Substrate specificity of human ABCC4 (MRP4)-mediated cotransport of bile acids and reduced glutathione

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Rius, Maria, Johanna Hummel-Eisenbeiss, Alan F. Hofmann, and Dietrich Keppler. Substrate specificity of human ABCC4 (MRP4)-mediated cotransport of bile acids and reduced glutathione. Am J Physiol Gastrointest Liver Physiol 290: G640–G647, 2006. First published November 10, 2005; doi:10.1152/ajpgi.00354.2005.—The multidrug resistance protein ABCC4 (MRP4), a member of the ATP-binding cassette superfamily, mediates ATP-dependent unidirectional efflux of organic anions out of cells. Previous studies showed that human ABCC4 is localized to the sinusoidal membrane of hepatocytes and mediates, among other substrates, the cotransport of reduced glutathione (GSH) with bile acids. In the present study, using inside-out membrane vesicles, we demonstrated that human ABCC4 in the presence of physiological concentrations of GSH has a high affinity for the taurine and glycine conjugates of the common natural bile acids as well as the unconjugated bile acid cholate. Chenodeoxycholyltaurine and chenodeoxycholylglycine were the GSH cosubstrates with the highest affinities for ABCC4, with $K_{m}$ values of 3.6 and 5.9 µM, respectively. Ursodeoxycholyltaurine and ursodexoxycholylglycine were cotransported together with GSH by ABCC4 with $K_{m}$ values of 7.8 and 12.5 µM, respectively, but no transport of ursodeoxycholic acid and deoxycholic acid was observed. The simultaneous transport of labeled GSH and cholate or cholyglycine was demonstrated in double-labeled cotransport experiments with a bile acid-to-GSH ratio of ~1:2. $K_{m}$ values of the bile acids for ABCC4 were in a range similar to those reported for the canalicular bile salt export pump ABCB11. Under physiological conditions, the sinusoidal ABCC4 may compete with canalicular ABCB11 for bile acids and thereby play a key role in determining the hepatocyte concentration of bile acids. In cholestatic conditions, ABCC4 may become a key pathway for efflux of bile acids from hepatocytes into blood.

Bile acids are synthesized from cholesterol in hepatocytes. After their biosynthesis, bile acids undergo enterohepatic circulation mediated by both hepatocyte and enterocyte transporters (18). In the hepatocyte, several members of the human ATP-binding cassette (ABC) superfamily have been identified as bile acid transporters, including the bile acid (salt) export pump ABCB11 (BSEP), which is the major canalicular bile salt transporter in hepatocytes (4, 24), and the multidrug resistance protein ABCC4 (MRP4), which functions as a bile acid efflux pump at the sinusoidal membrane of human hepatocytes (29). Although ABCC3 (MRP3) also is localized to the sinusoidal membrane (16), it has been reported to be a relatively poor transporter for bile acids (1, 37). In addition to the export pumps, several transporters in the sinusoidal membrane of hepatocytes mediate the uptake of bile acids (9), including the Na+-taurocholate cotransporting polypeptide NTCP (SLC10A1) and the sodium-independent organic anion transport polypeptides OATP1B1 (OATP-C/OATP2) and OATP1B3 (OATP8) (18).

Hepatocytes are the major source of reduced glutathione (GSH) in plasma. As a physiological antioxidant, GSH is involved in many biochemical processes and serves as the major transport form of cysteine (5, 23). Therefore, GSH needs to be transported across the sinusoidal membrane of the hepatocyte to enable GSH delivery to other tissues (2). Three members of the human multidrug resistance protein family (ABCC/MDR) have been identified as GSH export pumps. The first is ABCB1 (MRP1; Refs. 20, 22), which is not expressed in hepatocytes in detectable amounts (14). The second is ABCC2 (MRP2), a low-affinity GSH transporter (25), which is localized to the canalicular membrane of hepatocytes (14). The third is ABCC4, which is localized to the sinusoidal membrane of hepatocytes and has been shown to function as a cotransporter for GSH and the conjugated bile acid cholate, cholate (29). In the efflux by ABCB1 and ABCC4, GSH can be replaced by several GSH analogs, including 5-methyl-glutathione and ophthalmate (22, 29).

