

The Prediction of Human Pharmacokinetic Parameters from Preclinical and *In Vitro* Metabolism Data

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ABSTRACT

We describe a comprehensive retrospective analysis in which the abilities of several methods by which human pharmacokinetic parameters are predicted from preclinical pharmacokinetic data and/or *in vitro* metabolism data were assessed. The prediction methods examined included both methods from the scientific literature as well as some described in this report for the first time. Four methods were examined for their ability to predict human volume of distribution. Three were highly predictive, yielding, on average, predictions that were within 60% to 90% of actual values. Twelve methods were assessed for their utility in predicting clearance. The most successful allometric scaling method yielded clearance predictions that were, on average, within 80% of actual values. The best methods in which *in vitro* metabolism data from human liver microsomes were scaled to *in vivo* clearance values yielded predicted clear-

ance values that were, on average, within 70% to 80% of actual values. Human $t_{1/2}$ was predicted by combining predictions of human volume of distribution and clearance. The best $t_{1/2}$ prediction methods successfully assigned compounds to appropriate dosing regimen categories (e.g., once daily, twice daily and so forth) 70% to 80% of the time. In addition, correlations between human $t_{1/2}$ and $t_{1/2}$ values from preclinical species were also generally successful (72–87%) when used to predict human dosing regimens. In summary, this retrospective analysis has identified several approaches by which human pharmacokinetic data can be predicted from preclinical data. Such approaches should find utility in the drug discovery and development processes in the identification and selection of compounds that will possess appropriate pharmacokinetic characteristics in humans for progression to clinical trials.

The process by which new drug candidates are discovered and developed is both time consuming and expensive (DiMasi, 1994; DiMasi *et al.*, 1994). This is due in part to the high rate of attrition of drug candidates that enter clinical development, such that only ~10% of drug candidates that are selected for clinical development eventually become marketed drugs. In analyzing the reasons for attrition of drug candidates that enter clinical development, it has been reported that the clinical development of 40% of drug candidates was discontinued due to unacceptable pharmacokinetic properties (Prentis *et al.*, 1988).

These observations strongly suggest that the process by which new drugs are discovered and developed could benefit greatly if drug candidates were advanced to clinical development when predicted human pharmacokinetic characteristics were deemed to be acceptable (e.g., oral bioavailability and duration of exposure are projected to be appropriate for conducting pivotal efficacy studies). Thus, the development

and application of reliable methods to predict human drug disposition may decrease the overall attrition of drug candidates during clinical development by decreasing the number of candidates lost due to unacceptable pharmacokinetic characteristics. Furthermore, the eventual clinical utility as well as market success of a newly approved drug could be maximized by selecting for development only those compounds with optimal, rather than acceptable, pharmacokinetic characteristics for the intended therapeutic use.

The best described technique to predict human pharmacokinetics from *in vivo* preclinical pharmacokinetic data is allometric scaling. In its original form, allometry was a technique developed to explain observed relationships between organ size and body weight of mammals (Dedrick *et al.*, 1970; Mordenti, 1986). Additional studies demonstrated further relationships between mammalian body weight and physiological parameters. Considerations of the relationship between drug elimination and physiological parameters such as hepatic or renal blood flow inevitably led to the application of allometric scaling in correlating human pharmacokinetics

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ABBREVIATIONS: f_{ut} , fraction unbound in tissues; f_u , unbound fraction in plasma (or serum); VD_{ss} , steady state volume of distribution (in liters/kg); V_p , plasma volume (in liters/kg); V_e , extracellular fluid volume (in liters/kg); V_r , "remainder of the fluid" volume (in liters/kg); R_d/i , ratio of binding proteins in extracellular fluid (except plasma) to binding proteins in plasma; CL, clearance; F, oral bioavailability; MLP, maximum lifespan potential.

with pharmacokinetic parameters in preclinical species (Boxenbaum, 1982, 1984). Allometric scaling of pharmacokinetic data typically focuses on interspecies relationships between clearance or volume of distribution of unbound drug and species body weight; the relationships for these parameters established in preclinical species are then extrapolated to humans, allowing for predictions of human clearance and volume of distribution. Although a number of physiologically rather than allometrically based approaches have also been developed for interspecies scaling of pharmacokinetic data (Iwatsubo *et al.*, 1996; Suzuki *et al.*, 1995), allometry continues to be the most widely used approach due to its simplicity.

In recent years, there has been a resurgence in the use of allometric scaling to establish relationships among preclinical species and humans for both compounds that are metabolically and nonmetabolically cleared (Boxenbaum and DiLea, 1995; Mahmood and Balian, 1995, 1996a, 1996b). The major drawback in allometric scaling is its empirical nature. For example, traditional allometric scaling of plasma clearance does not allow for an understanding of species differences in pathways of metabolic clearance that may have significant impact on the ability to accurately extrapolate human clearance from preclinical data. However, recent publications have proposed novel methods of combining allometric scaling with knowledge of species differences in metabolism derived from *in vitro* metabolism data to improve the utility of allometry for compounds prone to major species differences in metabolism (Lave *et al.*, 1995, 1996a, 1996b; Ubeaud *et al.*, 1995).

