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Use of Sandwich-Cultured Hepatocytes To Evaluate Impaired Bile Acid Transport as a Mechanism of Drug-Induced Hepatotoxicity

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Abstract: Drug-induced liver toxicity is a significant problem in drug development and clinical practice, yet its mechanisms are not well understood. Growing evidence suggests that inhibition of bile acid transport may be one mechanism of hepatotoxicity. A number of hepatic transporters work in concert to transport bile acids and xenobiotics from blood to bile, and many drugs have been shown to perturb this process with detrimental consequences. Hepatocytes cultured in a sandwich configuration maintain transporter activity and liver-specific metabolic functions; thus, the sandwich-cultured hepatocyte model represents a useful tool for evaluating hepatotoxicity caused by interference with hepatic transporters. As an example, the peroxisome proliferator-activated receptor γ (PPARγ) agonist troglitazone is one such drug that has been shown to inhibit bile acid transport *in vitro*. Data presented in this manuscript indicate that troglitazone inhibits both basolateral uptake and canalicular excretion of taurocholate in a concentration-dependent manner in both sandwich-cultured and suspended human and rat hepatocytes. These data confirm both the interaction of troglitazone with bile acid transporters in hepatocytes and the utility of the sandwich-cultured hepatocyte model to study such interactions.

Keywords: Sandwich-cultured hepatocytes; bile acid transport; hepatotoxicity; troglitazone; BSEP/*ABCB11*; NTCP/*SLC10A1*

I. Drug-Induced Hepatotoxicity

Drug-induced hepatotoxicity can lead to acute liver failure, and even death, and is the most common adverse event leading to the removal of pharmaceuticals from clinical use.[1] Idiosyncratic toxicity, or rare hepatotoxic reactions of unknown etiology occurring in a small subset of patients, is a frequent reason for attrition of drug candidates in the development pipeline. Unfortunately, the mechanisms of drug-induced hepatotoxicity are varied and poorly understood, and current models used for safety assessment in drug development do not accurately predict hepatotoxicity in humans. While research into mechanisms of drug-induced hepatotoxicity has focused primarily on the formation of reactive metabolites and cellular damage from stable adduct formation, more recent evidence has led to the hypothesis that inhibition of normal bile acid transport is another important mechanism of hepatotoxicity.[2–4]

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II. Overview of Hepatic Bile Acid Transport Proteins

Bile acid uptake into hepatocytes from sinusoidal blood and excretion of bile acids from hepatocytes into the bile canaliculi is dependent on a number of transport proteins. A thorough review of these proteins is beyond the scope of this work; the reader is referred to several recent reviews for more details.[5–9] The sodium taurocholate co-transporting polypeptide NTCP (*SLC10A1*) is the only known sodium-dependent basolateral (sinusoidal) bile acid transport protein in the human hepatocyte; NTCP transports conjugated and unconjugated bile acids from the blood into the hepatocyte.[5,7,10] The basolateral organic anion-transporting polypeptides (OATPs/*SLCO*s), generally considered hepatic uptake transporters, translocate bile acids and a variety of organic anions and select organic cations in a sodium-independent manner into the hepatocyte.[5] The multidrug resistance-associated proteins MRP3 (MRP3/*ABCC3*) and MRP4 (MRP4/*ABCC4*) are involved in the basolateral excretion of bile acids from the hepatocyte back into the blood.[11–14] Apical (canalicular) bile acid transport proteins include the bile salt export pump BSEP (*ABCB11*), which transports conjugated and unconjugated bile acids into the bile canaliculi,[6,15] and MRP2 (*ABCC2*), which transports glucuronidated and sulfated bile acids, tauroursodeoxycholate, and reduced glutathione.[5,16–18] The heteromeric organic solute transporter OSTα-OSTβ is a more recently identified bile acid efflux transporter localized on the basolateral surface of cholangiocytes.[19]