ABCC4 has the lowest number of amino acids of the MRP family and transports a broad range of structurally diverse substances, including sulfated steroids as well as several drugs (17, 35). It is widely distributed in human epithelial cells, and in most of them, ABCC4 is localized to the basolateral membrane (19, 29, 30). An exception is the renal proximal tubular cell, in which ABCC4 has an apical localization (33). The ABCC4-mediated transport was potently inhibited by a widely used inhibitor of ATP-dependent ABC transporters, the quinoline derivative MK571, as well as by known ABCC4 substrates, such as dehydroepiandrosterone 3-sulfate, methotrexate, and folate (29).

The localization of ABCC4 to the sinusoidal membrane of human hepatocytes, together with the identification of C-tau and GSH as cosubstrates for ABCC4, suggest important functions of this export pump in the physiology and pathophysiology of the liver (29). We previously proposed that ABCC4 mediates the cycling of bile acids across the sinusoidal membrane and along the sinusoid (29). The cycling of bile acids may act to regulate the intrahepatocyte concentration of bile acids, whereas the efflux of GSH serves as a source of GSH for other tissues. Bile acid efflux also could be of major importance in case of impaired bile acid transport into bile.

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The aim of the present study was to define the substrate specificity of ABCC4 toward common natural conjugated and unconjugated bile acids, as well as to quantify the stoichiometry of bile acid-GSH cotransport.

MATERIALS AND METHODS

Materials. [22,23-3H]deoxycholylglycine (DC-gly; 1.1 TBq/mmol), [22,23-3H]cholylglycine (CG; 0.4 TBq/mmol), [22,23-3H]cholyltaurine (CT; 0.4 TBq/mmol), [22,23-3H]taurine (T; 0.4 TBq/mmol), [22,23-3H]urodeoxycholylglycine (UDC-gly; 0.4 TBq/mmol), [22,23-3H]urodeoxycholyltaurine (UDC-tau; 0.4 TBq/mmol), [22,23-3H]cholate (0.9 TBq/mmol), [22,23-3H]ursodeoxycholyltaurine (UDCA; 0.4 TBq/mmol), and [22,23-3H]deoxycholate (1.1 TBq/mmol) were synthesized at the University of California, San Diego as described previously (32). [3H(G)-cholyltaurine (C-tau; 129.5 GBq/mmol), [24-14C]C-tau (1.7 GBq/mmol), [Glycine-1-14C]cholesteryl cholate (CHC; 1.1 TBq/mmol), and [glycine-2-3H]glutathione (GSH; 1.9 TBq/mmol) were obtained from PerkinElmer Life Science (Boston, MA). [3H]deoxycholate (1.1 TBq/mmol) were prepared by rinsing with incubation buffer and were centrifuged at 400 g for 3 min at 4°C immediately before use. Aliquots (10 μl) of the incubations were taken at the times indicated and diluted with liquid scintillation fluid, and counted for radioactivity. For the counting in the double-labeling experiments, a program was used that measures [14C] and [3H] radioactivity simultaneously. With the use of this program, the spill-over of [14C] into the [3H] channel and vice versa was <5% and 0.01%, respectively. The transport rates were calculated as mentioned above.

For determination of kinetic constants, transport rates were measured at five different substrate concentrations (2–50 μM bile acid). K_{in} values were determined as the substrate concentration at half-maximal velocity of transport under these conditions with the use of double-reciprocal plots and direct curve fitting to the Michaelis-Menten equation.

RESULTS

ATP-dependent transport of bile acids into membrane vesicles from transfected V79 cells. The ATP-dependent accumulation of [3H]-labeled bile acid at the standard concentration of 5 μM in membrane vesicles from ABCC4- and vector-transfected cells was negligible in the absence of GSH compared with the presence of GSH for all tested bile acids (Fig. 1, B, D, and F, and Fig. 2B). The presence of 5 mM GSH caused an increase of the ATP-dependent [3H]-labeled bile acid accumulation in membrane vesicles from ABCC4-transfected cells (Fig. 1, A, C, and E and Fig. 2A). Although membrane vesicles from vector-transfected cells also showed an ATP-dependent bile acid transport in the presence of GSH, transport mediated by the ABCC4-containing membrane vesicles was at least twofold higher, as shown in Table 1. No stimulation was observed in the presence of 1 mM DTT (not shown). Transport by the control membrane vesicles was consistent with the presence of endogenous hamster Abcc4 in the V79 cells. Although hamster Abcc4 in V79 cells could not be detected at the protein level by currently available antibodies, the presence of hamster Abcc4 mRNA in V79 cells was detected with RT-PCR using Abcc4-specific primers (not shown).

