Bile-salt hydrophobicity is a key factor regulating rat liver plasma-membrane communication: relation to bilayer structure, fluidity and transporter expression and function

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Bile-salt hydrophobicity regulates biliary phospholipid secretion and subselection. The aim of this study was to determine whether bile salts can influence liver plasma membrane phospholipids and fluidity in relation to the ATP-dependent transporter. Rats were depleted of bile salts by overnight biliary diversion and then sodium taurocholate was infused intravenously at a constant rate (200 nmol/min per 100 g of body weight), followed by infusion of bile salts with various hydrophobicities (taurochenodeoxycholate, tauroursodeoxycholate, tauro-β-muricholate, tauro-α-muricholate at 200 nmol/min per 100 g of body weight). The hydrophobicity of the infused bile salts correlated with that of biliary phospholipids, but was inversely related to that of the canalicular membrane bilayer. Canalicular membrane fluidity (estimated by 1,6-diphenyl-1,3,5-hexatriene fluorescence depolarization) and expression of multidrug-resistance proteins (Mrp2, Mrp3) and apical Na^+-dependent bile-salt transporter (ASBT) were increased by hydrophilic bile salts, although there was no marked change in the expression of P-glycoprotein subfamilies (Mdr2). Bile-salt export pump (Bsep) expression was increased along with increasing bile-salt hydrophobicity. Bile salts modulate canalicular membrane phospholipids and membrane fluidity, as well as the ATP-dependent transporter expression and function, and these actions are associated with their hydrophobicity. The cytoprotective effect of hydrophilic bile salts seems to be associated with induction of Mrp2, Mrp3 and ASBT.

Key words: ASBT, Bsep, canalicular membrane fluidity, Mrp2, Mrp3.

INTRODUCTION

Previous studies performed by our laboratory [1,2] and others [3–5] have shown that alterations in the physical state of plasma-membrane lipids (i.e. fluidity and lateral packing) can influence the function of membrane carriers that mediate the transport of various nutrients, electrolytes and drugs, including membrane-bound enzymes. In fact, the carrier-mediated transport of taurocholate (TC) into canalicular membrane vesicles (CMVs) has been shown to be partly modulated by membrane fluidity [6]. Excretion of a large variety of endogenous and exogenous compounds from hepatocytes into the bile is an ATP-dependent process, predominantly performed by members of the P-glycoprotein (Pgp) subfamily and the multidrug-resistance protein subfamily of the ATP-binding cassette (ABC) protein superfamily [7–10]. In this regard, there have been several reports that the expression of these proteins is modulated by drugs causing intrahepatic cholestasis, as well as by bile-duct ligation [11–16]. However, the mechanism(s) of such regulation of the ABC transporters is yet to be established.

We recently demonstrated [1] that bile-salt hydrophobicity regulates the subselection of phospholipid (PL) species secreted into bile and thereby modulates bile metastability, and that these changes are associated with alterations of the PL species within cell-membrane bilayers. We suggested [1] that the function of canalicular membrane transporters may be modulated by such changes of the lipid bilayer in association with alterations of membrane fluidity. Accordingly, bile-salt hydrophobicity may play an important role in the regulation of hepatic excretory systems. Therefore, the aim of this study was to investigate whether changes of bile-salt hydrophobicity affect the fluidity and lipid composition of the canalicular membrane, as well as the function of liver plasma-membrane transporters.

