Defective canalicular transport and toxicity of dietary ursodeoxycholic acid in the abcb11<sup>−/−</sup> mouse: transport and gene expression studies

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Wang R, Liu L, Sheps JA, Forrest D, Hofmann AF, Hagey LR, Ling V. Defective canalicular transport and toxicity of dietary ursodeoxycholic acid in the abcb11<sup>−/−</sup> mouse: transport and gene expression studies. Am J Physiol Gastrointest Liver Physiol 305: G286–G294, 2013. First published June 13, 2013; doi:10.1152/ajpgi.00082.2013.—The bile salt export pump (BSEP), encoded by the abcb11 gene, is the major canalicular transporter of bile acids from the hepatocyte. BSEP malfunction in humans causes bile acid retention and progressive liver injury, ultimately leading to end-stage liver failure. The natural, hydrophilic, bile acid ursodeoxycholic acid (UDCA) is efficacious in the treatment of cholestatic conditions, such as primary biliary cirrhosis and cholestasis of pregnancy. The beneficial effects of UDCA include promoting bile flow, reducing hepatic inflammation, preventing apoptosis, and maintaining mitochondrial integrity in hepatocytes. However, the role of BSEP in mediating UDCA efficacy is not known. Here, we used abcb11 knockout mice (abcb11<sup>−/−</sup>) to test the effects of acute and chronic UDCA administration on biliary secretion, bile acid composition, liver histology, and liver gene expression. Acutely infused UDCA, or its taurine conjugate (TUDC), was taken up by the liver but retained, with negligible biliary output, in abcb11<sup>−/−</sup> mice. Feeding UDCA to abcb11<sup>−/−</sup> mice led to weight loss, retention of bile acids, elevated liver enzymes, and histological damage to the liver. Semi-quantitative RT-PCR showed that genes encoding Mrp4 (basolateral) and Mrp3 (canalicular) as well as Mdr1a and Mdr1b (canalicular) were upregulated in abcb11<sup>−/−</sup> mice. We concluded that infusion of UDCA and TUDC failed to induce bile flow in abcb11<sup>−/−</sup> mice. UDCA fed to abcb11<sup>−/−</sup> mice caused liver damage and the appearance of biliary tetra- and penta-hydroxy bile acids. Supplementation with UDCA in the absence of Bsep caused adverse effects in abcb11<sup>−/−</sup> mice.

UDCA, in contrast, showed no toxicity in abcb11<sup>−/−</sup> mice and humans have been sought in differences between the two species in bile acid metabolism and/or the physiology of bile formation (12). The primary bile acids in humans (cholic and chenodeoxycholic acids) are retained in the hepatocytes of patients with PFIC2 with BSEP deficiency and cause damage, either by their direct cytotoxicity (13), or by inducing the production of proinflammatory cytokines (1, 41), or both. The abcb11<sup>−/−</sup> mouse, however, appears to have an efficient mechanism for converting its primary bile acids (β-muricholic and cholic) into less toxic, polyhydroxy bile acids that can then be transported from the hepatocyte through an alternative transport system(s) (18, 38).

Ursodeoxycholic acid (UDCA) has been recommended as a primary treatment for patients with BSEP deficiency (5) although it has been indicated that UDCA is ineffective in infants with severe BSEP deficiency (34). UDCA is also widely used in the treatment of primary biliary cirrhosis (3, 12, 37) and intrahepatic cholestasis of pregnancy (4, 19, 25, 37). Like other natural bile acids, UDCA is conjugated with either glycine or taurocholate in the hepatocyte and induces bile flow in wild-type animals.

We were interested in knowing whether or not administration of UDCA would benefit abcb11<sup>−/−</sup> mice and also whether the alternative bile acid transport system(s) in abcb11<sup>−/−</sup> mice (18, 38) also would transport UDCA conjugates into bile. UDCA has a variety of effects, including reducing the cytotoxicity of circulating bile acids (3, 37). UDCA also has anti-inflammatory (16, 23, 24) and antiapoptotic properties (2, 11, 14, 28) and helps to maintain mitochondrial integrity (2, 3, 20, 28). Orally administered UDCA accumulates in the entero-hepatic circulation in a dose-dependent manner, eventually making up 30–50% of circulating bile acids (25, 37).

