Phosphatidylinositol 3-kinase-dependent signaling modulates taurochenodeoxycholic acid-induced liver injury and cholestasis in perfused rat livers

Christian Rust, Kris Bauchmuller, Peter Fickert, Andrea Fuchsbichler, and Ulrich Beuers

Department of Medicine II, Grosshadern, University of Munich, Munich, Germany; and Departments of Medicine and Pathology, Medical University, Graz, Austria

Submitted 1 October 2004; accepted in final form 24 February 2005

IN CHOLESTATIC LIVER DISEASES, bile acids accumulate within the liver and promote the development of liver cirrhosis and liver failure (15). Hydrophobic bile acids are known to induce both hepatocellular apoptosis and necrosis, thereby providing a cellular mechanism for bile acid-mediated liver injury (22, 25, 34). However, not all bile acids are toxic in vivo, and the rate and progression of liver injury in cholestasis likely reflects a balance between the effects of potentially toxic and nontoxic bile acids. In humans, bile acids are conjugated to glycine or taurine with the glycine conjugates predominating (14). Glycochenodeoxycholic acid (GCDCA) induces hepatocyte apoptosis by a Fas death receptor-dependent process independent of Fas ligand (8) and sensitizes hepatocytes to TRAIL-mediated apoptosis by upregulating death receptor 5/TRAIL–receptor 2 expression in vitro systems (13). In contrast, the taurine conjugate of chenodeoxycholic acid, taurochenodeoxycholic acid (TCDA), simultaneously activates a phosphatidylinositol 3-kinase (PI3-K)-dependent survival pathway that blocks the inherent toxicity of this bile acid in a rat hepatoma cell line (24). In this model, PI3-K mediates its antiapoptotic effects by activating an atypical protein kinase isoform, PKC-ζ, and the nuclear transcription factor NF-κB (24). The TCDA-induced PI3-K survival signal blocks Fas-mediated apoptosis by preventing caspase-8 activation and Bid mitochondrial translocation (29). In primary rat hepatocytes, TCDA failed to induce formation of the death-inducing signaling cascade in the absence but not in the presence of the PI3-K inhibitors (21). Thus conjugates of chenodeoxycholic acid (CDCA) exert differential effects on hepatocellular signaling cascades in vitro, which alter their inherent cytotoxicity. However, it is unclear whether taurine and glycine conjugates of CDCA exert differential effects on liver integrity also in the intact liver and, likewise, whether inhibition of PI3-K aggravates liver damage and cholestasis induced by TCDA. This study was, therefore, designed to investigate the differential effects of GCDCA and TCDA in the presence or absence of the PI3-K inhibitor wortmannin on liver injury and bile secretion in the model of the isolated perfused rat liver.

MATERIALS AND METHODS

Reagents. Anti-phospho PKB (Ser-473) antibody was from BD Pharmingen (Heidelberg, Germany), and anti-PKB antibody was from Cell Signaling (Beverly, MA). A goat anti-rabbit IgG antibody was from Bio-Rad (Munich, Germany). Immobilon-P membranes were from Millipore (Eschborn, Germany). A chemiluminescence reagent was from PerkinElmer Life Sciences (Boston, MA). Molecular weight markers were from Santa Cruz Biotechnology (Santa Cruz, CA), and Biomax MR film was from Eastman Kodak (Rochester, NY). 1-Chloro-2,4-dinitrobenzene (CDNB) was from ICN Biomedicals (Aurora, OH). Wortmannin, GCDCA, TCDA, DMSO, and all other reagents were obtained from Sigma (St. Louis, MO) and were of highest purity available.

Animals. Male Sprague-Dawley rats (229 ± 16 g) were obtained from Charles River (Sulzfeld, Germany). They were subjected to a...
12:12-h light-dark rhythm with unlimited access to rodent food and water. All animals received humane care. The study was registered with the local animal welfare committee.

