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MODULATION OF HEPATIC CANALICULAR OR BASOLATERAL TRANSPORT PROTEINS ALTERS HEPATOBILIARY DISPOSITION OF A MODEL ORGANIC ANION IN THE ISOLATED PERFUSED RAT LIVER*

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ABSTRACT

This study examined the impact of hepatic transport protein modulation on the hepatobiliary disposition of a non-metabolized probe substrate, 5 (and 6)-carboxy-2',7'dichlorofluorescein (CDF) in rat isolated perfused livers (IPLs). *In vivo* treatment with modulators (100- and 200-mg/kg/day clofibric acid, 80-mg/kg/day phenobarbital, and 25-mg/kg/day dexamethasone) was used to alter the expression of hepatic transport proteins [organic anion transporting polypeptide (Oatp)1a1, multidrug resistance-associated protein (Mrp)3, and Mrp2] governing the disposition of CDF. The basolateral and biliary excretion of CDF was measured in single-pass IPLs from control and treated rats. Modulators increased the percentage of CDF eliminated into perfusate of IPLs from treated rats (~20-35%) compared to controls (~10%); CDF biliary excretion was decreased in the treated groups. These observations are consistent with modulator-associated increases in the first-order rate constant governing CDF excretion from the hepatocytes into perfusate ($k_{perfusate}$) or decreases in the first-order rate constant governing CDF excretion into bile (k_{bile}). Pharmacokinetic modeling of the data and subsequent simulations revealed that the routes of CDF excretion were most sensitive to changes in $k_{perfusate}$. In contrast, hepatic accumulation of CDF was most sensitive to k_{bile}. The differential sensitivity of CDF excretory routes and hepatic accumulation to these rate constants is a function of intrahepatic distribution kinetics, which must be taken into consideration in assessing the potential impact of altered hepatobiliary transport processes.

INTRODUCTION

A complete understanding of the hepatobiliary transport properties of compounds is not only important for development of drug candidates, but also may be useful in predicting alterations in hepatobiliary disposition due to drug interactions or disease states. Transport systems are subject to modulation by various endogenous and exogenous compounds, and alterations in these systems may have significant implications for the hepatobiliary disposition, and consequently systemic exposure, of drugs.

The ATP-dependent multidrug resistance-associated proteins [Mrp/MRP, (*Abcc/ABCC*)] play an important role in the hepatic excretion of organic anions. Of the nine members in this subfamily, seven are involved in the elimination of compounds from the liver (Chandra and Brouwer, 2004). The canalicular transport protein Mrp2 is responsible for the biliary excretion of organic anions, including leukotriene C_4, divalent bile salts, and glutathione, glucuronide and sulfate conjugates (Konig et al., 1999a). The basolateral organic anion transporter Mrp3 (*Abcc3*) mediates the hepatic excretion of monovalent (*e.g.,* taurocholate and glycocholate) and sulfated bile salts, as well as other organic anions such as 5 (and 6)-carboxy-2',7'dichlorofluorescein (CDF) and glucuronide conjugates such as acetaminophen glucuronide (Xiong et al., 2000). Mrp3/MRP3 is expressed at low levels in normal liver, but is induced by phenobarbital, cholestatic conditions, and also in rats (*e.g.*, TR$^-$ and EHBR) and humans (Dubin-Johnson Syndrome) with hereditary defects in biliary excretion of organic anions (*i.e*., Mrp2 deficiency/mutation) (Jansen et al., 1987; Paulusma et al., 1996; Konig et al., 1999b).

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Upregulation of basolateral Mrp3/MRP3 compensates for diminished Mrp2/MRP2 transport capacity into bile.

Alterations in hepatobiliary disposition also may occur by modulation of transport proteins. Acute chemical treatment may inhibit protein function. Treatment of sandwich-cultured rat hepatocytes with the P-glycoprotein (P-gp) inhibitor GF120918 decreased the biliary excretion index of rhodamine 123 and digoxin (Annaert et al., 2001). Similarly, after GF120198 administration to isolated perfused rat livers, a significant decrease in the biliary excretion of the anti-cancer agent doxorubicin was observed (Booth et al., 1998). More prolonged treatment with chemical modulators may alter protein expression and/or function. For example, phenobarbital treatment of rats impaired the biliary excretion of the glucuronide and sulfate conjugates of acetaminophen, with a concomitant increase in basolateral excretion of the glucuronide conjugate both *in vivo* and in isolated perfused rat livers (Studenberg and Brouwer, 1992; Slitt et al., 2003). Induction of basolateral Mrp3 by phenobarbital accounts, in part, for the increased basolateral transport of acetaminophen glucuronide (Xiong et al., 2002). Competitive inhibition of Mrp2 by the phenobarbital metabolite *p*-hydroxy-phenobarbital glucuronide also may impair biliary excretion and redirect the route of excretion of glucuronide conjugates into sinusoidal blood.

