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Species Differences in Biliary Clearance and Possible Relevance of Hepatic Uptake and Efflux Transporters Involvement

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Abbreviations:

BCRP: breast cancer resistance protein

Cp,ss: plasma concentration of drug at steady state

Cp,mid: plasma concentration of drug at the mid-point of the bile collection interval

MRP2: multidrug resistance-associated protein 2

OAT: organic anion transporter

OATP: organic anion transporting polypeptide

OCT: organic cation transporter

P-gp: P-glycoprotein

Abstract

From a search of the available literature, a database of twenty two drugs of all charge types and several different therapeutic classes was compiled in order to compare rat and human biliary clearance data. Dog biliary excretion data was also found for nine of the drugs. For nineteen of the twenty two drugs (86%), rat unbound biliary clearance values, when normalized for body weight, exceeded those for man by factors ranging from 9 to over 2500-fold, whereas human/dog differences were much less dramatic. It was possible to define hepatic uptake and efflux transporter involvement for many of the drugs. Based on the findings it is postulated that regardless of the biliary efflux transporters implicated, when drugs do not require active hepatic uptake to access the liver there may be fairly insignificant differences in rat, dog and human biliary clearance. Conversely, when the organic anion-transporting polypeptide drug transporters are involved, one may expect at least a ten-fold discrepancy in rat to human biliary clearance normalized for body weight and corrected for plasma protein binding.

Introduction

Predicting human pharmacokinetics from pre-clinical data is a primary goal of Drug Discovery DMPK scientists, since therapeutic success can be compromised by poor human PK. Accurate predictions of hepatic metabolic clearance can be made from *in vitro* data providing appropriate attention to detail is made (Sohlenius-Sternbeck et al., 2012; Obach, 2011; Grime & Riley, 2006), but besides being eliminated from the body by metabolism, drugs can be eliminated directly into the urine or bile. Both routes are complex and involve passive and active cellular uptake and efflux transport processes. Biliary excretion can involve the hepatic uptake transporters organic anion transporting polypeptide (OATP), organic anion transporter (OAT) and organic cation transporter (OCT) and the canalicular efflux transporters breast cancer resistance protein (BCRP), P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 (MRP2) (Kusuhara et aand Sugiyama, 2010; Shitara et al., 2006). Similarly, active renal excretion of drugs can involve several transporters in the basolateral and apical membranes (Brown et al., 2008). Despite the complexity, an effective prediction method based on dog renal clearance after correction for differences in plasma protein binding and kidney blood flow has emerged (Paine et al., 2010). There has not been a wealth of literature on the subject of predicting human biliary clearance of drug candidates, perhaps because of the scarcity of relevant clinical data (Lavé et al., 2009) and as such, in terms of human clearance predictions, biliary stands apart as a largely unresolved problem.

Sandwich-cultured hepatocytes maintain liver-specific functions for several days after cell isolation and exhibit the formation of bile canaliculi and the localization of efflux transporters on the canalicular membrane (LeCluyse et al., 1994).

Advancements have been made in using this *in vitro* technique for predicting biliary clearance (Abe et al., 2008) but the robustness of the method is still to be extensively tested with a wide range of drugs. However, appreciation of the intracellular drug concentrations and calculation of an *in vitro* biliary intrinsic clearance may see the method emerge as a truly predictive tool (Nakakariya et al., 2012). Nonetheless, pre-clinical *in vivo* data retains an extremely important role in facilitating the understanding and contextualization of the risks associated with human pharmacokinetic predictions. A variety of inter-species allometric scaling approaches have been assessed specifically for biliary clearance (Mahmood 2005; Mahmood and Sahajwalla 2002; Sawada et al., 1984) but given the low number of drugs used in the analyses, the fact that some of the examples used involved total drug related material excreted rather than parent drug (Scatina et al., 1989) and that allometry under-predicts human biliary clearance for some drugs but not others (Påhlman et al., 1998; Sawada et al., 1984), a more extensive analysis has been required.