Isolated rat liver perfusion. The technical procedure used has been described previously (4). Livers were perfused in a nonrecirculating fashion with Krebs-Ringer bicarbonate solution at 37°C at a constant flow rate of 4.0–4.5 ml.min⁻¹.g liver⁻¹ for 90 min. After 20 min, the PI3-K inhibitor wortmannin (or the carrier DMSO only, 0.001%, vol/vol) was continuously infused for 70 min to reach a final concentration of 100 nM in the portal vein. After 30 min, the bile acids TCDCA or GCDCA or the carrier DMSO only (0.1%, vol/vol) were infused for 60 min at a continuous rate to reach a final concentration of 25 μM in the portal vein (0.1 ml.min⁻¹.g liver⁻¹). After 40 min, CDNB, the precursor of 2,4-dinitrophenyl-5-glutathione (DNP-GS), was infused for 10 min to reach a final concentration of 30 μM in the portal vein, at which saturation of biliary DNP-GS secretion was observed in the perfused rat liver (32).

Hepatopatuitic efflux of lactate dehydrogenase (LDH) and alanine aminotransferase (ALT) as indicators of liver cell damage were measured by standard enzymatic tests (2). Biliary secretion of the model Mrp2 substrate, DNP-GS, was determined photometrically (3); in brief, 5 μl of bile were added to 1,000 μl of H₂O in a cuvette. Absorption was measured at 335 nm, and biliary DNP-GS levels (nM) were calculated. The low background absorption at 335 nm of the bile sample collected just before infusion of CDNB was set as 0. Bile flow was measured gravimetrically in pretared tubes.

Immunoblot analysis. Shock-frozen tissue was homogenized in ice-cold lysis buffer (20 μM Tris-HCl, 150 μM NaCl, 1% Triton X-100, 1 μM EDTA, 1 μM EGTA, 2.5 μM Na₃P₂O₇, 1 μM glyceroatte, 200 μM PMSF, 100 μM Na₃VO₄, 500 μM okadaic acid, 5 μl aprotinin, 23.8 μl leupeptin; pH 7.5) and centrifuged for 5 min at 16,500 g. The supernatant was resolved by 10% SDS-PAGE, transferred to Immobilon-P membranes, and probed with phospho-PKB (AktSer-473) antibodies at a dilution of 1:1,000 overnight. Membranes were stripped and reprobed with a PKB/Akt antibody (1:1,000) and then visualized using a chemiluminescence reagent (Hy-Q). Horseradish peroxidase-conjugated goat anti-rabbit IgG antibody was incubated at a dilution of 1:1,000 overnight. Membranes were stripped and reprobed with a PKB/akt antibody (1:1,000) to detect total PKB/akt in an identical procedure. Peroxidase-conjugated goat anti-rabbit IgG antibody was incubated at a dilution of 1:1,000 overnight.

Immunofluorescence microscopy for activated caspase-3 and cytokeratin intermediate filament alterations. Activated caspase-3 and cytokeratin intermediate filament (CK-IF) alterations typical for apoptotic cell death were studied on frozen liver sections as described previously (9). For quantification, caspase-3-positive hepatocytes with concomitant CK-IF breakdown were counted in 20 different high-power fields per sample and expressed as x-fold increase over control.

Determination of bile acids. CDCA concentrations in the hepatopatuitic effluvate as well as cholic acid and muricholic acid concentrations in rat bile were determined as described previously (28). Briefly, bile acids in hepatopatuitic effluvates were extracted with Bond-Elut C18 cartridges (Analytichem International, San Diego, CA). In rat bile samples, bile acids were extracted with ethanol. The remainder of the procedure was identical for all samples. Enzymatic hydrolysis was performed to deconjugate bile acid amidates. Deconjugated bile acids were isolated by extraction on Lipidex 1000 (Packard Instruments, Groningen, The Netherlands) and were then methylated and trimethylsilylated for gas chromatography. Capillary gas chromatography was performed using a Carlo Erba Fractovap 4160 gas-chromatograph (Carlo Erba Instruments, Hofheim, Germany). Bile acid derivaties were separated on a fused silica capillary CP Sil 19 CB column (Chrompack, Middelburg, The Netherlands). Eluting bile acid derivaties were detected by a flame ionization detector.