III. Inhibition of Bile Acid Transport as a Mechanism of Hepatotoxicity

Compounds that inhibit one or more of the proteins responsible for bile acid excretion may cause the intracellular accumulation of bile acids in hepatocytes and subsequent toxicity due to detergent effects on cellular membranes, mitochondrial dysfunction, and cellular necrosis.[20–23] A number of drugs, including cyclosporin, glibenclamide, rifampin, and troglitazone, have been shown to inhibit rat Bsep-mediated taurocholate biliary excretion *in vitro,*[2,3,5,24] while bosentan has been reported to inhibit both rat and human isoforms.[25–27] Ritonavir, saquinavir, and efavirenz,

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three antiretroviral drugs associated frequently with hepatotoxicity in patients infected with human immunodeficiency virus, also inhibit hepatic bile acid transport.[28] Kostrubsky et al. demonstrated that cyclosporin A, bosentan, CI-1034, glyburide, erythromycin estolate, and troleandomycin inhibit bile acid excretion in a concentration-dependent manner in sandwich-cultured human hepatocytes, and to a lesser extent in conventionally cultured human hepatocytes.[29] Similarly, the same laboratory reported that the antidepressant nefazodone, unlike its analogs buspirone and trazodone, potently inhibits BSEP in membrane vesicle transport assays and taurocholate excretion in sandwich-cultured human hepatocytes, consistent with clinical findings of hepatotoxicity and liver failure associated with nefazodone use.[30]

IV. Sandwich-Cultured Hepatocyte Model

Sandwich-cultured hepatocytes are a promising tool for exploring the mechanisms of drug-induced toxicity. Unlike hepatocytes cultured using conventional techniques which lose polarity and metabolic function,[31,32] hepatocytes cultured between two layers of gelled collagen in a sandwich configuration maintain polarity, morphology, and liver-specific metabolic activity.[33–36] Over time in sandwich culture, hepatocytes develop functional bile canalicular networks sealed by tight junctions, and hepatic transport proteins are expressed and localized to the correct membrane domains allowing for assessment of transport function.[33–36] In contrast to *in vivo* studies in which the bile canaliculi are inaccessible, the hepatocytes and canalicular networks of cells in a sandwich-cultured configuration are directly accessible, making it possible to quantitatively measure substrates excreted into bile. Cryopreserved sandwich-cultured human hepatocytes also form bile canalicular networks, and the function of multiple uptake and excretory transport proteins is similar to that of fresh hepatocytes.[37] This technology is applicable to hepatocytes from species commonly used in toxicological testing such as rat, dog, and monkey,[38] as well as from humans, and therefore represents an effective *in vitro* approach for studying drug-induced hepatotoxicity.

IV. 1. Experimental Approaches. Transport studies in sandwich-cultured rat hepatocytes reported by Liu et al.[34–36] utilized these polarized cells to measure both the uptake of substrate across the basolateral membrane of the hepatocyte and excretion of substrate into the canalicular space. In Ca^{2+}-containing standard buffer (Hanks' balanced salt solution),[36] the tight junctions that serve as a barrier between the canalicular lumen (intercellular junctions) and the extracellular space are maintained, and substrate is excreted into and contained within the bile canaliculi. The cumulative uptake of substrate across the basolateral surface of the hepatocyte is represented by the sum of substrate in the cytosol and that excreted into the bile canalicular networks. Depletion of Ca^{2+} results in disruption of the tight junctions and opens

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the canalicular spaces; thus, incubation of cells in Ca^{2+}-free buffer (Hanks' balanced salt solution without calcium chloride and magnesium sulfate, containing 1 mM EGTA)[36] releases the contents of the bile canaliculi into the incubation medium. The amount of substrate excreted into the canalicular networks is calculated by subtracting the amount of substrate in cells preincubated in Ca^{2+}-free buffer (cellular accumulation) from the accumulated amount of substrate in cells preincubated in Ca^{2+}-containing buffer (cellular plus biliary accumulation). The biliary excretion of substrates can be expressed as a biliary excretion index (BEI), which is the percentage of accumulated substrate residing within the bile canaliculi. The BEI is calculated from the following equation: $BEI = [(accumulation_{standard\ buffer} - accumulation_{Ca^{2+}\text{-free buffer}})/accumulation_{standard\ buffer}] \times 100\%$.[34] The *in vitro* biliary clearance ($Cl_{biliary}$) is calculated using the following equation: $Cl_{biliary} = (accumulation_{standard\ buffer} - accumulation_{Ca^{2+}\text{-free buffer}})/(AUC_{incubation\ buffer})$, where AUC represents the area under the substrate concentration-time profile in the incubation buffer. A comparison of the biliary clearance of the model substrates inulin, salicylate, methotrexate, [D-pen^{2,5}]enkephalin, and taurocholate determined in sandwich-cultured rat hepatocytes revealed an excellent correlation ($r^2 = 0.99$) with rat biliary clearance measured *in vivo*.[34] More recently, Ghibellini et al. reported a clear relationship between the human *in vivo* biliary clearance of three different compounds (piperacillin, Tc-99m sestamibi, and Tc-99m mebrofenin, which exhibit low, intermediate, and high biliary clearance, respectively) with that predicted from *in vitro* studies with sandwich-cultured human hepatocytes.[39] This study established the relevance of sandwich-cultured hepatocytes for prediction of human *in vivo* biliary clearance.