Recently a database of eighteen drugs having known rat and human biliary clearance was published, representing the most extensive dataset to date (Morris et al., 2012). From this it was evident that, when considering unbound clearance (corrected for plasma protein binding), simple allometry using an exponent of 0.66 gave reasonable human predictions for some drugs, but for others rat over-estimated human biliary clearance by in excess of one order of magnitude. Based on a limited number of six drugs, multiple species allometry using unbound biliary clearance data gave good predictions, but it should be reflected on that inter-species allometry may to an extent afford better clinical predictions because of the smoothing out of data

from species where there is a large discrepancy with the human data. Indeed, in a drug discovery setting, the most likely scenario is that a poor prediction of rat clearance from *in vitro* hepatic metabolic data would precipitate a renal and biliary excretion study if the compound is of sufficient interest as a potential human therapeutic agent. From such information accurate decisions must be made about the suitability of the compound to progress further and therefore, understanding rat to human differences in biliary clearance is vital.

To investigate this further we compiled an even more extensive rat and human biliary clearance data set than that of Morris *et al*., for twenty two drugs of all charge types and several different therapeutic classes. For nine of the drugs it was also possible to find data for dog biliary clearance. An extensive literature search was performed to define the hepatic uptake and bile canalicular efflux drug transporters involved, with the aim of elucidating further the reason for inter-species differences and with the specific intention of helping DMPK scientists make effective decisions with early pre-clinical data.

Materials and Methods

No new data was generated for this work - all data used was obtained from the scientific literature and the references used for this are detailed in Tables 1 and 2.

For rat and dog data, the methods in the publications all refer to biliary clearance estimated in the same way, as follows: Surgically prepared (bile duct cannulated) animals were used and the amount of parent drug eliminated into bile was quantified. Biliary clearance was then calculated from the product of the total systemic clearance and the fraction of dose accounted for in bile as parent drug (Table 3).

For the clinical studies, the literature references detailed the following methods for collecting the bile (see Table 4): In the majority of studies, patients had a temporary bile shunt (T-tube) inserted. The T-tube diverts part of the bile flowing from the liver to a port for external collection. Other studies used duodenal aspiration or drainage, in which biliary secretions were withdrawn from the duodenum. Parent drug was quantified in the bile samples, according to the methods detailed in the individual references and human biliary clearance was then calculated in one of three ways:

1. From the amount of parent drug in the bile divided by the area under the plasma concentration-time curve (Amount in bile/AUC, Table 4)
2. From the ratio of the rate of biliary excretion and the plasma concentration, either steady state or mid-point of the bile collection period (bil. excr. rate /$C_{p,ss}$ or bil. excr. rate /$C_{p,mid}$, Table 4)
3. From the product of the total systemic clearance (CL) and the fraction of dose accounted for in bile as parent drug (CL x %bile, Table 4) or in the same way

but corrected for bioavailability (F) when the dose was administered orally (CL/F* %bile, Table 4).

In the source references for the human bile data (Table 1), the biliary clearance was not always calculated (in some of the publications, only the amount of parent drug excreted in to the bile was recorded). In these instances, we calculated the human biliary clearance by method 3 (above) using clearance and bioavailability values obtained from literature (Goodman and Gilman, 1990; Obach et al., 2008).

For the purposes of this present analysis (to assess the cross species differences in biliary clearance normalized for body weight and plasma protein binding and to determine if definition of the drug transporters involved help in elucidating the differences), the following steps were taken to supply the information in Table 1:

1. The biliary clearance was expressed in mL/min/kg terms, assuming rat, dog and human body weights of 0.3, 15 and 70 kg respectively. The body weight normalized biliary clearance (mL/min/kg) was divided by the fraction of each drug unbound in plasma (fup) to obtain the unbound biliary clearance. The following plasma protein binding data (rat, dog, human) were obtained from the scientific literature (where dog fup data was not required for the analysis, a dash (-) is given): Doxorubicin (0.34, 0.34, 0.28), digoxin (0.70, 0.49, 0.7), erythromycin (0.78, 0.53, 0.16), cefazolin (0.12, 0.74, 0.18), cefamandole (0.17, -, 0.25), cephalexin (0.76, -,0.86), cefotetan (0.70, 0.61, 0.15), cefixime (0.14, -,0.31), ceftriaxone (0.22, 0.86, 0.075), cefpiramide (0.54, 0.7, 0.04), cefoperazone (0.74, -, 0.07), valsartan (0.03, -, 0.04), moxalactam (0.51, -, 0.39), methotroxate (0.77, 0.63, 0.37), pravastatin (0.35, -, 0.5), diclofenac

(0.009, -, 0.005), ranitidine (0.9, -, 0.95), vincristine (0.39, -, 0.4), methadone (0.36, -, 0.21), fexofenadine (0.19, -, 0.35), ciprofloxacin (0.57, -, 0.7) and napsagatran (0.33, 0.52, 0.55).