Statistics. Results from at least three independent experiments are expressed as means (SD). Differences between groups were compared using an ANOVA for repeated measures and a post hoc Bonferroni analysis to test for multiple comparisons.

RESULTS

Do GCDCA and TCDCA differently induce liver injury in isolated perfused rat livers? TCDCA caused a moderate increase of LDH and ALT efflux (P < 0.05 vs. control; each; Fig. 1). GCDCA, however, induced a marked increase of LDH and ALT efflux, which was 6.5- and 5.4-fold, respectively, that of TCDCA-induced enzyme release (P < 0.01 vs. TCDCA, each; Fig. 1).

In TCDCA-perfused livers, apoptotic hepatocytes were 3.1-fold increased over controls (Fig. 2A). In contrast, extensive hepatocellular apoptosis with marked caspase-3 activation and characteristic breakdown of the cytokeratin intermediate filament network was observed in livers exposed to GCDCA making a reliable quantitation impossible (>100-fold above controls; Fig. 2A).

Do CDCA conjugates activate PI3-K activity in the intact liver? Because TCDCA activates a PI3-K-dependent survival signal in vitro (24) we studied whether this lipid kinase is also activated in the intact liver. PKB (PKB/Akt) is a downstream target of PI3-K, and PKB/Akt phosphorylation, at its Ser-473 residue, has been shown to be a sensitive readout for PI3-K activity (10, 33); PKB/Akt phosphorylation was, therefore, used to assess PI3-K activity. Indeed, TCDCA readily activated PI3-K as demonstrated by PKB/Akt phosphorylation. TCDCA increased PKB/Akt phosphorylation to 298% of control levels (P < 0.05, n = 6) whereas wortmannin, a potent PI3-K inhibitor (30), did not affect PKB/Akt activity by itself (Fig. 3). Wortmannin completely blocked the TCDCA-induced stimulation of PI3-K, demonstrating the specificity of the assay. GCDCA tended to increase PKB/Akt activity (160% of control), and wortmannin tended to counteract this effect. However, these changes did not reach statistical significance. Thus TCDCA increases PI3-K activity in liver tissue to a greater extent than GCDCA, providing a possible explanation for the lower cytotoxicity of TCDCA in the used model.

Fig. 1. Taurochenodeoxycholic acid (TCDCA) and glycochenodeoxycholic acid (GCDCA) differently induce liver injury in isolated perfused rats. Rat livers were perfused with 25 μM of each bile acid or the carrier DMSO only for 60 min (starting at minute 30). Lactate dehydrogenase (LDH) and alanine aminotransferase (ALT) release into the hepatopatuitic effluvate were determined photometrically after 55 min of bile acid administration. GCDCA induced significantly more LDH and GPT release than TCDCA (*P < 0.01 vs. control; #P < 0.05 vs. control; + P < 0.01 vs. TCDCA). Results are expressed as the means (SD) of 6 independent experiments.
Does PI3-K modulate TCDCA-induced cytotoxicity? We next tested whether TCDCA-induced PI3-K activity might explain its reduced cytotoxicity compared with GCDCA in the intact liver. The PI3-K inhibitor wortmannin (100 nM) was administered 10 min before and during TCDCA treatment. Wortmannin alone did not induce liver injury (Fig. 4). However, when wortmannin was administered together with TCDCA, TCDCA-induced liver injury was markedly aggravated: LDH and ALT release in livers treated with TCDCA and wortmannin were up to 242 and 180%, respectively, of livers treated with TCDCA alone ($P < 0.01$, each; $n = 6$; Fig. 4). Hepatocellular apoptosis increased 3.3-fold over TCDCA alone and 10.4-fold over control (Fig. 2B). These data suggest that TCDCA is inherently toxic in the intact liver, and its cytotoxicity is partly blocked by activation of a PI3-K-dependent survival signal. Consequently, the effect of PI3-K inhibition on GCDCA-induced liver injury was investigated. Wortmannin reduced liver damage and hepatocyte apoptosis in livers treated with GCDCA: LDH was reduced by 39%, ALT by 43% ($P < 0.05$, each; $n = 6$; Fig. 4) and hepatocyte apoptosis by ~50% compared with GCDCA alone (Fig. 2B).