CDF, a model anionic substrate, is taken up by hepatocytes via organic anion transporting polypeptide 1 (Oatp1a1; previously Oatp1) and possibly Oatp1b2 (Zamek-Gliszczynski et al., 2003; previously Oatp4), and is excreted across the hepatic basolateral membrane by Mrp3 and into bile by Mrp2 (Zamek-Gliszczynski et al., 2003). An excellent review of the old and new Oatp nomenclature has been published recently

by Hagenbuch and Meier (2004). The fact that CDF is fluorescent, not metabolized, and is a substrate for both Mrp3 and Mrp2 makes it an ideal model compound for studying transport modulation. Removal of anionic drugs from hepatocytes generally occurs either as a result of basolateral excretion into blood (for subsequent renal elimination) or excretion into bile. In this study, it was hypothesized that modulating basolateral Mrp3 or canalicular Mrp2 protein by *in vivo* chemical treatment would influence the hepatobiliary disposition and extent of elimination of CDF into perfusate (blood) or bile. Western blot analysis was employed to characterize treatment-associated perturbations in CDF transport processes, and pharmacokinetic modeling was used to predict how the direction (*e.g.*, induction vs. downregulation) and magnitude of changes in the relative transport rates for basolateral and biliary excretion influence the route and extent of CDF elimination from the hepatocyte and hepatic accumulation.

MATERIALS AND METHODS

Chemicals. CDF was obtained from Molecular Probes (Eugene, OR). Clofibric acid, dexamethasone, and phenobarbital were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were of analytical grade or higher and were readily available from commercial sources.

Animals. Male Wistar and Sprague-Dawley rats (250-300 g; Charles River Laboratories, Inc., Raleigh, NC) were used for isolated perfused liver (IPL) studies. Rats were kept in a constant alternating 12-h light-dark cycle with access to food and water *ad libitum*. All procedures were approved by the University's Institutional Animal Care and Use Committee. Rats were treated with vehicle or modulators for 4 days, with a 1-day washout, as follows: 100 or 200 mg/kg/day clofibric acid (90% propylene glycol/10% ethanol vehicle) via a jugular vein cannula; 80 mg/kg/day phenobarbital (saline vehicle) i.p.; or 25 mg/kg/day dexamethasone (corn oil vehicle) i.p. The dosing regimens for the modulators were selected based on reports from the literature (Cherrington et al., 2002; Ogawa et al., 2000).

Isolated Perfused Liver Studies. Rats were anesthetized (ketamine:xylazine, 60:12 mg/kg, i.p.), the liver was isolated, and the bile duct and portal vein were cannulated and perfused *in situ* in a single-pass manner using previously described techniques (Brouwer and Thurman, 1996). After transferring the liver to a 37°C perfusion chamber, the liver and perfusate were allowed to equilibrate for 15 min prior to perfusion with 1 µM CDF in oxygenated 37°C Krebs-Ringer bicarbonate buffer containing 16.5 µM taurocholate for

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35 min (steady-state levels were reached). The perfusate was switched to CDF-free buffer for a 25-min washout phase. Bile and perfusate samples were collected from 0-60 min and frozen at -20°C until analysis. On thawing, samples were appropriately diluted with water and analyzed for CDF by fluorescence spectroscopy using a FL600 microplate fluorescence reader (Bio-Tek Instruments, Inc.; Winooski, VT) at wavelengths of 505 nm (excitation) and 523 nm (emission). A standard curve was prepared in water at the time of analysis and was linear in the range of 5-100 nM; the lower limit of detection was 5 nM. Perfusion pressure and bile flow were used to assess liver viability. Livers were blotted dry, weighed, snap-frozen in liquid N_2 and stored at -80°C for immunoblot analysis.

Immunoblot Analysis. Liver samples were thawed in 2 ml of 1x Complete Protease Inhibitor cocktail (Roche, Indianapolis, IN) in 100 mM Tris HCl and homogenized at 30,000 rpm for 1 min and sonicated for 30 sec. Homogenates were centrifuged at 1500 g for 10 min. The supernatant was subsequently centrifuged at 100,000 g for 30 min, and the resultant pellet was resuspended in protease inhibitor solution. Protein concentration of resuspended liver membrane fractions was determined using the bicinchoninic acid method (Smith et al., 1985). Samples for Western blots were prepared according to the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA) and 50 μg of total protein was loaded onto 4-12% Bis-Tris gels without heating. After electrophoresis, proteins were transferred to polyvinylidene fluoride membranes. Membranes were probed with anti-Mrp2 antibody (M_2III-6, Alexis Biochemicals; 1:1500), anti-Mrp3 antibody (kindly donated by Dr. Yuichi Sugiyama; 1:2000), or anti-Oatp1a1 antibody (kindly donated by Dr. Peter Meier; 1:1500), washed 3 times and then probed with horseradish peroxidase

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conjugated anti-mouse or anti-rabbit IgG secondary antibody. Detection was via enhanced chemiluminescence reagents (SuperSignal/West Dura; Pierce, Rockford, IL).

Pharmacokinetic Modeling. Compartmental modeling was utilized to describe the hepatobiliary disposition of CDF in IPLs from control and modulator-treated rats. The compartmental model that best described the perfusate outflow rate and biliary excretion rate vs. time data for CDF in the single-pass IPL is shown in Fig.1. Several alternative compartmental models were evaluated before the final selection was made. The final model included a compartment representing the sinusoidal space of the liver (X_{Sin}) which was perfused at a flow rate of 30 ml/min (Q) with concentrations of CDF in the inflow and outflow perfusate of C_{in} and C_{out}, respectively. The sinusoidal space was assumed to be in equilibrium with the first of two compartments representing the hepatocellular space of the liver (L_1 and L_2), with subsequent unidirectional excretion into bile from the second liver compartment (k_{bile}). All processes were assumed to be linear, and are represented as either an uptake clearance from the sinusoidal space (CL_{up}) or a first-order rate constant (k_{ij}). It also was assumed that no metabolism of CDF occurred in this model system. A first-order process, representing the non-instantaneous mixing of CDF-free buffer with CDF-containing buffer in the IPL tubing leading into the liver, was included to account for the time lag associated with initial accumulation (when the perfusate was switched from CDF-free to CDF-containing) and washout (when perfusate was switched from CDF-containing to CDF-free). The rate of exit of CDF from the liver (outflow rate data) was calculated as the product of the flow and perfusate concentration during each collection interval. CDF biliary excretion rates similarly were calculated as the product of

