**Changes in Bile Acid Concentrations after Administration of Ketoconazole or Rifampicin to Chimeric Mice with Humanized Liver**

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Drug-induced liver injury (DILI) is a common side effect of several medications and is considered a major factor responsible for the discontinuation of drugs during their development. Cholestasis is a DILI that results from impairment of bile acid transporters, such as the bile salt export pump (BSEP), leading to accumulation of bile acids. Both in vitro and in vivo studies are required to predict the risk of drug-induced cholestasis. In the present study, we used chimeric mice with humanized liver as a model to study drug-induced cholestasis. Administration of a single dose of ketoconazole or rifampicin, known to potentially cause cholestasis by inhibiting BSEP, did not result in elevated levels of alkaline phosphatase (ALP), which are known hepatic biomarkers. The concentration of taurodeoxycholic acid increased in the liver after ketoconazole administration, whereas rifampicin resulted in increased tauromuricholic acid and taurocholic acid (TCA) levels in the liver and plasma. Furthermore, rifampicin resulted in an increase in the uniform distribution of a compound with m/z 514.3, presumed as TCA through imaging mass spectrometry. The mRNA levels of bile acid-related genes were also altered after treatment with ketoconazole or rifampicin. We believe these observations to be a part of a feedback mechanism to decrease bile acid concentrations. The changes in bile acid concentrations results may reflect the initial responses of the human body to cholestasis. Furthermore, these findings may contribute to the screening of drug candidates, thereby avoiding drug-induced cholestasis during clinical trials and drug development.

**Key words** bile acid; chimeric mouse; humanized liver; bile salt export pump; cholestasis

**INTRODUCTION**

Drug-induced liver injury (DILI) poses a major challenge to the drug development industry, often leading to discontinuation of drug candidates and withdrawal of marketed drugs. DILI is classified as a hepatocellular, cholestatic, or a mixed-pattern injury. Approximately, half of 784 cases of DILI reviewed by the Swedish adverse drug reactions advisory committee between 1970 and 2004 had either cholestatic or mixed cholestatic injury.1 A Japanese study identified cholestatic and mixed-patterned injury to constitute 36% of all hepatic adverse drug reactions in 307 DILI cases analyzed from 2010 to 2018.2

Drug-induced cholestasis results from the inhibitory effects of drugs on bile acid transporters, which disrupt the homeostasis of the enterohepatic circulation of bile acids. For example, cyclosporine A and troglitazone inhibit the bile salt export pump (BSEP)-mediated biliary excretion of bile acids, such as amino acid-conjugated bile acids, resulting in liver toxicity due to their accumulation.3–6 However, detailed mechanisms of toxicity leading to disturbance of bile acid homeostasis are not yet completely understood.

Optimization of drug candidates during clinical trials is influenced by between-species differences in inhibition of bile acid transporters. For instance, differences in inhibitory effects of troglitazone on BSEP were observed between humans and rodents.7 To reliably predict cholestatic injury, several in vitro models have been developed. Sandwich-cultured hepatocyte is a commonly used tool, owing to its ability to develop functional bile duct network for several days, which is highly efficient in studying the hepatobiliary transport.7,8 Accurate in vivo prediction of cholestasis in humans needs to be validated by similar studies using animal models. Currently, there is a dearth of literature on human models for the in vivo study of cholestasis. In the present study, we used chimeric mice with humanized liver in which mouse liver is largely replaced with human hepatocytes. These are generated by transplanting human hepatocytes into immunodeficient mice having liver dysfunction. Several host mice are widely used to produce chimeric mice, such as urokinase-type plasminogen activator (uPA)/severe combined immunodeficient (SCID) mouse model, Fah−/−, Rag2−/−, Il2rγ−/− (FRG) model, and NOD/Shi-scid IL2 receptor γ null mice with the herpes simplex virus 1 thymidine kinase (TK-NOG) model.9,10 Moreover, various human drug-metabolizing enzymes and transporters, such as CYP, uridine 5′-diphosphate (UDP)-glucuronosyltransferase, BSEP, and multidrug resistance-associated protein 2 (MRP2) are expressed in the liver.11 Therefore, chimeric mice have proved to be a useful animal model to study drug metabolism and pharmacokinetics.12,13 Furthermore, these could also be used to predict the risk of DILI, including cholestasis during drug development.