Annaert et al. utilized sandwich-cultured rat hepatocytes to confirm the role of P-glycoprotein (P-gp/*ABCB1*) in the biliary excretion of the model substrates rhodamine 123 (Rh123) and digoxin, and reported that treatment with the P-gp inhibitor GF120918 (elacridar) reduced biliary excretion of both substrates.[40] The biliary clearance values of Rh123 and digoxin predicted by the sandwich-cultured rat hepatocyte model were consistent with those reported in isolated perfused rat liver and *in vivo* studies. Annaert and Brouwer further explored the utility of sandwich-cultured rat hepatocytes to determine the effect of potential drug interactions on P-glycoprotein-mediated Rh123 biliary excretion.[41] The effects on BEI and *in vitro* $Cl_{biliary}$ of the P-gp inhibitors verapamil and progesterone, the P-gp activator quercetin, and the P-gp inducers dexamethasone and rifampin were assessed. Comparisons of BEI and $Cl_{biliary}$ values in the absence and presence of modulators were used to localize transport interactions to the basolateral or canalicular membrane, thus elucidating potential mechanisms of drug interactions in hepatic transport. For example, a change in BEI implies that canalicular transport is altered, while an alteration in $Cl_{biliary}$, with no change in BEI, suggests that the interaction is localized to the basolateral membrane.

The sandwich-cultured hepatocyte system also has been used to study xenobiotic disruption of bile acid transport using a modified experimental approach. In the method developed by Kostrubsky et al., sandwich-cultured hepatocytes can be used to determine effects on canalicular transport by measuring bile acid excretion into standard and Ca^{2+}-free media.[29] Sandwich-cultured hepatocytes are incubated with a model radiolabeled bile acid (most commonly [^{3}H]taurocholic acid) in the presence and absence of inhibitor and treated in parallel with standard or Ca^{2+}-free buffer; excretion is quantified by measuring the amount of substrate in the buffer. Bile acids that have accumulated in canaliculi are determined by subtracting the amount of radioactive bile acid in standard buffer from the amount of radioactivity in Ca^{2+}-free buffer. The difference in excretion in the presence and absence of Ca^{2+}, in the absence of inhibitor, is defined as 100% excretion.

IV. 2. Examples of the Use of Sandwich-Cultured Hepatocytes To Identify Inhibitors of Bile Acid Transport. The sandwich-cultured hepatocyte model has been employed successfully to predict the potential of a number of known cholestatic agents to cause hepatotoxicity. A study by McRae et al. demonstrated that the hepatotoxic antiretroviral drugs ritonavir, saquinavir, and efavirenz inhibited bile acid transport in sandwich-cultured human and rat hepatocytes.[28] The BEI of taurocholate, primarily a measure of BSEP function, was decreased 55% by ritonavir, 39% by saquinavir, and 20% by efavirenz in sandwich-cultured human hepatocytes. Similarly, in sandwich-cultured rat hepatocytes, the BEI of taurocholate was decreased 100% by ritonavir and 94% by saquinavir. Data generated in sandwich-cultured hepatocytes suggested that these antiretroviral drugs inhibit bile acid uptake in addition to biliary excretion of bile acids. Further studies in suspended rat hepatocytes revealed that ritonavir, saquinavir, and efavirenz significantly decreased the sodium-dependent and independent initial uptake rates of taurocholate. Consistent with these findings, taurocholate transport by recombinant NTCP and Ntcp was inhibited by these antiretroviral drugs with IC_{50} values for human and rat of 2.1 and 6.4 μM for ritonavir, 6.7 and 20 μM for saquinivir, and 43 and 97 μM for efavirenz, respectively. Nevirapine had no significant effect on transport of bile acids using any of the *in vitro* systems.

