NIH Public Access
Author Manuscript
Toxicol In Vitro. Author manuscript; available in PMC 2011 July 5.

Published in final edited form as:
Toxicol In Vitro. 2010 February ; 24(1): 297–309. doi:10.1016/j.tiv.2009.08.009.

Use of Cassette Dosing in Sandwich-Cultured Rat and Human Hepatocytes to Identify Drugs that Inhibit Bile Acid Transport

Kristina K. Wolf[1], **Sapana Vora**[1], **Lindsey O. Webster**[2], **Grant T. Generaux**[2], **Joseph W. Polli**[2], and **Kim L. R. Brouwer**[1]

[1]Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

[2]Preclinical Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, Inc., Research Triangle Park, NC 27709

Abstract

Hepatocellular accumulation of bile acids due to inhibition of the canalicular bile salt export pump (BSEP/*ABCB11*) is one proposed mechanism of drug-induced liver injury (DILI). Some hepatotoxic compounds also are potent inhibitors of bile acid uptake by Na^+-dependent taurocholate cotransporting polypeptide (NTCP/*SLC10A1*). This study used a cassette dosing approach in rat and human sandwich-cultured hepatocytes (SCH) to determine whether known or suspected hepatotoxic drugs inhibit bile acid transport individually or in combination. [^{3}H]-Taurocholate served as the NTCP/BSEP probe substrate. Individually, cyclosporin A and rifampin decreased taurocholate *in vitro* biliary clearance ($Cl_{biliary}$) and biliary excretion index (BEI) by more than 20% in rat SCH, suggesting that these drugs primarily inhibited canalicular efflux. In contrast, ampicillin, carbenicillin, cloxacillin, nafcillin, oxacillin, carbamazepine, pioglitazone, and troglitazone decreased the *in vitro* $Cl_{biliary}$ by more than 20% with no notable change in BEI, suggesting that these drugs primarily inhibited taurocholate uptake. Cassette dosing (n=2–4 compounds per cassette) in rat SCH yielded similar findings, and results in human SCH were consistent with rat SCH. In summary, cassette dosing in SCH is a useful *in vitro* approach to identify compounds that inhibit the hepatic uptake and/or excretion of bile acids, which may cause DILI.

Keywords

sandwich-cultured hepatocytes; hepatobiliary transport; taurocholate; high-throughput screening; hepatotoxicity; *in vitro* models

INTRODUCTION

Drug-induced liver injury (DILI), a clinical problem worldwide (reviewed in Lucena *et al*., 2008; Norris *et al*., 2008), is a leading reason for withdrawal of approved drugs from clinical use or mandated usage restrictions on drug labels, and frequently contributes to attrition of

Corresponding Author: Kim L. R. Brouwer, PharmD, PhD, Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill CB, #7569, Kerr Hall Room 3205, Chapel Hill, NC 27599, Telephone: 919-962-7030, Fax: 919-962-0644, kbrouwer@unc.edu.

CONFLICT OF INTEREST

K.L.R.B. is a co-founder and Chair of the Scientific Advisory Board of Qualyst, Inc. Qualyst, Inc. has exclusively licensed the sandwich-cultured hepatocyte technology for quantification of biliary excretion (B-CLEAR®).

This work was presented at the Society of Toxicology 47th Annual Meeting and ToxExpo™ in Seattle, WA, March 2008.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

drug candidates during development (Bakke *et al*., 1995; Kaplowitz, 2005; Kola and Landis, 2004). Numerous pathophysiological mechanisms underlie hepatotoxicity but remain poorly understood, and many cases of DILI are categorized as idiosyncratic (*i.e*., rare hepatotoxic reactions of unknown etiology). In addition, DILI is difficult to predict with existing animal models and only approximately half of the drugs that cause human hepatotoxicity can be identified during preclinical testing in rodents, dogs, and monkeys (Olson *et al*., 2000).

Cholestasis is marked by the accumulation of bile acids and bile constituents within hepatocytes; toxicity ensues due, in part, to the detergent-like effects of bile acids, which cause cellular apoptosis or necrosis and mitochondrial dysfunction (reviewed in Sokol *et al*., 2006). Many drugs and/or metabolites inhibit transport proteins responsible for the hepatobiliary transport of bile acids (reviewed in Kosters and Karpen, 2008; Pauli-Magnus and Meier, 2006). In addition, genetic and environmental factors that modulate the expression and/or activity of these transport proteins have been linked directly to cholestasis or to an increased susceptibility to its development (reviewed in Kosters and Karpen, 2008; Pauli-Magnus and Meier, 2006; Trauner and Boyer, 2003).

Transport proteins are increasingly recognized as pivotal in determining the pharmacokinetics and the pharmacological or toxicological effects of a number of drugs (Hirano *et al*, 2006; Szakács *et al*., 2008). Numerous transport proteins are responsible for the movement of bile acids into and out of hepatocytes (reviewed in Kosters and Karpen, 2008; Trauner and Boyer, 2003; Zollner *et al*., 2006). The sodium-taurocholate cotransporting polypeptide (NTCP/Ntcp; *SLC10A1*) and the bile salt export pump (BSEP/Bsep; *ABCB11*) are the predominant proteins involved in bile acid transport and homeostasis in humans and rats. On the basolateral/sinusoidal membrane of hepatocytes, NTCP/Ntcp mediates the uptake of taurine- and glycine-conjugated (*i.e*., taurocholate) and unconjugated bile acids from the blood in a sodium-dependent manner. Organic anion-transporting polypeptides (OATPs/Oatps; *SLCO*s) transport predominantly unconjugated bile acids in a sodium-independent manner. Multidrug resistance-associated proteins (MRP/Mrp) 3 (*ABCC3*) and 4 (*ABCC4*), and organic solute transporter (OST) α/β efflux bile acids from hepatocytes back into the blood. On the canalicular/apical membrane, BSEP/Bsep effluxes taurine- and glycine-conjugated bile acids into bile canaliculi, serving as a driving force for bile salt-dependent bile flow and for the concentration of bile acids in bile; MRP2/Mrp2 effluxes glucuronidated and sulfated bile acids.

Several *in vitro* models exist for assessing compound toxicity due to inhibition of transport proteins. Human and rat sandwich-cultured hepatocytes (SCH) are physiologically relevant *in vitro* models that maintain many *in vivo* structural and functional characteristics, including canalicular and basolateral membrane domains, expression and localization of liver-specific proteins, and functional bile excretion into sealed canalicular networks (LeCluyse *et al*., 1994, 2000; Liu *et al*., 1998, 1999a,b). These models have been used previously to study the inhibitory effects of drugs (*e.g*., troglitazone, nefazadone, antiretroviral compounds) on bile acid uptake and biliary excretion (Kemp *et al*., 2005; Kostrubsky *et al*., 2006; Marion *et al*., 2007; McRae *et al*., 2006).

Because of increased attention to DILI (EMEA, 2008; FDA, 2007), a robust assay utilizing SCH for inhibition of bile acid transport would be beneficial during drug development, allowing early identification of drug candidates that may cause cholestasis in humans. Traditionally, compounds are administered individually in preclinical drug development studies, a time-consuming, labor-intensive, and cost-ineffective practice. Cassette dosing, or the simultaneous administration of multiple compounds, was developed as an alternative screening method. This strategy has been used widely *in vitro* and *in vivo* to study the pharmacokinetics and metabolism of drug candidates (reviewed in White and Manitpisitkul,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2001; Zhou *et al*., 2004). To our knowledge, there are no reports in the literature regarding the use of cassette dosing to identify inhibitors of hepatic bile acid transport. The purpose of the present study was two-fold. First, inhibition of bile acid (taurocholate) transport by hepatotoxic drugs was assessed in rat and human SCH to determine whether it was a common mechanism of hepatotoxicity. Second, the feasibility of using a cassette dosing approach with rat and human SCH to screen for inhibition of taurocholate transport was investigated.

MATERIALS AND METHODS

Materials

Fetal bovine serum (FBS), dexamethasone (Dex), Hanks' balanced salts solution (HBSS) supplemented with (H-1387) or without (H-4891) calcium chloride, phenytoin, minocycline, azlocillin, nafcillin, ticarcillin, cloxacillin, carbenicillin, oxacillin, ampicillin, amoxicillin, haloperidol, chlorpromazine, valproic acid, pentamidine, prednisone, ibuprofen, etoposide, ceftriaxone, cefuroxime, carbamazepine, indomethacin, clotrimazole, bezafibrate, bifonazole, colchicine, hydralazine, menadione, piroxicam, isoniazid, sodium taurocholate, methanol, and Triton X-100 were obtained from Sigma-Aldrich (St. Louis, MO). Rifampin was obtained from Spectrum Chemical and Laboratory Products, Inc. (Gardena, CA). Troglitazone was obtained from Cayman Chemical (Ann Arbor, MI). Tienilic acid was purchased from BD Gentest (Woburn, MA). Pioglitazone was purchased from ChemPacific Corporation (Baltimore, MD). Cyclosporin A was obtained from Calbiochem (La Jolla, CA). GlaxoSmithKline, Inc. Chemical Registry supplied cefalexin. Collagenase (type 1, class 1) was purchased from Worthington Biochemicals (Lakewood, NJ). ITS+™ (insulin/transferrin/selenium) culture supplement, BioCoat™ collagen I plates, and Matrigel™ basement membrane matrix were purchased from BD Biosciences Discovery Labware (Bedford, MA). Dulbecco's modified Eagle's medium (DMEM) without phenol red, minimal essential medium (MEM) nonessential amino acids solution (100x), penicillin G-streptomycin solution, insulin, and L-glutamine were obtained from Invitrogen (Carlsbad, CA). [^{3}H]-Taurocholate (5 Ci/mmol, >97% purity) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Bio-Safe II™ liquid scintillation cocktail was obtained from Research Products International (Mt. Prospect, IL). Bicinchoninic acid (BCA) protein assay reagents and bovine serum albumin for the protein assay standard were purchased from Pierce Chemical Co. (Rockford, IL). Absolute ethanol (USP) was purchased from Aaper Alcohol and Chemical Co., Inc. (Shelbyville, KY). Dimethyl sulfoxide and chloroform were obtained from Fisher Scientific Co. (Pittsburgh, PA). The Cytotoxicity Detection Kit (LDH [Lactate Dehydrogenase]) was purchased from Roche Applied Science (Indianapolis, IN). All other chemicals and reagents were of analytical grade and available from commercial sources.

Animals

Male Wistar rats (200–300 g) from Charles River Laboratories, Inc. (Raleigh, NC) were used as liver donors for hepatocyte isolation. Animals were allowed to acclimate for at least five days prior to surgery. Rats were maintained in a controlled environment with a 12-h light/dark cycle and allowed free access to food and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of The University of North Carolina at Chapel Hill (UNC).

Isolation and Culture of Primary Rat and Human Hepatocytes

Rat (Wolf *et al*., 2008) and human (Ghibellini *et al*., 2007) SCH were prepared and maintained as described previously. Human tissue was obtained from liver resections performed by qualified medical staff in the Department of Surgery at the UNC School of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Medicine; hepatocytes were isolated from normal tissue surrounding the tumor that would have otherwise been discarded. Donor consent and approval of the UNC Biomedical Investigational Review Board was acquired prior to the surgery. Information on the liver donors is provided in Supplementary Table 1. Canalicular network development in rat and human SCH was monitored daily by phase contrast microscopy, as described previously (Ghibellini *et al*., 2007), and experiments were performed when canalicular network formation was optimal (day 4 for rat and either day 9 or 10 for human SCH).

Selection of Compounds and Test Concentrations

The compounds for this study were selected based on literature reports indicating that they were hepatotoxic or that they increased liver enzymes in the plasma/serum of rats and/or humans, as presented in Supplementary Table 2. Some hepatotoxic events associated with selected compounds were idiosyncratic and rare, while others were more common. The expected maximal therapeutic blood concentration and unbound fraction of each drug in humans was obtained from the literature (Lacy *et al*., 2002; Micromedex® Healthcare Series, Thomson Corporation, Stamford, CT; Thummel and Shen, 2001). The unbound concentration of each drug in the blood was determined as the product of the total concentration and the unbound fraction. Drug concentrations selected for this study were approximately 10 times the expected therapeutic unbound drug concentration in the blood. Selected compounds were tested in both rat and human SCH to determine the suitability of rat SCH in screening for bile acid transport inhibition in humans.

Transport Studies

Bile acid transport was investigated in SCH as described previously by Kemp *et al*. (2005), with modifications. Briefly, hepatocytes were rinsed twice with 2 ml warm HBSS containing Ca^{2+} (standard buffer) to maintain tight junction integrity and sealed bile canalicular networks, or with Ca^{2+}-free HBSS to disrupt tight junctions and open bile canalicular networks (Figure 1). Subsequently, incubation of cells was continued with either standard or Ca^{2+}-free buffer containing vehicle(s) (water, dimethyl sulfoxide, methanol, ethanol, or chloroform, individually or in combination) or drug(s) of interest (individually or in cassette) for 10 min at 37°C. The final concentration of each vehicle was ≤0.1% (v/v) of the treatment solution. After removal of the buffer, hepatocytes were incubated for 10 min at 37°C with 1.5 ml of [^{3}H]-taurocholate (1 μM), and vehicle(s) or drug(s) of interest in standard buffer. Following this incubation, hepatocytes were rinsed three times with 2 ml ice-cold standard buffer. Cells were lysed by the addition of 1 ml 0.5% (v/v) Triton X-100 in phosphate-buffered saline to each well, and the plate was shaken for a minimum of 20 min at room temperature.

Sample Analysis

Samples were analyzed by liquid scintillation spectroscopy using a Packard Tri-Carb scintillation counter (PerkinElmer Life and Analytical Sciences). The protein concentrations of SCH were determined in duplicate using BCA protein assay reagents and bovine serum albumin as the reference standard (0.2 – 1 mg/ml), as instructed by the manufacturer. Nonspecific binding of [^{3}H]-taurocholate was determined in Matrigel™-overlaid BioCoat™ plates without hepatocytes. Data were corrected for nonspecific binding and normalized to protein content.