2. A literature search was performed to identify the human hepatic uptake and canalicular efflux drug transporters implicated in the *in vivo* biliary clearance. The references are given in Table 2.

Results

In the analysis presented here, rat, dog and human differences in biliary clearance were explored by assembling, from the scientific literature, the most extensive database available to date. Additional definition of the uptake and efflux drug transporters involved was incorporated, with the intention of providing greater insight into the likely extent of cross-species differences. From this it is hoped that more enlightened early decision making may be possible in a Drug Discovery setting.

As with previous literature analyses, to overcome species differences in plasma protein binding, unbound biliary clearances were compared. In the analysis presented here, biliary clearance values were also normalized for body weight and presented in units of mL/min/kg body weight to allow a simpler cross-species comparison. Comparing rat, dog and human hepatic blood flow values of 72, 55 and 20 mL/min/kg (Barter et al., 2007; Taylor et al., 2007; McEntee et al 1996), one may expect a 3.6-fold difference between rat and human and a 2.8-fold difference between dog and human hepatic clearance in the absence of any mechanistic differences at the level of drug transport and metabolism. In this context, the most striking thing about the data set (Table 1, figure 1) is that for nineteen of the drugs (86%), rat unbound biliary clearance values exceed those for man by factors ranging from 9 to over 2500-fold.

Discussion

Of the nineteen drugs with a rat/human unbound biliary clearance ratio of approximately one order of magnitude or greater, it was possible to find literature references for hepatic uptake transporter involvement for nine (Table 2, figure 2a). However, since four of those drugs with un-defined hepatic uptake mechanisms (cefixime, cefamandole, cefotetan and cefpiramide) have similar properties to ceftriaxone, cefazolin, cephalexin and cefoperazone (structurally similar acidic drugs of the same antibiotic class with low $logD_{7.4}$ values and similar biliary efflux transporter substrate recognition) it is perhaps not unreasonable to speculate that the uptake transporters may also be similar – in other words the organic anion transporting polypeptides may be involved. It should however be acknowledged that the most lipophilic of these, ceftriaxone, is a substrate for OATP1B3 (Yamaguchi et al., 2011) but is not apparently a substrate for rat oatp1a4 (Nakakariya et al., 2008a). Biliary efflux transporter involvement could be defined for the majority of the twenty two drugs but no obvious relationship between the transporter definition and the rat/human discrepancy in unbound biliary clearance was apparent (Table 2, figure 2b).

Methadone, cefazolin and moxalactam (0.001, 0.001 and 0.003 mL/min/kg) had by far the lowest measured total human biliary clearance values and the rat/human unbound biliary clearance differences were calculated to range from approximately 500 to 2500. With the human biliary clearance values being so low and patient numbers being limited (one, five and six for the three drugs respectively), the absolute values should be treated with caution. Methadone is of particular concern since it has the highest estimated rat/human discrepancy and yet is a basic drug of

moderate to low lipophilicity. It is therefore unlikely to be an OATP substrate and the human biliary clearance value we found in the literature (Kreek et al., 1980) may not be a true representation in a wider patient group.

Interestingly, for the only three drugs where unbound rat, human and dog biliary clearance values were found to be approximately equal (in mL/min/kg), namely erythromycin, doxorubicin and digoxin (Table 1, figure 2a), active uptake into the liver has been reported to be at best very slow or only partially responsible for the permeability of the sinusoidal membrane or indeed non-existent (Yabe et al., 2011; Taub et al., 2011; Bi et al., 2006; Sun et al., 2004; Hilmer et al., 2004). Therefore, whilst there are dramatic species differences in the expression and activity of canalicular transporters that maybe up to ten-fold for rat/human Mrp2/MRP2 (Ishizuka et al., 1999; Li et al., 2009), the fact that erythromycin, doxorubicin and digoxin are substrates for P-gp or MRP2, along with many of the other drugs for which rat biliary clearance is considerably greater than for man, suggests that the key determinant in the biliary clearance species difference may indeed be the uptake step from the blood into the liver. It is not a new suggestion that sinusoidal active uptake from the blood into the liver may often be the rate limiting step for drugs cleared by biliary excretion (Yamazaki et al., 1996) and a similar inference comes from a recent study of 123 compounds showing significant excretion into rat bile and having considerable overlap in the physicochemical space occupied by human OATP and rat oatp substrates (Varma et al., 2012). Given this, the observation that rat biliary clearance exceeds that of human once normalized for body weight is perhaps not surprising, since functionally rat hepatic uptake transporters are more efficient than their human counterparts whilst dog hepatic uptake rates appear

similar or lower than for human when one compares hepatocyte uptake data for the same actively transported drugs (Gardiner and Paine, 2011; Wilby et al., 2011; Grime et al., 2008; Soars et al., 2007).

