Use of Segregated Hepatocyte Scaling Factors and Cross-Species Relationships to Resolve Clearance Dependence in the Prediction of Human Hepatic Clearance

D. Hallifax and J.B. Houston

Centre for Applied Pharmacokinetic Research, Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, United Kingdom

ABSTRACT

Human and rat hepatocytes have a strong tendency to underpredict hepatic intrinsic clearance (CL_{int}) and the extent of underprediction increases with increasing observed CL_{int}. In this study, application of the log average rat hepatocyte–rat in vivo empirical scaling factor (ESF) of 4.2 to human hepatocyte prediction 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 CL_{int} (log average range, 0.12−2.1) improved human prediction precision but only modestly reduced bias. However, rat in vivo CL_{int} was several-fold greater than human in vivo CL_{int} and this was reflected in greater rat hepatocyte and microsome CL_{int}, 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 CL_{int} ratio, which was consistent regardless of CL_{int} (log average 3.5), both accuracy and precision were improved, providing both a means of mitigating clearance dependence and reaffirming the potential role of rat hepatocytes for prediction of human metabolic CL_{int}. 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.

Introduction

A recent article by Wood et al. (2017) demonstrated that both rat and human hepatic in vitro systems (hepatocytes and microsomes) tend to underpredict in vivo intrinsic clearance (CL_{int}) in a clearance-dependent way. The reason for this is not yet clear but in vitro factors including cofactor exhaustion (Swales and Utesch, 1998; Hengstler et al., 2000; Wang et al., 2005) and a rate-limiting unstirred water layer (Wood et al., 2018) may play significant roles. Underprediction of hepatic CL_{int} using standard in vitro systems, particularly human, has been recognized for more than a decade but the bias has thus far been generally assumed to be imprecise and irrespective of in vivo CL_{int}. 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 recognized the clearance dependence (Ito and Houston, 2005; Poulin et al., 2012; Sohlenius-Sternbeck et al., 2012; Yamagata et al., 2017).

In the early 2000s, it was considered that prediction of clearance from rat hepatocytes was relatively successful compared with 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 CL_{int} using the single drug observed/predicted CL_{int} ratio (single drug empirical scaling factor [ESF_{sd}]) 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 nonspecific binding, a lack of equilibrium of blood binding (in vivo), involvement of bound drug in uptake, or heterogeneous distribution of transporters and metabolic enzymes in the liver. Since then, considerable evidence has accumulated confirming that human hepatocytes generally underpredict in vivo CL_{int} (Shibata et al., 2002; Hallifax et al., 2005; Ito and Houston, 2005; Riley et al., 2005; Brown et al., 2007; Chiba et al., 2009), attributed to unavoidable donor phenotypic variability, potentially detrimental processing (preparation and storage), and in vitro lability of metabolizing enzymes and uptake transporters, all of which are 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 (ESF_{av}) but the lack of any mechanistic basis for this correction limits its use prospectively.

A recent study by De Bruyn et al. (2018) investigated the utility of monkey hepatocytes to improve prediction of human clearance for a range of drugs cleared by hepatic uptake transporters. The authors found that individual drug scaling factors from monkeys applied to human data improved prediction. However, they also found that use of a monkey ESF_{av} 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 maximize human clearance prediction from human hepatocytes has prompted further investigation of the clearance dependence of prediction bias in humans and rats using the dataset of Wood et al. (2017). Our aim

ABBREVIATIONS: CL_{int}, intrinsic clearance; ESF, empirical scaling factor; ESF_{av}, average empirical scaling factor; ESF_{sd}, single drug empirical scaling factor; ESF_{seg}, segregated empirical scaling factor; fu_b, fraction unbound in blood; GMFE, geometric mean fold error; RMSE, root mean squared error.

was to establish whether the clearance dependence in human hepatic CL_{int} prediction from hepatocytes could be corrected by scaling from rat hepatocyte CL_{int} based on common system performance; several scaling approaches, including conventional ESFs (ESF_{av}, ESF_{sd}) 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.

Materials and Methods

Data Source. Datasets for human ($n = 101$ for hepatocytes and 83 for microsomes) and rat ($n = 128$ for hepatocytes and 71 for microsomes) in vitro CL_{int} and complementary in vivo CL_{int} were taken from Wood et al. (2017), in turn based on examination of published datasets, including both approved pharmaceuticals and investigatory proprietary compounds in which blood clearance or plasma clearance (CL_p) was determined from intravenous dosing and where blood clearance did not exceed hepatic blood flow. This included in vitro CL_{int} 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) assays without the addition of serum albumin. Some data were mean values of several independent publications. The datasets were considered to predominantly comprise highly permeable drugs, of which the majority would be expected to be cleared by metabolism without rate limitation by transport. All CL_{int} values had been corrected for unbound drug and physiologically scaled to the whole liver using standard methods. The in vivo CL_{int} values taken for this study were those calculated from the well stirred liver model, after correction for renal clearance where applicable. The fraction unbound in blood (fu_b) was also taken from this dataset.

Correlation Analysis. Predictions of in vivo CL_{int} from human hepatocytes were compared with observed in vivo CL_{int} for the whole datasets and for the subset of drugs common to both species, as in Wood et al. (2017). The human predicted CL_{int} values were compared with corresponding in vivo reported values directly (approach 1) and then corrected using a series of ESFs based on rat predictions of CL_{int}, as listed in Table 1. First, previously advocated ESFs calculated from rat predictions were applied (approaches 2 and 3 for ESF_{av} and ESF_{sd}, respectively).