Foster et al.14 reported that the expression of BSEP and MRP2 which excretes glucuronide and sulfate conjugates of
bile acids was attenuated in human hepatocytes but not in the residual mouse region after repeated administration of troglitazone for 7d to chimeric mice with humanized liver. Additionally, Xia et al. reported elevation of alanine aminotransferase (ALT) levels as a clinical indicator of hepatotoxicity and total bile acid concentration in chimeric mice after repeated administration of bosentan for 28d. This effect was not observed in control mice. These findings suggested that species differences exist in troglitazone- and bosentan-induced cholestasis between mice and humans. However, the validity of the chimeric mice as a model for cholestasis is still insufficient and requires further investigation through use of increased number of test compounds. Moreover, we believe a single administration rather than long-term repeated administration to chimeric mice should enhance its utility in drug development. A study reported elevated serum alkaline phosphatase (ALP) levels in patients receiving ketoconazole therapy (clinical dose; 200mg) as an indicator of cholestasis toxicity. Another study reported that rifampicin (clinical dose; 900mg) significantly increases human serum bile acid concentrations. These compounds are also known to exert inhibitory effects on BSEP and are associated with related hepatotoxicity in humans. In the present study, we used ketoconazole and rifampicin as model compounds. Chimeric mice with humanized liver were treated with a single administration of these drugs to study the initial responses of drug-induced cholestasis and changes in concentrations of various bile acids in plasma and the liver using LC-MS/MS. We have previously assessed amiodarone-induced phospholipidosis by analyzing the localization of phosphatidylcholine in liver sections using imaging mass spectrometry (IMS) after administration of amiodarone to chimeric mice. We have used the same approach in the current study to evaluate perturbations in bile acid concentrations in the liver sections of chimeric mice. The findings of the present study could pave the way for the identification of novel biomarkers, in addition to ALP, to assess the diseased state and predict the possibility of drug-induced cholestasis during drug development.

**MATERIALS AND METHODS**

**Materials** Following chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.): 9-aminoacridine (9-AA), carbamazepine, sodium glycochenodeoxycholate (GCDCa), and tamoxifen citrate. Cholic acid (CA) was bought from Nacalai Tesque, Inc. (Kyoto, Japan). Chenodeoxycholic acid (CDCA), ketonezole, lithocholic acid (LCA), rifampicin, sodium glycocholate hydrate (GCA), tauroursodeoxycholic acid dihydrate (TUdCA), and ursodeoxycholic acid (UDCA) were procured from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sodium deoxycholate (DCA) and sodium taurocholate (TCA) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Tauro-ß-muricholic acid sodium salt (TMCA) was obtained from Cayman Chemical (MI, U.S.A.). Taurochenodeoxycholic acid sodium salt (TCDCA) and taurodeoxycholic acid sodium salt (TDCa) were purchased from Merck KGaA (Darmstadt, Germany). Nor-deoxycholic acid (Nor-DCA) was procured from Toronto Research Chemicals (ON, Canada). Other chemicals were of analytical or high grade.

**Animals** We used 12–13 week-old, male chimeric mice (FXB-mouse®, PhoenixBio Co., Ltd., Hiroshima, Japan) developed by transplantation of commercially available human hepatocytes into urokinase-type plasminogen activator cDNA-transgenic/severe combined immunodeficient (cDNA-uPAwlds/+; SCID) mice.

Ketoconazole or rifampicin were suspended in 0.5% methylcellulose (10mL/kg; FUJIFILM Wako Pure Chemical Corporation) and orally administered to chimeric mice at 200mg/kg. Methylcellulose (0.5%) alone was administered to the control group (each group consisted of three mice). Replacement index (RI) of human hepatocytes in the ketoconazole and the control groups was 89.7 ± 12% (mean ± standard deviation (S.D.), n = 6). RI in the rifampicin and the control groups was 74.4 ± 5.0% (mean ± S.D., n = 6). The values indicated the occupancy ratio of human hepatocytes in the mice liver and were estimated by calculating the human albumin concentrations. At 6h after administration, blood and liver samples were collected. The animal study was approved by the Animal Care and Use Committee of the PhoenixBio Co., Ltd. and Hiroshima University.