Based on these observations we postulate that when drugs do not require active hepatic uptake to access the liver, there may be fairly insignificant differences in rat, dog and human biliary clearance once normalized for body weight and plasma protein binding differences. Conversely, when the organic anion-transporting polypeptide drug transporters are involved, one may expect at least a ten-fold discrepancy in rat to human biliary clearance (figure 2a). Additionally, based on very limited dog to human findings, one may expect very little dog/human discrepancy in biliary clearance regardless of the processes involved. Ultimately this may infer that *in vitro* hepatocyte active uptake intrinsic clearance data may be used to predict total hepatic clearance without the need to define the final fate of the drug, be it metabolized or excreted in the bile. This certainly appears to be the case for pravastatin (Soars et al., 2007). Although this is only an emerging perspective based on an analysis of the available literature, we believe it to be a useful indicator to Drug Discovery DMPK scientists handling rat biliary clearance data in a pre-clinical setting when rapid but educated decisions are required. Towards drug candidate selection at the pre-clinical / clinical development interface, biliary clearance data from multiple species will of course help in minimizing the risk of a poor human prediction (Morris et al., 2012) and a pharmacokinetic modeling/simulation approach, taking into account all the relevant processes and *in vitro* data to facilitate more precise predictions of human biliary clearance should be a strong consideration (Kusuhara and Sugiyama 2010; Swift et al., 2010). Future

work to further understanding should certainly involve a detailed package of *in vitro* work, with the drugs discussed here, to define the active uptake intrinsic clearance values in rat, dog and human hepatocytes and to more fully define the transporter information.

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Authorship Contributions

Participated in research design: Ken Grime and Stuart Paine

Performed data analysis: Ken Grime and Stuart Paine

Wrote or contributed to the writing of the manuscript: Ken Grime

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Figure Legends

Figure 1: Relationship between human and animal (rat, closed circles and dog, open squares) unbound biliary clearance. The solid line is the line of unity and the dashed line is ten-fold from unity)

Figure 2: Relationship between human and rat unbound biliary clearance and the involvement of human hepatic drug transporters: A, hepatic uptake transporter definition with closed black circles representing drugs with no/slow active uptake, closed red circles representing substrates for OATP1B1/3, open red circles representing drugs (cefixime, cefamandole, cefotetan and cefpiramide) that are likely to be substrates for OATP1B1/3 (based on drug class, structural and physico-chemical similarity to cefixime, cefamandole, cefotetan and cefpiramide) but for which no literature evidence was found, the closed blue circle is ranitidine, an OCT-1 substrate and the open circles represent drugs for which literature evidence was not found to define the transporters involved; B, hepatic canalicular efflux transporter definition with green circles representing P-gp substrates, open triangles representing MRP2 substrates, green triangles representing MRP2 and P-gp substrates, blue circles representing BCRP substrates, blue triangles representing BCRP and MRP2 substrates and crosses representing drugs where no efflux transporter reference was found in the literature.

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TABLE 1: Human, rat and dog biliary clearance, presented in units of mL/min/kg as total (CL_{bile}) and unbound ($CL_{bile,u}$) after adjusted for plasma protein binding