To investigate the mechanistic origin of the clearance-dependent bias in prediction, direct comparison between species in vivo (approaches 4 or 5) or the in vitro system (approaches 6–9) was examined using subsets of common drugs within the whole dataset. In addition, approaches 10 and 11 explore correlations between human hepatocyte and human microsome CL_{int} (common drugs in humans) and between human hepatocytes 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 CL_{int} values (ESF_{seg}) 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 CL_{int} (five levels: 10–10,000 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) (eq. 1). The root mean squared error (RMSE) (eq. 2) was used as a measure of precision.

$$GMFE = 10^{\frac{\sum \log \frac{Predicted}{Observed}}{n}} \qquad (1)$$

$$RMSE = \sqrt{\frac{1}{n}\sum (Predicted - Observed)^2} \qquad (2)$$

where n is the number of predictions.

Residuals (log 10 predicted/observed) of predictions were examined graphically and the percentage of CL_{int} predictions within 2-fold of in vivo was used as an indicator of predictive accuracy, consistent with previous publications (Obach, 1999; Sohlenius-Sternbeck et al., 2012; Chan et al., 2013).

Calculation of ESFs. The ESFs required to equate predicted CL_{int} with observed CL_{int} for either whole datasets or subsets (ESF_{av}) or for individual compounds (ESF_{sd}) within each dataset were calculated using eqs. 3 and 4, respectively.

$$ESF_{av} = 10^{\frac{\sum \log \frac{Observed}{Predicted}}{n}} \qquad (3)$$

$$ESF_{sd} = \frac{Observed\ CL_{int,u}}{Predicted\ CL_{int,u}} \qquad (4)$$

Results

Human Hepatocyte In Vitro−In Vivo Correlation. Human in vivo CL_{int} was predominantly underpredicted in hepatocytes (Fig. 1, A and B); the average fold-underprediction (GMFE) was 4.2. The overall prediction precision, represented by an RMSE value of

TABLE 1

Correlation analysis methods performed for comparison of in vitro–in vivo, in vivo–in vivo, and in vitro–in vitro CL_{int} and f_{ub} for humans and rats

The order shown corresponds to the order of presentation of each correlation in the Results.

| Approach Number	System Comparison	Human Parameter (y)	Applied ESF	Human or Rat Parameter (x)	Figure
1	In vitro–in vivo	Hepatocyte predicted CL_{int}	None	Human in vivo CL_{int}	1, A and B
2		Hepatocyte predicted CL_{int}	Rat average (ESF_{av})	Human in vivo CL_{int}	1, C and D
3		Hepatocyte predicted CL_{int}	Rat individual (ESF_{sd})	Human in vivo CL_{int}	1 E and F
4	In vivo–in vivo	CL_{int}	N/A	Rat in vivo CL_{int}	2
5		fu_b	N/A	Rat fu_b	3
6	In vitro–in vitro	Hepatocyte predicted CL_{int}	N/A	Rat hepatocyte predicted CL_{int}	4A
7		Microsome predicted CL_{int}	N/A	Rat microsome predicted CL_{int}	4B
8		Hepatocyte absolute CL_{int}	N/A	Rat hepatocyte absolute CL_{int}	4C
9		Microsome absolute CL_{int}	N/A	Rat microsome absolute CL_{int}	4D
10		Hepatocyte predicted CL_{int}	N/A	Human microsome predicted CL_{int}	5A
11		Hepatocyte predicted CL_{int} (same donor)	N/A	Human microsome predicted CL_{int} (same donor)	5B
12	In vitro–in vivo	Hepatocyte predicted CL_{int}	Rat segregated (ESF_{seg})	Human in vivo CL_{int}	6A
13		Hepatocyte predicted CL_{int}	Rat ESF_{seg} × [human ESF_{av}/rat ESF_{av}]	Human in vivo CL_{int}	6C
14		Hepatocyte predicted CL_{int}	Human ESF_{seg}	Human in vivo CL_{int}	6E