Methods by which *in vivo* clearance can be predicted from *in vitro* data were first described ~20 years ago (Rane *et al.*, 1977). The methodologies and mathematics behind approaches to predict *in vivo* clearance from intrinsic clearance data have been summarized in a recent review by Houston (1994). Although the data described by Houston are from rat, the principles described are applicable to other species, including humans (Iwatsubo *et al.*, 1997). In the seminal work by Rane *et al.* (1977), it was demonstrated that the extent of hepatic extraction of several drugs in rats could be estimated from enzyme kinetic parameters of the oxidative biotransformation of these drugs in rat liver microsomes. The concept of an *in vitro/in vivo* correlation that included data from both human and preclinical species was reduced to practice for felodipine 10 years later (Baarnhielm *et al.*, 1986). Various *in vitro* systems are available to obtain hepatic intrinsic clearance data; those most commonly used are liver microsomes, hepatocytes and precision-cut liver slices. Each system possesses unique advantages and disadvantages in both ease of use and accuracy and completeness of the data obtained. In general, for kinetic experiments, such as determination of intrinsic clearance, the body of data available suggest that hepatocytes are a superior method with regard to accurate predictions of *in vivo* data, with microsomes also providing good data (Ashforth *et al.*, 1995; Hayes *et al.*, 1995; Vickers *et al.*, 1993; Zomorodi *et al.*, 1995).

In this article, we describe a comprehensive retrospective analysis of preclinical pharmacokinetic and *in vitro* metabolism data accrued over a 14-year period for Pfizer proprietary compounds. The compounds in the data set used for this analysis cover a broad range of small-molecule (*e.g.*, molecular weight <600) organic compounds designed for therapeutic use in a variety of disease states. Thus, use of this data set

presents a great challenge to pharmacokinetic prediction methods because each method must not only be applicable to a close-in homologous series of compounds but also be broadly applicable to compounds of all types and physicochemical properties. These data were used in several methods, described herein, designed to predict the pharmacokinetics (clearance, volume of distribution, $t_{1/2}$ and oral bioavailability) of drugs in humans. The methods include a battery of *in vitro*, *in vivo* and combined *in vivo/in vitro* approaches both obtained from the scientific literature and described for the first time here. A comparison of the predicted values to authentic human pharmacokinetic data was made to compare the accuracies and uses of these prediction methods.

Methods

Sources of Pharmacokinetic and *In Vitro* Data

The original pool of compounds included in this analysis were all of those brought into preclinical development at Pfizer over a 14-year period from 1981 through 1994 ($n = 83$). From this set, those compounds for which no human data were available were removed ($n = 30$). Another three were excluded because they were developed as prodrugs. Thus, the data used in this analysis included all available preclinical pharmacokinetic and *in vitro* metabolism data for those compounds for which a minimum of a human *in vivo* $t_{1/2}$ value was available ($n = 50$; table 1). The amount of preclinical data available for each compound ranged from extensive (in which case, all prediction methods could be tested) to scant (in which case, only one or two prediction methods could be applied). Human *in vivo* clearance and oral bioavailability data used for a given compound were from the lowest dose in which sufficient plasma concentration-*vs.*-time data were available to adequately describe the terminal phase. This was done to minimize the potential of including CL and F values that could be confounded by saturation of CL and/or F or limitations on oral absorption at high doses.

Methods for Predicting Human Volume of Distribution

Four methods were examined for their ability to accurately and successfully predict human volume of distribution (table 2): (1) a method in which an average fraction unbound in tissue in preclinical species is used with human plasma protein binding data to calculate human VD_{ss} (method V1), (2) a method in which a proportionality is established between VD_{ss} and f_u in dog and human (method V2) and (3) allometric scaling without (method 3a) and with (method 3b) considerations for interspecies differences in plasma protein binding. This yielded a total of four methods, which are further described below.

Average fraction unbound in tissues method (method V1).

In this method, experimentally determined values for volume of distribution (in units of liters/kg) and plasma protein binding for each species were used, along with standard values for extracellular fluid volumes, plasma volumes and so forth, to calculate the fraction unbound in tissues in animal species. The following equation, which is a rearranged form of one previously described by Oie and Tozer (1979), was used to calculate the fraction unbound in tissues for each preclinical species for each compound:

$$f_{ut} = \frac{V_r f_u}{[VD_{ss} - V_p - (f_u V_e)] - \left[(1 - f_u) \frac{R_e}{i} V_p \right]} \quad (1)$$

Table 3 contains the values used for each of these parameters in preclinical species and humans in method V1.

After f_{ut} was calculated for each of the preclinical species, all values for a given compound were averaged. This averaged animal

TABLE 1
Summary of pharmacokinetic and physicochemical properties of 50 compounds examined^a