Here, we report the effects of acute and chronic UDCA treatment on biliary secretion, bile flow, liver histology, and liver gene expression in abcb11<sup>−/−</sup> mice.

**MATERIALS AND METHODS**

**Chemicals and reagents.** Bile acids standards were obtained from Sigma and Steraloids (Steraloids, Newport, RI). UDCA used in dietary supplementation was obtained from Axcan (Axcan Pharma Mont-Saint-Hilaire, Québec, Ontario, Canada). Assay kits for bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) assays were purchased from Biotron Diagnostic (Hemat, CA). SYBR Green PCR Master Mix was purchased from Applied Biosystems (Foster City, CA). 22,23-[<sup>3</sup>H]-tauroursodeoxycholate was prepared by reductive tritiation of the Δ<sup>22–23</sup> derivative of UDCA as described (33). The [3H] label has been shown to be stable during hepatic transport (6).
Animals. Mice with a deficiency in the abcb11 gene (abcb11−/− mice) used in this study were generated in Vancouver (40). Animals were maintained in a 12-h:12-h light/dark cycle at 23°C with free access to food and water. The mice were fed a regular diet, except where specified in the results. These experiments were performed using protocols approved by the Committee on Animal Care, University of British Columbia, according to the guidelines of the Canadian Council on Animal Care.

Acute administration of TUDC or UDCA to the anesthetized biliary fistula mouse. Mice were anesthetized by intraperitoneal injection of ketamine (112.5 mg/kg) and xylazine (11.3 mg/kg) after 2–4 h of fasting. The abdomen was opened and the gall bladder cannulated using a PE-10 catheter after distal common bile duct ligation (32, 40). After 20 min of bile flow equilibration, bile was collected into tared tubes in 5-min batches for 10 min. A bolus of 22,23-[3H]-tauroursodeoxycholic acid (TUDC) together with carrier UDCA (100 μmol/kg body wt) was then infused into the tail vein over a 20-s interval. In a second experiment, UDCA (65 μmol/kg body wt) was infused similarly. Bile was then collected through the cannula in 2-min batches for 10 or 20 min, followed by 10-min batches for 20 or 30 min.

UDCA feeding studies. Mice were fed a diet supplemented with 0.02% or 1% (wt/wt) UDCA. The mice were weighed on day 0 and every 3 days thereafter and monitored. Body weights and liver weights were recorded on the last day when mice were killed and sampled. Urine, bile, plasma, and liver samples were collected, snap-frozen in liquid N2, and kept at −80°C. Liver slices were fixed in 10% neutral buffered formalin solution (EMS, Fort Washington, PA) for paraffin sectioning.

High-performance liquid chromatography. Bile samples were collected from mice through bile duct cannulation and stored at −80°C with minimal freeze-thaw cycles. Before analysis, samples were thawed on ice and mixed well. The bile was then diluted 1:4 in MeOH and centrifuged for 2 min at 16,000 g.

High-performance liquid chromatography (HPLC) analysis of biliary-conjugated bile acids was carried out in Vancouver with a Waters 600 pump and controller and a Waters 486 UV detector. Separation was performed on a Waters Spherisorb S5 ODS2 C-18 (5-μm particle size, 250 mm × 4 mm) reverse-phase analytical column preceded by guard APLolumn (Nova-Pack; Waters, Milford, MA). Integration of peaks was carried out using Millennium 2010 software. The bile acids were separated at ambient temperature over 48 min at a flow rate of 0.6 ml/min and 3,200 psi. The mobile phase consisted of solvent A (MeOH) and solvent B (60:40 MeOH:0.01 M potassium phosphate, 0.02 M sodium phosphate pH 5.35), modified from Refs. 10 and 29. Initial conditions were held at 100% B for the first 25 min. There was a linear gradient to 30% B over the next 10 min, which was then held constant for 5 min, followed by a linear gradient to 100% A over 8 min. The system was then flushed with 100% MeOH, followed by equilibration back to the initial conditions. The effluent was monitored at 210 nm. Each metabolite was quantified by interpolation from a standard curve constructed for each metabolite. Some metabolites could not be quantified accurately due to lack of standards, and a first approximation was made using the standard curve of a commercially available bile acid standard with a similar retention time. HPLC of biliary bile acids was also performed in San Diego as described (29).

