The Prediction of Drug Metabolism, Tissue Distribution, and Bioavailability of 50 Structurally Diverse Compounds in Rat Using Mechanism-Based Absorption, Distribution, and Metabolism Prediction Tools

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ABSTRACT:
The aim of this study was to assess a physiologically based modeling approach for predicting drug metabolism, tissue distribution, and bioavailability in rat for a structurally diverse set of neutral and moderate-to-strong basic compounds \((n = 50)\). Hepatic blood clearance \((\text{CL}_{\text{h}})\) was projected using microsomal data and shown to be well predicted, irrespective of the type of hepatic extraction model \((80\% \text{ within 2-fold})\). Best predictions of \(\text{CL}_{\text{h}}\) were obtained disregarding both plasma and microsomal protein binding, whereas strong bias was seen using either blood binding only or both plasma and microsomal protein binding. Two mechanistic tissue composition-based equations were evaluated for predicting volume of distribution \((V_{\text{dss}})\) and tissue-to-plasma partitioning \((P_{\text{tp}})\). A first approach, which accounted for ionic interactions with acidic phospholipids, resulted in accurate predictions of \(V_{\text{dss}}\) \((80\% \text{ within 2-fold})\). In contrast, a second approach, which disregarded ionic interactions, was a poor predictor of \(V_{\text{dss}}\) \((60\% \text{ within 2-fold})\). The first approach also yielded accurate predictions of \(P_{\text{tp}}\) in muscle, heart, and kidney \((80\% \text{ within 3-fold})\), whereas in lung, liver, and brain, predictions ranged from 47% to 62% within 3-fold. Using the second approach, \(P_{\text{tp}}\) prediction accuracy in muscle, heart, and kidney was on average 70% within 3-fold, and ranged from 24% to 54% in all other tissues. Combining all methods for predicting \(V_{\text{dss}}\) and \(\text{CL}_{\text{h}}\) resulted in accurate predictions of the in vivo half-life \((70\% \text{ within 2-fold})\). Oral bioavailability was well predicted using \(\text{CL}_{\text{n}}\) data and Gastroplus Software \((80\% \text{ within 2-fold})\). These results illustrate that physiologically based prediction tools can provide accurate predictions of rat pharmacokinetics.

Obtaining rapid information regarding the pharmacokinetics (PK) of new drug candidates can be a bottleneck in early drug discovery. Considerable resources are required to assess the PK properties of potential drug candidates in vivo in animals. To optimize the use of such in vivo testing, there has been a growing interest in predicting the PK behavior of drug candidates as early as possible (Norris et al., 2000; van de Waterbeemd and Gifford, 2003). If sufficiently reliable, such simulations could also help to select the best candidates for development and to reject those with a low probability of success.

The characterization of a drug’s PK requires elucidation of each of the coincident processes of absorption, distribution, metabolism, and elimination (ADME). A large number of methodologies have been established for this purpose, including empirical and physiologically based methods (Boxenbaum and Ronfeld, 1983; Theil et al., 2003). Until recently, PK prediction has been predominantly descriptive, using empirical methods. Although in some cases these methods give good predictions, their physiological basis is low and inaccurate results can be obtained, in particular when there are large interspecies differences in metabolic clearance (Lave et al., 1995; Zuegge et al., 2001; Shiran et al., 2006). To improve on these predictive approaches, it is necessary to explore more mechanistic methods, such as physiologically based pharmacokinetic (PBPK) models (Theil et al., 2003). PBPK models are derived from the knowledge of the underlying physiology of the species and the behavior of drugs within this system. As a result, PBPK models are generic and can be applied to a wide array of structurally diverse compounds (Poulin and Theil, 2002b; Nestorov, 2003). Physiologically based models can be applied for estimation of single in vivo PK parameters (e.g., metabolic clear-

