JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1181–1190

Received January 8, 2007
Accepted March 20, 2007
DOI 10.1211/jpp.59.9.0001
ISSN 0022-3573

Review Article

Prediction of human pharmacokinetics – evaluation of methods for prediction of volume of distribution

Urban Fagerholm

Abstract

The aim was to evaluate and review methods for prediction of the steady-state volume of distribution ($V_{D,ss}$) of xenobiotics in man. For allometry, ~30–40% of predictions are classified as incorrect, humans and animals belong to different $V_{D,ss}$ categories for ~30% of the compounds, maximum prediction errors are large (>10-fold), the b-exponent ranges between –0.2 and 2.2 (averaging ~0.8–0.9), and >2-fold prediction errors are found for 35% of the substances. The performance is consistent with species differences of binding in and outside the vasculature. The largest errors could potentially lead to very poor prediction of exposure profile and failure in clinical studies. A re-evaluation of allometric scaling of unbound tissue volume of distribution demonstrates that this method is less accurate (27% of predictions >2-fold errors) than a previous evaluation demonstrated. By adding molecular descriptor information, predictions based on animal $V_{D,ss}$ data can be improved. Improved predictions (~1/10 of allometric errors) can also be obtained by using the relationship between unbound fraction in plasma ($f_{u,pl}$) and $V_{D,ss}$ for each substance (method suggested by the author). A physiologically-based 4-compartment model (plasma, red blood cells, interstitial fluid and cell volume) together with measured tissue–plasma partitioning coefficients in rats, $f_{u,pl}$, interstitial–plasma concentration ratio of albumin, organ weight and blood flow data has been successfully applied. Prediction errors for one basic and one neutral drug are only 3–5%. The data obtained with this comparably laboratory-intensive method are limited to these two compounds. A similar approach where predicted tissue partitioning is used, and a computational model, give prediction errors similar to that of allometry. Advantages with these are the suitability for screening and avoidance of animal experiments. The evaluated methods do not account for potential active transport and slow dissociation rates.

Introduction

Good predictions of gastrointestinal fraction absorbed (f_a), clearance (CL) and volume of distribution (V_D) in man are required/desired for choosing the most suitable candidate drugs, and for assuring safe and effective dosing in man. To reach this goal, an understanding of the determinants of these parameters and species similarities and differences is needed. Methods for prediction of gastrointestinal f_a and hepatic CL have recently been reviewed (Fagerholm 2007a, b).

The V_D is determined by the volumes and binding capacities of blood, organs and tissues, and sometimes is also influenced by permeation and dissociation rates (Berezhkovskiy 2004a, b). In addition, the metabolic capacity of binding organs (where distribution is, or may be, a part of the elimination) could also be of importance. In general, estimation of the V_D is based on plasma exposure.

Most of the binding of acidic drugs in the circulation can be accounted for by association with albumin (electrostatic and hydrophobic bonds), while α_1-acid glycoprotein plays a major role in the binding of several basic drugs (Tillement et al 1984). Most basic and neutral drugs also bind to albumin (Tillement et al 1984). The degree of binding to plasma proteins is to some extent dependent on the drug lipophilicity (log D) (Figure 1). The binding to plasma proteins appears to be higher at high log D. For compounds in this data set with log D > 2, the unbound fraction in plasma ($f_{u,pl}$) was maximally ~0.1, and highest $f_{u,pl}$-values (~0.6–1) were observed for compounds with log D < 0–0.5. There was no apparent correlation between log D and blood-to-plasma concentration ratios (C_{bl}/C_{pl}) (Figure 2). Data in these figures were collected or extracted from Sawada et al (1984, 1985), Obach (1999),

Clinical Pharmacology, AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden

Urban Fagerholm

Correspondence: U. Fagerholm, Clinical Pharmacology, AstraZeneca R&D Södertälje, S-151 85 Södertälje, Sweden. E-mail: urban.fagerholm@astrazeneca.com

Acknowledgments: My colleagues at AstraZeneca R&D Södertälje for inspiration and reviewing the manuscript.

Note: This paper includes personal opinions of the author, which do not necessarily represent the views or policies of AstraZeneca.

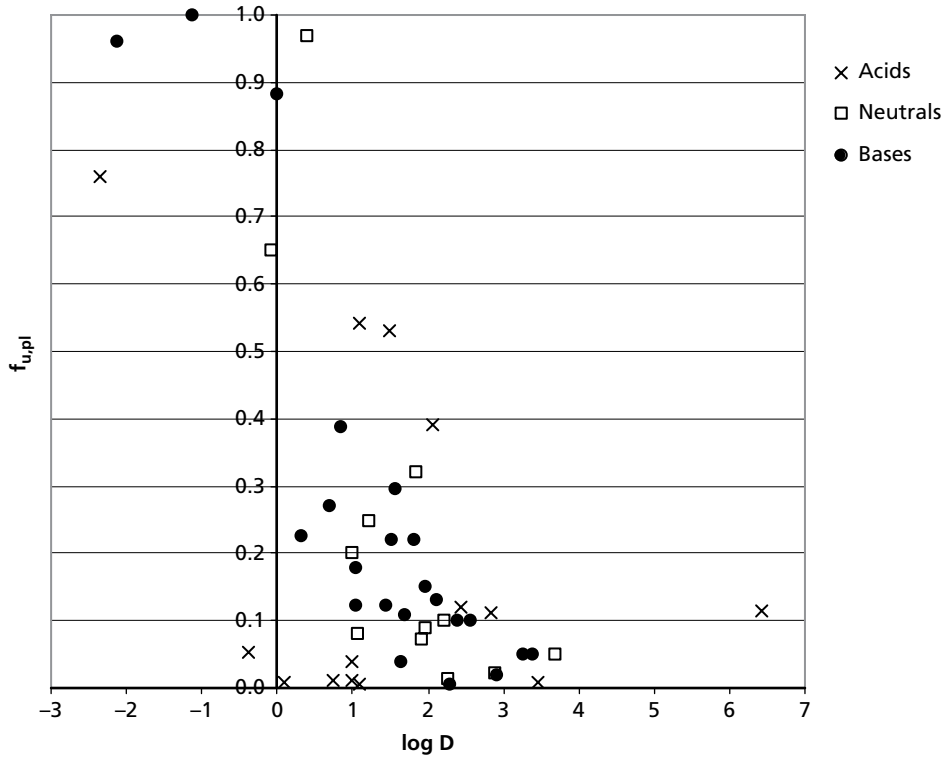

Figure 1 The relationship between log D at pH 7.4 and $f_{u,pl}$ for 48 acidic, neutral and basic compounds in man. Data were collected from Sawada et al (1984, 1985), Obach (1999), Poulin & Theil (2002), Shibata et al (2002), Doran et al (2005) and Fagerholm & Björnsson (2005).

Poulin & Theil (2002), Shibata et al (2002), Doran et al (2005) and Fagerholm & Björnsson (2005).