Kostrubsky et al. studied the hepatotoxic potential of a group of compounds that are eliminated preferentially by biliary excretion but were not predicted to show clinical hepatotoxicity based on results of testing in preclinical

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species.[29] The effects of these compounds on taurocholate transport in hepatocytes cultured in conventional and collagen-sandwich configuration were evaluated. Cyclosporin A, bosentan, CI-1034, glyburide, erythromycin estolate, and troleandomycin inhibited bile acid excretion in a concentration-dependent manner in sandwich-cultured human hepatocytes, and to a lesser extent in human hepatocytes cultured in conventional configuration, while the macrolide antibiotics were much less potent inhibitors of taurocholate excretion. Cyclosporin A, CI-1034, glyburide, and bosentan also inhibited uptake of taurocholate in a concentration-dependent manner. As a result of these findings, Kostrubsky and coworkers suggested a strategy for using transport assays to rank compounds according to their hepatotoxic potential. Kostrubsky et al. also used data generated in sandwich-cultured hepatocytes, in conjunction with membrane vesicles isolated from Sf9 cells transfected with BSEP, and from *in vivo* studies in intact rats to compare the effects of the antidepressant nefazodone and the structural analogs buspirone and trazodone on hepatobiliary transport of taurocholate.[30] Nefazodone potently inhibited BSEP in membrane vesicles and inhibited taurocholate excretion in sandwich-cultured human hepatocytes; in intact rats, nefazodone administration caused a transient increase in serum bile acids. Buspirone and trazodone, in contrast, did not affect biliary transport in any of the models tested. These findings are consistent with clinical observations of hepatotoxicity and liver failure associated with nefazodone use, and suggest that *in vitro* inhibition of bile acid transport may be predictive of hepatotoxicity *in vivo*.

The peroxisome proliferator-activated receptor γ (PPARγ) agonist troglitazone, first in a new class of thiazolidinedione antidiabetic agents, was approved by the FDA in 1997 for the treatment of type II diabetes but was subsequently withdrawn from the market in 2000 after numerous reports of liver failure.[42] Preclinical toxicological testing of troglitazone in animals failed to predict this hepatotoxicity, and subsequent research efforts have focused on elucidating the mechanism(s) of troglitazone-associated hepatotoxicity. While the precise mechanism(s) remain(s) unclear, several hypotheses have been suggested, including metabolism of troglitazone to electrophilic reactive intermediates, mitochondrial injury and induction of mitochondrial permeability transition, induction of apoptosis, PPARγ-mediated steatosis, and inhibition of BSEP.[42,43]

Recent studies have generated a wealth of data supporting a potential role for altered hepatic transport processes in troglitazone's hepatotoxicity. Troglitazone decreased bile secretion rates in isolated perfused rat livers,[44] while both troglitazone and troglitazone sulfate, a major metabolite excreted into bile, competitively inhibited rat Bsep in isolated canalicular rat liver plasma membrane vesicles.[24,45] Work by Kostrubsky et al.[46] demonstrated that inhibition of troglitazone sulfation resulted in increased cytotoxicity in human and porcine hepatocyte cultures, presumably due to accumulation of unmetabolized parent compound; in addition, Kostrubsky et al.[47] showed that the biliary excretion of troglitazone sulfate and troglitazone glucuronide in rats was mediated by Mrp2 (*Abcc2*). Kemp et al. examined the effect of troglitazone on bile acid transport mechanisms in sandwich-cultured rat hepatocytes.[26] Troglitazone (10 μM) decreased the total accumulation of taurocholate in cells plus canalicular networks approximately 3-fold; troglitazone also significantly decreased the BEI of taurocholate with an IC_{50} of 0.91 ± 0.12 μM. Further investigations in suspensions of freshly isolated rat hepatocytes revealed that 10 μM troglitazone decreased the initial rate of taurocholate uptake by $\sim$3-fold.

