Role of Farnesoid X Receptor in the Enhancement of Canalicular Bile Acid Output and Excretion of Unconjugated Bile Acids: A Mechanism for Protection against Cholic Acid-Induced Liver Toxicity

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ABSTRACT

Mice lacking the farnesoid X receptor (FXR) involved in the maintenance of hepatic bile acid levels are highly sensitive to cholic acid-induced liver toxicity. Serum aspartate aminotransferase (AST) activity was elevated 15.7-fold after feeding a 0.25% cholic acid diet, whereas only slight increases in serum AST (1.7- and 2.5-fold) were observed in wild-type mice fed 0.25 and 1% cholic acid diet, respectively. Bile salt export pump mRNA and protein levels were increased in wild-type mice fed 1% cholic acid diet (2.1- and 3.0-fold) but were decreased in FXR-null mice fed 0.25% cholic acid diet. The bile acid output rate was 2.0- and 3.7-fold higher after feeding of 0.25 and 1.0% cholic acid diet in wild-type mice, respectively. On the other hand, no significant increase in bile acid output rate was observed in FXR-null mice fed 0.25% cholic acid diet in contrast to a significant decrease observed in mice fed a 1.0% cholic acid diet in spite of the markedly higher levels of hepatic tauro-conjugated bile acids. Unconjugated cholic acid was not detected in the bile of wild-type mice fed a control diet, but it was readily detected in wild-type mice fed 1% cholic acid diet. The ratio of biliary unconjugated cholic acid to total cholic acid (unconjugated cholic acid and tauro-conjugated cholic acid) reached 30% under conditions of hepatic taurine depletion. These results suggest that the cholic acid-induced enhancement of canalicular bile acid output rates and excretion of unconjugated bile acids are involved in adaptive responses for prevention of cholic acid-induced toxicity.

Hepatic bile acid levels are tightly regulated through manipulation of canalicular output, basolateral uptake, and biosynthesis. Bile salt export pump (Bsep), a bile canalicular ATP-binding cassette transporter, mediates canalicular bile acid excretion. Drugs causing intrahepatic cholestasis, such as cyclosporine A, rifampicin, and glibenclamide, are known to inhibit Bsep/BSEP-mediated taurocholate transport (Stieger et al., 2000; Byrne et al., 2002; Noe et al., 2002). Bsep-null mice exhibit decreased levels of total bile acid output (30% of wild-type mice). Thus, the canalicular bile acid secretion through Bsep is believed to be one of the determinants for maintenance of hepatic bile acid homeostasis. Furthermore, bile acid synthesis and hepatic bile acid uptake from plasma involving cholesterol 7α-hydroxylase (CYP7A1) and Na+-taurocholate cotransporting polypeptide (NTCP), respectively, might also control homeostasis.