**ABBREVIATIONS:** PK, pharmacokinetic(s); ADME, absorption, distribution, metabolism, and elimination; AUC, area under the curve; AUMC, area under the first moment curve; \(\text{CL}_{\text{h}}\), hepatic blood clearance; \(\text{CL}_{\text{plasma}}\), hepatic plasma clearance; \(\text{CL}_{\text{int}}\), intrinsic clearance; \(\text{CL}_{\text{oral}}\), oral blood clearance; \(\text{CL}_{\text{repl}}\), renal clearance; \(\text{CL}_{\text{tot}}\), total blood clearance; \(E/P\), erythrocyte-to-plasma concentration ratio; \(F\), absolute oral bioavailability; \(F_{\text{oral}}\), fraction of dose that has reached the portal vein; \(f_{\text{unb}}\), fraction unbound in microsomes; \(f_{\text{pl}}\), fraction unbound in plasma; \(Ht\), hematocrit content in blood; PBPK, physiologically based pharmacokinetics; \(D_{\text{vow}}\), vegetable oil:buffer partition coefficient of both the non-ionized and ionized species at pH 7.4; \(P_{\text{ovw}}\), n-octanol:water partition coefficient of the non-ionized species; \(P_{\text{tp}}\), tissue-to-plasma partition coefficient; \(P_{\text{vow}}\), vegetable oil:water partition coefficient of the non-ionized species; \(Q_{\text{h}}\), hepatic blood flow; \(R_{\text{pl}}\), blood-to-plasma concentration ratio; \(S_{\text{w}}\), aqueous solubility; \(V_{\text{dss}}\), volume of distribution at steady state.
In vivo pharmacokinetic data of the 50 compounds studied in rat are summarized in Table 2. Plasma concentration versus time profiles were obtained from at least three male rats for each route of administration. Test compound was given intravenously by bolus injection through a catheter implanted into the jugular vein and orally by gavage. Noncompartmental analysis was performed using WinNonLin version 4.01 (Pharsight, Mountain View, CA) to calculate the total blood clearance (CLint) from the relationship $CL_{int} = Dose/AUC$, and $V_{ss}$ was determined from $CL_{int} = Dose \cdot AUMC/(AUC)^2$. Absolute oral bioavailability ($F$) was calculated as the ratio of dose-normalized AUC after oral and intravenous administration using the mean of individual AUCs. Experimental $P_{ty}$ values have been determined under in vivo conditions (single oral or intravenous dose) as the ratio of the AUC (calculated over a minimum of five time points), assuming pseudo-equilibrium.

### Prediction of Hepatic Blood Clearance (CL$_h$) from In Vitro $t_{1/2}$ Data

The in vitro $t_{1/2}$ of each compound was determined in pooled male rat liver microsomes (protein concentration 1 mg/ml, substrate concentration 5 μM) using a typical screening assay as described previously (Kantharaj et al., 2003). Conversion of in vitro $t_{1/2}$ data (minutes) to the intrinsic clearance (CL$_{int}$, ml/min/kg) was performed as follows:

$$CL_h = \frac{0.693}{t_{1/2}^{\text{in vitro}}} \cdot \text{ml incubation} \cdot \frac{45 \text{ mg microsomes}}{\text{g liver}} \cdot \frac{40 \text{ g liver}}{\text{kg b.wt.}}$$

where $t_{1/2}^{\text{in vitro}}$ is the biological half-life of each compound in microsomes, and $40 \text{ g liver/kg b.wt.}$ is a typical rat liver concentration. The scale-up factors from protein to grams of liver and from grams of liver to kilograms body weight (kg b.wt.) are $45 \text{ mg/g liver}$ (Houston, 1994) and $40 \text{ g liver/kg b.wt.}$ (Narimoto et al., 2001), respectively. Conversion of CL$_{int}$ to CL$_h$ involved the use of either the well stirred model (eq. 2) or the parallel tube model (eq. 3) (Wilkinson, 1987):

$$CL_h = Q_h \cdot \left( f_{um} + \frac{CL_{int} f_{um}}{Q_h} \right) \cdot \frac{CL_{int} f_{um}}{Q_h}$$

$$CL_h = Q_h \cdot \left( 1 - \frac{1}{1 + f_{um} Q_h CL_{int}} \right)$$

where $Q_h$ is the hepatic blood flow ($55.2 \text{ ml/min/kg}$) (Davies and Morris, 1993) and $f_{um}$ is the fraction unbound in microsomes. Both models were examined using three different variations: 1) assuming that no binding parameter has an impact on clearance ($f_{um} = f_{um} = 1$); 2) incorporating binding to blood constituents only ($f_{um} = 1$); and 3) incorporating both blood and microsomal binding. For microsomal protein binding, $f_{um}$ in the incubation mixture at 1 mg/ml microsomal protein was calculated by eq. 4 (Austin et al., 2002):

$$\log(1 - f_{um} Q_h) = 0.53 \log P_{ty} - 1.42$$

### Calculation of CL$_h$ from In Vivo Data

After oral dosing, CL$_h$ values were determined from eq. 5 by use of the apparent oral blood clearance ($CL_{oral}$) values (Iwatsubo et al., 1997). For all calculations, renal clearance (CLR) was considered to be negligible and the fraction of dose that has reached the portal vein ($F_{PD}$) was taken to be 1. For compounds INJ5, INJ115, INJ16, INJ27, and INJ28, where only CL$_{int}$ was available, CL$_h$ was assumed to be equal to CL$_{oral}$:

$$CL_h = CL_{oral} - CL_{int}$$

$$CL_h = \frac{CL_{oral} \cdot PD_h - CL_{int}}{1 + CL_{oral} / Q_h}$$

### Prediction of $P_{ty}$ and V$_{ss}$ According to Rodgers et al. (2005a) (Method Vd1)

A detailed derivation of the equations for predicting the $P_{ty}$ values of moderate-to-strong bases is provided in the article by Rodgers et al. (2005a). In brief, it is assumed that electrostatic interactions of moderate-to-strong bases with acidic phospholipids is the primary factor controlling the distribution of these drugs within the body. In addition, moderate-to-strong bases also dissolve in tissue water, and the un-ionized form can partition into neutral lipids and neutral phospholipids, so that $P_{ty}$ of unbound drug ($P_{tyu}$) can be calculated using the following equation:

$$P_{tyu} = \frac{V_{ss}}{1 + 10^{10^{-6} - pK_{um} \cdot \nu_{wu}}} + \frac{10^{10^{-6} - \nu_{wu} \cdot K_{um} \cdot \alpha \cdot P_{tyu} \cdot \nu_{wu}}}{1 + 10^{10^{-6} - \nu_{wu}}} + \frac{P_{ty} + (0.3P_{ty} + 0.7)}{1 + 10^{10^{-6} - \nu_{wu}}}$$

