Ursodeoxycholate modulates bile flow and bile salt pool independently from the cystic fibrosis transmembrane regulator (Cftr) in mice

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Bodewes FAJA, Wouthuizen-Bakker M, Bijnvels MJ, Havinga R, de Jonge HR, Verkade HJ. Ursodeoxycholate modulates bile flow and bile salt pool independently from the cystic fibrosis transmembrane regulator (Cftr) in mice. Am J Physiol Gastrointest Liver Physiol 302: G1035–G1042, 2012. First published February 2, 2012; doi:10.1152/ajpgi.00258.2011.—Cystic fibrosis liver disease (CFLD) is treated with ursodeoxycholate (UDCA). Our aim was to evaluate, in cystic fibrosis transmembrane regulator knockout (Cftr−/−) mice and wild-type controls, whether the supposed therapeutic action of UDCA is mediated via choleretic activity or effects on bile salt metabolism. Cftr−/− mice and controls, under general anesthesia, were intravenously infused with tauroursodeoxycholate (TUDCA) in increasing dosage or were fed either standard or UDCA-enriched chow (0.5% wt/wt) for 3 wk. Bile flow and bile composition were characterized. In chow-fed mice, we analyzed bile salt synthesis and pool size of cholate (CA). In both Cftr−/− and controls intravenous TUDCA stimulated bile flow by ~250% and dietary UDCA by ~500%, compared with untreated animals (P < 0.05). In non-UDCA-treated Cftr−/− mice, the proportion of CA in bile was higher compared with that in controls (61 ± 4 vs. 46 ± 4%; P < 0.05), accompanied by an increased CA synthesis (16 ± 1 vs. 10 ± 2 mmol·h−1·100 g body wt (BW)−1; P < 0.05) and CA pool size (28 ± 3 vs. 19 ± 1 mmol/100 g BW; P < 0.05). In both Cftr−/− and controls, UDCA treatment drastically reduced the proportion of CA in bile below 5% and diminished CA synthesis (2.3 ± 0.3 vs. 2.2 ± 0.4 mmol·day−1·100 g BW−1; nonsignificant) and CA pool size (3.6 ± 0.6 vs. 1.5 ± 0.3 mmol/100 g BW; P < 0.05). Acute TUDCA infusion and chronic UDCA treatment both stimulate bile flow in cystic fibrosis conditions independently from Cftr function. Chronic UDCA treatment reduces the hydrophobicity of the bile salt pool in Cftr−/− mice. These results support a potential beneficial effect of UDCA on bile flow and bile salt metabolism in cystic fibrosis conditions.

ursodeoxycholic acid; cystic fibrosis liver disease; mouse model; bile salt kinetics

Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (Cftr) gene, which result in dysfunction of the CFTR protein (7, 23). CFTR is a cAMP-activated chloride channel and is present in the apical membrane of various epithelial and nonepithelial tissues (26, 27, 46). Cirrhotic CF liver disease (CFLD) develops in ~5–10% of CF patients and is the second leading cause of mortality in CF patients (10, 28, 36). Absence of functional CFTR in biliary epithelium is thought to initiate abnormal secretin/cAMP-stimulated chloride and bicarbonate secretion, leading to decreased bile flow and bile duct plugging by thickened secretion. Secondary cholangiocyte and hepatocyte injury can ultimately lead to the development of cirrhosis (14).

Treatment with ursodeoxycholate (UDCA) is applied for different cholangiopathies, including CFLD. Although routinely applied in CF patients with increased levels of liver function parameters in serum, the therapeutic action of UDCA in CF conditions remains unclear. It has been hypothesized that UDCA is beneficial by its choleretic activity and/or its capacity to correct aberrant bile salt metabolism (21). Indeed, it has been shown that UDCA stimulates biliary secretion of bile acids in patients with primary biliary cirrhosis and primary sclerosing cholangitis (29, 30). However, there are only a few trials assessing the efficacy of UDCA for the treatment of CFLD, and currently there is insufficient evidence to justify the routine use of UDCA in CF (5).