**Measurement of Biochemical Parameters** Plasma ALT, aspartate aminotransferase (AST), ALP, and total bilirubin (T-Bil) were measured using the FUJI DRI-CHEM analyzer (FUJIFILM; Tokyo, Japan).

**Measurement of Ketoconazole Concentration in the Plasma and Liver by LC-MS/MS** The plasma and liver homogenates were mixed with methanol including carbamazepine as an internal standard, and centrifuged. Supernatants obtained were diluted with 0.1% formic acid and subjected to LC-MS/MS. The Nexera HPLC system (Shimadzu; Kyoto, Japan) equipped with an Inertil ODS-3 column (5µm, 2.1 × 50mm; GL Sciences Inc., Tokyo, Japan) with column oven temperature of 40°C was used. The mobile phases were 0.1% formic acid (A) and methanol (B). The gradient conditions (A:B) were: 65 : 35 (0 min), 5 : 95 (5–8 min), and 65 : 35 (8.01–11 min) at a flow rate of 200µL/min. The MS/MS measurement was performed using the LCMS-8050 system (Shimadzu). The precursor and product ions (m/z) of ketoconazole and carbamazepine under positive-ion mode were m/z = 531.25 to 82.20 and m/z = 237.10 to 194.10, respectively. While calculating the hepatic concentration, 1g of liver was assumed as 1mL.

**Measurement of Rifampicin Concentration in the Plasma and Liver by LC-MS/MS** The plasma and liver homogenates were mixed with acetone/10% methanol including tamoxifen as an internal standard, and centrifuged. Supernatants obtained were diluted with 10mM ammonium acetate and subjected to LC-MS/MS. The same LC-MS/MS system as in the case of ketoconazole was used. The column was also the same as in the case of ketoconazole. The mobile phases were 10mM ammonium acetate (A) and acetone/10% (B). The gradient conditions (A:B) were: 65 : 35 (0min), 5 : 95 (5–8min), and 65 : 35 (9.01–12min) at a flow rate of 200µL/min. The MS/MS measurement was performed using the LC-MS-8050 system (Shimadzu). The precursor and product ions (m/z) of rifampicin and tamoxifen under positive-ion mode were m/z = 823.45 to 399.10 and m/z = 372.20 to 72.15, respectively.

**Measurement of Bile Acid Concentrations in the Plasma and Liver by LC-MS/MS** The plasma and liver homogenates were mixed with methanol including Nor-DCA as an internal standard, and centrifuged. Supernatants obtained were diluted with 10mM ammonium acetate and subjected to LC-MS/MS. The mobile phases were 10mM ammonium acetate (A) and acetone/10% (B). The gradient conditions (A:B) were: 65 : 35 (0min), 5 : 95 (5–8min), and 65 : 35 (9.01–12min) at a flow rate of 200µL/min. The MS/MS measurement was performed using the LC-MS-8050 system (Shimadzu). The precursor and product ions (m/z) of ketoconazole and carbamazepine under positive-ion mode were m/z = 531.25 to 82.20 and m/z = 237.10 to 194.10, respectively. While calculating the hepatic concentration, 1g of liver was assumed as 1mL.
were diluted with 10 mM ammonium acetate and subjected to LC-MS/MS. The same LC-MS/MS system was used as in the case of ketoconazole and rifampicin. The Inertsil ODS-3 column (5 μm, 2.1 × 100 mm, GL Sciences Inc.) with column oven temperature of 40°C was used. The mobile phases were 10 mM ammonium acetate (A) and acetonitrile (B). The gradient conditions (A:B) were: 75:25 (0 min), 60:40 (12 min), and 5:95 (16–20 min), and 75:25 (20.1–23 min) at a flow rate of 200 μL/min. TMCA (α plus β forms) was measured using TMCA (β form) standard. The precursor and product ions of bile acids under negative-ion mode were: TMCA, m/z = 514.20 to 80.05; GCA, m/z = 464.30 to 74.20; TUDCA, m/z = 498.30 to 80.05; CA, m/z = 407.25 to 407.25; TCA, m/z = 514.30 to 80.05; UDCA, m/z = 391.25 to 391.25; GCDCA, m/z = 448.25 to 74.20; TCDCA, m/z = 498.25 to 80.10; TDCA, m/z = 498.20 to 80.05; CDCA, m/z = 391.25 to 391.25; DCA, m/z = 391.25 to 391.25; LCA, m/z = 375.25 to 375.25; and Nor-DCA, m/z = 377.20 to 377.20.