Lipid solubility, degree of ionization, molecular size and hydrogen-bonding ability have been identified as the main determinants for uptake by red blood cells (RBCs) (Schanker et al 1961; Naccache & Sha'afi 1973). Drugs generally enter RBCs more rapidly when the lipophilicity and degree of unionized form are high, and molecular size and ability to form hydrogen bonds are low (Schanker et al 1961; Naccache & Sha'afi 1973). Partially ionized lipid-soluble bases usually penetrate RBCs so rapidly that the time for equilibration cannot be accurately determined (Sahin & Rowland 2004). It may take several hours to reach steady state between RBCs and plasma for compounds with low lipophilicity (low permeability; P_e) (Schanker et al 1961). Such slow binding is likely to influence their distribution and elimination. Basic drugs, in particular, bind non-specifically and to α-adrenergic receptors on these cells (Tillement et al 1984). In addition, they have also been shown to bind to leucocytes and platelets, and to α-adrenergic receptors on platelets, granulocytes and lymphocytes (Tillement et al 1984). RBCs have a comparably small volume (about half of the blood volume) and a binding capacity vs plasma proteins that is generally not very high. C_{bl}/C_{pl} values in Figure 2 range from ~0.55 (~1 – the haematocrit) to 1.5. The lipophilic basic compounds that bind extensively to these cells are likely to bind extensively to other tissues (Figure 3), and hydrophilic acids generally appear to bind more extensively to plasma proteins than to RBCs (Figure 1, 2). On this basis, the binding to RBCs is generally not anticipated to contribute significantly to the total plasma-based V_D.

Drug-binding plasma proteins, such as albumin, globulins, lipoproteins and α1-acid glycoprotein, are also present extravascularly (Mansor et al 1991; Poulin & Theil 2002; Björkman et al 2001). This can explain the correlation that exists between the degree of binding in plasma and tissues. Figure 4 demonstrates the relationship between $f_{u,pl}$ and unbound fraction in tissues ($f_{u,T}$) for neutral and basic compounds (n = 120; data were extracted from Lombardo et al (2004)), and it is apparent that compounds of these classes generally bind more extensively to components outside the circulation than to plasma proteins. The lowest and average tissue/plasma binding ratio (ratio of bound fractions) was 1.0 and 2.0, respectively; about 6% of the compounds had ratios of at least 5, and the largest ratio was 17. In contrast, many acids (which mainly bind to albumin in plasma) have low tissue/plasma binding ratios and low apparent steady-state V_D ($V_{D,ss}$). Basic lipophilic drugs are often characterized by a high $V_{D,ss}$ (the relationship between log D at pH 7.4 and $f_{u,pl}/f_{u,T}$ in Figure 3 shows a trend insofar as the extent of tissue binding increases with increasing lipophilicity, especially for bases). These bind extensively to cell components, especially to mitochondria and lysosomes (Siebert et al 2004; Rodgers et al 2005a). The binding to membrane phospholipids and microsomal protein is non-specific, whereas the accumulation

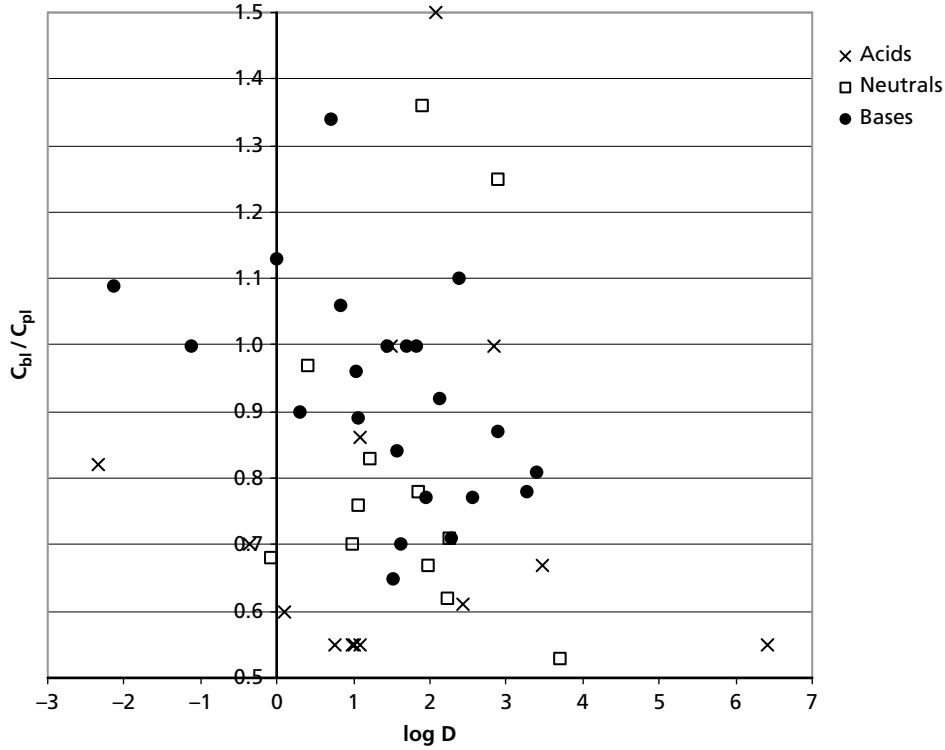

Figure 2 The relationship between log D at pH 7.4 and C_{bl}/C_{pl} for 48 acidic, neutral and basic compounds in man. Data were collected from Sawada et al (1984, 1985), Obach (1999), Poulin & Theil (2002), Shibata et al (2002), Doran et al (2005) and Fagerholm & Björnsson (2005).