N/A, not applicable.
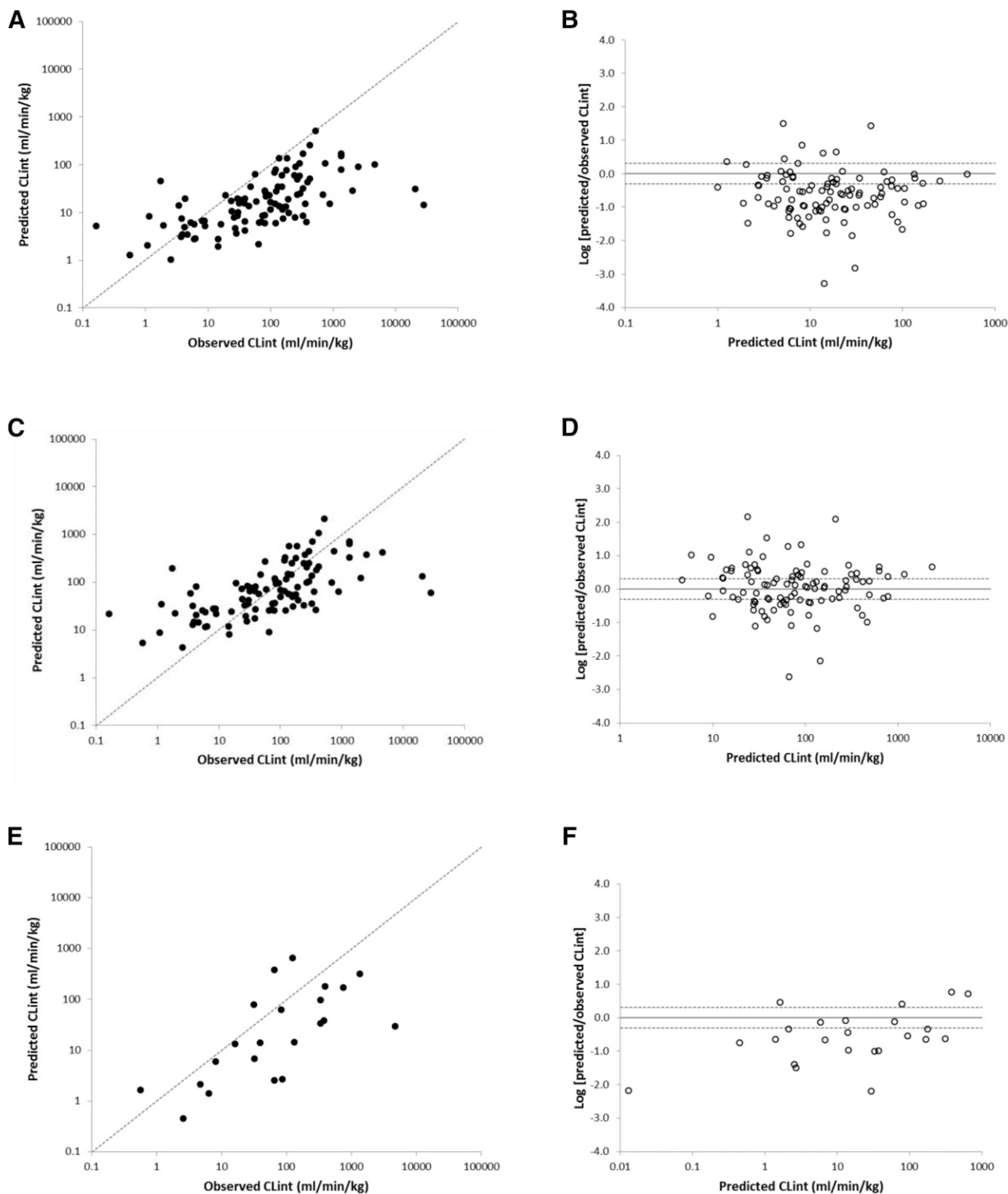
Fig. 1. (A, C, and E) Correlation of in vitro CL_{int} in human hepatocytes either uncorrected (A), adjusted with average rat scaling factor (C), or adjusted using individual drug rat scaling factors (E) with human in vivo CL_{int} for 101 drugs (A and C) and 23 drugs (E). Dashed lines represent unity. (B, D, and F) Corresponding residual plots of log predicted/observed CL_{int} with predicted CL_{int} are shown, respectively.

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 CL_{int} predictions than hepatocytes (human and rat); human hepatocyte predicted CL_{int} ranged from approximately 1 to 1000 ml/min/kg

compared with approximately 0.1 to 10,000 ml/min/kg in microsomes (Wood et al., 2017). In rats, predicted CL_{int} was limited to approximately 10,000 ml/min/kg in hepatocytes, whereas it reached 100,000 ml/min/kg in microsomes (Wood et al., 2017). Prediction of CL_{int} was clearance dependent in both species (and systems), with negative bias increasing

TABLE 2

Accuracy and precision of human hepatocyte prediction of in vivo CL_{int} using rat and human in vitro–in vivo scaling factors, as represented by GMFE, RMSE, and the percentage of predictions that fall within 2-fold of the observed in vivo CL_{int}

Parameter	Uncorrected Prediction		Corrected Prediction Using a Scaling Factor				
	Total Human Dataset	Human Drugs Common to Rats	Rat Average (ESF_{av})	Rat Individual Drug (ESF_{sd})	Rat Segregated Average (ESF_{seg})	Rat Segregated Average × Human/Rat Ratio (Human ESF_{av}/Rat ESF_{av})	Human Segregated Average (ESF_{seg})
Rats, n of drugs	N/A	23	128	23	128	128	128
Humans, n of drugs	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	N/A	35.6	13.0	30.7	45.5	49.5
Figure	1A	N/A	1C	1E	6A	6C	6E

N/A, not applicable.

with increasing CL_{int}. The intercept of the prediction trend line with the line of unity occurred at about 10 ml/min/kg for humans and about 100 ml/min/kg for rats, signifying generally greater rates in rats compared with humans.

Cross-Species Correction of Bias: Rat−Human Scaling Factors. The similar GMFE prediction bias between humans (4.2; Table 2) and rats (4.7; Wood et al., 2017) indicated potential for consistent cross-species correction for hepatocyte prediction. By applying this simple factor (rat ESF_{av}), bias in human prediction was effectively abolished; the rat-corrected human hepatocyte GMFE was 0.896 (Table 2). However, as expected, precision was virtually unaffected (RMSE = 3530); consequently, clearance-dependent bias remained (Fig. 1, C and D).

Alternatively, using the ESFs for individual drugs (ESF_{sd}), 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 with the common drug dataset predictions when no ESF was applied (Fig. 1, E and F; Table 2).

Rat−Human Correlation In Vivo. Comparison of in vivo CL_{int} for common drugs between humans and rats revealed that human in vivo CL_{int} is on average several-fold less than for rats (Fig. 2), similar to the average bias in human hepatocyte prediction of CL_{int} (Table 2). By visual inspection, there was no clearance dependence in this bias, although the dataset was considerably smaller ($n = 23$) than the in vitro–in vivo datasets for all drugs ($n = 101$ for humans and 128 for rats).