Detection of Bile Acids by IMS Frozen liver tissue blocks were frozen in liquid nitrogen under isopentane. The liver sections of 10 μm thickness were prepared with a cryostat. Imaging profiles of liver sections mounted on indium tin oxide (ITO) glass plate (Matsunami Glass Ind., Ltd., Osaka, Japan) were analyzed using iMScope (Shimadzu). The glass plate was placed in a matrix holder and covered by 9-AA powder as an analytical matrix using iMLayer (Shimadzu). Following this, the glass plate was placed in a container with a paper containing 5% methanol and heated at 37°C. It was then vacuum dried to recrystallize 9-AA. Automated laser scanning of the liver slices was performed using the Imaging MS Solution software (Shimadzu), and 50 consecutive laser shots at a frequency of 1000 Hz were collected at each spot. The laser step size was 20 μm. The MS spectra were obtained in negative ion mode within the m/z range of 350 to 600. The detector and sample voltage were maintained consistently at 2.1 and 3.0 kV, respectively.

Quantification of mRNA Expression Using Real-Time RT-Quantitative(q)PCR The mRNA levels of bile acid-related genes, namely BSEP, MRP2, CYP7A1, and CYP27A1 were calculated using RT-qPCR. The liver samples were permeated in the RNA later solution (Thermo Fisher Scientific; MA, U.S.A.). Total RNA was extracted using the TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer’s instructions. The RNA was treated with Dnase I, Amplification Grade (Thermo Fisher Scientific). After addition of dNTP mix (Thermo Fisher Scientific) and Oligo (dT) 15 Primer (Promega, Fitchburg, WI, U.S.A.), the cDNA was synthesized from total RNA using reverse transcriptase, ReverTra Ace® (Toyobo Co., Ltd., Osaka, Japan). Next, primers (forward and reverse) and the Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent Technologies; Tokyo, Japan) were added, and qPCR was performed using a PikoReal Real-Time PCR system (Thermo Fisher Scientific). The expression level of each target gene was calculated using the ΔΔCT method and normalized against the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard gene. The following primers were used in the present study.

BSEP forward: 5'-CACGTCCTCAAGCTGCAAGAGGAT-3';
BSEP reverse: 5'-CTGCGCTAGCTACCCTTGGT-3';
MRP2 forward: 5'-ACCGAGATGCTATCATGCTTCT-3';
MRP2 reverse: 5'-TGGTCATCCATGAGCTTCT-3';
CYP7A1 forward: 5'-TCCAGGCAGCTTCTGAGTT-3';
CYP7A1 reverse: 5'-TGCGCTTCAAGCTGACCTTT-3';
CYP27A1 forward: 5'-AGACGGGGGATGGTGAATG-3';
and CYP27A1 reverse: 5'-CAGCTGCAGCTTCTCAG-3'.

Statistical Analysis The statistical analysis was performed using Student’s t-test. A p-value <0.05 is considered as statistically significant.

RESULTS

Changes in Biochemical Parameters after Administration of Ketoconazole to Chimeric Mice with Humanized Liver Mice in both the control and ketoconazole-treated groups did not show any changes in the plasma levels of ALT, AST, and ALP. However, in both the groups, the level of T-BiL was lower than the quantification limit (0.2 mg/dL) (Fig. 1).

Ketoconazole Concentration in Chimeric Mice with Humanized Liver after Its Administration Concentrations of ketoconazole in the liver and plasma of mice at 6 h after administration of ketoconazole measured by LC-MS/MS were 49.3 ± 16.4 nmol/g and 41.8 ± 15.9 μM (mean ± S.D., n = 3), respectively. Distribution of ketoconazole in the liver was confirmed.