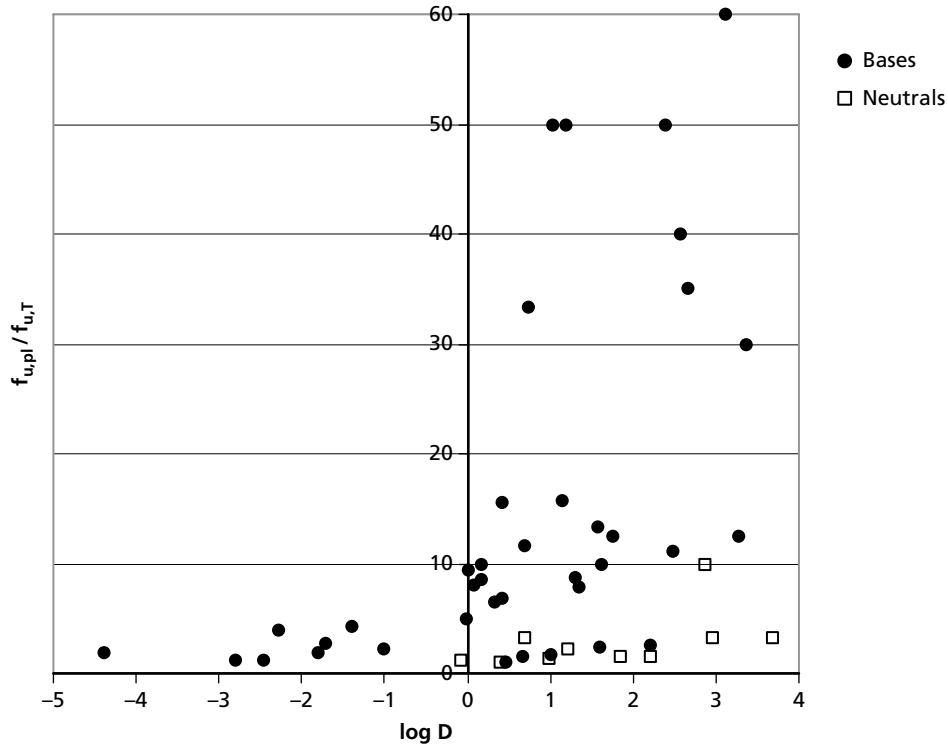

Figure 3 The relationship between log D at pH 7.4 and $f_{u,pl}/f_{u,T}$ for 51 basic and neutral compounds in man. Data were collected from Austin et al (2002), Poulin & Theil (2002), Lombardo et al (2004), Doran et al (2005) and Fagerholm & Björnsson (2005).

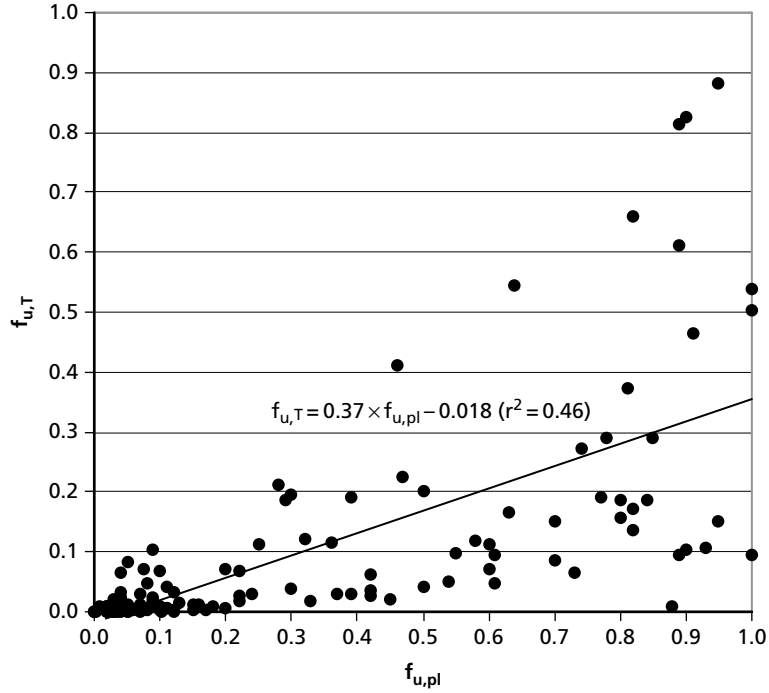

Figure 4 The relationship between $f_{u,pl}$ and $f_{u,T}$ in man for 120 neutral and basic compounds. Data were extracted from Lombardo et al (2004).

in mitochondria and lysosomes is believed to be a result of the pH difference with the cytoplasm (pH 4–5 and 6.7–7.0 in lysosomes and mitochondria vs pH 7.2–7.3 in the cytoplasm), or so called ion-trapping (Siebert et al 2004). The extent of ion-trapping is determined by the lipophilicity and pK_a of the drug, and the pH of and fraction of hepatocyte volume occupied by these acidic organelles (Roberts et al 2002). Liver (mitochondria and lysosomes occupy approximately 20 and 1% of the hepatocyte volume, respectively), lungs and kidneys are lysosyme-rich organs (Roberts et al 2002; Siebert et al 2004). α1-Acid glycoprotein is also a potentially important intra-hepatocellular binding protein for cationic drugs (Mansor et al 1991). Rodgers et al (2005a) demonstrated that the regional tissue distribution of a series of basic beta blockers in the rat correlated well with tissue acidic phospholipid concentrations. They summarized that plasma protein binding and acid phospholipid concentrations in blood cells and tissues appear to be the predominant factors controlling the tissue distribution of basic compounds. The binding to fat tissue, which contains approximately 80% lipids, has been reported to increase with drug lipophilicity (Poulin & Theil 2001). Björkman (2002) found that fat accounted for a median of 65% (range 12–82%) of the $V_{D,ss}$ for basic drugs (n = 17). The corresponding number for acids was 34% (range 9.0–82%) (n = 18). The binding of bases and acids to muscles accounted for a median of 19% (range 7.1–51%) and 35% (range 8.4–58%), respectively. In addition to non-specific binding, the binding to many sites (such as to receptors) could be specific. Different distribution patterns could be found for such substances.

The concentrations and characteristics of blood/plasma components differ among species (Davies & Morris 1993; Lin 1995), and this often causes species differences in the extent of blood and tissue binding and $V_{D,ss}$. Figure 5 demonstrates the relationship between the in-vitro equilibrium $f_{u,pl}$ for 50 drugs with various physicochemical properties in rats and man (human $f_{u,pl} = 0.73 \bullet$ rat $f_{u,pl}$; $r^2 = 0.67$). The maximum human/rat and rat/human $f_{u,pl}$ ratios were 4 and 17, respectively, and for about half of the compounds there was less than 2-fold difference between the species. Data were collected from the literature and correlated (Sawada et al 1984, 1985; Obach 1999; Saiakhov et al 2000; Shibata et al 2000, 2002; Hardman et al 2001; Poulin & Theil 2002; Ito & Houston 2004; Fagerholm & Björnsson 2005). After this data collection and analysis had been done, Tang & Mayersohn (2005) published a similar comparison (n = 61). The results are in good agreement with findings in this paper. The maximum human/rat and rat/human $f_{u,pl}$ ratios in their data set were 3 and 20, respectively, and >2-fold differences between the species were found for 28% of the compounds. Similar comparisons and findings were made for dog and human $f_{u,pl}$ data for 15 substances (data taken from Boxenbaum (1982), Sawada et al (1984) and Lin (1995)). The human $f_{u,pl} = 0.55 \bullet$ dog $f_{u,pl}$ ($r^2 = 0.43$), and the maximum human/dog and dog/human $f_{u,pl}$ ratios were both 4. A weak correlation also exists between in-vitro equilibrium C_{bl}/C_{pl} in man and rat for a limited number of substances (n = 14) (human $C_{bl}/C_{pl} = 0.54 \bullet$ rat C_{bl}/C_{pl}; $r^2 = 0.43$). A limited number of available plasma protein and blood cell binding data for other animals (dogs, mice, monkeys, rabbits, sheep) also demonstrates similar trends (Sawada et al 1984).