Electrospray ionization tandem mass spectrometry. Electrospray mass spectrometry was performed in San Diego using a Perkin-Elmer Sciex API-III instrument (Perkin-Elmer, Edmonon, Alberta, Canada) as described previously (17). Chemical identity of peaks was confirmed by the fragmentation pattern of selected ions (Q3 mode) using argon collision gas. The presence of conjugated bile acids was confirmed by selection for m/z 74 (glycine), m/z 97 (sulfate), and m/z 124 (taurine).

Quantitative reverse-transcription PCR. RNA samples were prepared from the mouse liver as previously described (39). Complementary DNA (cDNA) was synthesized from 5–10 ng of total RNA from each sample and used for PCR reactions in a PRISM 7900HT Sequence Detection System (Applied Biosystems). The “Standard Curve Method” (ABI PRISM User Bulletin 2) was used for evaluating the results, using the primers reported previously (39). The mRNA expression of ribosomal protein S15 (Rps15) was used to normalize the mRNA expression of all genes.

Liver histology. For light microscopy, mice were killed with an overdose of anesthetics after fasting for 2–4 h. Livers were immediately sliced and transferred into 10% neutral buffered formalin followed by paraffin sectioning and hematoxylin-eosin staining (Wax-it Histology Services, British Columbia, Vancouver, Canada).

Statistical analysis. All numbers are expressed as means ± SD (n). The statistical significance of differences between groups of mice was assessed using the two-tailed Student’s t-test. Where results from only three animals are available, mean values are given.

RESULTS

Hepatic transport of TUDC. To confirm whether TUDC could stimulate bile flow in the absence of Bsep, we infused a bolus of TUDC by tail vein injection and measured bile flow rate via gallbladder cannulation in wild-type and abcb11−/− mice (see MATERIALS AND METHODS). As seen in Fig. 1A, before infusion of TUDC, the basal bile flow rate in wild-type mice was about threefold higher than in abcb11−/− mice. Infused TUDC resulted in an immediate and marked increase in bile flow in wild-type mice that lasted for about 10 min. In contrast, infused UDCA caused only a small, transient increase in bile flow in the abcb11−/− mice (Fig. 1B).

The distribution of TUDC in mice was also examined by determining the fate of an intravenous dose (100 μmol) of [3H]-TUDC over a 30-min period (Fig. 1, C–E). Similar to the kinetics observed in Fig. 1B, radiolabeled TUDC was rapidly extracted by the liver and secreted into bile in wild-type mice but not in abcb11−/− mice (Fig. 1, C and D). After 30 min, it was seen that the majority of labeled TUDC was in the bile in wild-type mice, but, in abcb11−/− mice, [3H]-TUDC was retained in the liver (Fig. 1E). We also examined the capacity of unconjugated UDCA (65 μmol/kg body wt) to stimulate bile flow in these mice. Infused UDCA behaved similarly to TUDC (data not shown).

Physiological and biochemical responses to UDCA feeding. To assess the effects of UDCA in abcb11−/− mice, we challenged the mice with a diet containing 1% UDCA. Figure 2 shows the relative body weight change in wild-type and abcb11−/− mice fed 1% UDCA for 72 days. Both abcb11−/− and wild-type mice suffered progressive weight loss at this high dosage. After 24 days, the wild-type mice began to regain weight slowly, but the abcb11−/− mice did less well. Neither group regained all of their lost weight, even after 72 days.

Liver biochemical indicators also confirmed the adverse effects of 1% UDCA feeding, with abcb11−/− mice showing a greater effect. As seen in Table 1, UDCA-fed abcb11−/− mice were severely jaundiced with an increase in serum bilirubin over fourfold greater than that of wild-type mice given the same treatment. The mutant mice also had elevated serum ALP and ALT levels, compared with wild-type mice (Table 1). This biochemical profile and progressive body weight loss (Fig. 2) were similar to those seen in abcb11−/− mice fed a lower dose (0.5%) of cholic acid (39), a more toxic bile acid.

