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Prediction of human pharmacokinetics – improving microsome-based predictions of hepatic metabolic clearance

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Abstract

Physiologically based methods generally perform poorly in predicting in-vivo hepatic CL (CL_H) from intrinsic clearance (CL_{int}) in microsomes in-vitro and unbound fraction in blood ($f_{u,bl}$). Various strategies to improve the predictability have been developed, and inclusion of an empirical scaling factor (SF) seems to give the best results. This investigation was undertaken to evaluate this methodology and to find ways to improve it further. The work was based on a diverse data set taken from Ito and Houston (2005). Another objective was to evaluate whether rationalization of CL_H predictions can be made by replacing blood/plasma-concentration ratio (C_{bl}/C_{pl}) measurements with SFs. There were apparently no or weak correlations between prediction errors and lipophilicity, permeability (compounds with low permeability missing in the data set) and main metabolizing CYP450s. The use of CL_{int} class (high/low) and drug class (acid/base/neutral) SFs (the CD-SF method) gives improved and reasonable predictions: 1.3-fold median error (an accurate prediction has a 1-fold error), 76% within 2-fold-error, and a median absolute rank ordering error of 2 for CL_H ($n = 29$). This approach is better than the method with a single SF. Mean ($P < 0.05$) and median errors, fraction within certain error ranges, higher percentage with most accurate predictions, and ranking were all better, and 76% of predictions were more accurate with this new method. Results are particularly good for bases, which generally have higher CL_H and the potential to be incorrectly selected/rejected as candidate drugs. Reasonable predictions of $f_{u,bl}$ can be made from plasma f_u ($f_{u,pl}$) and empirical blood cell binding SFs (B-SFs; 1 for low $f_{u,pl}$ acids; 0.62 for other substances). Mean and median $f_{u,bl}$ prediction errors are negligible. The use of the CD-SF method with predicted $f_{u,bl}$ (the BCD-SF method) also gives improved and reasonable results (1.4-fold median error; 66% within 2-fold-error; median absolute rank ordering error = 1). This new empirical approach seems sufficiently good for use during the early screening; it gives reasonable estimates of CL_H and good ranking, which allows replacement of C_{bl}/C_{pl} measurements by a simple equation.

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Introduction

Appropriate selection of candidate drugs (CDs) requires accurate prediction and ranking of hepatic clearance (CL_H). A 2-fold prediction error (+100% and −50%) has been proposed as acceptable (an accurate prediction has a 1-fold error.) However, an error of this size could lead to incorrect selection or rejection of a CD, misinterpretations and unnecessary studies. It has recently been proposed that maximum acceptable prediction errors for compounds with low CL_H (corresponding to a liver extraction ratio (E_H) of a few %) and high CL_H should be ~50% and ~10%, respectively (Fagerholm 2007). In-vitro intrinsic clearance (CL_{int}) data are commonly used together with a physiologically based in-vitro–in-vivo (PB-IVIV) method to assess the in-vivo stability potential and to predict CL_H. Predictions based on hepatocyte CL_{int} data demonstrate that acceptable predictions and ranking of CL_H are possible (Shibata et al 2000, 2002; Fagerholm 2007). It appears that the methods with the lowest prediction errors also give the best ranking of compounds (Fagerholm 2007). PB-IVIV predictions with microsome CL_{int} data appear less suitable for prediction of human CL_H than hepatocyte CL_{int}-based predictions. Reasons for this include lack of complete sets of membranes to permeate through, cell components to bind to, presence of metabolizing and transporting enzymes, and low metabolic activity (Fagerholm 2007). PB-IVIV predictions