MATERIALS AND METHODS

Chemicals

Sodium salts of TC, taurochenodeoxycholate (TCDC), tauroursodeoxycholate (TUDC), tauro-β-muricholate (TβMC) and tauro-α-muricholate (TαMC) were provided generously by Tokyo Tanabe (Tokyo, Japan). These salts were $> 99\%$ pure when examined by HPLC. HPLC-grade acetonitrile and propan-2-ol were obtained from Wako Pure Chemical Industries (Osaka, Japan) and 1,6-diphenyl-1,3,5-hexatriene (DPH) was obtained from Molecular Probes (Eugene, OR, U.S.A.). Polyclonal rabbit antibodies against multidrug-resistance protein Mrp2 (K3, dilution 1:1000) and polyclonal antibodies against bile-salt export pump (Bsep; K12, dilution 1:1000) were kindly provided by Dr M. Muller (Groningen Institute for Drug Studies, University of Groningen, Groningen, The Netherlands). Mouse monoclonal antibody C219 (dilution 1:1000; Signet Laboratories, Dedham, MA, U.S.A.) was used to detect all Pgps. Mouse monoclonal antibody 6/1G (dilution 1:300; Chemicon International, Temecula, CA, U.S.A.) was used to detect multidrug-resistance protein Mdr2. Rat antibodies against Mrp3 (dilution 1:1000) were prepared at the Graduate School of Pharmaceutical Sciences, University of Tokyo (Tokyo, Japan). Rat antibodies

Abbreviations used: TC, taurocholate; TCDC, taurochenodeoxycholate; TUDC, tauroursodeoxycholate; TβMC, tauro-β-muricholate; TαMC, tauro-α-muricholate; PL, phospholipid; CMV, canalicular membrane vesicle; DPH, 1,6-diphenyl-1,3,5-hexatriene; Pgp, P-glycoprotein; ABC, ATP-binding cassette; Bsep, bile-salt export pump; ASBT, apical sodium-dependent bile-salt transporter; S/U ratio, ratio of saturated to unsaturated fatty acid; FXR, farnesoid X receptor; PC, phosphatidylcholine.

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against the apical sodium-dependent bile-salt transporter (ASBT; RIBMAL1, dilution 1:1000) was provided by Dr W. Kramer (DG Metabolic Diseases, Aventis Pharma Deutschland GmbH, Frankfurt, Germany).

Animal model and experimental protocol

Adult male Sprague–Dawley rats (250–300 g; Hiroshima Jikken Doubutsu, Hiroshima, Japan) were housed together in cages and were allowed free access to food and water before the study. The rats were anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal). After opening the abdomen, the bile duct was cannulated with a polyethylene tube to allow sampling of the bile and the left femoral vein was catheterized for administration of solutions. The catheters used (PE10) were purchased from Nippon Becton Dickinson (Tokyo, Japan). After surgery, the rats were placed in restraining cages and intravenous infusion of 0.9 % NaCl was started at a rate of 0.4 ml/h per 100 g of body weight. After the endogenous bile-salt pool had been drained for 15 h, the animals were intravenously infused with TC at a constant rate (200 nmol/min per 100 g of body weight). After 2 h, the rats were infused with TCDC, TUDC, TβMC or TαMC (200 nmol/min per 100 g of body weight) for 2 h. Bile was collected into pre-weighed tubes at intervals until the end of the experiment. Subsequently, the rats were killed by an intravenous injection of pentobarbital and their livers were excised, weighed and prepared for the examination of crude liver homogenate and liver plasma membranes. The study protocol was approved by the Hiroshima Animal Care and Use Committee, and the animals received humane care in compliance with the directives of the National Research Council as outlined in [16a].

Bile-salt hydrophobicity

The hydrophilic/hydrophobic balance of bile salts was determined by the retention times on reversed-phase HPLC (Shimadzu LC-6A system; Shimadzu Industruments, Tokyo, Japan) as described previously [17]. The hydrophobic index was calculated from the retention time of each bile salt.

Isolation of canalicular membranes

Liver crude membrane vesicles and CMVs were isolated using the sucrose-gradient centrifugation technique described by Takenaka et al. [18]. Then the membrane fraction was stored at −70 °C until use.

The activity of two CMV marker enzymes, leucine aminopeptidase and γ-glutamyltranspeptidase, was measured with commercially available kits (LAP C-test Wako and γ-GTP C-test Wako; Wako Junyaku Kogyo, Osaka, Japan). Compared with the crude liver homogenate, the activities of the respective enzymes showed 55-fold and 80-fold increases in CMVs.