The possibility remained that a lower dosage of UDCA could still be beneficial to abcb11−/− mice because UDCA has multiple hepatoprotective effects. We tested this by feeding...
mice a diet containing 0.02% UDCA equivalent to 30 mg/day per kilogram of body weight (mice consume about 1.5 g diet per 10 g body wt per day, on average). This dietary level corresponds to a high therapeutic dosage in humans (25). At the 0.02% dietary dosage, there was a clear difference in the abilities of wild-type and mutant mice to gain body weight (Fig. 3). Both wild-type and heterozygous mice grew significantly better than \( abcb11^{-/-} \) mice. It is interesting to note that there was a small net weight loss in male \( abcb11^{-/-} \) mice, whereas the female mutants appeared to be less affected. A similar sex dimorphism in response to exogenously administered cholic acid in mice was observed earlier (39).

Liver biochemical tests showed that wild-type and heterozygous mice behaved similarly under UDCA loading (Table 1). In the \( abcb11^{-/-} \) mice, with the 0.02% UDCA diet there was a dramatic drop in serum bilirubin, compared with the 1% diet.

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**Fig. 1.** Bile flow rate (BFR) and tauroursodeoxycholic acid (TUDC) excretion after a bolus infusion of TUDC into wild-type (WT) and \( abcb11^{-/-} \) male mice. A: BFR as the function of liver weight after a bolus infusion of TUDC. B: TUDC excretion as a function of liver weight in WT and \( abcb11^{-/-} \) male mice, as measured by high-performance liquid chromatography. C: rates of TUDC excretion over 30 min after infusion of \( [\text{H}] \)-TUDC (100 \( \mu \text{mol/kg} \)), as determined by distribution of radioactivity by percentage (left axis) or pmol (right axis) in the bile over the course of 30 min after a bolus infusion of \( [\text{H}] \)-TUDC. D: cumulative recovery of TUDC as determined by radioactivity in the bile over the course of 30 min after a bolus infusion of \( [\text{H}] \)-TUDC. E: cumulative radioactivity in the bile, liver, or plasma 30 min after a bolus infusion of \( [\text{H}] \)-TUDC. In each of these experiments, a bolus of TUDC (100 \( \mu \text{mol/kg} \) body wt) was infused into the tail vein over a 20-s interval. Results are presented as the means ± SD.

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**Fig. 2.** Initial body weight (A) and relative body weight (B) of adult (2–4 mo) female mice fed a diet containing 1% ursodeoxycholic acid (UDCA) over a 72-day period. Relative body weight was calculated as a function of body weight at the beginning of UDCA feeding. WT, wild-type. Each point represents the average of 3 to 5 animals. Error bars represent standard deviation.
DEFECTIVE TRANSPORT OF UDCA IN THE ABCB11−/− MOUSE

Table 1. Levels of plasma bilirubin and enzymes in abcb11−/−, abcb11+/−, and WT mice on 1% and 0.02% UDCA-supplemented diets for 108 days

<table>
<thead>
<tr>
<th>UDCA</th>
<th>Genotype</th>
<th>Bilirubin, mg/dl</th>
<th>ALP, U/l</th>
<th>ALT, U/l</th>
<th>AST, U/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>abcb11−/−</td>
<td>9.14 ± 0.377†</td>
<td>328 ± 290†</td>
<td>42.6 ± 2.73†</td>
<td>158 ± 40.0</td>
</tr>
<tr>
<td>♂</td>
<td>WT</td>
<td>1.95 ± 0.832</td>
<td>134.8 ± 6.08</td>
<td>20.1 ± 1.09</td>
<td>86.6 ± 38.7</td>
</tr>
<tr>
<td>0.02%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>abcb11−/−</td>
<td>0.706 ± 0.208*</td>
<td>395 ± 53.8‡</td>
<td>341 ± 144*</td>
<td>637 ± 322</td>
</tr>
<tr>
<td>♂</td>
<td>abcb11+/−</td>
<td>0.387 ± 0.097</td>
<td>89.2 ± 5.69</td>
<td>37.7 ± 8.38</td>
<td>191 ± 87.1</td>
</tr>
<tr>
<td>♂</td>
<td>WT</td>
<td>0.317 ± 0.0942</td>
<td>85.6 ± 17.5</td>
<td>40.1 ± 18.8</td>
<td>264 ± 81.5</td>
</tr>
<tr>
<td>♀</td>
<td>abcb11−/−</td>
<td>1.44</td>
<td>285.7</td>
<td>1430.2</td>
<td>1049.5</td>
</tr>
<tr>
<td>♀</td>
<td>WT &amp; abcb11+/−</td>
<td>0.37</td>
<td>65.9</td>
<td>52.0</td>
<td>161.9</td>
</tr>
</tbody>
</table>