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bile flow and biliary concentration during each collection interval. Differential equations

based on the mass balance of CDF in each compartment were fit simultaneously to the

perfusate and biliary excretion data by nonlinear least-squares regression (WinNonlin Pro

v4.2, Pharsight Corp., Mountain View, CA). A weighting scheme of $1/(Y_{predicted})$ and

$3/(Y_{predicted})$ was used for the outflow rate and biliary excretion rate data, respectively.

The 3-fold higher weight for the bile data was incorporated to offset the approximately 3-

fold larger number of data points in perfusate as compared to bile. The goodness of fit for

each model to the data was determined based on visual examination of the distribution of

residuals, the condition number, coefficient of variation of parameter estimates,

correlation matrices, and Akaike's information criterion (Akaike, 1976). The differential

equations used to describe the disposition of CDF in this system are described below:

$$\frac{dX_{\sin}}{dt} = Q \cdot C_{in} - Q \cdot C_{out} + k_{perfusate} \cdot X_{L1} - CL_{up} \cdot C_{out} \qquad X_{\sin}^0 = 0$$

$$\frac{dX_{L1}}{dt} = CL_{up} \cdot C_{out} - k_{perfusate} \cdot X_{L1} - k_{12} \cdot X_{L1} + k_{21} \cdot X_{L2} \qquad X_{L1}^0 = 0$$

$$\frac{dX_{L2}}{dt} = k_{12} \cdot X_{L1} - k_{21} \cdot X_{L2} - k_{bile} \cdot X_{L2} \qquad X_{L2}^0 = 0$$

where the variables and parameters are defined as in Fig. 1.

Simulations were performed in order to evaluate the effects of perturbations in the

parameters representing the basolateral excretion, biliary excretion, and uptake clearance

processes (*i.e.*, $k_{perfusate}$, k_{bile} and CL_{up}) on the extent of CDF elimination into blood and

bile, and hepatic accumulation using the model structure in Fig. 1. The effect of either

increasing or decreasing each individual parameter 3-, 10-, or 100-fold on CDF

basolateral excretion rate, biliary excretion rate and hepatic content (sum of CDF in L1

and L2) was examined. A constant rate of CDF presentation to the liver (100 nmol/min)

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was used for all simulations with parameter estimates based on the values from control rats (Table 2). The infusion was terminated at 240 min, at which time steady-state excretion rates were obtained in every modulation scenario.

Data Analysis. Three rats per treatment group were used in the IPL studies. All data are expressed as mean ± SD. Statistically significant differences between the generated parameters for each treatment were determined by Student's t-test or analysis of variance (ANOVA) and appropriate post-hoc pairwise comparison procedures. Statistical significance was determined at the $\alpha=0.05$ level.

RESULTS

The model displayed schematically in Fig. 1 was capable of describing the CDF perfusate outflow rate- and biliary excretion rate-time data in all treatment groups. Representative descriptions of the data and model fits are displayed in Fig. 2 for IPLs from a control and clofibric acid treatment group.

Clofibric acid treatment resulted in dose-dependent increases in Oatp1a1 and decreases in Mrp2 expression, while Mrp3 levels remained unchanged (Fig. 3A). Treatment with phenobarbital or dexamethasone decreased hepatic Oatp1a1 expression and increased Mrp2 and Mrp3 protein levels (Fig. 3B).

Pharmacokinetic analysis of the outflow rate and biliary excretion rate data revealed that pretreatment with clofibric acid significantly decreased k_{bile}; no alteration in $k_{perfusate}$ was evident (Table 1). Clofibric acid also appeared to decrease CL_{up} in a dose-dependent manner although the difference relative to control was significant only at the highest dose. Phenobarbital treatment significantly decreased CL_{up} and increased $k_{perfusate}$, while k_{bile} was unchanged relative to control (Table 2). Pretreatment with dexamethasone significantly decreased CL_{up} and k_{bile}, while $k_{perfusate}$ increased relative to control (Table 3).

The percentage of CDF eliminated into the perfusate of single-pass IPLs during the washout phase is depicted in Fig. 4 for each treatment group. Clofibric acid (100- and 200-mg/kg/day) decreased CDF excretion in bile (expressed as a percentage of the total amount recovered) from control values of $90.6 \pm 1.3\%$ to $84.3 \pm 1.4\%$ and $78.2 \pm 5.1\%$, respectively. Likewise, phenobarbital decreased CDF biliary excretion from $84.7 \pm 1.9\%$

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to $69.8 \pm 1.6\%$; dexamethasone decreased CDF biliary excretion from $90.0 \pm 0.5\%$ to $64.1 \pm 4.1\%$.

