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Title page

Use of segregated hepatocyte scaling factors and cross-species relationship to resolve clearance-dependency in prediction of human hepatic clearance

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Running title page

Running title

Segregated hepatocyte scaling factors in prediction of clearance

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Non-standard abbreviations: CLb blood clearance; CLint (intrinsic clearance); CLp (plasma clearance); ESF (empirical scaling factor); ESFav (average empirical scaling factor); ESFsd (single drug empirical scaling factor); ESFseg (segregated empirical scaling factor); fub (fraction unbound in blood); GMFE (geometric fold error); PBPK (physiologically based pharmacokinetic); Q_H (hepatic blood flow)

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Abstract

Human and rat hepatocytes have a strong tendency to underpredict hepatic intrinsic clearance (CLint) and the extent of under prediction increases with increasing observed CLint (Wood et al., 2017, Drug Metab and Dispos 45: 1178–1188). In the present study, application of the log average rat hepatocyte–rat in vivo empirical scaling factor (ESF) of 4.2 to human hepatocyte prediction (from the Wood et al database) successfully removed bias but did not improve precision. An analogous method, using individual drug rat ESFs only achieved marginal improvement in accuracy but not precision. A novel approach to resolve clearance-dependent prediction, involving rat ESFs calculated for particular (order of magnitude) ranges of observed CLint (log average range 0.12–2.1), improved human prediction precision but only modestly reduced bias. However, rat in vivo CLint was several-fold greater than human in vivo CLint and this was reflected in greater rat hepatocyte and microsome CLint, suggesting that rat metabolic enzymes are more efficient than their human counterparts, by several-fold. By applying the segregated rat ESFs followed by the human–rat CLint ratio, which was consistent regardless of CLint (log average 3.5), both accuracy and precision were improved, providing both a means of mitigating clearance-dependency and re-affirming the potential role of rat hepatocytes for prediction of human metabolic CLint. These cross-species observations indicate that underprediction from human in vitro systems may be predominantly consequential of an intrinsic property of the in vitro system rather than individual drug properties.

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Introduction

In a recent article by Wood et al (2017), it was demonstrated that both rat and human hepatic in vitro systems (hepatocytes and microsomes) tend to underpredict in vivo CLint in a clearance-dependent way. The reason for this is not yet clear but in vitro factors including co-factor exhaustion (Swales and Utesch, 1998; Hengstler et al, 2000; Wang et al, 2005) and rate limiting unstirred water layer (Wood et al, 2018) may play significant roles. Underprediction of hepatic CLint using standard in vitro systems, particularly human, has been recognised for more than a decade but the bias has so far been generally assumed to be imprecise and irrespective of in vivo CLint. Over this time, pragmatic empirical correction of bias has frequently been advocated and although this has not been limited to the use of a simple average scaling factor, it has not recognised the clearance-dependency (Ito and Houston 2005; Poulin et al., 2012; Sohlenius-Sternbeck et al., 2012; Yamagata et al., 2016).

In the early 2000's, it was considered that prediction of clearance from rat hepatocytes was relatively successful compared to the equivalent results from the then emerging use of human hepatocytes (Naritomi et al 2001, 2003). From this perspective, Naritomi et al (2003) found that scaling human hepatocyte CLint using single drug observed/predicted CLint ratio (ESFsd) from rat hepatocytes improved human predictions to mostly within 5-fold for nine model compounds. They speculated that each compound had an intrinsic scaling factor due to either in vitro non-specific binding, lack of equilibrium of blood binding (in vivo), involvement of bound drug in uptake or heterogeneous distribution of transporters and metabolic enzyme in the liver. Since then, considerable evidence has accumulated confirming that human hepatocytes generally underpredict in vivo CLint (Shibata et al, 2002; Hallifax et al, 2005; Ito and Houston, 2005; Riley et al, 2005; Brown et al, 2007; Chiba et al, 2009) reflecting unavoidable donor phenotypic variability, potentially detrimental processing (preparation and storage) and in vitro lability of metabolising enzymes and uptake transporters; all potential sources of variation and possibly bias. The removal of this bias can be achieved by applying an average hepatocyte–in vivo empirical scaling factor (ESFav) but the lack of any mechanistic basis for this correction limits its use prospectively.

A recent study by De Bruyn et al (2018) investigating the utility of monkey hepatocytes to improve prediction of human clearance for a range of drugs cleared by hepatic uptake transporters, found that individual drug scaling factors from monkey applied to human data improved prediction. However,

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they also found that use of a monkey ESFav gave similar improvements in prediction, which confounds the hypothesis that cross-species scaling facilitates common factors related to specific drug properties. This recent attempt at a cross-species approach to maximise human clearance prediction from human hepatocytes has prompted further investigation of the clearance-dependency of prediction bias in human and rat using the dataset of Wood et al (2017). Our aim was to establish if the clearance-dependency in human hepatic CLint prediction from hepatocytes could be corrected by scaling from rat hepatocyte CLint based on common system performance; several scaling approaches including conventional ESFs (ESFav, ESFsd) and a novel, multi-ESF approach, were compared (see Table 1).

