit IgG (1:2000 dilution, ab6721; Abcam) for anti-CYP3A4 and anti-GAPDH, respectively. The primary and secondary antibodies were diluted with Can Get Signal Solution (TOYOBO, Osaka, Japan). CYP3A4 and GAPDH proteins were detected by an enhanced chemiluminescence method (GE Healthcare, Buckinghamshire, United Kingdom) and LAS-4000 (Fujifilm, Tokyo, Japan). Intensities of bands were determined using ImageQuant TL (GE Healthcare).

**Pharmacokinetic Analysis.** CYP3A-MAC/hPXR and hCYP3A-MAC mice were orally treated with TRZ (1 mg/kg) after oral administration of RIF (30 mg/kg per day) or a vehicle (0.5% carboxymethyl cellulose) for 3 consecutive days. Blood samples were collected from suborbital veins at 15, 30, 60, 120, 240, and 360 minutes after TRZ administration. Plasma was separated by centrifugation and stored at −80°C. The concentrations of TRZ, 1'-hydroxy TRZ and 4-hydroxy TRZ were determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described elsewhere (Kazuki et al., 2019).

The area under the plasma concentration−time curve (AUC) from 0 to the last data point (AUC0–t) was calculated using the linear trapezoidal method. AUC0–t was calculated by combining AUC0–t and AUCextra. AUCextra represents an extrapolated value obtained by Ct/kel, where Ct is the plasma concentration at the last data point and kel represents the terminal elimination rate constant determined by log-linear regression analysis of the measured plasma concentrations of the terminal elimination phase. Maximum plasma concentration (Cmax) and time to Cmax (tmax) were obtained directly from the observed values. Apparent half-life was obtained as ln2/kel.

**Mass Spectrometry Imaging.** The hCYP3A-MAC/hPXR mice were orally treated with RIF (10 mg/kg) or a vehicle (0.5% carboxymethyl cellulose) for 3 consecutive days. Blood samples were collected from suborbital veins at 15, 30, 60, 120, 240, and 360 minutes after TRZ administration. Plasma was separated by centrifugation and stored at −80°C. The concentrations of TRZ, 1'-hydroxy TRZ and 4-hydroxy TRZ were determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described elsewhere (Kazuki et al., 2019).

The area under the plasma concentration−time curve (AUC) from 0 to the last data point (AUC0–t) was calculated using the linear trapezoidal method. AUC0–t was calculated by combining AUC0–t and AUCextra. AUCextra represents an extrapolated value obtained by Ct/kel, where Ct is the plasma concentration at the last data point and kel represents the terminal elimination rate constant determined by log-linear regression analysis of the measured plasma concentrations of the terminal elimination phase. Maximum plasma concentration (Cmax) and time to Cmax (tmax) were obtained directly from the observed values. Apparent half-life was obtained as ln2/kel.
cellulose) for 3 consecutive days. On day 4, the mice were orally administered TRZ (10 mg/kg). At 10 minutes after TRZ administration, the liver and small intestine were quickly removed and frozen in liquid nitrogen.

Serial sections were sliced to a thickness of 20 μm using a cryostat (CM 1950; Leica Microsystems, Wetzlar, Germany). Sections were thaw-mounted onto an indium-tin-oxide-coated glass slide (Sigma-Aldrich, St. Louis, MO) and dried at room temperature. Before the mass spectrometry imaging, the samples were kept at −80°C. We used α-cyano-4-hydroxycinnamic acid as the matrix-assisted laser desorption ionization matrix, deposited by iMLayer (Shimadzu Corp., Kyoto, Japan). Mass imaging data were obtained using iMScope TRIO (Shimadzu). The mass range was set to m/z 300–400 with a spatial resolution of 20 μm.

Analysis of CYP3A-Dependent Induction in the Intestine. CYP3A-MAC/hPXR and Cyp3a-KO/hPXR mice were orally treated with TRZ (1 mg/kg) after oral administration of RIF (30 mg/kg per day) or a vehicle (0.5% carboxymethyl cellulose) for 3 consecutive days. Blood samples were collected from portal veins at 10 minutes after TRZ administration. Plasma was separated by centrifugation and stored at −80°C. The concentrations of TRZ, 1'-hydroxy TRZ, and 4-hydroxy TRZ were determined by LC-MS/MS as described elsewhere (Minegishi et al., 2019).

Statistical Analysis. Data are expressed as mean with S.D. Data were analyzed by using Statcel 4 (OMS, Saitama, Japan) and R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Comparisons of two groups were performed using a two-tailed t test. Comparisons of multiple groups were performed by multivariate ANOVA followed by one-way ANOVA with a post hoc test. P < 0.05 was considered statistically significant.