Data Analysis

The biliary excretion index (BEI, representing the percent of accumulated compound that resides in bile canaliculi) and the intrinsic *in vitro* biliary clearance (*in vitro* $Cl_{biliary}$; ml/

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

min/kg) of taurocholate were calculated using B-CLEAR® technology (Qualyst, Inc., Raleigh, NC; Liu *et al*., 1999a) and the following equations:

$$\text{BEI}=\frac{\text{Accumulation}_{\text{cells+bile}}-\text{Accumulation}_{\text{cells}}}{\text{Accumulation}_{\text{cells+bile}}}\times 100 \quad (1)$$

$$\textit{InVitro}\ \text{Cl}_{\text{biliary}}=\frac{\text{Accumulation}_{\text{cells+bile}}-\text{Accumulation}_{\text{cells}}}{\text{AUC}_{\text{medium}}} \quad (2)$$

where substrate accumulation in cells+bile was determined in hepatocytes preincubated in standard buffer, cellular accumulation of substrate was determined in hepatocytes preincubated in Ca^{2+}-free buffer, and AUC_{medium} was determined as the product of the taurocholate concentration in medium and the incubation time. Because the taurocholate medium concentration at the end of the incubation did not differ by more than 10% from the concentration at the start of the incubation, the concentration of taurocholate in the medium was defined as the initial concentration. Rat *in vitro* $Cl_{biliary}$ values were converted to ml/min/kg based on 200 mg protein/g of liver and 40 g liver/kg of rat body weight (Seglen, 1976). Human *in vitro* $Cl_{biliary}$ values were converted to ml/min/kg based on 71 mg protein/g of liver and 25.7 g liver/kg of human body weight (Davies and Morris, 1993).

Accumulation in cells+bile and cells, BEI, and *in vitro* $Cl_{biliary}$ results are reported as a percentage of control data. An arbitrary cutoff was set at 80% of control to detect small changes that may have occurred in taurocholate hepatobiliary disposition; any treatment that produced a mean value at or below this cutoff was considered to have an inhibitory effect. The clinical translation of this cutoff is unknown. This work serves as an initial benchmarking exercise towards determining a future inhibition cutoff value that can be used as a trigger to further assess the cholestatic potential of a drug.

Cytotoxicity Testing

To assess the toxicity of each treatment that inhibited bile acid transport, rat SCH were incubated with 2 ml standard buffer or Ca^{2+}-free buffer containing the drug(s) of interest for 10 min at 37°C. The buffer was removed, and the hepatocytes were incubated for an additional 10 min at 37°C with 1.5 ml standard buffer containing the drug(s) of interest and non-radiolabelled taurocholate (1 μM). The culture medium was collected and LDH leakage was measured using the Cytotoxicity Detection Kit, according to the manufacturer's instructions. The degree of LDH release was expressed as a percentage of maximum cellular LDH release, determined by incubation of rat SCH with 2% (v/v) Triton X-100.

$$\text{Cytotoxicity}=\frac{\text{LDH}_{\text{sample}}-\text{LDH}_{\text{blank}}}{\text{LDH}_{\text{TritonX-100}}-\text{LDH}_{\text{blank}}}\times 100 \quad (3)$$

The individual drugs and drug cassettes that caused a notable inhibition of taurocholate accumulation, BEI, and *in vitro* $Cl_{biliary}$ in rat SCH were tested for toxicity: ampicillin; nafcillin; rifampin; cyclosporin A; pioglitazone; troglitazone; troglitazone and rifampin; troglitazone and ampicillin; troglitazone, phenytoin, pioglitazone, and cyclosporin A; troglitazone, pioglitazone, and cyclosporin A; pioglitazone and troglitazone; cyclosporin A, phenytoin, and troglitazone; cyclosporin A and troglitazone; troglitazone, phenobarbital, and indomethacin; minocycline, cyclosporin A, and pioglitazone; oxacillin and troglitazone; minocycline and troglitazone; and troglitazone and phenobarbital. Each drug was

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

administered at the same concentration that was used in the prior experiments to determine the effect on taurocholate hepatobiliary disposition (Table 1). None of the treatments were toxic as measured by release of LDH into the culture medium (data not shown).

RESULTS

Hepatobiliary Disposition of Taurocholate in Rat and Human SCH

The accumulation in cells+bile, accumulation in cells, BEI, and *in vitro* $Cl_{biliary}$ of taurocholate in untreated rat and human SCH is illustrated in Figure 2. Human SCH accumulated taurocholate in cells to a greater extent than rat SCH; however, human SCH appeared to have a lower BEI for taurocholate than rat SCH. The *in vitro* $Cl_{biliary}$ of taurocholate was similar between rat and human SCH.

Modulation of Taurocholate Hepatobiliary Disposition in Rat SCH

Table 1 summarizes the effects of known and suspected hepatotoxic drugs from various treatment classes on four measures of taurocholate hepatobiliary disposition in rat SCH: taurocholate accumulation in cells+bile, accumulation of taurocholate in cells, BEI, and *in vitro* $Cl_{biliary}$. None of the vehicle controls had an effect on any of these disposition parameters (data not shown). Of the 34 drugs tested, two altered all four measures (rifampin and cyclosporin A), six altered three measures (ampicillin, nafcillin, piroxicam, carbamazepine, pioglitazone, troglitazone), four altered two measures (carbenicillin, cloxacillin, oxacillin, pentamidine), two altered one measure (ticarcillin, prednisone), and 20 had no notable effects (<20% change from control). Compared to control, rifampin and cyclosporin A decreased taurocholate accumulation in cells+bile, BEI, and *in vitro* $Cl_{biliary}$, and increased cellular accumulation. Ampicillin, carbenicillin, cloxacillin, nafcillin, oxacillin, carbamazepine, pioglitazone, and troglitazone decreased taurocholate accumulation in cells+bile and *in vitro* $Cl_{biliary}$ with no notable change in BEI compared to control, suggesting that uptake of taurocholate was mainly inhibited. In addition, many of these drugs (ampicillin, nafcillin, carbamazepine, pioglitazone, and troglitazone) also decreased cellular accumulation of taurocholate. Ticarcillin only decreased cellular taurocholate accumulation. Conversely, pentamidine and piroxicam increased accumulation of taurocholate in cells+bile and *in vitro* $Cl_{biliary}$ compared to control, while piroxicam also increased cellular taurocholate accumulation. Prednisone increased only cellular accumulation of taurocholate.

Cassette Dosing of Hepatotoxic Drugs in Rat SCH

The utility of the administration of drugs in cassette to determine the effects on taurocholate hepatobiliary disposition in rat SCH was investigated. The effects of numerous cassettes on the same four measures of taurocholate hepatobiliary disposition (accumulation in cells +bile, accumulation in cells, BEI, and *in vitro* $Cl_{biliary}$) were compared to the effects of an anchor compound (a single compound utilized in each of the cassettes tested). The anchor compounds were selected in order to provide a range of effects on taurocholate disposition to which combinations of compounds could be compared for additive or synergistic effects.

Menadione-containing cassettes—Menadione served as a negative control because it had no effect on taurocholate disposition on its own. When menadione was administered in cassette with prednisone and bifonazole, there were no notable effects (<20% change from control) on taurocholate accumulation in cells+bile (Figure 3A), BEI (Figure 3B), or on *in vitro* $Cl_{biliary}$ (Figure 3B) compared to menadione alone. The increase in cellular taurocholate accumulation in the menadione cassette containing prednisone was similar to the effect caused by prednisone alone (27% increase compared to control; Table 1). No

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

notable effects on the four measures of taurocholate hepatobiliary disposition were observed with menadione, isoniazid, and clotrimazole in cassette (Figure 3A and 3B).

Phenobarbital-containing cassettes—Phenobarbital also served as a negative control. Compared to control, phenobarbital and troglitazone in cassette decreased taurocholate accumulation in cells+bile and cells (77% and 55%, respectively; Figure 4A), and *in vitro* $Cl_{biliary}$ (80%; Figure 4B); there was no notable effect on the BEI of taurocholate (Figure 4B). These changes were similar to the effect caused by troglitazone alone. Likewise, phenobarbital in cassette with troglitazone and indomethacin also decreased accumulation of taurocholate in cells+bile and cells (76% and 62%, respectively; Figure 4A), and *in vitro* $Cl_{biliary}$ (78%; Figure 4B), with no notable effect on BEI (Figure 4B). However, there was no further change in any of the parameters compared to the phenobarbital and troglitazone cassette, indicating that indomethacin does not alter taurocholate disposition, similar to what was found when indomethacin was administered alone (Table 1). Overall, these results for taurocholate hepatobiliary disposition when troglitazone was added to the cassette were similar to the results observed when troglitazone, but not phenobarbital, was administered alone (Table 1, Figures 4A and 4B).

Oxacillin-containing cassettes—Oxacillin affected two of the four parameters of taurocholate disposition and was selected as an anchor compound to determine if additional parameters would be affected when administered in cassette. Compared to control, treatment with oxacillin and cloxacillin decreased the accumulation of taurocholate in cells+bile (30%) and cells (30%; Figure 5A), and *in vitro* $Cl_{biliary}$ (30%; Figure 5B); there was no notable change in BEI (Figure 5B). Oxacillin in cassette with cloxacillin and azlocillin had no effects (Figure 5A and 5B). Treatment with oxacillin in cassette with cloxacillin and ampicillin decreased the cellular accumulation of taurocholate (25%; Figure 5A). Oxacillin and ampicillin in cassette did not affect any of the four measures of taurocholate hepatobiliary disposition (Figure 5A and 5B), unlike the decrease in taurocholate accumulation in cells+bile and cells, and *in vitro* $Cl_{biliary}$ ampicillin caused when administered alone (Table 1). Taurocholate accumulation in cells+bile (31%; Figure 5A) and *in vitro* $Cl_{biliary}$ (33%; Figure 5B) were decreased by treatment with the cassette containing oxacillin, ampicillin, and cyclosporin A. Treatment with oxacillin, ampicillin, and nafcillin decreased taurocholate accumulation in cells+bile (56%) and cells (47%; Figure 5A), and *in vitro* $Cl_{biliary}$ (56%; Figure 5B), with no notable change in BEI (Figure 5B). These decreases were slightly more compared to treatment with oxacillin and nafcillin (40%, 41%, and 40%, respectively; Figures 5A and 5B). Treatment with oxacillin in cassette with ampicillin and amoxicillin decreased taurocholate accumulation in cells+bile (29%) and cells (35%; Figure 5A), and *in vitro* $Cl_{biliary}$ (29%; Figure 5B); there was no notable change in BEI (Figure 5B). In contrast, oxacillin and amoxicillin alone did not decrease any of the four measures of taurocholate hepatobiliary disposition (Figures 5A and 5B). Treatment with oxacillin in cassette with carbenicillin decreased the accumulation of taurocholate in cells+bile (24%) and cells (40%; Figure 5A), and *in vitro* $Cl_{biliary}$ (22%; Figure 5B); there was no notable change in BEI (Figure 5B). Oxacillin in cassette with azlocillin and ticarcillin decreased the accumulation of taurocholate in cells+bile and cells (23% and 20%, respectively; Figure 5A), and *in vitro* $Cl_{biliary}$ (23%; Figure 5B), with no notable effect on BEI (Figure 5B). Oxacillin in cassette with troglitazone decreased taurocholate accumulation in cells+bile and cells (90% and 59%, respectively; Figure 5A), BEI (30%; Figure 5B), and *in vitro* $Cl_{biliary}$ (93%; Figure 5B). In summary, the effects on the hepatobiliary disposition of taurocholate that were observed with treatment of oxacillin-containing cassettes were similar to effects produced by treatment with oxacillin alone (Table 1, Figures 5A and 5B) when variability was taken into account. However, cassettes containing either nafcillin or troglitazone further

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

decreased taurocholate accumulation in cells+bile and cells, BEI, and *in vitro* $Cl_{biliary}$ compared to oxacillin alone (Table 1, Figures 5A and 5B).

Nafcillin-containing cassettes—Nafcillin affected three of the four parameters of taurocholate disposition and was selected as an anchor compound to determine if the parameters would be further affected when administered in combination. The five treatments utilizing cassettes containing nafcillin decreased taurocholate accumulation in cells+bile and cells (Figure 6A), and *in vitro* $Cl_{biliary}$ (Figure 6B) compared to control: nafcillin and ticarcillin (31%, 21%, and 32%, respectively); nafcillin and cloxacillin (53%, 31%, and 55%, respectively); nafcillin and oxacillin (40%, 41%, and 40%, respectively; as noted previously from Figure 5); nafcillin, oxacillin, and ampicillin (56%, 47%, and 56%, respectively); and nafcillin and ampicillin (51%, 54%, and 51%, respectively). There were no notable effects on BEI (Figure 6B). The effects produced by nafcillin-containing cassettes on taurocholate hepatobiliary disposition were similar to those produced by treatment with nafcillin alone (Table 1, Figures 6A and 6B).

Pioglitazone-containing cassettes—Pioglitazone also affected three out of the four measures of taurocholate hepatobiliary disposition to a greater degree than nafcillin but to a lesser extent than troglitazone. Compared to control, the five treatments utilizing pioglitazone in cassette decreased taurocholate accumulation in cells+bile and cells (Figure 7A), and *in vitro* $Cl_{biliary}$ (Figure 7B): pioglitazone and cyclosporin A (61%, 48%, and 63%, respectively); pioglitazone, cyclosporin A, and phenytoin (62%, 28%, and 65%, respectively); pioglitazone, cyclosporin A, phenytoin, and troglitazone (90%, 57%, and 94%, respectively); pioglitazone and troglitazone (85%, 76%, and 86%, respectively); and pioglitazone and ampicillin (68%, 60%, and 68%, respectively). The only cassette to decrease the BEI of taurocholate contained pioglitazone, cyclosporin A, phenytoin, and troglitazone (38%; Figure 7B). The effects produced by pioglitazone-containing cassettes were similar to those produced by treatment with pioglitazone alone (Table 1, Figures 7A and 7B), except there was a further decrease in taurocholate accumulation in cells+bile and *in vitro* $Cl_{biliary}$ caused by the two pioglitazone cassettes containing troglitazone, and a decrease in BEI caused by the cassette containing pioglitazone, cyclosporin A, phenytoin, and troglitazone.