The use of a cross-species ESF has the potential advantage of being an independent measure of isolated hepatocyte functionality with respect to in vivo activity and hence differs from the conventional ESF which applies within the same species. Hence it might be used prospectively together with the capacity scalers (hepatocellularity and/or proteomic measurements) to boost confidence in human prediction from either rat or human hepatocytes by way of providing a measure of in vitro functional activity.

Methods

Data source

Datasets for human (n= 101, hepatocytes; n= 83, microsomes) and rat (n= 128 hepatocytes; n= 71, microsomes) in vitro CLint and complimentary in vivo CLint were taken from Wood et al (2017), in turn based on examination of published datasets, including both approved pharmaceuticals and investigatory proprietary compounds where blood clearance (CLb) or plasma clearance (CLp) was determined from intravenous dosing and where CLb did not exceed hepatic blood flow (Q_H). This included in vitro CLint determined from either metabolite formation (over a range of substrate concentrations), or from substrate depletion from single substrate concentration depletion time profiles. All hepatocyte data were from suspended cell (fresh or cryopreserved) assay without addition of serum of albumin. Some data were mean values of several independent publications. The datasets were considered to predominantly comprise highly permeable drugs of which the vast majority would be expected to be cleared by metabolism without rate limitation by transport. All CLint values had

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been corrected for unbound drug and physiologically scaled to whole liver using standard methods. The in vivo CLint values taken for this study were those calculated from the well-stirred liver model, after correction for renal clearance where applicable. Fraction unbound in blood (fub) was also taken from this dataset.

Correlation analysis

Predictions of in vivo CLint from human hepatocytes were compared with observed in vivo CLint for the whole datasets and for the sub-set of drugs common to both species, as in Wood et al, 2017. The human predicted CLints were compared to corresponding in vivo reported values directly (Approach 1) and then corrected using a series of empirical scaling factors (ESFs) based on rat predictions of CLint, as listed in Table 1. Firstly, previously advocated empirical scaling factors calculated from rat predictions were applied (Approaches 2 and 3 for ESFav and ESFsd, respectively).

To investigate the mechanistic origin of the clearance-dependent bias in prediction, direct comparison between species in vivo (Approaches 4/5) or in vitro system (Approaches 6–9) was examined using sub-sets of common drugs within the whole dataset. In addition, approaches 10 and 11 explore correlations between human hepatocyte and human microsome CLint (common drugs in human) and between human hepatocyte and human microsomes from the same donor livers from a specific study (Foster et al, 2011).

Subsequently, a novel prediction correction approach using ESFs based on prediction data segregated according to a particular range of CLint values (ESFseg) was examined as a potential means of correcting for clearance-dependent bias. Approaches 12–14 investigate human and rat predictions segregated according to each order of magnitude of observed CLint, (five levels: 10–10000 ml/min/kg).

Assessment of accuracy and precision of predictions

The bias in predictions was assessed by calculation of the geometric mean fold error (GMFE) (Equation 1). Root mean squared error (RMSE) (Equation 2) was used as a measure of precision.

$$GMFE = 10^{\frac{\sum \log \frac{predicted}{observed}}{n}} \quad (1)$$

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$$\text{RMSE} = \sqrt{\frac{1}{n}\sum(\text{predicted-observed})^2} \quad (2)$$

where n = number of predictions.

Residuals (log 10 predicted/observed) of predictions were examined graphically and the percentage of CLint predictions within two-fold of in vivo was used as indicator of predictive accuracy, consistent with previous publications (Obach, 1999; Sohlenius-Sternbeck et al., 2012; Chan et al., 2013).

Calculation of empirical scaling factors

The empirical scaling factors (ESFs) required to equate predicted CLint with observed CLint for either whole datasets or sub-sets (ESFav) or for individual compounds (ESFsd) within each dataset were calculated using Equations 3 and 4, respectively.

$$\text{ESFav} = 10^{\frac{\sum \log \frac{\text{observed}}{\text{predicted}}}{n}} \quad (3)$$

$$\text{ESFsd} = \frac{\text{observed } CL_{int,u}}{\text{predicted } CL_{int,u}} \quad (4)$$

Results

Human hepatocyte in vitro–in vivo correlation

Human in vivo CLint was predominantly underpredicted in hepatocytes (Figure 1 A and B); average fold-underprediction (GMFE) was 4.2. The overall prediction precision, represented by an RMSE value of 3550 provides a benchmark against which subsequent predictions, where correction factors are applied, can be compared (Table 2).