The fractional volumes of plasma, RBCs, interstitial fluid and cells in the rat organs/tissues are reported to be 0.012 (adipose and skin) to 0.34 (lungs), 0.007 (adipose and skin) to

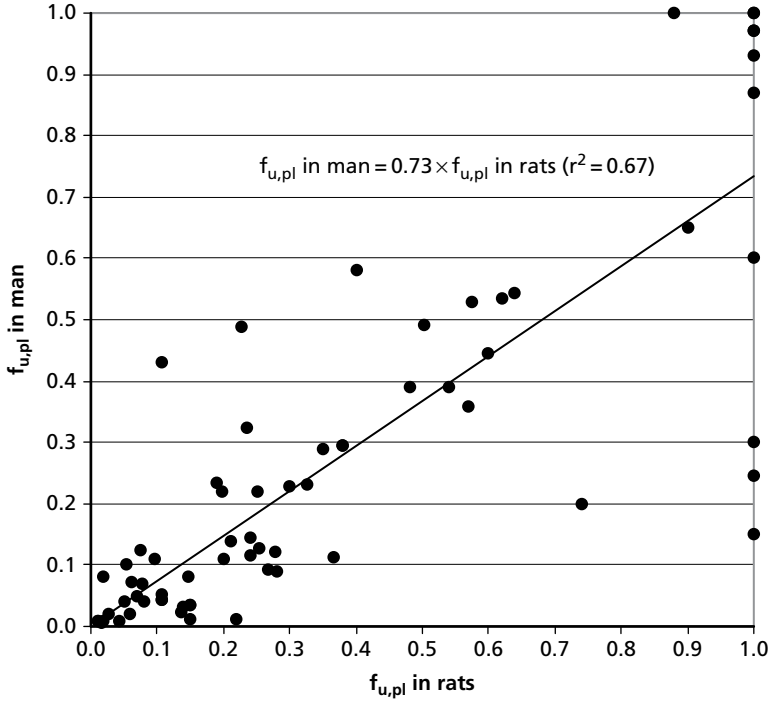

Figure 5 The relationship between $f_{u,pl}$ in rats and man (n = 50). Data were collected from Sawada et al (1984, 1985), Obach (1999), Saiakhov et al (2000); Shibata et al (2000), Hardman et al (2001), Poulin & Theil (2002), Shibata et al (2002), Ito & Houston (2004) and Fagerholm & Björnsson (2005).

0.18 (lungs), 0.10 (including brain and adipose) to 0.30 (skin) and 0.56 (liver) to 0.88 (adipose), respectively (Björkman et al 2001). The fractional volumes of plasma, RBCs, interstitial fluid and cells in adipose (85% fat cells) and muscles, which account for a large fraction of the body mass and are most important for drug binding, are quite similar, 0.012 and 0.017, 0.007 and 0.009, 0.10 and 0.12, and 0.88 and 0.85, respectively (Björkman et al 2001). The interstitial-to-plasma concentration ratio of albumin in these tissues was 0.5–0.6 (Björkman et al 2001). A similar ratio has been proposed for globulins and lipoproteins (Poulin & Theil 2002).

Organ volumes generally appear to follow the allometric principle. Allometric slope factors for volumes of plasma, RBCs, muscles, kidneys, liver, gut, heart, lungs, spleen and marrow are 0.84–1.10 (Mordenti 1986; Davies & Morris 1993). The human brain is, however, comparably large (2.2, 0.75 and 0.39 % of body weight in humans, rats and rabbits, respectively) (Kawakami et al 1994), and a normal weight human has about 3.5-fold higher adipose volume per kg body weight than a rat (Davies and Morris 1993).

The protein binding of small drug molecules is usually rapidly reversible (Berezhkovskiy 2004a), but there are compounds that are slowly dissociated from blood or tissue binding sites. Slow dissociation from tissues may result in a comparably long half-life and large $V_{D,ss}$, and slow dissociation for blood components may limit drug distribution and elimination (Berezhkovskiy 2004a, b).

The P_e across cells and endothelia could potentially also play an important role in drug distribution and elimination. The sets and capacities of transport-proteins differ among species, and this is only one of the factors that make prediction of the impact of active transport difficult. The P_e of compounds with low and moderate passive P_e are generally anticipated to be more sensitive to the active influx and efflux. Highly (passive) permeable compounds are, or are expected to be, less sensitive to active transport, and to have virtually unlimited organ uptake and re-distribution (back to the circulation). An exception is the uptake of high P_e-drugs by organs with short passage time for blood, such as the brain. They are also anticipated to have no, or low, renal and bile excretion. The high enterohepatic (a low metabolic hepatic CL and moderate/high intestinal P_e are required) and renal redistribution capacities of such compounds could result in enhanced $V_{D,ss}$ and prolonged half-life.

The objective was to evaluate and review methods for prediction of the $V_{D,ss}$ of xenobiotics in man.

Evaluation of methods for prediction of volume of distribution

Methods used to predict the $V_{D,ss}$ include: extrapolation and allometric scaling from animal $V_{D,ss}$ or unbound tissue volume ($V_{u,T}$) data; physiologically based (PB) prediction using tissue–plasma partitioning, tissue volumes and blood flows, and $f_{u,pl}$-data; predictions from molecular descriptors, alone or in combination with $f_{u,pl}$-data; and predictions from animal $V_{D,ss}$ and molecular descriptors (Sawada et al 1984, 1985; Iwatsubo et al 1996; Mahmood & Balian 1996; Obach et al 1997; Mahmood 1998; Björkman et al 2001; Boobis et al

2002; Poulin & Theil 2002; Theil et al 2003; Wajima et al 2003; Caldwell et al 2004; Lombardo et al 2004; Ward & Smith 2004; van de Waterbeemd 2005). A summary of the performances of these methods is shown in Table 1. A 2-fold prediction error means +100% and −50%, and an accurate prediction is defined as a 1.0-fold error. It should be noted that different sets of compounds and experimental conditions (including different doses, sampling schemes and limits of quantificant (LOQs)) have been used for the different methods, and that this potentially has influenced the direct comparisons of their prediction accuracies.

Allometric scaling and interspecies extrapolation

Based upon species differences in characteristics and concentrations of drug binding proteins and RBCs, it is not surprising to find that allometric scaling of $V_{D,ss}$ is associated with rather high predictions errors for a considerable fraction of compounds, and that a correction for differences in $f_{u,pl}$ leads to improvements. Reasons for the poor performances of some compounds may also include differences in experimental conditions, body composition, active transport and dissociation rates. The largest errors could potentially lead to very poor predictions of exposure profiles and failures in clinical studies.