	Human CL_{bile}	Human $CL_{bile,u}$	Rat CL_{bile}	Rat $CL_{bile,u}$	Dog CL_{bile}	Dog $CL_{bile,u}$
Doxorubicin	2.035 [a]	7.268	3.68 [b]	10.82	1.720 [c]	5.059
Digoxin	1.595 [d]	2.279	3.03 [e]	4.329	1.313 [f]	2.680
Erythromycin	1.147 [g]	7.169	17.4 [h]	22.38	2.376 [i]	4.483
Cefazolin	0.001 [j]	0.007	1.40 [k]	11.24	0.066 [l]	0.089
Cefamandole	0.012 [m]	0.048	5.00 [n]	29.41		
Cephalexin	0.0134 [o]	0.016	1.07 [o]	1.408		
Cefotetan	0.048 [o]	0.320	6.14 [o]	8.771	0.696 [o]	1.141
Cefixime	0.140 [p]	0.452	1.00 [q]	7.143		
Ceftriaxone	0.070 [r]	0.933	0.82 [s]	12.73	2.5 [s]	2.907
Cefpiramide	0.040 [t]	1.000	5.22 [u]	9.667	0.979 [v]	1.399
Cefoperazone	0.149 [w]	2.129	16.3 [x]	22.03		
Valsartan	0.431 [y]	10.775	12.5 [z]	378.8		
Moxalactam	0.003 [aa]	0.008	2.19 [ab]	4.294		
Methotrexate	0.048 [o]	1.108	9.60 [o]	12.47	0.217 [o]	0.344
Pravastatin	3.105 [ac]	6.210	27.5 [ad]	78.57		
Diclofenac	0.042 [ae]	8.400	0.85 [af]	94.44		
Ranitidine	0.153 [ag]	0.161	1.40 [o]	1.556		
Vincristine	0.209 [ah]	0.523	33.1 [ai]	84.87		
Methadone	0.001 [aj]	0.006	5.22 [ak]	14.33		
Fexofenadine	1.400 [al]	4.000	8.00 [am]	42.11		
Ciprofloxacin	0.059 [an]	0.084	3.50 [ao]	6.140		
Napsagatran	4.357 [o]	7.922	34.7 [o]	105.2	32.75 [o]	62.98

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TABLE 2: Rat/human and dog/human unbound biliary clearance ratios and assignment of hepatic drug transporter involvement

	Rat / human $CL_{bile,u}$	Dog / human $CL_{bile,u}$	hepatic uptake transporter	biliary efflux transporter	Chemo -type
Doxorubicin	1	1	-	MRP2; P-gp [a,b,c]	b
Digoxin	2	1	-	P-gp [d]	n
Erythromycin	3	1	-	MRP2; P-gp [e]	b
Cefazolin	1685	13	OATP1B1, 1B3 [f]	BCRP [g]	a
Cefamandole	613		*	MRP2, BCRP [h]	a
Cephalexin	90		OATP1B1, 1B3 [f]	*	a
Cefotetan	27	4	*	BCRP [g]	a
Cefixime	16		*	*	a
Ceftriaxone	14	3	OATP1B3 [f, h]	MRP2 [g]	a
Cefpiramide	10	1	*	MRP2, BCRP [g]	a
Cefoperazone	10		OATP1B1, 1B3 [h, i]	MRP2, BCRP [g]	a
Valsartan	35		OATP1B1, 1B3 [j]	MRP2 [j]	a
Moxalactam	507		*	*	a
Methotrexate	11	3	OATP1B1 [k]	MRP2 [l]	a
Pravastatin	13		OATP1B1 [m]	MRP2 [n]	a
Diclofenac	11		*	*	a
Ranitidine	10		OCT-1 [o]	P-gp [p]	b
Vincristine	162		*	MRP2; P-gp [q, r]	b
Methadone	2508		*	P-gp [s]	b
Fexofenadine	11		OATP1B1, 1B3 [t, u]	P-gp [u]	z
Ciprofloxacin	73		*	*	z
Napsagatran	13	8	*	*	z

- Literature indicates no or inefficient active hepatic uptake;

* Indicates that no literature reference was found;

a, b, n, z in the final column indicates acid, base, neutral and zwitterionic drug.

Literature references for the transporter definition: a. Fujiwara et al., 2011; b. Hidemura et al., 2003; c. Koike et al., 1997; d. Bi et al., 2006; e. Hariharan et a., 2009; f. Nakakariya et al., 2008a; g. Kato et al., 2008; h. Yamaguchi et al., 2011; i. Nakakariya et al., 2008b; j. Yamashiro et al., 2006; k. Gui et al., 2010; l. Choi et a., 2009; m. Kusuhara & Sugiyama 2010; n. Watanabe et al., 2009; o. Bourdet et al., 2005; p. Huang et al., 2005; q. van Zanden et al., 2005; r. Watanabe et al., 1995; s. Rodriguez et al, 2004; t. Matsushima et al., 2008; u. Cvetkovic et al., 1999.