The averages ± SD of the liver enzyme values are presented. Statistical significance in comparison with wild-type (WT) controls for the same treatment, *P < 0.05, †P < 0.01, ‡P < 0.001. The sample sizes of the male abcb11−/− and WT & abcb11+/− were 2 and 3, respectively. UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

(0.706 vs. 9.14 mg/dl) but little change in serum ALP (395 vs. 328 U/l) (Table 1). There was a significant elevation of ALT and AST levels in abcb11−/− mice fed a 0.02% UDCA diet compared with wild-type (Table 1).

Histological examination of the livers of female abcb11−/− mice fed 0.02% UDCA revealed severe ductular proliferation (Fig. 4, A and C), which was absent in the livers of wild-type (Fig. 4B), heterozygous (abcb11+/−), and male abcb11−/− mice under the same treatment, as well as in abcb11−/− mice fed a normal diet.

We were interested in determining the products of UDCA metabolism that could be found in the bile of wild-type and mutant animals after feeding with UDCA for 2 wk. Table 2 shows biliary bile acid composition, as determined by electrospray ionization tandem mass spectrometry (ESI-MS/MS). In wild-type and heterozygous mice, the major peak was at m/z 498, which corresponds to a taurine-conjugated dihydroxy bile acid, presumably TUDC, accounting for over 80% of total bile acids. This observation is consistent with what we observed in a short-term intravenous infusion of TUDC, where more than 80% of infused TUDC was recovered in the bile of wild-type mice after 30 min (see Fig. 1, D and E). However, in abcb11−/− mice, only a very low proportion of dihydroxy bile acid was detected in the bile, indicating that TUDC secretion was minimal. The major bile acids in the bile of abcb11−/− mice were trihydroxy and tetrahydroxy bile acids. Pentahydroxy bile acids were also present. Alternative bile acid transporters have been postulated to be responsible for transporting the tetrahydroxy bile acids that dominate the bile of untreated abcb11−/− mice (12, 18, 22, 38). Our conclusion is that these

Fig. 3. Initial body weight (left) and relative body weight (right) of adult (2–4 mo old) female (A) and male (B) mice fed a diet containing 0.02% UDCA. Relative body weight was calculated as a function of starting body weight at the beginning of UDCA feeding. Each point represents the average of 3 to 5 animals. Error bars represent standard deviation.
alternative transport systems were ineffective in transporting TUDC in abcb11−/− mice.

There were no peaks corresponding to monohydroxy taurine conjugates, indicating a lack of the taurine conjugate of lithocholic acid in the bile of UDCA-fed mice. Unconjugated UDCA itself was present only in trace amounts.

Gene expression. To examine in greater detail the molecular responses in mice fed a 0.02% UDCA diet, we examined the expression profiles of key liver genes using real-time PCR in wild-type, abcb11+/− and abcb11−/− mice. These included genes encoding the cytochrome P450 enzymes and those associated with cholesterol and lipoprotein metabolism, with nuclear receptors, and with basolateral and canalicular transporters (Table 3). A number of changes was observed, but in general the data obtained for the abcb11+/− heterozygous mice were not significantly different from those obtained for wild-type mice. However, the abcb11−/− mice had a twofold higher expression of Cyp3a11 and Cyp3a16 and significantly reduced levels of Cyp3a44 and Cyp2b10, compared with values in the wild-type mice, when both were fed a normal chow diet. Moreover, when mice were fed the 0.02% UDCA diet, Cyp3a11 and Cyp3a16 mRNAs increased sixfold, or more, relative to wild-type. We speculate that these upregulated P450 enzymes represented a compensatory detoxification response and that they were responsible for the observed increase in the amount and variety of polyhydroxy bile acids observed in the bile of mutant mice (Table 2) (39, 40). There also appeared to be a concomitant downregulation of Cyp4a41, Cyp3a44, and Cyp2b10 with UDCA feeding. How the expression of these downregulated enzymes relates to the upregulated Cyp enzymes is not known, but they may represent part of an overall P450 enzyme detoxification response.