Compound No.	Molecular weight	Acid, base or neutral	Lipophilicity	CL	VD _{ss}	t _{1/2}	F	Plasma f _u	Urinary excretion
			<i>clogP</i>	<i>ml/min/kg</i>	<i>liter/kg</i>	<i>hr</i>	<i>%</i>		<i>%</i>
1	454	Base	6.99			16		0.01	
2	241	Base	2.91			0.9			
3	222	Base	1.48	4.0	0.7	3.5	20	0.12	<2
4	311	Base	3.90			3.8		0.03	
5	412	Base	4.42	12	2.3	2.8	59	0.001	<1
6	296	Base	3.46	15	6.6	4.7	1.0	0.19	<1
7	404	Acid	0.91			1.9		0.51	60
8	380	Base	4.10			7.4		0.07	
9	321	Neutral	5.10			1.2			
10	387	Base	5.97			30			<1
11	339	Acid	4.80			1.3		0.09	
12	262	Neutral	0.62			40		0.55	
13	291	Acid	2.67			5.5		0.02	
14	369	Acid	1.56			2.3		0.01	
15	620	Neutral	4.31			1.5		0.11	
16	740	Neutral	1.83			45			
17	329	Base	0.19	21	1.5	1.1	4.6	0.60	<1
18	327	Base	1.81	16	5.5	4.3		0.60	
19	375	Base	4.37			41		0.08	
20	414	Base	5.50			1.0		0.07	
21	236	Neutral	0.64			43			
22	419	Acid	2.35			27		0.006	
23	749	Base	1.83			68		0.93	6
24	342	Base	5.35			26		0.02	>1
25	320	Acid	4.69	0.1	0.1	26	89	0.001	<2
26	331	Acid	2.70			45		0.007	10
27	338	Acid	4.84			45		0.001	<1
28	452	Base	-0.56			11	70	0.28	10
29	373	Acid	5.59			25		0.005	<1
30	428	Acid	5.53			400		0.005	<1
31	465	Acid	4.61			30		0.01	<1
32	318	Neutral	2.06			2.3		0.08	<1
33	299	Base	6.09			1.0		0.004	<1
34	451	Base	3.82	1.2	1.0	11	69	0.01	<1
35	283	Acid	2.04	7.6	0.4	0.6	70	0.16	47
36	408	Base	2.78	7.0	21.0	35	64	0.03	<1
37	306	Neutral	-0.11	0.3	0.7	26	80	0.89	72
38	283	Acid	4.02			0.9			
39	395	Base	4.00	8.0	15.1	27		0.02	<1
40	253	Base	1.69	3.2	1.5	5.4	93	0.43	20
41	376	Base	1.53	5.9	9.0	2.4		0.02	59
42	441	Base	1.58	4.3	2.8	7.6	83	0.36	65
43	399	Acid	0.18	2.3	3.4	1.6		0.01	61
44	474	Base	2.28	9.8	1.5	4.0	41	0.04	1
45	439	Base	2.03			3.2		0.12	<1
46	418	Base	3.08	5.9	2.1	4.1	46	0.12	8
47	497	Acid	7.21			16		0.001	<1
48	582	Base	5.22			2.5		0.01	
49	415	Base	5.44			33		0.002	
50	426	Base	3.66			3.0		0.08	<1

^a A blank entry indicates no data available.

value for f_{ut} is assumed to be equal to f_{ut} in humans and, along with the value experimentally determined for human f_u (fraction unbound in human serum/plasma), was used in the prediction of human VD_{ss} (in units of liters/kg) using the following equation (rearranged version of equation 1) and using appropriate human values for V_p, R_e/i and so forth:

$$VD_{(human\ prediction)} = V_p + [f_{u(human)} \cdot V_e] + \left\{ \left[1 - f_{u(human)} \right] \cdot \frac{R_e}{i} \cdot V_p \right\} + V_r \cdot \frac{f_{u(human)}}{f_{u(average)}} \quad (2)$$

Proportionality (method V2). This method simply states that a proportionality could be set up between the free-fraction of drug in

plasma in dog and human and the volume of distribution in these two species. [In other words, free VD_(human) = free VD_(dog).] Implicit to this method was the assumption that tissue binding of drugs is similar in dogs and humans and that physiological parameters, such as extracellular fluid volumes, are similar between the two species on a per-weight basis. Solving for the human volume of distribution (in units of liters/kg) yielded the following equation:

$$VD_{(human\ prediction)} = \frac{f_{u(human)} \cdot VD_{(dog)}}{f_{u(dog)}} \quad (3)$$

where the term f_u designated the fraction of drug unbound in the plasma (or serum) of dog or human, and VD_(dog) represented the volume of distribution at steady state in dog (in units of liters/kg).