Results

Human PXR-Dependent Induction of Hepatic CYP3A in hCYP3A-MAC/hPXR Mice. We treated hCYP3A-MAC/hPXR and hCYP3A-MAC mice intraperitoneally with the human PXR ligand RIF and rodent PXR ligand PCN. After PCN treatment, CYP3A4 mRNA was significantly increased in the liver of hCYP3A-MAC mice but not in hCYP3A-MAC/hPXR mice (Fig. 1A). By contrast, RIF treatment did not increase CYP3A4 mRNA in hCYP3A-MAC mice, whereas an increase of CYP3A4 mRNA was found in hCYP3A-MAC/hPXR mice, though the increase was not statistically significant.

Effect of RIF on CYP3A4 Expression in the Liver and Intestine of hCYP3A-MAC/hPXR Mice. Next, we examined the induction of CYP3A4 in the liver and intestine of hCYP3A-MAC/hPXR mice after oral treatment with RIF. A significant increase of CYP3A4 mRNA levels induced by RIF
was observed in the liver of hCYP3A-MAC/hPXR mice (Fig. 2A). Hepatic CYP3A activity was also significantly induced by RIF in hCYP3A-MAC/hPXR mice (Fig. 2B). In the intestine, a significant increase of CYP3A activity was observed, but the magnitude of increase was less than that in the liver. No increase of CYP3A4 mRNA with RIF treatment was found in the intestine (Fig. 2A), although CYP3A4 protein was marginally increased by RIF in the intestine of CYP3A-MAC/hPXR mice (Supplemental Fig. 1).

Effect of RIF on Pharmacokinetics of TRZ in hCYP3A-MAC/hPXR Mice. To assess whether RIF can affect the pharmacokinetics of TRZ and its metabolites in hCYP3A-MAC/hPXR (A and B) and hCYP3A-MAC (C and D) in hCYP3A-MAC/hPXR (A and C) and hCYP3A-MAC (B and D) mice. Mice (N = 3/group for hCYP3A-MAC/hPXR mice and N = 4/group for hCYP3A-MAC mice) were given an oral dose of the vehicle or RIF (30 mg/kg) for 3 days followed by a 1 mg/kg oral dose of TRZ. Values for the vehicle are indicated as open circles and those for RIF are indicated as closed circles. Each point represents the mean with S.D.

<table>
<thead>
<tr>
<th>Group</th>
<th>$t_{\text{max}}$</th>
<th>$C_{\text{max}}$</th>
<th>$\text{HalfLife}$</th>
<th>$\text{AUC}_{0-\infty}$</th>
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<tr>
<td></td>
<td>h</td>
<td>nM</td>
<td>h</td>
<td>nM h</td>
</tr>
<tr>
<td>hCYP3A-MAC/hPXR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.42 ± 0.14</td>
<td>515 ± 190</td>
<td>1.10 ± 0.14</td>
<td>1050 ± 242</td>
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<tr>
<td>RIF</td>
<td>0.50 ± 0.43</td>
<td>235 ± 55</td>
<td>0.39 ± 0.05*</td>
<td>250 ± 71*</td>
</tr>
<tr>
<td>hCYP3A-MAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.38 ± 0.14</td>
<td>229 ± 81</td>
<td>1.08 ± 0.13</td>
<td>448 ± 157</td>
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<tr>
<td>RIF</td>
<td>0.44 ± 0.13</td>
<td>180 ± 22</td>
<td>1.21 ± 0.44</td>
<td>370 ± 82</td>
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</table>

$\text{AUC}_{0-\infty}$, area under the plasma concentration–time curve from time 0 to infinity; hCYP3A-MAC, human cytochrome P450 3A enzyme mouse artificial chromosome; PXR, pregnane X receptor; RIF, rifampicin.

*P < 0.01 compared with the vehicle control (Student’s t test).
TABLE 2
Pharmacokinetic parameters of 1'-hydroxy TRZ in hCYP3A-MAC/hPXR and hCYP3A-MAC mice

<table>
<thead>
<tr>
<th>Group</th>
<th>t_{max} (h)</th>
<th>C_{max} (μM)</th>
<th>Half-life (h)</th>
<th>AUC_{0-∞} (μg h/mL)</th>
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<td>hCYP3A-MAC/hPXR</td>
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<tr>
<td>Vehicle</td>
<td>1.33 ± 0.58</td>
<td>978 ± 200</td>
<td>3.25 ± 0.50</td>
<td>4893 ± 1062</td>
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<td>RIF</td>
<td>0.58 ± 0.38</td>
<td>1250 ± 414</td>
<td>0.64 ± 0.14</td>
<td>1737 ± 270</td>
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<tr>
<td>hCYP3A-MAC</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.63 ± 0.25</td>
<td>747 ± 165</td>
<td>2.19 ± 0.43</td>
<td>2618 ± 738</td>
</tr>
<tr>
<td>RIF</td>
<td>0.88 ± 0.25</td>
<td>712 ± 47</td>
<td>2.07 ± 0.38</td>
<td>2313 ± 562</td>
</tr>
</tbody>
</table>

AUC_{0-∞} = area under the concentration-time curve from time 0 to infinity; hCYP3A-MAC, human cytochrome P450 3A enzyme mouse artificial chromosome; hPXR, human pregnancy X receptor; RIF, rifampicin. *P < 0.01 compared with the vehicle control (Student’s t test for half-life and Welch’s t test for AUC). **P < 0.05 compared with the vehicle control (Student’s t test for half-life and Welch’s t test for AUC).