Vesicles from ABCC4-transfected V79 cells transported the following bile acids in a GSH-dependent manner as summarized in Table 1: for DC-gly, the rate was 31.6 pmol·mg protein^{-1}·min^{-1} (Fig. 1C); for CDC-gly, 35.7 pmol·mg protein^{-1}·min^{-1} (Fig. 1E); and for CDC-tau, 51.9 pmol·mg protein^{-1}·min^{-1}. For UDC-gly, the rate was 33.8 pmol·mg protein^{-1}·min^{-1}, and for UDC-tau, 36.8 pmol·mg protein^{-1}·min^{-1} (Fig. 2A). For trihydroxy bile acids, the rate for C-gly was 19.4 pmol·mg protein^{-1}·min^{-1}, and that for C-tau was 47.1 pmol·mg protein^{-1}·min^{-1}. The unconjugated bile acid cholate was transported at a rate of 8.9 pmol·mg protein^{-1}·min^{-1} (Fig. 1A). However, no transport could be
detected for the unconjugated dihydroxy bile acids UDCA and deoxycholate in either ABCC4-containing vesicles or control vesicles under our experimental conditions. Transport rates of vesicles from vector-transfected V79 cells for the corresponding bile acids in the presence of GSH were much smaller, as summarized in Table 1.

Kinetic analysis of ABCC4-mediated ATP-dependent bile acid transport in the presence of glutathione. To further characterize the affinity of ABCC4 for the different bile acids, we determined \( K_m \) and \( V_{max} \) values in ABCC4-containing vesicles for each bile acid in a concentration range from 2 to 50 \( \mu \)M and in the presence of 5 mM GSH (Table 2). CDC-tau was the bile acid with the lowest \( K_m \) value of 3.6 \( \mu \)M, followed by CDC-gly with a \( K_m \) value of 5.9 \( \mu \)M. DC-gly and C-tau showed \( K_m \) values of 6.7 and 7.7 \( \mu \)M, respectively. The taurine and glycine conjugates of UDCA exhibited different \( K_m \) values, with higher affinity of ABCC4 for UDC-tau (7.8 \( \mu \)M) than for UDC-gly (12.5 \( \mu \)M; Table 2 and Fig. 2C). The lowest affinity was observed for cholate (14.8 \( \mu \)M) and C-gly (25.8 \( \mu \)M; Table 2). Because membrane vesicles with similar transport rates for all bile acids, and thus similar \( V_{max} \) values, were used, the transport efficiency could be calculated for each bile acid (Table 2). The ratio \( V_{max}/K_m \) obtained for the various bile acids resulted in the following ranking from high to low transport efficiency: CDC-tau > C-tau > DC-gly > UDC-tau > CDC-gly > UDC-gly > C-gly > cholate (Table 2).

Ursodeoxycholate conjugate-mediated inhibition and its kinetic characterization. ATP-dependent transport of the unconjugated bile acid UDCA into membrane vesicles from transfected cells was not detectable under our experimental conditions. However, ABCC4-mediated ATP-dependent transport of its taurine and glycine conjugates was observed in the presence of 5 mM GSH as noted above. Because UDCA is used widely in clinical practice to treat cholestatic liver disease, and because its dominant metabolites in humans are its glycine and taurine conjugates, we tested whether UDCA as well as UDC-
Bile acid transport in ABCC4-containing vesicles and in control vesicles

<table>
<thead>
<tr>
<th>Bile acid + 5 mM GSH</th>
<th>Transport in ABCC4-Containing Vesicles, pmol/mg protein min⁻¹</th>
<th>Transport in Control Vesicles, pmol/mg protein min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholate</td>
<td>8.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Cholylglycine</td>
<td>19.4</td>
<td>10.3</td>
</tr>
<tr>
<td>Cholyltaurine</td>
<td>47.1</td>
<td>22.1</td>
</tr>
<tr>
<td>Deoxycholylglycine</td>
<td>31.6</td>
<td>13.4</td>
</tr>
<tr>
<td>Chenodeoxycholylglycine</td>
<td>35.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Chenodeoxycholyltaurine</td>
<td>51.9</td>
<td>25.4</td>
</tr>
<tr>
<td>Ursodeoxycholylglycine</td>
<td>33.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Ursodeoxycholyltaurine</td>
<td>36.8</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Bile acid transport in the presence of 5 mM GSH was calculated from the ATP-dependent bile acid transport obtained between 1 and 10 min in ABCC4-containing vesicles and in control vesicles obtained from hamster V79 cells. Bile acid transport in control vesicles is likely due to endogenous hamster Abcc4 detected by RT-PCR. Data represent mean (SD) values from a triplicate determination reproduced independently at least once.