Simulation studies examined the effect of either increasing or decreasing $k_{perfusate}$ or k_{bile} on biliary and basolateral excretion (Fig. 5A and B, respectively) and hepatic content (Fig. 6) of CDF. Decreasing $k_{perfusate}$ resulted in a substantial increase in the proportion of CDF undergoing biliary excretion (Fig. 5A). When $k_{perfusate}$ was increased, the primary route of elimination was shifted from bile to perfusate (Fig. 5B), and accumulation of CDF in the liver was modestly decreased (Fig. 6). A decrease in k_{bile} reduced CDF biliary excretion and redirected elimination into the perfusate, although the system was not as sensitive to changes in k_{bile} compared to changes in $k_{perfusate}$. As k_{bile} increased, the percentage of the dose excreted in bile reached a limiting value of ~80% (Fig. 5A). A 100-fold decrease in k_{bile} from the control value markedly increased the hepatic content of CDF (Fig. 6). As expected, modulating CL_{up} did not result in a change in the proportion of CDF eliminated into bile or perfusate relative to control. However, increases in CL_{up} substantially enhanced hepatic accumulation (data not shown).

DISCUSSION

In this study, four treatment protocols were utilized to modulate expression of hepatic transport proteins governing the disposition of CDF. On the basolateral membrane, Oatp1a1 (and perhaps Oatp1b2) is responsible for the uptake of CDF from perfusate (representing blood), while egress from the liver occurs via basolateral Mrp3 and canalicular Mrp2 into perfusate and bile, respectively (Zamek-Gliszczynski et al., 2003).

Clofibric acid increased Oatp1a1 expression, and decreased Mrp2 expression, in a dose-dependent manner. Mrp3 levels were not affected by this modulator. Based on pharmacokinetic modeling, the rate constants associated with excretion of CDF into perfusate and bile ($k_{perfusate}$ and k_{bile}, respectively) were consistent with the changes in protein expression ($k_{perfusate}$ unchanged and k_{bile} decreased). In contrast, although Oatp1a1 expression increased in response to clofibric acid treatment, CL_{up} was decreased in livers from clofibric acid-treated rats. It is possible that the induced Oatp1a1 protein may not have been functional due to factors such as improper localization in the membrane or impaired glycosylation of the protein (Cai et al., 2002), leading to the apparent disconnect between expression and function. A similar observation has been reported for Mrp2 in sandwich-cultured rat hepatocytes (Zhang et al., 2004).

Treatment with phenobarbital decreased Oatp1a1 expression with a concomitant decrease in the CL_{up} of CDF. Significant induction of basolateral Mrp3 was observed following phenobarbital treatment, with a consequent increase in excretion of CDF across the basolateral membrane (increased $k_{perfusate}$). The lack of an effect of phenobarbital on k_{bile} despite an increase in Mrp2 protein was not surprising. Other investigators have

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reported unaltered or even decreased Mrp2-mediated transport into bile after *in vivo* phenobarbital treatment (Patel et al., 2003), presumably due to competition for transport by phenobarbital metabolites. An alternative explanation is that phenobarbital increases sequestration of CDF in compartment L2.

Changes in CL_{up} and $k_{perfusate}$ in response to dexamethasone correlated with changes in protein expression of Oatp1a1 and Mrp3, respectively. However, the CDF biliary excretion rate constant (k_{bile}) was decreased despite induction of hepatic Mrp2, as demonstrated by Western blot analysis. This may have resulted from defective protein phosphorylation or impaired cellular trafficking after exposure to dexamethasone (Kain et al., 1992; Rao et al., 2001; Zhang et al., 2004), but the underlying mechanism requires further investigation.

The percentage of CDF that was eliminated into perfusate during the washout phase is shown in Fig. 4. For a compound such as CDF which is not excreted avidly from the hepatocyte into perfusate (~10-15%), a large increase in $k_{perfusate}$ (dictating excretion of CDF from the liver into sinusoidal blood) and a large decrease in k_{bile} (governing excretion of CDF from the liver into bile), as occurs with dexamethasone treatment, resulted in a marked increase in the percentage of CDF excreted into perfusate. It is interesting to note that each modulator examined in this experiment resulted in an increased proportion of CDF eliminated into perfusate during the washout phase, consistent with either increased $k_{perfusate}$ or decreased k_{bile} (Tables 1-3).

Simulation experiments were performed to provide a framework for understanding the physiologic implications of the altered pharmacokinetic parameters associated with each of the chemical modulators. As expected, the percentage of CDF

excreted into bile (74%) and across the basolateral membrane into blood (26%) under steady-state conditions did not vary from the control condition when CDF uptake was perturbed (data not shown). In contrast, an increase in the percentage of CDF eliminated into bile can be achieved by either decreasing $k_{perfusate}$ or increasing k_{bile}. For example, a 10-fold reduction in $k_{perfusate}$ increased the percentage of CDF excreted into bile from 74% (*i.e.*, the fractional excretion observed in control rat livers) to 97% (Fig. 5A). In contrast, increasing k_{bile} by as much as 100-fold resulted in only a marginal increase in the amount of CDF in bile (74% to 78%). The more significant influence of $k_{perfusate}$, as compared to k_{bile}, on the biliary excretion of CDF is a function of the intrahepatic distribution of the substrate. The driving force for biliary excretion is the amount of CDF in compartment L2. Large increases in k_{bile} do not substantially influence the flux of CDF into L2, as this process is rate-limited by k_{12}. However, large increases in $k_{perfusate}$ compete with k_{12} for CDF flux into L2, reducing presentation of substrate to the site of biliary excretion.