There was some discrepancy between human and rat fu_b, although there was no evidence of bias between the species (Fig. 3). Where fu_b was less than 0.1, differences between rats and humans ranged several-fold, indicating potentially considerable differences in CL_{int} for highly bound drugs.

Rat−Human Correlation In Vitro. Predicted CL_{int} from human hepatocytes was consistently lower than from rat hepatocytes and was clearance dependent (Fig. 4A). Prediction from human microsomes was also consistently lower than from rats; in contrast, there was no clear evidence of clearance dependence (Fig. 4B). More direct assessment of CL_{int} between the species at the cellular and subcellular levels was enabled by comparison of unscaled CL_{int} (Fig. 4, C and D); in this case, only marginal convergence due to the differences in physiologic scaling factors could be seen and hence the above observations were found to hold.

Human Hepatocyte−Microsome Correlation. Human hepatocyte predictions of CL_{int} were marginally lower on average than human microsome predictions, with a tendency for bias to increase with CL_{int} (Fig. 5A). This trend was corroborated by a discrete dataset of CL_{int} for a series of benzodiazepines in hepatocytes and microsomes from the same livers, from four donors (Foster et al., 2011) (Fig. 5B). In this more specific dataset, hepatocyte bias was less negative for all substrates in those donors showing overall lower CL_{int} 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 CL_{int} (Table 3), so that predictions within each human level (one order of magnitude of in vivo CL_{int}) were corrected using the particular equivalent rat CL_{int} level average ESF (ESF_{seg}), clearance dependence was effectively eliminated (Fig. 6, A and B). However, considerable bias remained (GMFE = 2.82) although precision was increased (RMSE = 3030) compared with uncorrected predictions (Table 2). The ESF_{seg} values are given in Table 3. To remove the consistent bias, each rat ESF_{seg} 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; Fig. 6, E and F).

In comparison, using human ESF_{seg} to correct human predictions completely eliminated the bias, as expected (GMFE = 1.00) (Fig. 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 ESF_{seg} (together with the ratio of human ESF_{av}/rat ESF_{av}, as above) was similar, with an RMSE of 2100.

Discussion

It has become clear in recent years that physiologic scaling of CL_{int} obtained from suspended human hepatocytes not only underpredicts in vivo CL_{int} on average (Ito and Houston, 2005; Riley et al., 2005;
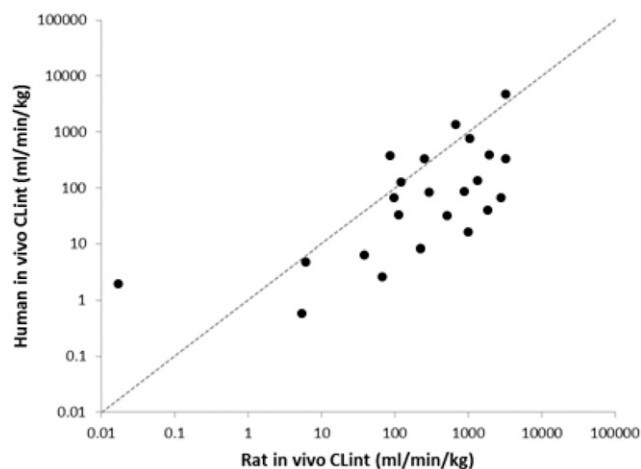

Fig. 2. Correlation of human and rat in vivo CL_{int} for 23 drugs. Dashed lines represent unity.
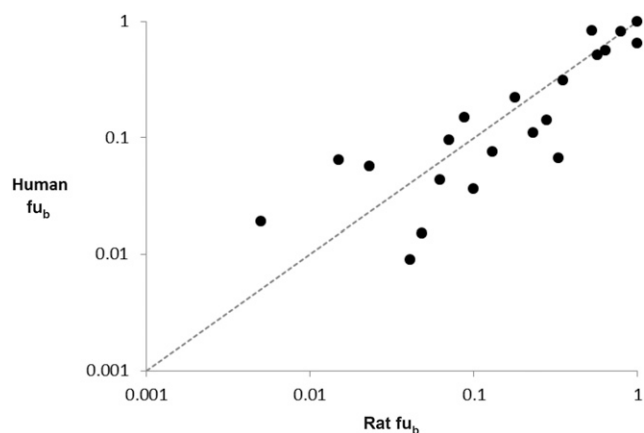
Fig. 3. Correlation of rat and human fu_b for 23 drugs. Dashed lines represent unity.

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 dependence 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, 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 CL_{int}.

