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Relationship between drug/metabolite exposure and impairment of excretory transport function

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Abbreviations: f_e, fraction of clearance mediated by a specific transport pathway; f_m fraction of clearance mediated by a specific metabolic pathway; Cl, clearance; [I], inhibitor concentration; K_I, binding affinity of inhibitor; K_M, Michaelis-Menten constant; WT, wild-type; KO, knock-out; Mrp, multidrug resistance-associated protein; Bcrp, breast cancer resistance protein; P-gp, P-glycoprotein; 4MUG, 4-methylumbelliferyl glucuronide

Abstract

The quantitative impact of excretory transport modulation on the systemic exposure to

xenobiotics and derived metabolites is poorly understood. This manuscript presents

fundamental relationships between exposure and loss of a specific excretory process that

contributes to overall clearance. The mathematical relationships presented herein were

explored based on hepatic excretory data for polar metabolites formed in the liver of

various transporter-deficient rodents. Experimental data and theoretical relationships

indicated that the fold change in exposure is governed by the relationship, $1/(1-f_e)$, where

f_e is the fraction excreted by a particular transport protein. Loss-of-function of a transport

pathway associated with an f_e lower than 0.5 will have minor consequences (< 2 fold) on

exposure, but exposure will increase exponentially in response to loss-of-function of

transport pathways with an f_e greater than 0.5. These mathematical relationships may be

extended to other organs, such as the intestine and kidney, as well as systemic drug

exposure. Finally the relationship between exposure and f_e is not only applicable to

complete loss-of-function of a transport pathway, but can be extended to partial inhibition

scenarios by modifying the equation with the ratio of the inhibitor concentration and

inhibition constant.

Introduction

Quantitative methods to predict the change in drug exposure resulting from drug-drug

interactions occurring at the level of excretory transport have not been fully developed.

A fundamental understanding of the effect of excretory transport modulation on

drug/metabolite exposure presently is lacking. Kinetic approaches originally employed

to examine issues related to drug metabolism, such as the relative activity factor method,

are now being applied to transport processes (Hirano et al., 2004). In this manuscript, the

equation describing the effect of inhibition of drug metabolism on systemic

concentrations (Rowland and Matin, 1973) is adapted to explore the effect of transport

modulation on exposure.

Hepatobiliary excretion data for polar metabolites generated in perfused livers of various

transporter-deficient animals primarily is used in this commentary as supporting evidence

for the theoretical equations. However, the relationships presented are applicable to any

organ such as the intestine and kidney in which the formed metabolites can be excreted

into two distinct compartments. Most importantly, the relationship describing the fold

change in exposure is not only applicable to an isolated organ, but also to the systemic

exposure of drugs and metabolites when systemic clearance is mediated by excretory

transport. This manuscript provides proof that the maximum fold change in exposure to a

drug or metabolite cleared by active excretion is dictated by the relationship $1/(1-f_e)$,

where f_e is the fraction of total clearance mediated by the ablated transport protein.

Although the presented equations may appear theoretically obvious, extensive

experimental proof of these relationships currently does not exist in the literature. The

relationship, $1/(1-f_e)$, forms the basis for predicting the importance of a given excretory

transport pathway as a potential site for drug-drug interactions.

Results

Clearance-Exposure Relationship

The fold increase (fold Δ) in systemic drug exposure caused by inhibition of metabolism

has been described by Rowland and Matin (1973) as:

$$fold \quad \Delta = \frac{1}{\frac{f_m}{(1+[I]/K_I)} + (1-f_m)} \qquad \text{eq. 1}$$

where f_m is the fraction of total clearance mediated by the relevant enzyme, [I] is the

inhibitor concentration, and K_I is the inhibitor concentration at which enzyme activity is

impaired 50% when substrate concentrations are below the Michaelis-Menten constant

(K_M).

When [I] >> K_I, the increase in systemic drug exposure is only a function of f_m in the

uninhibited condition:

$$fold \quad \Delta = \frac{1}{1 - f_m} \qquad \text{eq. 2}$$

This relationship is equally valid for complete genetic deficiency of an enzymatic

pathway, where f_m is determined at "normal" expression of the enzyme. Eq. 2 indicates

that at most, a 2-fold increase in systemic exposure will be observed when f_m is ≤ 0.5,

even when inhibition of the relevant enzyme is complete. In contrast, systemic exposure

will increase exponentially in response to complete inhibition or knockout of the enzyme

as f_m increases from 0.5 to 1.

In the case of drug transport, the relevant parameter is f_e, the fraction of total clearance

mediated by a particular excretory transport protein, as opposed to f_m. However, the

mathematical relationships are analogous. When a particular route of transport is

inhibited completely or genetically ablated,

$$fold \quad \Delta = \frac{1}{1 - f_e} \qquad \qquad \text{eq. 3}$$

Metabolism and excretory transport function in concert to clear drugs from the body. In

cases where both metabolic and transport processes contribute to the overall clearance,

the sum of f_m and f_e must equal one, such that these fractions are calculated relative to

total clearance and not metabolic or excretory clearance in isolation.