Shimokura et al. (40) demonstrated in biliary cells that UDCA, in pharmacological concentrations, increased intracellular calcium and induced chloride efflux. These scientists speculated that UDCA increased bile flow by direct stimulation of ductular secretion, which could be of therapeutic benefit for patients with CF who have impaired cyclic AMP-dependent biliary secretion (40). However, Fiorotto et al. (13) suggested that UDCA induced bile flow in a Cftr-dependent manner. They showed that UDCA stimulated cholangiocyte fluid secretion in vitro in bile duct units and in isolated perfused livers from wild-type, but not from Cftr, knockout mice. UDCA induced a Cftr-dependent ATP release, which, by activating the purinergic signaling pathway, induced cholangiocyte secretion by stimulation of calcium-activated chloride channels (CaCC). These in vitro and ex vivo observations clearly supported the concept that UDCA-induced fluid secretion is Cftr dependent and that, in CF conditions, UDCA-induced cholangiocyte cholestasis cannot be achieved.

An alternative beneficial effect of UDCA in CF conditions could be related to changes in bile salt metabolism. It is known that the size and composition of the bile salt pool is critical for adequate bile formation (18). In addition, a more hydrophobic bile salt composition is suggested to be toxic to the hepatobiliary tract and might contribute to the development of CFLD (1). Freudenberg et al. (16) reported an increased biliary hydrophobicity index in Cftr508/508 mice compared with controls. Different effects are published on the influence of UDCA on bile salt metabolism. Frenkiel et al. (15) reported that UDCA reduced the cholate pool size and cholate synthesis rate in patients with gallstones and decreased the hydrophobicity of bile. In contrast, Beuers et al. (2) described that UDCA did not decrease the bile salt pool size in patients with cholestatic liver disease. In UDCA-treated patients with CFLD, duodenal bile became enriched with the conjugated species of UDCA (ac-
counting for 3–12% of the total biliary bile salts), indicating an increased hydrophilic bile composition (8). Thus far, the effect of UDCA on bile salt metabolism under CF conditions has not been conclusive.

We have reasoned that insights in the effects of UDCA on bile production, cholate biosynthesis rate, and cholate pool size under controlled in vivo CF conditions have been lacking. In the present study, we aimed to overcome this knowledge gap by exploiting recently developed techniques, allowing the study of these parameters in small experimental animals (22, 39). We determined the effect of UDCA on bile flow and bile salt metabolism in Cfrt knockout mice and wild-type littermates during acute intravenously tauroursodeoxycholate (TUDCA) infusion and after chronic dietary administration of UDCA. Although biliary phenotypes have been described in different CF mouse models, the C57Bl/6; 129 Cfrt–/– mice are not reported to exhibit related gallbladder or liver abnormalities (12, 16, 38). The lack of hepatobiliary abnormalities allows us to exclusively investigate the action UDCA under complete Cfrt null conditions, without the secondary interference of cholestasis on biliary parameters.

MATERIAL AND METHODS

Animals and diets. C57Bl/6;129 Cfrt–/– and Cfrt+/+ littermates were bred and accommodated at the Animal Experimental Center of the Erasmus Medical Center in Rotterdam, The Netherlands. Southern blotting of tail-clip DNA was performed to verify the genotype of individual animals (38). In accordance with previous studies, the Cfrt–/– mice did not exhibit CF liver or gallbladder abnormalities (data not shown). Mice were housed in a light-controlled (lights on 6 AM to 6 PM) and temperature-controlled (21°C) facility and were allowed access to tap water and a semisynthetic chow diet (SRM-A; Hope Farms BV Woerden, The Netherlands) from the time of weaning. All experiments were performed on female and male animals of 10–20 wk of age. Group size varied per experiment from five to nine animals per genotype. Experimental protocols were approved by the Ethical Committee for Animal Experiments of the Erasmus Medical Center.