Recent data from our laboratory demonstrated that troglitazone inhibited taurocholate uptake and excretion in sandwich-cultured human hepatocytes, in addition to rat hepatocytes. In agreement with the findings of Kemp et al.[26] in sandwich-cultured rat hepatocytes, 0.1–10 μM troglitazone decreased taurocholate cellular accumulation and cellular plus biliary accumulation in a concentration-dependent manner (Figure 1A). The BEI of taurocholate also was reduced to 71.0%, a reduction of 17.2% compared with the control value, at the highest concentration of troglitazone studied (10 μM), suggestive of inhibition of Bsep-mediated taurocholate excretion into the canalicular lumen. The biliary clearance of taurocholate was reduced in a concentration-dependent manner by 1−10 μM troglitazone from 74.9% to 21.5% of control values, respectively. Similar results were observed in sandwich-cultured human hepatocytes; 1 and 10 μM troglitazone decreased taurocholate cellular accumulation, and cellular plus biliary accumulation, as well as the BEI value (Figure 1B). The biliary clearance of taurocholate in human hepatocytes was decreased to 75.0% and 11.6% of control, respectively, at 1 and 10 μM troglitazone.

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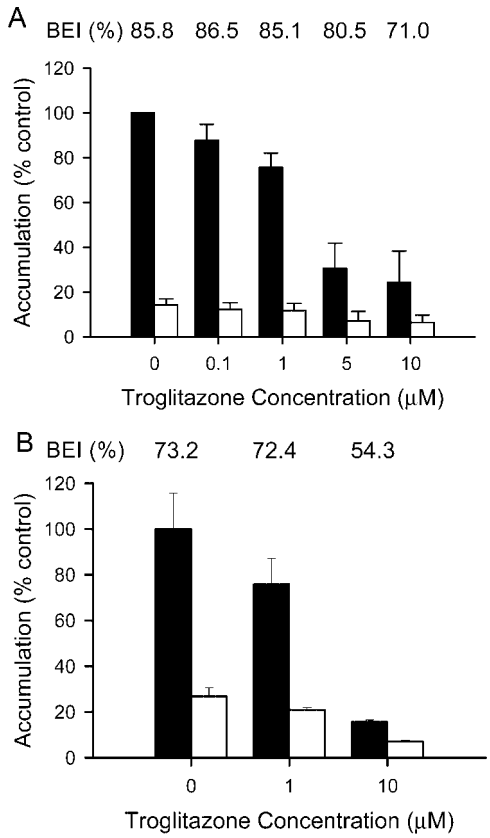

Figure 1. Taurocholate uptake and excretion by sandwich-cultured rat (A) and human (B) hepatocytes in the presence and absence of troglitazone. On day 4 (rat) or day 10 (human) of culture, hepatocytes were preincubated for 10 min in the presence of Ca^{2+} (cells plus bile, solid bars) or absence of Ca^{2+} (cells only, white bars), followed by incubation with [^{3}H]taurocholate (1 μM; 60 nCi/ml) in standard buffer at 37 °C. [^{3}H]Taurocholate accumulation by rat hepatocytes and human hepatocytes was measured at 10 min, in the presence of increasing concentrations of troglitazone. The biliary excretion index (BEI) is defined as the percentage of accumulated substrate residing within the bile canaliculi. Results in (A) are presented as the mean ± SEM of triplicate determinations obtained in three independent experiments. Results in (B) are presented as the mean ± SD of triplicate determinations in one of two experiments; similar results were observed in the second experiment.