Analytical procedures

Total biliary bile salts were measured enzymically using 3-α-hydroxysteroid dehydrogenase [19]. The biliary cholesterol concentration was measured using a commercial kit after lipid extraction with diethyl ether [20], and the PL concentration was measured directly as described by Bartlett [21]. Fatty acid composition of lecithin was measured by GLC. Lecithin hydrophobicity was estimated by the ratio of saturated fatty acid to unsaturated fatty acid, the S/U ratio.

Membrane lipids were extracted for analysis by the method of Bligh and Dyer [22]. The chloroform layers were pooled, washed with an equal volume of methanol/water (1:1, v/v), and evaporated to dryness. After being redissolved in 100 μl of 10 % Triton X-100, aliquots (20 μl) were taken for the determination the PLs and total cholesterol using a commercial enzymic method. Total protein levels in the liver homogenates and membrane subfractions were measured with a commercially available kit (Coomassie Brilliant Blue dye-binding assay; Bio-Rad Laboratories, Richmond, CA, U.S.A.) [23].

DPH polarization value

Membrane fluidity was estimated by the DPH fluorescence depolarization method. In brief, 2×10^{-3} M DPH in tetrahydrofuran was first diluted 1000-fold with vigorous stirring for 10 min at 25 °C and a stable aqueous dispersion of 2×10^{-6} M DPH was obtained. One volume of CMV suspension (100 μg of protein/ml) was mixed with one volume of the DPH dispersion and was incubated with gentle shaking at 37 °C in darkness for 45 min (the time required to reach the maximal fluorescence intensity). Steady-state fluorescence polarization measurements were performed using a Hitachi F2000 spectrofluorometer (Hitachi, Tokyo, Japan) equipped with a circulating-water bath to maintain the temperature at 37 °C. The excitation and emission wavelengths were 360 and 430 nm, respectively. The emission intensity of vertically polarized light was detected by an analyser oriented parallel (Ivv) or perpendicular (Ivh) to the excitation plane and fluorescence polarization (P) was calculated by the following equation: $P = (\mathrm{Ivv} - \mathrm{Ivh})/(\mathrm{Ivv} + \mathrm{Ivh})$. The calculated values were corrected for light scatter according to the method of Lentz et al. [24] and were used as an indicator of membrane fluidity, with increased anisotropy indicating a decrease of PL acyl chain motion within the membrane [25]. All determinations were performed in triplicate.

Western-blot analysis

Crude liver homogenate and liver plasma-membrane proteins (50 μg) were separated by SDS/PAGE [26] and were transferred to nitrocellulose membranes (Millipore, Bedford, MA, U.S.A.) using a tank-blotting system according to the manufacturer's instructions (Bio-Rad Laboratories). After transfer, the membranes were blocked for 2 h at room temperature with 5 % powdered skimmed milk dissolved in Tris-buffered saline containing 0.05 % Tween 20, and they were then incubated overnight at 4 °C with the first antibody. Immune complexes were detected using alkaline phosphatase-conjugated anti-rabbit IgG or rabbit anti-mouse IgG (1:3000 dilution) according to the manufacturer's instructions (Bio-Rad Laboratories). Detection of membrane transporters was performed by comparison with the following standards: myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), BSA (66 kDa) and ovalbumin (43 kDa). The immunoreactive bands on the membranes were quantified by densitometric analysis.

Statistical analysis

Statistical differences were determined by one-way ANOVA, followed by a multiple-comparisons test. The significance level was $P < 0.05$.

RESULTS

Bile-salt hydrophobicity

The hydrophobicity of the various bile salts was ranked in the following order: TCDC (2.13) > TC (1) > TUDC (0.46) > TβMC (0.21) > TαMC (0.16).

