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In vitro-in vivo extrapolation and hepatic clearance dependent underprediction

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Abstract

Accurately predicting the hepatic clearance of compounds using *in vitro* to *in vivo* extrapolation (IVIVE) is crucial within the pharmaceutical industry. However several groups have recently highlighted the large error in the process. While empirical or regression-based scaling factors may be used to mitigate the common underprediction, they provide unsatisfying solutions since the reasoning behind the underlying error has yet to be determined. One previously noted trend was intrinsic clearance-dependent underprediction, highlighting the limitations of current *in vitro* systems. When applying these generated *in vitro* intrinsic clearance values during drug development and making first-in-human dose predictions for new chemical entities though, hepatic clearance is the parameter that must be estimated using a model of hepatic disposition such as the well-stirred model. Here we examine error across hepatic clearance ranges and find a similar hepatic clearance-dependent trend, with high clearance compounds not predicted to be so, demonstrating another gap in the field.

Keywords

Clearance; Hepatic clearance; In Vitro/In Vivo (IVIVC) Correlation(s)

Introduction

Given that many drugs are primarily eliminated by metabolism, the accurate prediction of hepatic clearance (CL_H) is crucial for both evaluating and optimizing new chemical entities as well as estimating first-in-human doses. Successful predictions could help reduce the high attrition[1] associated with the current drug discovery and development process. While allometric scaling may be attempted for prediction, it is more accurate for renally cleared compounds[2,3]. Alternatively, *in vitro* to *in vivo* extrapolation (IVIVE) is commonly used to predict hepatic clearance.

When implementing IVIVE, microsomes or hepatocytes can be used to determine an *in vitro* intrinsic clearance (CL_{int}). The *in vitro* value is then scaled to an *in vivo* CL_{int} using physiologically based parameters such as microsomal protein content/hepatocellularity and

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liver weight. Ultimately the scaled value is input into a model of hepatic disposition such as the well-stirred model to estimate hepatic clearance.

Several publications have examined the accuracy of IVIVE predictions with rat[4–6] and human[7–11] data and further comparisons have been made with data generated in microsomes vs. hepatocytes[12–14]. One review found that on average, human microsomes underpredict clearance by 9 fold, while human hepatocytes underpredict by 3–6 fold[15]. This would be expected given that hepatocytes contain transporters, both phase I and II enzymes, and the natural localization of organelles and cofactors, unlike microsomes. However, examining a larger quantity of data, groups have recently reported the error between the two systems to be more comparable[16,17].

Several hypotheses have been proposed to account for the systematic underprediction observed. Concerns with hepatocyte cryopreservation have been expressed, however studies have shown no significant differences between cryopreserved and fresh cells[4,8,13,18]. Similarly, the impact of donor variability is frequently discussed[18], however both over-and underprediction would be expected[15] and many groups now use pooled microsomes and hepatocytes. Other proposed reasons for the inaccuracy have included differences in liver sample viability and preparation[19], differences in the use of binding terms[7,20], inaccuracies in the measurement of fraction unbound[21,22], the presence of inhibitory long-chain unsaturated fatty acids in microsomal incubations[23,24], ignoring extra-hepatic metabolism[15,25], and simplifying the complex interplay between uptake, metabolism, biliary secretion, and efflux[26].

When exploring reasons for error, groups have also considered clearance-dependent trends. While reducing the clearance of compounds is often a goal to facilitate lower dosage requirements and longer half-lives, measuring low clearance *in vitro* is experimentally challenging. Stringer et al.[27] found that of compounds with an *in vivo* CL_{int} of 1–10 ml/min/kg, only 8% had a measurable value in microsomes and 13% in hepatocytes. Given that enzyme activity begins declining in microsomes after 1 hour of incubation, and cell viability begins decreasing in hepatocytes at 4–6 hours, a low turnover compound can have large uncertainty in its clearance and first dose estimations[28]. A study examining predictions in hepatocyte preparations from four species found poorer accuracy with low clearance compounds[4]. However newer methods such as the hepatocyte relay method[29,30], and hepatocyte culture systems containing flow and/or cell coculture[31,32], have been developed to try to address the error.