Note that all presented equations assume first-order kinetics. Transport proteins and drug

metabolizing enzymes are inherently capacity limited, and therefore nonlinear. All the

relationships presented herein assume approximate linear kinetic behavior, and thus all

substrate concentrations are assumed to be below the K_M of the relevant transport protein.

In the case of hepatic transport [*i.e.,* excretion from the hepatocyte across the basolateral

membrane into blood or across the canalicular membrane into bile (Fig. 1)], this

fundamental relationship can be illustrated most clearly by considering the excretion of

polar metabolites formed in the liver. In this case, the resultant influence of changes in

excretory transport on systemic exposure is not confounded by the kinetics of drug

uptake into the liver or passive membrane permeability. Furthermore, it is assumed that

all probe substrates are not further metabolized, such that excretion is the only

mechanism of clearance from the liver.

Unlike f_m, the impact of f_e on changes in exposure differs when the knocked-out or inhibited transport protein mediates or opposes the excretory route (apical or basolateral) of interest. For example, knockout or inhibition of a canalicular transport protein will result in decreased biliary metabolite recovery, whereas knockout or inhibition of a basolateral excretory transport protein will result in a higher driving force for biliary excretion (*i.e.,* increased hepatic metabolite concentration), and therefore increased biliary metabolite recovery, assuming that a compensatory protein exists on the canalicular membrane with adequate affinity and capacity to transport the metabolite into bile.

Decrease in Excretion via Inhibition/Knockout of Direct Transport

When considering knockout or inhibition of a transport protein mediating metabolite excretory clearance in the direction of interest, f_e is calculated based on the decrease in the excretory clearance across the relevant membrane:

$$f_{e,Bile} = \frac{Cl_{Bile}^{WT} - Cl_{Bile}^{KO}}{Cl_{Bile}^{WT}} \qquad\qquad \text{eq. 4.1}$$

$$f_{e,B/L} = \frac{Cl_{B/L}^{WT} - Cl_{B/L}^{KO}}{Cl_{B/L}^{WT}} \qquad\qquad \text{eq. 4.2}$$

where $f_{e,Bile}$ and $f_{e,B/L}$ refer to the fraction excreted by a canalicular or basolateral transport protein of interest, Cl_{Bile} and $Cl_{B/L}$ are the biliary and basolateral intrinsic excretory clearances, and WT and KO refer to the wild-type and transporter knockout/deficient animals, respectively. In other words, f_e is a function of the fraction of vectorial flux mediated by the transport protein of interest.

Compensatory Increase in Excretion via Inhibition/Knockout of Opposing Transport

When a competing transport mechanism on the opposite plasma membrane domain is knocked out or inhibited, the increase in excretion in the opposite direction is a function of the fraction of total hepatic excretory clearance (*i.e.,* the sum of Cl_{Bile} and $Cl_{B/L}$) that is mediated by the impaired competing transport protein:

$$f_{e,Bile} = \frac{Cl_{B/L}^{WT} - Cl_{B/L}^{KO}}{Cl_{Bile}^{WT} + Cl_{B/L}^{WT}} \qquad \text{eq. 5.1}$$

$$f_{e,B/L} = \frac{Cl_{Bile}^{WT} - Cl_{Bile}^{KO}}{Cl_{Bile}^{WT} + Cl_{B/L}^{WT}} \qquad \text{eq. 5.2}$$

In this case, the change in f_e is not caused directly by the impaired transport in the opposing direction, but rather by the decrease in total excretory clearance, which elicits an increase in hepatic concentrations of the metabolite that serve as the driving force for increased excretion in the direction of interest.

Effect of Excretory Transport Inhibition/Knockout on Organ Exposure

The increase in hepatic metabolite concentrations in response to transport inhibition is governed not by the f_e of a particular transport pathway, but by the change in total hepatic excretory clearance. The relationship also would be governed by the f_e (eq. 5) if other transport processes were not altered in the knockout animal, or if inhibitors were specific enough not to affect other transporters. However, rodents genetically deficient in transporters may exhibit alterations in other pathways, and inhibitors often affect multiple processes (for review see Zamek-Gliszczynski et al., 2006a). Thus, the change in hepatic exposure to a derived metabolite is governed by the total fraction excreted ($f_{e,Total}$):

$$f_{e,Total} = \frac{Cl_{Total}^{WT} - Cl_{Total}^{KO}}{Cl_{Total}^{WT}} \quad or \quad \frac{Cl_{Total}^{KO} - Cl_{Total}^{WT}}{Cl_{Total}^{KO}} \qquad \text{eq. 6}$$

where Cl_{Total} is the sum of the biliary and basolateral excretory clearances. Total

excretory clearance in an animal genetically deficient in a transport protein may actually

be higher than in the corresponding wild-type, and in this case the relationship on the

right should be used to maintain the $f_{e,Total}$ between zero and unity.