### Materials and Methods

#### Sources of In Vitro, In Silico and In Vivo Parameters

The compounds used in the analysis were taken from those brought into early development at Johnson & Johnson Pharmaceutical Research and Development (Beershe, Belgium). Compounds ($n = 50$) were selected based on the availability of historical data on experimental studies in vitro data: fraction unbound in plasma ($f_{um}$), dissociation constant ($pK_a$), n-octanol:water partition coefficient of the non-ionized species (log $P_{um}$), aqueous solubility ($S_a$) at defined pH, in vitro half-life in liver microsomes (in vitro $t_{1/2}$), and blood-to-plasma concentration ratio ($R_{bio}$) (Table 1). All experimental values of $f_{um}$ were obtained using DIANORM equilibrium dialysis (Pacifici and Viant, 1992). Solubility data were obtained at 20°C after overnight incubation of drug powder in water or dosing vehicle at defined pH conditions between 4.0 and 7.5. All structure-based predictions of log $P_{um}$, $pK_a$, and jejunal permeability were obtained using ADMET Predictor version 1.3.2 (Simulations Plus Inc., Lancaster, CA).
where \( V \) is the fractional tissue volume content of extracellular water (EW), intracellular water (IW), neutral lipids (NL), and neutral phospholipids (NP). \( [AP] \) is concentration of acidic phospholipids in tissue. Values for \( pH_p \) (pH of the plasma) and \( pH_{IW} \) were taken to be 7.4, and 7.0, respectively. \( pK_a \) represents the dissociation constant of the monoprotic base (cutoff in this study, \( pK_a \approx 6.8 \)). \( pK_w \) was calculated from \( pK_a \) (as follows (Leo et al., 1971)):

\[
\log pK_w = 1.15 \log pK_a - 1.35
\]

\( K_a \) is the association constant of the compound with the acidic phospholipids, and is calculated from eqs. 8 to 10:

\[
P_{\text{predic}} = \frac{1 + 10^{pK_a - pH_p}}{1 + 10^{pK_a - pH_w}} \cdot \frac{V_{IW,BC}}{(P \cdot V_{SN,BC} + (0.35 + 0.7) \cdot V_{SN,BC})} \cdot \left( \frac{1 + 10^{pK_a - pH_p}}{[AP]_{BC} \cdot 10^{pK_a - pH_p}} \right)
\]

\[
P_{\text{predic}} = E \cdot P_{tpu}
\]

\( E = (R_h - (1 - Ht)) / Ht \)

where BC is the red blood cell, E:P the erythrocyte-to-plasma concentration ratio, and Ht the hematocrit content in blood (assumed to be 45%). Values for \( pH_p \), \( pH_{IW} \), and \( pH_{BC} \) were taken to be 7.4, 7.0, and 7.22, respectively. In eq.

\[
K_a = \frac{pK_w}{pH_{IW} - pH_{WS}}
\]
8, $P$ was taken to be the $P_{\text{cumeq}}$. It is important to note that the use of $P_{\text{cumeq}}$ in eq. 8 yielded negative $K_{\text{dss}}$ values (and thus negative $P_{\text{cumeq}}$) for compounds having both a low $R_0$ value and a high $P_{\text{cumeq}}$ value. This problem was overcome by using $P_{\text{cumeq}}$ instead of $P_{\text{cumeq}}$ for calculation of $K_{\text{dss}}$ as well as for calculation of $P_{\text{cumeq}}$ of both adipose and nonadipose tissues (eq. 6).

For calculation of $V_{\text{dss}}$, each predicted $P_{\text{cumeq}}$ value for bone, brain, intestine, heart, kidney, liver, lung, muscle, skin, spleen, and adipose tissue was multiplied by $R_0$ to obtain the corresponding $P_{\text{cumeq}}$ value for each tissue. $V_{\text{dss}}$ equals the plasma volume ($V_p$) in addition to the sum of each $P_{\text{cumeq}}$ multiplied with its respective tissue volume (Sawada et al., 1984):

$$V_{\text{dss}} = (\Sigma V_{\text{p}}) + (V_{\text{cumeq}} E:P) + V_p \quad (11)$$

where $V$ is the fractional body volume in l/kg tissue (t), erythrocyte (e), and plasma (p). All tissue-specific input information on $V_c$, $V_t$, and $V_p$ was obtained from the literature (Rodgers et al., 2005a). It is important to note that the use of $P_{\text{cumeq}}$ in both eqs. 6 and 8 was not found to affect the overall $V_{\text{dss}}$ prediction accuracy compared with Rodgers et al. (2005a), who have used $P_{\text{cumeq}}$ and $D_{\text{cumeq}}$ (i.e., vegetable oil/buffer partition coefficient of both the non-ionized and ionized species at pH 7.4) as input parameters for calculation of nonadipose and adipose $P_{\text{cumeq}}$ respectively (data not shown).