As noted previously, microsomes display a wider range of CLint predictions than hepatocytes (human and rat); human hepatocyte predicted CLint ranged approximately 1–1,000 ml/min/kg compared to approximately 0.1–10,000 ml/min/kg in microsomes (Wood et al, 2017). Whereas in rat, predicted CLint was limited to approximately 10,000 ml/min/kg in hepatocytes, while reaching

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100,000 ml/min/kg in microsomes (Wood et al, 2017). Prediction of CLint was clearance-dependent in both species (and systems) with negative bias increasing with increasing CLint. The intercept of the prediction trend line with the line of unity occurred at about 10 ml/min/kg for human and about 100 ml/min/kg for rat, signifying generally greater rates in rat compared to human.

Cross-species correction of bias: rat–human scaling factors

The similar GMFE prediction bias between human (4.2, Table 2) and rat (4.7, Wood et al., 2017) indicated potential for consistent cross-species correction for hepatocyte prediction. By applying this simple factor, rat ESFav, bias in human prediction was effectively abolished; rat-corrected human hepatocyte GMFE was 0.896 (Table 2). However, as expected, precision was virtually unaffected (RMSE = 3530); consequently, clearance-dependent bias remained (Figure 1 C and D).

Alternatively, using the ESFs for individual drugs (ESFsd), bias was only marginally reduced (GMFE from 4.35 to 3.82). For this drug specific approach, the precision remained about the same (RMSE = 1010) compared to the common drug dataset predictions when no ESF was applied (Table 2) (Figure 1 E and F).

Rat–human correlation in vivo

Comparison of in vivo CLint for common drugs between human and rat revealed that human in vivo CLint is on average several-fold less than for rat (Figure 2), similar to the average bias in human hepatocyte prediction of CLint (Table 2). By visual inspection, there was no clearance-dependency in this bias, although the dataset was considerably smaller (n=23) than the in vitro–in vivo datasets for all drugs (n=101 for human and 128 for rat).

There was some discrepancy between human and rat fub, although there was no evidence of bias between the species (Figure 3). Where fub was less than 0.1, differences between rat and human ranged several fold indicating potentially considerable differences in CLint for highly bound drugs.

Rat–human correlation in vitro

Predicted CLint from human hepatocytes was consistently lower than from rat hepatocytes and was clearance-dependent (Figure 4 A). Prediction from human microsomes was also consistently lower than from rat but, in contrast, there was no clear evidence of clearance-dependency (Figure 4 B).

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More direct assessment of CLint between the species at the cellular and subcellular level was enabled by comparison of unscaled CLint (Figure 4 C and D); in this case, only marginal convergence due to the differences in physiological scaling factors could be seen and hence the above observations were found to hold.

Human hepatocyte–microsome correlation

Human hepatocyte predictions of CLint were marginally lower on average than human microsome predictions with a tendency of bias to increase with CLint (Figure 5 A). This trend was corroborated by a discrete dataset of CLint for a series of benzodiazepines in hepatocytes and microsomes from the same livers, from four donors (Foster et al, 2011) (Figure 5 B). In this more specific dataset, hepatocyte bias was less negative for all substrates in those donors showing overall lower CLint for all pathways.

Rat–human correction of bias: clearance dependent scaling factors

When both the human and rat datasets were segregated into several discrete levels of in vivo CLint (Table 3), so that predictions within each human level (one order of magnitude of in vivo CLint) were corrected using the particular equivalent rat CLint level average ESF (ESFseg), clearance-dependency was effectively eliminated (Figure 6 A and B). However, considerable bias remained (GMFE = 2.82) although precision was increased (RMSE = 3030) compared to uncorrected predictions (Table 2). The ESFseg values are given in Table 3. To remove the consistent bias, each rat ESFseg was multiplied by the ratio of log average human ESF to log average rat ESF; this two-step scaling approach achieved effective elimination of bias (GMFE = 0.802, Figure 6 E and F).

In comparison, using human ESFseg to correct human predictions completely eliminated the bias, as expected (GMFE = 1.00) (Figure 6 C and D). The precision was greater than any other method applied to this dataset (RMSE = 1990), providing a representation of the optimum precision obtainable by this kind of empirical correction for this particular dataset. The precision achieved using the rat ESFseg (together with the ratio of human ESFav/ rat ESFav, as above) was similar, with RMSE = 2100.

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Discussion

It has become clear in recent years that physiological scaling of CLint obtained from suspended human hepatocytes not only underpredicts in vivo CLint on average (Ito and Houston, 2005; Riley et al., 2005; Brown et al., 2007; Bowman and Benet 2016) but does so in a clearance-dependent way (Hallifax et al 2010; Wood et al 2017). Although the reasons for this dependency remain unclear – but could involve effects of the unstirred water layer and/or cofactor depletion in vitro (Hengstler et al., 2000; Hewitt et al., 2000; Hewitt and Utesch, 2004; Hallifax et al., 2010; Foster et al., 2011; Wood et al 2017; Wood et al 2018) – achieving resolution of this clearance-dependent bias from the considerable prediction uncertainty has provided an opportunity to apply better targeted empirical correction. Because prediction from rat hepatocytes has now been shown to be clearance-dependent in the same manner as human hepatocytes (Wood et al 2017), there appears to exist a basis for renewed potential in the utility of rat hepatocytes for prediction of human in vivo CLint.