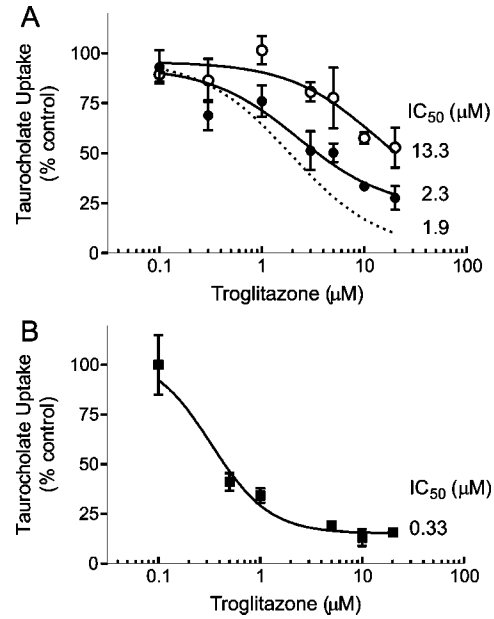

Figure 2. Taurocholate uptake by rat and human primary suspended hepatocytes in the presence and absence of troglitazone. Freshly isolated hepatocytes were suspended in Na^+-containing or Na^+-free/choline-containing HBSS at $1x10^6$ cells/ml and incubated with [^{3}H]taurocholate (1 μM; 60 nCi/ml) at 37 °C. (A) [^{3}H]Taurocholate uptake by rat hepatocytes in the presence (●) and absence (○) of Na^+ was measured at 30 s, in the presence of increasing concentrations of troglitazone. Na^+-dependent uptake (broken line) was determined by subtracting [^{3}H]taurocholate uptake in the absence of Na^+ from uptake in the presence of Na^+. Symbols represent mean ± SD obtained in four independent experiments, with triplicate determinations in each experiment. (B) [^{3}H]Taurocholate uptake by human hepatocytes was measured at 45 s, in the presence of increasing concentrations of troglitazone. Na^+-independent uptake was negligible for these human hepatocyte preparations. Symbols represent mean ± SD of triplicate determinations obtained in one of two experiments (IC_{50} for the second experiment was 0.69 μM).

While the sandwich-cultured hepatocyte model is useful for measuring overall alterations in the hepatobiliary disposition of bile acids, and especially for examining alterations in bile acid excretion, suspended hepatocytes may be preferred for analysis of alterations in bile acid uptake due to the technical ease of determining initial rates of uptake. Thus, suspended hepatocytes are a useful tool for further characterization of xenobiotic-induced alterations in bile acid uptake observed in sandwich-cultured hepatocytes.

The effect of troglitazone on taurocholate uptake was assessed previously in rat hepatocytes at a single troglitazone concentration.[26] Figure 2 shows the decrease in taurocholate uptake as a function of troglitazone concentration in rat and human suspended hepatocytes. As expected, Na^+-dependent uptake was the predominant mechanism of taurocholate uptake by both rat and human hepatocytes, and accounted for 68% and 97% of taurocholate uptake, respectively. The effect of troglitazone on taurocholate uptake by rat hepatocytes was assessed in the presence and absence of Na^+ (Figure 2A). Transport in the presence of Na^+ was inhibited more potently (IC_{50} of 2.3 μM) than in the absence of Na^+ (IC_{50} of 13.3 μM), suggesting that Na^+-dependent Ntcp (*Slc10a1*) is more sensitive to troglitazone inhibition than the Na^+-independent Oatps (*Slcos*). The overall Na^+-dependent uptake (i.e., uptake in the presence of Na^+ minus uptake in the absence of Na^+) of taurocholate by rat