Discussion

We previously reported an hCYP3A-MAC mouse model with a mouse PXR background (Kazuki et al., 2019; Minegishi et al., 2019). In contrast to the hCYP3A-MAC mouse model, the present hCYP3A-MAC/hPXR mouse model was improved as a mouse line with a human PXR-like response. Therefore, the hCYP3A-MAC/hPXR mouse model would be a useful tool for the study of CYP3A4 induction by human PXR activators.

The present study clearly showed that plasma concentrations of TRZ were decreased by RIF only in the hCYP3A-MAC/hPXR mice (Fig. 3, A and B), suggesting that oral clearance of TRZ was enhanced in response to hPXR activation by RIF. In hCYP3A-MAC/hPXR mice, hepatic CYP3A4 mRNA levels and TRZ metabolism in the liver microsomes were increased by oral treatment with RIF (Fig. 2), which was consistent with previous results obtained from humanized mouse models for PXR accomplished using different strategies (Ma et al., 2008; Hasegawa et al., 2011). Plasma elimination of TRZ after oral dosing was also enhanced by RIF in hCYP3A-MAC/hPXR mice (Table 1). These findings suggested induction of CYP3A4-mediated metabolism of TRZ in the liver of hCYP3A-MAC/hPXR mice. In addition, TRZ metabolism and CYP3A4 protein in the intestinal microsomes were also increased by oral treatment with RIF (Fig. 2B; Supplemental Fig. 1). The results of mass spectrometry imaging analysis demonstrated that hydroxy TRZ in the sliced intestine of hCYP3A-MAC/hPXR mice was increased by treatment with RIF (Fig. 4A).

Moreover, the plasma concentrations of TRZ and its metabolites in portal blood were decreased and increased, respectively, by RIF in hCYP3A-MAC/hPXR mice (Fig. 4E, F). Although metabolites found in blood of the portal vein were not always derived from the mesenteric vein, the concentrations of metabolites at 10 minutes after oral administration would reflect the intestinal metabolism. Because these experiments were performed for hCYP3A-MAC/hPXR mice, we cannot exclude the possibility that the induction of intestinal metabolism is hPXR-independent. However, plasma concentrations of 1'-hydroxy TRZ at the early phase after TRZ dosing were increased by RIF treatment in hCYP3A-MAC/hPXR mice but not in hCYP3A-MAC mice (Fig. 3, C and D). In addition, RIF could not induce CYP3A in Pxr-null mice (Ma et al., 2007). These findings suggested that TRZ metabolism in the intestine of hCYP3A-MAC/hPXR mice was enhanced in response to hPXR activation by RIF. Therefore, our study has shown that the overall metabolism of TRZ in the liver and intestine was functionally enhanced by oral administration of RIF in hCYP3A-MAC/hPXR mice.

In agreement with previously reported findings in huPXR/ huCAR/huCYP3A4/3A7 mice (Hasegawa et al., 2011), the...
AUC₀₋∞ value and half-life of 1'-hydroxy TRZ were also decreased by RIF treatment in hCYP3A-MAC/hPXR mice (Table 2). Notably, our results indicated a trend for plasma concentrations of 1'-hydroxy TRZ at 15 and 30 minute after TRZ dosing to be increased by RIF treatment in hCYP3A-MAC/hPXR mice (Fig. 3C). These results take together with results showing enhanced formation of 1'-hydroxy TRZ in the intestine of hCYP3A-MAC/hPXR mice treated with RIF (Fig. 4) suggested that formation of 1'-hydroxy TRZ in intestinal metabolism was enhanced and subsequently further metabolism of 1'-hydroxy TRZ was enhanced in the liver of hCYP3A-MAC/hPXR mice treated with RIF.