Fig. 2 demonstrates the applicability of eq. 3 in predicting the fold change in biliary,

systemic, or hepatic exposure to polar metabolites formed in the liver. These data were

generated in single-pass liver perfusion experiments in which the biliary and perfusate

(systemic) recovery of substrates could be quantified directly (Takenaka et al., 1995;

Xiong et al., 2002; Nezasa et al., 2006; Zamek-Gliszczynski et al., 2006b; Zamek-

Gliszczynski et al., 2006c). Rodents genetically deficient in a specific transport protein

were used to examine the impact of complete abrogation of the transporter on

exposure/cumulative recovery. In this experimental paradigm, biliary and perfusate

recovery serve as surrogates for intestinal and systemic metabolite exposure, respectively.

Altered exposure/recovery of 4-methylumbelliferyl glucuronide (4MUG) in Mrp3- and

Bcrp-knockout mice (Table 1) will be used to illustrate the application of the theoretical

relationships. In mice, 4MUG is excreted into bile by Bcrp and across the hepatic

basolateral membrane into blood by Mrp3 (Zamek-Gliszczynski et al., 2006a), therefore

biliary excretion of 4MUG would be expected to be impaired in Bcrp-knockout mice but

would be elevated in mice lacking Mrp3, a transport pathway that opposes the excretory

route of interest. Since Bcrp is directly involved in transporting 4MUG into bile, using

eq. 4.1 and excretory clearance values ($Cl_{Bile}^{WT} = 88; Cl_{Bile}^{KO} = 24$ μL/min/g liver) reported by

Zamek-Gliszczynski et al. (2006c), $f_{e, Bile}$ of Bcrp is calculated to be 0.73. Equation 3

predicts the decrease in biliary recovery of 4MUG to be 3.7 fold; experimentally a 2.1-

fold decrease was observed. In contrast, Mrp3 functions in the opposite direction from

biliary excretion, so using eq. 5.1 and excretory clearance values ($Cl_{B/L}^{WT} = 259; Cl_{Bile}^{WT} =$

$30; Cl_{B/L}^{KO} = 36$ μL/min/g liver) reported by Zamek-Gliszczynski et al. (2006b), $f_{e, Bile}$ of

Mrp3 is calculated to be 0.77. Equation 3 predicts the increase in biliary recovery of

4MUG to be 4.4 fold; experimentally a 5.4-fold increase was observed. Using eq. 6 and

the same dataset ($Cl_{Total}^{WT} = 259+30; Cl_{Total}^{KO} = 36+59$ μL/min/g liver), $f_{e, Total}$ of Mrp3 is

calculated to be 0.67. Equation 3 predicts that hepatic 4MUG exposure in Mrp3-

knockout mice will be 3.0-fold higher relative to wild-type mice; experimentally an

increase of 2.4-fold was observed.

Discussion

The fold change in exposure to polar metabolites in response to modulation of hepatic excretory transport is described by eq. 3, $1/(1-f_e)$. Although the change in metabolite exposure resulting from modulation of hepatic excretory transport can be predicted by complex physiologically-based models of hepatobiliary pharmacokinetics (Chiba and Pang, 1993; Liu and Pang, 2006), the theoretical relationships presented in this manuscript provide a simple and reliable prediction of the impact of excretory transport modulation on exposure. Hepatobiliary disposition data were used to support the relationship between exposure and f_e, because of the existing wealth of data in the liver. However, as demonstrated below, the relationships presented herein are applicable to overall systemic drug/metabolite exposure as well as any organ.

The relationship, $1/(1-f_e)$, is applicable to systemic exposure of parent drug. This point is exemplified by elevated pravastatin exposure in Mrp2-deficient rats. In normal bile duct-cannulated rats, 81% of the intravenous pravastatin dose was excreted in bile (Adachi et al., 1996). Mrp2 was shown to transport pravastatin *in vitro* (Sasaki et al., 2002). If it is assumed that Mrp2 entirely mediated the biliary excretion of pravastatin, and assigned it an f_e value of 0.81, then the expected increase in drug exposure in rats lacking this transporter would be approximately 5.3 fold (eq. 3). Relative to wild-type rats, Mrp2-deficient rats exhibited 4.7- and 6.1-fold higher drug exposure following intravenous and oral administration of pravastatin, respectively (Kivisto et al., 2005).