**Prediction of $P_{\text{cumeq}}$ and $V_{\text{dss}}$ According to Poulin and Theil (Method Vd2)**

(Poulin and Theil, 2000; Poulin et al., 2001). A detailed derivation of the tissue composition-based equations for predicting the $P_{\text{cumeq}}$ in adipose and
nonadipose tissue is provided by Poulin and Theil (Poulin and Theil, 2000; Poulin et al., 2001). In brief, it is assumed that a drug distributes homogeneously into each tissue (and plasma) by passive diffusion. Consequently, the drug partitions between lipids and water and binds reversibly to common proteins present in plasma and tissue interstitial space:

\[
P_{tp\,\text{nonadipose}} = \frac{P_{ow}(V_{NLt} + 0.3 \, V_{PHt}) + ([V_{Wt} + 0.7 \, V_{PHt}])}{[P_{ow}(V_{NLp} + 0.3 \, V_{PHp}) + ([V_{Wp} + 0.7 \, V_{PHp}])] / RA}
\]

where \( V \) is fractional tissue (t) or plasma (p) volume content of neutral lipids (NL), phospholipids (PH), and water (W). The data on rat tissue volumes was obtained from the literature (Poulin and Theil, 2000; Poulin et al., 2001). To obtain \( D_{\text{vow}} \), log \( P_{\text{vow}} \) was first estimated from data on log \( P_{\text{pow}} \) using eq. 7, and log \( P_{\text{vow}} \) was subsequently converted to log \( D_{\text{vow}} \) using the Henderson-Hasselbalch equations (Poulin and Theil, 2002a). The values of the fraction unbound in tissue (\( f_{ut} \)) were estimated from data on \( f_{wm} \), as follows:

\[
f_{ut} = 1/(1 + (((1 - f_{wm}))/f_{wm}) / RA)
\]

where RA is the ratio of albumin concentration found in tissue over plasma. For adipose tissue, RA was set to 0, whereas for nonadipose tissue, RA was set to 0.50 (Poulin and Theil, 2002a).

For calculation of \( V_{\text{dss}} \), \( P_{\text{o}} \) values were determined in bone, brain, intestine, heart, kidney, liver, lung, muscle, skin, spleen, and adipose using eqs. 12 and 13, and subsequently inserted into eq. 11 as described under Method Vd1.

**Prediction of in Vivo Half-Life (in Vivo \( t_{1/2} \):** Both method Vd1 and method Vd2 for predicting \( V_{\text{dss}} \) were combined with the predicted hepatic plasma clearance values (CL \( _{\text{h,plasma}} = \frac{\text{CL}_{\text{h}} \cdot R_{\text{f}}}{\text{Q}_{\text{h}}}) \) from both the well stirred (Fig. 1, A–C) and parallel tube models (Fig. 1, D–F) to generate predictions of in vivo \( t_{1/2} \) using the following formula:

\[
\text{Predicted in vivo } t_{1/2} = 0.693 \times \frac{\text{Predicted } V_{\text{dss}}}{\text{Predicted } \text{CL}_{\text{h,plasma}}}
\]

**Prediction of \( F \):** The first method (method F1) used for predicting \( F \) was based on eq. 16 and only accounts for hepatic first pass metabolism and thus accounts neither for potential limitations on absorption nor potential first pass metabolism by the gut (i.e., \( F_{\text{Dp}} = 1 \)). CL \( _{\text{h}} \) values were obtained using the well stirred model, assuming that \( f_{\text{ut}} = f_{wm}/R_{\text{h}} \) (See also Fig. 1A).

\[
F = \frac{\text{FDp} \cdot (1 - \frac{\text{CL}_{\text{h}}}{\text{Q}_{\text{h}}})}{\frac{\text{CL}_{\text{h,plasma}}}{\text{Q}_{\text{h}}}}
\]

In the second method (method F2), Gastropus Simulation (version 5.1.0; Simulations Plus Inc.) was used. For all simulations, Gastropus was provided with experimentally derived data (Table 1) on log \( P_{\text{vow}}^{-1} \), pK \( _{a} \), S \( _{w} \) at defined pH, or solubility in dosing vehicle at defined pH, dose administered (Table 2), and first pass metabolism. First pass metabolism was assumed to occur only in the liver and was calculated from the CL \( _{\text{h}} \) as described under method F1. As no experimental data were available on permeability estimates, in silico estimates of human jejunal permeability (Table 1) were obtained by the artificial neural network model in ADMETPredictor (version 1.3.2; Simulations Plus Inc.) and subsequently converted to rat permeability by the built-in correlation of Gastropus. The variation of solubility and permeability within different regions of the gut was assumed to depend on the pH of that region and was calculated from the pK \( _{a} \) and logD values of the drug (Gastropus Opt logD model).