Bile acid-induced apoptosis requires uptake of bile acids into hepatocytes. Thus, to indirectly assess bile acid uptake in the experimental conditions under study, levels of CDCA were determined in the hepatovenous effluvate. Wortmannin tended to reduce bile acid uptake in livers perfused with both TCDCA and GCDCA as demonstrated by increased CDCA concentrations in the hepatovenous effluvate at minute 65 (Fig. 5). A similar trend was observed at minute 45 (data not shown).

**Fig. 2**. Wortmannin differently modulates bile acid-induced hepatocyte apoptosis in isolated perfused rat livers. Hepatocyte apoptosis was determined in liver tissue after 60 min by immunohistochemistry of activated caspase-3 (red), cytokeratin (CK) 18 (green), or both (yellow; due to colocalization of caspase-3 and CK 18). A: GCDCA-treated livers show massive hepatocellular activation of caspase-3 in cells with CK intermediate filament breakdown and granular cytoplasmic condensation (characteristic of apoptotic hepatocytes). In contrast, immunoreaction for activated caspase-3 and CK 18 breakdown is scarce in livers perfused with TCDCA. B: immunoreaction for activated caspase-3 is minimal in livers perfused with wortmannin (WT). In contrast, TCDCA in combination with WT markedly increased activated caspase-3 and CK 18 filament breakdown in hepatocytes, whereas GCDCA in combination with wortmannin decreased hepatocyte apoptosis. Representative pictures of 6 independent experiments are shown (magnification $\times 250$).

**Fig. 3**. TCDCA induces phosphatidylinositol 3-kinase (PI3-K)-dependent activation of protein kinase B (PKB)/Akt. PKB/Akt activity was determined in shock-frozen liver tissue using a specific pPKB (Ser-473) antibody and immunoblot analysis. In parallel, total PKB/Akt mass was determined on each blot using a nonselective PKB/Akt antibody to prove that the total amount of PKB/Akt was identical in each lane. A representative immunoblot is shown in A of which the top band represents pPKB (Ser-473), and the bottom band represents total PKB/Akt under different experimental conditions. B: activated PKB/Akt in liver tissue was quantitated by densitometry of pPKB (Ser-473). Results are expressed as means (SD) of 6 independent experiments (*$P < 0.05$ vs. control).
Fig. 4. The PI3-K inhibitor WT aggravates TCDCA-induced and alleviates GCDCA-induced liver injury. Rat livers were perfused with 25 μM TCDCA, 25 μM GCDCA, or the carrier DMSO for 60 min (starting at minute 30) in the absence or presence of 100 nM WT (starting at minute 20). After 55 min of bile acid administration, LDH and ALT activities in the hepatovenous effluent were determined photometrically. TCDCA in combination with wortmannin resulted in significantly increased LDH and GPT releases. In contrast, GCDCA-induced LDH and GPT release was alleviated by simultaneous administration of WT (P < 0.01 vs. TCDCA; +P < 0.01 vs. GCDCA).