Troglitazone-containing cassettes—Troglitazone was one of three compounds (troglitazone, cyclosporin A, rifampin) that had the greatest effects on taurocholate disposition. Cassettes containing troglitazone and cyclosporin A decreased taurocholate accumulation in cells+bile and cells (Figure 8A), BEI (Figure 8B), and *in vitro* $Cl_{biliary}$ (Figure 8B) compared to control: troglitazone and cyclosporin A (91%, 67%, 27%, and 94%, respectively); troglitazone, cyclosporin A, and phenytoin (92%, 59%, 55%, and 97%, respectively); and troglitazone, cyclosporin A, phenytoin, and pioglitazone (90%, 57%, 38%, and 94%, respectively; as noted previously from Figure 7). Troglitazone in cassette with either oxacillin, rifampin, or with minocycline plus phenytoin also decreased taurocholate accumulation in cells+bile (90%, 95%, and 91%, respectively) and cells (59%, 45%, and 58%, respectively; Figure 8A), BEI (30%, 100%, and 43%, respectively; Figure 8B), and *in vitro* $Cl_{biliary}$ (93%, 100%, and 95%, respectively; Figure 8B). Treatment with troglitazone and minocycline in cassette decreased taurocholate accumulation in cells+bile and cells (77% and 71%, respectively; Figure 8A), and *in vitro* $Cl_{biliary}$ (78%; Figure 8B); this treatment caused no notable change in BEI (Figure 8B). As noted previously from Figure 7, troglitazone and pioglitazone in cassette decreased taurocholate accumulation in cells+bile and cells (85% and 76%, respectively; Figure 8A), and *in vitro* $Cl_{biliary}$ (86%; Figure 8B) with no notable effect on BEI (Figure 8B). Treatment with troglitazone and phenobarbital in cassette decreased taurocholate accumulation in cells+bile and cells (77%

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and 55%, respectively; Figure 8A), and *in vitro* $Cl_{biliary}$ (80%; Figure 8B), as noted previously from Figure 4. Also noted previously from Figure 4, treatment with troglitazone, phenobarbital, and indomethacin in cassette also decreased taurocholate accumulation in cells+bile and cells (76% and 62%, respectively; Figure 8A), and *in vitro* $Cl_{biliary}$ (78%; Figure 8B). Troglitazone in cassette with valproic acid and ibuprofen decreased the accumulation of taurocholate in cells+bile and cells (85% and 68%, respectively; Figure 8A), and *in vitro* $Cl_{biliary}$ (87%; Figure 8B). Overall, the results of taurocholate accumulation in cells+bile and cells, and *in vitro* $Cl_{biliary}$ are similar to those obtained when troglitazone was administered alone (Table 1, Figures 8A and 8B).

Cyclosporin A-containing cassettes—Cyclosporin A affected all four measures of taurocholate disposition and was selected as an anchor compound to determine if any parameters would be further affected when administered in combination with other compounds. Treatment with cyclosporin A in cassette with troglitazone decreased taurocholate accumulation in cells+bile and cells (91% and 67%, respectively; Figure 9A), BEI (27%; Figure 9B), and *in vitro* $Cl_{biliary}$ (94%; Figure 9B) compared to control, as noted previously from Figure 8. Similar decreases in the four measures were observed when two drugs were added to the cyclosporin A-troglitazone cassette (Figures 9A and 9B): phenytoin (92%, 59%, 55%, and 97%, respectively), and phenytoin plus pioglitazone (90%, 57%, 38%, and 94%, respectively). As noted previously from Figure 7, cyclosporin A in cassette with pioglitazone alone decreased taurocholate accumulation in cells+bile and cells (61% and 48%, respectively; Figure 9A), and *in vitro* $Cl_{biliary}$ (63%; Figure 9B) with no notable change in BEI (Figure 9B). Treatment with cyclosporin A, pioglitazone, and phenytoin in cassette decreased the accumulation of taurocholate in cells+bile and cells (62% and 28%, respectively; Figure 9A), and *in vitro* $Cl_{biliary}$ (65%; Figure 9B); there was no notable change in BEI (Figure 9B). Cyclosporin A in cassette with phenytoin and minocycline decreased taurocholate accumulation in cells+bile (25%; Figure 9A), BEI (27%; Figure 9B), and *in vitro* $Cl_{biliary}$ (39%; Figure 9B), and increased cellular taurocholate accumulation (64%; Figure 9A). Cyclosporin A in cassette with ampicillin only decreased *in vitro* $Cl_{biliary}$ (20%; Figure 9B), while treatment with cyclosporin A in cassette with ampicillin and oxacillin decreased taurocholate accumulation in cells+bile (31%; Figure 9A) and *in vitro* $Cl_{biliary}$ (33%; Figure 9B), as noted previously from Figure 5. Cyclosporin A in cassette with only amoxicillin decreased taurocholate accumulation in cells+bile (38%; Figure 9A) and *in vitro* $Cl_{biliary}$ (44%; Figure 9B). Cassettes containing cyclosporin A and one of the glitazones further decreased accumulation of taurocholate in cells+bile and cells, and *in vitro* $Cl_{biliary}$ compared to cyclosporin A alone; the decrease was greater with troglitazone than with pioglitazone (Table 1, Figures 9A and 9B).

Modulation of Taurocholate Hepatobiliary Disposition by Hepatotoxic Drugs in Human SCH

The effects of hepatotoxic drugs, administered individually, on the hepatobiliary disposition of taurocholate in human SCH are presented in Table 1. Compared to control, troglitazone decreased taurocholate accumulation in cells+bile and cells, BEI, and *in vitro* $Cl_{biliary}$. Pioglitazone decreased taurocholate accumulation in cells+bile and cells, and *in vitro* $Cl_{biliary}$. Nafcillin only decreased cellular taurocholate accumulation, while cyclosporin A only decreased taurocholate *in vitro* $Cl_{biliary}$. Additionally, as seen in Figure 10, tienilic acid decreased taurocholate accumulation in cells+bile and cells, and *in vitro* $Cl_{biliary}$.

Figure 10 illustrates the effects of hepatotoxic drugs administered in cassette on taurocholate hepatobiliary disposition in human SCH. Two drug cassettes, troglitazone combined with tienilic acid, and cloxacillin combined with nafcillin, were studied. The troglitazone and tienilic acid cassette decreased taurocholate accumulation in cells+bile (82%) and cells

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(68%), BEI (23%), and *in vitro* $Cl_{biliary}$ (86%) compared to control; these results were similar to those following incubation with troglitazone alone (Table 1, Figure 10). The cloxacillin and nafcillin cassette decreased cellular taurocholate accumulation (38%) compared to control, similar to results following incubation with nafcillin alone (Table 1, Figure 10).

DISCUSSION

Modulation of hepatobiliary transport proteins may be an important mechanism underlying DILI. In humans and rodents, BSEP/Bsep and NTCP/Ntcp are largely responsible for hepatic bile acid transport, and are subject to xenobiotic interactions and genetic mutations (reviewed in Kosters and Karpen, 2008; Pauli-Magnus and Meier, 2006; Trauner and Boyer, 2003). Inhibition of BSEP/Bsep can decrease biliary excretion of bile acids, and the detergent-like effects of accumulated bile acids can cause toxicity (reviewed in Sokol *et al*., 2006). A number of drugs, including cyclosporin A, troglitazone, and bosentan, inhibit BSEP/Bsep and also have been reported to cause clinical cases of cholestasis (reviewed in Kosters and Karpen, 2008). A robust assay to identify potentially cholestatic compounds early in drug development would be beneficial.

In vitro $Cl_{biliary}$ in SCH reflects both the uptake and efflux of a compound. The BEI primarily reflects excretion of substrate across the canalicular domain. Pioglitazone, troglitazone, ampicillin, carbenicillin, cloxacillin, nafcillin, oxacillin, and carbamazepine inhibited the *in vitro* $Cl_{biliary}$ of taurocholate without affecting the BEI, suggesting that they primarily inhibited the uptake of bile acids into rat SCH at the concentrations investigated (Table 1). Inhibition of *in vitro* $Cl_{biliary}$ varied from ~25% to 92%. Because taurocholate is primarily transported by Ntcp (reviewed in Kosters and Karpen, 2008; Trauner and Boyer, 2003), these drugs most likely impair bile acid transport via Ntcp inhibition. Additional studies are required to distinguish Ntcp inhibition from Oatp inhibition, similar to those performed by Marion *et al*. (2007) in suspended hepatocytes utilizing Na^+-containing and Na^+-free buffers to distinguish between Ntcp- and Oatp-mediated taurocholate uptake. The key observation with these compounds was that the majority of bile acid inhibition was associated with inhibition of hepatic uptake rather than canalicular efflux, which suggests that increased plasma bile acid concentrations may be driven more likely by attenuation of hepatic uptake than by Bsep inhibition. These compounds may inhibit Bsep at higher concentrations than those used in this study; however, drug concentrations achieved within hepatocytes are unknown. Furthermore, a metabolite of the compound may be a more potent inhibitor of either bile acid uptake and/or excretion than the parent compound, as is the case with troglitazone (Funk *et al*., 2001); the short incubation time used in this study may not have been sufficient for appreciable metabolite formation.

Cyclosporin A and rifampin inhibited both the *in vitro* $Cl_{biliary}$ and BEI of taurocholate (Table 1), indicating that both uptake of taurocholate into hepatocytes and subsequent excretion into bile canaliculi were inhibited. Importantly, intracellular taurocholate accumulation increased with both of these drugs, possibly due to greater inhibition of efflux. This situation could potentially result in cholestasis with cellular damage which is, by definition, mixed hepatic injury (Lucena *et al*., 2008).

The overall findings from administration of a single hepatotoxic drug to rat SCH are in agreement with previous studies characterizing the inhibition of Ntcp and/or Bsep by some of these compounds in different *in vitro* models. Troglitazone, used as a positive control in our study, decreased taurocholate uptake in both canalicular and basolateral liver plasma membranes (Snow and Moseley, 2007). Kemp *et al*. (2005) and Marion *et al*. (2007) reported that troglitazone inhibited taurocholate accumulation in a dose-dependent manner

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

with a marginal to no decrease in BEI in rat SCH. Using canalicular liver plasma membranes, Stieger *et al*. (2000) reported that rifampin and cyclosporin A inhibited Bsep-mediated taurocholate transport. In conventionally-cultured primary rat hepatocytes, cyclosporin A competitively inhibited the uptake and efflux of taurocholate in a concentration-dependent manner (Kukongviriyapan and Stacey, 1988). Fattinger *et al*. (2000) reported that Na^+-independent uptake of taurocholate via Oatps was inhibited by low concentrations of rifampin, while higher concentrations also inhibited Na^+-dependent uptake via Ntcp in short-term conventional cultures of primary rat hepatocytes. Finally, Horikawa *et al*. (2003) reported that high concentrations of cloxacillin, chlorpromazine, and colchicine inhibited taurocholate uptake by rat canalicular liver plasma membranes via Bsep. Colchicine reportedly causes cholestasis in rats (Crocenzi *et al*., 1997); therefore, colchicine was expected to inhibit Bsep and decrease taurocholate efflux from hepatocytes into bile. However, in the present study, colchicine did not alter any of the four measures of taurocholate hepatobiliary disposition (Table 1). A longer incubation time with colchicine may be necessary to impair taurocholate hepatobiliary disposition since the mechanism of action of colchicine is to disrupt microtubule action and trafficking of Bsep to the membrane. Piroxicam causes cholestatic jaundice in humans (Hepps *et al*., 1991), suggesting impaired bile acid transport. In rat SCH, increased accumulation of taurocholate in cells+bile and cells, and increased *in vitro* $Cl_{biliary}$ with no change in BEI supports perturbation of bile acid transport in rats. This perturbation may be due to inhibition of bile acid efflux by transport proteins located on the basolateral membrane.

Results following administration of hepatotoxic drugs in cassettes to rat SCH were similar to those observed when hepatotoxic drugs were administered singly, suggesting that a single potent inhibitor defined the inhibition profile. Drugs that did not inhibit the *in vitro* $Cl_{biliary}$ of taurocholate alone showed no inhibition when administered in cassette; the same occurred for the BEI of taurocholate. In general, drugs that inhibited the *in vitro* $Cl_{biliary}$ and/or BEI when administered alone inhibited the corresponding parameter(s) when administered in cassette. When multiple drugs that inhibited *in vitro* $Cl_{biliary}$ and/or BEI were administered in cassette, increased inhibition of taurocholate transport usually resulted. However, the increased inhibition was neither additive nor synergistic, except in the case of troglitazone and rifampin administered in cassette (Figure 8). One possible reason for this is that the drugs administered in cassette may all preferentially inhibit Oatps rather than Ntcp or vice versa. Further studies are required to define the specific mechanism(s) of inhibition. Overall, however, these results indicate that the cassette dosing approach with rat SCH is a useful model system to study the ability of a compound to inhibit bile acid transport.