Unconjugated bile acids are conjugated with amino acids in the liver by bile acid-CoA synthetase (BACS) and bile acid-CoA:amino acid N-acyltransferase (BAT) before secretion into bile (Schersten et al., 1967; Czuba and Vessey, 1981; Solaas et al., 2000). The bile acids are converted into tauro- or glyco-conjugated forms in humans and almost exclusively to tauro-conjugated forms in mice (Falany et al., 1997). The ATP binding cassette transporter Bsep transports conjugated bile acids into the bile. However, transport of unconjugated bile acids such as cholic acid has not been detected in rodent and humans Bsep-expressing cell lines (Gerloff et al.,...
Serum AST and Alkaline Phosphatase (ALP) Activities and Determination of Bile Acid. Serum AST activity was estimated by the POP-TOOS [pyruvate oxidase-N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine] method using a commercial kit, Transaminase CII-B-test Wako (Wako Pure Chemicals, Osaka, Japan). Serum ALP activity was estimated by the Bessey-Lowry method using Alkaliphospha B-test Wako (Wako Pure Chemicals). Bile, liver, and serum 3a-hydroxy bile acid concentrations were estimated by an enzymecolorimetric method using the Total bile acid-test kit (Wako Pure Chemicals). The liver 3a-hydroxy bile acid contents were measured by HPLC as described previously (Kitada et al., 2003). A portion (100 µl) of liver homogenate was mixed with 1 ml of ethanol containing 2 nmol of androstane and treated at 85°C for 1 min, and then centrifuged at 1000g for 5 min. After the supernatant was isolated, the precipitate was extracted twice with 1 ml of ethanol and combined extracts were dried and redissolved in 200 µl of methanol. HPLC analyses were performed with a Jasco intelligent model PU-980 pump (Jasco), Waters M-45 pump (Waters, Milford, MA), and FP-920S fluorescence detector (Jasco). The bile acid extracts were separated at 35°C with an L-column ODS (2.1 × 150 mm) (Kagakuhinnkensakyoukai). The eluates were mixed with a NAD+ solution before introduction of 3a-hydroxysteroid dehydrogenase immobilized on Enzymepak 3a-HSD column. NADH produced was measured by fluorescence using an excitation wavelength of 365 nm and an emission wavelength of 470 nm. The separation was started at 0.5 ml/min with a 60-min linear gradient of solution A/solution B mixture (25: 75) to solution A/solution B mixture (55:45), and then continued with solution A/solution B mixture (55:45) for 25 min [solution A: 10 mM phosphate buffer (pH 7.2) + acetoniitrile (60:40); and solution B: 30 mM phosphate buffer (pH 7.2)+acetoniitrile (80:20)]. The eluates were passed through a 3a-HSD column after mixing with solution C (1:1) [solution C: 10 mM phosphate buffer (pH 7.2), 1 mM EDTA, 0.05% 2-mercaptoethanol, and 0.3 mM NAD+].

Hepatic ATP and Taurine Levels. Hepatic ATP levels were measured by the luciferase assay (Lundin and Thore, 1975) using a commercial kit, ATP Assay System LL-100-1 (Toyo Ink, Tokyo, Japan). Hepatic taurine levels were determined by an HPLC method with fluorometric detection (Waterfield, 1994). Taurine was derivatized with o-phthalaldehyde (Sigma-Aldrich) before injection onto COSMOSIL C18 Econopak.

Immunoblot Analysis. Mouse Bsep cDNA containing carboxy terminal region (bp 3195–4070 in AF133903) was subcloned into a commercial kit, ATP Assay System LL-100-1 (Toyo Ink, Tokyo, Japan). Hepatic taurine levels were determined by an HPLC method with fluorometric detection (Waterfield, 1994). Taurine was derivatized with o-phthalaldehyde (Sigma-Aldrich) before injection onto COSMOSIL C18 Econopak.

Materials and Methods

Materials. Cholic acid, deoxycholic acid, taurocholic acid, taurochenodeoxycholic acid, and taurodeoxycholic acid were purchased from Sigma-Aldrich (St. Louis, MO). HPLC column, Chemosorb 5-ODS-H (6.0 × 150 mm) was purchased from Chemco Scientific Co. (Tokyo, Japan). L-column ODS (2.1 × 150 mm) was obtained from Kagakuhinknkensakyoukai (Tokyo, Japan). Enzymepak 3a-HSD column. NADH produced was measured by fluorescence using an excitation wavelength of 365 nm and an emission wavelength of 470 nm. The separation was started at 0.5 ml/min with a 60-min linear gradient of solution A/solution B mixture (25: 75) to solution A/solution B mixture (55:45), and then continued with solution A/solution B mixture (55:45) for 25 min [solution A: 10 mM phosphate buffer (pH 7.2)+ acetoniitrile (60:40); and solution B: 30 mM phosphate buffer (pH 7.2)+acetoniitrile (80:20)]. The eluates were passed through a 3a-HSD column after mixing with solution C (1:1) [solution C: 10 mM phosphate buffer (pH 7.2), 1 mM EDTA, 0.05% 2-mercaptoethanol, and 0.3 mM NAD+].