Caldwell et al (2004) compared human and rat $V_{D,ss}$ data for 144 compounds and found the following relationship: log human $V_{D,ss} = 0.83 \bullet \log \text{rat } V_{D,ss} - 0.18$ (L kg^{-1}). This relationship and rat data were then used to predict the human $V_{D,ss}$. The average-fold and maximum errors were 1.82- and 9-fold, respectively. More than 2-, 3- and 4-fold prediction errors were found for 35, 19 and 9% of the substances, respectively. Similar results were obtained when a fixed allometric b-exponent (0.93) was used. Ward & Smith (2004) used various allometric approaches to predict the human $V_{D,ss}$ from rat, dog and monkey $V_{D,ss}$ for 103 compounds, and their findings are consistent with those of Caldwell et al (2004). About 30–40% of the predictions were classified as incorrect, man and animals belonged to a different $V_{D,ss}$ categories in about 30% of the cases, maximum prediction errors were large (>10-fold), and the allometric b-exponent ranged between −0.2 and 2.2 (average ~0.8) (Ward & Smith 2004). These observations are also in agreement with earlier reports by Sawada et al (1984, 1985), Mahmood & Balian (1996), Obach et al (1997) and Mahmood (1998).

Sawada et al (1984, 1985) correlated $V_{u,T}$ data between rats and man for 15 drugs with different properties ($V_{D,ss}$ ranging from ~0.2 to ~30 L kg^{-1}) and found a significant ($P < 0.001$; $r = 0.96$) and linear relationship (human $V_{u,T}$ (L kg^{-1}) = rat $V_{u,T}^{0.95}$ (L kg^{-1})). The $V_{u,T}$ values for many compounds were accurately predicted and the prediction errors were within 2-fold. A poorer relationship was observed for V_D ($P < 0.001$; $r = 0.85$; human $V_D = 0.53 \bullet$ rat $V_D^{0.85}$). These results indicate that this could be an appropriate and useful approach for scaling of the $V_{D,ss}$ in man. There are, however, reasons to question this approach and the results. For some substances where human C_{bl}/C_{pl} data were unavailable the human value was set equal to that of the rat. Many of the human C_{bl}/C_{pl} data that have become available later are different from those of the rat. Furthermore, the partitioning coefficient between whole blood (which includes plasma) and plasma was used as a measure of the partitioning between blood cells and plasma. A re-calculation of $V_{u,T}$ data (using the RBC-to-plasma partition ratio, and human C_{bl}/C_{pl} data) for 11 substances was performed (complete data information for the remainding compounds are lacking). The mean and maximum errors, and percentage with less than 30% and 2-fold prediction errors were 1.4-fold, 2.3-fold, 36% and 73%, respectively. The slope of the relationship between rat and human values was 1.48 ($r^2 = 0.98$). When excluding the compound with the highest $V_{u,T}$ (which was an apparent outlier), the numbers were improved slightly. Thus, the performance of the method is not as good as indicated previously. The slope of rat vs human $V_{u,T}$ obtained from data of 10 compounds was lower than unity (0.79), indicating that binding to human tissues generally is somewhat greater than in rats. This finding is in good agreement with that for plasma protein binding (Figure 5; slope = 0.73 for $f_{u,pl}$).

The human $V_{D,ss}$ (based on plasma) could also be estimated from the relationship between $f_{u,pl}$ and $V_{D,ss}$ (L kg^{-1}) for each substance. This approach could be useful, especially when $V_{D,ss}$ and $f_{u,pl}$ show large interspecies differences. By doing so, it is possible to compensate for vascular and

Table 1 The performance of methods for prediction of human $V_{D,ss}$

Method	Error (fold[a])			n	References
	Average	<2	Maximum		
Rat vs man relationship ($V_{D,ss}$)	1.8	65%	9	144	Caldwell et al (2004)
Rat vs man relationship ($V_{u,T}$)	1.4	73%	2.3	11	Sawada et al (1984,1985)
Allometry	n.d.	62–70%[b]	>10	103	Ward & Smith (2004)
Allometry + molecular descriptors	n.d.	78%	5	64	Wajima et al (2003)
$F_{u,pl}$ vs $V_{D,ss}$ relationship	1.2	100%	1.4	5	This paper
PB (using measured rat tissue binding)	1.04	100%	1.05	2	Björkman et al (2001,2004)
PB (using predicted tissue binding)	1.06	80%	20	123	Poulin & Theil (2002)
In-silico	2.2	72%	10	120	Lombardo et al (2004)

[a]Two-fold error = +100% or −50% error, and an accurate prediction is defined as a 1.0-fold error. [b]With acceptable errors. n.d.; not determined.

extravascular binding to "plasma" proteins. Available animal and human data from Sawada et al (1984) were used to evaluate the performance of the $f_{u,pl}$ vs $V_{D,ss}$ approach. Five basic compounds with data in at least two animal species were found. This proposed method gave average and maximum prediction errors of 1.21- and 1.37-fold, respectively. Compared to allometry (2.6-fold average error and 3.7-fold maximum error), this was a considerable improvement. The good performance was confirmed by predictions for a number of AstraZeneca compounds under development.

Physiologically based prediction

The PB approach has a sound rationale and with such methodology it is possible to reach good predictions of $V_{D,ss}$. Limitations with available PB methods include the assumptions that active transport is negligible and dissociation rates are rapid.

Björkman et al (2001) pointed out the importance of drug binding to albumin in interstitial fluid when scaling and predicting the $V_{D,ss}$. They used tissue–plasma partitioning coefficients from rats, human $f_{u,pl}$, organ weight and blood flow data, and developed a PB 4-compartment model (plasma, RBCs, interstitial fluid and cell volume) for prediction of the human $V_{D,ss}$. It was assumed that the tissue–plasma partitioning coefficients for unbound drug in rats and man were similar. The body composition of each patient in the study was estimated based on gender, body weight, height and age. With this approach they predicted the individual $V_{D,ss}$ for midazolam (neutral) and theophylline (basic) with good accuracy and precision (Björkman et al 2001; Björkman 2004). In the first study (Björkman et al 2001), the correlation between predicted and observed values for midazolam in adult patients was 0.82 ($P < 0.001$), and the mean prediction error was only 3.4% (range −24 to +39%). In the second study (Björkman 2004), this approach was applied to predict the $V_{D,ss}$ of midazolam and theophylline in infants, children and adults. The calculated $V_{D,ss}$ of midazolam fell mostly within the range of reported values, and predictions for theophylline had a median bias and imprecision of only 3.4 and 5.2%, respectively. The methodology and results demonstrate the potential of this approach for accurate prediction of the $V_{D,ss}$. Limitations, other than those mentioned above and the sparse data, are that this method is comparably laboratory-intensive and the assumption that tissue–plasma partitioning coefficients in rats and man are similar (this might not necessarily always be true).

Ballard et al (2003) compared in-vivo and in-vitro tissue-to-unbound plasma distribution coefficients for 9 barbiturates and found a good agreement between the parameters. The exception was for the most lipophilic compounds (with log P = 4), where the in-vitro model underestimated the in-vivo tissue binding by almost a magnitude. Björkman (2002) found with his approach that the $V_{D,ss}$ of both acidic and basic compounds in various animals can be predicted based on two tissue-to-plasma partition coefficients only (muscle-to-plasma and fat-to-plasma partition). For bases, the partition coefficients for all organs, except lungs and fat, were set equal to that of muscles. Partition coefficients for lungs and fat were set similar to that for fat. For acids, the partition coefficients for all organs/tissues, except fat, were set equal to that of muscles. Neutral compounds were not included in the study. Björkman (2003) showed that a reduction of full set of tissue-to-plasma partition coefficients to those obtained in two (fat and muscles) or three tissues (fat, muscles and lungs) did not result in a loss of power to predict the $V_{D,ss}$.