of in-vitro unbound fraction (f_u) in blood ($f_{u,bl}$) and microsome CL_{int} data give on average 5–9-fold underprediction of CL_H, and maximum underpredictions may reach more than 100-fold (Fagerholm 2007). The potential consequence of such great underpredictions of CL_H (and overprediction of exposures, half-life and safety limits) is failure to reach the desired effect profiles. Predictions may be improved by the use of an empirical scaling factor (SF), inclusion of non-specific microsomal binding, correction for predicted/observed CL_{int} ratio in the rat, or ignoring $f_{u,bl}$ for basic and neutral compounds (Obach 1999; Ito & Houston 2005; Fagerholm 2007). With the use of an empirical SF (in that particular case 6.2; Ito & Houston 2005), the average and maximum prediction errors are still 2- and 6-fold, respectively, and ~70% of predictions are within 2-fold (n = 52) (Ito & Houston 2005; Fagerholm 2007). The approach presented by Ito and Houston (2005) seems to be the one with the overall best performance of various microsome-based methods (Fagerholm 2007). Corresponding numbers for the average and maxium prediction errors when the f_u in the incubation mixture ($f_{u,inc}$) is considered are 3- and 11-fold, respectively, and ~50% of predictions are within 2-fold (Obach 1999; Fagerholm 2007). The rationale for ignoring $f_{u,bl}$ and incorporating $f_{u,inc}$ does not appear physiologically relevant, however. This is because $f_{u,inc}$ is a determinant for both the CL_{int} and the volume of distribution of the incubation medium. The extent of binding to microsomes and hepatocytes could differ by up to at least an order of magnitude (whole cells have additional binding sites) (Austin et al 2005), and the bound fraction in cells could be bound to the metabolizing site (and be degraded) or dissociate to be a part of the unbound fraction.

The main objectives of the current study were to evaluate microsome-based PB-IVIV predictions of in-vivo CL_{int} and CL_H and to find possible ways to improve predictions further. The work was based on a data set taken from Ito and Houston (2005), which comprised 52 acids, bases and neutrals with a wide range of CL_{int} and physicochemical properties. One aim was to evaluate whether CL_H predictions can be rationalized by replacing blood/plasma-concentration ratio (C_{bl}/C_{pl}) measurements with SFs.

Methods

Predicted and observed data for microsome CL_{int} presented in Ito and Houston (2005) were used. Relationships were investigated between prediction errors for CL_{int} and lipophilicity (log D at pH 7.4; log $D_{7.4}$), permeability (P_e), main metabolizing enzymes, drug class (acids/bases/neutrals) and CL_{int} class (low/intermediate/high). Log $D_{7.4}$ and in-vitro artificial membrane P_e data were taken from Kasim et al (2004), Willmann et al (2004), Doran et al (2005) and Cerep website. A data set with substrates for cytochrome P450s for CYP1A2, 2C19, 2D6 and 3A4, and UDP-glucuronosyltransferases (UGTs) (n = 31 compounds) presented in McGinnity et al (2004) was used for further investigations of potential relationships between main metabolizing enzymes and prediction errors (with human hepatocytes). The intention was to consider such relationships (if found) in the predictions of CL_H.

In-vivo CL_H was predicted using available in-vitro $f_{u,bl}$ and CL_{int} data, and the well-stirred liver extraction model. The blood flow rate was set to 1.5 L min^{-1} (an average estimate for a 70 kg person). Observed (calculated) in-vivo CL_{int} estimates presented by Ito and Houston (2005) were used for validation of predictions. Data for 10 acidic compounds, 9 basic compounds and 10 neutral compounds for which data on CL_{int}, $f_{u,pl}$ and $f_{u,bl}$ were available were used in this part of the analysis. CL_{int} values were multiplied by various SFs: 1; no SF, SF = 6.2 (as Ito & Houston 2005) or SF obtained from established relationships, for use in the well-stirred model equation.

I have also evaluated whether $f_{u,bl}$ can be estimated from plasma f_u ($f_{u,pl}$) without measuring and using C_{bl}/C_{pl} data. Drug characteristics/classes were also considered.

Statistical analysis

Student's paired *t*-test was used to assess statistical differences between methods. Other comparisons include ranking, fraction with less than 1.3- and 2-fold prediction errors (an accurate prediction has a 1-fold error), and % of predictions that are superior to the SF = 6.2 (reference) method. The method without SF was not included in this analysis.