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Immunoblot Analysis. Mouse Bsep cDNA containing carboxy terminal region (bp 3195–4070 in AF133903) was subcloned into a prokaryotic expression vector, pQE30 (QIAGEN, Valencia, CA). Recombinant proteins were expressed in Escherichia coli, M15 (pREP4) strain, and purified by SDS-polyacrylamide gel electrophoresis. Japanese white rabbits were immunized twice by intradermal injection of 100 µg of the purified protein. Antisera were obtained 1 week after the boost. Specific IgG was purified by affinity column chromatography with Sepharose 4B-bound purified Bsep protein. Crude membranes were prepared from livers by a modification of the method described by Lee et al. (1993). Liver was homogenized in buffer A (100 mM Tris, 100 mM potassium chloride, and 1 mM EDTA) with a protease inhibitor cocktail (20 µM butylated hydroxytoluene and 2 mM phenylmethanesulfonyl fluoride). Nuclei and cell debris were removed by centrifugation at 1000g for 15 min. The supernatant was spun at 10,000g for 5 min, and the resulting pellet was resuspended in membrane storage buffer (100 mM potassium phosphate, pH 7.4, 1.0 mM EDTA, 20% glycerol, 1 mM diithiothreitol, and the protease inhibitor cocktail). The crude membrane proteins were loaded onto a 6% polyacrylamide gel, isolated, and transferred to nitrocellulose filters. The filter was immunostained with anti-Bsep IgG (1:1000 dilution), alkaline phosphatase-conjugated goat anti-rabbit IgG (1: 3000 dilution), 5-bromo-4-chloro-3-indolyphosphate, and nitro blue tetrazonium as described previously; Honma et al., 2002).

Analysis of mRNA Levels. Messenger RNA levels of differentially expressed genes were analyzed using reverse transcription-polymerase chain reaction (RT-PCR). Single-strand cDNAs were constructed using an oligo(dT) primer with the Ready-to-Go You-Prime First-strand

Animal Treatment and Sample Collection. FXR-null mice (Sinal et al., 2000) were housed under standard 12-h light/12-h dark cycle. Before the administration of special diets, mice were fed standard rodent chow (CE-2; CLEA, Tokyo, Japan) and water ad libitum for acclimation. Experimental diets contained 0.1, 0.25, 0.5, or 1.0% (w/w) cholic acid mixed with the control diet (CE-2). Age-matched groups of 8- to 12-week-old animals were used for all experiments and were allowed access to water ad libitum. Bile, blood, and tissue samples were taken for biochemical assays after 5 days of feeding special diets. Total RNA was prepared from livers using the ULTRASPEC II RNA isolation system (Biotexc Laboratories, Houston, TX), and RNA concentrations were determined by measuring the absorbance at 260 nm using a spectrophotometer (Beckman DU 640). Biliary excretion was monitored by measuring the absorbance at 260 nm using a spectrophotometer (Beckman DU 640). Biliary excretion was monitored by measuring the absorbance at 260 nm using a spectrophotometer (Beckman DU 640). Biliary excretion was monitored by measuring the absorbance at 260 nm using a spectrophotometer (Beckman DU 640). Biliary excretion was monitored by measuring the absorbance at 260 nm using a spectrophotometer (Beckman DU 640).
Beads kit (Amersham Biosciences AB, Uppsala, Sweden). These cDNAs provided templates for PCRs using specific primers at a denaturation temperature of 94°C for 30 s, an annealing temperature of 58°C for 30 s, and an elongation temperature of 72°C for 30 s in the presence of deoxynucleoside-5'-triphosphates and Taq polymerase. The PCR cycle numbers were titrated for each primer pair to ensure amplification in linear range. The reaction was completed by a 7-min incubation at 72°C. PCR products were analyzed in 2% agarose gel (w/v) containing ethidium bromide for visualization. The specific forward and reverse primers for the genes examined by PCR were the following: Bsep (bp 2094–2517 in AF133903) sense, 5'-ACAGCTTACAGTTACGG-3'; antisense, 5'-TCATTGCTCAAGGACCAATGTCG-3'; Tct (bp 71–671 in U95131) sense, 5'-ACACTGCCTCCAGCTCATTC-3'; and antisense, 5'-GCCGAGGAATGTTGTTGATG-3'; Mrp2 (bp 838–1022 in NM 013806) sense, 5'-GGTGCCTCTGCTGTTGAGCTG-3'; and antisense, 5'-GCGACTGAGATTACAAAACAC-3'; Oatp1 (bp 711–1036 in AF148218) sense, 5'-TGATAACCCGCTGGTCGTGC-3'; and antisense, 5'-GCTGCTCCAGGTATTTGGGC-3'; BAT (bp 222–713 in U95215) sense, 5'-CTCTTGTTGTGATGACTGCTGC-3' and antisense, 5'-AACTCCATCAATCCACCACG-3'; Bacs sense, 5'-ACCTGGATCAGCTCCTGGAT-3' and antisense, 5'-GTCTCCAGCAGCCAGTGGG-3'; and GAPDH (bp 487–682 in NM 009320) sense, 5'-GCTGCTCCAGGTATTTGGGC-3'; antisense, 5'-CAGCTGAGGATTCAGAAACAAA-3'; and antisense, 5'-GCCAGTAAGTGTGGTGTCATG-3'. To more precisely quantify Bsep mRNA level, real-time PCR was carried out with SYBR Green by using ABI PRISM 7000 (Applied Biosystems, Foster City, CA). To calculate Bsep mRNA level, a standard curve was generated by plotting the threshold cycle value versus the log of the amount of mouse Bsep cDNA. Bsep mRNA levels were normalized from the GAPDH mRNA levels.