Table 1 Effects of bile salts on biliary lipid secretion and on lipid composition of CMVs

The effects of bile salt on PC, cholesterol (CH), CH/PL ratio and S/U ratio are expressed as the mean percentage ± S.D. of the value during infusion with TC alone. The output of PC and cholesterol were significantly reduced by the hydrophilic bile salts whereas the CH/PL ratio was significantly increased ($P < 0.05$). In contrast, PC concentration was significantly increased by the hydrophilic bile salts ($P < 0.05$) whereas the CH/PL ratio had a tendency to be reduced (not significant). The S/U ratio in bile was significantly decreased by the hydrophilic bile salts. In contrast, the S/U ratio in CMVs was significantly increased by the hydrophilic bile salts ($P < 0.05$).

Retention factor (hydrophobic index) ...	TCDC 2.13	TC 1.00	TUDC 0.46	TβMC 0.21	TαMC 0.16
Bile					
PC secretion (nmol/min per 100 g of body weight)	40.3 ± 8.77*	31.35 ± 6.76	23.15 ± 5.01*	14.89 ± 3.21*	12.54 ± 3.55*
CH secretion (nmol/min per 100 g of body weight)	4.82 ± 0.88*	3.91 ± 0.49	3.01 ± 0.85*	2.8 ± 0.51*	2.83 ± 0.42*
CH/PL ratio	0.162 ± 0.04*	0.203 ± 0.05	0.223 ± 0.06*	0.232 ± 0.04*	0.23 ± 0.05*
PC S/U ratio	1.09 ± 0.06	0.97 ± 0.05	0.8 ± 0.12*	0.74 ± 0.07*	0.71 ± 0.05*
CMVs					
PC (nmol/mg of protein)	232.3 ± 86.7*	298.1 ± 127.9	432.3 ± 36.9*	425.2 ± 20.1*	428.4 ± 36.9*
CH (nmol/mg of protein)	214.8 ± 63.5	263.7 ± 117.9	312 ± 45.6	332.5 ± 24.2	330.3 ± 23.4
CH/PL ratio	0.942 ± 0.23	0.903 ± 0.19	0.606 ± 0.38	0.685 ± 0.16	0.64 ± 0.26
PC S/U ratio	3.32 ± 0.21	3.61 ± 0.28	3.58 ± 0.16	3.85 ± 0.09*	3.72 ± 0.12*

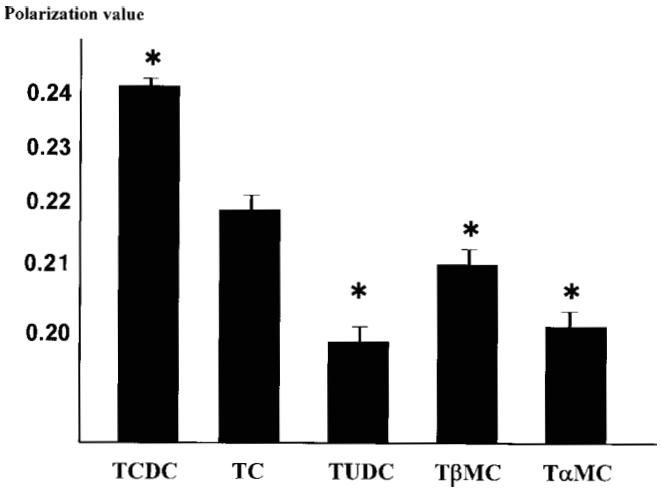

Figure 1 Effects of bile acid on canalicular membrane fluidity in rats

Rats were treated after their endogenous bile-salt pools had been drained for 15 h and were infused intravenously with TC, TCDC, TUDC, Tβ MC or TαMC (200 nmol/min per 100 g of body weight) for 2 h. They were killed 120 min after the intravenous infusion and livers were isolated from each rat group. Canalicular membrane fluidity was estimated by DPH fluorescence depolarization. Steady-state fluorescence polarization measurements were performed by using a spectrometer. The polarization value of DPH fluorescence, shown on the y axis, is taken as a measure of membrane fluidity, increased anisotropy indicating a decreased extent of PL acyl chain motion within the membrane. Values are means ± S.D. from four rats. *$P < 0.05$, compared with TC alone (control).