TABLE 2

Summary of pharmacokinetic prediction methods

Method	Abbreviation in text	Data required	Underlying assumptions
A. Volume of distributions			
Average fraction unbound in tissues	V1	Plasma protein binding in two or more species and human Intravenous pharmacokinetics in two or more species	Average $f_{ut(\text{preclinical species})} = f_{ut(\text{human})}$ R_e/i is uniform across species and is the same for all binding proteins
Dog-human proportionality	V2	Plasma protein binding in dog and human Intravenous pharmacokinetics in dog	$f_{ut(\text{dog})} = f_{ut(\text{human})}$
Allometric scaling, excluding interspecies protein binding differences	V3a	Intravenous pharmacokinetic data in two or more species	No intrinsic differences in plasma protein or tissue binding across preclinical species and human
Allometric scaling, including interspecies protein binding differences	V3b	Intravenous pharmacokinetic data in two or more species Plasma protein binding in two or more species and human	No intrinsic differences in tissue binding across preclinical species and human
B. Clearance			
<i>In vitro</i> $t_{1/2}$, excluding protein binding, well-stirred model	C1a	Turnover rate in human <i>in vitro</i> system	<i>In vitro</i> rates and activities are representative of those that occur <i>in vivo</i>
<i>In vitro</i> $t_{1/2}$, including protein binding, well-stirred model	C1b	Plasma protein binding in human Turnover rate in human <i>in vitro</i> system	Liver is major organ of CL $CL_{\text{metabolism}} \gg CL_{\text{renal}} + CL_{\text{biliary}}$ Oxidative microsomal metabolism $\gg$ other metabolism
<i>In vitro</i> $t_{1/2}$, excluding protein binding, parallel tube model	C1c	Turnover rate in human <i>in vitro</i> system	$f_{u(\text{incubation matrix})} = \text{unity}$ $[S] < K_M$
<i>In vitro</i> $t_{1/2}$, including protein binding, parallel tube model	C1d	Plasma protein binding in human Turnover rate in human <i>in vitro</i> system	No inactivation of enzyme Equilibrium not approached
Enzyme kinetics, excluding f_u , well-stirred model	C2a	Substrate saturation experiment in human <i>in vitro</i> system (V_{max}/K_M)	<i>In vitro</i> rates and activities are representative of those that occur <i>in vivo</i>
Enzyme kinetics, including f_u , well-stirred model	C2b	Substrate saturation experiment in human <i>in vitro</i> system (V_{max}/K_M) Plasma protein binding in human	Liver is major organ of CL $CL_{\text{metabolism}} \gg CL_{\text{renal}} + CL_{\text{biliary}}$
Enzyme kinetics, excluding f_u , parallel tube model	C2c	Substrate saturation experiment in human <i>in vitro</i> system (V_{max}/K_M)	Oxidative microsomal metabolism $\gg$ other metabolism
Enzyme kinetics, including f_u , parallel tube model	C2d	Substrate saturation experiment in human <i>in vitro</i> system (V_{max}/K_M) Plasma protein binding in human	$f_{u(\text{incubation matrix})} = \text{unity}$ No inactivation of enzyme
Allometric scaling, including interspecies f_u and MLP differences	C3a	Plasma protein binding in two or more species and human Intravenous pharmacokinetics in two or more species	Mechanism of CL is similar across species Assumes no interspecies differences in intrinsic CL
Allometric scaling, excluding interspecies f_u differences, including MLP differences	C3b	Intravenous pharmacokinetics in two or more species	
Allometric scaling, including interspecies f_u differences, excluding MLP differences	C3c	Plasma protein binding in two or more species and human Intravenous pharmacokinetics in two or more species	
Allometric scaling, excluding interspecies f_u and MLP differences	C3d	Intravenous pharmacokinetics in two or more species	
C. $t_{1/2}$ and oral bioavailability			
Human vs. monkey	T1	Intravenous pharmacokinetics in monkey	Empirical approach; assumes uniform intrinsic properties between preclinical species and humans
Human vs. dog	T2	Intravenous pharmacokinetics in dog	
Human vs. rat	T3	Intravenous pharmacokinetics in rat	
Combinations of volume and CL predictions	$T_{v(x)c(x)}$	Data for particular CL and volume prediction methods	Same assumptions for individual VD and CL prediction methods VD _{ss} prediction inappropriate for $t_{1/2}$ prediction if multicompartmental pharmacokinetic behavior is anticipated
Corresponding CL methods	$F_{c(x)}$	Data for particular CL methods	Same assumptions for individual CL prediction methods Fraction absorbed is unity and no first-pass extraction by intestinal mucosa

TABLE 3
Values used for physiological constants in selected preclinical species and humans

Species	V_p	V_e	V_r	R_e/i^a	Body weight	$\log_{10}$ body weight	MLP
		liters/kg			kg		years
Mouse	N.A.	N.A.	N.A.	N.A.	0.02	-1.70	2.7
Rat	0.0313	0.265	0.364	1.4	0.25	-0.60	4.7
Guinea pig	0.0313	0.265	0.364	1.4	0.5	-0.30	6.7
Rabbit	0.0314	0.179	0.322	1.4	3.0	0.48	8.0
Monkey	0.0448	0.208	0.485	1.4	3.5	0.54	20
Dog	0.0515	0.216	0.450	1.4	12.5	1.10	20
Human	0.0436	0.151	0.380	1.4	70	1.84	93

Some values were from Davies and Morris (1993) and Oie and Tozer (1979).
NA, not available.

^a R_e/i was assumed to be 1.4 for all species and all binding proteins.

Allometry without protein binding (method V3a). In allometric scaling of volume of distribution, the physiological parameter used in the scaling was total body weight (Boxenbaum, 1982). In this method, plots were constructed of total volume of distribution in preclinical species (in units of liters per animal) *vs.* animal body weight (table 3) on a log-log scale for each compound in the analysis. Allometric equations in the form:

$$\log_{10} VD = a \cdot \log_{10} \text{body weight}_{(\text{kg})} + b \quad (4)$$

were obtained by linear regression of the data points to determine the values a and b for each compound. These were then used, along with a standard value for human body weight (70 kg), to predict human volumes of distribution.