The main purpose of the present study was to compare conventional empirical scaling factors (ESFs) between rat and human hepatocyte CLint with a novel ESF scaling approach to deal with clearance-dependency. At the same time, we have examined the relationships between rat and human CLint both in vivo and in vitro and between human hepatocytes and microsomes for further mechanistic insight into species-dependent and species-independent performance in vitro. This might offer a means of empirical enhancement of conventional scaling which would take account of factors affecting inherent functional activity in vitro, in addition to physiological capacity.

For these datasets, comprising drugs primarily cleared by metabolism without permeability limitation, use of the rat average ESF clearly removes the average bias for human prediction but without increasing precision, leaving unsatisfactory prediction uncertainty. Despite the considerable imprecision among the predictions, clearance-dependency has been previously resolved (Hallifax et al, 2010; Wood et al 2017) and as such it is clear that a single scaling factor is inappropriate. Scaling factors for individual drugs is a concept that gained some traction after studies by Naritomi et al (2001, 2003) showed prediction improvement (from 12–199-fold to within about 5-fold for hepatocytes) which was attributed to drug-specific factors. But according to the literature, this approach does not appear to have become widely adopted; rather, various groups have pursued a

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variety of alternative, empirical and semi-mechanistic approaches to the problem of underprediction of clearance (Sohlenius-Sternbeck et al., 2012; Yamagata et al., 2016). Recently, Wood et al (2017), in examining literature prediction datasets for common drugs between human and rat (as used in the present study), found no correlation of individual ESF between rat and human – which does not support a drug-specific premise. Using the same data, we examined the relationship between human and rat hepatocyte predictions rather than individual ESFs and although there was a correlation – which was biased in favour of rat – it was clearance-dependent. So there remains little evidence that drug specific ESFs offer an improvement in prediction uncertainty. This agrees with recent findings for prediction of transporter-mediated clearance where application of individual drug ESFs from monkey hepatocytes to human hepatocyte CLint did not markedly improve prediction of in vivo clearance compared to use of an average monkey hepatocyte ESF (De Bruyn et al 2018). It is apparent that other factors in the in vitro-scaling processes need to be considered and resolved.

The subset of predictions for drugs common to both human and rat hepatocyte predictions showed that in vitro CLint tends to be greater in rat by several-fold, although with considerable imprecision – similar to in vitro–in vivo predictions. From a broad inter-species scaling perspective, it is expected that rat hepatic clearance exceeds human due to greater liver relative volume and greater relative hepatic blood flow. However, when focussing specifically on the CLint parameter, which is normalised to hepatic blood flow, the remaining inter-species discrepancy would signify that rat liver is inherently more efficient at clearing drugs than its human counterpart. As apparent in this study, human hepatocyte CLint is less than rat in a clearance-dependent way; at high CLint (>100 ml/min/kg) there is a tendency towards more than 10-fold difference between the species. However, while the generally lower CLint in human hepatocytes is reflected in the equivalent comparison for microsomes, there does not appear to be a clearance-dependent relationship in this sub-cellular system. Therefore, a consistent difference between rat and human hepatic metabolic CLint, of several-fold, is indicated. This appears to reflect the general difference in in vivo CLint between the species, suggesting a generally greater capacity and/or affinity of drug metabolising enzymes in rat hepatocytes. As hepatocytes from both species give clearance-dependent prediction (Wood et al 2017), the consistency in microsomes indicates existence of both a species-dependent factor (difference in metabolic efficiency) and a species-independent factor underlying a relatively greater inability of

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human hepatocytes to process high-clearance drugs compared to rat hepatocytes. Thus the inter-species examination described appears to resolve factors underlying the performance of hepatocytes in vitro, despite the uncertainty recognised among predictions, and this may support attempts at a general, empirical, approach to address clearance-dependency in prediction.