Experimental procedures. To evaluate the effect of UDCA on bile production and bile composition, we used a mouse bile duct cannulation experimental model. This model provides the option to measure bile production over an extended period of time. The animals were placed in a temperature and humidity controlled incubator. Bile was collected after surgical ligation of the common bile duct and cannulation of the gallbladder using polyethylene tubing under intraperitoneal anesthesia with fentanyl-fluanisone 1 mg/kg BW and diazepam (10 mg/g BW), as previously described (25). Bile secretions were collected in 15-min fractions during the stepwise dose increase phase and in 10-min fraction at the highest dose (600 nmol/min) for 60 min. Bile samples were used for bile salt composition analysis. Bile flow rate was assessed gravimetrically, assuming a density of 1 g/ml.

Acute intravenous TUDCA administration. We performed an acute bile salt infusion experiment to evaluate the dose-response effect of acute UDCA supplementation on bile flow and bile salt composition in Cfrt–/– and control mice. Acute TUDCA administration provides the possibility to measure the direct choleretic effects of bile salts without the possible interfering effects of adaptations to long-term bile salt administration. We infused taurine-conjugated UDCA (TUDCA) for our acute infusion experiment, to closely mimic the physiological condition in which bile salts are secreted into bile almost exclusively in conjugated form. An intravenous line was placed in the jugular vein, and the gallbladder was cannulated. After equilibration of the bile flow for 5–10 min, spontaneous bile production was assessed for 30 min, i.e., without TUDCA infusion. Subsequently, TUDCA solution (43 mM dissolved in phosphate-buffered saline, pH 7.4) was administered using an intravenous pump (47). The TUDCA dosage was increased every 30 min in a stepwise manner (dosage steps 150, 300, 450, and 600 nmol/min). The maximal dosage was given for 60 min. During TUDCA infusion, bile was collected in 15-min fractions.

Chronic dietary UDCA administration. In CF patients, UDCA supplementation is given chronically via the enteral route (8). Analogous to the human situation, we evaluated the effect of chronic enteral (dietary) UDCA treatment in Cfrt–/– and control mice compared with untreated animals. Mice were fed either a control diet consisting of standard chow or the same diet enriched with UDCA (0.5% w/wt) for 3 wk. Body weight was measured after the diet period. After gallbladder cannulation, spontaneous bile production was determined by bile collection for 30 min. We additionally determined cholate synthesis and pool size, using a previously developed and validated stable isotope dilution technique (22). In short, 3.0 mg of [3H]cholate in a solution of 0.5% NaHCO3 in phosphate-buffered saline was slowly injected via the penile vein during isoflurane anesthesia. Blood samples were taken before injection and at 12, 24, 36, 48, 60, and 72 h after injection. Blood samples (100 μl) were collected by tail bleeding. Blood was collected in EDTA tubes and centrifuged to obtain plasma. After centrifuging (3,000 rpm for 10 min at 4°C), plasma was stored at −20°C until analysis. At the last day of the experiment (72 h), mice were anesthetized and equipped with a catheter in the bile duct, as described above. Subsequently, bile was collected for 30 min, after an initial equilibration period of 5–10 min. Animals were euthanized by heart puncture.

Analytic procedures. Biliary bile salt concentrations were determined by an enzymatic fluorimetric assay (32). Biliary bile salt composition was determined by capillary gas chromatography (24). The hydrophobicity of bile salts in bile was calculated according to the Heunen index, based on the fractional contribution of the major murine bile salt species cholate, chenodeoxycholate, deoxycholate,ursodeoxycholate, α-muricholate, and β-muricholate (17). Alanine transaminase (ALT) was determined in plasma samples. Plasma and bile samples were prepared for gas-liquid chromatography-mass spectrometry analysis on a Finnigan SSQ7000 Quadrupole GC-MS machine, as described previously by Stellard et al. (44). The isotope dilution technique is based on the dilution of a labeled tracer into the pool of the metabolite of interest. It has been demonstrated to result in virtual identical cholate synthesis rates, as obtained with a 14C-cholesterol bile salt synthesis measuring methods (34). The tracer is administered as a bolus, which mixes into the pool. Shortly after mixing, the isotopic enrichment is highest. Thereafter, the enrichment decreases due to dilution with unlabeled molecules introduced by de novo synthesis. Enrichment of [3H]cholate in plasma was determined as the increase of the M4-/M0-cholate, relative to baseline measurements, and was expressed as the natural logarithm of atom percent excess (ln APE). From the decay curve of ln APE (calculated by linear regression analysis), daily fractional turnover rate (equals the slope of the regression line) and pool size ([administered amount of label × isotopic purity × 100)/intercept of the ln APE curve] of cholate were calculated. Multiplying the pool size with the fractional turnover rate results in a value for the absolute turnover rate. In the steady-state situation, the absolute turnover rate equals the synthesis rate (22, 34, 43).