Allometry corrected for protein binding (method V3b). An identical approach was taken as described above except that animal volume of distribution values were corrected for plasma protein binding using the following equation:

$$VD_{\text{free}} = \frac{VD_{\text{total}}}{f_u} \quad (5)$$

to yield free volumes of distribution. These values were then plotted as in method V3a to determine the allometric relationship for free volume of distribution *vs.* total body weight. The projected human free volume of distribution was then converted to total volume of distribution by $VD_{\text{free(human)}} \cdot f_{u(\text{human})}$.

Methods for Predicting Human Clearance

Three approaches were examined for their ability to accurately and successfully predict human CL, with each approach possessing important variations, leading to a total of 12 prediction methods (table 2): (1) methods in which first-order consumption of parent drug was monitored in liver microsomal incubations to yield *in vitro* $t_{1/2}$ values (methods C1a–C1d), (2) methods in which V_{max} and K_{Mapp} were determined and used in the calculation of CL'_{int} (methods C2a–C2d) and (3) allometric scaling methods with and without considerations of interspecies differences in plasma protein binding and/or MLP (methods C3a–C3d).

***In vitro* $t_{1/2}$ methods.** With methods C1a, C1b, C1c and C1d, values for intrinsic CL (CL'_{int}) were calculated from *in vitro* $t_{1/2}$ data obtained in an appropriate system (*e.g.*, liver microsomes), which were then scaled up to represent the CL expected in an entire organism. The fundamental basis behind this simple approach lies in the derivation of the integrated Michaelis-Menten equation (Segel, 1975):

$$V_m \cdot dt = - \frac{K_{\text{Mapp}} + [S]}{[S]} \cdot d[S] \quad (6)$$

Over one $t_{1/2}$ (*i.e.*, when $[S] = 0.5[S]_{t=0}$, the following equation applies:

$$\frac{V_m \cdot t_{1/2}}{K_{\text{Mapp}}} = 0.693 + \frac{0.5[S]_{t=0}}{K_{\text{Mapp}}} \quad (7)$$

A necessary assumption in this approach, which is included in the experimental design, is that the substrate concentration used is well below the K_{Mapp} value, such that:

$$\frac{0.5[S]}{K_{\text{Mapp}}} \ll 0.693 \quad (8)$$

Thus, the equation degenerates to:

$$\frac{V_m \cdot t_{1/2}}{K_{\text{Mapp}}} = 0.693 \quad (9)$$

$$\frac{V_m}{K_{\text{Mapp}}} = \frac{0.693}{t_{1/2}} = CL'_{\text{int}} \quad (10)$$

The *in vitro* $t_{1/2}$ is incorporated into the following equation:

$$CL'_{\text{int}} = \frac{0.693 \cdot \text{liver weight}}{\text{in vitro } t_{1/2} \cdot \text{liver in incubation} \cdot f_{u(\text{inc})}} \quad (11)$$

where *in vitro* $t_{1/2}$ is in min, liver weight is in g/kg of body weight and liver in incubation refers to the g of liver/ml in the incubation, resulting in units of ml/min/kg for CL'_{int} . The “liver in incubation” value was calculated from the amount of protein in the incubation and a scale-up factor from protein to g of liver. [For microsomes, this scale-up factor is 45 mg/g of liver (Houston, 1994).] This equation indicates that a value for binding to protein in the incubation be included, however, in this treatment, it was assumed to be zero (*i.e.*, $f_{u(\text{inc})} = 1$; see Discussion). Thus, the intrinsic CL values calculated were based on total concentrations, not free concentrations in the incubation. Full expansion of equation 11 yields the following:

$$CL'_{\text{int}} = 0.693 \cdot \frac{1}{t_{1/2}(\text{min})} \cdot \frac{\text{g of liver weight}}{\text{kg of body weight}} \cdot \frac{\text{ml incubation}}{\text{mg of microsomal protein}} \cdot \frac{45 \text{mg of microsomal protein}}{\text{g of liver weight}} \quad (12)$$

Conversion of intrinsic CL to CL involved the use of equations describing the well-stirred (equation 13) and parallel tube (equation 14) models of hepatic CL (Pang and Rowland, 1977; Wilkinson and Shand, 1975):

$$CL_p = \frac{Q \cdot f_u \cdot CL'_{\text{int}}}{Q + f_u \cdot CL'_{\text{int}}} \quad (13)$$

$$CL_p = Q \cdot \left(1 - e^{-\frac{CL'_{\text{int}} \cdot f_u}{Q}} \right) \quad (14)$$

where Q is hepatic blood flow, and f_u is the free fraction in blood. Values of 20 ml/min/kg for hepatic blood flow and 20 g of liver/kg of body weight were used in these calculations. Also, when the blood/plasma ratio was known to significantly differ from unity, plasma (or serum) CL values were converted to blood CL values by correcting with the blood/plasma ratio:

$$CL_{\text{bl}} = \frac{CL_p}{B/P} \quad (15)$$

where CL_{bl} represents CL in whole blood, and B/P is the blood to plasma concentration ratio.