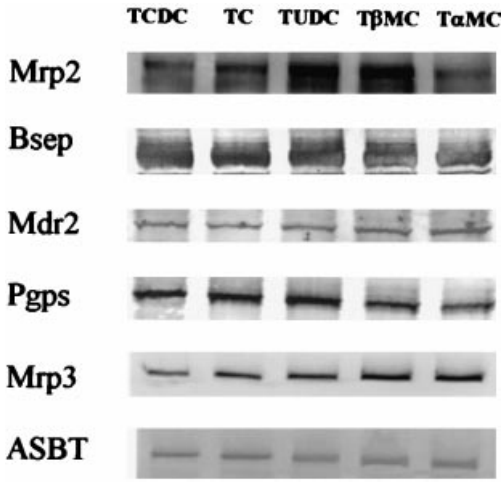

Figure 2 Immunoblot analyses of transporters after administration of bile acid in rats

Rats were treated after their endogenous bile-salt pools had been drained for 15 h and were infused intravenously with TC, TCDC, TUDC, Tβ MC or TαMC (200 nmol/min per 100 g of body weight) for 2 h. They were killed 120 min after the intravenous infusion and livers were isolated from each rat group. Membrane proteins (50 μg) were resolved by SDS/PAGE (7.5% gel), and immunoblots were performed using primary antibodies: polyclonal rabbit antibodies against Pgps (C219, dilution 1:300), Mrp2 (K3, dilution 1:500) and Bsep (K12, dilution 1:500), monoclonal mouse antibody against Pgp specifically recognizing *mdr2*-encoded gene product (6/1G, dilution 1:300) and rat monoclonal antibody against Mrp3 (dilution 1:1000). Rat antibodies against ASBT (RIMBAL1, dilution 1:1000).

Effect of bile-salt infusion on biliary lipid secretion and lipid composition of CMVs

After 15 h of bile diversion, the biliary secretion of cholesterol and lecithin was low. When rats were subsequently infused with the various bile salts after diversion, the output of lecithin and cholesterol increased rapidly and then remained constant. Lecithin output increased with increasing bile-salt hydrophobicity. Cholesterol secretion also increased with increasing bile-salt hydrophobicity. In contrast, the biliary cholesterol/lecithin ratio decreased in proportion to bile-salt hydrophobicity. In the CMVs, phosphatidylcholine (PC) concentration decreased with increasing bile-salt hydrophobicity, whereas the cholesterol/PL ratio had a tendency to be increased (Table 1).

Effect of bile-salt infusion on biliary and CMV lecithin species

The hydrophobicity of the lecithin species was dependent on the hydrophobicity of the infused bile salt. Relative hydrophobicity of the lecithin species was defined from the retention times on HPLC, and the hydrophobic index of each PC was calculated.

It was found that the hydrophobic index of biliary lecithin increased in proportion to the bile-salt hydrophobic index, whereas the hydrophobicity of lecithin in CMVs decreased. The S/U ratio in bile was significantly decreased by hydrophobic bile salts. In contrast, the S/U ratio in CMVs was significantly increased by hydrophilic bile salts (Table 1).