Changes in Bile Acid Concentrations after Administration of Ketoconazole to Chimeric Mice with Humanized Liver A comparison of concentrations of bile acids in the liver of the control and the ketoconazole-treated groups revealed TDCA concentration to be significantly higher in the treated group (Fig. 2A). Concentrations of other taurine-conjugated bile acids were also found to be elevated in the treated group; however, the change was insignificant. Similarly, elevated concentrations of DCA (p = 0.064) and TDCA (p = 0.061) were found in the plasma of mice in the treated group; however, the change was insignificant (Fig. 2B).

Visualization of Bile Acids after Administration of Ketoconazole to Chimeric Mice with Humanized Liver by IMS The liver tissue collected from chimeric mice was sectioned, coated with 9-AA and analyzed using IMS. As a result, peaks were mainly detected at m/z of 498.3 and m/z of 514.3 [M–H]−. Shimada et al. reported the detection of TCDCA and TCA, which corresponded to these m/z values, using IMS in the mouse liver. Therefore, in the present study, it was presumed that m/z of 498.3 and m/z of 514.3 were TCDCA and TCA, respectively. The distribution of m/z of 498.3 and m/z of 514.3 in the liver sections was visualized using the imaging MS solution software. The IMS analysis revealed individual differences in imaging profiles, with no significant variation between the control and ketoconazole-treated groups (Fig. 3).

Changes in mRNA Expression of Bile Acid-Related Genes after Ketoconazole Administration to Chimeric Mice with Humanized Liver The mRNA expression levels of bile-acid related genes in the liver were compared between the control and ketoconazole-treated groups. We did not observe any change in the mRNA expression of BSEP, MRP2, and CYP27A1 encoding for a bile acid biosynthesis enzyme; whereas, the expression of CYP7A1 mRNA, encoding a rate limiting enzyme of bile acid biosynthesis, was found to decrease in the treated group (Fig. 4).

Changes in Biochemical Parameters after Administration of Rifampicin in Chimeric Mice with Humanized Liver
Liver A comparison of the concentrations of plasma ALT, AST, and ALP did not increase in rifampicin-treated groups. However, a significant increase in the concentration of T-BiL was observed in the treated group (Fig. 5).

Concentration of Rifampicin in Chimeric Mice with Humanized Liver after Its Administration Concentrations of rifampicin in the liver and plasma at 6h after administration of rifampicin measured by LC-MS/MS were $91.9 \pm 15.1$ nmol/g and $32.4 \pm 12.1$ µM (mean ± S.D., n = 3), respectively. Distribution of rifampicin in the liver was also confirmed. The plasma concentration of rifampicin likely increased in dose-dependent manner when compared with that reported by Kakuni et al.,[22]
although the administration routes were different.

**Changes in Bile Acid Concentrations after Rifampicin Administration to Chimeric Mice with Humanized Liver**

Concentrations of bile acids in the liver of the control and rifampicin-treated groups were studied. We found a significant elevation in the concentrations of TMCA and TCA in both the liver and plasma of the treated group. The concentration of DCA was also found to increase significantly in the plasma. These bile acids contributed a significant increase to the total bile acid concentration in the liver and plasma after administration of rifampicin (Figs. 6A, B).

**Visualization of Bile Acids after Administration of Rifampicin to Chimeric Mice with Humanized Liver by IMS**

Imaging profiles of bile acids in the liver were compared between the control and rifampicin-treated groups by IMS. Also in this case, peaks were mainly detected at m/z of 498.3 and m/z of 514.3 [M−H]−. The compounds with m/z values 498.3 and 514.3 were also presumed to be TCDCA and TCA, respectively, considering the reports of Shimada et al. We also found m/z 514.3 to be widely distributed in the liver of the treated group as compared with the control group, whereas no change in the levels of m/z 498.3 was observed (Fig. 7).

**Changes in mRNA Expression of Bile Acid-Related Genes after Administration of Rifampicin to Chimeric Mice with Humanized Liver**

The mRNA expression levels of bile-acid related genes in the liver were compared between
the control and rifampicin-treated groups. The expression of BSEP and MRP2 mRNAs was found to be increased in the treated group. However, no change in the expression of CYP7A1 and CYP27A1 mRNAs was observed (Fig. 8).