**Results**

**Cholitic Acid-Induced Liver Damage and Hepatic Bile Acids.** Under control diet feeding, serum AST activity was 2.6-fold higher in FXR-null mice than in the wild-type mice. No significant difference in serum ALP activity was observed between both lines of mice (Table 1). Because FXR-null mice exhibited some mortality during 1% cholic acid feeding for 5 days, the mice were thereafter fed a 0.25% cholic acid diet in the present study. Serum ALP activity increased to 4.9-fold in FXR-null mice fed a 0.25% cholic acid diet, whereas the increase was minimal (1.6-fold) in the wild-type mice even after a 1.0% cholic acid diet. Serum AST activity increased 15.7-fold in FXR-null mice fed a 0.25% cholic acid diet, compared with the mice fed a control diet. Slight increases (1.7- and 2.5-fold) were observed in wild-type mice fed either 0.25 or 1.0% cholic acid diets.

Feeding 0.25% cholic acid resulted in clear increases in serum and hepatic bile acid levels in FXR-null mice. Similar experiments on wild-type mice revealed only slight increases in hepatic bile acid levels and no significant increases in the serum bile acid level. A significant correlation ($r^2 = 0.92$) was observed between hepatic bile acid concentrations and AST activity in individual mice (Fig. 1).

**Bile Salt Export Pump Protein and mRNA Levels.** Decreased levels of canalicular bile acid transporter, Bsep represents one possible mechanism for cholestasis. Changes in hepatic mRNA levels of Bsep were assessed in cholic acid-fed mice by RT-PCR (Fig. 2A). The mRNA levels were increased in the wild-type mice fed cholic acid diet. In contrast, no clear increase in the Bsep mRNA levels was observed in FXR-null mice after feeding 0.25% cholic acid diet. Furthermore, hepatic mRNA levels of Bsep were quantified by real-time PCR revealing 2.0-fold higher levels of specific Bsep mRNA in control diet-fed wild-type mice compared with control diet-fed FXR-null mice (Fig. 2B). Bsep mRNA levels became 2.1-fold higher in wild-type mice after 1.0% cholic acid feeding. Consistent with Bsep mRNA levels, Bsep protein levels assessed by immunoblotting were higher in the wild-type mice fed control diets than in FXR-null mice and became 3.0-fold higher in wild-type mice after 1.0% cholic acid feeding (Fig. 2C), whereas no significant increases in protein levels were found in FXR-null mice fed 0.25 and 1.0% cholic acid diets and in wild-type mice fed 0.1 and 0.5% cholic acid diets (data not shown).