The main purpose of this study was to compare conventional ESFs between rat and human hepatocyte CL_{int} with a novel ESF scaling approach to deal with clearance dependence. At the same time, we examined the relationships between rat and human CL_{int} 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 that would take account of factors affecting inherent functional activity in vitro, in addition to physiologic 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 dependence was previously resolved (Hallifax et al., 2010; Wood et al., 2017); as such, it is clear that a single scaling factor is inappropriate.
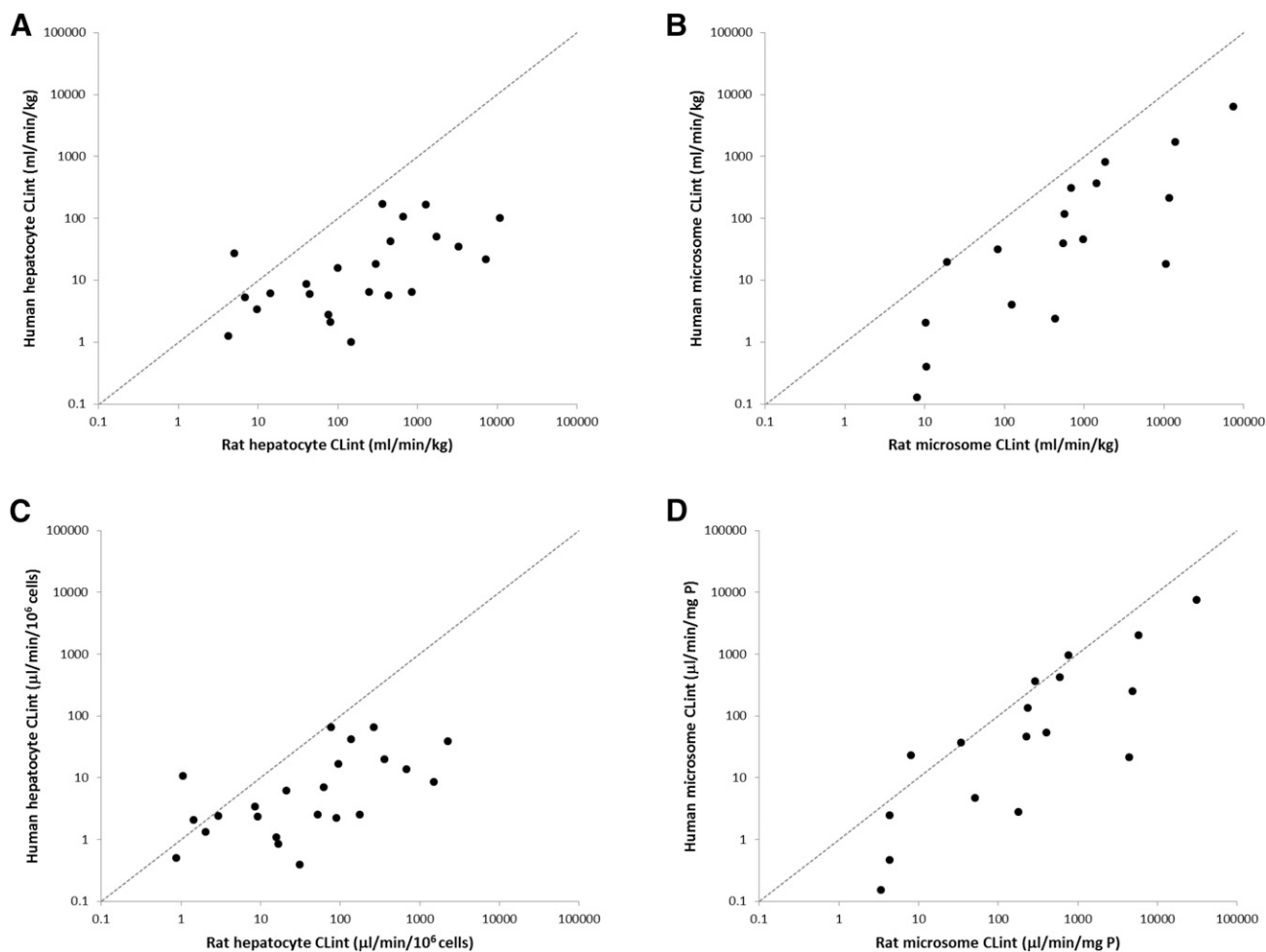
Fig. 4. (A–D) Correlation of physiologically scaled in vitro CL_{int} in human and rat hepatocytes (A) and microsomes (B) and unscaled in vitro CL_{int} in human and rat hepatocytes (C) and microsomes (D). Dashed lines represent unity.
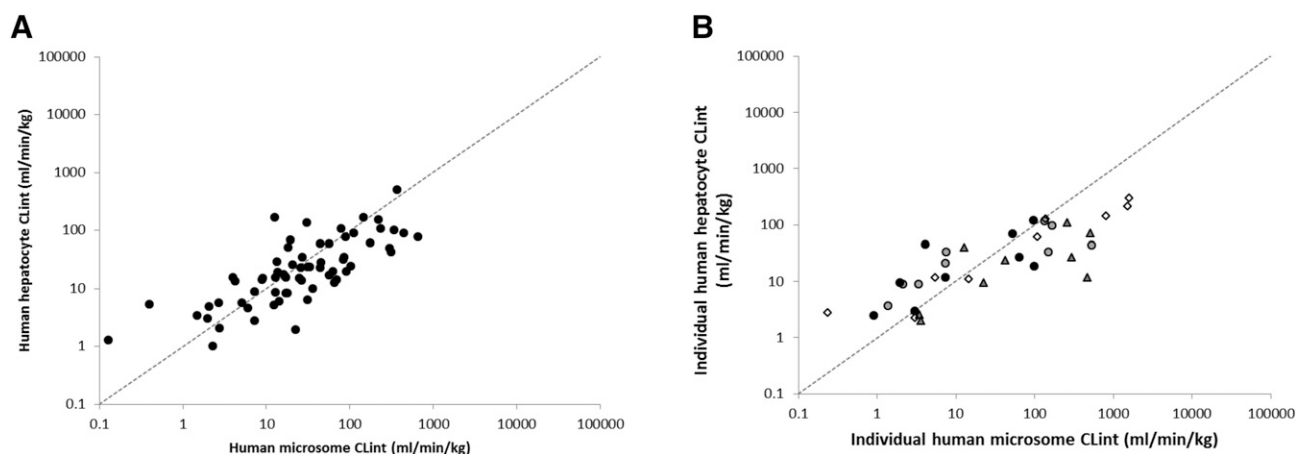
Fig. 5. (A and B) Correlation of physiologically scaled in vitro CL_{int} in human hepatocytes and human microsomes for a dataset of 23 common drugs (A) and for hepatocytes and microsomes from the same donor livers (B), where different symbols represent the different donors. Dashed lines represent unity.