Lastly, the utility of cassette dosing in human SCH was investigated. Pioglitazone, troglitazone, and tienilic acid inhibited taurocholate cellular accumulation and *in vitro* $Cl_{biliary}$, while troglitazone also decreased the BEI. These compounds may primarily inhibit bile acid uptake into hepatocytes, although troglitazone additionally inhibits the canalicular efflux of taurocholate. Results observed after 5 μM troglitazone administration are in good accordance with previously published data (Marion *et al*., 2007) in which troglitazone decreased cellular taurocholate accumulation and BEI in a dose-dependent manner in human SCH. In contrast to results herein, Kostrubsky *et al*. (2003) reported that both 1 and 10 μM cyclosporin A inhibited taurocholate uptake and canalicular efflux in human SCH in a dose-dependent manner following a 15-min preload and a 10-min efflux with taurocholate and cyclosporin A. Horikawa *et al*. (2003) reported that cloxacillin (75 μM) and cyclosporin A (1 μM) inhibited taurocholate uptake by human canalicular liver plasma membranes, indicating inhibition of taurocholate canalicular efflux by BSEP. However, consistent with our findings of decreased *in vitro* $Cl_{biliary}$, Mita *et al*. (2006) demonstrated that inhibition of NTCP rather than BSEP was more important in the inhibition of bile acid transport by cyclosporin A in NTCP- and BSEP-expressing LLC-PK1 cells. Some of the differences

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

observed in these studies may be due to differential expression and/or activity of NTCP and/or BSEP between hepatocytes from different donors; other differences may be dependent on the concentration of drug or the *in vitro* model.

When comparing the results between rat and human SCH treated with the same compound, a few similarities and differences were observed (Table 1). Minocycline had no effect on taurocholate hepatobiliary disposition in either rat or human SCH, while pioglitazone appeared to primarily inhibit taurocholate uptake into both rat and human SCH at the concentrations tested. In contrast, cloxacillin, nafcillin, and oxacillin inhibited taurocholate uptake into rat SCH but had no effect in human SCH. Cyclosporin A prominently inhibited the uptake and biliary efflux of taurocholate in rat SCH, with a modest decrease in taurocholate biliary clearance at the concentration examined (3 μM) in human SCH. Troglitazone, on the other hand, appeared to only inhibit taurocholate uptake in rat SCH, but inhibited both taurocholate uptake and biliary efflux in human SCH at the concentration tested. The differences observed in taurocholate hepatobiliary disposition between rat and human SCH may be due to differences in the sensitivity of transport proteins (*i.e*. Ntcp/NTCP, Oatps/OATPs, Bsep/BSEP) to inhibition caused by the individual drugs, as previously reported for Ntcp/NTCP inhibition. Leslie *et al*. (2007) found Ntcp to be more sensitive to inhibition by bosentan than NTCP. Marion *et al*. (2007) reported that NTCP is more sensitive to inhibition by troglitazone than Ntcp, and McRae *et al*. (2006) found the same to be true for ritonavir, saquinivir, and efavirenz. These findings emphasize the differences between species in hepatic transport protein function. In addition, there could also be differences in the sensitivity of a transport protein to inhibition between humans because of polymorphisms.

The cutoff set at 80% of control was selected arbitrarily to detect small changes in taurocholate hepatobiliary disposition. However, changes in biliary disposition of this magnitude may not result in clinically-detectable cholestasis. A higher threshold (*i.e*., >20% change from control) may be more relevant and would take into account polymorphisms that occur in transport proteins, which may render a certain population more susceptible to perturbation of bile acid transport. A cutoff set at 50% would exclude many compounds, including some β-lactam antibiotics that have been reported to cause rare cases of cholestasis in humans. Using the 80% cutoff, results from this study suggest that inhibition of bile acid transport was not a function of drug class, except for the glitazones and some penicillin analogs.

In summary, the present work demonstrates the utility of a novel *in vitro* approach, rat and human SCH in combination with cassette dosing, to increase throughput when screening compounds (*i.e*., potential drug candidates) for inhibition of bile acid transport. Compounds should first be screened in combination, and then follow-up studies of the cassettes causing inhibition would be required to identify which individual component(s) caused inhibition. These subsequent single-agent studies also would minimize false positives. False negatives could be minimized by utilizing a compound in multiple combinations and at more than one concentration. Furthermore, exposure of rat and human SCH to compounds for an adequate period of time to allow formation of metabolites prior to assessing taurocholate transport could provide additional information about whether the metabolite(s) and/or the unchanged drug is responsible for inhibition of bile acid transport. Overall, results indicate that the majority of hepatotoxic drugs do not inhibit bile acid transport at clinically relevant concentrations. For those drugs that did affect bile acid transport *in vitro*, the majority appear to decrease only the *in vitro* $Cl_{biliary}$, reflecting an inhibition of bile acid uptake from the medium into hepatocytes as opposed to an inhibition of bile acid efflux from hepatocytes into bile canaliculi. The drugs that only inhibit bile acid uptake may cause hepatotoxicity by another mechanism and/or cause toxicity elsewhere in the body due to increased plasma

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

levels of bile acids. In contrast, the few drugs that altered taurocholate hepatobiliary disposition to the greatest extent (*i.e.*, troglitazone and rifampin) were those which have been associated with the most reports of clinical hepatotoxicity. Thus, this approach is useful in detecting compounds that inhibit the hepatic uptake and/or excretion of bile acids, and which may cause cholestasis or hepatotoxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Yi-Wei Rong for her technical expertise in the isolation of rat and human hepatocytes, and Tracy Marion and Dr. Elaine Leslie for review of the manuscript. This work was supported by the National Institutes of Health grants GM41935 (K.L.R.B.) and CA106101 (K.L.R.B.), and by GlaxoSmithKline, Inc. K.K.W. was funded by a postdoctoral fellowship from GlaxoSmithKline, Inc.

Abbreviations

BEI	biliary excretion index
DILI	drug-induced liver injury
HBSS	Hanks' balanced salts solution
***in vitro* $Cl_{biliary}$**	*in vitro* biliary clearance
LDH	lactate dehydrogenase
SCH	sandwich-cultured hepatocytes

REFERENCES

Bakke OM, Manocchia M, de Abajo F, Kaitin KI, Lasagna L. Drug safety discontinuations in the United Kingdom, the United States, and Spain from 1974 through 1993: a regulatory perspective. Clinical Pharmacology and Therapeutics. 1995; 58:108–117. [PubMed: 7628177]

Bénichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. Journal of Hepatology. 1990; 11:272–276. [PubMed: 2254635]

Crocenzi FA, Sisti A, Pellegrino JM, Roma MG. Role of bile salts in colchicine-induced hepatotoxicity. Implications for hepatocellular integrity and function. Toxicology. 1997; 121:127–142. [PubMed: 9230445]

Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharmaceutical Research. 1993; 10:1093–1095. [PubMed: 8378254]

EMEA. Non-clinical guideline on drug-induced hepatotoxicity. 2008. Available at: http://www.emea.europa.eu/pdfs/human/swp/15011506en.pdf

Fattinger K, Cattori V, Hagenbuch B, Meier PJ, Stieger B. Rifamycin SV and rifampicin exhibit differential inhibition of the hepatic rat organic anion transporting polypeptides, Oatp1 and Oatp2. Hepatology. 2000; 32:82–86. [PubMed: 10869292]

FDA. Drug-induced liver injury: Premarketing clinical evaluation. 2007. Available at: http://www.fda.gov/Cder/guidance/7507dft.htm

Funk C, Pantze M, Jehle L, Ponelle C, Scheuermann G, Lazendic M, Gasser R. Troglitazone-induced intraheptic cholestasis by an interference with the hepatobiliary export of bile acids in male and female rats. Correlation with the gender difference in troglitazone sulfate formation and the inhibition of the canalicular bile salt export pump (Bsep) by troglitazone and troglitazone sulfate. Toxicology. 2001; 167:83–98. [PubMed: 11557132]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Ghibellini G, Vasist LS, Leslie EM, Heizer WD, Kowalsky RJ, Calvo BF, Brouwer KLR. In vitro-in vivo correlation of hepatobiliary drug clearance in humans. Clinical Pharmacology and Therapeutics. 2007; 81:406–413. [PubMed: 17235333]

Hepps KS, Maliha GM, Estrada R, Goodgame RW. Severe cholestatic jaundice associated with piroxicam. Gastroenterology. 1991; 101:1737–1740. [PubMed: 1955140]

Hirano H, Kurata A, Onishi Y, Sakurai A, Saito H, Nakagawa H, Nagakura M, Tarui S, Kanamori Y, Kitajima M, Ishikawa T. High-speed screening and QSAR analysis of human ATP-binding cassette transporter ABCB11 (bile salt export pump) to predict drug-induced intrahepatic cholestasis. Molecular Pharmaceutics. 2006; 3:252–265. [PubMed: 16749857]

Horikawa M, Kato Y, Tyson CA, Sugiyama Y. Potential cholestatic activity of various therapeutic agents assessed by bile canalicular membrane vesicles isolated from rats and humans. Drug Metabolism and Pharmacokinetics. 2003; 18:16–22. [PubMed: 15618715]

Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nature Reviews Drug Discovery. 2005; 4:489–499.

Kemp DC, Zamek-Gliszczynski MJ, Brouwer KLR. Xenobiotics inhibit hepatic uptake and biliary excretion of taurocholate in rat hepatocytes. Toxicological Sciences. 2005; 83:207–214. [PubMed: 15509663]

Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nature Reviews Drug Discovery. 2004; 3:711–715.

Kosters A, Karpen SJ. Bile acid transporters in health and disease. Xenobiotica. 2008; 38:1043–1071. [PubMed: 18668439]

Kostrubsky VE, Strom SC, Hanson J, Urda E, Rose K, Burliegh J, Zocharski P, Cai H, Sinclair JF, Sahi J. Evaluation of hepatotoxic potential of drugs by inhibition of bile-acid transport in cultured primary human hepatocytes and intact rats. Toxicological Sciences. 2003; 76:220–228. [PubMed: 12944587]

Kostrubsky SE, Strom SC, Kalgutkar AS, Kulkarni S, Atherton J, Mireles R, Feng B, Kubik R, Hanson J, Urda E, Mutlib AE. Inhibition of hepatobiliary transport as a predictive method for clinical hepatotoxicity of nefazodone. Toxicological Sciences. 2006; 90:451–459. [PubMed: 16410371]

Kukongviriyapan V, Stacey NH. Inhibition of taurocholate transport by cyclosporin A in cultured rat hepatocytes. The Journal of Pharmacology and Experimental Therapeutics. 1988; 247:685–689. [PubMed: 3183963]

Lacy, CF.; Armstrong, LL.; Goldman, MP.; Lance, LL. Drug Information Handbook. 10th ed.. Ohio: Lexi-Comp Inc; 2002.

LeCluyse EL, Audus KL, Hochman JH. Formation of extensive canalicular networks by rat hepatocytes cultured in collagen-sandwich configuration. The American Journal of Physiology – Cell Physiology. 1994; 266:C1764–C1774.

LeCluyse EL, Fix JA, Audus KL, Hochman JH. Regeneration and maintenance of bile canalicular networks in collagen-sandwiched hepatocytes. Toxicology In Vitro. 2000; 14:117–132. [PubMed: 10793290]

Leslie EM, Watkins PB, Kim RB, Brouwer KLR. Differential inhibition of rat and human Na^+-dependent taurocholate cotransporting polypeptide (NTCP/*SLC10A1*) by bosentan: a mechanism for species differences in hepatotoxicity. The Journal of Pharmacology and Experimental Therapeutics. 2007; 321:1170–1178. [PubMed: 17374746]

Liu X, Brouwer KLR, Gan LS, Brouwer KR, Stieger B, Meier PJ, Audus KL, LeCluyse EL. Partial maintenance of taurocholate uptake by adult rat hepatocytes cultured in a collagen sandwich configuration. Pharmaceutical Research. 1998; 15:1533–1539. [PubMed: 9794494]

Liu X, LeCluyse EL, Brouwer KR, Gan LS, Lemasters JJ, Stieger B, Meier PJ, Brouwer KLR. Biliary excretion in primary rat hepatocytes cultured in a collagen-sandwich configuration. The American Journal of Physiology – Gastrointestinal and Liver Physiology. 1999a; 277:G12–G21.

Liu X, LeCluyse EL, Brouwer KR, Lightfoot RM, Lee JI, Brouwer KLR. Use of Ca2+ modulation to evaluate biliary excretion in sandwich-cultured rat hepatocytes. The Journal of Pharmacology and Experimental Therapeutics. 1999b; 289:1592–1599. [PubMed: 10336557]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Lucena MI, García-Cortés M, Cueto R, Lopez-Duran J, Andrade RJ. Assessment of drug-induced liver injury in clinical practice. Fundamental and Clinical Pharmacology. 2008; 22:141–158. [PubMed: 18353109]

Marion TL, Leslie EM, Brouwer KLR. Use of sandwich-cultured hepatcoytes to evaluate impaired bile acid transport as a mechanism of drug-induced hepatotoxicity. Molecular Pharmaceutics. 2007; 4:911–918. [PubMed: 17963355]

McRae MP, Lowe CM, Tian X, Bourdet DL, Ho RH, Leake BF, Kim RB, Brouwer KLR, Kashuba ADM. Ritonavir, saquinavir, and efavirenz, but not nevirapine, inhibit bile acid transport in human and rat hepatocytes. The Journal of Pharmacology and Experimental Therapeutics. 2006; 318:1068–1075. [PubMed: 16720753]

Mita S, Suzuki H, Akita H, Hayashi H, Onuki R, Hofmann AF, Sugiyama Y. Inhibition of bile acid transport across Na^+/taurocholate contransporting polypeptide (SLC10A1) and bile salt export pump (ABCB11)-coexpressing LLC-PK1 cells by cholestasis-inducing drugs. Drug Metabolism and Disposition. 2006; 34:1575–1581. [PubMed: 16760228]

Norris W, Paredes AH, Lewis JH. Drug-induced liver injury in 2007. Current Opinions in Gastroenterology. 2008; 24:287–297.

Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regulatory Toxicology and Pharmacology. 2000; 32:56–67. [PubMed: 11029269]

Pauli-Magnus C, Meier PJ. Hepatobiliary transporters and drug-induced cholestasis. Hepatology. 2006; 44:778–787. [PubMed: 17006912]

Seglen PO. Preparation of isolated rat liver cells. Methods in Cell Biology. 1976; 13:29–83. [PubMed: 177845]

Snow KL, Moseley RH. Effect of thiazolidinediones on bile acid transport in rat liver. Life Sciences. 2007; 80:732–740. [PubMed: 17126857]

Sokol RJ, Devereaux M, Dahl R, Gumpricht E. “Let there be bile”—understanding hepatic injury in cholestasis. Journal of Pediatric Gastroenterology and Nutrition. 2006; 43:S4–S9. [PubMed: 16819400]

Stieger B, Fattinger K, Madon J, Kullak-Ublick G, Meier P. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (bsep) of rat liver. Gastroenterology. 2000; 118:422–430. [PubMed: 10648470]

Szakács G, Váradi A, Ozvegy-Laczka C, Sarkadi B. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). Drug Discovery Today. 2008; 13:379–393. [PubMed: 18468555]

Thummel, KE.; Shen, DD. Design and optimization of dosage regimens: pharmacokinetic data. In: Hardman, JG.; Limbird, LE.; Gliman, AG., editors. Goodman and Gilman’s The Pharmacological Basis of Therapeutics. 10th ed.. New York: The McGraw-Hill Companies, Inc; 2001. p. 1917-2023.

Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. Physiological Reviews. 2003; 83:633–671. [PubMed: 12663868]

White RE, Manitpisitkul P. Pharmacokinetic theory of cassette dosing in drug discovery screening. Drug Metabolism and Disposition. 2001; 29:957–966. [PubMed: 11408361]

Wolf KK, Brouwer KR, Pollack GM, Brouwer KLR. Effect of albumin on the biliary clearance of compounds in sandwich-cultured rat hepatocytes. Drug Metabolism and Disposition. 2008; 36:2086–2092. [PubMed: 18653747]

Zhou H, Tong Z, McLeod JF. “Cocktail” approaches and strategies in drug development: valuable tool or flawed science? Journal of Clinical Pharmacology. 2004; 44:120–134. [PubMed: 14747420]

Zollner G, Marschall HU, Wagner M, Trauner M. Role of nuclear receptors in the adaptive response to bile acids and cholestasis: pathogenetic and therapeutic considerations. Molecular Pharmaceutics. 2006; 3:231–251. [PubMed: 16749856]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

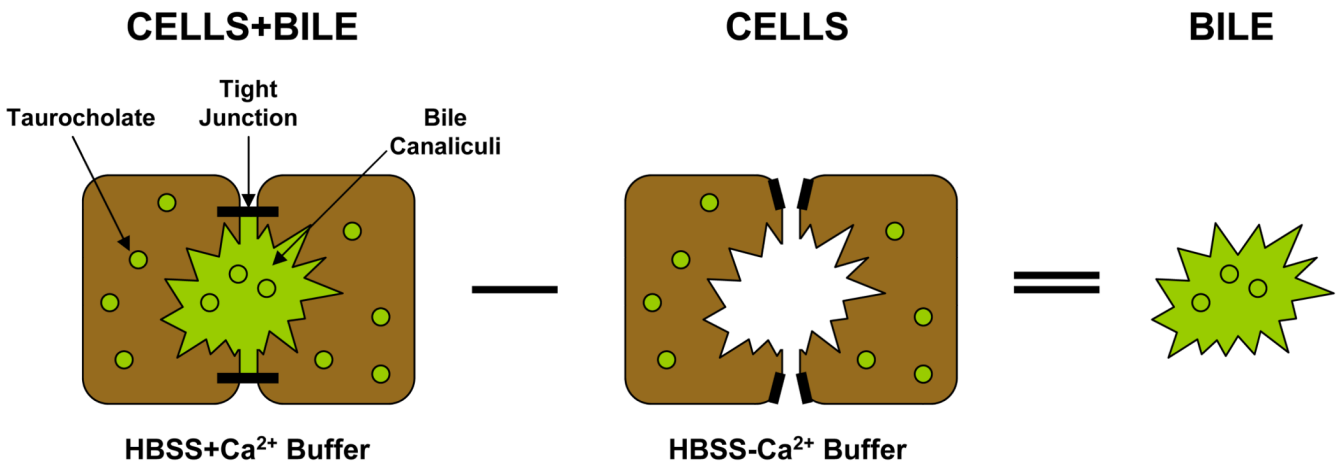


Figure 1. Schematic depicting method of calculating taurocholate accumulation in hepatocytes and bile canaliculi in SCH

SCH were preincubated in HBSS+Ca^{2+} buffer (standard buffer), or HBSS-Ca^{2+} buffer (Ca^{2+}-free buffer) to disrupt tight junctions sealing the bile canalicular space (Liu *et al*., 1999b). Subsequently, cultures were rinsed and SCH were incubated in standard buffer with substrate for 10 min. In SCH preincubated with standard buffer, [^{3}H]taurocholate (circles) was taken up into SCH and excreted into the bile canaliculi; substrate accumulation (cells +bile) was determined at 10 min. In adjacent wells where SCH were preincubated with HBSS-Ca^{2+} buffer to open tight junctions, [^{3}H]taurocholate excreted into the bile compartment was released into the medium; substrate accumulation (cells) was determined at 10 min. The mass of [^{3}H]taurocholate excreted into the bile during the 10-min incubation was estimated as the difference in accumulation in SCH with intact and disrupted tight junctions [(cells+bile) minus cells].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

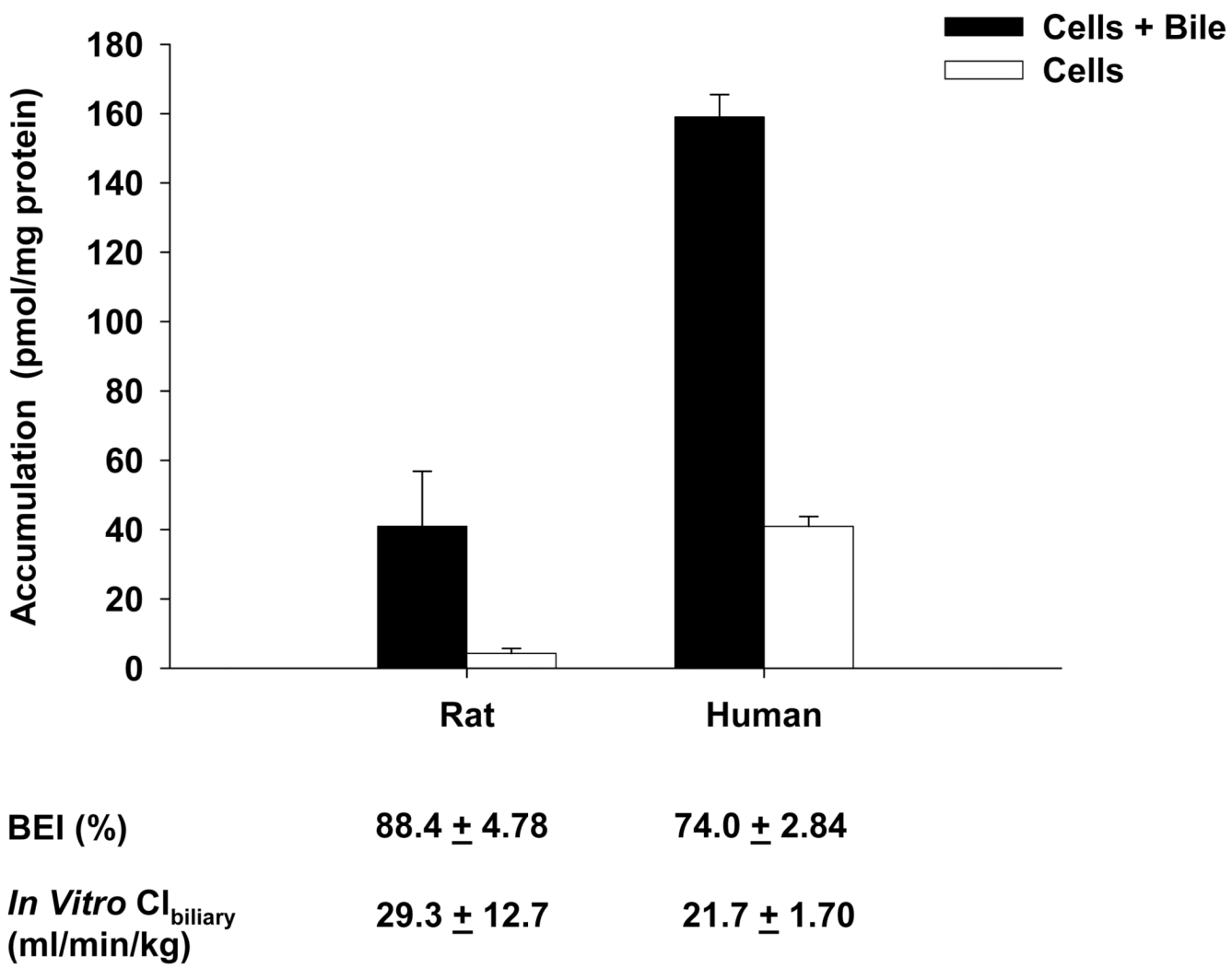


Figure 2. Hepatobiliary disposition of taurocholate *in vitro*

Rat and human SCH were incubated with [^{3}H]-taurocholate (1 μM) for 10 min following incubation in either standard or Ca^{2+}-free buffer. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± SD for n=9 livers in triplicate for rat SCH, and mean ± ½ of the range for n=2 livers in triplicate for human SCH.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

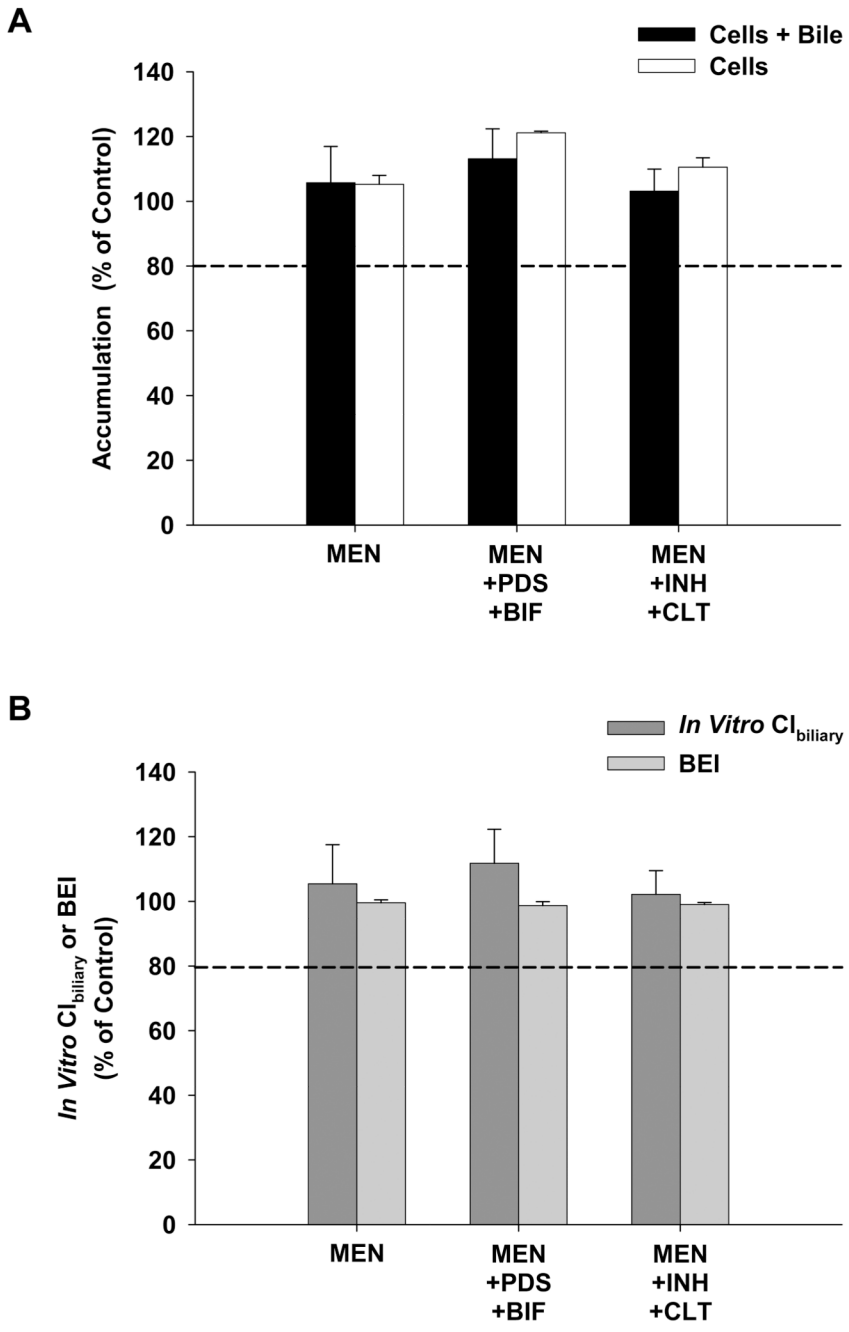


Figure 3. Effect of menadione-containing cassettes on the hepatobiliary disposition of taurocholate in rat SCH

Rat SCH were incubated with menadione (MEN) alone or in cassette with prednisone (PDS) and bifonazole (BIF), or isoniazid (INH) and clotrimazole (CLT), in either standard or Ca^{2+}-free buffer for 10 min, followed by an additional 10-min incubation with [^{3}H]-taurocholate (1 μM) and the drug(s) of interest. **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± ½ of the range for n=2

livers in duplicate. Data for treatment with menadione alone also were presented in Table 3 and serve as a reference in this figure.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