Results and Discussion

Relationship between CL_{int} prediction errors and drug characteristics

There were no apparent correlations or relationships between CL_{int} underprediction error and log $D_{7.4}$ (log $D_{7.4}$ range −1.5 to 3.7) ($r^2 = 0.03$; n = 32), in-vitro artificial membrane P_e (10-fold range) ($r^2 = 0.16$; n = 20) or metabolizing enzymes. However, the data set did not include compounds with low P_e and was limited to 17 substrates for CYPs 1A2, 2C9, 2C19, 2D6 and 3A4. Re-evaluation of the data set presented in McGinnity et al (2004) (substrates for CYPs 1A2, 2C19, 2D6 and 3A4, and UGTs; n = 31) showed no such trend between human hepatocyte CL_{int} prediction errors and main metabolic enzymes.

Re-evaluation of the data in Ito & Houston (2005) shows that the mean underprediction errors for acidic, basic and neutral compounds are 10.5 (median 7.4; maximum 36-fold), 15 (15; 38-fold) and 15 (6.7; 106-fold), respectively. Thus, it appears that there is a relationship between CL_{int} prediction error and drug class (smaller error for acids). There was also an apparent relationship between CL_{int} and CL_{int} prediction error. Prediction errors for acidic, basic and neutral compounds that had an observed in-vivo CL_{int} below 3500 mL min^{-1} (< 50 mL min^{-1} kg^{-1}) were generally 3–4 times lower than those for compounds with CL_{int} above 3500 mL min^{-1}. Apparently, microsomes have a reduced capacity to metabolize the metabolically most unstable compounds.

Estimated CD-SFs (CL_{int} and drug class scaling factors; see below) for low-CL_{int} acids, neutrals and bases were 4.7, 3.4 and 3.2, respectively and for high CL_{int} acids, neutrals and bases were 13, 8.1 and 15, respectively.

Predictions of CL_{int} considering differences in drug characteristics

By using the median errors for acids, bases and neutrals as drug class SFs (D-SF method), the mean errors for CL_{int} were reduced to 2.8-, 2.4- and 3.2-fold, respectively, and the maximum errors to 5.4-, 10- and 16-fold, respectively. The percentage of errors that were within 2-fold were 50, 67 and 47% for these classes, respectively. Apparently, the improvement was most pronounced for the basic compounds (which generally have higher CL_{int}). Corresponding estimates for bases obtained with SF = 6.2 (Ito & Houston 2005) were 2.9-fold (mean error) and 6.1-fold (maximum error), and 27% of errors within 2-fold. Changes for acids and neutrals were negligible, which is not surprising as their D-SFs are close to 6.2.

The CD-SF approach, which involved application of both CL_{int} class SF (C-SF; low/high CL_{int}) and drug-class SF (D-SF; acids/bases/neutrals), resulted in further improvements in prediction of CL_{int}. Mean errors for in-vivo CL_{int} for acids, bases and neutrals were 2.6-, 1.9- ($P < 0.05$) and 4.0-fold, respectively; maximum errors were 7.7-, 4.1- and 31-fold, respectively; 50, 67 and 53% of the errors were within 2-fold, respectively.

Prediction of $f_{u,bl}$ using $f_{u,pl}$ and empirical scaling factors

There was a strong correlation/relationship between $f_{u,bl}$ and $f_{u,pl}$ ($r^2 = 0.95$; n = 58), and it seems that the $f_{u,bl}$ can be quite well predicted using $f_{u,pl}$ data and blood cell binding SF (B-SF): $f_{u,bl} \approx \text{B-SF} \times f_{u,pl}$.