Methods C1b and C1d use equations 13 and 14, respectively, as written above. Methods C1a and C1c use equations 16 and 17, which represent variations on equations 13 and 14 in which fraction unbound (f_u) was removed:

$$CL_p = \frac{Q \cdot CL'_{int}}{Q + CL'_{int}} \quad (16)$$

$$CL_p = Q \cdot \left(1 - e^{-\frac{CL'_{int}}{Q}}\right) \quad (17)$$

Enzyme kinetic methods. With methods C2a, C2b, C2c and C2d, the enzyme kinetic parameters K_{Mapp} and V_{max} measured in liver microsomal incubations were used to define intrinsic CL as:

$$CL'_{int} = \frac{V_{max}}{K_{Mapp}} \quad (18)$$

Intrinsic CL was scaled-up to predictions of CL as described above. Both the well-stirred and parallel tube models of hepatic CL (equations 13, 14, 16 and 17) were applied. Methods C2a and C2c disregarded the impact of protein binding (equations 16 and 17, respectively), whereas methods C2b and C2d included this parameter in the prediction (equations 13 and 14, respectively). As with the *in vitro* $t_{1/2}$ methods, a standard value of 45 mg of microsomal protein/g of liver weight was used in the scale-up of *in vitro* intrinsic CL data, and values of 20 g of liver/kg of body weight and 20 ml/min/kg hepatic blood flow were also used.

Allometric scaling with protein binding and MLP correction factor (method C3a). In allometric scaling of CL, the physiological parameter used in the scaling was total body weight. In the case of this method, corrections for interspecies differences in both plasma protein binding and MLP (Boxenbaum, 1982) were applied. For plasma protein binding, free CL is defined as:

$$CL_{p(free)} = \frac{CL_{p(total)}}{f_u} \quad (19)$$

The values of $CL_{p(free)}$ were then corrected for interspecies differences in MLP: $[CL_{p(free)}/MLP]$ for the various species. A list of MLP values used for the species are given in table 3. The $\log_{10}[CL_{p(free)}]$ (in units/MLP) was plotted *vs.* $\log_{10}(\text{body weight})$ for each individual compound. The functions obtained for each compound were subject to linear regression (to obtain the expression $\log_{10}CL_p = a \cdot \log_{10}\text{body weight} + b$), the values for $CL_{p(free)}$ for human, per MLP, were projected from the regression, and the total CL values were calculated using the values for plasma protein binding in humans and human MLP.

Allometric scaling without protein binding and without MLP correction factor (method C3b). This allometric method was carried out as described above using total CL and body weight, with no correction for interspecies differences in MLP.

Allometric scaling with protein binding and without MLP correction factor (method C3c). This allometric method was carried out as described in C3b using free CL values and body weight, with no correction for interspecies differences in MLP.

Allometric scaling without protein binding and with MLP correction factor (method C3d). This allometric method was carried out as described in C3a, except that CL values were not converted to free CL values before regression.

Methods for Predicting Human $t_{1/2}$

Two approaches were examined for their ability to accurately and successfully predict human $t_{1/2}$ (table 2): (1) methods that rely on direct correlations between animal and human $t_{1/2}$ values (methods T1–T3) and (2) methods in which individual volume of distribution

and CL predictions are combined to yield $t_{1/2}$ predictions (methods T_{VIC1a} , T_{VIC1b} and so forth).

Animal correlations (methods T1–T3). Assessment of animal/human $t_{1/2}$ correlations were undertaken with a data set containing both data for in-house proprietary compounds and data from the scientific literature for which $t_{1/2}$ data was available for rat, dog, monkey and human. Only compounds with $t_{1/2}$ data for all four species were used in these analyses. To construct correlations, measured $t_{1/2}$ values in rat, dog or monkey were plotted *vs.* human $t_{1/2}$ values, and functions were derived from $1/x$ -weighted linear regression. The predictions of human $t_{1/2}$ were then obtained by inserting the animal $t_{1/2}$ value into the regression equation.

Combinations of human volume and clearance predictions [methods $T_{v(x)c(x)}$]. In this approach, each method for predicting the volume of distribution was combined with each method of predicting CL to generate predictions of human $t_{1/2}$ using the following formula:

$$\text{Predicted human } t_{1/2} = \frac{0.693 \cdot \text{predicted human VD}}{\text{Predicted human } CL_p} \quad (20)$$

All volume and CL combinations were tested, regardless of whether the individual volume and CL methods were originally from different types of approaches (*e.g.*, volume predictions from allometry were combined with CL predictions from *in vitro* data). This provided a total of 48 $t_{1/2}$ prediction methods (four volume prediction methods $\times$ 12 CL prediction methods).

Methods for Predicting Human Oral Bioavailability (Methods F_{C1a} – F_{C3d})

The methods for predicting human oral bioavailability used those described for CL (table 2), with a rearranged equation that accounted only for first-pass hepatic CL and accounted for neither the potential limitations on absorption from the GI tract (*i.e.*, fraction absorbed, F_a , was assumed to be unity) nor potential first pass extraction by the gut wall tissue ($F_g = 1$):

$$F = F_a \cdot F_g \cdot \left(1 - \frac{CL_p}{Q}\right) = 1 \cdot 1 \cdot \left(1 - \frac{CL_p}{Q}\right) \quad (21)$$

Thus, the number of oral bioavailability methods is equal to the number of CL methods (12).