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- to 199-fold to within about 5-fold for hepatocytes), which was attributed to drug-specific factors. Yet according to the literature, this approach does not appear to have become widely adopted; rather, various groups have pursued a variety of alternative, empirical, and semi-mechanistic approaches to the problem of underprediction of clearance (Sohlenius-Sternbeck et al., 2012; Yamagata et al., 2017). Wood et al. (2017) examined literature prediction datasets for common drugs between humans and rats (as used in our study) and found no correlation of individual ESFs between rats and humans, 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; although there was a correlation, which was biased in favor of the rat, it was clearance dependent. Thus 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, in which the application of individual drug ESFs from monkey hepatocytes to human hepatocyte CL_{int} did not markedly improve prediction of in vivo clearance compared with the 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 CL_{int} tends to be greater in rats by several-fold, although with considerable imprecision, similar to in vitro–in vivo predictions. From a broad interspecies scaling perspective, it is expected that rat hepatic clearance exceeds that of humans due to greater liver relative volume and greater relative hepatic blood flow. However, when focusing specifically on the CL_{int}

parameter, which is normalized to hepatic blood flow, the remaining interspecies discrepancy would signify that the rat liver is inherently more efficient at clearing drugs than its human counterpart. As apparent in this study, human hepatocyte CL_{int} is less than that of rats in a clearance-dependent way; at high CL_{int} (>100 ml/min/kg), there is a tendency toward a more than 10-fold difference between the species. However, while the generally lower CL_{int} in human hepatocytes is reflected in the equivalent comparison for microsomes, there does not appear to be a clearance-dependent relationship in this subcellular system. Therefore, a consistent difference between rat and human hepatic metabolic CL_{int} (of several-fold) is indicated. This appears to reflect the general difference in in vivo CL_{int} between the species, suggesting a generally greater capacity and/or affinity of drug-metabolizing enzymes in rat hepatocytes. As hepatocytes from both species give clearance-dependent prediction (Wood et al., 2017), the consistency in microsomes indicates the existence of both a species-dependent factor (difference in metabolic efficiency) and a species-independent factor underlying a relatively greater inability of human hepatocytes to process high-clearance drugs compared with rat hepato-cytes. Thus, the interspecies examination described appears to resolve factors underlying the performance of hepatocytes in vitro, despite the uncertainty recognized among predictions, and this may support attempts at a general, empirical approach to address clearance de-pendence 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. In addition to accommodating a more comprehensive set of metabolic pathways than microsomes (Engtrakul et al., 2005; Riley et al., 2005; Hallifax et al., 2010), hepatocytes allow potentially influential uptake as well as the overall disposition processes to be assessed, which is increasingly important given trends in drug discovery toward larger molecules with limited permeability. To address the clearance dependence in prediction (at least empirically), it is clear that ESFs need to be clearance dependent as well. In addition, the possibility emerges that such scaling factors from the rat, as a key preclinical 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 (ESF_{seg}) was calculated based on the separation of paired predicted and observed values, according to different ranges of observed CL_{int}. A relatively simple segregation, by order of magnitude of observed CL_{int}, was found adequate to achieve effective removal of clearance dependence. Although bias between

TABLE 3

Segregated scaling factors for rat and human hepatocytes as given by log observed/predicted CL_{int}

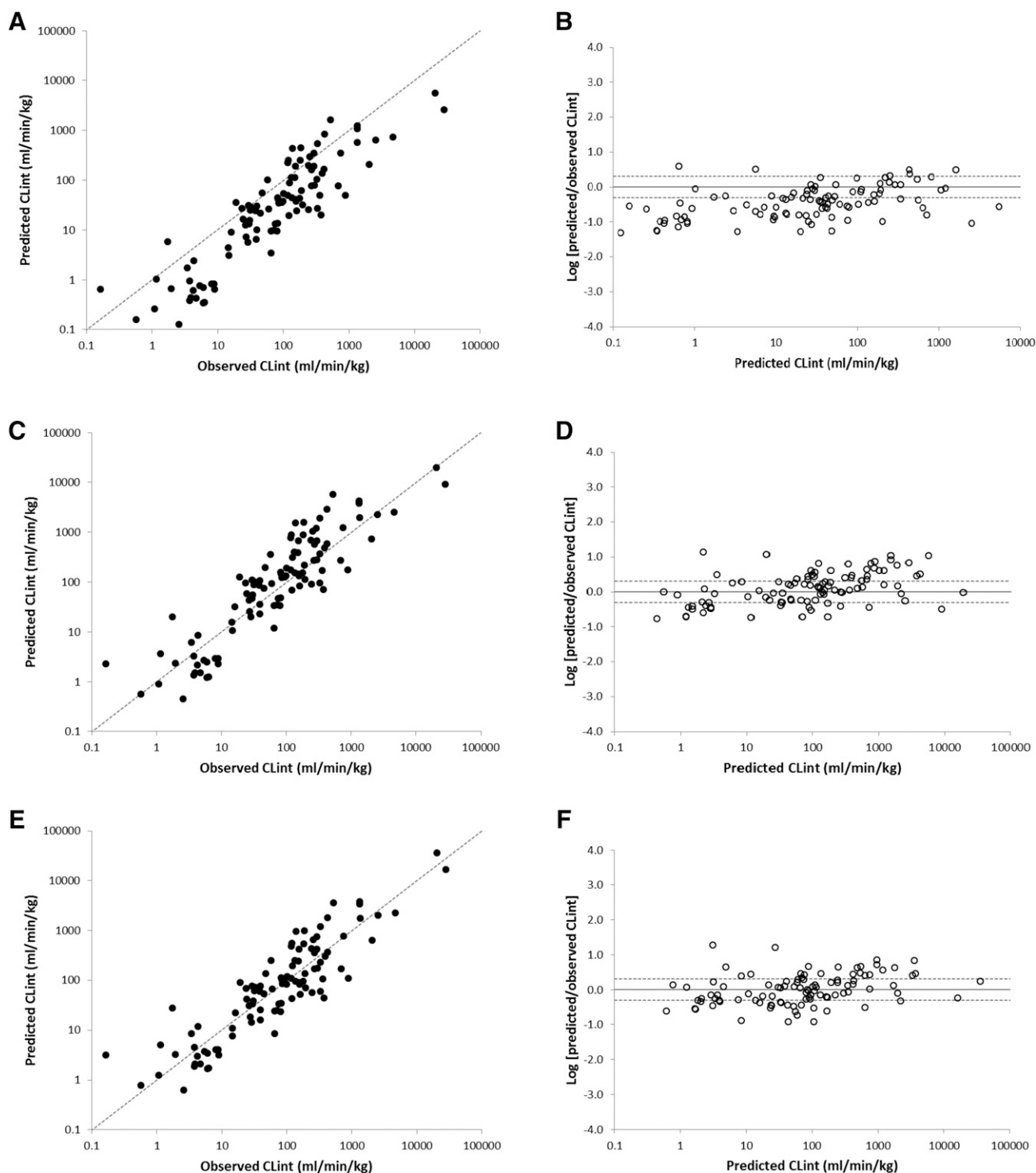| Species	Log average Observed/Predicted for Segregated Observed CL_{int}				
	<10	10–100	100–1000	1000–10,000	>10,000
	ml/min/kg				
Human	0.609	3.94	7.14	22.2	1150
Rat	0.125	1.57	3.24	7.17	179
Human/rat	4.88	2.50	2.20	3.10	6.46