**Bile Flow and Bile Acid Output Rate.** Bile acid secretion across the canalicular membrane is the rate-limiting step of enterohepatic circulation under normal dietary conditions. Bile flow and bile acid output rate (3α-hydroxy bile acid excretion rate), which correlate with bile acid excretion rates to the biliary duct, were determined in both mice fed a control diet or a cholic acid diet, to determine the biliary excretion capacity (Fig. 3, A and B). Contrary to the Bsep protein levels, bile acid output rate was significantly higher in FXR-null mice fed control diets than in the wild-type mice (Fig. 3B). Hepatic bile acid concentrations were 2.5-fold higher in FXR-null mice than that in the wild-type controls. An increase in bile acid output rate and hepatic bile acid concentration was observed in the wild-type mice fed the cholic acid diet. Feeding a 1% cholic acid diet to wild-type mice increased the bile flow and bile acid output rate by 3- and 3.7-fold, respectively. In FXR-null mice, no significant difference in bile flow and bile acid output rate was observed between control diet and cholic acid (0.1 and 0.25%) diet in spite of the marked accumulation of hepatic bile acids (2.0 μmol/l liver) in FXR-null mice fed 0.25% CA diet (Fig. 3B). Furthermore, a decrease in bile flow and bile acid output

**TABLE 1**

Liver diagnostic markers and levels of 3α-hydroxy bile acid

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Wild-Type Mice</th>
<th>FXR-Null Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.25%</td>
</tr>
<tr>
<td>Serum 3α-OH bile acid (μM)</td>
<td>5 ± 2</td>
<td>8 ± 5</td>
</tr>
<tr>
<td>Liver 3α-OH bile acid (nmol/g liver)</td>
<td>147 ± 35</td>
<td>206 ± 35a</td>
</tr>
<tr>
<td>AST activity (IU/l)</td>
<td>23 ± 5</td>
<td>40 ± 12a</td>
</tr>
<tr>
<td>ALP activity (IU/l)</td>
<td>28 ± 1</td>
<td>22 ± 5</td>
</tr>
</tbody>
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3α-OH, 3α-hydroxy; ND, not determined.

*p < 0.05 versus control.

*p < 0.01 versus control.
rates were observed in FXR-null mice fed a 1% cholic acid diet, compared with FXR-null mice fed the control diet (data not shown).

**Tauro-Conjugated and Unconjugated Bile Acid Levels in Liver and Bile.** Biliary bile acids are conjugated with taurine before their excretion in control mice. This is consistent with reports showing no transport activity of unconjugated bile acids such as cholic acid in Bsep-expressing cells (Gerloff et al., 1998). BACS and BAT are involved in tauro-conjugation. Thus, the amount of both unconjugated and tauro-conjugated forms of hepatic bile acids in cholic acid-fed wild-type and FXR-null mice was determined by HPLC (Fig. 4A). Cholic acid, taurocholic acid, deoxycholic acid, and taurodeoxycholic acid were detected as major components in the livers of FXR-null and the wild-type mice after feeding of 0.25% cholic acid diet. Taurocholic acid was the major component in livers of FXR-null mice fed 0.25% cholic acid diet (Fig. 4B). Hepatic taurodeoxycholic acid level was also significantly higher in FXR-null mice than in wild-type mice after feeding of 0.25% cholic acid diet. Taurocholic acid was the major component in livers of FXR-null mice fed 0.25% cholic acid diet (Fig. 4B). Hepatic taurodeoxycholic acid level was also significantly higher in FXR-null mice than in wild-type mice after feeding the 0.25% cholic acid diet. In contrast, high levels of unconjugated cholic acid were detected in the bile of wild-type mice fed a 1% cholic acid diet (Fig. 5A). However, unconjugated deoxycholic acid levels were very low in the bile. In contrast to the control diet, the biliary ratio of unconjugated cholic acid to total cholic acid was increased to 32.9% in wild-type mice fed a 1% cholic acid diet, whereas it accounted only for 0.6 and 0.4% in the wild-type and FXR-null mice fed a 0.25% cholic acid diet, respectively (Fig. 5B).