Similarly, an increase in the percentage of CDF eliminated via basolateral excretion into perfusate can be achieved by either increasing $k_{perfusate}$ or decreasing k_{bile}. For example, in order for the basolateral and biliary processes to each account for 50% excretion of CDF, $k_{perfusate}$ could be increased 3-fold or k_{bile} could be reduced 10-fold relative to control (Fig. 5B). As was the case for biliary excretion, basolateral excretion of CDF is more sensitive to changes in $k_{perfusate}$ as compared to k_{bile}, again as a consequence of the intra-organ distribution kinetics of this substrate.

The effect of varying $k_{perfusate}$ or k_{bile} on hepatic content of CDF is shown in Fig. 6. In contrast to the fractional excretion of CDF in bile vs. sinusoidal blood, hepatic content

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of CDF was more sensitive to changes in k_{bile} than to changes in $k_{perfusate}$, as the bile represents the "preferred" route of CDF excretion under basal conditions. When k_{bile} is decreased, CDF tends to accumulate in liver (predominantly in compartment L2) due to the fact that other flux processes are relatively slow. On the other hand, decreases in $k_{perfusate}$ do not have a substantial effect on hepatic accumulation because the preferred route of elimination remains intact.

While the influence of changes in the two routes of excretion on hepatic accumulation of CDF are clear, the potential impact of such changes on the biologic activity of drug substrates in liver requires further consideration. The liver represents the primary target organ for therapeutic effect (*e.g.,* HMG-CoA reductase inhibitors) or toxicity (*e.g.,* troglitazone) of some drugs. In the case of CDF, the liver is best represented by two segregated spaces in equilibrium rather than a homogeneous distributional space. For drugs with distributional kinetics similar to CDF, the impact of hepatic accumulation on therapeutic or toxic effects may be dependent on the site of biologic activity within the liver (*e.g.,* compartment L1 vs. L2).

Modulation of hepatic transport proteins may have clinical relevance. If excretion through a particular pathway is desirable, alterations in hepatic transport could produce a favorable clinical outcome. For example, the anticancer drug irinotecan is metabolized to SN38G (the glucuronide conjugate) prior to biliary excretion (Mick et al., 1996). Excretion via the bile leads to high intestinal exposure and, as a consequence, results in the dose-limiting toxicity of severe diarrhea (Gupta et al., 1994). If SN38G excretion from the liver could be redirected into blood (and the amount excreted into bile decreased), the severity of the dose-limiting toxicity may be reduced.

The pharmacokinetic model that best described CDF disposition in the single-pass IPL incorporated two compartments to represent the hepatocellular space (Fig. 1). There is precedence for this type of two-compartment hepatic behavior for certain compounds, with distribution into different subcellular spaces (*e.g.,* subapical compartment, endoplasmic reticulum, cytosol) required before elimination into bile can occur (Levine and Singer, 1972). Since it is known that Mrp2 can reside in the membranes of subapical compartments near the canalicular membrane (Roelofsen et al., 1998) it is possible that compartment L2 could represent Mrp2-containing organelles. To test this hypothesis, CDF disposition was evaluated in single-pass IPLs from Mrp2-deficient TR$^-$ rats. Both 1- and 2-compartment hepatic models were fit to the data, and standard model selection criteria were applied to identify the superior model. If the kinetics of intrahepatic distribution of CDF were a function of Mrp2, the model that best describes CDF disposition in TR$^-$ livers would consist of a single homogeneous unit representing the liver. The results of this modeling exercise indicated that a 2-compartment hepatic model was required for Mrp2-deficient livers, suggesting that intrahepatic distribution of CDF is not dependent on the presence of Mrp2 (data not shown). Further experimentation is required to determine the mechanisms underlying the hepatic distributional behavior of CDF, and the degree to which this system might generalize to other agents.

In summary, this study demonstrates how *in vivo* chemical treatment can modulate transport protein expression and the disposition of a model organic anion to varying degrees. Such modulation may have clinical relevance; however, as illustrated in the current studies, additional or unrecognized intracellular processes may be important determinants of substrate disposition, thus requiring full characterization.

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DMD #3665

FOOTNOTES

FIGURE LEGENDS

Fig. 1. Model scheme depicting the disposition of CDF in IPLs from control and modulator-treated rats. Q, flow; X_{Sin}, amount of CDF in sinusoidal space; C_{in}, concentration of CDF in inflow perfusate; C_{out}, concentration of CDF in outflow perfusate; CL_{up}, uptake clearance into the liver; $k_{perfusate}$, first-order rate constant for basolateral excretion; X_{L1}, amount of CDF in liver compartment 1; X_{L2}, amount of CDF in liver compartment 2; k_{12}, first-order rate constant for distribution into liver compartment 2; k_{21}, first-order rate constant for distribution from liver compartment 2 to liver compartment 1; k_{bile}, first-order rate constant for biliary excretion; X_{bile}, amount of CDF in bile.

Fig. 2. Representative fit of the model scheme depicted in Fig. 1 to CDF perfusate outflow rate (o) and biliary excretion rate (●) data obtained from a control (upper panel) and a clofibric acid-treated (200-mg/kg/day; lower panel) rat. Lines represent fit of the model to observed data. Outflow rate and biliary excretion rate data were fit simultaneously for each data set.