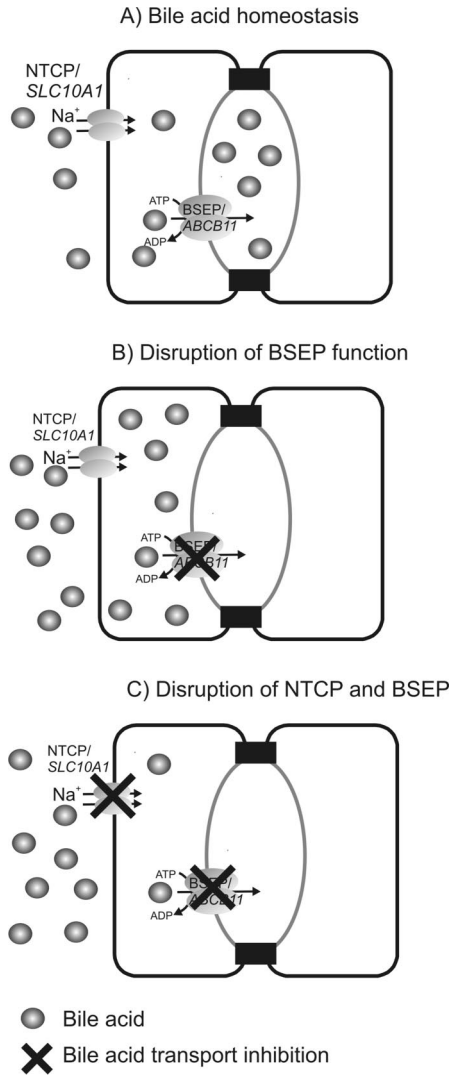

Figure 3. Potential implications of NTCP and BSEP inhibition in plasma, hepatocyte, and bile compartments: (A) NTCP- and BSEP-mediated transport of bile acids under homeostatic conditions is shown; (B) disruption of BSEP/Bsep function can result in bile acid accumulation in the hepatocyte and the plasma; (C) inhibition of bile acid basolateral uptake (NTCP/Ntcp) and canalicular excretion (BSEP/Bsep) may result in less intra-hepatocyte accumulation of bile acids. Plasma bile acid concentrations would increase regardless of whether hepatic bile acid uptake or excretion is impaired.

hepatocytes was inhibited potently by troglitazone (IC_{50} of 1.9 μM). Interestingly, troglitazone was an even more potent inhibitor of Na^+-dependent taurocholate uptake in human hepatocytes (IC_{50} of 0.33 μM) (Figure 2B). This difference in Ntcp/NTCP inhibition could have implications for species differences in susceptibility to troglitazone-induced hepatotoxicity (Figure 3).

Isolated membrane vesicles from canalicular and basolateral plasma membranes represent another *in vitro* model that has been employed to identify inhibitors of bile acid transport. Although the preparation of highly enriched basolateral or canalicular membranes for vesicle transport studies is technically challenging, such data can reveal useful information about transport mechanisms across a specific membrane that is not confounded by the presence of competing metabolic pathways or transport proteins on the opposite membrane. Inhibition of taurocholate transport by troglitazone reported in sandwich-cultured and suspended hepatocytes also has been confirmed by membrane vesicle experiments. Snow and Moseley reported that troglitazone and two analogues, rosiglitazone and ciglitazone, inhibited ATP-dependent taurocholate transport in rat liver plasma membrane vesicles.[48] In order to determine whether the hepatotoxicity associated with troglitazone involved canalicular bile acid transport alone, and whether hepatotoxicity was a class effect of thiazolidinediones or limited to troglitazone alone, the effects of troglitazone, rosiglitazone, and ciglitazone on taurocholate transport in rat basolateral and canalicular liver plasma membrane vesicles were investigated. In canalicular liver plasma membrane vesicles, taurocholate uptake was inhibited significantly by 100 μM troglitazone, rosiglitazone, and ciglitazone. Similarly, in rat basolateral plasma membrane vesicles, 100 μM troglitazone, rosiglitazone, and ciglitazone also significantly inhibited Na^+-dependent taurocholate transport. These results point to a class effect among the thiazolidinediones on hepatic bile acid transport inhibition.

Like troglitazone, bosentan also has been shown to inhibit bile acid uptake and excretion using multiple model systems. Inhibition of BSEP resulting in bile acid accumulation in the hepatocyte has been proposed as a mechanism of bosentan-induced hepatotoxicity. However, this mechanism is challenged by the observation that bosentan does not induce hepatotoxicity in rats, although inhibition of both human and rat BSEP/Bsep by bosentan has been demonstrated *in vitro* using sandwich-cultured human and rat hepatocytes and BSEP/Bsep enriched membrane vesicles.[3,25–27,29] In sandwich-cultured rat and human hepatocytes, bosentan inhibited uptake, in addition to excretion, of taurocholate.[26,27] This led to the hypothesis that the balance between inhibition of bile acid uptake and excretion processes could be very important for initiation of hepatotoxic events (Figure 3). Further analysis using rat and human suspended hepatocytes revealed that bosentan inhibited Na^+-dependent taurocholate uptake, suggesting that Ntcp/NTCP was inhibited.[26,27] This observation was confirmed by examining the effect of bosentan on taurocholate uptake by HEK293 cells overexpressing rat Ntcp or human NTCP.[27] Interestingly, suspended hepatocytes and the HEK293 model system revealed that rat Ntcp was inhibited more potently by bosentan than human NTCP. These data suggest that bosentan could cause toxicity in humans due to the inhibition of BSEP and less potent inhibition of NTCP, resulting in accumulation of bile acids in human hepatocytes; in rats, bile acids may not accumulate in hepatocytes because both the uptake and excretion of bile acids are inhibited, thus protecting the hepatocyte from