At the transporter gene expression level, there were only small changes in the abcb11−/− mice fed 0.02% UDCA, compared with the levels expressed under the regular chow diet. The observed fourfold increase in the canalicular efflux transporters Mdr1a and Mdr1b and threefold increase in the basolateral bile acid efflux transporter Mrp4 are consistent with the hypothesis that these transporters work in concert to reduce the levels of toxic bile acids accumulating in the hepatocytes of abcb11−/− mice. As noted above, we have previously demonstrated (38) that the Mdr1 genes likely play a major role as alternative transporters for the polyhydroxy bile acids.

Similarly, with respect to the cholesterol, lipoprotein metabolism, and nuclear receptor genes, few additional expression changes were observed in abcb11−/− mice, compared with wild-type mice, upon UDCA feeding (Table 3). For example, the approximately sixfold increase in expression of Hmgcr, the rate-limiting enzyme in cholesterol synthesis, observed in the abcb11−/− mice relative to wild-type on the regular chow diet, was not significantly altered in the UDCA-fed mice. The major hydroxylases Cyp3a11 and Cyp3a16 were among the liver genes stimulated by feeding with 0.02% UDCA.

DISCUSSION

The experiments reported here sought to determine whether UDCA is of therapeutic value in mice with defective Bsep. It was not. In fact, UDCA feeding caused frank hepatotoxicity in female abcb11−/− mice, even at a relatively low dietary dosage of 0.02%. This toxicity manifested as ductular proliferation in the liver (Fig. 4), elevation of serum bilirubin, elevation of ALP and ALT enzymes indicative of hepatocellular damage (Table 1), and a greatly reduced body weight gain, compared with wild-type mice (Fig. 3). From our results, it is evident that UDCA and its taurine conjugate TUDC require a functional Bsep for transport into the bile. Induction of bile flow in wild-type mice after infusion of TUDC was directly associated with the biliary secretion of TUDC across the canalicular membrane via Bsep (Fig. 1B). The observed differences in the steady-state bile flow rates and the induced rates in wild-type and mutant mice are completely consistent with the notion that the major driver of bile flow under UDCA treatment in mice is dependent on a functional Bsep mediating the transport of bile.

### Table 2. Biliary bile acid composition (%) of 4 individual mice after 2 wk of 1% UDCA feeding, measured by ESI/MS/MS

<table>
<thead>
<tr>
<th>abcb11 Genotype</th>
<th>T(OH)</th>
<th>T(OH)2</th>
<th>T(OH)3</th>
<th>T(OH)4</th>
<th>(OH)5</th>
<th>(OH)6</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>47</td>
<td>498</td>
<td>514</td>
<td>530</td>
<td>546</td>
<td>407</td>
</tr>
<tr>
<td>a</td>
<td>85</td>
<td>94</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>abcb11+/−</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>57</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>abcb11−/−</td>
<td>0</td>
<td>2</td>
<td>48</td>
<td>48</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

m/z: mass-to-charge ratio; subscripts indicate the number of hydroxyl groups in a bile acid.

For m/z 482, 498, 514, 530, 546, and 407, the data are reported as the percentage of the total bile acid peak area.

Fig. 4. Histopathological changes in the livers of mice fed a 0.02% UDCA diet for 108 days. A and C: liver sections from an abcb11−/− female mouse, where evident ductular proliferation can be seen (arrows). B: liver section of WT female control showing normal morphology after UDCA feeding. Hematoxylin and eosin stain. Magnifications: A and B, ×10; C, ×40.
acids across the canaliculus. In abcb11−/− mice, TUDC was largely retained in the liver (Fig. 1E), causing hepatotoxicity.