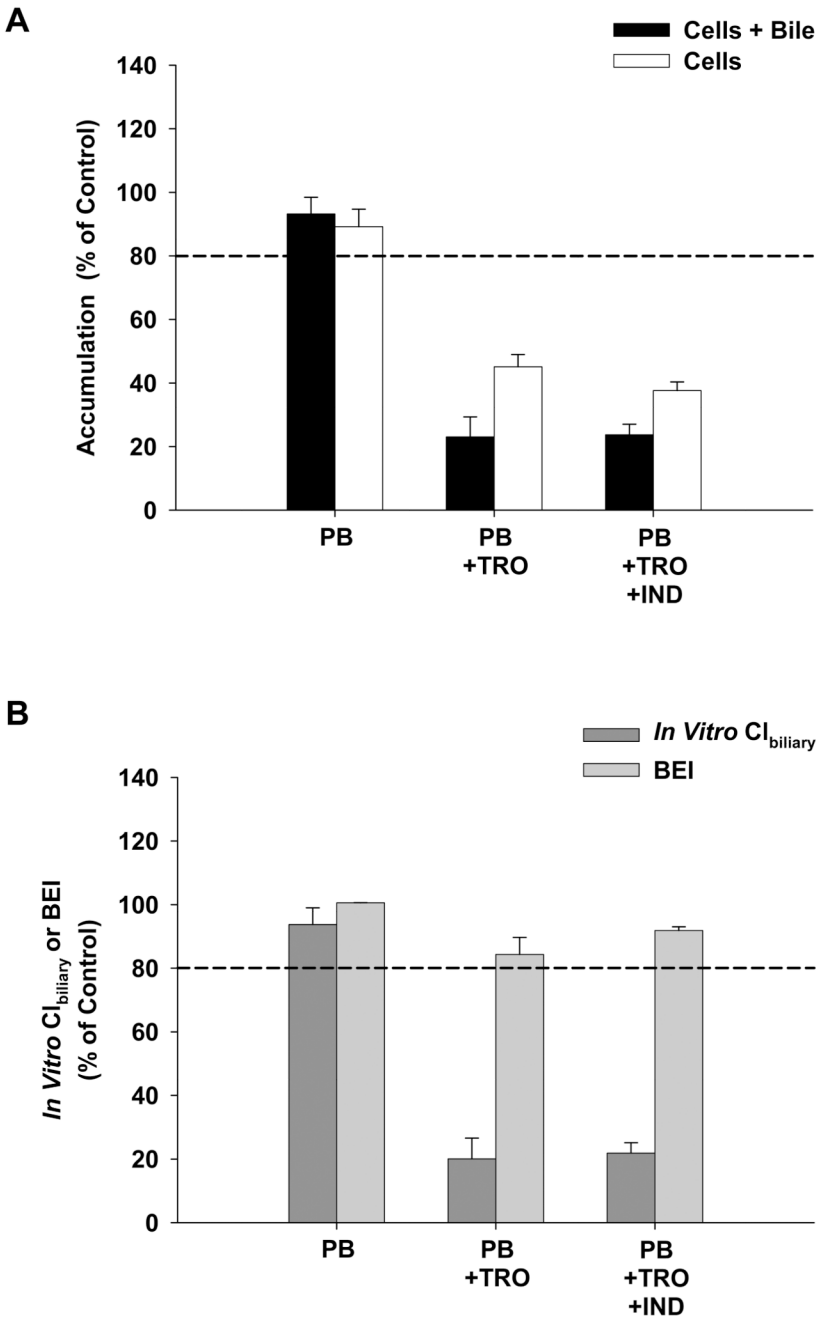


Figure 4. Effect of phenobarbital-containing cassettes on the hepatobiliary disposition of taurocholate in rat SCH

Rat SCH were incubated with phenobarbital (PB) alone or in cassette with troglitazone (TRO) with or without indomethacin (IND) in either standard or Ca^{2+}-free buffer for 10 min, followed by an additional 10-min incubation with [^{3}H]-taurocholate (1 μM) and the drug(s) of interest. **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± ½ of the range for n=2 livers in duplicate. Data

for treatment with phenobarbital alone also were presented in Table 3 and serve as a reference in this figure.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

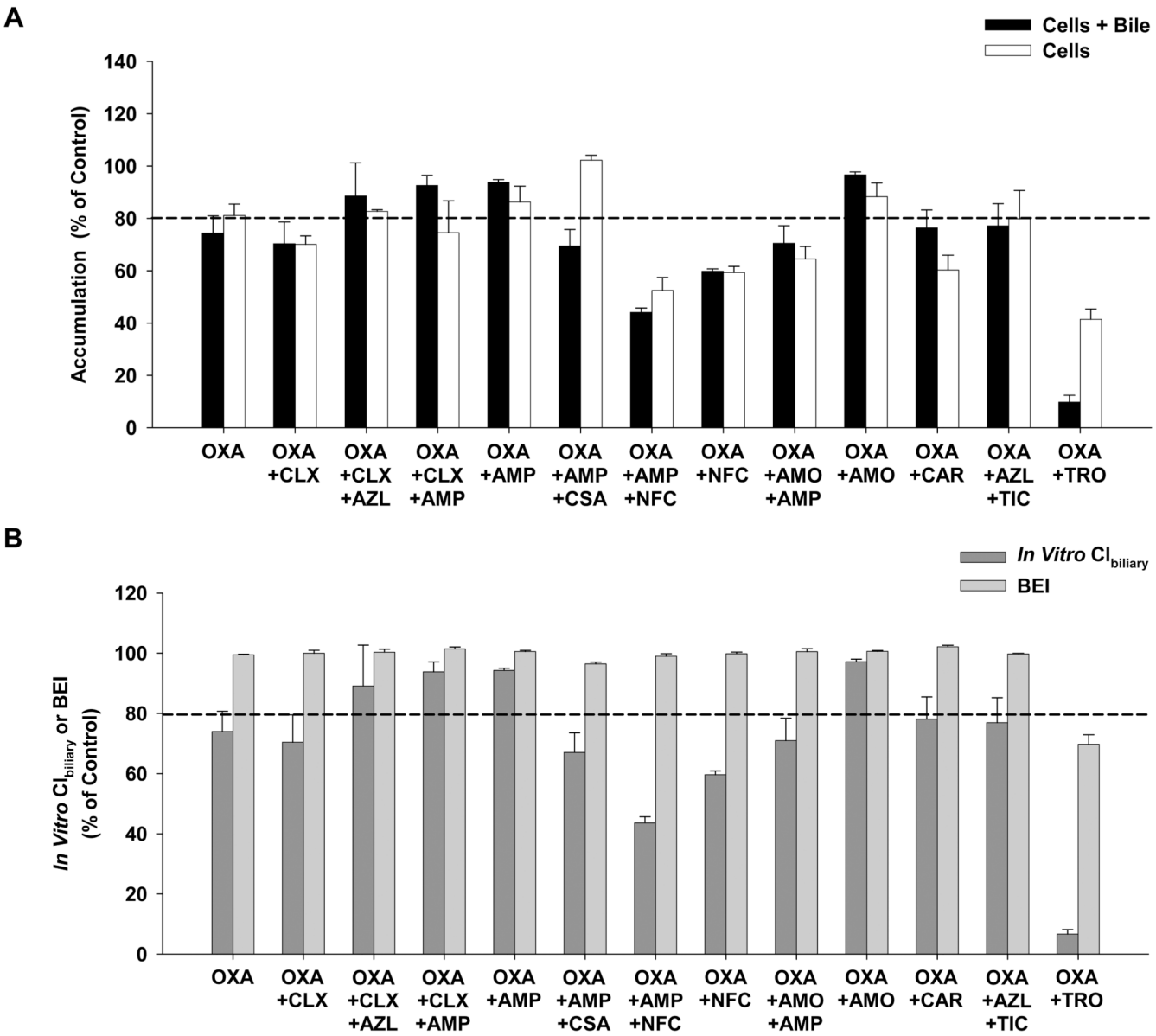


Figure 5. Effect of oxacillin-containing cassettes on the hepatobiliary disposition of taurocholate in rat SCH

Rat SCH were incubated with oxacillin (OXA) alone or in cassette with cloxacillin (CLX), azlocillin (AZL), ampicillin (AMP), cyclosporin A (CSA), nafcillin (NFC), carbenicillin (CAR), amoxicillin (AMO), ticarcillin (TIC), and/or troglitazone (TRO) in either standard or Ca^{2+}-free buffer for 10 min, followed by an additional 10-min incubation with [^{3}H]-taurocholate (1 μM) and the drug(s) of interest. **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± ½ of the range for n=2 livers in duplicate. Data for treatment with oxacillin alone also were presented in Table 3 and serve as a reference in this figure.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

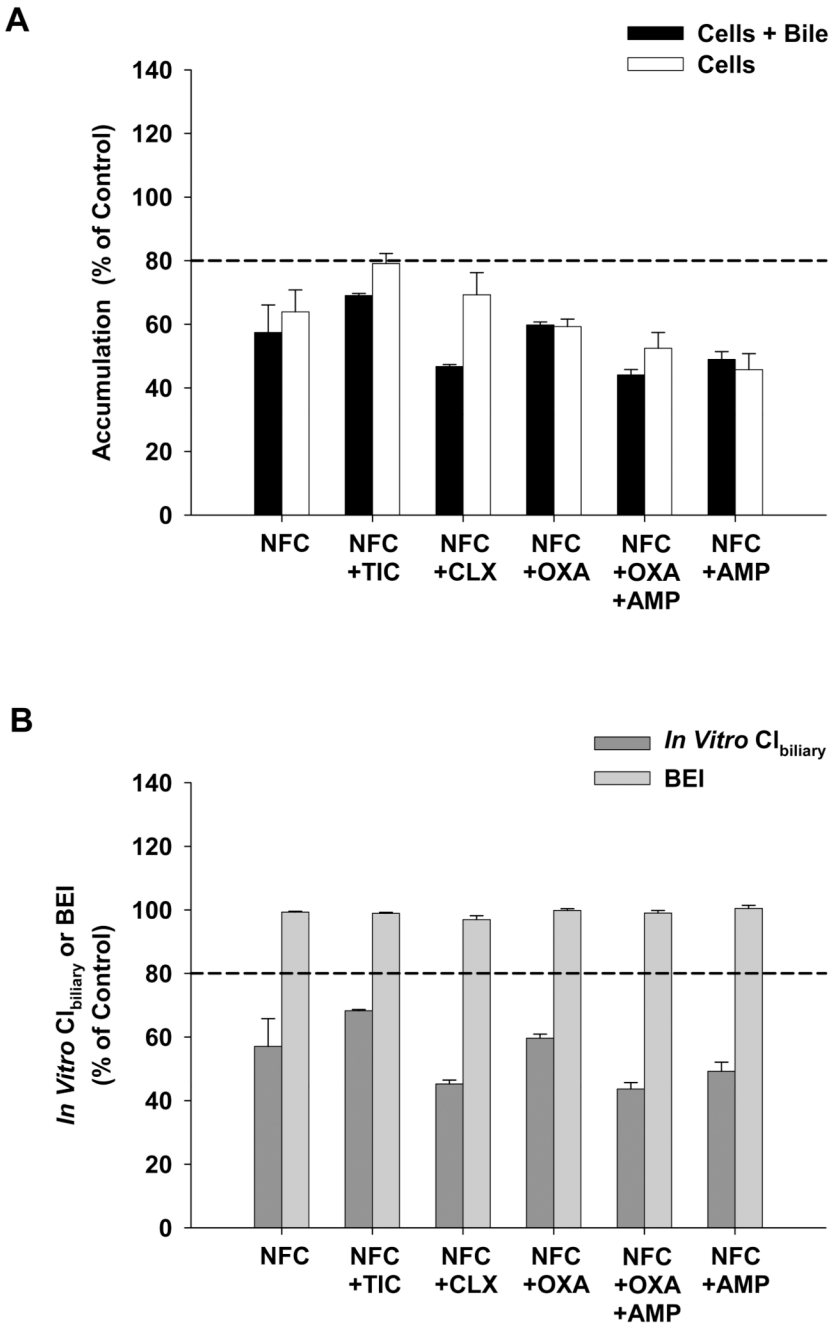


Figure 6. Effect of nafcillin-containing cassettes on the hepatobiliary disposition of taurocholate in rat SCH

Rat SCH were incubated with nafcillin (NFC) alone or in cassette with ticarcillin (TIC), cloxacillin (CLX), oxacillin (OXA), and/or ampicillin (AMP) in either standard or Ca^{2+}-free buffer for 10 min, followed by an additional 10-min incubation with [^{3}H]-taurocholate (1 μM) and the drug(s) of interest. **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± ½ of the range for n=2 livers in duplicate. Data for treatment with nafcillin alone also were presented in Table 3 and

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

serve as a reference in this figure. Data for treatment of nafcillin in cassette with oxacillin with or without ampicillin also were presented in Figure 4 for comparison to treatment with oxacillin alone.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