At the other extreme, studies have seen an increase in error with increasing *in vivo* CL_{int} in hepatocytes[17,33,34] and microsomes[17] in both human and rat preparations[17]. Suggested reasons for this trend include endogenous cofactor depletion, loss of enzymatic activity, permeability limitation, and rate limiting diffusion through the unstirred water layer[13,33,34,35].

While recognizing CL_{int} trends are important for determining the limitations of the cell systems currently utilized, ultimately, an accurate scaled CL_H is needed for new chemical entities and first-in-human dose predictions. Hepatic clearance is directly related to other

pharmacokinetic parameters including half-life, bioavailability, and exposure, which drive the dosing regimen and efficacy/toxicity profiles of potential compounds. Here we explore the accuracy of hepatic clearance predictions across extraction ratio ranges to determine where the most improvement is needed.

Materials and Methods

The large database, including human (n=101, hepatocytes; n=83, microsomes) and rat (n=128 hepatocytes; n=71 microsomes) values, which was recently compiled by Wood et al. [17], was utilized for this analysis. Hepatic clearance was calculated using the well-stirred model

$$CL_H = \frac{Q_H \cdot \left(\frac{f_{u,B}}{f_{u,inc}}\right) \cdot CL_{int}}{Q_H + \left(\frac{f_{u,B}}{f_{u,inc}}\right) \cdot CL_{int}} \quad (1)$$

where Q_H is liver blood flow and $f_{u,B}$ and $f_{u,inc}$ are fraction unbound in the blood and incubation, respectively. Physiologically based scaling factors, not empirical or regression-based factors were used. Details on the specific values and scaling factors can be found in the original source[17].

The coefficient of determination, R^2, was used to examine the potential of clearance-dependent error. The overall bias in predictions was measured by calculating the average fold error (AFE) and precision was measured with the root mean squared error (RMSE) as follows:

$$\text{AFE} = 10^{\frac{1}{N}\sum \log\left(\frac{observed}{predicted}\right)} \quad (2)$$

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum \left(predicted - observed\right)^2} \quad (3)$$

Additionally, the accuracy of predictions was determined based on whether the predictions fell within 2-fold of the true *in vivo* values, as has been a standard cutoff in previous studies[8,12,36]. As was done by Wood et al.[17], an empirical scaling factor (ESF) was calculated to determine the error associated with each prediction

$$\text{ESF} = \frac{observed\, CL_H}{predicted\, CL_H} \quad (4)$$

The data were divided into difference clearance ranges: low extraction ratio (ER) (<30% of liver blood flow (LBF)), intermediate (30–70%), and high (>70%) where LBF was assumed to be 20.7 and 100 ml/min/kg for human and rat, respectively[17].

Results and Discussion

When working with new chemical entities, CL_H is the parameter that would be used for predicting first-in-human doses and deciding whether to move a compound forward. Therefore, while a compound may have high CL_{int}, which could imply a likely error based on the CL_{int} trend[17,33,34], sizable error may not carry over for CL_H predictions. For instance, considering lorcainide and its human microsome data, its predicted CL_{int} is 449 vs. its observed value of 2559 ml/min/kg leads to a 5.7 fold difference[17]. However, when actually developing this compound, its predicted CL_H would have been 16.3, a value only 1.2 fold off from its 20.0 ml/min/kg observed CL_H. Table 1 highlights different *in vivo* CL_{int} ranges and the number of these compounds in each *in vivo* CL_H ER range. Given that not all low CL_{int} compounds have low *in vivo* CL_H for instance, it is crucial to examine potential CL_H dependent trends too.

When visually examining *in vivo* CL_H vs. ESF in Figure 1, a clearance-dependent trend does not strongly appear and the R^2 values are very low. However, this is expected as any clearance dependency would be suppressed due to the blood flow limitation at higher CL. Despite the potential suppression, the AFE moderately increased from low to high ER in all cases, with the largest AFEs for the human and rat hepatocyte data (Table 2). The lower number of high ER drugs particularly for rats should be noted though. The larger RMSE values for the rat data could be attributed to the higher CL range for the species, and the larger RMSE values noted in every case for the high ER drugs could be due to fewer compounds in this range.