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

the species remained, this was now relatively consistent, reflecting some improvement in precision. Having removed the clearance dependence, comparison of ESF_{seg} between rats and humans by CL_{int} level revealed a relatively constant cross-species factor for hepatocytes, as anticipated in the earlier species comparisons. Therefore, a second ESF (human ESF_{av}/rat ESF_{av}) 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 that offers an overall improvement of predictions of human CL_{int} from rat hepatocyte CL_{int}. For these drugs (metabolically cleared without permeability limitation), it can be seen that a human ESF at one

particular level of CL_{int} is approximately matched by a rat ESF at the CL_{int} level one order of magnitude greater. This displacement appears to equal an interspecies factor of about 3-fold (as discussed above), which when factored by the clearance-dependent ESF (ESF_{seg}) allows consistent correction between these two species. Therefore, sets of predictions of human CL_{int} via rat hepatocytes might be more confidently undertaken using the rat scaling factors described above, in addition to the direct use of human ESF_{seg} to human hepatocyte CL_{int}. While it is tempting to assume that CL_{int} for any individual drug in rats can be successfully scaled to the human situation by this factor, the observation that specific drug hepatocyte CL_{int} appears not to be linearly related between humans and rats means that a species differential may not hold consistently for individual drugs.

When CL_{int} was directly compared between hepatocytes and microsomes from the same human liver donors, there was a tendency for CL_{int} in both systems to be relatively low or high between individuals (Foster et al., 2011). Because this phenomenon holds for low and high CL_{int} pathways for the same drug, a consistent liver factor may be invoked that feeds through to a difference between hepatocyte and microsome CL_{int} according to the level of CL_{int}. For individuals with relatively high hepatic activity compared with others, there is thus a tendency for the highest CL_{int} pathways to give lower CL_{int} in hepatocytes compared with microsomes (and vice versa). Therefore, it appears that the CL_{int} level, rather than the individual drug, is the driver of this scaling phenomenon. Notwithstanding inevitable contributions from drug-specific factors, a predominantly system-specific phenomenon would explain why drug-specific ESFs (ESF_{sd}) are not particularly successful, as alluded to above. It is also notable that this relationship prevails at low CL_{int} where for human prediction (<10 ml/min per kilogram), 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 are 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 the 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 cytochrome P450 enzymes or transporters resulting in differences in capacity and/or affinity. In addition, differences in the extent of binding to plasma proteins could reflect significant species differences in protein. Hence, there is potential for recategorization of a drug's clearance level between rats and humans and factors specific between drug- and species-specific proteins may further explain why drug-specific ESFs are not particularly successful. In addition, 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 CL_{int} 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 CL_{int} 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 physiologically based pharmacokinetic modeling.

Authorship Contributions

Participated in research design: Hallifax, Houston.

Performed data analysis: Hallifax, Houston.

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

References

Bowman CM and Benet LZ (2016) Hepatic clearance predictions from in vitro-in vivo extrapolation and the biopharmaceutics drug disposition classification system. *Drug Metab Dispos* **44**:1731–1735.

Brown HS, Griffin M, and Houston JB (2007) Evaluation of cryopreserved human hepatocytes as an alternative in vitro system to microsomes for the prediction of metabolic clearance. *Drug Metab Dispos* **35**:293–301.

Chan TS, Yu H, Moore A, Khetani SR, and Tweedie D (2013) Meeting the challenge of predicting hepatic clearance of compounds slowly metabolized by cytochrome P450 using a novel hepatocyte model, HepatoPac [published correction appears in *Drug Metab Dispos* (2014) 42:200 and *Drug Metab Dispos* (2019) 47:58–66]. *Drug Metab Dispos* **41**:2024–2032.