Statistical analysis. Statistical analysis was performed using SPSS version 16.0 for Windows (SPSS, Chicago, IL). All results are reported as means ± SE. Differences between genotypes or diet groups were evaluated using the Mann-Whitney U-test. The level of significance was set at a P value of <0.05.
RESULTS

The physiological state of the enterohepatic circulation in Cftr−/− mice. We evaluated bile production during the first 30 min after acute interruption of the enterohepatic circulation, i.e., closely reflecting the physiological state (represented by time points 15 and 30 min in Fig. 1, A–C). We found that bile flow [4.5 ± 0.6 vs. 4.0 ± 0.4 μl·min⁻¹·100 g BW⁻¹, nonsignificant (NS); biliary bile salt concentration (67 ± 12 vs. 54 ± 5 mM, NS), and biliary bile salt output (313 ± 72 vs. 208 ± 25 mmol·min⁻¹·100 g BW⁻¹, NS) were similar in Cftr−/− and control mice, respectively, indicating no quantitative differences in the choleretic capacity between Cftr−/− mice and their controls at baseline.

TUDCA administration increased bile flow equally in Cftr−/− and control mice in a dose-dependent manner. Infusions with TUDCA increased bile flow to a similar extent in Cftr−/− mice and controls (+ ~250%; Fig. 1A). The results indicate that TUDCA is capable of generating a bile salt-induced bile flow, independent of the expression of CFTR. The biliary bile salt concentration increased in parallel to the infused bile salt dosage (Fig. 1B). The bile salt secretion rates, i.e., the product of flow (Fig. 1A) and concentration (Fig. 1B), increased in equal pace with an increased TUDCA intravenous dosage and was not different between Cftr−/− and control mice (Fig. 1C). We performed Mann-Whitney U-tests for all individual time points during the TUDCA infusion experiments. There was no significant difference between Cftr−/− and Cftr+/+ mice at any of the individual time points for bile flow, bile salt concentration, and bile salt secretion rate. To determine the choleretic capacity of TUDCA in both Cftr−/− and control mice, we related the bile flow to the bile salt secretion rate (Fig. 1D). The choleretic capacity of TUDCA was similar in Cftr−/− and control mice, as was the estimated bile salt-dependent bile flow, based on the Y-intercepts (4.6 vs. 5.3 μl·min⁻¹·100 g BW⁻¹, NS). The increase in bile flow was linearly related to the bile salt output (control: R² 0.9, slope 0.003 μmol/mmol; Cftr−/− R² 0.9, slope 0.003 μmol/mmol).

UDCA treatment increased bile flow equally in Cftr−/− and control mice. Chronic UDCA treatment for 3 wk increased bile flow with ~500% compared with untreated mice (P < 0.05). This increase was comparable for Cftr−/− and control mice (29.0 ± 2.6 vs. 31.0 ± 1.9 μl·min⁻¹·100 g BW⁻¹; NS; Fig. 2A). Chronic UDCA treatment decreased the total biliary bile salt concentration in both genotypes, but to a lower extent in Cftr−/− mice (Cftr−/+ −51% vs. Cftr−/− mice −37%), resulting in significantly higher bile salt concentration in Cftr−/− mice compared with controls (42 ± 3 vs. 26 ± 3 mM, P < 0.05, Fig. 2B). The bile salt output was significantly higher in Cftr−/− mice compared with controls during chronic UDCA treatment (Cftr−/−, 1,232 ± 147 vs. control, 827 ± 113 μmol-min⁻¹·100 g BW⁻¹, P < 0.05, Fig. 2C). A significant reduced growth of the Cambridge Cftr−/− mice compared with wild-type animals has been reported (38). Since the findings of the differences in the bile composition on treatment with UDCA may be affected by...
the nutritional status, we measured body weight of the treated and untreated mice (Fig. 2D). In our present experiment, the phenotype of the Cftr<sup>−/−</sup> mice includes a decreased body weight compared with controls; however, this is not affected by UDCA treatment.