These findings may have important implications for the clinical use of UDCA as a choleretic and anti-inflammatory agent. UDCA is one of the most commonly used medications in patients with PFIC2, where BSEP is impaired (26). Although it is not known whether UDCA conjugates are transported exclusively by BSEP protein in humans, in severe PFIC2 cases where BSEP protein is not detected by immunochemical staining, UDCA treatment was found to be ineffective (34). In these cases, UDCA administration resulted in very high serum bile acid levels (>1,000 μmol/l), which led in turn to increased urinary clearance of its sulfate conjugate (15). On the other hand, in patients carrying milder ABCC11 mutations, such as some missense point mutations, UDCA was effective in slowing the progression of cholestasis (36), suggesting that UDCA treatment works more effectively in patients with residual BSEP activities. Thus the clinical observations are consistent with our findings in mice, indicating that BSEP function is the major requirement for efficient biliary secretion of UDCA N-acylamilide conjugates. The requirement for a functional BSEP for transport of UDCA and its conjugates suggests that a more detailed understanding of the effect of different BSEP mutations on UDCA transport may help predict the efficacy of UDCA in patients with PFIC2 and in other conditions where BSEP function is impaired. The use of UDCA in patients without BSEP function may be contraindicated. Notably, when UDCA was used at a high dose, in a clinical trial for primary sclerosing cholangitis, patients displayed significantly higher mortality, leading to premature termination of the trial (21, 31).

When the abcb11−/− mice were generated, we were surprised to find that they displayed only a mild cholestasis (12,
This mild phenotype was attributed to two factors: first, the mice seem to have an efficient mechanism for bile acid hydroxylation, reducing bile acid toxicity by converting cholic and muricholic acids into tetrahydroxy bile acids (possibly via the induced P450 enzymes Cyp3a11 and Cyp3a16, noted in Table 3); and second, an alternative canicular transport system (P-glycoprotein, the product of the Mdr1 genes) was upregulated (39), preferentially transporting tetrahydroxy bile acids and \( \beta \)-muricholate and allowing for a significant level of residual bile acid-dependent bile flow (18, 40, 41). These two factors, reducing the toxicity of accumulated bile acids through hydroxylation and canicular secretion of the polyhydroxy bile acids by an alternative transport system, resulted in a mild phenotype in the \( abcb11^{-/-} \) mice compared with their human genetic disease equivalent, PFIC2. A recent report by Zhang et al. (42) suggests that other factors, such as deficiency in \( \beta \)-oxidation of fatty acids, may contribute to the more progressive cholestatic phenotype observed in their strain of \( abcb11^{-/-} \) mice, developed on a pure C57Bl/6 genetic background.

Because of the known ability of ABC transporters to interact with multiple substrates (see Ref. 30, for example), a number of ABC transporters in the canicular membrane should also be regarded as having the potential to act as alternative transporters of bile acids. Megaraj et al. (22) reported that \( 3\alpha,6\alpha,7\alpha,12\alpha \)-tetrahydroxy bile acid (6-0H-TC) could be transported by several murine hepatic transporters and that Mrp2 displayed a lower apparent \( K_m \) for this substrate than did Mdr1a or Bsep in SF9 insect membrane vesicles (22). Decreased biliary secretion of 6-0H-TC was also observed in mrp2 \(-/-\) mice, and this could be further inhibited by the Mdr1 inhibitor GF120918, suggesting that both ABC transporters have broad implications for the use of UDCA in cholestatic diseases where BSEP function is impaired.

In conclusion, data presented in the current study indicate that cholestatic stress in \( abcb11^{-/-} \) mice was not alleviated by feeding them UDCA. Instead, the opposite occurred: UDCA supplementation caused adverse effects. These findings may have broad implications for the use of UDCA in cholestatic diseases where BSEP function is impaired.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: R.W. and V.L. conception and design of research; R.W., L.L., D.N.F., and L.R.H. performed experiments; R.W., L.L., D.N.F., and L.R.H. analyzed data; R.W., J.A.S., A.F.H., and V.L. interpreted results of experiments; R.W. and D.N.F. prepared figures; R.W. drafted manuscript; R.W., J.A.S., A.F.H., and V.L. edited and revised manuscript; R.W. and V.L. approved final version of manuscript.

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