Poulin & Theil (2002) used a similar method to Björkman et al (2001) for prediction of the rat and human $V_{D,ss}$ for 123 acidic, neutral and basic compounds. The major methodological differences compared with Björkman et al (2001) were that Poulin & Theil did not account for binding to stomach, intestines or carcass (about 12% of total body weight, or slightly less than half of the weight of adipose tissue), and that they used predicted (and not measured) tissue partitioning coefficients (from octanol–water and olive oil–water partitioning coefficients at pH 7.4) and pK_a as determinants. This makes this method easier to apply than the method of Björkman et al. Another advantage is that animal experiments are not required. When such predicted partitioning coefficients are used, ionic interactions between drugs and charged cell membrane lipids, and pH gradients between intra- and extracellular spaces are neglected. Furthermore, it is assumed that there is a good relationship between octanol–water and olive oil–water partitioning coefficients vs the binding to all kinds of cells. Both Björkman et al (2001) and Poulin & Theil (2002) have predicted the $V_{D,ss}$ of midazolam. The prediction errors for this compound by Björkman et al (2001) and Poulin & Theil (2002) were on average 1.03- and 1.64-fold, respectively. In the study by Poulin & Theil (2002), the average ratio of predicted/observed $V_{D,ss}$ (data from both species) was 1.06 (s.d. = 0.82; r = 0.78), the prediction error was more than 2-fold for 20% of all substances, and the maximum over- and under-predictions were ~6- and ~20-fold, respectively. Overall, this approach appears to have a predictability similar to that found for allometric scaling and extrapolation of $V_{D,ss}$ from animals to man (see above). Considerable under-predictions (up to 10-fold) were found for many cationic-amphiphilic compounds (log oil–water partitioning coefficient > 3; $pK_a > 8.6$). This might have been due to the neglect of ionic interactions between compounds and charged tissue lipids. Ballard et al (2003) compared binding data for barbiturates (acids) obtained by this method with those after intravenous infusion in-vivo (in rats), and found a slight over-prediction trend (1.4-fold on average; ~40% with >2-fold over-prediction) with the approach by Poulin & Theil (2002). Further limitations of this approach are assumptions that active transport plays no role, predicted and true tissue–plasma partitioning coefficients are similar, and dissociation from binding sites is rapid.

Rodgers et al (2005b) extended the method of Poulin & Theil (2002) by incorporating the electrostatic interactions with tissue acidic phospholipids. The predictions of binding to 13 rat tissues for 28 moderate-to-strong bases were significantly more accurate than those obtained with the method used by Poulin & Theil (2002). With the new approach, 68 and 88% of predictions of binding to adipose, bone, gut, heart, kidney, liver, muscle, pancreas, thymus, skin and spleen deviated less than 2- and 3-fold from the measured values, respectively. Corresponding numbers for the method by Poulin & Theil (2002) were 25 and 45%, respectively. The predictions for lung and brain were less successful, and this was probably

due to involvement of additional processes. Later, Rodgers & Rowland (2006) applied their approach for acids, very weak bases, neutrals and zwitterions. Sixty-three and eighty-four percent of predicted tissue-to-plasma water partitioning coefficients had a prediction error within 2- and 3-fold, respectively. The potential of this approach for predicting the $V_{D,ss}$ in man remains to be demonstrated. The improvement of prediction of tissue binding compared with Poulin & Theil (2002) indicates that predictions of $V_{D,ss}$ could potentially be better than for allometric scaling. The prediction errors of tissue binding with this methodology (65% (for all studied compounds) within 2-fold error; maximum 2-fold mis-prediction) and allometric scaling (65% within 2-fold error; maximum 10-fold over- and under-predictions) indicate, however, that these approaches may generate similar prediction errors.

Predictions from molecular descriptors without or with animal $V_{D,ss}$ data

Predictions obtained from molecular descriptors also neglect potential active transport and slow dissociation rates. Advantages are that animal experiments are not required and the possibility for rapid screening. The performances with currently available methodologies may reach that obtained with simple allometry, which makes the most accurate of them suitable for screening of $V_{D,ss}$ for lead compounds and candidate drugs.

In a review by Smith et al (1996), relationships between log D (at pH 7.4) and human $V_{D,ss}$ for acidic, neutral and basic compounds are shown. The $V_{D,ss}$ for neutral and basic substances appeared (trend) to increase with increasing log D, and bases generally had higher $V_{D,ss}$ than either neutral or acidic drugs. A figure presented in that paper shows that log D is a crude predictor the $V_{D,ss}$. For example, two basic compounds could have a similar $V_{D,ss}$ despite a 5-fold difference in log D. The acids (log D range −2 to 2) had $V_{D,ss}$ values between ~0.1 and ~0.3 L kg^{-1}. Just as for lipophilic neutral and basic compounds, acids with high lipophilicity could have $V_{D,ss}$ reaching several L kg^{-1} (Poulin & Theil 2002).

Lombardo et al (2004) used estimates of log D and fraction ionized at pH 7.4, and $f_{u,pl}$ to predict the $V_{D,ss}$ of 120 neutral and basic compounds in man. Seventy-two and ninety-three percent of predictions were within 2- and 3-fold of the observed values, respectively, and the maximum prediction error was ~10-fold. Predictions were also made for a test set of 18 compounds, and the result was 56 and 89% of predictions <2- and <3-fold of the observed values, respectively. The corresponding numbers for allometry with correction for interspecies $f_{u,pl}$ differences were 63 and 88%, respectively.

In 2006, Lombardo and colleagues developed a computational approach with 31 computed descriptors (including lipophilicity, ionization, molecular volume and various molecular fragments) (Lombardo et al 2006). The model was trained using human $V_{D,ss}$ data for 384 compounds (acids inclusively). The ability of this method to predict the $V_{D,ss}$ was essentially identical to animal-based approaches.

Wajima et al (2003) developed a regression equation for prediction of the $V_{D,ss}$ in man. In this model, rat and dog $V_{D,ss}$ for 64 drugs and molecular descriptors such as molecular weight, calculated partition coefficients and number of hydrogen bond acceptors, were used for prediction. The best of a number of tested methods gave 78 and 92% of drugs with less than 2- and 3-fold prediction errors, respectively. These numbers were slightly better than those found for allometric scaling, 72 and 88%, respectively. Maximum prediction errors with these two approaches were ~5- and ~10-fold, respectively.