The B-SF for acids with very low $f_{u,pl}$ (< 0.01) was estimated to be 1 (they do not appear to bind significantly to red blood cells) and for other compounds to be 0.62 (~ the divine proportion − 1). Figure 1 demonstrates the predictability for 17 acidic, 14 neutral and 27 basic compounds. The $f_{u,bl}$ data were calculated (using the equation below) from $f_{u,pl}$ and C_{bl}/C_{pl} data taken from Sawada et al (1984, 1985), Obach (1999), Poulin & Theil (2002), Shibata et al (2002) and Fagerholm & Björnsson (2005). The haematocrit (Hct) is 0.44 in man (Davies & Morris 1993).

$$f_{u,bl} = ([1 - Hct] \cdot f_{u,pl}) / (C_{bl}/C_{pl}).$$

This equation assumes that the f_u in red blood cells belongs to the cell compartment (and not to the plasma compartment) and is not directly available for hepatic absorption and disposition. Forty-one, 64, 79 and 93% of predictions had less than 1.1-, less than 1.2-, less than 1.3- and less than 1.5-fold errors, respectively. The mean, median and maximum errors were 1.2-, 1.1- and 1.7-fold, respectively. There was no apparent relationship between drug class (acid/base/neutral) or lipophilicity (log $D_{7.4}$) and prediction error. The $f_{u,bl}$ for acids with low $f_{u,pl}$ was generally more well predicted (78% within 1.07-fold error). Errors are considerably smaller than for the microsome-based in-vivo CL_{int} predictions (see above). It is therefore suggested that this simple approach can replace in-vitro C_{bl}/C_{pl} measurements (commonly used to estimate $f_{u,bl}$) during early screening. Instead, C_{bl}/C_{pl} could be measured in-vitro at a later stage.

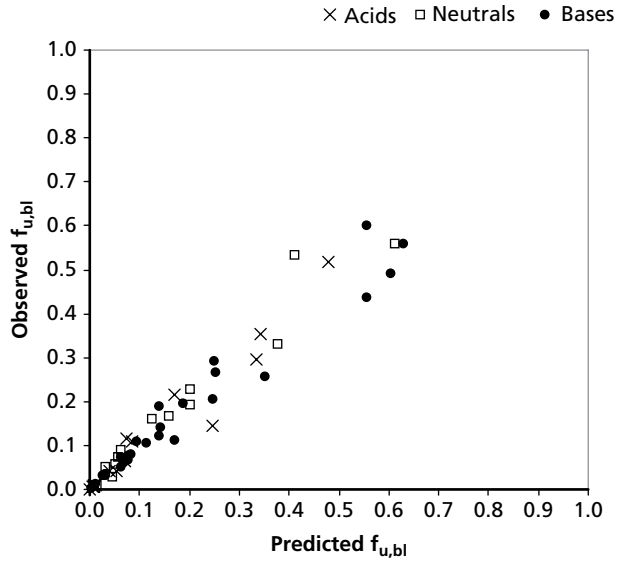

Figure 1 The relationship between predicted and observed unbound fraction in blood ($f_{u,bl}$) for 17 acidic, 14 neutral and 27 basic compounds in humans. For highly bound acids $f_{u,bl}$ was set to equal the unbound fraction in plasma ($f_{u,pl}$); for other compounds $f_{u,bl}$ was set to equal $0.62 \times f_{u,pl}$. $F_{u,pl}$ and blood/plasma concentratrion data (C_{bl}/C_{pl}) used for the estimation of $f_{u,bl}$ (using $f_{u,bl} = ([1 - \text{haematocrit}] \times f_{u,pl}) / [C_{bl}/C_{pl}]$) were collected from Sawada et al (1984 & 1985), Obach (1999), Poulin & Theil (2002), Shibata et al (2002), and Fagerholm & Björnsson (2005).

Different approaches for prediction of CL_H

Five different microsome-based methods to predict CL_H estimates were investigated and compared:

- without SF, with measured $f_{u,bl}$
- SF = 6.2, with measured $f_{u,bl}$
- D-SF method, with measured $f_{u,bl}$
- CD-SF method, with measured $f_{u,bl}$
- BCD-SF method (CD-SF method with predicted $f_{u,bl}$).