Chiba M, Ishii Y, and Sugiyama Y (2009) Prediction of hepatic clearance in human from in vitro data for successful drug development. *AAPS J* **11**:262–276.

De Bruyn T, Ufuk A, Cantrill C, Kosa RE, Bi YA, Niosi M, Modi S, Rodrigues AD, Tremaine LM, Varma MVS, et al. (2018) Predicting human clearance of organic anion transporting polypeptide substrates using cynomolgus monkey: in vitro-in vivo scaling of hepatic uptake clearance. *Drug Metab Dispos* **46**:989–1000.

Engtrakul JJ, Foti RS, Strelevitz TJ, and Fisher MB (2005) Altered AZT (3′-azido-3′-deoxythymidine) glucuronidation kinetics in liver microsomes as an explanation for underprediction of in vivo clearance: comparison to hepatocytes and effect of incubation environment. *Drug Metab Dispos* **33**:1621–1627.

Foster JA, Houston JB, and Hallifax D (2011) Comparison of intrinsic clearances in human liver microsomes and suspended hepatocytes from the same donor livers: clearance-dependent relationship and implications for prediction of in vivo clearance. *Xenobiotica* **41**:124–136.

Hallifax D, Foster JA, and Houston JB (2010) Prediction of human metabolic clearance from in vitro systems: retrospective analysis and prospective view. *Pharm Res* **27**:2150–2161.

Hallifax D, Rawden HC, Hakooz N, and Houston JB (2005) Prediction of metabolic clearance using cryopreserved human hepatocytes: kinetic characteristics for five benzodiazepines. *Drug Metab Dispos* **33**:1852–1858.

Hengstler JG, Utesch D, Steinberg P, Platt KL, Diener B, Ringel M, Swales N, Fischer T, Biefang K, Gerl M, et al. (2000) Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. *Drug Metab Rev* **32**:81–118.

Hewitt NJ, Fischer T, Zuehlke U, Oesch F, and Utesch D (2000) Metabolic activity of fresh and cryopreserved cynomolgus monkey (Macaca fascicularis) hepatocytes. *Xenobiotica* **30**:665–681.

Hewitt NJ and Utesch D (2004) Cryopreserved rat, dog and monkey hepatocytes: measurement of drug metabolizing enzymes in suspensions and cultures. *Hum Exp Toxicol* **23**:307–316.

Ito K and Houston JB (2005) Prediction of human drug clearance from in vitro and preclinical data using physiologically based and empirical approaches. *Pharm Res* **22**:103–112.

Klopf W and Worboys P (2010) Scaling in vivo pharmacokinetics from in vitro metabolic stability data in drug discovery. *Comb Chem High Throughput Screen* **13**:159–169.

Naritomi Y, Terashita S, Kagayama A, and Sugiyama Y (2003) Utility of hepatocytes in predicting drug metabolism: comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. *Drug Metab Dispos* **31**:580–588.

Naritomi Y, Terashita S, Kimura S, Suzuki A, Kagayama A, and Sugiyama Y (2001) Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with liver microsomes from animals and humans. *Drug Metab Dispos* **29**:1316–1324.

Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos* **27**:1350–1359.

Poulin P, Kenny JR, Hop CECA, and Haddad S (2012) In vitro-in vivo extrapolation of clearance: modeling hepatic metabolic clearance of highly bound drugs and comparative assessment with existing calculation methods. *J Pharm Sci* **101**:838–851.

Riley RJ, McGinnity DF, and Austin RP (2005) A unified model for predicting human hepatic metabolic clearance from in vitro intrinsic clearance data in hepatocytes and microsomes. *Drug Metab Dispos* **33**:1304–1311.

Shibata Y, Takahashi H, Chiba M, and Ishii Y (2002) Prediction of hepatic clearance and availability by cryopreserved human hepatocytes: an application of serum incubation method. *Drug Metab Dispos* **30**:892–896.

Sohlenius-Sternbeck AK, Jones C, Ferguson D, Middleton BJ, Projean D, Floby E, Bylund J, and Afzelius L (2012) Practical use of the regression offset approach for the prediction of in vivo intrinsic clearance from hepatocytes. *Xenobiotica* **42**:841–853.

Swales NJ and Utesch D (1998) Metabolic activity of fresh and cryopreserved dog hepatocyte suspensions. *Xenobiotica* **28**:937–948.

Wang Q, Jia R, Ye C, Garcia M, Li J, and Hidalgo IJ (2005) Glucuronidation and sulfation of 7-hydroxycoumarin in liver matrices from human, dog, monkey, rat, and mouse. *In Vitro Cell Dev Biol Anim* **41**:97–103.

Wood FL, Houston JB, and Hallifax D (2017) Clearance prediction methodology needs fundamental improvement: trends common to rat and human hepatocytes/microsomes and implications for experimental methodology. *Drug Metab Dispos* **45**:1178–1188.

Wood FL, Houston JB, and Hallifax D (2018) Importance of the unstirred water layer and hepatocyte membrane integrity in vitro for quantification of intrinsic metabolic clearance. *Drug Metab Dispos* **46**:268–278.

Yamagata T, Zanelli U, Gallemann D, Perrin D, Dolgos H, and Petersson C (2017) Comparison of methods for the prediction of human clearance from hepatocyte intrinsic clearance for a set of reference compounds and an external evaluation set. *Xenobiotica* **47**:741–751.

Address correspondence to: D. Hallifax, Centre for Applied Pharmacokinetic Research, Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester M13 9PT, UK. E-mail: david.hallifax@manchester.ac.uk