The role of Bcrp in renal excretion of the sulfate metabolite of E3040 was studied in mice

(Mizuno et al., 2004). Renal excretory clearance of E3040 sulfate was decreased from

2.35 to 0.59 mL/min/kg in Bcrp-knockout mice. Thus, the f_e of Bcrp in urinary excretion

of E3040 sulfate is 0.75 (eq. 4), and so the maximal expected decrease in urinary

recovery of this metabolite in knockout mice is 4 fold (eq. 3). Experimentally, urinary

recovery of E3040 sulfate was decreased approximately 3 fold.

The relationships presented in this commentary are based on data generated in rodents

genetically deficient in specific transport proteins, but eq. 3 could be modified as follows

to describe transport inhibition:

$$fold \quad \Delta = \frac{1}{\dfrac{f_e}{(1+[I]/K_I)} + (1-f_e)} \qquad \text{eq. 7}$$

The change in exposure as a function of f_e at various $[I]/K_I$ ratios is presented in Fig. 3.

In the case of inhibition, the inhibitor concentration must exceed K_I for a transport

pathway representing $f_e > 0.5$ in order to observe appreciable (> 2-fold) changes in

drug/metabolite exposure. Note that the fold change in systemic and biliary exposure in

the presence of an inhibitor may be oversimplified by eq. 7, because many inhibitors

impair multiple transport mechanisms on both plasma membrane domains (Zamek-

Gliszczynski et al., 2006a). In contrast, the $f_{e,Total}$, which determines the fold change in

hepatic exposure accounts for the non-specific effects of inhibitors.

Systemic clearance of methotrexate in rats is partially mediated by biliary excretion via

Mrp2; the f_e of this transport protein in systemic elimination of methotrexate is

approximately 0.5 (Chen et al., 2003). In Mrp2-deficient rats, Equation 3 predicts a 2-fold increase in systemic exposure to methotrexate, which is in good agreement with the 1.9-fold increase observed by Chen et al. (2003). However, if Mrp2 function is not lost altogether, but instead inhibited to a limited extent, a less pronounced increase in exposure would be expected. The K_I of probenecid for Mrp2 was determined to be 180 μM *in vitro*, and this inhibitor was intravenously infused to four different steady-state concentrations in wild-type rats to study its effect on methotrexate systemic exposure (Ueda et al., 2001). Equation 7 predicts a 1.1-, 1.4-, 1.4-, and 1.5-fold increase in exposure at circulating probenecid concentrations of 243, 633, 704, and 905 μM, respectively. This prediction is in good agreement with the observed 1.2- and 1.3-fold increase in methotrexate exposure at 243 and 633 μM probenecid concentrations. However, 1.9- and 2.1-fold observed increase in drug exposure at 704 and 905 μM inhibitor concentrations exceeded the prediction, because at these higher concentrations, probenecid also was appreciably inhibiting hepatic uptake of methotrexate in addition to its biliary excretion (Ueda et al., 2001).

Theoretical relationships supported by experimental data indicate that a considerable change in drug or metabolite exposure will occur only when an appreciable excretory transport pathway ($f_e > 0.5$) is inhibited substantially or knocked out. In order to elicit a considerable change in exposure with an inhibitor, f_e of the impaired pathway must exceed 0.5 and the $[I]/K_I$ ratio must exceed 1. As the f_e of the extensively-inhibited or knocked-out transport pathway increases above 0.5 and approaches unity, exposure will increase in an exponential manner.

References

Adachi Y, Okuyama Y, Miya H, Matsusita H, Kitano M, Kamisako T and Yamamoto T
(1996) Pravastatin transport across the hepatocyte canalicular membrane requires
both ATP and a transmembrane pH gradient. *J Gastroenterol Hepatol* **11:**580-585.

Chen C, Scott D, Hanson E, Franco J, Berryman E, Volberg M and Liu X (2003) Impact
of Mrp2 on the biliary excretion and intestinal absorption of furosemide,
probenecid, and methotrexate using Eisai hyperbilirubinemic rats. *Pharm Res*
20:31-37.

Chiba M and Pang KS (1993) Effect of protein binding on 4-methylumbelliferyl sulfate
desulfation kinetics in perfused rat liver. *J Pharmacol Exp Ther* **266:**492-499.

Hirano M, Maeda K, Shitara Y and Sugiyama Y (2004) Contribution of OATP2
(OATP1B1) and OATP8 (OATP1B3) to the hepatic uptake of pitavastatin in
humans. *J Pharmacol Exp Ther* **311:**139-146.

Kivisto KT, Grisk O, Hofmann U, Meissner K, Moritz KU, Ritter C, Arnold KA,
Lutjoohann D, von Bergmann K, Kloting I, Eichelbaum M and Kroemer HK
(2005) Disposition of oral and intravenous pravastatin in MRP2-deficient TR- rats.
Drug Metab Dispos **33:**1593-1596.

Liu L and Pang KS (2006) An integrated approach to model hepatic drug clearance. *Eur J
Pharm Sci* **29:**215-230.