Does inhibition of PI3-K reduce CDCA-induced cholestasis? Bile flow was 1.31 ± 0.31 μl·min⁻¹·g liver⁻¹ (n = 6) after 20 min before bile acids, wortmannin, or their carrier DMSO (0.1% vol/vol) were infused, indicating an adequate secretory capacity of livers under these experimental conditions. Addition of CDNB (30 μM for 10 min, minutes 40–50) led to a transient increase of bile flow under all experimental conditions due to the choleretic potential of the CDNB-glutathione conjugate, DNP-GS, in rat livers. In controls, bile flow was 71.5 ± 10.5 μl·50 min⁻¹·g liver⁻¹ (n = 6) after CDNB infusion. Wortmannin (100 nM) did not significantly affect bile flow (108% of controls), whereas TCDCA (25 μM) markedly reduced bile flow to 39% of controls (P < 0.05; Fig. 6A; Table 1). Wortmannin (100 nM) tended to further decrease bile flow in TCDCA-treated livers to 26% of controls (Fig. 6A; Table 1).

GCDCA (25 μM) reduced bile flow to 17% of controls (P < 0.01); this effect was significantly more pronounced than that of TCDCA (P < 0.01 vs. GCDCA). Wortmannin (100 nM) tended to further decrease bile flow in GCDCA (25 μM)-treated livers to 10% of controls (not significant vs. GCDCA; Table 1). However, this effect was less pronounced, because GCDCA alone already severely impaired bile flow and organic anion secretion. Therefore, GCDCA was used at half the concentration (12.5 μM) to further evaluate the effect of wortmannin on GCDCA-induced cholestasis. In this setting, wortmannin (100 nM) significantly aggravated GCDCA (12.5 μM)-induced reduction of bile flow (P < 0.01; Fig. 7A).

Biliary secretion of DNP-GS, a model Mrp2 substrate, was 900 ± 117 nmol·50 min⁻¹·g liver⁻¹ (n = 6) after administration of CDNB (30 μM) for 10 min as described above. Wortmannin (100 nM) did not affect basal DNP-GS secretion (103% of controls; Fig. 6B). In contrast, TCDCA (25 μM) markedly reduced DNP-GS secretion to 23% of controls (P < 0.05). Wortmannin (100 nM) further decreased DNP-GS secretion in TCDCA-treated livers from 23 to 11% of controls (P = 0.05; Fig. 6B; Table 1). GCDCA (25 μM) reduced DNP-GS secretion to 4% of controls (P < 0.01 vs. control and TCDCA); GCDCA (25 μM) in combination with wortmannin further decreased DNP-GS secretion to 0.6% of controls (P < 0.05 vs. GCDCA; Table 1). Similarly, GCDCA at half the concentration (12.5 μM) in combination with wortmannin (100 nM) also worsened DNP-GS secretion (P < 0.05 vs. GCDCA; Fig. 7B). Thus, compared with TCDCA, GCDCA had a significantly higher cholestatic potency at equimolar concentrations.

The present study shows that TCDCA induces significantly less liver damage by hepatocyte apoptosis and is less cholestatic than GCDCA when administered at low equimolar con-
concentrations in the intact liver. In addition, we demonstrated that marked activation of PI3-K by TCDCA, but not GCDCA, in the experimental model under study might, in part, explain these differences. Pharmacological inhibition of PI3-K by wortmannin markedly aggravated TCDCA- but not GCDCA-induced liver damage and hepatocyte apoptosis as well as TCDCA-induced cholestasis as demonstrated by a decrease of bile flow and secretion of the model Mrp2 substrate DNP-GS.

Previous in vitro studies (21, 23, 34) have demonstrated that GCDCA induces hepatocyte apoptosis. In our model, administration of GCDCA at low micromolar concentrations (0.1 mM) induced extensive liver injury by hepatocyte apoptosis, indicating that bile acid-induced hepatocyte apoptosis may play an important role in cholestatic liver injury of rats. Bile-acid induced hepatocyte apoptosis in vivo has been investigated by feeding a bile acid-enriched diet and by bile duct ligation (BDL) of rodents. In rats, a diet enriched with 0.4% deoxycholic acid induced significant hepatocyte apoptosis after 10 days (22). However, results of BDL studies are inconsistent: in mice, BDL has been demonstrated to cause Fas-dependent hepatocyte apoptosis in one study (20), whereas others could not find hepatocyte apoptosis as a major mechanism of liver injury after BDL (9).