The percentage of predictions falling within two-fold of observed data was generally consistent between ranges (Fig. 2) and surprisingly slightly increased across ER ranges in every system except human hepatocytes (Table 3). There were more underpredictions than overpredictions or accurate predictions in almost every case. While there appears to be consistent percentage accuracy between ER ranges, examining human microsome data for promethazine as an example, it has an accurate (within-two fold) *in vitro* prediction of 9.4 vs. the observed 16, but the prediction would be deemed an intermediate, not high ER compound. Correct determination of extraction ratio is crucial to understand if a compound will be sensitive to changes in protein binding, blood flow, and/or intrinsic clearance[37].

When examining the classification accuracy across ER ranges, similar trends were seen with both human and rat microsomes and hepatocytes (Table 4). The great majority of low ER drugs, >90% in all cases, were accurately predicted to be low ER drugs. However, the majority of intermediate and high ER drugs were also predicted to be low ER drugs. High ER drugs had the poorest accuracy, with ≤25% of high ER drugs predicted to have a high ER.

The predictions in Table 4 were made assuming the well-stirred model. Since it is generally believed that high ER drugs are better described by the dispersion and parallel tube models and it is known that for these latter models predicted ER values will always be greater than those predicted values from the well-stirred model[15], we also did the calculations for the human hepatocyte data using the parallel tube model. In essence, there is no improvement

seen in Table 4 for human hepatocytes. One observed low ER drug is now predicted to be high ER; one observed intermediate drug is now predicted to be high ER; and two observed high ER drugs predicted to be low ER with the well-stirred model are now predicted to be intermediate ER.

Determining the mechanisms behind the likely multifactorial IVIVE error is crucial for moving the field forward and improving the efficiency of the drug discovery and development process. While several reasons have been proposed over the years and new technologies are being created to help combat extrinsic issues such cell viability and enzyme activity loss, systematic underprediction still remains. One phenomenon recently focused upon is CL_{int}-dependent underprediction, highlighting the limitations of current *in vitro* systems. When applying these generated *in vitro* values during drug development though, CL_H is the parameter that must be estimated. Here we show a similar trend of CL_H-dependent underprediction. This underprediction could be due to the CL_{int} error previously noted, errors in protein binding measurements or the understanding of protein binding if protein-facilitated uptake is occurring[38], or yet to be discovered mechanisms. The majority of high ER drugs are not predicted to have high or even intermediate ERs, highlighting a need for improved prediction methodologies especially in this range.

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Abbreviations:

AFE	average fold error
CL_{int}	intrinsic clearance
CL_H	hepatic clearance
ER	extraction ratio
ESF	empirical scaling factor
fu_b	fraction unbound in blood
IVIVE	*in vitro* to *in vivo* extrapolation
LBF	liver blood flow
RMSE	root mean squared error

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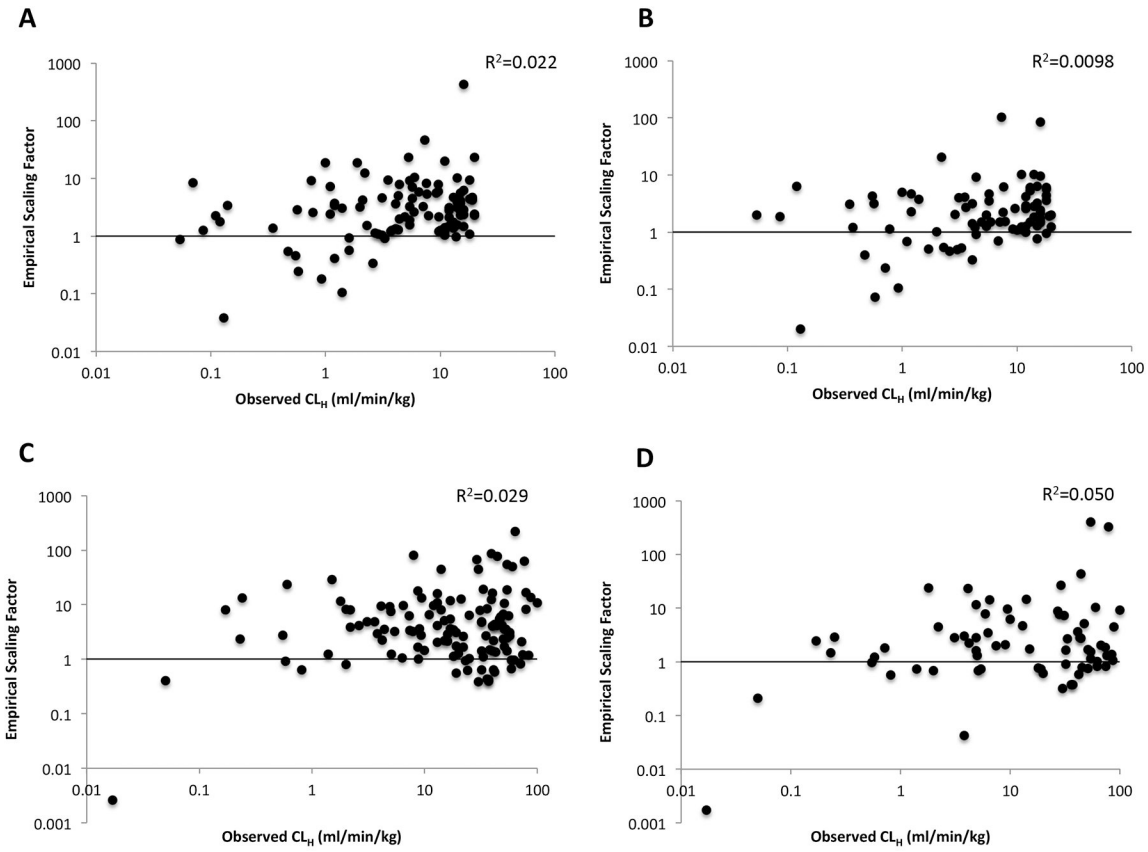