To assess the relationship between depletion of taurine and the increased ratio of unconjugated cholic acid in liver and bile of wild-type mice fed a cholic acid diet, hepatic taurine levels were determined in both wild-type and FXR-null mice fed a cholic acid diet. Hepatic taurine level was clearly decreased upon cholic acid feeding FXR-null and the wild-type mice. The level in wild-type mice fed a 1% cholic acid diet was reduced to 10% of the control diet fed mice (Fig. 6A). It is noteworthy that ATP is necessary for the Bsep-mediated bile acid transport as well as taur-conjugation. Since depletion of hepatic ATP affects the transport activity, hepatic ATP levels were quantified in cholic acid-fed mice. Hepatic ATP levels were, however, not significantly decreased in wild-type and FXR-null mice fed the cholic acid diet, compared with the control diet (Fig. 6B).

**Specific mRNA Levels of Bile Acid Transporters and Amino Acid Conjugation Enzymes.** To explore the involvement of bile acid-related genes other than Bsep in protection against hepatic bile acid accumulation, changes in the hepatic mRNA levels of bile acid transporters and amino acid conjugation enzymes were analyzed by RT-PCR (Fig. 7). Hepatic Cyp7a1 mRNA was higher in FXR-null mice fed control diet than in wild-type mice. The level was decreased in cholic acid-fed wild-type and FXR-null mice. Mrp2 mRNA level was slightly increased in cholic acid-fed wild-type and FXR-null mice. On the other hand, the major bile acid uptake transporters Ntcp and Oatp1 mRNA levels were decreased upon...
cholic acid-feeding of wild-type mice, whereas only Oatp1 mRNA levels were decreased in cholic acid-fed FXR-null mice. Hepatic mRNA levels of BACS and BAT that mediate tauro-conjugation of bile acids were not significantly changed in FXR-null mice or after cholic acid feeding. Specific mRNA level of the taurine transporter was significantly increased in both mice fed the cholic acid diet.

**Discussion**

The aim of the present study was to determine the in vivo mechanism for FXR-mediated protection against extraordinarily high bile acid levels by use FXR-null and wild-type mice fed cholic acid diets. A clear correlation was found between individual hepatic bile acid concentrations and AST activities. Individual hepatic bile acid concentrations and AST activities suggest that major hepatic bile acid taurocholic acid but not unconjugated cholic acid is involved in hepatotoxicity in FXR-null mice fed a cholic acid diet. Bile flow and bile acid output rates of wild-type mice fed a cholic acid diet increased with increasing hepatic bile acid concentration (from 0.1–0.5 μmol/g liver), whereas those of FXR-null mice did not increase, but decreased in spite of the marked accumulation of hepatic bile acids (2.0 μmol/g liver) after feeding a 0.25% cholic acid diet. The data on bile acid output rates and hepatic bile acid concentrations indicate that targeted disruption of FXR results in the impairment of the regulation of canalicular bile acid excretion to maintain a low level of hepatic bile acids.

Consistent with previous reports (Schuetz et al., 2001; Kok et al., 2003; Lambert et al., 2003; Zollner et al., 2003), Bsep mRNA and protein levels were lower in FXR-null mice fed a control diet than that in the wild-type mice in spite of higher bile acid output rates in FXR-null mice. Bsep protein levels were not correlated with bile acid output rates between FXR-null and the wild-type mice fed control diets. These results suggest a higher efficacy for Bsep-mediated excretion in FXR-null mice. It is known that the functional activity of ABC transporters, including Bsep and Mdr1 are regulated by several posttranslational processes such as protein phosphorylation and cellular localization (Kullak-Ublick et al., 2004). Because hepatic bile acid concentrations were 2.5-fold higher in FXR-null mice fed control diets compared with wild-type mice, this discordance might reflect an adaptive response of Bsep to high levels of hepatic bile acids. In wild-type mice fed a cholic acid diet, the adaptive enhancement of canalicular bile acid excretion capacity dependent on the increase in both the Bsep protein levels and possibly the rates of Bsep-mediated excretion is likely one of the critical determinants for the suppression of hepatic bile acid accumulation.