Table 2. Kinetic constants for ABCC4-mediated bile acid cotransport in the presence of 5 mM GSH

<table>
<thead>
<tr>
<th>Bile Acid + 5 mM GSH</th>
<th>$K_m$, μM</th>
<th>$V_{max}$, pmol/min/mg protein⁻¹</th>
<th>$V_{max}/K_m$, μmol/min⁻¹/mg protein⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholate</td>
<td>14.8 ± 0.8</td>
<td>75 ± 15</td>
<td>5.1</td>
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<tr>
<td>Cholylglycine</td>
<td>25.8 ± 0.8</td>
<td>175 ± 37</td>
<td>6.8</td>
</tr>
<tr>
<td>Cholyltaurine</td>
<td>7.7 ± 0.3</td>
<td>154 ± 10</td>
<td>20</td>
</tr>
<tr>
<td>Deoxycholylglycine</td>
<td>6.7 ± 0.6</td>
<td>116 ± 16</td>
<td>17.3</td>
</tr>
<tr>
<td>Chenodeoxycholylglycine</td>
<td>5.9 ± 0.3</td>
<td>93 ± 8</td>
<td>15.8</td>
</tr>
<tr>
<td>Chenodeoxycholyltaurine</td>
<td>3.6 ± 0.1</td>
<td>83 ± 2</td>
<td>23.1</td>
</tr>
<tr>
<td>Ursodeoxycholylglycine</td>
<td>12.5 ± 0.5</td>
<td>130 ± 15</td>
<td>10.4</td>
</tr>
<tr>
<td>Ursodeoxycholyltaurine</td>
<td>7.8 ± 0.6</td>
<td>133 ± 19</td>
<td>17.1</td>
</tr>
</tbody>
</table>

V79-ABCC4 membrane vesicles from the same membrane preparation were used for kinetic analysis of several bile acids together with 5 mM GSH. The $K_m$ values were calculated from double-reciprocal plots. Data represent mean (SD) values from 3 determinations each performed in duplicate.

Double-labeling experiments for measuring simultaneous ATP-dependent transport of [3H]glutathione and [14C]-labeled bile acids into vesicles from transfected V79 cells. To demonstrate the cotransport of GSH and bile acids, we measured the simultaneous ATP-dependent transport of 5 mM [3H]GSH with 5 μM [14C]C-tau or that of 5 mM [3H]GSH with different concentrations of [14C]C-gly (Fig. 3). High background of the
GSH transport into vesicles from vector-transfected cells was obtained, which is probably due to other endogenous GSH efflux mechanisms present in all cells.

The following transport rates were obtained in a 20-min time period for 5 mM GSH and 5 μM C-gly in double-labeling experiments: for GSH transport, 122.9 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 41.4 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells (Fig. 3A); and for C-gly transport, 7.1 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 3.4 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells (Fig. 3B). From these transport rates, ABCC4-mediated transport rates were subsequently obtained: 81.5 pmol·mg protein⁻¹·min⁻¹ for GSH and 3.7 pmol·mg protein⁻¹·min⁻¹ for C-gly, resulting in an approximate C-gly-to-GSH ratio of 1:22. When double-labeling experiments were performed at 5 mM GSH and 10 μM C-gly, the approximate C-gly-to-GSH ratio was maintained at 1:19 when the ABCC4-mediated transport was measured within the first 20 min: 157.6 pmol·mg protein⁻¹·min⁻¹ for GSH and 8.2 pmol·mg protein⁻¹·min⁻¹ for C-gly. At higher C-gly concentrations, the relative amounts of GSH and C-gly coefflux were not constant; however, much more GSH than C-gly was consistently cotransported.

When C-gly was replaced with C-tau, the cotransport with GSH was corroborated by the following transport rates: for GSH transport, 110.2 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 9.7 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells; and for C-tau transport, 10.3 pmol·mg protein⁻¹·min⁻¹ in vesicles from ABCC4-transfected V79 cells and 5.5 pmol·mg protein⁻¹·min⁻¹ in vesicles from vector-transfected V79 cells (not shown). The resulting ABCC4-mediated C-tau-to-GSH ratio was 1:21. Under the same conditions, ATP-dependent transport of 5 mM [³H]GSH in the absence of bile acids into membrane vesicles from vector- and ABCC4-transfected V79 cells was detected with similar transport rates: 3.2 pmol·mg protein⁻¹·min⁻¹ for vector-transfected cells and 2.0 pmol·mg protein⁻¹·min⁻¹ for ABCC4-transfected cells (not shown).