Despite the complication of clearance-dependent prediction from human and rat hepatocytes, this whole cell in vitro system should remain the preferred choice compared to hepatic microsomes; as well as accommodating a more comprehensive set of metabolic pathways than microsomes (Engtrakul et al., 2005; Riley et al., 2005; Hallifax et al., 2010), it allows potentially influential uptake as well as the overall disposition processes to be assessed, which is increasingly important given trends in drug discovery towards larger molecules with limited permeability. But to address the clearance-dependency in prediction – at least empirically – it is clear that ESFs need to be clearance-dependent as well. But, in addition, the possibility emerges that such scaling factors from rat, as a key pre-clinical species, can be applied to the human situation prospectively for improved prediction among sets of test compounds. To this end, a panel of segregated rat ESFs (ESFseg) was calculated based on separation of paired predicted and observed values, according to different ranges of observed CLint. A relatively simple segregation – by order of magnitude of observed CLint – was found adequate to achieve effective removal of clearance-dependency. Although bias between the species remained, this was now relatively consistent, reflecting some improvement in precision. Having removed the clearance-dependency, comparison of ESFseg between rat and human by CLint level revealed a relatively constant cross-species factor for hepatocytes, as anticipated in the earlier species comparisons. Therefore, a second ESF (human ESFav/rat ESFav) was introduced, to correct this apparently inherent difference in metabolic rate between the two species. By applying these ESFs in sequence, human hepatocyte predictions became largely free of bias with improved precision – virtually identical to the human internally corrected predictions. Hence, a simple basis for cross-species scaling was established which offers an overall improvement of predictions of human CLint from rat hepatocyte CLint. For these drugs (metabolically cleared without permeability limitation), it can be seen that a human ESF at one particular level of CLint is approximately matched by a rat ESF at the CLint level one order of magnitude greater. This displacement appears to equal an inter-species factor of about 3-fold (as discussed above) which when factored by the clearance-

dependency ESF (ESFseg), allows consistent correction between these two species. Therefore, sets of predictions of human CLint via rat hepatocytes might be more confidently undertaken using the rat scaling factors described above, in addition to the direct use of human ESFseg to human hepatocyte CLint. While it is tempting to assume that CLint for any individual drug in rat can be successfully scaled to the human situation by this factor, the observation that specific drug hepatocyte CLint appears not to be linearly related between human and rat means that a species differential may not hold consistently for individual drugs.

When CLint was directly compared between hepatocytes and microsomes from the same human liver donors, there was a tendency for CLint in both systems to be relatively low or high between individuals (Foster et al 2011). Because this phenomenon holds for low and high CLint pathways for the same drug, a consistent liver factor may be invoked which feeds through to a difference between hepatocyte and microsome CLint according to the level of CLint. So, for individuals with relatively high hepatic activity compared to others, there is a tendency for the highest CLint pathways to give lower CLint in hepatocytes compared to microsomes (and vice versa). Therefore it appears that the CLint level, rather than individual drug, is the driver of this scaling phenomenon. Nothwithstanding inevitable contributions from drug-specific factors, a predominantly system-specific phenomenon would explain why drug specific ESFs (ESFsd) are not particularly successful, as alluded to above. It is also notable that this relationship prevails at low CLint where, for human prediction (<10 ml/min/kg), the incidence of underprediction is more or less negated by the incidence of overprediction (in terms of bias). The reasons for this system specific scaling phenomenon is not clear although some difference in metabolic capacity between rat and human hepatocytes is indicated in this study.

Beyond the system-specific factor, there are species differences between hepatocytes whose impact can be dependent on drug, such as the shaking of hepatocyte incubations (Wood et al 2018). Other, drug specific species differences in hepatic clearance include metabolic and transporter pathways, not least due to species differences in CYP enzymes/transporters resulting in differences in capacity and/or affinity. Additionally, differences in the extent of binding to plasma proteins could reflect significant species differences in protein. Hence, there is potential for re-categorisation of a drug's clearance level between rat and human and factors specific between drug and species-specific

proteins may further explain why drug-specific ESFs are not particularly successful. Also, experimental methodology may confound species comparison, including failure to identify high affinity clearance pathways due to inadequately low substrate concentration (Klopf and Worboys 2010; Wood et al 2018).

The relationship between human and rat CLint both in vitro and in vivo has been explored for historical datasets of drugs comprising drugs predominantly cleared by metabolism without permeability limitation and, based on the species-dependent and species-independent factors revealed, viable empirical improvement of human CLint prediction from either human or rat hepatocytes is indicated. Nevertheless, further experimental examination of the sources of uncertainty are advocated, including potentially influential drug specific uncertainties, due to the need for more accurate in vitro pharmacokinetic parameter values for confident PBPK modelling.

Author contributions

Participated in research design: Hallifax and Houston.

Performed data analysis: Hallifax and Houston.

Wrote or contributed to the writing of the manuscript: Hallifax and Houston.

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Footnote

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Figure legends

Figure 1. Correlation of in vitro CLint in human hepatocytes (A, C and E) either uncorrected (A), adjusted with average rat scaling factor (C) or adjusted using individual drug rat scaling factors (E) with human in vivo CLint for 101 drugs (A and C) and 23 drugs (E). Dashed lines represent unity. Corresponding residual plots of log predicted/observed with predicted CLint are shown in panels B, D and F, respectively.

Figure 2. Correlation of human and rat in vivo CLint for 23 drugs. Dashed lines represent unity.

Figure 3. Correlation of rat and human fub for 23 drugs. Dashed lines represent unity.

Figure 4. Correlation of physiologically scaled in vitro CLint in human and rat hepatocytes (A) and microsomes (B) and unscaled in vitro CLint in human and rat hepatocytes (C) and microsomes (D). Dashed lines represent unity.