PI3-K induces a variety of cellular responses including the regulation of gene expression and the activation of signaling kinases, which function in membrane ruffling, endocytosis, glucose transport, DNA synthesis, and cell survival in response induced liver damage and hepatocyte apoptosis as well as TCDCA-induced cholestasis as demonstrated by a decrease of bile flow and secretion of the model Mrp2 substrate DNP-GS.

Fig. 6. TCDCA-induced cholestasis is increased by the PI3-K inhibitor WT. DMSO (0.1% vol/vol; ☩), TCDCA (25 μM; ◦), WT (100 nM; ■), and TCDCA (25 μM) + WT (100 nM; ●) were administered for 60 (bile acid) and 70 min (WT), respectively. After 40 min, 1-chloro-2,4-dinitrobenzene (CDNB), the precursor of 2,4-dinitrophenyl-S-glutathione (DNP-GS), was infused for 10 min. WT tended to reduce TCDCA-induced bile flow and significantly worsened DNP-GS efflux (P < 0.05 vs. TCDCA alone). Results are expressed as the means (SD) of 6 experiments.

Table 1. Effects of TCDCA and GCDCA ± wortmannin on bile flow and organ anion secretion in isolated perfused rat livers

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<th>Bile flow, μl/min</th>
<th>DNP-GS secretion, nmol/min</th>
<th>g liver⁻¹</th>
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<tr>
<td>Control</td>
<td>71.5 ± 10.5</td>
<td>900.1 ± 177.3</td>
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<tr>
<td>Wortmannin 100 nM</td>
<td>78.1 ± 9.7</td>
<td>928.6 ± 115.9</td>
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<tr>
<td>TCDCA 25 μM</td>
<td>28.3 ± 10.1*</td>
<td>204.9 ± 76.7*</td>
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<tr>
<td>TCDCA 25 μM + wortmannin 100 nM</td>
<td>19.4 ± 8.2</td>
<td>102.5 ± 83.0</td>
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<tr>
<td>GCDCA 25 μM</td>
<td>11.5 ± 5.2*§</td>
<td>38.9 ± 16.4*§</td>
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<tr>
<td>GCDCA 25 μM + wortmannin 100 nM</td>
<td>7.1 ± 1.0</td>
<td>5.0 ± 4.1§</td>
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Values are means (SD) of 6 experiments. Livers were treated with glycochenodeoxycholic acid (GCDCA) (25 μM) and taurochenodeoxycholic acid (TCDCA) (25 μM) in the presence or absence of the phosphatidylinositol 3-kinase inhibitor wortmannin (100 nM) or the carrier DMSO (0.1% vol/vol control) only. Wortmannin was administered from minute 20 to 90, bile acids from minute 30 to 90. *P < 0.01 vs. control; †P = 0.05 vs. TCDCA; ‡P < 0.05 vs. GCDCA; §P < 0.01 vs. TCDCA.

Fig. 7. GCDCA-induced cholestasis is increased by the PI3-K inhibitor WT. DMSO (0.1% vol/vol; ☩), GCDCA (12.5 μM; ◦), WT (100 nM; ■), and GCDCA (12.5 μM) + WT (100 nM; ●) were administered for 60 (bile acid) and 70 min (WT), respectively. After 40 min, CDNB, the precursor of DNP-GS, was infused for 10 min. WT aggravated GCDCA-induced cholestasis by significantly reducing bile flow (P < 0.01) and DNP-GS efflux (P < 0.05 vs. GCDCA alone). Results are expressed as the means (SD) of 3 experiments.
to various apoptotic stimuli (5, 6, 19). PI3-K has been implicated as an activator of different antiapoptotic signaling effectors including PKB/Akt and the atypical isoforms of PKC (PKC-ζ and PKC-λ/ι) (7, 11). PKB/Akt suppresses apoptotic cell death induced by various apoptotic stimuli in a number of cell types (6) and is activated by the PI3-K product, phosphatidylinositol (3,4,5)-trisphosphate (27). PI3-K-dependent PKC-ζ and NF-κB activation were responsible for the reduction of TDCA-induced toxicity in McNtcp.24 cells, a rat hepatoma cell line, although PKB/Akt activation was not detected (24). In contrast to these in vitro studies, we observed a distinct PI3-K-dependent PKB/Akt activation in livers treated with TDCA, suggesting that activation of the PI3-K/Akt signaling pathway reduces the inherent toxicity of TDCA in the intact liver.