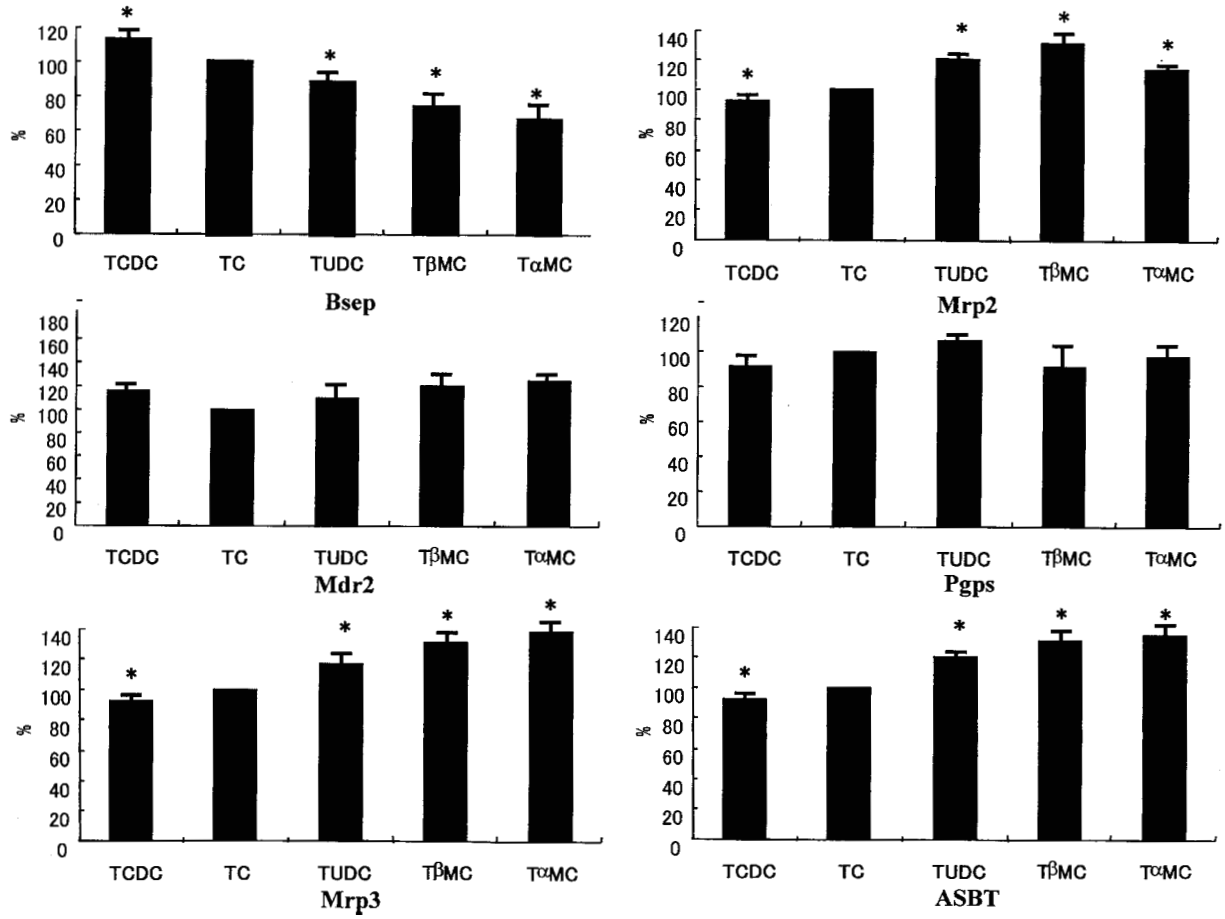

Figure 3 Densitometric analysis of liver transporters protein levels

Immunoblots of liver crude homogenate and liver plasma-membrane vesicle from TC (control) and TCDC, TUDC, TαMC and TβMC were prepared as described in the Materials and methods section. Autoradiographs were quantified by densitometry. Data are means $\pm$ S.D. from four rats. $^*P < 0.05$ compared with TC alone.

Effect of bile salts on canalicular membrane fluidity

Canalicular membrane fluidity was estimated by the DPH fluorescence depolarization method. DPH fluorescence was taken as an indication of membrane fluidity, with increased anisotropy indicating a decrease of PL acyl chain motion within the membrane. Infusion of hydrophilic bile acids resulted in significantly lower polarization values than infusion of hydrophobic bile acids. In particular, canalicular membrane fluidity was increased markedly by TUDC (polarization values, -20%). These results indicated that hydrophilic bile acids could increase canalicular membrane fluidity (Figure 1).