Figure 5. Correlation of physiologically scaled in vitro CLint in human hepatocytes and human microsomes for a dataset of 23 common drugs (A) and for hepatocytes and microsomes from the same donor livers (B) where different symbols represent the different donors. Dashed lines represent unity.

Figure 6. Correlation of in vitro CLint in human hepatocytes (A, C and E) adjusted using rat scaling factors for segregated in vivo CLint (A), adjusted using rat scaling factors for segregated in vivo CLint multiplied by log average segregated human ESF/log average segregated rat ESF (C) or using human scaling factors for segregated in vivo CLint (E). Dashed lines represent unity. Corresponding residual plots of log predicted/observed with predicted CLint are shown in panels B, D and F, respectively.

Table 1. Correlation analysis methods performed for comparison of in vitro-in vivo, in vivo-in vivo and in vitro-in vitro CLint and fub for human and rat [a].

Correlation analysis					
Approach no.	System comparison	Human parameter (y)	Applied ESF	Human or rat parameter (x)	Figure
1	in vitro–in vivo	hepatocyte predicted CLint	none	human in vivo CLint	1 A,B
2		hepatocyte predicted CLint	rat average (ESFav)	human in vivo CLint	1 C,D
3		hepatocyte predicted CLint	rat individual (ESFsd)	human in vivo CLint	1 E,F
4	in vivo–in vivo	CLint	n/a	rat in vivo CLint	2
5		fub	n/a	rat fub	3
6	in vitro–in vitro	hepatocyte predicted CLint	n/a	rat hepatocyte predicted CLint	4 A
7		microsome predicted CLint	n/a	rat microsome predicted CLint	4 B
8		hepatocyte absolute CLint	n/a	rat hepatocyte absolute CLint	4 C
9		microsome absolute CLint	n/a	rat microsome absolute CLint	4 D
10		hepatocyte predicted CLint	n/a	human microsome predicted CLint	5 A

11		hepatocyte predicted CLint (same donor)	n/a	human microsome predicted CLint (same donor)	5 B
12	in vitro–in vivo	hepatocyte predicted CLint	rat segregated (ESFseg)	human in vivo CLint	6 A
13		hepatocyte predicted CLint	rat ESFseg x [hu ESFav/rat ESFav]	human in vivo CLint	6 C
14		hepatocyte predicted CLint	human ESFseg	human in vivo CLint	6 E

[a] order shown corresponds to order of presentation of each correlation in Results

Table 2. Accuracy and precision of human hepatocyte prediction of *in vivo* CLint using rat and human in vitro-in vivo scaling factors, as represented by GMFE, RMSE and percentage of predictions that fall within two-fold of observed *in vivo* CLint; n = number of drugs.

	Uncorrected predictions		Corrected predictions				
			Scaling factor				
	Total human dataset	Human drugs common to rat	Rat average (ESFav)	Rat individual drug (ESFsd)	Rat segregated average (ESFseg)	Rat segregated averages x human/rat ratio (hu ESFav/rat ESFav)	Human segregated average (ESFseg)
n (rat)	-	23	128	23	128	128	128
n (human)	101	23	101	23	101	101	101
GMFE	4.20	4.35	0.896	3.82	2.82	0.802	1.00
RMSE	3550	1000	3530	1010	3030	2100	1990
% within 2-fold	22.8	-	35.6	13.0	30.7	45.5	49.5
Figure	1 A	-	1 C	1 E	6 A	6 C	6 E

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Table 3. Segregated scaling factors for rat and human hepatocytes as given by log observed/predicted CLint.

	Log average observed/predicted CLint				
	Segregated observed CLint (ml/min/kg)				
	<10	10–100	100–1000	1000–10000	>10000
Human	0.609	3.94	7.14	22.2	1150
Rat	0.125	1.57	3.24	7.17	179
Human/rat	4.88	2.50	2.20	3.10	6.46

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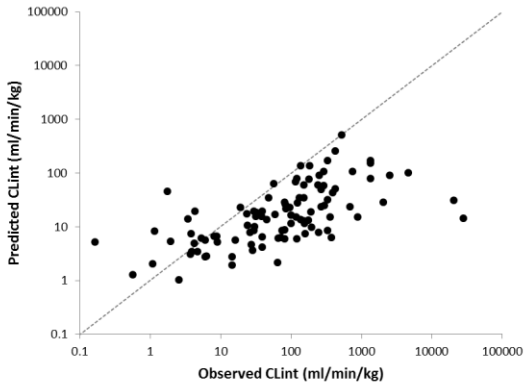
Figure 1