Several factors, including hepatic bile acid levels and ATP levels, were shown to modulate canalicular bile acid excretion. Amino acid conjugation is one of the factors involved in canalicular bile acid excretion. Human BAT and BACS, involved in the conjugation of bile acids to taurine and glycine, respectively, are positively regulated by FXR (Pircher et al., 2003). As shown in Figs. 4 and 5A, nearly all of the cholic acids were detected as tauro-conjugated derivatives in bile and liver of FXR-null mice fed a 0.25% cholic acid diet. The data on bile acid output rates and hepatic bile acid concentrations indicate that targeted disruption of FXR results in the impairment of the regulation of canalicular bile acid excretion to maintain a low level of hepatic bile acids.

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More than 30% of the total cholic acid content was detected as unconjugated cholic acid in the bile of wild-type mice fed a 1% cholic acid diet. The increased ratio of biliary unconjugated cholic acid to total cholic acid suggests the existence of
an unconjugated cholic acid excreting system in the wild-type mice. The excreting system may become evident as an adaptive response under the condition of hepatic taurine depletion. Amino acid conjugation is not necessarily the obligatory step for the excretion. Although the system involved in unconjugated cholic acid excretion has not been identified in the present study, it remains a possibility that there is an alternative transport system other than Bsep. Thus, it cannot be excluded that acyl glucuronides of cholic acid are formed and excreted, followed by hydrolysis in bile.

Although bile acids excreted by Bsep are the major osmotic driving force generating bile flow, no significant decrease in bile flow was found in Bsep-null mice (Wang et al., 2001). Bsep-null mice still have a capacity to excrete the bile acid into bile (30% of wild-type mice), suggesting the existence of an alternative bile acid transport system. Furthermore, bile flow and bile acid output rates of Bsep-null mice increase after feeding a 0.5% CA diet (Wang et al., 2003), a result that differs from those of FXR-null mice fed a 0.5% cholic acid diet. These results suggest that a Bsep-independent bile acid excretion system was induced in Bsep-null mice fed a cholic acid diet. This excretion system is unlikely to be enhanced in FXR-null mice fed a cholic acid diet. An FXR-mediated adaptive bile acid excretion system other than Bsep might also be involved in the increase in bile acid output rate, including excretion of the unconjugated cholic acid in the wild-type mice.
mice fed a cholic acid diet, although the increase in Bsep protein levels likely explains at least in part the increase in bile acid output rate in wild-type mice fed a cholic acid diet.

Disruption of FXR enhances the sensitivity to cholic acid-induced toxicity. The enhancement of bile acid output rate mediated by FXR is likely a critical determinant for protection against cholic acid-induced liver toxicity. In contrast to cholic acid-induced toxicity, induction of toxicity by the secondary bile acid lithocholic acid is ameliorated by hydroxylation of bile acid St2a induced through PXR (Sonoda et al., 2001). Nuclear receptor-mediated protective mechanisms of cholic acid-induced toxicity might be different from that of lithocholic acid-induced toxicity. Recently, it was reported that constitutive androstane receptor is also involved in protection against cholic acid-induced toxicity by using PXR-FXR double null mice (Guo et al., 2003). Role of the nuclear receptor interaction among FXR, PXR, and constitutive androstane receptor in the protection against bile acid-induced toxicity remains to be fully clarified.

The present study demonstrated that the adaptive enhancement of canaliculal bile acid excretion is one of the critical protective mechanisms for cholic acid-induced toxicity. Furthermore, the transport system of unconjugated cholic acid is at least in part involved in protection against hepatic cholic acid accumulation under the condition of hepatic taurine depletion. Ideal mRNA levels of apical sodium-dependent bile acid transporter was 3.0-fold higher in FXR-null mice fed a cholic acid diet, compared with that of the wild-type mice fed the same diet (Maeda et al., 2004). It was also reported that the absolute amounts of bile acids reabsorbed from the intestine is enhanced by 2-fold in FXR-null mice (Kok et al., 2003). Although the analysis of the absorption process in intestine is necessary to explore the precise mechanism for protection against hepatic bile acid accumulation, FXR-null mice fed a cholic acid diet might be useful model to evaluate the possible involvement of Bsep in cholestasis.

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References
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