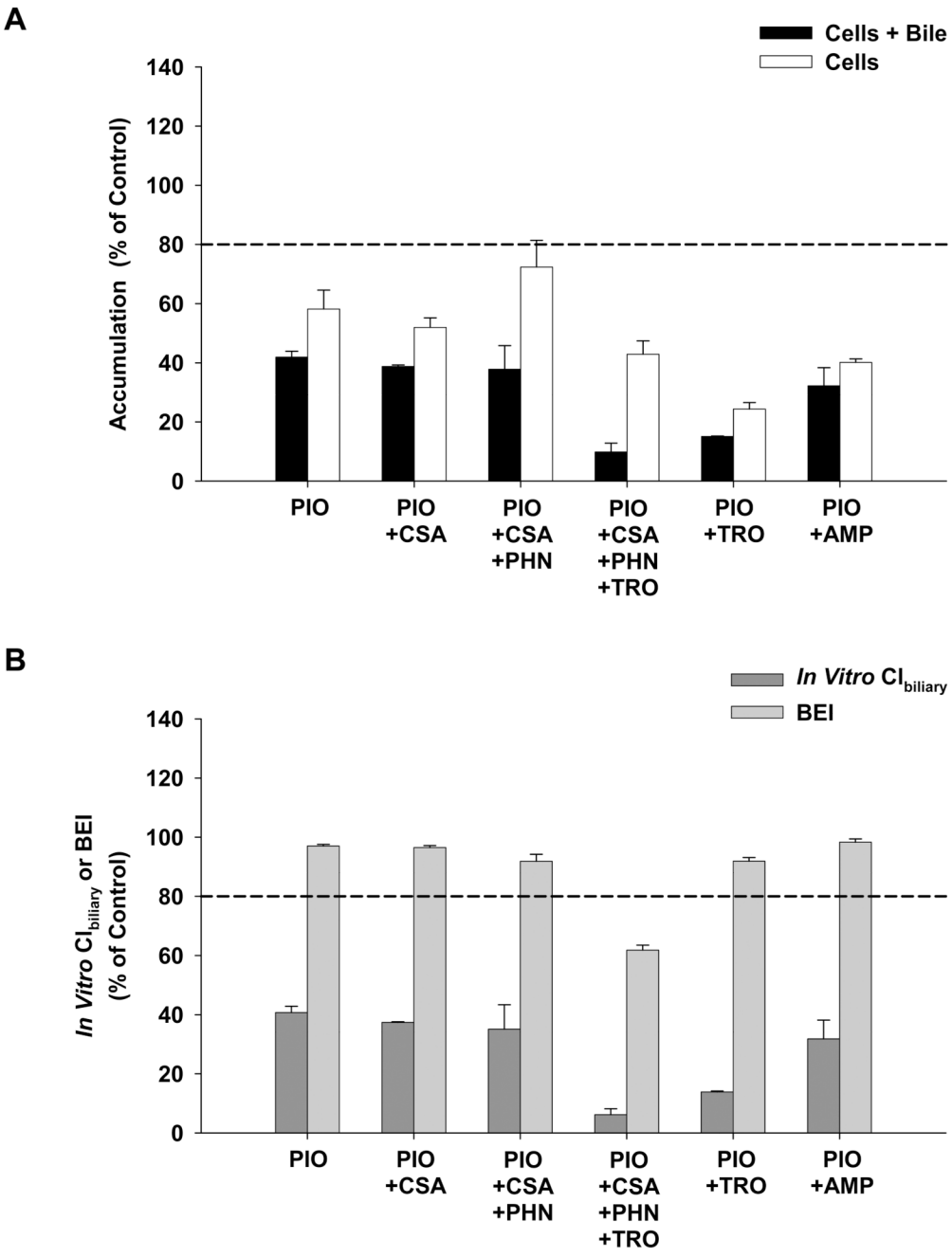


Figure 7. Effect of pioglitazone-containing cassettes on the hepatobiliary disposition of taurocholate in rat SCH

Rat SCH were incubated with pioglitazone (PIO) alone or in cassette with cyclosporin A (CSA), phenytoin (PHN), troglitazone (TRO), and/or ampicillin (AMP) in either standard or Ca^{2+}-free buffer for 10 min, followed by an additional 10-min incubation with [^{3}H]-taurocholate (1 μM) and the drug(s) of interest. **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± ½ of the range for n=2

livers in duplicate. Data for treatment with pioglitazone alone also were presented in Table 3 and serve as a reference in this figure.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

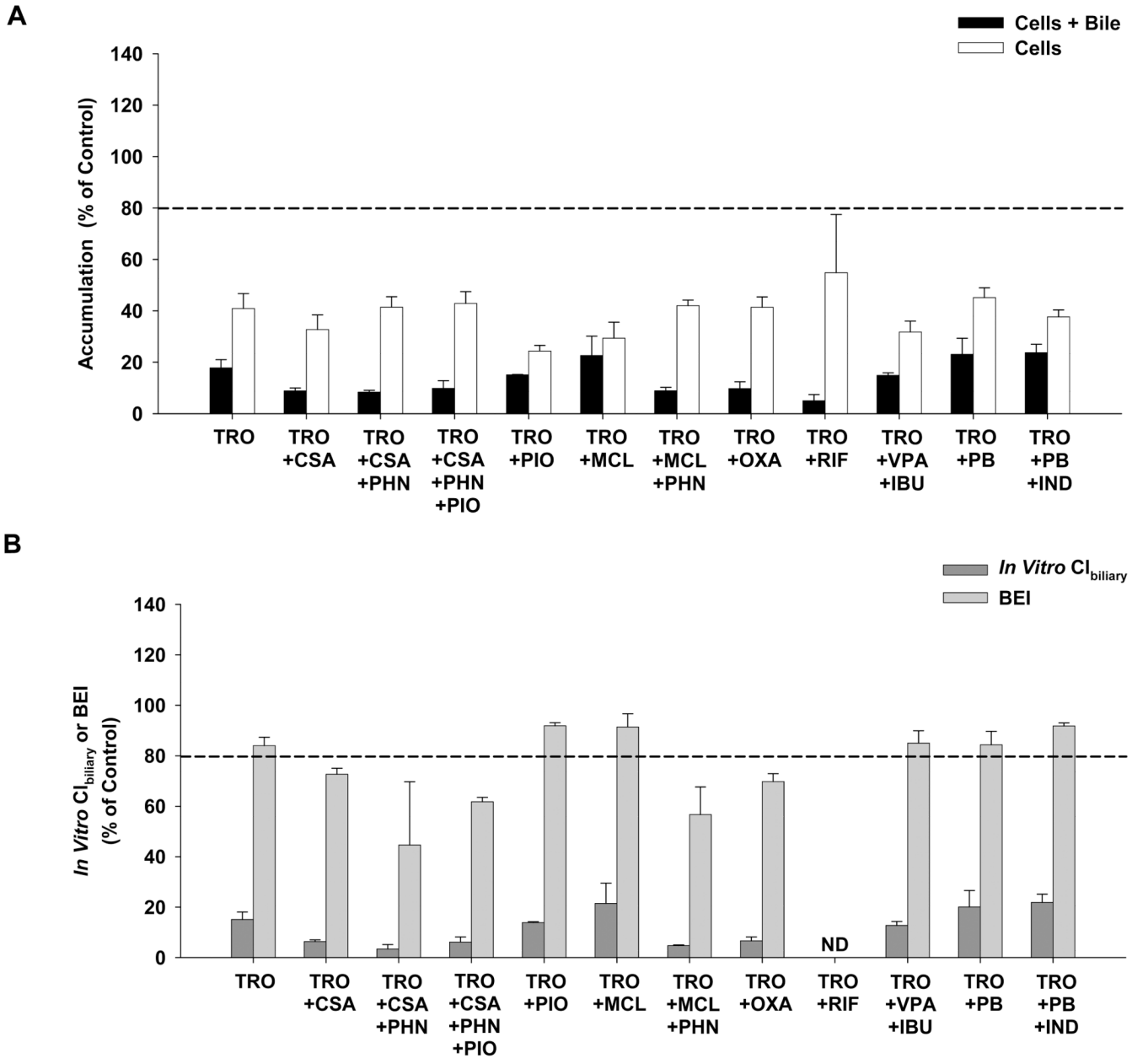


Figure 8. Effect of troglitazone-containing cassettes on the hepatobiliary disposition of taurocholate in rat SCH

Rat SCH were incubated with troglitazone (TRO) alone or in cassette with cyclosporin A (CSA), phenytoin (PHN), pioglitazone (PIO), minocycline (MCL), oxacillin (OXA), rifampin (RIF), valproic acid (VPA), ibuprofen (IBU), phenobarbital (PB), and/or indomethacin (IND) in either standard or Ca^{2+}-free buffer for 10 min, followed by an additional 10-min incubation with [^{3}H]-taurocholate (1 μM) and the drug(s) of interest. **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± ½ of the range for n=2 livers in duplicate, except for troglitazone (mean ± SD for n=3 livers in duplicate). Data for treatment with troglitazone alone also were presented in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 3 and serve as a reference in this figure. Data for treatment of troglitazone in cassette with phenobarbital with or without indomethacin also were presented in Figure 3 for comparison to treatment with phenobarbital alone. Data for treatment of troglitazone in cassette with oxacillin also were presented in Figure 4 for comparison to treatment with oxacillin alone. Data for treatment of troglitazone in cassette with cyclosporin A, phenytoin, and/or pioglitazone also were presented in Figure 6 for comparison to treatment with pioglitazone alone.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

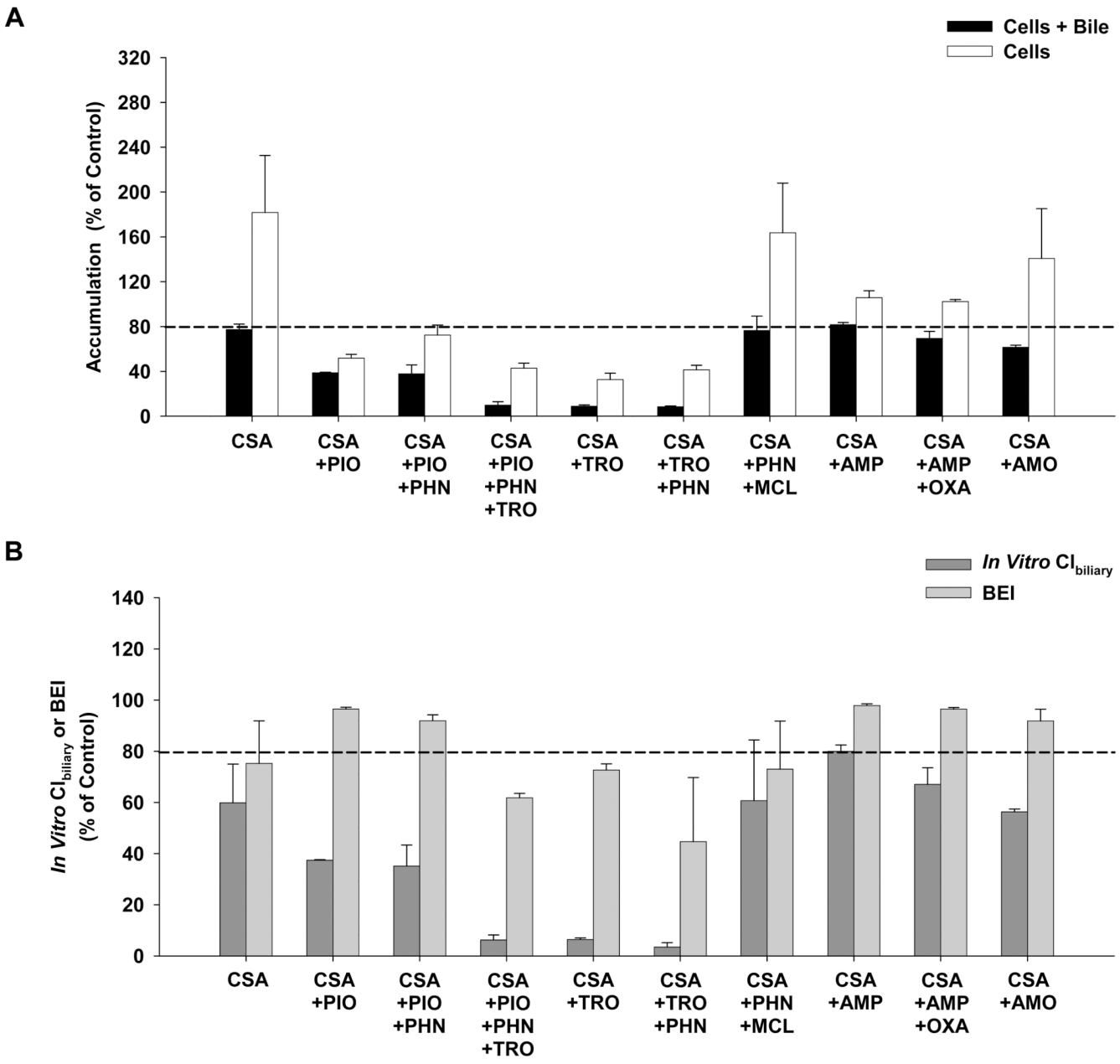


Figure 9. Effect of cyclosporin A-containing cassettes on the hepatobiliary disposition of taurocholate in rat SCH

Rat SCH were incubated with cyclosporin A (CSA) alone or in cassette with pioglitazone (PIO), phenytoin (PHN), troglitazone (TRO), minocycline (MCL), ampicillin (AMP), oxacillin (OXA), and/or amoxicillin (AMO) in either standard or Ca^{2+}-free buffer for 10 min, followed by an additional 10-min incubation with [^{3}H]-taurocholate (1 μM) and the drug(s) of interest. **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as mean ± ½ of the range for n=2 livers in duplicate, except for cyclosporin A (mean ± SD for n=3 livers in duplicate). Data for treatment with cyclosporin A alone also were presented in Table 3 and serve as a reference in this figure.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