Fig. 3. Representative Western blot of Oatp1a1, Mrp3, and Mrp2 after 4-day treatment of rats with A) 100 or 200 mg/kg/day clofibric acid and B) 80 mg/kg/day phenobarbital or 25 mg/kg/day dexamethasone. CA = clofibric acid; PB = phenobarbital; DEX = dexamethasone.

Fig. 4. Percentage of CDF eliminated into perfusate during the washout phase for each treatment group and its respective control. Mean $\pm$ SD; n = 3. C = control; CA = clofibric acid; PB = phenobarbital; DEX = dexamethasone. * $p<0.05$ relative to control group.

Fig. 5. Effect of modulating $k_{perfusate}$ ($\bullet$) or k_{bile} (o) on CDF elimination in bile (A) or across the basolateral membrane (B) at steady-state. Parameter estimates from the control group in Table 2 were used for all simulations; the rate of CDF presentation to the liver was 100 nmol/min.

Fig. 6. Effect of modulating $k_{perfusate}$ ($\bullet$) or k_{bile} (o) on hepatic CDF content (sum of CDF in compartments L1 and L2) at steady-state. Parameter estimates from the control group in Table 2 were used for all simulations; the rate of CDF presentation to the liver was 100 nmol/min.

Table 1. Mean parameter estimates are shown for CL_{up}, $k_{perfusate}$, k_{12}, k_{21} and k_{bile} for CDF disposition in single-pass IPLs from control (90% propylene glycol/10% ethanol vehicle) and clofibric acid-treated rats using the model scheme shown in Fig. 1 (mean ± SD; n=3). CA = clofibric acid. * p<0.05 relative to control group.

Parameter	Control (mean ± SD)	CA 100 (mean ± SD)	CA 200 (mean ± SD)
CL_{up} (ml/min)	8.4 ± 1.2	7.0 ± 0.7	4.2 ± 0.4 *
$k_{perfusate}$ (min^{-1})	0.014 ± 0.007	0.006 ± 0.005	0.019 ± 0.015
k_{12} (min^{-1})	0.061 ± 0.004	0.20 ± 0.14	0.22 ± 0.03
k_{21} (min^{-1})	0.031 ± 0.026	0.10 ± 0.04	0.084 ± 0.006
k_{bile} (min^{-1})	0.21 ± 0.04	0.073 ± 0.009 *	0.035 ± 0.006 *

Table 2. Mean parameter estimates for CL_{up}, $k_{perfusate}$, k_{12}, k_{21} and k_{bile} for CDF disposition in single-pass IPLs from control (saline vehicle) and phenobarbital-treated rats using the model scheme shown in Fig. 1 (mean $\pm$ SD; n=3). * p<0.05 relative to control group.

Parameter	Control (mean $\pm$ SD)	Phenobarbital (mean $\pm$ SD)
CL_{up} (ml/min)	6.7 ± 0.6	4.5 ± 0.5 *
$k_{perfusate}$ (min^{-1})	0.022 ± 0.004	0.075 ± 0.010 *
k_{12} (min^{-1})	0.077 ± 0.013	0.24 ± 0.13
k_{21} (min^{-1})	0.060 ± 0.064	0.028 ± 0.007
k_{bile} (min^{-1})	0.25 ± 0.11	0.074 ± 0.014

Table 3. Mean parameter estimates for CL_{up}, $k_{perfusate}$, k_{12}, k_{21} and k_{bile} for CDF disposition in single-pass IPLs from control (corn oil vehicle) and dexamethasone-treated rats using the model scheme shown in Fig. 1 (mean $\pm$ SD; n=3). * p<0.05 relative to control group.

Parameter	Control (mean $\pm$ SD)	Dexamethasone (mean $\pm$ SD)
CL_{up} (ml/min)	8.1 ± 0.5	5.2 ± 0.4 *
$k_{perfusate}$ (min^{-1})	0.016 ± 0.005	0.10 ± 0.02 *
k_{12} (min^{-1})	0.097 ± 0.009	0.33 ± 0.17
k_{21} (min^{-1})	0.004 ± 0.004	0.060 ± 0.025 *
k_{bile} (min^{-1})	0.25 ± 0.04	0.068 ± 0.011 *