Mizuno N, Suzuki M, Kusuhara H, Suzuki H, Takeuchi K, Niwa T, Jonker JW and
Sugiyama Y (2004) Impaired renal excretion of 6-hydroxy-5,7-dimethyl-2-
methylamino-4-(3-pyridylmethyl) benzothiazole (E3040) sulfate in breast cancer

resistance protein (BCRP1/ABCG2) knockout mice. *Drug Metab Dispos* **32:**898-901.

Nezasa K, Tian X, Zamek-Gliszczynski MJ, Patel NJ, Raub TJ and Brouwer KL (2006) Altered hepatobiliary disposition of 5 (and 6)-carboxy-2',7'-dichlorofluorescein in Abcg2 (Bcrp1) and Abcc2 (Mrp2) knockout mice. *Drug Metab Dispos* **34:**718-723.

Rowland M and Matin SB (1973) Kinetics of drug-drug interactions. *J Pharmacokinet Biopharm* **1:**553-567.

Sasaki M, Suzuki H, Ito K, Abe T and Sugiyama Y (2002) Transcellular transport of organic anions across a double-transfected Madin-Darby canine kidney II cell monolayer expressing both human organic anion-transporting polypeptide (OATP2/SLC21A6) and Multidrug resistance-associated protein 2 (MRP2/ABCC2). *J Biol Chem* **277:**6497-6503.

Takenaka O, Horie T, Kobayashi K, Suzuki H and Sugiyama Y (1995) Kinetic analysis of hepatobiliary transport for conjugated metabolites in the perfused liver of mutant rats (EHBR) with hereditary conjugated hyperbilirubinemia. *Pharm Res* **12:**1746-1755.

Ueda K, Kato Y, Komatsu K and Sugiyama Y (2001) Inhibition of biliary excretion of methotrexate by probenecid in rats: quantitative prediction of interaction from in vitro data. *J Pharmacol Exp Ther* **297:**1036-1043.

Xiong H, Suzuki H, Sugiyama Y, Meier PJ, Pollack GM and Brouwer KL (2002) Mechanisms of impaired biliary excretion of acetaminophen glucuronide after

acute phenobarbital treatment or phenobarbital pretreatment. *Drug Metab Dispos* **30:**962-969.

Zamek-Gliszczynski MJ, Hoffmaster KA, Nezasa K, Tallman MN and Brouwer KL (2006a) Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. *Eur J Pharm Sci* **27:**447-486.

Zamek-Gliszczynski MJ, Nezasa K, Tian X, Bridges AS, Lee K, Belinsky MG, Kruh GD and Brouwer KLR (2006b) Evaluation of the role of Mrp3 and Mrp4 in hepatic basolateral excretion of sulfate and glucuronide metabolites of acetaminophen, 4-methylumbelliferone, and harmol in Abcc3(-/-) and Abcc4(-/-) mice. *J Pharmacol Exp Ther* **319:**1485-1491.

Zamek-Gliszczynski MJ, Nezasa K, Tian X, Kalvass JC, Patel NJ, Raub TJ and Brouwer KLR (2006c) The important role of Bcrp (Abcg2) in the biliary excretion of sulfate and glucuronide metabolites of acetaminophen, 4-methylumbelliferone, and harmol in mice. *Mol Pharmacol* **70:**2127-2133.

Footnote

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Figure Legends

Figure 1 Scheme depicting the bidirectional hepatic excretion of metabolites. All supporting data were generated in single-pass liver perfusion experiments in which the livers were perfused with parent compounds, and the recovery of relevant metabolites in bile and perfusate, as well as steady-state hepatic metabolite concentrations, were quantified directly.

Figure 2 (A) Fold change in biliary recovery as a function of the fraction excreted $(f_{e,Bile})$. (B) Fold change in perfusate recovery (a surrogate of systemic exposure) as a function of the fraction excreted $(f_{e,B/L})$. (C) Fold change in hepatic metabolite concentration as a function of total fraction excreted $(f_{e,Total})$. Solid line represents the theoretical relationship (eq. 3) $\pm$ 50% represented by dashed lines. Presented data is summarized in corresponding panels of Table 1.

Figure 3 Fold change in exposure as a function of f_e at various $[I]/K_I$ ratios as described by eq. 7.

Table 1A. Fraction excreted ($f_{e,Bile}$) by the ablated transport pathway in each animal

model and the corresponding experimentally-observed fold change in biliary recovery.