Increased hydrophobic bile salt composition of Cftr<sup>−/−</sup> mice compared with control mice. In non-UDCA-treated Cftr<sup>−/−</sup> mice, the fractional biliary cholate content was higher compared with control mice (61.4 ± 1.5 vs. 46.5 ± 3.8%, P < 0.05; Table 1). The natural biliary UDCA enrichment was ~50% lower in Cftr<sup>−/−</sup> mice compared with controls (2.7 ± 0.4 vs. 6.0 ± 0.5%, P < 0.05; Fig. 2A). In non-UDCA-treated mice, β-muricholate is the major hydrophilic biliary bile salt in both Cftr<sup>−/−</sup> and control mice (42 ± 3.3 vs. 33 ± 1.6%; NS). Based on the fractional contribution of the bile salt, we calculated the biliary hydrophobicity index, according to Heuman et al. (17). The bile salt composition of non-UDCA-treated Cftr<sup>−/−</sup> mice was significantly more hydrophobic than that of the control mice (0.05 ± 0.002 vs. −0.07 ± 0.005; P < 0.01; Fig. 3B).

UDCA treatment decreased the biliary hydrophobicity of Cftr<sup>−/−</sup> mice. After UDCA treatment, the biliary bile salt composition changes extensively (Table 1). After treatment, the bile salt composition consisted of more than ~80% of UDCA in both Cftr<sup>−/−</sup> mice and controls (Fig. 3A). UDCA treatment drastically reduced the fraction of cholate in the bile to <5% in both Cftr<sup>−/−</sup> mice and controls. However, the fraction of β-muricholate was also reduced in Cftr<sup>−/−</sup> and control mice compared with the untreated animals (6.5 ± 1.8 vs. 8.7 ± 1.6%; NS). Taken together, UDCA treatment decreased the bile salt hydrophobicity index of Cftr<sup>−/−</sup> mice to wild-type levels (~0.08 ± 0.003 vs. ~0.08 ± 0.002; NS; Fig. 3B). To evaluate the potential hepatotoxic effects of UDCA and differences in biliary bile hydrophobicity, we measured plasma ALT levels in normal diet and in UDCA-treated animals (Fig. 3C). Under control diet conditions, although not significant, ALT was higher in CF than in control mice (40 ± 8 vs. 60 ± 13 U/l; NS). UDCA decreased ALT in both CF and control mice (31 ± 9 vs. 15 ± 1 U/l; NS). UDCA treatment significantly lowered plasma ALT levels in Cftr<sup>−/−</sup> mice compared with Cftr<sup>−/−</sup> on a control diet (15 ± 1 vs. 60 ± 13 U/l; P < 0.05).

UDCA treatment decreased cholate synthesis and pool size in Cftr<sup>−/−</sup> and control mice. Non-UDCA-treated Cftr<sup>−/−</sup> mice had a higher cholate synthesis rate (16 ± 1 vs. 10 ± 2 μmol·day<sup>−1</sup>·100 g BW<sup>−1</sup>; P < 0.05) and larger cholate pool size (28 ± 3 vs. 19 ± 1 μmol/100 g BW; P < 0.05) compared with controls (Fig. 4). Chronic UDCA treatment reduced the cholate pool size by ~90% in both Cftr<sup>−/−</sup> and control mice (P < 0.05). Nevertheless, the pool size of Cftr<sup>−/−</sup> mice remained higher compared with that of controls (3.6 ± 0.6 vs. 1.5 ± 0.3 μmol/100 g BW; P < 0.05). Under the assumption that steady-state conditions of the bile salt kinetics were obtained after 3 wk of treatment, we found that UDCA decreased the cholate synthet-
sis rate by ~85% in both Cfr<sup>-/-</sup> mice and controls compared with that in untreated mice (P < 0.05; Fig. 4B). Interestingly, UDCA treatment straightened out the difference in synthesis rate between Cfr<sup>-/-</sup> and control mice (2.3 ± 0.3 vs. 2.2 ± 0.4 μmol·h<sup>-1</sup>·100 g BW<sup>-1</sup>; NS).