Success criteria. For volume of distribution and CL predictions, success was assessed by the geometric mean of the ratio of predicted and actual values. Thus:

$$\text{Average-fold error} = 10^{\left| \frac{\sum \log \frac{\text{Predicted}}{\text{Actual}}}{N} \right|} \quad (22)$$

This approach prohibited poor overpredictions from being canceled out by equally poor underpredictions; underpredictions were of equal value to overpredictions. It also did not allow any single outlier prediction from biasing conclusions concerning a particular prediction method. A method that predicted all actual values perfectly would have a value of 1; one that made predictions that were on average 2-fold off (100% above or 50% below) would have a value of 2 and so forth. A prediction method with an average -fold error ≤ 2 was considered successful.

For $t_{1/2}$, a similar calculation was made. In addition, a second success criterion was applied that was applicable to drug development and compound selection. In this criterion, the success rate of correctly placing compounds into an appropriate $t_{1/2}$ zone was measured. These predetermined zones were based on dosing regimens associated with half-lives (when considerations of disparate PK/PD relationships and wide therapeutic indices are ignored). The zones were 0 to 4 hr (three times daily), 4 to 12 hr (twice daily), 12 to 48 hr (once daily) and >48 (once daily or less often). In addition, if a compound was predicted to have a value that was outside of the

appropriate zone but the prediction was still within 2-fold of the actual $t_{1/2}$, the prediction was also considered to be successful. The success rate of a $t_{1/2}$ prediction method is simply the number of compounds successfully predicted by the method divided by the total number of predictions made using the method and then multiplied by 100.

For oral bioavailability, success of prediction methods was assessed by the percentage of compounds that were appropriately predicted to be <10%, 10% to 30% and >30%; these zones represent categories of unacceptable, intermediate and satisfactory, respectively, as defined by general decision making criteria typically used in drug discovery and development processes.

Results

Predictions of volume of distribution. There were 16 compounds that had adequate preclinical data and human intravenous pharmacokinetic data suitable for assessment of VD predictions. Predictions of VD by methods V1, V2, V3a and V3b are presented in figure 1, A–D, respectively, and the identities of outlier compounds are indicated.

Method V1 predicted human VD within 2-fold of actual for 14 of 16 compounds (88%; fig. 1A). The simplest approach, method V2, predicted human VD within 2-fold of actual for 13 of 16 compounds (81%; fig. 1B). The accuracy of predictions using V1 or V2 was typically much better than 2-fold as indicated by a geometric mean accuracy value of 1.56 for each method (table 4). Method V3a was the poorest predictor of human VD (fig. 1C) in this analysis in that only 8 of 15 predictions (53%) were within 2-fold of actual. The geometric mean prediction accuracy value for method V3a was 2.78. Method V3b predicted human VD within 2-fold of actual for 10 of 13 compounds (77%; fig. 1D). The geometric mean prediction accuracy value for method V3b was 1.83. No similarities (e.g., physicochemical properties, VD, f_u and so forth)

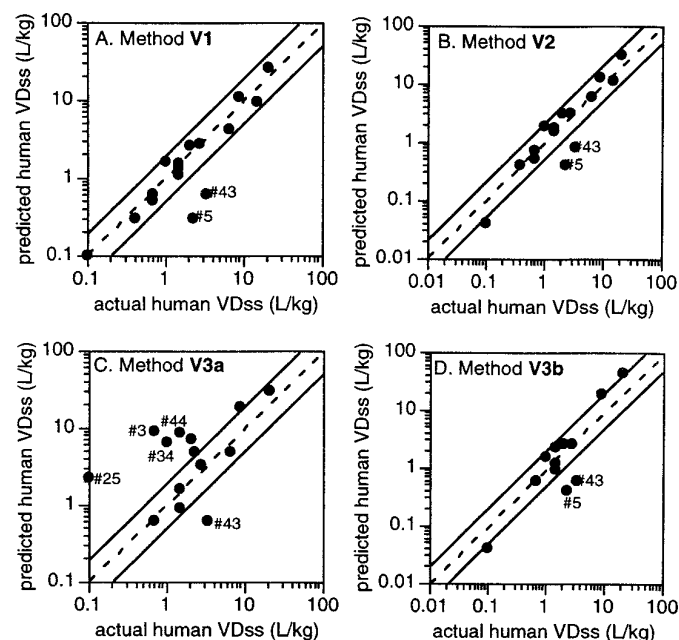


Fig. 1. Plots of predicted human volume of distribution values vs. actual values measured after intravenous administration. A, Method V1. B, Method V2. C, Method V3a. D, Method V3b. Dashed lines represent lines of unity, and the area between the solid lines represents an area within 2-fold error. The identity of outlier compounds are indicated.

TABLE 4

Accuracy of human clearance and volume of distribution prediction methods

A value of unity would indicate that the prediction method was 100% accurate for all predictions made; a value of 2 would indicate that the method is, on average, 2-fold in error. The n values refer to the number of predicted values that were compared with *in vivo* pharmacokinetic data obtained after intravenous administration.

Method	n	Average-fold error ($X_{\text{predicted}}/X_{\text{actual}}$)
V1	16	1.56
V2	16	1.56
V3a	14	2.78
V3b	12	1.83
C1a	7	1.95
C1b	7	9.28
C1c	7	1.81
C1d	7	9.39
C2a	8	1.63
C2b	8	8.12
C2c	8	1.67
C2d	8	7.81
C3a	12	3.36
C3b	14	1.91
C3c	12	1.79
C3d	14	2.67

were readily apparent for the compounds for which poor predictions of VD were obtained.