Western blotting of liver plasma-membrane transporters after bile-salt infusion and densitometric analysis

The changes in membrane transporters were quantified by densitometry, and the transporter levels in CMVs (Mrp2, Bsep, Mdr2 and Pgps), liver crude membrane vesicle (Mrp3) and crude liver homogenate (ASBT) were expressed as percentages of the corresponding control values. The expression of Mdr2 and Pgps was not altered by bile-salt infusion, whereas increased expression of Mrp2, Mrp3 and ASBT and decreased expression of Bsep were evident after infusion of hydrophilic bile salts (Figures 2 and 3).

DISCUSSION

Bile salts are important for stimulating bile flow and biliary lipid secretion [27]. In the bile, cholesterol is solubilized in PC vesicles and in mixed micelles that are composed of bile salts, PC and cholesterol. Formation of such micelles serves to attenuate the deleterious detergent action and potential cytotoxicity of hydrophobic bile salts on biliary epithelial cells [17,28]. The present study was performed to determine whether bile salts could influence the PC species of the biliary canalicular membrane in association with altering bile-solute-transporter expression and function, and to clarify whether the cytoprotective action of hydrophilic bile salts was associated with such changes in cell-membrane bilayers.

This study yielded several interesting findings. First, infusion of hydrophilic bile salts (TUDC, TβMC and TαMC) significantly lowered the polarization value, which indicated that canalicular membrane fluidity was increased, whereas a reduction of canalicular membrane fluidity was evident after infusion of hydrophobic bile salts. Canalicular membrane fluidity was the most dramatically increased by TUDC. Similarly, Bellentani et al. [29]

have reported that TUDC increases canalicular membrane fluidity. Our previous study demonstrated that hydrophilic bile salts increased the hydrophobicity of PL species in canalicular membranes and thus enhanced the lateral packing density, thereby decreasing the release of canalicular membrane enzymes [1,2], raising the possibility of increased canalicular membrane fluidity being modulated by such changes of PL composition. Hofmann et al. [30] suggested that the diffusion rate of bile salts through (model) membranes is not only dependent on hydrophobicity, but also on bile-salt di- (and poly-)meric associations and on membrane-lipid composition [30]. Similarly, our data also revealed that membrane fluidity is not exactly correspondent with bile-salt hydrophobicity. Taken together, these findings suggest that bile-salt hydrophobicity at least partly regulates the physiological function of canalicular membranes during bile secretion by altering its membrane structure.

Another interesting finding was that the expression of ABC transporters was affected by bile salts. Expression of Mrp2 was enhanced by hydrophilic bile salts, and Bsep expression was correlated with the hydrophobicity of the bile salts. Since Mrp2 is the major driving force for bile-salt-independent bile flow [31] as well as transporting organic anions including bilirubin glucuronides, its enhanced expression may relate to the increased excretion of such molecules into the bile. The fact that Bsep, the major driving force for bile-salt-dependent bile flow, was increased by hydrophobic bile salts could be considered to be a homoeostatic process occurring in the liver to induce the biliary excretion of cytotoxic bile salts having a high hydrophobicity. In hepatocytes accumulation of bile salts is known to cause mitochondrial damage and, ultimately, apoptosis and necrosis [32,33]. The farnesoid X receptor (FXR) is critical for bile-salt homoeostasis by virtue of its role as intracellular bile-salt sensor and transcriptional regulator, suggesting the regulatory relationship between FXR and Bsep expression [34]. In the present study, the expression of ABC transporters such as Bsep and Mrp2 was altered by bile-salt infusion for several hours, although mRNA levels might not be affected in such a short period. Thus these changes could be explained by delivery of transporter proteins from somewhere else. In fact, Kipp et al. [35–37] recently indicated that ABC transporters cycle between the bile canaliculus and large intrahepatic ABC transporter pools. Similarly, we previously showed that cyclosporin A up-regulates Mdr2 protein expression for several hours [38]. Nevertheless, a hydrophobic bile salt such as chenodeoxycholate, a ligand for FXR, presumably stimulates Bsep expression by up-regulating FXR, and thus further investigations are certainly needed to confirm this hypothesis.