Conclusion

With allometry about 30–40% of predictions ($n > 100$) are classified as incorrect, man and animals belong to different $V_{D,ss}$ categories for about 30% of the compounds, maximum prediction errors are large (>10-fold), the b-exponent ranges between −0.2 and 2.2 and averages ~0.8–0.9, and more than 2-, 3- and 4-fold prediction errors are found for 35, 19 and 9% of the substances, respectively. The performance is consistent with species differences of binding in and outside the vasculature. The largest prediction errors could potentially lead to very poor predictions of exposure profiles and failures in clinical studies. Reasons for such poor performances may include differences in experimental conditions (such as different doses, sampling schemes and LOQs), active transport and dissociation rates. With allometric scaling of V_{uT} from rat to man for 11 compounds, the mean and maximum prediction errors were 1.4- and 2.3-fold, respectively. Twenty-seven percent of these predictions had >2-fold prediction error. Thus, this approach appears less accurate than a previous evaluation demonstrated. By adding molecular descriptor information, predictions based on animal $V_{D,ss}$ data can be improved.

The $V_{D,ss}$ is well predicted from $f_{u,pl}$ vs $V_{D,ss}$ relationships. With the limited amount of available data obtained with this proposed method, average and maximum prediction errors are 1.2- and 1.4-fold, respectively. These errors are only ~1/10 of those obtained with allometry.

A PB 4-compartment model (plasma, RBCs, interstitial fluid and cell volume), together with measured tissue–plasma partitioning coefficients in rats, human $f_{u,pl}$, interstitial–plasma concentration ratio of albumin, organ weight and blood flow data, has been successfully applied to predict the $V_{D,ss}$. The prediction errors for one basic and one neutral drug are only 3–5%. Data for this comparably laboratory-intensive method are limited to these two drugs. With this approach, the $V_{D,ss}$ can be predicted based on two (fat and muscles) or three (fat, muscles and lungs) tissue-to-plasma partition coefficients only. A similar approach, where predicted tissue partitioning is used (no need for animal experiments), gives prediction errors similar to those of allometry. Limitations with the available PB methods include the assumptions that active transport is negligible, predicted and rat tissue–plasma partitioning coefficients are similar to those in man in-vivo, and dissociation from binding sites is rapid.

The predictability of computational (in-silico) methods has also been demonstrated to be essentially identical to simple allometry. Just like the other methods, these do not account for active processes and assume that dissociation is rapid. Advantages include the avoidance of animal studies and suitability for rapid screening.

References

Austin, R. P., Barton, P., Cockroft, S. L., Wenlock, M. C., Riley, R. J. (2002) The influence of non-specific microsomal binding on apparent intrinsic clearance, and its prediction from physicochemical properties. *Drug Metab. Dispos*. **30**: 1497–1503

Ballard, P., Leahy, D. E., Rowland, M. (2003) Prediction of *in vivo* tissue distribution from *in vitro* data. 3. Correlation between *in vitro* and *in vivo* tissue distribution of a homologous series of nine 5-n-alkyl-5-ethyl barbituric acids. *Pharm. Res*. **20**: 864–872

Berezhkovskiy, L. M. (2004a) Determination of volume of distribution at steady with complete consideration of the kinetics of protein and tissue binding in linear pharmacokinetics. *J. Pharm. Sci*. **93**: 364–374

Berezhkovskiy, L. M. (2004b) Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. *J. Pharm. Sci*. **93**: 1628–1640

Björkman, S. (2002) Prediction of the volume of distribution of a drug: which tissue-plasma partition coefficients are needed? *J. Pharm. Pharmacol*. **54**: 1237–1245

Björkman, S. (2003) Reduction and lumping of physiologically based pharmacokinetic models: Prediction of the disposition of fentanyl and pethidine in humans by successively simplified models. *J. Pharmacokinet. Pharmacodyn*. **30**: 285–307

Björkman, S. (2004) Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: theophylline and midazolam as model drugs. *Br. J. Clin. Pharmacol*. **59**: 691–704

Björkman, S., Wada, D. R., Berling, B.-M., Benoni, G. (2001) Prediction of the disposition of midazolam in surgical patients by a physiologically based pharmacokinetic model. *J. Pharm. Sci*. **90**: 1226–1241

Boobis, A., Gundert-Remy, U., Kremers, P., Macheras, P., Pelkonen, O. (2002) *In silico* prediction of ADME and pharmacokinetics. Report of an expert meeting organized by COST B15. *Eur. J. Pharm. Sci*. **17**: 183–193

Boxenbaum, H. (1982) Interspecies scaling, allometry, physiological time, and ground plan of pharmacokinetics. *J. Pharmacokinet. Biopharm*. **10**: 201–227

Caldwell, G. W., Masucci, J.A., Yan, Z., Hageman, W. (2004) Allometric scaling of pharmacokinetic parameters in drug discovery: can human CL, V_{ss} and tbe predicted from *in-vivo* rat data? *Eur. J. Drug Metab. Pharmacokinet*. **29**: 133–143

Davies, B., Morris, T. (1993) Physiological parameters in laboratory animals and humans. *Pharm. Res*. **10**: 1093–1099

Doran, A., Obach, R. S., Smith, B. J., Hosea, N. A., Becker, S., Callegari, E., Chen, C., Chen, X., Choo, E., Cianfrogna, J., Cox, L. M., Gibbs, J. P., Gibbs, M. A., Hatch, H., Hop, C. E., Kasman, I. N., Laperle, J., Liu, J., Liu, X., Logman, M., Maclin, D., Nedza, F. M., Nelson, F., Olson, E., Rahematpura, S., Raunig, D., Rogers, S., Schmidt, K., Spracklin, D. K., Szewc, M., Troutman, M., Tseng, E., Tu, M., Van Deusen, J. W., Venkatakrishnan, K., Walens, G., Wang, E. Q., Wong, D., Yasgar, A. S., Zhang, C. (2005) The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. *Drug Metab. Dispos*. **33**: 165–174

Fagerholm, U. (2007a) Prediction of human pharmacokinetics – evaluation of methods for prediction of hepatic metabolic clearance. *J. Pharm. Pharmacol*. **59**: 803–828

Fagerholm, U. (2007b) Prediction of human pharmacokinetics – gastrointestinal absorption. *J. Pharm. Pharmacol*. **59**: 905–916

Fagerholm, U., Björnsson, M. A. (2005) Clinical pharmacokinetics of the cyclooxygenase-inhibiting nitric oxide donating donator (CINOD) AZD3582. *J. Pharm. Pharmacol*. **57**: 1539–1554

Hardman, J. G., Limbird, L. E., Goodman, L. S, Gilman, A. G. (eds) *Goodman & Gilman's the pharmacological basis of therapeutics*. McGraw Hill, UK

Ito, K., Houston, J. B. (2004) Comparison of the use of liver models for predicting drug clearance using *in vitro* kinetic data from hepatic microsomes and isolated hepatocytes. *Pharm. Res*. **21**: 785–792

Iwatsubo, T., Hirota, N., Ooie, T., Suzuki, H., Sugiyama, Y. (1996) Prediction of *in vivo* drug disposition from *in vitro* data based on physiological pharmacokinetics. *Biopharm. Drug Dispos*. **17**: 273–310