**DISCUSSION**

In the present study, we have examined in detail the bile acid specificity for GSH-bile acid cotransport by human ABCC4, an export pump localized to the sinusoidal membrane of human hepatocytes. Transport studies in inside-out membrane vesicles demonstrated ATP-dependent transport into ABCC4-containing membrane vesicles for the glycine- and taurine-conjugated bile acids predominating in human biliary bile acids, as well as for the conjugates of UDCA and the unconjugated bile acid cholate in the presence of physiological GSH concentrations (Figs. 1 and 2, Table 1). Whether other known ABCC4 substrates can influence GSH transport or whether their transport is modulated by GSH is currently unknown.

The $K_m$ values obtained for all bile acids in the presence of GSH were in the micromolar range and thus in the range of physiological concentrations (Table 2). The highest affinity was observed for CDC-tau with a $K_m$ value of 3.6 μM and the lowest affinity for C-gly with a $K_m$ value of 25.8 μM (Table 2). UDC-gly and UDC-tau, the amidated conjugates of UDCA, showed $K_m$ values of 7.8 and 12.5 μM (Fig. 2C), respectively, which are in the range of $K_m$ values obtained for most of the naturally occurring bile acids in humans (Table 2). CDC-tau followed by C-tau had the highest transport efficiency in ABCC4-containing membrane vesicles (Table 2). Transport affinities of all bile acids were comparable and almost identical to those obtained for the canalicular bile salt export pump ABCB11 (4, 24) with the difference that ABCC4-mediated bile acid transport is a GSH-dependent transport process. Thus the kinetic analysis reveals that ABCC4 is able, at least in principle, to compete with ABCB11 for bile acids in hepatocytes.

UDCA is widely used for the treatment of cholestatic liver diseases (26). In the hepatocyte, UDCA is rapidly conjugated with glycine and taurine. These conjugates may protect the liver against hepatotoxicity of endogenous bile acids by a variety of mechanisms (3). Although UDCA is present in human bile only in trace amounts, treatment with UDCA increases its concentration in plasma and bile, and UDCA becomes the predominant circulating bile acid (3). Although our data indicate that ABCC4 mediates ATP-dependent transport of UDCA and UDC-gly (Fig. 2A and C), transport of labeled UDCA could not be demonstrated using membrane vesicles for reasons that need to be defined in future studies. However, UDCA was able to compete for the ABCC4-mediated ATP-dependent transport of C-gly (Fig. 2D), suggesting that UDCA also may be a substrate for the ABCC4-mediated GSH cotransport. Thus our results indicate that ABCC4 can...
contribute to the increase of UDCA conjugates, and possibly that of UDCA, in plasma during UDCA treatment of cholestatic liver diseases.

In an earlier study in our laboratory (29), measurements of ATP-dependent transport using $^3$H-labeled GSH in the presence of unlabeled C-tau suggested that ABCC4 cotransports GSH along with C-tau. Several other studies on ABCC1 (MRP1), which cannot be detected in human hepatocytes (14), have described the cotransport of GSH and several substances, including vinceristine (20, 21). It has been proposed for ABCC1 that the protein contains a bipartite binding site for hydrophobic and anionic moieties (10). In our present transport system, ABCC4 may bind GSH as an anionic moietiy, inducing a conformational change that allows the binding of bile acids to the other binding site, with this in turn leading to the transport of both cosubstrates. Direct evidence for cotransport has now been provided by double-labeling experiments, in which membrane vesicles were simultaneously incubated with both substrates, radiolabeled with different isotopes, i.e., in our study, $[^3]$H[GSH and $[^1]$C[C-tau or $[^1]$C[C-gly. These experiments demonstrated ABCC4-mediated simultaneous ATP-dependent transport of both compounds (Fig. 3, A and B). GSH cotransport was measured at a concentration of 5 mM, which is within the concentration range (1–10 mM) found in cells (2). This concentration was chosen on the basis of our earlier kinetic analysis (29), which indicated a $K_m$ value of 2.7 mM for GSH in the presence of 5 $\mu$M C-tau. Although glutathione S-transferases show dissociation constants between 10 and 200 $\mu$M for GSH (7), only a small proportion of hepatic GSH is used for glutathione S-transferase reactions (28), and the major proportion of hepatic GSH is effluxed across the basolateral membrane of hepatocytes (11).