**DISCUSSION**

In the present study, we assessed the changes in bile acid concentrations 6 h after single administration of ketoconazole or rifampicin (200 mg/kg) as test compounds to chimeric mice with humanized liver. A previous study reported the IC_{50} values of ketoconazole and rifampicin for BSEP-mediated transport of 3H-taurocholate in Sf9 cells to be 3.4 and 25.3 µM, respectively. The present study confirmed that the hepatic exposure levels of both ketoconazole and rifampicin at 6 h after administration exceeded the respective IC_{50} values. A comparison of the biochemical parameters of the control and treated groups revealed no increase in the concentrations of ALT, AST, and ALP in both ketoconazole- and rifampicin-treated groups (Figs. 1, 5). These observations indicated that chimeric mice had not reached the stage of extensive liver damage. On the other hand, T-BiL levels were found to be significantly elevated after administration of rifampicin, but not ketoconazole. In this regard, a previous study reported the IC_{50} values of ketoconazole and rifampicin for MRP2-mediated transport of 3H-estradiol-17β-D-glucuronide in Sf9 cells to be 76 and 18 µM, respectively. The elevated plasma levels of T-BiL could be attributed to the fact that rifampicin is a potent inhibitor of MRP2 activity. However, it is also necessary to note that ALT baseline is high in chimeric mice because host mice originally had a damaged liver before hepatocyte transplantation.

Cepa et al. reported that taurine-conjugated bile acids in plasma were significantly increased following a single administration of troglitazone to rats, a compound with inhibitory effects on BSEP. In the present study that evaluated 13 bile acids including principal non-conjugated and amino acid-conjugated bile acids, we found concentrations of several of these bile acids to increase in the liver and plasma of ketoconazole- and rifampicin-treated chimeric mice (Figs. 2, 6). In particular, the hepatic TDCA concentration increased significantly in the ketoconazole-treated group. Similarly, concentrations of TMCA and TCA showed a marked increase in both the liver and plasma of mice in the rifampicin-treated group. However, the variations in the bile acid profiles were greater in the rifampicin group than in the ketoconazole group. These variations were more pronounced in the rifampicin group, indicating a stronger effect on MRP2 activity compared to ketoconazole.
findings may be related to other factors as well as BSEP. On the other hand, Kuipers et al. previously reported that bile acid synthesis was transiently inhibited by treatment of ketoconazole in rats. Additionally, inhibition of cholesterol 7α-hydroxylase activity mediated by CYP7A1, the rate-limiting enzyme in bile acid synthesis was observed in rat liver microsomes. Furthermore, Princen et al. reported that ketoconazole inhibited bile acid synthesis in human hepatocytes as well. Therefore, changes in bile acid concentration induced by treatment with ketoconazole might have become smaller than those induced by treatment with rifampicin because ketoconazole has an inhibitory effect on bile acid synthesis.

Foster et al. reported that administration of troglitazone which induced to cholestasis to chimeric mice resulted in reduced expression of human BSEP and MRP as shown by immunostaining. Therefore, we examined the expression of bile acid-related genes including BSEP and MRP, evaluated by the change in mRNA (Figs. 4, 8). The administration of rifampicin led to elevated expression of BSEP and MRP2 genes. Taking into the consideration the observation that concentrations of bile acids increased in the rifampicin-treated group, we believe that a feedback mechanism is activated to regulate this change in the liver. In the case of ketoconazole, the expression of CYP7A1, a gene involved in the synthesis of bile acids, may be suppressed to reduce bile acid concentrations via a feedback mechanism.