**DISCUSSION**

The major physiological effect of UDCA is its capacity to increase bile flow. This property supports the therapeutic use of UDCA in a variety of cholangiopathies, including CFLD (37). In vitro and ex vivo studies indicated that the stimulatory effect of UDCA on cholangiocyte secretion depends on the presence of CFTR (13, 40). In the present study, we demonstrated that UDCA, in vivo, either during acute or chronic administration, induced a significant Cfr-independent increase of bile flow in mice. Therefore, our results indicate that a positive choleretic effect of UDCA can also be expected in CF conditions. UDCA treatment reduced the relative hydrophobic biliary bile salt composition in Cfr<sup>-/-</sup> mice by replacing the high percent contribution of the bile salt cholate by UDCA and by the quantitative reduction of the cholate pool size. These properties could contribute to the assumed beneficial effects of UDCA in CFLD.

Our present in vivo results are in apparent contrast to in vitro and ex vivo studies, which report on the interaction between Cfr and bile salt-stimulated biliary secretion (13, 40). In these studies, an important role is ascribed to the function of calcium-induced chloride channels in UDCA-stimulated bile flow, through the induction of purinergic signaling via Cfr-dependent ATP release. There can be several possibilities underlying the divergence of our in vivo results from the in vitro and ex vivo reported studies. First, the choleretic effect found in our in vivo studies probably predominantly reflects an osmotic, bile salt-induced canaliculare bile flow, rather than a major ductular bile flow. The canalicular bile flow may thereby predominate the Cfr-dependent secretion effect of UDCA at the level of the cholangiocytes. Second, the activation of Cfr-independent routes may differ between the in vitro and in vivo conditions. In vivo, UDCA may stimulate hepatocytes to secrete ATP into bile in a CFTR-independent manner and subsequently induce bicarbonate secretion via paracrine purinergic pathways linked to CaCC in the cholangiocytes (33). CaCCs have been suggested to play a more prominent role in epithelial fluid secretion in mice than in other species, including pigs (6). This might, therefore, explain the relatively mild phenotype in CF mouse models (31). Recently, Beuers et al. (3) postulated the “bicarbonate umbrella hypothesis” by suggesting that biliary bicarbonate secretion in humans serves to maintain an alkaline environment in the small intestine.

**Fig. 4.** Cholate pool size (A) and cholate synthesis (B) after chronic dietary UDCA administration. Cfr<sup>-/-</sup> knock mice (*Cfr<sup>-/-</sup>*) and control littersmotes (*Cfr<sup>+/+</sup>*) were intravenously injected with 2H<sub>4</sub>-cholate after a normal or 0.5% UDCA chow diet for 3 wk. Enrichment of the administered 2H<sub>4</sub>-cholate was determined in plasma until 72 h after the administration of the label. From the plasma decay curve, cholate pool size (A) and cholate synthesis rate (B) were calculated, as detailed in the MATERIALS AND METHODS. Values are means ± SE of 4–6 mice per group. *P value < 0.05.
pH near the apical surface of hepatocytes and cholangiocytes to prevent the uncontrolled membrane permeation of protonated glycine-conjugated bile. In this concept, the bile acid receptor TGR5 (G protein-coupled bile acid receptor-1), localized on the tip of the cilia of apical cholangiocyte membranes in mice and humans could trigger a Cfrt-independent signaling cascade resulting in cholangiocyte secretion.

During chronic UDCA administration, a CF biliary phenotype became apparent: an increased bile salt secretion rate (Fig. 2C, P < 0.05). The difference in secretion rate is based on the product of two measured parameters. Since the majority of biliary bile salts are derived from enterohepatic circulation, the most plausible explanation is an increased total bile salt pool size, which, during treatment, is predominantly accounted for by UDCA (Fig. 3A). It seems, therefore, logical to assume that the total amount of bile salts in the enterohepatic circulation is increased in CF mice. Indeed, this explanation seems to be supported by the ~50% higher cholate pool size in CF mice fed a normal diet (Fig. 5).