Please note that $f_{e,Bile}$ values for canalicular transporters pumping directly into the bile

were calculated using eq. 4.1, whereas $f_{e,Bile}$ for basolateral transporters pumping in the

opposite direction (into perfusate) were calculated using eq. 5.1. Positive fold Δ values

indicate an increase in exposure, whereas decreased exposure is denoted by a negative

fold Δ value. All data points were calculated using mean values reported in the literature

(Takenaka et al., 1995; Xiong et al., 2002; Nezasa et al., 2006; Zamek-Gliszczynski et al.,

2006b; Zamek-Gliszczynski et al., 2006c). Acetaminophen sulfate (AS), acetaminophen

glucuronide (AG), 4-methylumbelliferyl sulfate (4MUS), 4-methylumbelliferyl

glucuronide (4MUG), harmol sulfate (HS), harmol glucuronide (HG), E3040 sulfate

(E3040S), E3040 glucuronide (E3040G), carboxydichlorofluorescein (CDF), not

determined (ND).

Metabolite	Mrp2-deficient rat		Mrp2-knockout mouse		Bcrp-knockout mouse		P-gp-knockout mouse		Mrp3-knockout mouse		Mrp4-knockout mouse	
	$f_{e,Bile}$	fold Δ	$f_{e,Bile}$	fold Δ	$f_{e,Bile}$	fold Δ	$f_{e,Bile}$	fold Δ	$f_{e,Bile}$	fold Δ	$f_{e,Bile}$	fold Δ
AS	0.85	-3.4	0.00	1.2	0.87	-5.8	0.00	1.6	0.17	1.8	0.21	-1.0
AG	0.99	-511.8	0.10	-2.5	0.86	-4.5	0.29	1.5	0.96	7.3	0.00	1.4
4MUS	ND	ND	0.00	1.0	1.00	-631.3	0.00	1.1	0.35	1.4	0.44	-1.0
4MUG	ND	ND	0.33	-2.0	0.73	-2.1	0.00	1.3	0.77	5.4	0.00	1.2
HS	ND	ND	0.00	1.1	0.99	-66.4	0.00	1.1	0.13	1.2	0.21	-1.2
HG	ND	ND	0.21	-1.0	0.96	-15.0	0.09	1.2	0.26	1.6	0.09	1.3
CDF	ND	ND	1.00	-1740.7	0.00	1.4	ND	ND	ND	ND	ND	ND
E3040S	0.00	1.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E3040G	0.97	-36.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 1B. Fraction excreted ($f_{e,B/L}$) by the ablated transport pathway in each animal

model and the corresponding experimentally-observed fold change in perfusate recovery,

a surrogate of systemic exposure. Please note that $f_{e,B/L}$ values for basolateral transporters

pumping directly into perfusate were calculated using eq. 4.2, whereas $f_{e,B/L}$ for

canalicular transporters pumping in the opposite direction (into the bile) were calculated

using eq. 5.2.

Metabolite	Mrp2-deficient rat		Mrp2-knockout mouse		Bcrp-knockout mouse		P-gp-knockout mouse		Mrp3-knockout mouse		Mrp4-knockout mouse	
	$f_{e,B/L}$	fold Δ	$f_{e,B/L}$	fold Δ	$f_{e,B/L}$	fold Δ	$f_{e,B/L}$	fold Δ	$f_{e,B/L}$	fold Δ	$f_{e,B/L}$	fold Δ
AS	0.03	1.1	0.00	-1.0	0.05	-1.1	0.00	1.1	0.18	-1.6	0.22	-1.5
AG	0.49	2.4	0.00	1.4	0.01	-1.2	0.00	1.1	0.96	-5.9	0.00	1.1
4MUS	ND	ND	0.00	1.0	0.37	1.5	0.00	1.1	0.50	-1.6	0.64	-1.6
4MUG	ND	ND	0.04	1.2	0.08	1.0	0.00	1.1	0.86	-3.3	0.00	1.2
HS	ND	ND	0.00	-1.1	0.65	3.1	0.00	-1.2	0.28	-2.0	0.44	-2.2
HG	ND	ND	0.13	1.1	0.56	2.7	0.05	-1.2	0.62	-3.0	0.21	-1.3
CDF	ND	ND	0.57	2.6	0.00	-1.1	ND	ND	ND	ND	ND	ND
E3040S	0.00	-1.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E3040G	0.62	2.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 1C. Total fraction excreted ($f_{e,Total}$; eq. 6) by the ablated transport pathway in each animal model and the corresponding experimentally-observed fold change in hepatic metabolite concentration in animal models lacking various transporter proteins. Hepatic concentrations were not quantified for CDF (Nezasa et al., 2006) and were not reported for E3040S and E3040G (Takenaka et al., 1995).