The results are presented in Table 1 and Figures 2 and 3. The method without SF performed least well, and the CD-SF method with measured $f_{u,bl}$ data performed the best. Except for a one-third higher maximum error, this new CD-SF approach was ~20 to ~80% better than using SF = 6.2 for all compounds in several quality measurements. The CD-SF method was superior to the SF = 6.2 method for 76% of the compounds. With this new approach it was possible to reduce (compared with the SF = 6.2 method) the mean and median errors from 2.2- and 1.6-fold, respectively, to 1.8-fold ($P < 0.05$) and 1.3-fold, respectively. The fraction of compounds with more than 1.3-fold and more than 2-fold errors was reduced from 69% and 38%, respectively, to 52% and 24%, respectively. The rank ordering also improved. The mean, median and maximum absolute ranking errors were 1.8, 2 and 6, respectively. The CD-SF method appears to be particularly good for basic compounds: mean, median and maximum errors were 1.2-fold ($P < 0.05$), 1.1-fold and 1.9-fold,

Table 1 Performance of different microsome-based methods for prediction of hepatic clearance (CL_H) (n = 29 compounds)

Method[a]	Errors					Absolute ranking errors			% of predictions better than SF = 6.2[e]
	Mean[a]	Median[a]	Max[a]	% < 1.3[b]	% < 2[b]	Mean	Max	% ≤ 1 placings	
No SF[c]	7.8	5.2	36	3	21	2.6	8	38	21
SF = 6.2	2.2*	1.6	5.8	31	62	2.6	8	38	–
D-SF	2.0	1.4	5.4	41	76	2.0	5	45	59
CD-SF	1.8*	1.3	7.7	48	76	1.8	6	45	76
BCD-SF[d]	2.2	1.4	8.0	38	66	1.9	6	52	52

[a]SF; scaling factor, C-SF; CL_{int} class SF (low/high); D-SF; drug class SF (acid/base/neutral); CD-SF; combined C-SF and D-SF, BCD-SF; combined CD-SF and predicted unbound blood binding.
[b]fold-error; 2-fold error = +100% or −50%; an accurate prediction has a 1-fold error.
[c]not included in the statistical analysis.
[d]predicted fraction unbound in blood ($f_{u,bl}$) (measured $f_{u,bl}$ for other methods).
[e]% of predictions that are superior to the SF = 6.2 method.
*$P < 0.05$.

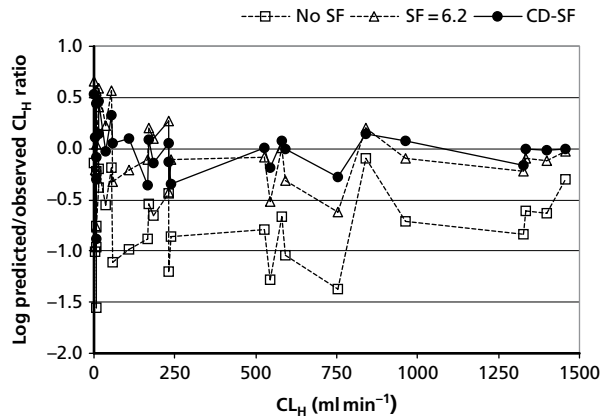

Figure 2 Measured hepatic clearance (CL_H) vs log predicted/observed CL_H ratio for 29 acidic, neutral and basic compounds obtained with three different prediction methods: without scaling factor (SF), SF = 6.2, and CD-SF (scaling factor based on drug class and intrinsic clearance (CL_{int}) classes). Measured data for unbound fraction in blood ($f_{u,bl}$) were used. Hepatic blood flow rate was set at 1.5 L min^{-1}; CL_{int} data used for the calculations were taken from Ito & Houston (2005).