Kawakami, J., Yamamoto, K., Sawada, Y., Iga, T. (1994) Prediction of brain delivery of ofloxacin, a new quinolone, in the human from animal data. *J. Pharmacokinet. Biopharm*. **22**: 207–227

Lin, J. H. (1995) Species similarities and differences in pharmacokinetics. *Drug Metab. Dispos*. **23**: 1008–1021

Lombardo, F., Obach, R. S., Shalaeva, M. Y., Gao, F. (2004) Prediction of human volume of distribution values for neutral and basic drugs. 2. Extended data set and leave-class-out statistics. *J. Med. Chem*. **47**: 1242–1250

Lombardo, F., Obach, R. S., DiCapua, F. M., Bakken, G. A., Potter, D. M., Gao, F., Miller, M. D., Zhang, Y. (2006) A hybrid mixture discriminant analysis — random forest computational model for the prediction of volume of distribution of drugs in human. *J. Med. Chem*. **49**: 2262–2267

Mahmood, I. (1998) Interspecies scaling: predicting volumes, mean residence time and elimination half-life. Some suggestions. *J. Pharm. Pharmacol*. **50**: 493–499

Mahmood, I., Balian, J. D. (1996) Interspecies scaling: a comparative study for the prediction of clearance and volume using two or more than two species. *Life Sci*. **59**: 579–585

Mansor, S. M., Ward, S. A., Edwards, G., Hoaksey, P. E., Breckenridge, A. M. (1991) The influence of alpha 1-acid glycoprotein on quinine and quinidine disposition in the rat isolated perfused liver preparation. *J. Pharm. Pharmacol*. **43**: 650–654

Mordenti, J. (1986) Man versus beast: pharmacokinetic scaling in mammals. *J. Pharm. Sci*. **75**: 1028–1040

Naccache, P., Sha'afi, R. I. (1973) Patterns of nonelectrolyte permeability in human red blood cell membrane. *J. Gen. Physiol*. **62**: 714–736

Obach, R. S. (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

Obach, R. S., Baxter, J. G., Liston, T. E., Silber, B. M., Jones, B. C., MacIntyre, F, Rance, D. J., Wastall, P. (1997) The prediction of human pharmacokinetic parameters from preclinical and *in vitro* metabolism data. *J. Pharmacol. Exp. Ther*. **283**: 46–58

Poulin, P., Theil, F.-P. (2001) Prediction of adipose tissue: plasma partition coefficients for structurally unrelated drugs. *J. Pharm. Sci*. **90**: 436–447

Poulin, P., Theil, F.-P. (2002) Prediction of pharmacokinetics prior to *in vivo* studies. 1. Mechanism-based prediction of volume of distribution. *J. Pharm. Sci*. **91**: 129–156

Roberts, M. S., Magnusson, B. M., Burczynski, F. J., Weiss, M. (2002) Enterohepatic circulation. Physiological, pharmacokinetic and clinical implications. *Clin. Pharmacokinet*. **41**: 751–790

Rodgers, T., Rowland, M. (2006) Physiologically based pharmacokinetic modelling 2: Predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *J. Pharm. Sci*. **95**: 1238–1257

Rodgers, T., Leahy, D., Rowland, M. (2005a) Tissue distribution of basic drugs: Accounting for enantiomeric, compound and regional differences amongst β-blocking drugs in rat. *J. Pharm. Sci*. **94**: 1237–1248

Rodgers, T., Leahy, D., Rowland, M. (2005b) Physiologically based pharmacokinetic modelling 1: Predicting the tissue distribution of moderate-to-strong bases. *J. Pharm. Sci*. **94**: 1259–1276

Sahin, S., Rowland, M. (2004) Effect of erythrocytes on the hepatic distribution kinetics of antipyrine. *Eur. J. Drug Metab. Pharmacokinet*. **29**: 37–41

Saiakhov, R. D., Stefan, L. R., Klopman, G. (2000) Multiple computer-automated structure evaluation model of the plasma protein binding of diverse drugs. *Perspect. Drug Disc. Design* **19**: 133–155

Sawada, Y., Hanano, M. Sugiyama, Y., Harashima, H., Iga, T. (1984) Prediction of the volumes of distribution of basic drugs in humans based on data from animals. *J. Pharmacokinet. Biopharm.* **12**: 587–596

Sawada, Y., Hanano, M., Sugiyama,Y., Iga T. (1985) Prediction of the disposition of nine weakly acidic and six weakly basic drugs in humans from pharmacokinetic parameters in rats. *J. Pharmacokinet. Biopharm*. **13**: 477–492

Schanker, L. S., Nafpliotis, P. A., Johnson, J. M. (1961) Passage of organic bases into human red cells. *J. Pharmacol. Ther*. **133**: 325–331

Shibata, Y., Takahashi, H., Ishii, Y. (2000) A convenient *in vitro* screening method for predicting *in vivo* drug metabolic clearance using isolated hepatocytes suspended in serum. *Drug Metab. Dispos*. **28**: 1518–1523

Shibata, Y., Takahashi, H., Chiba, M., 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

Siebert, G. A., Hung, D. Y., Chang, P., Roberts, M. S. (2004) Ion-trapping, microsomal binding, and unbound drug distribution in the hepatic retention of basic drugs. *J. Pharmacol. Exp. Ther*. **308**: 228–235

Smith, D. A., Jones, B. C., Walker, D. K. (1996) Design of drugs involving the concepts and theories of drug metabolism and pharmacokinetics. *Med. Res. Rev*. **16**: 243–266

Tang, H., Mayersohn, M. (2005) A novel model for prediction of human drug clearance by allometric scaling. *Drug Metab. Dispos*. **33**: 1297–1303

Theil, F.-P., Guentert, T. W., Haddad, S., Poulin, P. (2003) Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection. *Toxicol. Lett*. **138**: 29–49

Tillement, J.-P., Houin, G., Zini, R., Urien, S., Albengres, E., Barré, J., Lecomte, M., D'Athis, P., Sebille, B. (1984) The binding of drugs to blood plasma macromolecules: Recent advances and therapeutic significance. *Adv. Drug Res*. **13**: 59–94

van de Waterbeemd, H. (2005) Which *in vitro* screens guide the prediction of oral absorption and volume of distribution? *Basic Clin. Pharmacol. Toxicol*. **96**: 162–166

Wajima, T., Fukumura, K., Yano, Y., Oguma, T. (2003) Prediction of human pharmacokinetics from animal data and molecular structural parameters using multivariate regression analysis: volume of distribution at steady state. *J. Pharm. Pharmacol*. **55**: 939–949

Ward, K. W., Smith, B. R. (2004) A comprehensive quantitative and qualitative evaluation of extrapolation of intravenous pharmacokinetic parameters from rat, dog, and monkey to humans. II. Volume of distribution and mean residence time. *Drug. Metab. Dispos*. **32**: 612–619