Metabolite	Mrp2-deficient rat		Mrp2-knockout mouse		Bcrp-knockout mouse		P-gp-knockout mouse		Mrp3-knockout mouse		Mrp4-knockout mouse	
	$f_{e,Total}$	fold Δ	$f_{e,Total}$	fold Δ	$f_{e,Total}$	fold Δ	$f_{e,Total}$	fold Δ	$f_{e,Total}$	fold Δ	$f_{e,Total}$	fold Δ
AS	0.43	-1.1	0.03	-1.2	0.38	1.3	0.02	-1.3	0.14	-1.2	0.21	-1.1
AG	0.73	2.4	0.70	-3.0	0.44	1.2	0.14	1.1	0.95	6.1	0.31	-1.1
4MUS	ND	ND	0.43	-1.9	0.44	1.6	0.17	-1.4	0.40	1.5	0.60	1.8
4MUG	ND	ND	0.35	-1.4	0.43	1.2	0.42	-1.6	0.67	2.4	0.32	-1.3
HS	ND	ND	0.04	-1.2	0.23	1.3	0.22	-1.4	0.10	-1.4	0.28	-1.2
HG	ND	ND	0.15	1.1	0.23	1.2	0.22	1.0	0.10	1.0	0.06	1.1
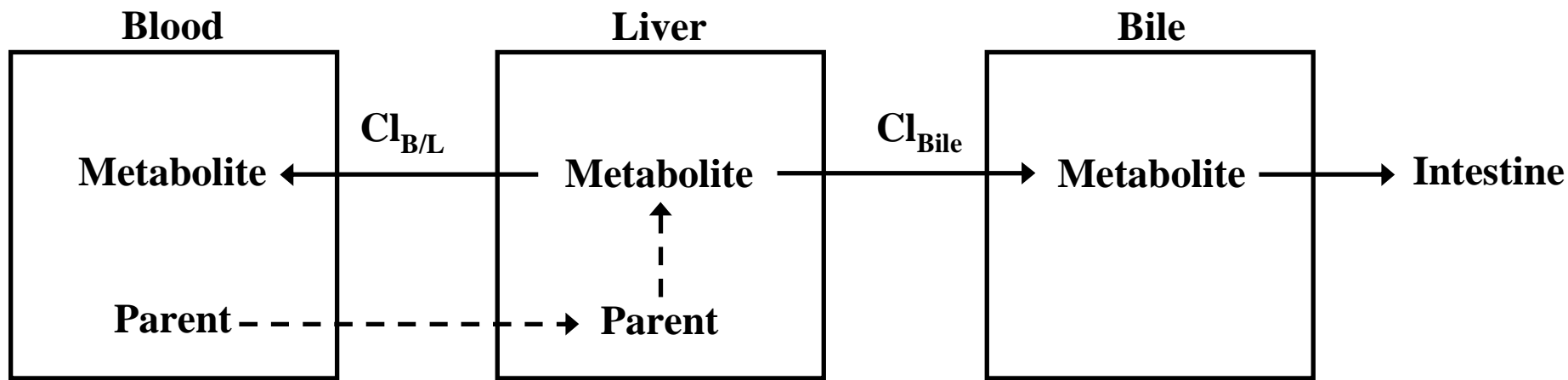
Figure 1
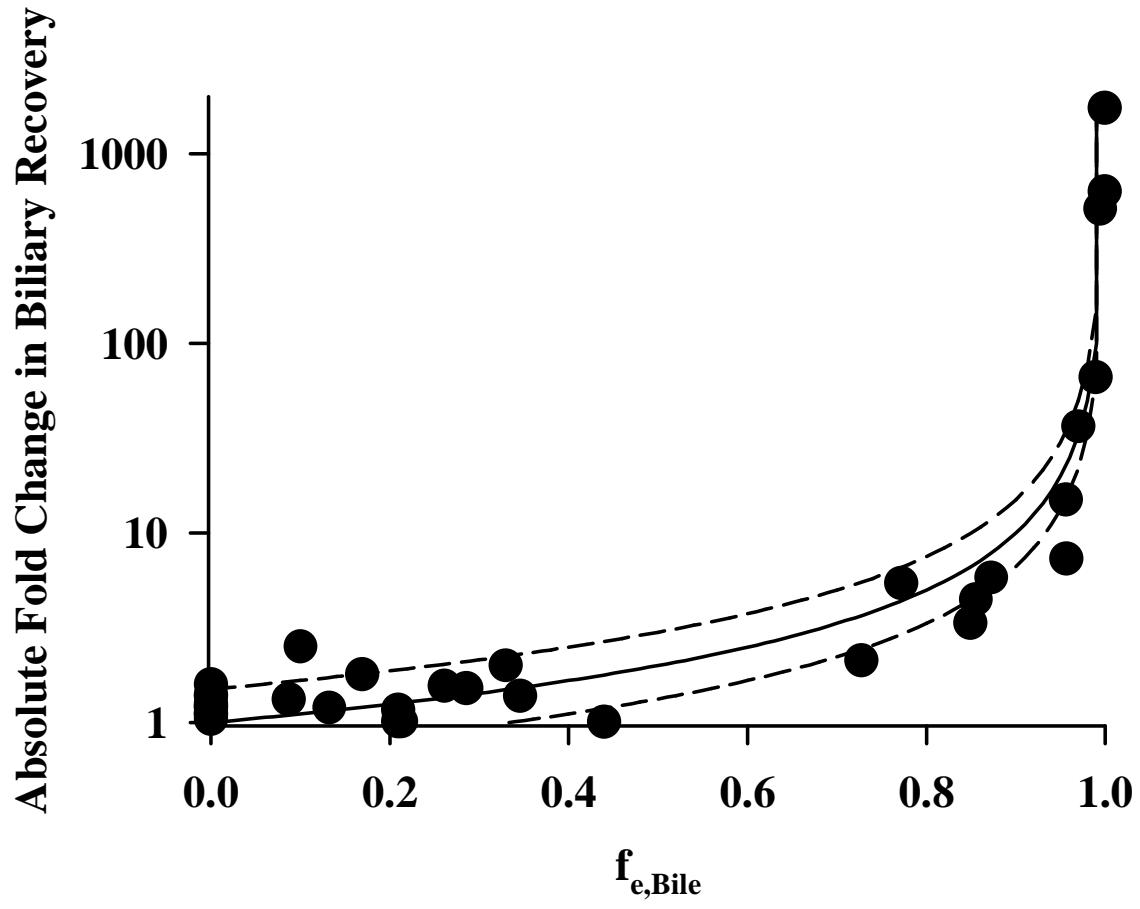
Figure 2A
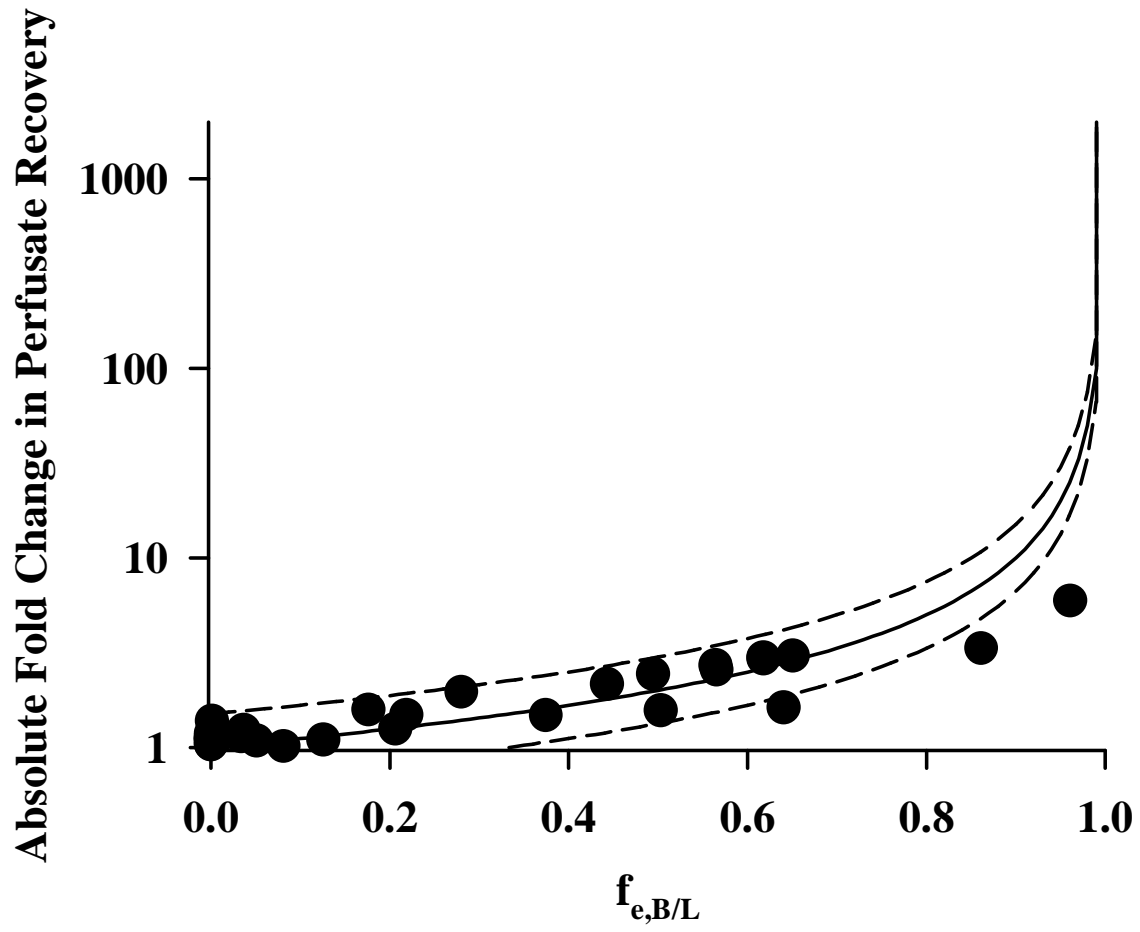
Figure 2B

Figure 2C

Figure 3