During bile salt treatment, however, CF and control mice were administered the same, supraphysiological dosages of TUDCA and UDCA in the acute and chronic experiment, respectively. Our laboratory previously reported an increased fecal loss of bile salts in the Cfrt−/− mice (4). Although UDCA could potentially influence intestinal fat malabsorption, we could not find an effect of UDCA treatment on the body weight in Cfrt−/− or control mice. Furthermore, we recently reported in rats that UDCA does not influence fecal fat excretion (11). Therefore, our results suggest a different bile salt kinetic steady state in CF mice, in which overcompensation of bile salt synthesis results in an enlargement of the bile salt pool. Since the higher bile salt secretion rate in CF mice was seen most prominently during chronic treatment, we speculate that CFTR is involved in adaptation of the enterohepatic circulation to chronic bile salt treatment, at the level either of the intestine, of the liver, or both.

Although differences exist in bile salt metabolism between mice and man (20), we found clear similarities between CF patients and Cfrt−/− mice in bile composition. Untreated Cfrt−/− mice (i.e., without UDCA treatment) have a more hydrophobic bile salt pool composition and an increased cholate synthesis rate and pool size compared with control mice. This is in line with the report by Freudenberg et al. (16) of the increased biliary hydrophobicity in their Cfrt−/− mice model. Similar results have been found in CF patients: Strandvik et al. (45) reported an increased proportion of primary bile salts in serum and bile of CF, which has been attributed to an enhanced bile salt biosynthesis in response to increased fecal bile salt disposal. The Cfrt−/− mice used in the present study are, therefore, comparable to human CF patients in this respect.

The relative hydrophobic bile salt composition in CF has been implied in the development of liver disease in CF conditions, but definitive proof for this concept is (still) lacking (42). Nevertheless, chronic UDCA treatment is apparently capable to partially correct the hydrophobic bile salt profile in CF mice. Additionally, UDCA treatment normalized the initially increased liver function tests in CF mice, in agreement with our hypothesis.

We investigated the effect of UDCA under CF conditions in the absence of CFLD. The advantage of this approach is the ability to exclusively examine UDCA effects on several bile salt parameters during CFTR-deficient conditions, without possible interference of secondary changes due to liver disease. The major induction of bile flow and alterations in bile salt composition could contribute to a preventive role of UDCA on the development of CFLD. It is unclear whether effects on bile flow and bile salt composition can be found under conditions of CFLD. Nakagawa et al. (35) evaluated duodenal bile salt...
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composition after a 2-mo UDCA treatment in nine CF patients with CFLD. Similar to our results, the percent contribution of UDCA increased, resulting in a more hydrophilic bile salt pool. The contribution of UDCA was not as high as in our study (25%, compared with ~80% in the present study), possibly due to the relatively low dosage of UDCA that was used (10–15 mg·kg·BW−1·day−1). In patients with primary sclerosing cholangitis UDCA treatment dosage of 25–30 mg·kg−1·day−1 resulted in UDCA comprising 74% of the bile salt pool, with a corresponding cholate reduction from 29% to 6% (41). In addition, a reduction in cholate pool size and cholate syntheses is also described in gallstone patients treated with 750 mg UDCA (19). Therefore, regarding the effect of UDCA on bile composition, we have no indication that the observed effects will be absent during CF-related cholestasis or other signs of CFLD.

In conclusion, our results in mice, in vivo, indicate that UDCA exerts a choleric effect and influences the bile salt profile and synthesis, independent of the presence of functional CFTR. When extrapolated to the human situation, this might imply that UDCA treatment results both in CF and in non-CF individuals in increased choleresis, reduced bile salt synthesis, and a more hydrophilic bile salt pool. Interpretation of the present results for the human (CF) condition needs to take into account the possibility of species specificity of the observed effects. However, the outcome of this study does provide a firm experimental basis to explain the beneficial effects of UDCA observed in CF patients.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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