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TABLE 3: Experimental rat and dog biliary excretion information used in the analysis

	Admin. Route (rat)	% dose excreted into bile (rat)	Admin. Route (dog)	% dose excreted into bile (dog)
Doxorubicin	i.v.	17	i.v.	10
Digoxin	i.v.	31	i.v.	7
Erythromycin	i.v.	30	i.v.	5
Cefazolin	i.v.	30	i.v.	2
Cefamandole	i.v.	33		
Cephalexin	i.v.	32		
Cefotetan	i.v.	48	i.v.	17
Cefixime	i.v.	41		
Ceftriaxone	i.v.	62	i.v.	63
Cefpiramide	i.v.	58	i.v.	19
Cefoperazone	i.v.	86		
Valsartan	i.v.	70		
Moxalactam	i.v.	25		
Methotrexate	i.v.	64		
Pravastatin	i.v.	40		
Diclofenac	i.v.	5		
Ranitidine	i.v.	5		
Vincristine	i.v.	43		
Methadone	i.v.	9		
Ciprofloxacin	i.v.	10		
Fexofenadine	i.v.	28		
Napsagatran	i.v.	61	i.v.	97

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TABLE 4: Experimental human biliary excretion information used in the analysis

	Calculation Method	% parent drug in bile	Admin. Route	Method	Collec-tion Interval
Doxorubicin	CL x %bile	14	i.v.	T-tube	24 hr
Digoxin	bil. excr. rate /Cp, mid	41	i.v	duodenal perf.	8 hr
Erythromycin	CL x %bile	13	i.v.	duodenal asp.	20 hr
Cefazolin	CL x %bile	0.1	i.v.	T-tube	
Cefamandole	CL x %bile	0.4	i.v.	T-tube	6 hr
Cephalexin	-				
Cefotetan	CL x %bile	11	i.v.	duodenal perf.	7 hr
Cefixime	Amount in bile/AUC	11	p.o	T-tube	24 hr
Ceftriaxone	bil. excr. rate /Cp,ss	30	i.v.	duodenal perf.	6-8 hr
Cefpiramide	Amount in bile/AUC	28	i.v.	T-tube	24 hr
Cefoperazone	CL x %bile	12	i.v.	T-tube	24 hr
Valsartan	CL x %bile	88	i.v.	T-tube	24 hr
Moxalactam	CL x %bile	0.5	i.v.	T-tube	8 hr
Methotrexate	CL x %bile	10	i.v.	T-tube	24 hr
Pravastatin	CL x %bile	23	i.v.	T-tube	24 hr
Diclofenac	CL x %bile	1	i.v.	T-tube	8 hr
Ranitidine	CL x %bile	1.5	i.v.	T-tube	24 hr
Vincristine	CL x %bile	10	i.v.	T-tube	72 hr
Methadone	CL/F x %bile	0.06	p.o	T-tube	24 hr
Ciprofloxacin	CL/F x %bile	0.5	p.o	T-tube	24 hr
Fexofenadine	CL/F x %bile	23	p.o	Fecal ext.	12 hr
Napsagatran	-	60	-	-	

Ratio of biliary excretion rate and the steady state plasma concentration or mid-point plasma concentration during the bile collection period = (bil. excr. rate /Cp,ss or bil. excr. rate /Cp,mid);

Percentage of parent drug accounted for in bile = %bile; F = bioavailability and AUC = area under the plasma drug concentration-time profile; - indicates that the precise details of the procedure were not available in the literature;

Duodenal perf. = duodenal perfusion;

Duodenal asp. = duodenal aspiration;

Fecal ext. = Fecal extraction.

Figure 1

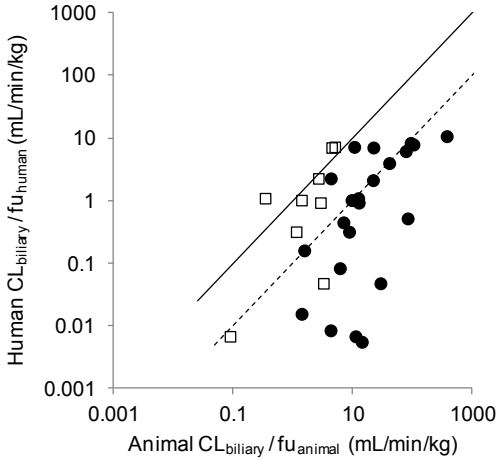

Figure 2