Fig 1
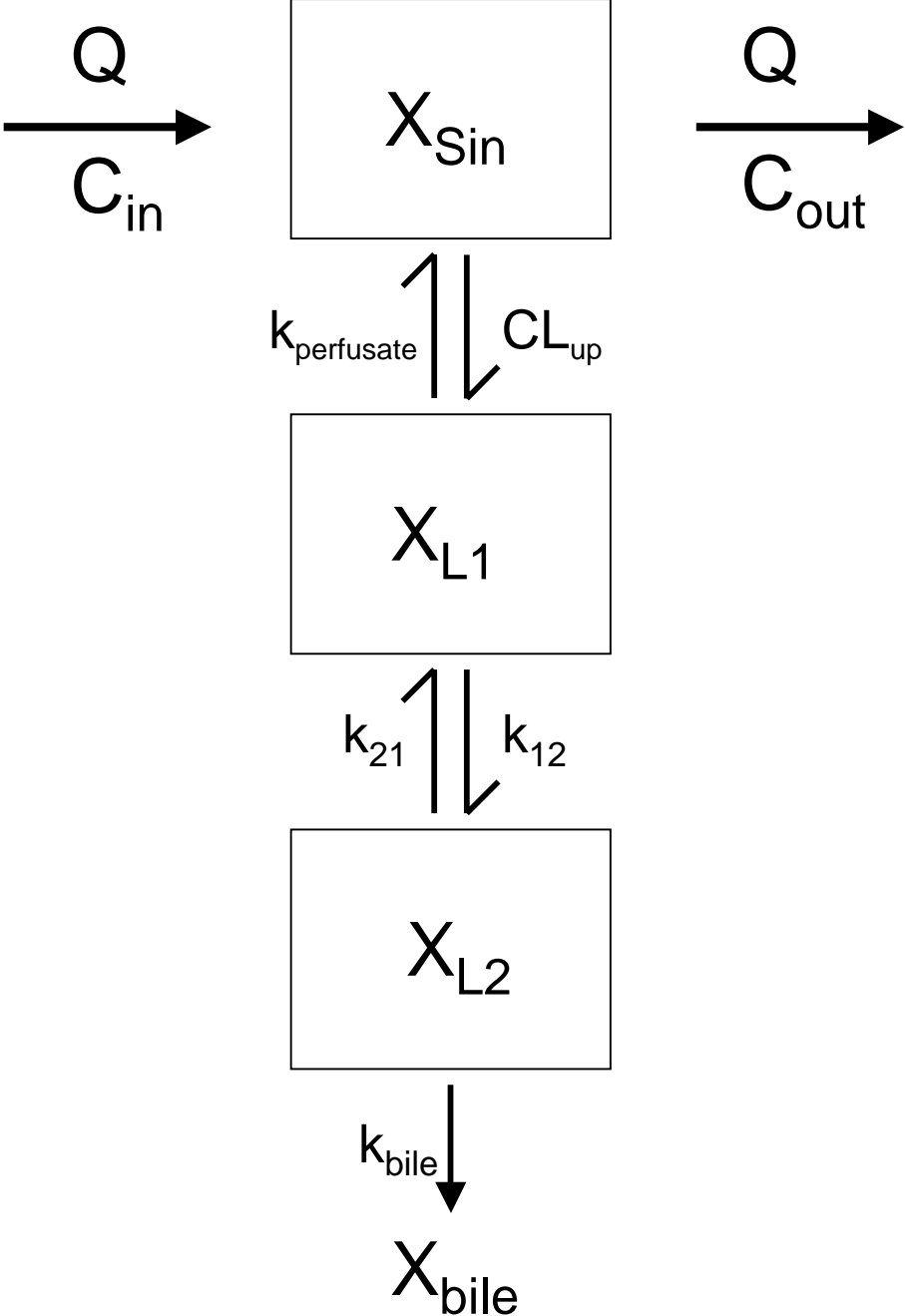
Fig 2A
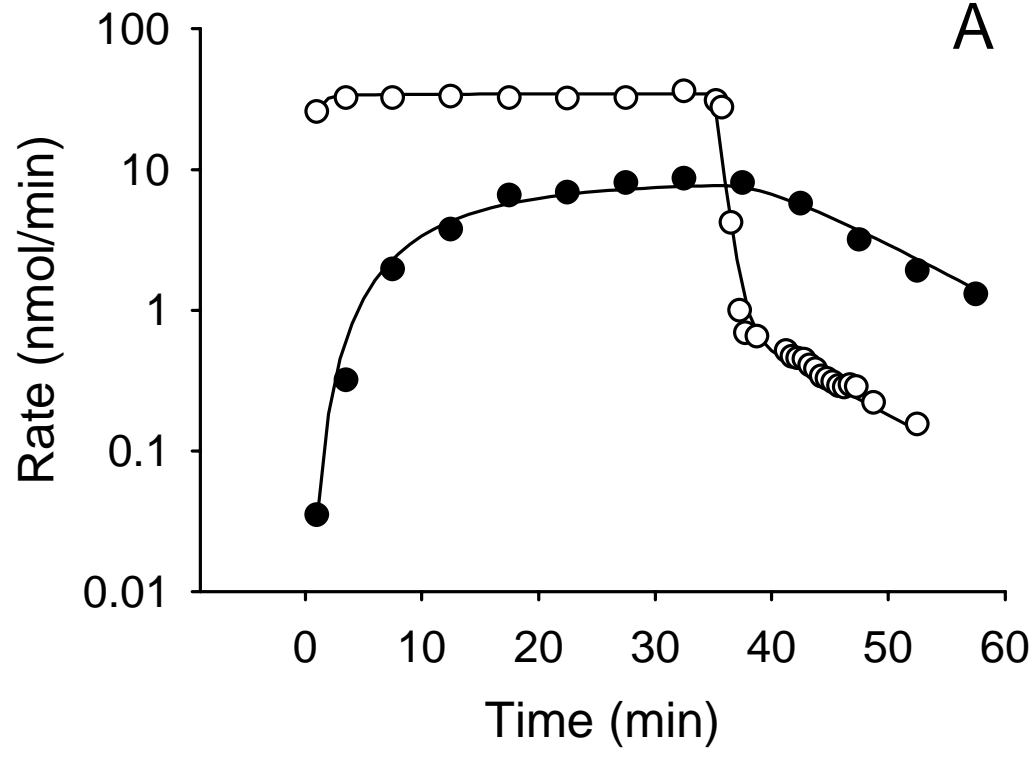
Fig 2B