Figure 1:

The relationship between ESF (ratio of observed to predicted hepatic clearance) and observed *in vivo* CL_H for hepatocytes (A and C) and microsomes (B and D) in human (A and B) and rat (C and D).

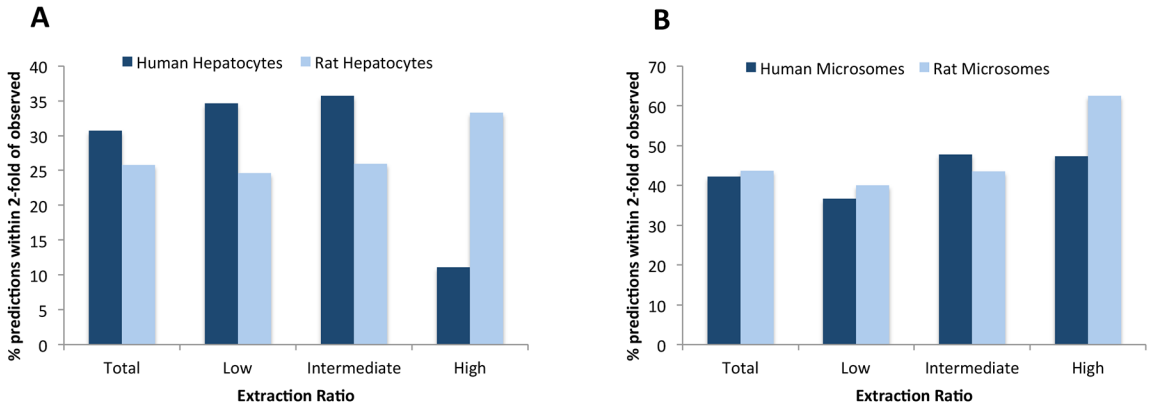

Figure 2:

The percentage of *in vitro* predictions falling within two-fold of observed *in vivo* values grouped by extraction ratio for hepatocytes (A) and microsomes (B).

Table 1:

Observed CL_{int} ranges and the number of compounds with observed low/intermediate/high ERs within those ranges.

	Human Hep.			Human Mic.			Rat Hep.			Rat Mic.		
CL_{int} (mL/min/kg)	**Low ER**	**Inter. ER**	**High ER**	**Low ER**	**Inter. ER**	**High ER**	**Low ER**	**Inter. ER**	**High ER**	**Low ER**	**Inter. ER**	**High ER**
<10–100	45	7	1	34	3	0	13	2	0	10	1	0
100–1000	10	19	11	7	16	11	43	23	1	25	8	1
1000->10,000	0	2	6	0	4	8	13	25	8	5	14	7

Table 2:

The AFE and RMSE for human and rat hepatocytes and microsomes according to level of observed CL_H.