For C-tau as well as for C-gly at concentrations between 5 and 10 $\mu$M and a constant concentration of 5 mM GSH, the bile acid-to-GSH ratio of $\sim 1:22$ indicates that GSH is cotransported along with bile acids at a far greater transport rate than the bile acids themselves. At higher C-gly concentrations, the bile acid-to-GSH ratio was no longer constant, but the amounts of GSH transported were invariably greater than the amounts of bile acid transported. Thus this transport process can be described as an obligatory coefflux of GSH and bile acids without a strict stoichiometry. Other cotransporters have been described with stoichiometries differing from 1:1, such as the human Na+-glucose cotransporter SGLT1, an apical membrane protein, which couples the transport of 2 Na$^+$ ions and 1 glucose molecule with $\sim 210$ water molecules (34).

Under physiological conditions in the hepatocyte, canicular ABCB11 mediates the secretion of bile acids into bile (Fig. 4). In addition, ABCC4 in the sinusoidal membrane of hepatocytes should mediate active efflux of GSH and bile acids into the space of Disse and thence into sinusoidal blood (Fig. 4). Bile acids can then reenter into more portalley situated hepatocytes by the Na$^+$-taurocholate cotransporting polypeptide NTCP or the sodium-independent organic anion transporting polypeptidse OATP1B1 and OATP1B3 (Fig. 4) (9). Such a reuptake of compounds from blood into the hepatocytes has been described in the isolated perfused rat liver (27). Phamacokinetic analysis of this model indicated that the net sinusoidal efflux rate of dibromosulfophthalein is the result of sinusoidal efflux and partial reuptake of dibromosulfophthalein transported into the medium. In addition, Proost et al. (27) argued that biliary excretion and sinusoidal efflux of a compound may occur at the same time because of the involvement of different intracellular compartments. Thus the study by Proost et al. supports our proposed model of bile acid percolation along the sinusoid by ABCC4 when at the same time bile acids are excreted into bile by ABCB11. The requirement of GSH for the bile acid transport by ABCC4, but not by ABCB11, may be part of a regulatory mechanism to control intracellular concentrations of bile acids. In addition, hepatic GSH is predominantly released across the basolateral membrane of hepatocytes into the blood circulation as the major source of plasma GSH, cysteine, and cystine. Thus ABCC4 can be considered as a new pathway for the efflux of GSH into the systemic circulation, as pointed out in our earlier publication (29).

Under cholestatic conditions, the concentration of substances, which are normally excreted into bile, may increase intracellularly and cause hepatotoxicity. One compensatory mechanism that prevents hepatotoxicity is the efflux of substances into blood across the sinusoidal membrane, as demonstrated for leukotriene metabolites formed in the hepatocytes of Mrp2-deficient mutant rats (8, 15) and presumably for bile acids that are excreted in urine in cholestatic liver disease.

The mechanisms that preserve hepatocytes from bile acid accumulation have been investigated in cholestatic animal models (6, 31). Mice lacking the farnesoid X receptor/bile acid receptor have a reduced expression of Abcb11 but an increased expression of Abcc4 mRNA as well as increased plasma concentrations and urinary excretion of bile acids (31). A similar adaptive response with increased Abcc4 protein expression has been observed in obstructive cholestasis in rats (6).
However, species differences must be taken into account. A recent study in patients with progressive familial intrahepatic cholestasis, who have elevated serum concentrations of bile acids, showed a marked upregulation of ABCB4 protein and ABCB4 mRNA in BSEP-deficient patients suffering from mutations in the ABCB11 gene, as well as in ABCB4 (MDR3)-deficient patients with mutations in the ABCB4 (MDR3) gene (12). On the other hand, protein expression of ABCC3, which has been proposed to mediate bile acid transport (36), was unaltered in liver samples from these cholestatic patients (12). Comparison of the affinities of both transporters, ABCC3 and ABCC4, appears to be a major player in buffering the intracellular concentration of bile acids in the hepatocyte, especially when canalicular secretion of bile acids is impaired. The molecular mechanisms by which ABCC4 is upregulated in cholestatic liver diseases are presently unknown.

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