**Success Criteria.** Success of predictions was assessed by the root mean squared prediction error (rmse) and the average-fold error (afe) as measures of precision and bias, respectively, with equal value to under- and overpredictions:
The objective of the experiments described herein is to test more exhaustively the possibility of predicting hepatic clearance of compounds that had adequate intravenous PK data for assessment of dss using methods Vd1 and Vd2, respectively. Method Vd1 resulted in a considerable bias with general underpredictions and overpredictions for bases and neutrals, respectively. The underpredictions were predominantly associated with strong bases (pK_a > 8.0), characterized by high lipophilicity (logPow > 4) or low protein binding (f_un > 0.5). The majority of the neutral compounds (11 of 12 compounds) showed high lipophilicity (logPow > 3.5).

To explore whether it was possible to reduce the number of experimentally determined input parameters, dss predictions (n = 35) using experimentally derived logPow and pK_a values were compared with those using their calculated counterparts generated by ADMET-Predictor. The various statistical parameters calculated are given in Table 4. Using computed input, for method Vd1 a considerable increase in bias (afe) and a decrease in accuracy (rmse) were seen compared with experimentally derived logPow and pK_a values.

### Results

**Physicochemical and Pharmacokinetic Properties of the Compounds.** The molecular weight ranged from 287 to 777. The lipophilicity was high, with logPow ranging between 1.1 and 5.5. The majority of the compounds (n = 36) were defined as bases with a basic pK_a ≥ 6.8, and the remainder (n = 14) were defined as neutrals with a basic pK_a < 6.8. The f_un ranged from 0.001 to 0.82. Solubility was highly variable with values ranging from <0.001 mg/ml to 192 mg/ml. Although this study was performed retrospectively, the physicochemical properties of our data set in Table 1 have remained representative of compounds currently being considered as preclinical development candidates (Table 1, JN137, JN138, JN144, IN145). The majority of compounds were moderate-to-strong bases, characterized by high lipophilicity and low free fraction in plasma.

**Prediction of CL_h in Rat.** The objective of the experiments described herein is to test more exhaustively the possibility of predicting CL_h by disregarding the fraction unbound in both blood (f_up/R_B) and microsomes (f_un) in eqs. 2 and 3. The validity of the assumption that binding to blood constituents in vivo would be similar to binding to microsomal protein in vitro (i.e., f_up/R_R is cancelled out by f_un) would greatly extend the use of hepatic clearance models in early drug discovery, where data on f_up, R_B, and f_un are usually not available. CL_h was predicted using both the well stirred (eq. 2) and parallel tube (eq. 3) models under three variations: 1) disregarding all binding values (f_up/R_B = 1 and f_un = 1), 2) including only blood binding (f_un = 1), and 3) including both blood and in vitro microsomal binding. Graphical comparisons of the prediction methods are shown in Fig. 1 (A–F), and the parameters for the accuracy of predictions are summarized in Table 3. In general, disregarding all binding in either model of hepatic extraction (Fig. 1, A and D) yielded the best agreement between observed and predicted CL_h values (approximately 85% within 2-fold of observed). Using only the blood binding value (Fig. 1, B and E) in each model of extraction yielded very poor predictions, with a strong bias toward underprediction (approximately 20% within 2-fold of observed). Including both binding factors improved significantly the accuracy of the predictions (approximately 60% within 2-fold of observed) compared with blood binding only; however, many of the underpredictions remained (Fig. 1, C and F).

The latter finding suggests that both binding factors would not necessarily cancel out. Poor predictions were obtained for compounds with an in vitro t_1/2 > 60 min (n = 13), with less than 50% of predicted values within 2-fold of observed (Table 3).

**Prediction of V_dss: Method Vd1 versus Vd2.** There were 47 compounds that had adequate intravenous PK data for assessment of dss predictions (Tables 1 and 2). Since method Vd1 is only to be applied to basic compounds, the utility and accuracy of both methods was first compared solely on all moderate-to-strong bases (basic pK_a ≥ 6.8, n = 35). Figure 2 illustrates the correlations between the observed and predicted values of dss using methods Vd1 and Vd2, respectively. Method Vd1 resulted in highly accurate predictions of dss with more than 80% within 2-fold of observed (Fig. 2A). In contrast, method Vd2 was a poor predictor of dss in this analysis in that only 60% of predictions were within 2-fold of observed (Fig. 2B). For the neutral compounds (n = 12), only method Vd2 was applicable and the results are also shown in Fig. 2B (open circles) and Table 4.

To assess the bias of the predictions, the prediction error (expressed as the log of predicted/observed ratio) was plotted as a function of dss using both method Vd1 (Fig. 2C) and method Vd2 (Fig. 2D), and various statistical parameters were calculated (Table 4). Remarkably, method Vd2 yielded a considerable bias with general underpredictions and overpredictions for bases and neutrals, respectively. The underpredictions were predominantly associated with strong bases (pK_a > 8.0), characterized by high lipophilicity (logPow > 4) or low protein binding (f_un > 0.5). The majority of the neutral compounds (11 of 12 compounds) showed high lipophilicity (logPow > 3.5).