A study reports increased serum bile acid concentrations after ketoconazole administration to rats. Rifampicin has also been reported to significantly increase total bile acid concentrations in both serum and the liver after single administration to mice at 6 h. A study reported that a single intraperitoneal administration of ketoconazole at 50 mg/kg administered to rats could increase the serum concentrations of CA, TCA, CDCA, GCA, GCDCA, GDCA, DCA, and TCDCA due to inhibitory effect of ketoconazole on rat hepatocellular uptake of bile acids. This finding suggested that changes in the dose of ketoconazole resulted in differences in the extent of change in bile acid concentrations between chimeric mice and rats although these profiles could not be directly compared owing to the dose and administration methods to be different from our study. Additionally, Kis et al. and Zhang et al. suggested that species differences existed in the Km values of substrates and IC50 of bile salt transport activity. Therefore, it is important to consider the species differences in drug-induced cholestasis.

Shimada et al. successfully detected TCA and TCDCA in the liver sections after administration of a single dose of carbon tetrachloride to mice, and TCA was found to be localized around the portal vein. In the present study, we could also detect compounds with m/z 498.3 and 514.3 in chimeric mouse liver, and presumed them to be TCDCA and TCA, respectively, according to what was reported by Shimada et al., despite the possibility of the presence of high concentrations of other taurine conjugates with the same m/z values (Figs. 3, 7). Furthermore, we found an increase in the uniform distribution of compound with m/z 514.3 after administration of rifampicin, which confirmed the results obtained by LC-MS/MS although quantitation using IMS may not be enough. On the other hand, hepatotoxicity related to increased bile acids could not be detected owing to a single administration. It was known that cytotoxicity contributes to hydrophobic bile acids such as LCA. The long-term administration of compounds may reveal the relationship between the lesion site and accumulation of key bile acids related to hepatotoxicity using IMS. However, to achieve this, it is also crucial to further improve the detection sensitivity of bile acids in IMS.

Chow et al. reported that the bile acid concentrations in chimeric mice with humanized liver were higher than in the non-transplanted host mice (FRGN mice). In mice, bile acids in the intestinal tract stimulate Fxr, leading to secretion of fibroblast growth factor 15 (Fgf15) from the intestinal tract. It is known that Fgf15 binds to the fibroblast growth factor...
receptor 4 (Fgfr4) of the mouse liver to inhibit Cyp7a1, which, in turn, leads to negative regulation of bile acid biosynthesis. However, since human version of FGFR4 is expressed in the liver of chimeric mouse, bile acid biosynthesis cannot be controlled, resulting in increased concentrations of bile acids. In the present study, inter-individual differences in the bile acid concentrations were partly observed. Differences in CYP7A1 activity and contribution of the remaining mouse liver in bile acid synthesis which can be based on RI differences, and effects of intestinal bacteria in chimeric mice may reflect individual differences in the bile acid profiles. Therefore, it is crucial to consider specific in vivo conditions of bile acids for studying cholestasis in chimeric mice. In this context, chimeric mice expressing the gene coding for human FGF19, an ortholog of mouse Fgf15, would be of great utility.

In summary, the findings of the present study confirmed that administration of rifampicin in particular to chimeric mice with humanized liver increased the levels of TMCA, TCA, and taurine-conjugated bile acids with no increase in the levels of ALP. Changes in bile acid concentrations may reflect the initial response of cholestasis in humans. However, ketoconazole is a strong BSEP inhibitor and is known to induce cholestasis, but the changes in bile acids were smaller in the ketoconazole group. Furthermore, it is essential to evaluate bile acid profiles and their related gene expression through long-term administration of several compounds including ketoconazole and rifampicin, while considering clinical dosages and other factors as well as BSEP inhibition. Woolbright et al. revealed an increase in some taurine conjugated glycine conjugated bile acids such as TCA and GCA in sera.
Fig. 8. The mRNA Expression of Bile Acid-Related Genes in the Liver after Administration of Rifampicin to Chimeric Mice with Humanized Liver

The expression level of each target gene was calculated using the ΔΔCT method and normalized against the expression level of GAPDH. Data are expressed as mean±S.D. (n=3). *p<0.05 vs. the control. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; S.D., standard deviation.

of patients with cholestasis. These profiles will be important indicators to show the validity of novel biomarkers in studies using chimeric mice with humanized liver after administration of various compounds. They are also expected to contribute to the screening of drug candidates, thereby avoiding drug-induced cholestasis during clinical trials and drug development.

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Conflict of Interest The present study was conducted in collaboration with the PhoenixBio Co., Ltd.

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