In contrast to TDCA, GCDCA-induced liver injury and apoptosis were reduced by inhibition of PI3-K. This is at variance with a recent study that showed that the PI3-K inhibitor LY294002 increased GCDCA-induced caspase-3 activity in primary rat hepatocytes (26). However, the specificity of that effect was somewhat questionable, because LY294002 also stimulated caspase-3 under basal conditions (26). The effects of PI3-K inhibitors on GCDCA-induced liver injury in the intact liver have not been studied before. Interestingly, liver injury induced by another hydrophobic bile acid, tauroholothic acid, was also alleviated by the PI3-K inhibitor wortmannin in perfused rat livers (4). Thus liver injury induced by different hydrophobic bile acids can be reduced by wortmannin in the intact liver. How can these novel results be explained? Three classes and various subclasses of PI3-K have been described, which can all be inhibited by wortmannin (31). However, their individual functions are poorly understood (1). Thus GCDCA might activate subclasses of PI3-K in the intact liver without markedly activating PKB/Akt. This explanation, however, remains speculative, because diagnostic methods or class-specific PI3-K inhibitors are not yet available to analyze PI3-K subclasses (1).

Bile acid uptake and membrane insertion of the sodium taurocholate cotransporting polypeptide (Ntcp) have been recently shown to be regulated by PI3-K in hepatocytes; insertion of Ntcp into sinusoidal membranes appears to be PKC-ζ dependent but may be PKB/Akt independent (18). Thus it could be speculated that PI3-K inhibition might reduce GCDCA-induced liver injury, in part, by reducing bile acid uptake via reduced Ntcp translocation. Indeed, bile acid concentration in the hepatovenous effluence measured at two different time points tended to be higher in livers treated with GCDCA and wortmannin than in those treated with GCDCA alone, indicating a trend toward a reduced bile acid uptake.

Intravenous administration of TCDA at low micromolar concentrations causes cholestasis in bile fistula rats (12, 17). In our model, 25 μM TCDA markedly decreased bile flow and organic anion secretion, suggesting that the cholestatic effect of this bile acid is mainly independent of a cytotoxic effect. Aggravation of TCDA-induced cholestasis by PI3-K inhibition could be explained by two independent mechanisms: 1) aggravation of TCDA-induced cytotoxicity by PI3-K inhibition; 2) reduction of insertion and/or stimulation of retrieval of canaliculared transporters into/out of the apical liver cell membrane (19).

Hepatic retention of potentially toxic bile acids is thought to play a key role in liver injury and cholestasis in humans (16, 25). Our data suggest that some bile acids attenuate their inherent cytotoxic and cholestatic effects by activating PI3-K-dependent signaling. Because glycine and taurine conjugates of CDCA are the predominant dihydroxy bile acids in cholestatic patients and have been held responsible for cholestasis-associated liver injury (25), the novel observations on a key role of PI3-K in CDCA-induced liver injury in the intact liver may have implications for future treatment of cholestatic liver diseases.

ACKNOWLEDGMENTS

The authors thank Ralf Wimmer for expert technical assistance.

GRANTS

This work was supported by the Deutsche Forschungsgemeinschaft Grant Ru 742/3–1 (to C. Rust) and Grant Be 1242/5–4 (to U. Beuers).

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