B
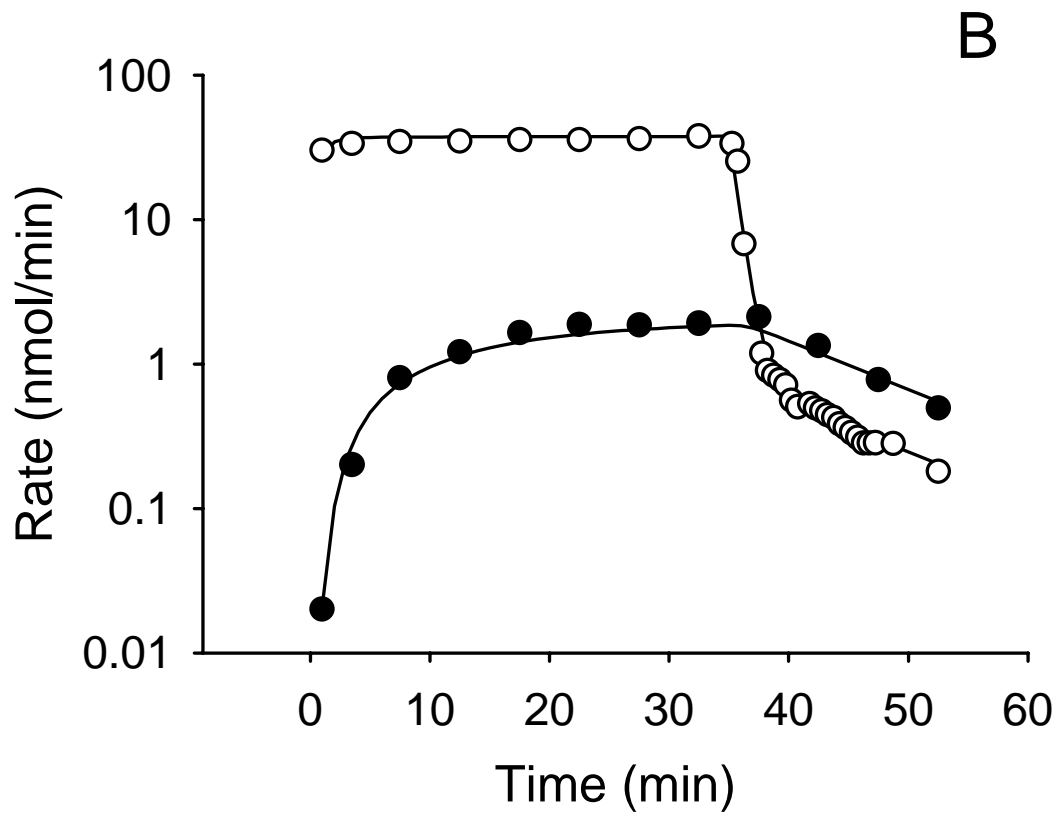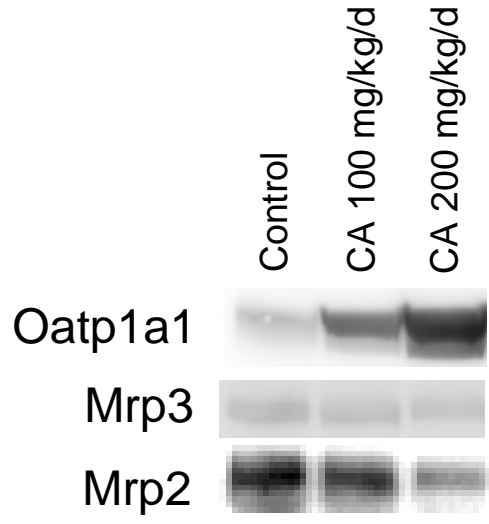
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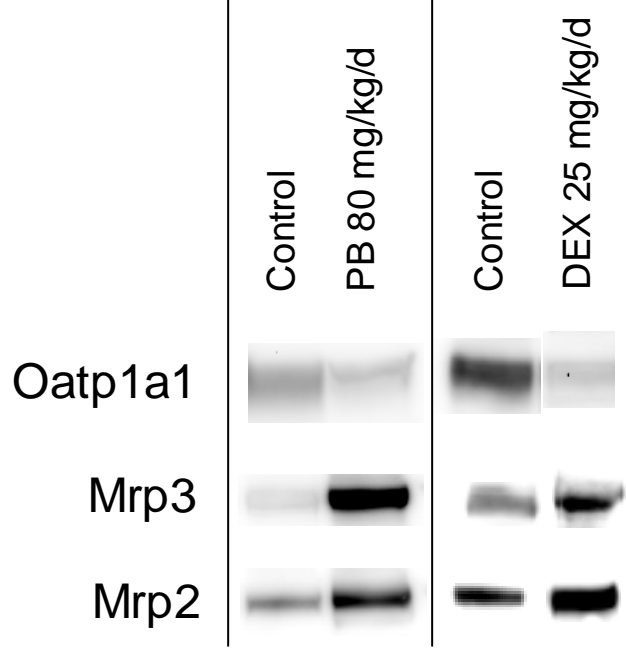
Fig 3B

Fig 4

Fig 5A

Fig 5B

Fig 6