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toxicity (Figure 3). To begin characterization of the species difference in bosentan inhbition of NTCP/Ntcp, two chimeric molecules were generated and expressed in HEK293 cells.[27] Thus, what began as an important observation in sandwich-cultured hepatocytes has led to the molecular characterization of drug-transport protein interactions.

V. Inhibition of Hepatic Uptake and Excretion of Bile Acids: Potential Implications

Inhibition of BSEP, leading to the intracellular accumulation of cytotoxic bile acids, has been hypothesized as one mechanism of drug-induced hepatotoxicity (Figure 3).[2–4] Progressive familial intra-hepatic cholestasis type 2 is a rare genetic disease caused by mutations in BSEP that results in severe cholestasis, rapid progression to cirrhosis, and liver failure.[6] These symptoms are consistent with certain types of drug-induced liver injury of a cholestatic nature. Individuals with less severe genetic variants of BSEP and other transport proteins that play a critical role in the hepatic excretion of bile acids (e.g., MRPs) may be predisposed to drug-induced hepatotoxicity.[49] For example, in Caucasian women suffering from intrahepatic cholestasis of pregnancy, polymorphisms in *ABCB11* (1457T>C → Val444Ala) and *ABCC2* (3600T>A → Val1188Glu and 4581G>A → Cys1515Tyr) were associated with decreased expression of hepatic BSEP and increased expression of MRP2;[50,51] BSEP expression was decreased in healthy human liver tissue with the Val444Ala mutation. Interestingly, the Val444Ala polymorphism in *ABCB11* was observed in 83% of patients with intrahepatic cholestasis of pregnancy versus 51% in control women.[52] The interplay between pharmacogenomics and drug-induced hepatotoxicity is the subject of ongoing investigations and is of critical relevance for understanding the inter-individual susceptibility to drug-induced liver injury.

Inhibition of bile acid uptake would be expected to disrupt normal bile acid homeostasis and lead to elevated plasma bile acid concentrations (Figure 3). Furthermore, alterations in bile composition due to decreased bile acid content could change the bile acid/phospholipid ratio resulting in supersaturation of bile with cholesterol and possibly the formation of intrahepatic sludge and cholesterol gallstones.[53] This may result in a cascade of events that predisposes the liver to cellular injury. Because the sandwich-cultured hepatocyte model can be used to study cellular events (e.g., intracellular trafficking)[54] in addition to direct inhibition of bile acid transport, this model may prove to be particularly useful in exploring functional consequences of altered hepatic transport of bile acids.

VI. Conclusions

Drug-induced hepatotoxicity is well recognized as a significant but poorly understood issue in drug development. Model systems that can be employed to elucidate factors predisposing patients to drug-induced liver injury, or to determine the hepatotoxic potential of drug candidates, are particularly relevant in current drug development. Sandwich-cultured hepatocytes represent a useful model for examining mechanisms of drug-induced hepatotoxicity. A number of drugs have been shown to impair bile acid transport using this system. Research from our laboratory demonstrates that sandwich-cultured human and rat hepatocytes can be utilized to show that troglitazone, a known hepatotoxin, inhibits bile acid transport in a concentration-dependent manner. Thus, the sandwich-cultured hepatocyte model confirms and supplements data from other experimental systems such as suspended hepatocytes and membrane vesicles. The potential exists to use this model as an efficient screening tool to identify compounds that alter bile acid uptake and/or excretion early in drug development. The cellular consequences of such transport modulation may be explored over days in culture in an effort to identify new mechanisms of drug-induced hepatotoxicity.

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