A



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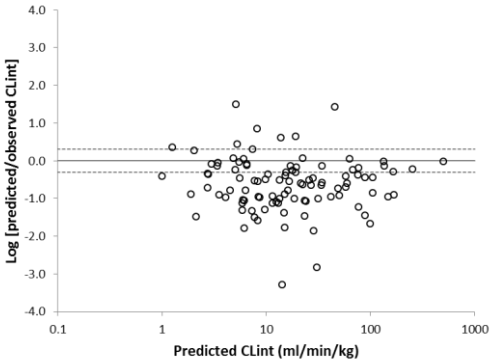

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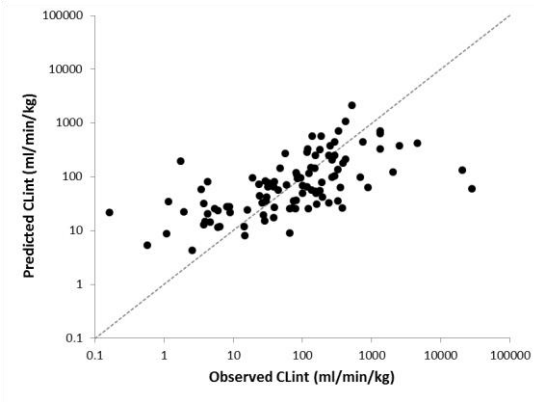

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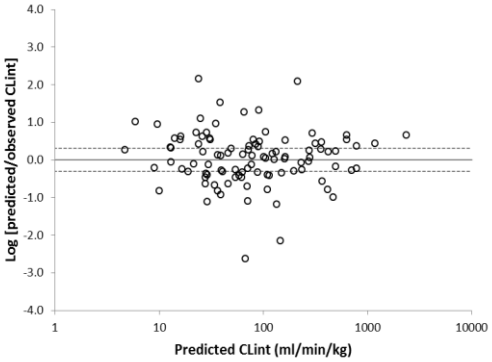

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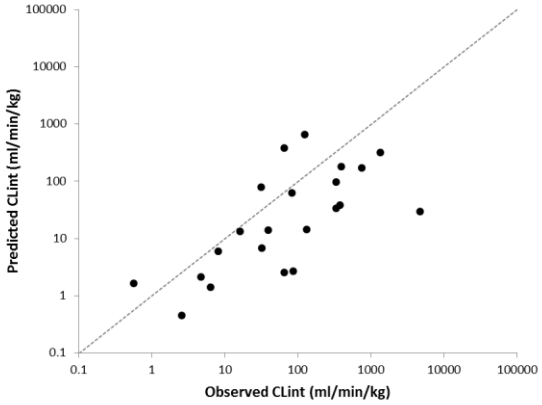

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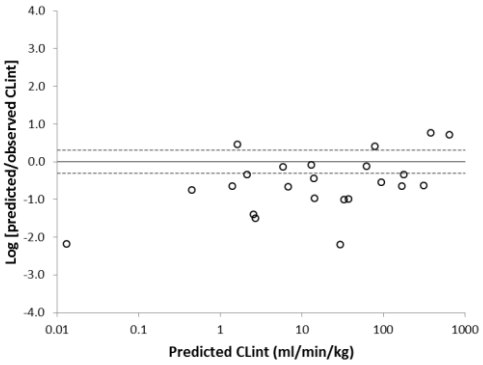