### Table 3

**Accurate of CL_h predictions from hepatic microsomal in vitro t_1/2 data**

<table>
<thead>
<tr>
<th>afe</th>
<th>rmse</th>
<th>Percentage within 1.5-fold Error</th>
<th>Percentage within 2-fold Error</th>
<th>Percentage within 2.5-fold Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well stirred model*&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No binding considered (f_un/R_B = f_un = 1)</td>
<td>1.17 (3.93)</td>
<td>15.0 (23.0)</td>
<td>77.8 (0.00)</td>
<td>86.1 (25.0)</td>
</tr>
<tr>
<td>Including only blood binding (f_un = 1)</td>
<td>13.5 (59.3)</td>
<td>34.1 (26.5)</td>
<td>11.1 (0.00)</td>
<td>16.7 (8.33)</td>
</tr>
<tr>
<td>Including both blood and microsomal binding</td>
<td>2.51 (13.5)</td>
<td>23.1 (25.4)</td>
<td>47.22 (0.00)</td>
<td>61.1 (8.33)</td>
</tr>
<tr>
<td>Parallel tube model*&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No binding considered (f_un/R_B = f_un = 1)</td>
<td>1.04 (3.68)</td>
<td>13.6 (38.1)</td>
<td>75.0 (16.7)</td>
<td>91.7 (33.3)</td>
</tr>
<tr>
<td>Including only blood binding (f_un = 1)</td>
<td>12.0 (58.2)</td>
<td>36.4 (44.5)</td>
<td>13.9 (0.00)</td>
<td>22.2 (8.33)</td>
</tr>
<tr>
<td>Including both blood and microsomal binding</td>
<td>2.16 (13.1)</td>
<td>26.6 (43.0)</td>
<td>50.0 (0.00)</td>
<td>63.9 (8.33)</td>
</tr>
</tbody>
</table>

* Values outside parentheses: statistical analysis on all compounds with an in vitro t_1/2 < 60 min (n = 36). Values in parentheses: statistical analysis on all compounds with an in vitro t_1/2 > 60 min (n = 13).

<sup>a</sup> For details on the well stirred and parallel tube models, see Materials and Methods.

\[
\text{mse} = \frac{1}{N} \sum \left(\text{Predicted} - \text{Observed}\right)^2, \text{rmse} = \sqrt{\text{mse}}
\]

\[
\text{afe} = \frac{1}{N} \sum \frac{1}{\left| \text{Predicted} / \text{Observed} \right|}
\]
and the parameters for the accuracy of predictions are summarized in Table 5.

Trends for $P_{tp}$ predictions were seen similar to those observed for $V_{dss}$ predictions. In general, method Vd1 yielded more accurate predictions compared with method Vd2, where there was a tendency to underpredict. For both methods, acceptable predictions were obtained in muscle and heart tissue, whereas underpredictions were most pronounced in liver and lung. In contrast, in brain tissue, overpredictions were obtained for both methods; however, the bias was significantly more pronounced using method Vd2.

**Estimation of in Vivo $t_{1/2}$ in Rat.** Combining both methods for predicting $V_{dss}$ (methods Vd1 and Vd2) with all those for predicting $CL_h$ (Fig. 1) resulted in a total of 12 $t_{1/2}$ calculation methods. To directly compare methods Vd1 and Vd2, in vivo $t_{1/2}$ predictions were performed on all moderate-to-strong bases, providing they had an in vitro $t_{1/2} < 60$ min (Table 1, $n = 25$); compounds with an in vitro $t_{1/2} > 60$ min were not included because $CL_h$ predictions were not found to be accurate (Table 3). The parameters for the accuracy of predictions are summarized in Table 6. Overall, in vivo $t_{1/2}$ calculations based on $CL_h$ predictions that included protein binding were generally more inaccurate than those based on $CL_h$ predictions that disregarded all protein binding. Among the latter, the best in vivo $t_{1/2}$ calculations were based on method Vd1 (Fig. 4), whereas those based on method Vd2 showed similar accuracy but higher bias, due to underpredictions of $V_{dss}$ (data not shown).

**Prediction of $F$.** Only compounds with experimental solubility data (Table 1) and an in vitro $t_{1/2} < 60$ min were considered for bioavailability ($F$) predictions ($n = 30$). The graphs of predicted versus observed $F$ are shown in Fig. 5, and the parameters for the accuracy of the predictions are given in Table 7. In the first method (method F1), hepatic first pass metabolism was considered as the sole determinant influencing $F$, thus assuming that all compounds were 100% absorbed in the portal vein ($FD_p = 1$). Despite its simplicity, this method yielded accurate predictions with more than 80% of predictions within 2-fold of observed (Fig. 5A). These results suggest that for most compounds within this analysis, $F$ was predominantly governed by hepatic first pass metabolism. This hypothesis was supported by the Gastroplus simulation (method F2) depicted in Fig. 5B. The
comparable results within both panels of Fig. 5 confirm that for most of the compounds within this analysis, neither permeability nor solubility rate-limited issues significantly affected the absorption process. However, it is important to note that for all simulations of Fig. 5B, the influence of solubility enhancers (cyclodextrin or polyethylene glycol), if any were present in the dosing vehicle, on Gastroplus solubility input data were taken into account (Table 7, Method F2 Simulation 2). When the effect of such additives on solubility was ignored (Table 7, Method F2 Simulation 1), Gastroplus strongly underpredicted $F$ for seven compounds (i.e., JNJ2, JNJ26, JNJ38, JNJ41, JNJ42, JNJ43, and JNJ47), resulting in an increased rmse and a decrease in number of accurate predictions within 2-fold of observed.