	Human Hepatocytes			Human Microsomes			Rat Hepatocytes			Rat Microsomes		
CL_H (ml/min/kg)	**AFE**	**n**	**RMSE**	**AFE**	**n**	**RMSE**	**AFE**	**n**	**RMSE**	**AFE**	**n**	**RMSE**
All	2.7	101	6.6	2.0	83	6.4	3.8	128	28	2.2	71	29
Low ER	**2.1**	**55**	**2.9**	**1.3**	**41**	**3.0**	**3.6**	**69**	**8.8**	**2.0**	**40**	**16**
Intermediate ER	**3.2**	**28**	**6.7**	**2.7**	**23**	**6.6**	**3.9**	**50**	**35**	**2.2**	**23**	**35**
High ER	**4.8**	**18**	**12**	**2.9**	**19**	**10**	**5.3**	**9**	**61**	**3.8**	**8**	**51**

Table 3:

The percentage of predictions falling within two-fold, below, and above for the Wood et al. (2017) datasets grouped by CL_H range.

	CL_H (ml/min/kg)	All	Low ER	Intermediate ER	High ER
Human Hepatocytes	**% within 2-fold (n)**	**30.7 (31)**	**34.6 (19)**	**35.7 (10)**	**11.1 (2)**
	% below (n)	62.4 (63)	52.7 (29)	64.3 (18)	88.9 (16)
	% above (n)	6.90 (7)	12.7 (7)	0.00 (0)	0.00 (0)
Human Microsomes	**% within 2-fold (n)**	**42.2 (35)**	**36.6 (15)**	**47.8 (11)**	**47.4 (9)**
	% below (n)	48.2 (40)	43.9 (18)	52.2 (12)	52.6 (10)
	% above (n)	9.60 (8)	19.5 (8)	0.00 (0)	0.00 (0)
Rat Hepatocytes	**% within 2-fold (n)**	**25.8 (33)**	**24.6 (17)**	**26.0 (13)**	**33.3 (3)**
	% below (n)	69.5 (89)	72.5 (50)	66.0 (33)	66.7 (6)
	% above (n)	4.70 (6)	2.90 (2)	8.00 (4)	0.00 (0)
Rat Microsomes	**% within 2-fold (n)**	**43.7 (31)**	**40.0 (16)**	**43.5 (10)**	**62.5 (5)**
	% below (n)	47.9 (34)	52.5 (21)	43.5 (10)	37.5 (3)
	% above (n)	8.40 (6)	7.50 (3)	13.0 (3)	0.00 (0)

Table 4:

The number of compounds (%) in each extraction ratio range that have correct classifications.

Human Hepatocytes		**Predicted to be Low ER**	**Predicted to be Intermediate ER**	**Predicted to be High ER**
Well-stirred model	**Observed Low ER**	53 (96.4%)	2 (3.6%)	0 (0.0%)
	Observed Intermediate ER	18 (64.3%)	10 (35.7%)	0 (0.0%)
	Observed High ER	12 (66.7%)	5 (27.8%)	1 (5.5%)
Parallel tube model	**Observed Low ER**	53 (96.4%)	1 (1.8%)	1 (1.8%)
	Observed Intermediate ER	17 (60.7%)	10 (35.7%)	1 (3.6%)
	Observed High ER	10 (55.6%)	7 (38.9%)	1 (5.5%)
Human Microsomes				
Well-stirred model	**Observed Low ER**	37 (90.2%)	4 (9.8%)	0 (0.0%)
	Observed Intermediate ER	14 (60.9%)	9 (39.1%)	0 (0.0%)
	Observed High ER	8 (42.1%)	8 (42.1%)	3 (15.8%)
Rat Hepatocytes				
Well-stirred model	**Observed Low ER**	67 (97.1%)	2 (2.9%)	0 (0.0%)
	Observed Intermediate ER	37 (74.0%)	7 (14.0%)	6 (12.0%)
	Observed High ER	5 (55.6%)	2 (22.2%)	2 (22.2%)
Rat Microsomes				
Well-stirred model	**Observed Low ER**	38 (95.0%)	1 (2.5%)	1 (2.5%)
	Observed Intermediate ER	10 (43.5%)	8 (34.8%)	5 (21.7%)
	Observed High ER	3 (37.5%)	3 (37.5%)	2 (25.0%)