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Figure 2

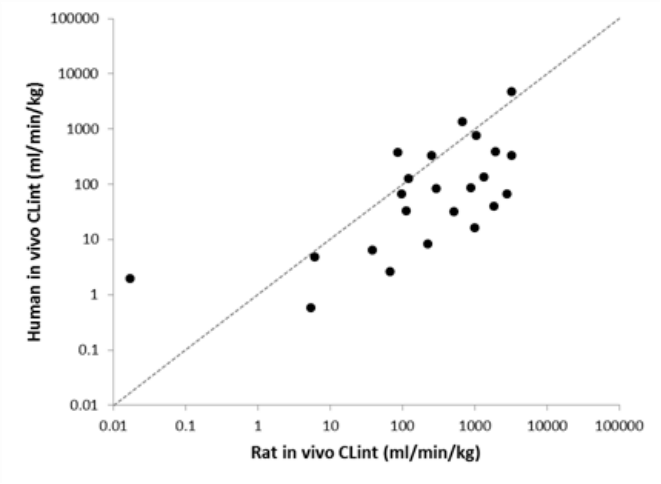

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Figure 3

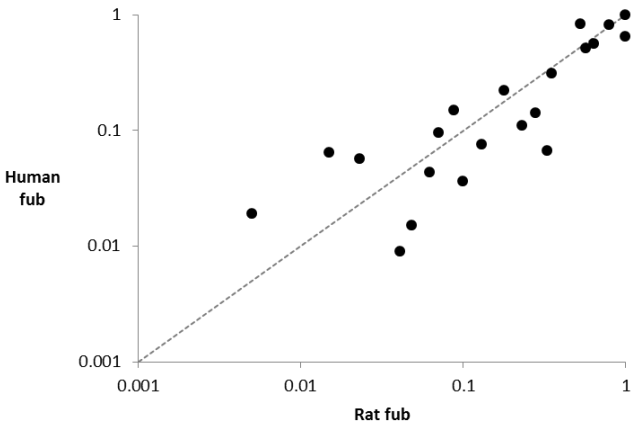

Figure 4

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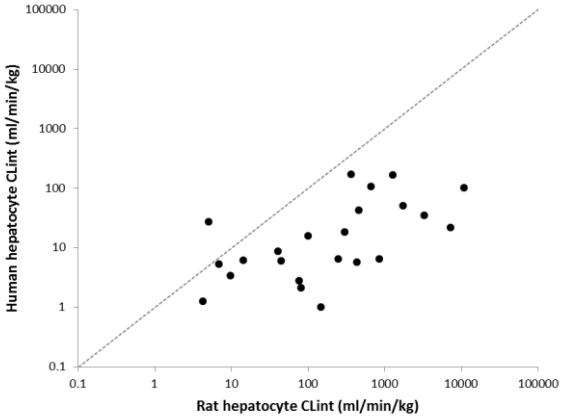

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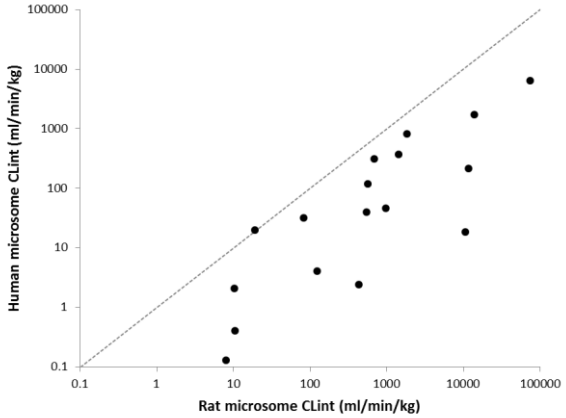

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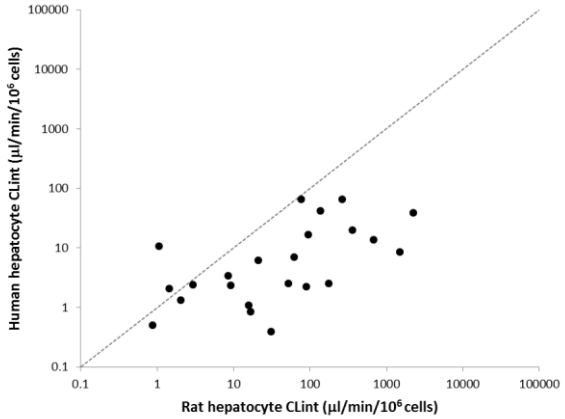

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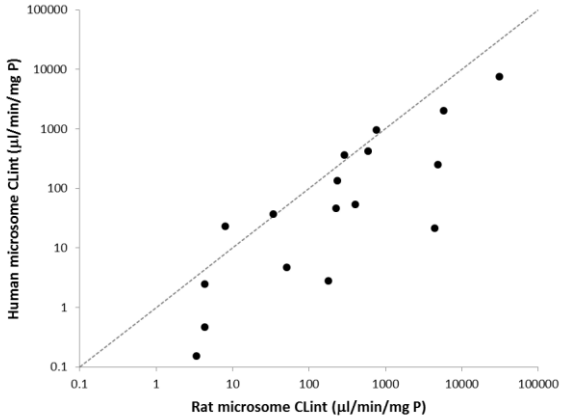

Figure 5

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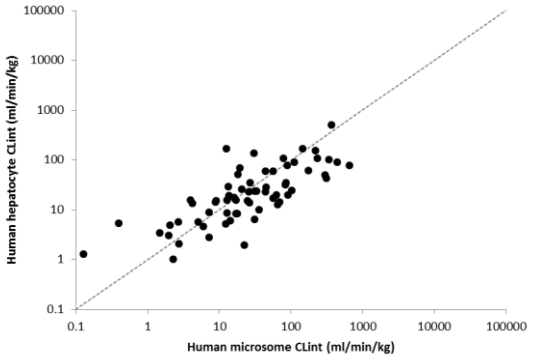

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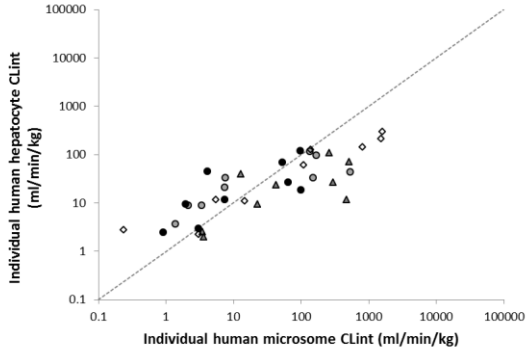

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Figure 6