### Discussion

In recent years, PBPK models and prediction tools based on in vitro and in silico input parameters have become more popular. However, the predictive utility of such tools within drug discovery has been on the whole poorly evaluated, with only a few reports in the literature (Parrott et al., 2005a,b). In general, the prediction accuracy as well as the body of in vitro data needed for prediction of a particular parameter will vary depending on the approach, the type of chemistry, and the prediction system used. Therefore, in our efforts to apply these methods in drug discovery mode, the objectives of this study were: 1) to assess the validity of various approaches toward binding factors when predicting CL$_h$ from microsomal data; 2) to perform a comparative evaluation of two recently published tissue composition-based equations for their accuracy and input requirements to predict tissue distribution ($P_{tp}$ and $V_{dss}$); 3) to explore the use of computed physicochemical input and predicted microsomal binding; and 4) to combine the aforementioned parameters and predict in vivo $t_{1/2}$ and $F$.

Overall, CL$_h$ was a well predicted process within this analysis, irrespective of the type of model of hepatic extraction used (Fig. 1). Because the differences between the two liver models are minimal, we suggest that the most commonly adapted well stirred model could continue to be applied within our in-house chemistry. Poor correlations were obtained for compounds that did not show significant turnover in vitro. Remarkably, literature reports usually do not include such compounds within their analyses, most probably because poor correlations are to be expected (e.g., enzyme saturation, phase 2 metabolism, etc.) Because compounds are often selected for further
development based on this property, such data should not be left out from correlation analysis.

The decision whether to incorporate plasma and microsomal protein binding in $\text{CL}_{\text{rat}}$ predictions remains controversial. In drug discovery, one is frequently faced with the situation in which data on $f_{\text{unb}}$ and $f_{\text{up}}$ are not available; therefore, it is an attractive option to assume that both parameters may cancel out. The inclusion of both unbound fractions has been suggested to be the general applicable approach. However, our results and those of others demonstrate that, in the case of some compound classes, especially basic ones, disregarding both binding values may yield the most accurate predictions (Obach, 1997, 1999). Nevertheless, one must recognize that accurate predictions from scaled microsomal data are only possible when the compound is mainly cleared by oxidative microsomal metabolism, and neither extrahepatic metabolism nor renal or biliary clearance significantly contributes to $\text{CL}_{\text{rat}}$. For some of the compounds within our analysis, multiple forms of elimination are known to be present (e.g., JNJ3, JNJ8, JNJ22, JNJ34, JNJ36). Some underpredictions might be explained by sensitivity analysis illustrating that $V_{\text{dss}}$ predictions become insensitive to $\log P_{\text{ow}}$ changes above 4 and $f_{\text{up}}$ changes below 0.01 (Poulin and Theil, 2002a; Parrott et al., 2005b). In this study, $V_{\text{dss}}$ prediction of all lipophilic strong bases ($\log P_{\text{ow}} > 4$, $pK_a > 8$) was blunted around 4 to 5 l/kg (e.g., JNJ4, JNJ6, JNJ11, JNJ26, JNJ29, JNJ32, JNJ37). Remarkably, $V_{\text{dss}}$ predictions of highly lipophilic strong bases with $f_{\text{up}}$ values of $\geq 0.05$ seemed to be more prone to underprediction (e.g., JNJ4, JNJ29, JNJ32, JNJ37).

The tissue composition-based equation of Rodgers et al. (2005a) (method Vd1) performed significantly better on the prediction of both $V_{\text{dss}}$ and $P_{\text{tp}}$, irrespective of lipophilicity (Fig. 2). This divergence in prediction accuracy between method Vd1 and Vd2 can be explained as ionic interactions between charged drugs and acidic phospholipids are neglected in method Vd2. Basic drugs tend to have larger $V_{\text{dss}}$ values due to favorable ion-pair interactions of the basic centers with the acidic head groups of the phospholipid membranes (Rodgers et al., 2005b). In the model of method Vd2 it is assumed that macromolecular binding of drugs in tissue interstitial fluids (i.e., $f_{\text{sat}}$) is driven by binding to macromolecules similar to those present in plasma; therefore, $f_{\text{sat}}$ is simply calculated from $f_{\text{up}}$ data (eq. 14). However, in the case of strong bases ($pK_a > 8$), the calculated $f_{\text{sat}}$ value will most likely underestimate tissue binding. In particular, for bases that have low protein binding, the calculated $f_{\text{sat}}$ can be expected to deviate more from actual. Overall, these results suggest that the model of Rodgers et al. (2005b) should be used for moderate-to-strong bases, and certainly for our in-house chemistry. However, the major disadvantage is the prerequisite for an additional input factor (i.e., $R_{\text{tp}}$). To reduce the required input, some efforts were devoted to explore computed parameters. Unfortunately, a decrease in accuracy was seen using computed parameters compared with experimentally derived ones (Table 4).