Previously, Hasegawa et al. (2011) reported that huPXR/huCAR/huCYP3A4/3A7 mice showed a CYP3A4 induction response to human PXR activators, but the induction of intestinal CYP3A4 mRNA expression was not significant when RIF was orally administered. Ma et al. (2007, 2008) also reported a PXR-humanized mouse model and a double transgenic mouse model expressing human PXR and CYP3A4, but they showed no data for the induction of CYP3A4 mRNA in the intestine. Consistent with these findings, our study using hCYP3A-MAC/hPXR mice also has shown that the induction of intestinal CYP3A4 mRNA expression was not significant when RIF was orally administered (Fig. 2A). On the other hand, expression of intestinal CYP3A protein was increased in hCYP3A-MAC/hPXR mice (Supplemental Fig. 1) as well as previous mouse models (Ma et al., 2007, 2008; Hasegawa et al., 2011) when RIF was orally administered. Therefore, it may be difficult to detect the induction of CYP3A4 at the mRNA level in the intestine of mouse models treated with RIF.

Metabolic activities of TRZ in intestinal microsomes were increased by RIF in hCYP3A-MAC/hPXR mice (Fig. 2B), but we could not exclude the possibility that TRZ was metabolized by other genes that were induced by RIF in hCYP3A-MAC/hPXR

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Fig. 4. Induction of TRZ metabolism in the small intestine of hCYP3A-MAC/hPXR mice. (A) Imaging mass spectrometry of TRZ and its hydroxy metabolites in the small intestine. We gave hCYP3A-MAC/hPXR mice (N = 1/group) an oral dose of the vehicle or RIF (30 mg/kg) for 3 days followed by a 10-mg/kg oral dose of TRZ. At 10 minutes after TRZ dosing, the small intestine was removed and analyzed as described in the Materials and Methods section. (B and C) Plasma concentrations of TRZ (B) and its metabolites 1'-hydroxy TRZ and 4-hydroxy TRZ (C) in portal blood of hCYP3A-MAC/hPXR mice. Mice (N = 4/group) were given an oral dose of the vehicle or RIF (30 mg/kg) for 3 days followed by a 1 mg/kg oral dose of TRZ. At 10 minutes after TRZ dosing, blood samples were collected from portal veins. Data are expressed as mean with S.D. Statistical analysis was performed with Student’s t test. *P < 0.05 compared with the vehicle control. ††P < 0.01 compared with 1'-OH of hCYP3A-MAC/hPXR mice treated with a vehicle. ‡‡P < 0.01 compared with 4-OH of hCYP3A-MAC/hPXR mice treated with a vehicle.
enzymes could be increased by KO of expression levels of some genes including mouse Cyp2c in Cyp3a-KO/hPXR mice. It has been reported that enzymes other than CYP3A contribute to TRZ metabolism and 4-hydroxy TRZ in the portal blood in Cyp3a-KO/hPXR only the with KO of other genes are needed. KO background, further modifications such as a combination were involved in TRZ metabolism in Cyp3a-KO/hPXR mice. Therefore, we investigated the induction of intestinal metabolism of CYP3A substrates via human PXR containing mice and hPXR. The hCYP3A-MAC/hPXR mice used in our study have not the statistical analysis. This research was partly performed at the Ohira at Tottori University for critical discussion and Dr. Akihiro Dr. Masaharu Hiratsuka, Dr. Tetsuya Ohbayashi, and Dr. Takahito Dr. Kan Chiba at Chiba University and Dr. Hiroyuki Kugoh, Eri Kaneda, Akiko Ashiba, and Dr. Kazuomi Nakamura at Tottori Yukako Sumida, Hiromichi Kohno, Masami Morimura, Kei Yoshida, Authorship Contributions

**Participated in research design:** Kobayashi, Kuze, Kazuki.

**Conducted experiments:** Kuze, Takehara, Minegishi, Igarashi, Kitajima, Kanno, Yamamoto.

**Performed data analysis:** Kobayashi, Kuze, Abe, Yamamoto.

**References**


Wrote or contributed to the writing of the manuscript: Kobayashi, Abe, Yamamoto, Oshimura, Kazuki.

In conclusion, our study demonstrated that oral treatment with Rif to hCYP3A-MAC/hPXR mice enhanced TRZ metabolism in the intestine as well as the liver in response to hPXR activation. The hCYP3A-MAC/hPXR mouse line will be a useful in vivo model for predicting the induction of hepatic and intestinal metabolism of CYP3A substrates via human PXR activation.

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**Authorship Contributions**

**Participated in research design:** Kobayashi, Kuze, Kazuki.

**Conducted experiments:** Kuze, Takehara, Minegishi, Igarashi, Kitajima, Kanno, Yamamoto.

**Performed data analysis:** Kobayashi, Kuze, Abe, Yamamoto.


**Address correspondence to:** Dr. Kaoru Kobayashi, Laboratory of DDS Design and Drug Disposition, Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan. E-mail: kaoruk@faculty.chiba-u.jp; or Dr. Yasuhiro Kazuki, Department of Biomedical Science, Institute of Regenerative Medicine and Biofunction, Graduate School of Medical Science, Tottori University, 86 Nishi-cho, Yonago, Tottori 683-8503, Japan. E-mail: kazuki@tottori-u.ac.jp