In contrast, Mdr2 expression was not significantly altered by the hydrophobic degree of bile salts infused in the present study. In this regard, Gupta et al. [39] showed, however, that taurine-conjugated bile salts (50 mM) increased Mdr2 mRNA levels in primary rat hepatocytes [39]. The difference between the two studies could be based upon the experimental design and the dose of bile salts used, although no direct information was provided in the present study to clarify this issue.

Canalicular membrane fluidity, lateral packing density and ABC transporter expression may be affected by changes in the PC species that comprise the canalicular membrane. No previous studies have shown that alterations in canalicular membrane fluidity can influence the expression of a number of important ABC transporters (Pgps, Mdr2, Mrp2 and Bsep). Canalicular membrane fluidity was increased in association with a lower cholesterol/PL ratio in the canalicular membrane lipid bilayer, which may account for the increased membrane fluidity. The increases in Mrp2 expression by hydrophilic bile salts and in Bsep expression by hydrophobic bile salts somehow suggest that the therapeutic effect of hydrophilic bile salts depends, in part, on enhancing biliary organic anion excretion, while self-protection against cytotoxic (hydrophobic) bile salts may induce the secretion of such bile salts.

The basolateral isoform, Mrp3, which is located on the basolateral membranes of hepatocytes and cholangiocytes [40,41], is up-regulated in mutant animals with a defect in Mrp2 and in extrahepatic cholestasis [42]. Mrp3 mediates the ATP-dependent transport of bile salt and anionic conjugates, particularly of glucuronides and sulphoconjugates, across the basolateral hepatocyte membrane into sinusoidal blood. The inverse relation in expression of Mrp3 and Mrp2 has been demonstrated under various conditions, which is explained as a compensatory role of Mrp3 in the hepatic secretion of bile salt and anionic conjugates during impaired transport into bile [43]. However, the expression of Mrp3 as well as Mrp2 was increased with decreasing bile-salt hydrophobicity. This may suggest that hydrophilic bile salt up-regulates canalicular and lateral membrane transporter proteins to mediate the excretion of bilirubin and bile salt.

Although bile-salt transport by bile-duct epithelial cells, or cholangiocytes, has been postulated, the details of this process remain unclear. The cholehepatic shunt hypothesis provides a possible scenario by which cholangiocytes may participate in the enterohepatic circulation of bile salts. This pathway was proposed by Hofmann and colleagues [44,45]. The hypothesis proposes that bile salt is protonated in the canaliculus, absorbed passively into the cholangiocyte, and then secreted at the basolateral cholangiocyte domain. From there, the bile-salt molecule enters the periductular capillary plexus and proceeds to the sinusoids to be reabsorbed by the hepatocyte where it can be resecreted into bile, promoting additional canalicular bile secretion. Lazaridis et al. [46] indicate that biliary epithelia actively transport conjugated bile salts at their apical domain via ASBT. ASBT may serve physiological functions. Its presence may help to regulate bile formation and promote water absorption, and to affect cholangiocyte signalling pathways, including protein kinase C, Mg^{2+} and cAMP [46]. In our study, hydrophilic bile salts up-regulate ASBT protein levels. This indicates that the hydrophilic bile salts strengthen the role of ASBT in the transport of xenobiotics into cholangiocytes, leading to their recycling to hepatocytes via a cholehepatic shunt pathway and/or to their further metabolism by biliary epithelia. Taken together, hepatocellular cytoprotection by hydrophilic bile salts is possibly based on increased membrane fluidity and enhanced organic anion secretion into bile.

In summary, bile salts can modulate canalicular membrane PL species and fluidity as well as transporters, and this action is associated with hydrophobicity. The cytoprotective effect of hydrophilic bile salts is seemingly associated with induction of Mrp2, Mrp3 and ASBT.

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