A similar trend between methods Vd1 and Vd2 was demonstrated for prediction of data on $P_{\text{tp}}$ (Fig. 3). With the exception of muscle and heart tissue, a poor accuracy and strong bias of method Vd2 was demonstrated. Bias resulted predominantly from pronounced underprediction. Despite the overall better performance of method Vd1, underpredictions still prevailed in lung and liver tissues. One possible explanation is lysosomal trapping, since these acidic organelles are...
abundant in lung and liver, and extensive distribution to these tissues has been reported for many basic drugs (MacIntyre and Cutler, 1988; Ishizaki et al., 1998). Importantly, significant lysosomal trapping may affect the first passage of a basic compound through the lung; thus, the estimation of \( V_{dss} \) in vivo from plasma concentration-time profiles may be biased. In liver tissue, potential clearance processes, enterohepatic circulation, or active uptake processes may also have influenced experimental \( P_{tp} \) values. In brain tissue, overpredictions were predominantly apparent for both methods. The main assumption with these equations is that passive diffusion governs tissue distribution. Brain penetration is, however, more restrictive and selective, and several active transport mechanisms are likely to play a key role. Inaccurate predictions will also prevail if in vivo terminal \( t_{1/2} \) is most likely to be underpredicted when using \( CL_h \) and \( P_{tp} \), if present in the dosing vehicle, were taken into account. A similar issue was found by Parrott et al. (2005b), who corrected for increased solubility that would occur in the presence of bile salts.

In combining \( CL_h \) and \( V_{dss} \) predictions to predict in vivo \( t_{1/2} \), all methods were combined to remain unbiased (Table 6). As expected, most successful combinations in predicting in vivo \( t_{1/2} \) consisted of those methods that were most successful in predicting the independent parameters. An important consideration in the prediction of in vivo \( t_{1/2} \) is the fact that experimental \( t_{1/2} \) values may exhibit multiphasic plasma concentration versus time profiles; therefore, terminal \( t_{1/2} \) is most likely to be underpredicted when using \( CL_h \) and \( V_{dss} \) predictions. However, in drug discovery it is not of tremendous importance to target prediction of in vivo \( t_{1/2} \) as absolute values but rather as an ability to place compounds in appropriate dosing regimens.

Predictions of \( F \) were generally successful, even when only hepatic metabolism was considered (Fig. 5A). It is acknowledged that such methods should be treated carefully since, by definition, they underestimate the potential impact of absorption and first pass metabolism limitations by the gut mucosa. More advanced PBPK models have now become available to predict \( FD_p \) and \( F \). For example, the model underlying Gastroplus Software is known as the Advanced Compartmental Absorption and Transit Model (ACAT model) (Agoram et al., 2001). Gastroplus takes drug-specific input data (solubility, permeability) as well as metabolic first pass estimates and uses these to predict \( FD_p \) and \( F \). In this study, Gastroplus simulation of \( F \) yielded results similar to those from predictions based solely on \( CL_h \) data (Fig. 5), suggesting that hepatic first pass metabolism was a major determinant influencing \( F \). Interestingly, accuracy of Gastroplus simulations seemed to improve when the influence of solubility enhancers, if present in the dosing vehicle, were taken into account. A similar issue was found by Parrott et al. (2005b), who corrected for increased solubility that would occur in the presence of bile salts.

In summary, we have compared various mechanism-based ADME prediction tools to predict \( CL_q \), tissue distribution \( (V_{dss} \text{ and } P_{tp}) \), in vivo \( t_{1/2} \), and \( F \) using a diverse data set covering many targets and chemotypes. Although such prediction tools are said to be generic and thus applicable to a wide array of structurally diverse compounds, our results and those obtained by others demonstrate that diverse physicochemical properties as well as invalid model assumptions may put compounds beyond the validity of the approach. Therefore, it is suggested that the utility of each prediction tool should be validated on a class-by-class basis. Physiologically based prediction of human PK with an initial validation in animals is currently under evaluation in our department.

References


TABLE 7

Accuracy of \( F \) predictions

<table>
<thead>
<tr>
<th>Method</th>
<th>( n )</th>
<th>rmse (%)</th>
<th>Percentage within 1.5-fold Error</th>
<th>Percentage within 2-fold Error</th>
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<td>43.3</td>
<td>63.3</td>
</tr>
</tbody>
</table>

* For details on methods F1 and F2, see Materials and Methods.

** Table 1: \( S_w \) for the prediction tool. \( S_w \) values are used to estimate \( P_{tp} \) values.


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