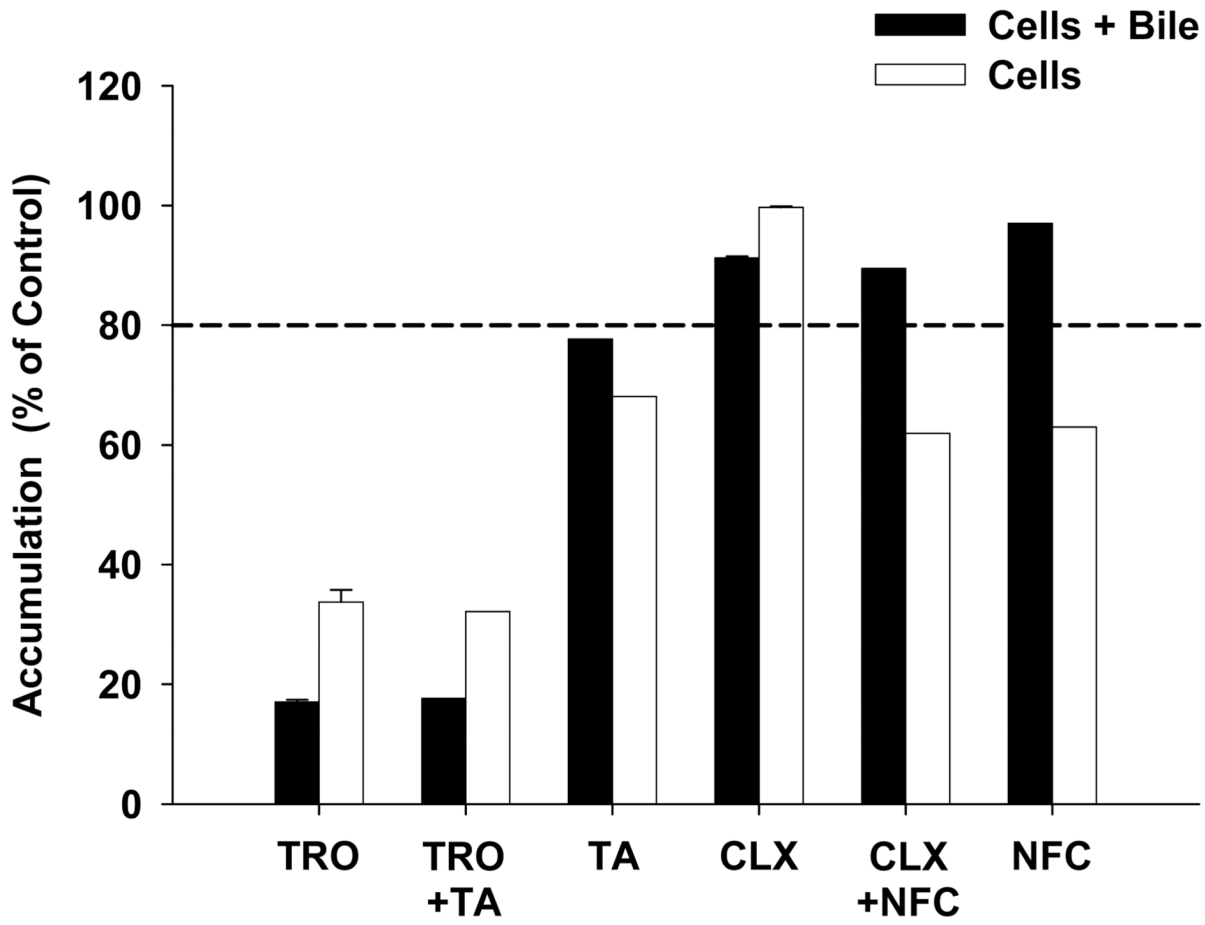
Data for treatment of cyclosporin A in cassette with oxacillin and ampicillin also were presented in Figure 4 for comparison to treatment with oxacillin alone. Data for treatment of cyclosporin A in cassette with pioglitazone, phenytoin, and/or troglitazone also were presented in Figure 6 and Figure 7 for comparison to treatment with pioglitazone or troglitazone alone, respectively.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

A



NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

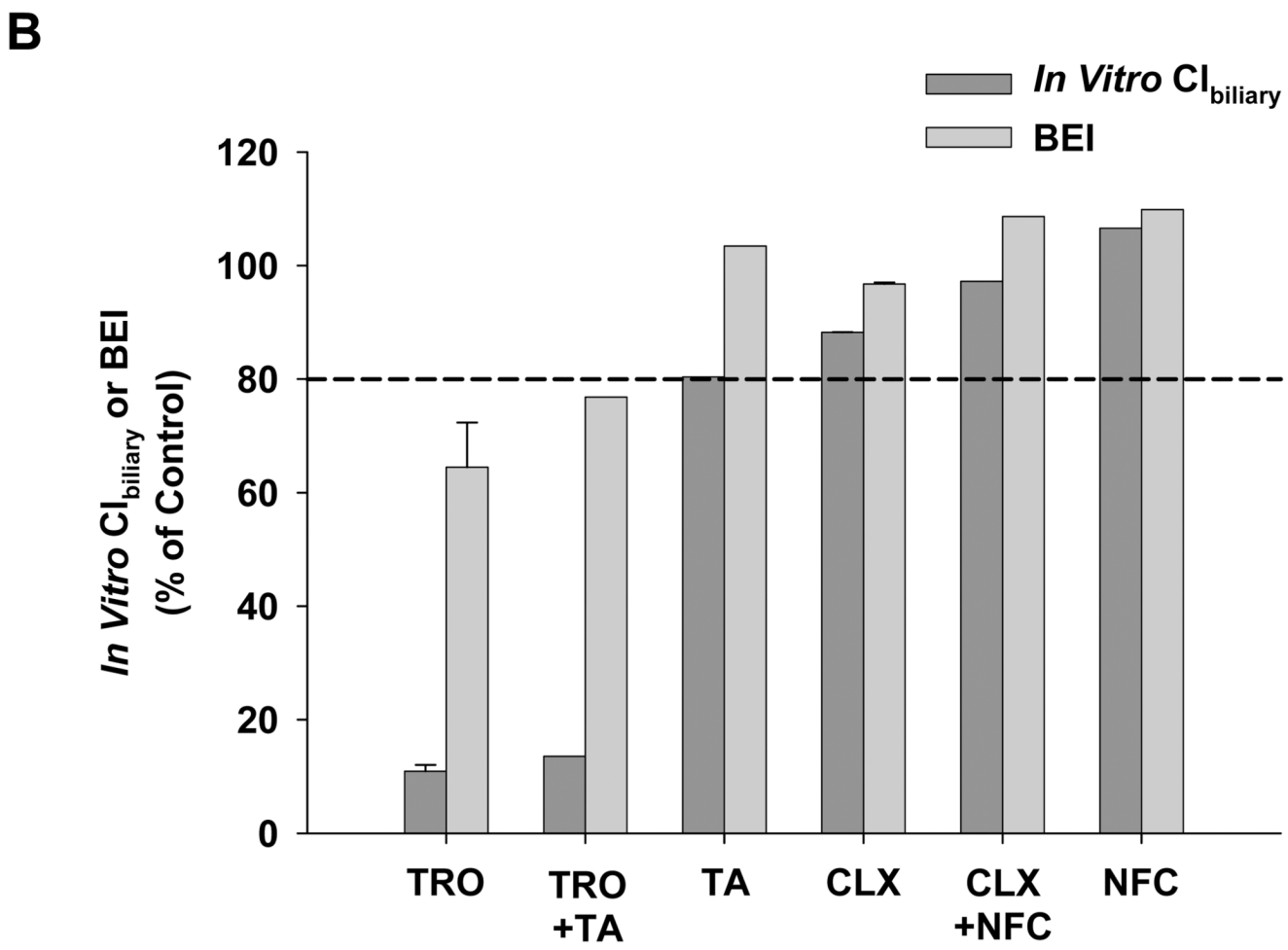


Figure 10. Effect of hepatotoxic compounds on the hepatobiliary disposition of taurocholate in human SCH

Human SCH were incubated with [^{3}H]-taurocholate and the hepatotoxic compound(s) of interest for 10 min following a 10-min incubation in standard or Ca^{2+}-free buffer containing the same hepatotoxic compound(s). **(A)** Taurocholate accumulation. Solid bars represent accumulation in hepatocytes and bile canaliculi (cells+bile). Open bars represent accumulation in hepatocytes (cells). **(B)** BEI and *in vitro* $Cl_{biliary}$ were calculated as described in *Materials and Methods*. Data are presented as the mean for n=1 liver in duplicate, except for troglitazone and cloxacillin (mean ± ½ of the range for n=2 livers in duplicate). TRO, troglitazone; TA, tienilic acid (3.00 μM); CLX, cloxacillin; NFC, nafcillin. Data for treatment with troglitazone, cloxacillin, and nafcillin alone also were presented in Table 3 and serve as a reference in this figure.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Effect of hepatotoxic compounds on [^{3}H]-taurocholate accumulation, biliary excretion index (BEI), and *in vitro* biliary clearance (*in vitro* $Cl_{biliary}$) in sandwich-cultured rat and human hepatocytes[a]

Treatment	Concentration In Buffer	Cells+Bile Accumulation (% of Control)		Cells Accumulation (% of Control)		BEI[b] (% of Control)	*In Vitro*$Cl_{biliary}$[c] (% of Control)	
ANTI-INFECTIVE AGENTS								
ANTIBIOTIC								
β-Lactams								
Penicillins								
Amoxicillin	220 μM	104±19		112±17		99.5±0.2	104±19	
Ampicillin	275 μM	63.2±9.5	↓	76.3±5.5	↓	98.7±0.5	62.4±9.7	↓
Azlocillin	120 μM	95.1±17.5		105±26		99.5±0.5	94.4±17.0	
Carbenicillin	1.65 mM	69.6±9.0	↓	86.4±0.9		98.5±0.8	68.7±9.4	↓
Cloxacillin								
RAT	20.0 μM	71.8±6.0	↓	96.8±15.5		97.9±0.8	70.2±5.3	↓
HUMAN	20.0 μM	91.2±0.3		99.7±0.2		96.8±0.3	88.3±0.0	
Nafcillin								
RAT	80.0 μM	57.4±8.7	↓	63.9±6.9	↓	99.3±0.2	57.1±8.8	↓
HUMAN	80.0 μM	97.0		63.0	↓	110	107	
Oxacillin								
RAT	70.0 μM	74.4±6.7	↓	81.1±4.4		99.5±0.2	74.0±6.8	↓
HUMAN	70.0 μM	91.0		82.0		104	94.9	
Ticarcillin	3.15 mM	82.0±13.6		71.4±12.2	↓	101±0	82.7±13.7	
Cephalosporins								
Cefalexin	700 μM	100±9		101±6		100±2	101±12	
Ceftriaxone	450 μM	116±8		102±7		102±0	118±7	
Cefuroxime	1.13 mM	96.3±6.1		108±7		97.9±0.4	94.2±5.6	
Minocycline								
RAT	3.00 μM	9±16		88.3±5.6		103±1	113±17	
HUMAN	3.00 μM	100		102		99.6	99.5	
ANTIPROTOZOAL								

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Treatment	Concentration In Buffer	Cells+Bile Accumulation (% of Control)		Cells Accumulation (% of Control)		BEI[b] (% of Control)	*In Vitro*$Cl_{biliary}$[c] (% of Control)	
Pentamidine	3.21 μM	121±5	↑	104±1		101±0	121±4	↑
ANTIFUNGAL								
Bifonazole	0.160 μM	105±2		105±6		100±0	105±2	
ANTITUBERCULOSIS								
Isoniazid	908 μM	114±7		105±2		101±1	115±7	
Rifampin	150 μM	21.0±6.8	↓	142±42	↑	41.1±6.0	7.82±1.53	↓
ANTI-INFLAMMATORY AGENTS								
Ibuprofen	30.0 μM	101±4		114±18		98.6±1.6	99.2±2.3	
Indomethacin	0.600 μM	94.8±0.3		99.3±1.3		99.4±0.1	94.2±0.2	
Piroxicam	3.62 μM	132±3	↑	133±0	↑	99.8±0.3	132±3	↑
Prednisone	0.680 μM	113±4		127±15	↑	99.1±0.8	112±3	
NEUROLOGICAL DISORDER AGENTS								
Carbamazepine	125 μM	72.4±0.9	↓	76.8±8.4	↓	99.3±1.4	71.8±0.1	↓
Chlorpromazine	0.500 μM	116±5		118±15		100±1	116±3	
Haloperidol	0.100 μM	103±1		98.8±7.3		101±1	104±0	
Phenobarbital	900 μM	93.2±5.3		89.2±5.5		101±0	93.7±5.3	
Phenytoin	3.00 μM	90.3±12.2		83.9±0.6		100±2	91.3±14.5	
Valproic Acid	2.70 mM	95.3±5.6		95.1±16.6		100±2	95.4±4.1	
CARDIOVASCULAR AGENTS								
Hydralazine	1.50 μM	104±7		99.4±8.2		101±0	105±7	
ANTIDIABETIC AGENTS								
Pioglitazone								
RAT	5.00 μM	41.9±2.0	↓	58.2±6.4	↓	97.0±0.5	40.7±2.2	↓
HUMAN	5.00 μM	54.1	↓	55.9	↓	98.6	53.4	↓
Troglitazone								
RAT	5.00 μM	17.8±6.4	↓	40.9±11.6	↓	84.0±6.7	15.1±5.9	↓
HUMAN	5.00 μM	17.0±0.4	↓	33.7±2.1	↓	64.5±7.9	10.9±1.1	↓
MISCELLANOUS AGENTS								
Bezafibrate	19.6 μM	91.4±2.8		97.8±5.9		99.4±0.2	90.8±2.6	
Colchicine	0.220 μM	102±1		104±1		99.8±0.1	102±1	

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Treatment	Concentration In Buffer	Cells+Bile Accumulation (% of Control)		Cells Accumulation (% of Control)		BEI[b] (% of Control)	*In Vitro*$Cl_{biliary}$[c] (% of Control)	
Cyclosporin A								
RAT	3.00 μM	77.3±9.8	↓	182±102	↑	75.3±33.1	59.9±30.2	↓
HUMAN	3.00 μM	84.3		95.9		94.1	79.4	↓
Etoposide	0.500 μM	109±10		113±15		99.7±0.6	109±9	
Menadione	4.90 μM	106±11		105±3		99.5±0.9	105±12	

[a] Data from rat SCH are presented as mean ± ½ of the range for two livers in duplicate, except for troglitazone (mean ± SD of six livers in duplicate) and cyclosporin A (mean ± SD of three livers in duplicate). Data from human SCH are presented as mean for one liver in duplicate, except for troglitazone and cloxacillin (mean ± ½ of the range for two livers in duplicate).

[b] BEI values were calculated according to equation 1 in *Materials and Methods*

[c] *In vitro* $Cl_{biliary}$ values were calculated according to equation 2 in *Materials and Methods*

↓/↑ decrease/increase of ≥20% relative to control (vehicle)