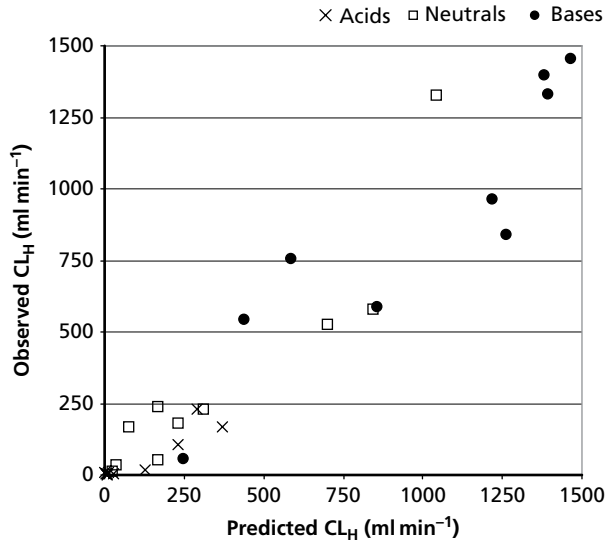

Figure 3 Predicted vs observed hepatic clearance (CL_H) in humans for 29 acidic, neutral and basic compounds. The predicted CL_H was estimated using the BCD-SF method (scaling factor based on drug class (acidic/neutral/base), intrinsic clearance (high/low) and predicted unbound fraction in blood.

respectively. Corresponding estimates for the SF = 6.2 method are 1.6-, 2.0-, and 4.1-fold, respectively (56% within 2-fold error). This improvement is particularly important because bases in general, and those in this data set in particular, have comparably high CL_{int}, $f_{u,bl}$ and CL_H values, and there is therefore greater potential for them to be incorrectly selected/rejected as CDs. E_H values for acids, bases and neutrals in the data set averaged 0.1, 0.6 and 0.3, respectively. The performance of this CD-SF method appears only slightly inferior to a similar hepatocyte (cryopreserved) CL_{int}-based method with a single SF (1.4-, 1.3- and 3.9-fold mean, median and maximum errors, respectively; 93% within 2-fold error) (Shibata et al 2002; Fagerholm 2007).

The performance of the BCD-SF method (CD-SF method with predicted $f_{u,bl}$) was numerically similar to or slightly better than that of the SF-based method with measured $f_{u,bl}$. The mean and median errors were 2.2- and 1.4-fold, respectively and and the fraction of compounds with more than 1.3-fold and more than 2-fold error were 62% and 34%, respectively. This approach gave the best rank ordering of all the tested methods (mean, median and maximum absolute ranking errors were 1.9, 1.0 and 6.0, respectively). Figure 3 shows the performance of this method. The acidic and neutral compounds in the data set generally have sufficiently low predicted and observed E_H to ensure high oral bioavailability. Predictions for bases with high CL_H also appear sufficiently good for reasonable CD selection/rejection. Thus, this

comparably simple approach without C_{bl}/C_{pl} measurements appears to be suitable for the early screening process.

Further improvements are also potentially achievable. These include the use of a new liver extraction model that assumes intermediate mixing/convection of hepatic blood, a new $f_{u,bl}$ estimation method which assumes that the f_u within red blood cells is not directly available for hepatic uptake and disposition (as assumed in the traditional method), and consideration of the different hepatic transit times for blood components and unbound and bound fractions (Fagerholm 2007; Fagerholm unpublished).

Conclusion

It was possible to improve microsome CL_{int}-based predictions of CL_H. The use of CL_{int} class (high/low) and drug class (acid/base/neutral) SFs (the CD-SF method) gives significant improvement and reasonable predictions and rank ordering, especially for basic compounds. A simple approach to predict $f_{u,bl}$ from $f_{u,pl}$ and blood binding SFs (without C_{bl}/C_{pl} measurements) was developed. This BCD-SF method – a combination of the $f_{u,bl}$ prediction method and the CD-SF method –gives comparably good predictions and rank ordering of CL_H. This new, simple and empirical method has the potential to improve CD screening, lead optimization and CD selection/rejection in terms of quality, time and costs.

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