Predictions of clearance. There were as many as 14 compounds that had adequate preclinical data and human intravenous pharmacokinetic data suitable for assessment of CL predictions.

Methods C1a to C1d used easily obtainable *in vitro* $t_{1/2}$ data in the calculation of *in vitro* CL'_{int} and scale-up to *in vivo* CL values. The four variations of this method applied the *in vitro* CL'_{int} values in the well-stirred and parallel tube models of hepatic extraction both with and without considerations for protein binding. Of these four methods, C1a and C1c, which use the well-stirred and parallel tube models, respectively, without considerations for plasma protein binding, yielded, on average, accurate predictions of human CL (table 4). In each case, predictions of the CL of six of seven compounds were within 2-fold of actual values (fig. 2, A and C). However, when protein binding values were included in the equations for the well-stirred or parallel tube models, large underpredictions of CL were obtained (fig. 2, B and D), resulting in an overall inaccuracy for methods C1b and C1d. The compounds that were underpredicted using C1b and C1d were all highly protein bound ($f_u < 0.04$).

The predictions of CL by commonly applied *in vitro* enzyme kinetic methods, using the well-stirred model (C2a and C2b) and the parallel tube model (C2c and C2d), are presented in figure 3, A–D, respectively. Methods C2a and C2c predicted human CL within 2-fold of actual CL for seven of eight compounds (88%). The geometric mean accuracy values for prediction methods C2a and C2c were 1.63 and 1.67, respectively (table 4). As with methods C1b and C1d, inclusion of plasma protein binding corrections into the well-stirred or parallel tube models for methods C2b and C2d resulted in significant underpredictions of CL for some compounds and thereby decreased the predictive power of these approaches. Thus, only four of eight compounds (50%) were within 2-fold of actual CL values for methods C2b and C2d, and geometric mean prediction accuracy was poor, with values of 8.12 and 7.81, respectively.

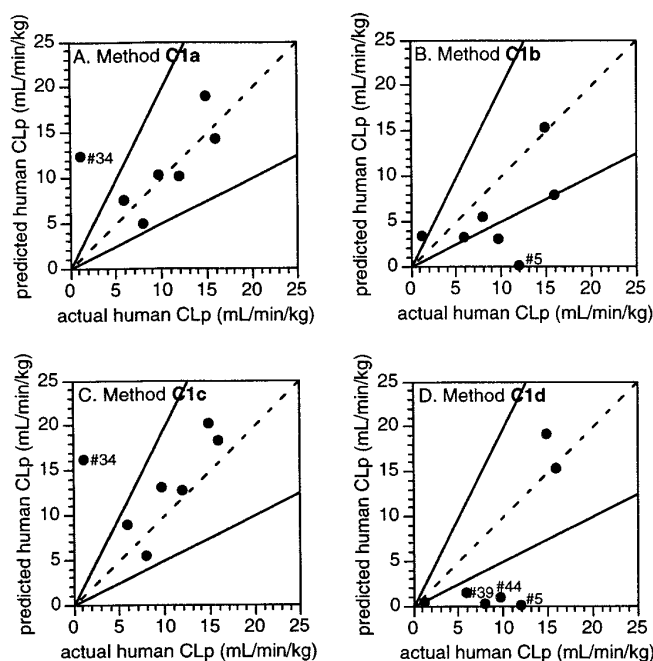


Fig. 2. Plots of human CL values predicted from *in vitro* $t_{1/2}$ data vs. actual values measured after intravenous administration. A, Method C1a. B, Method C1b. C, Method C1c. D, Method C1d. Dashed lines represent lines of unity, and the area between the solid lines represents an area within 2-fold error. The identity of outlier compounds are indicated.

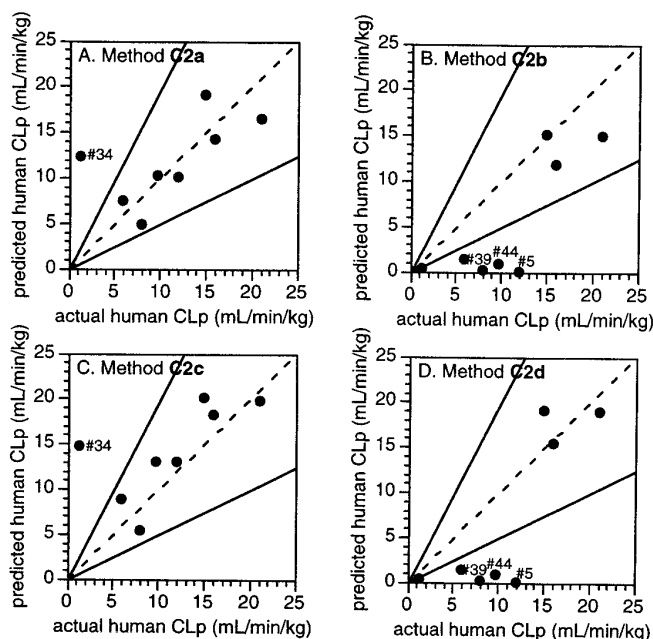


Fig. 3. Plots of human CL values predicted from *in vitro* enzyme kinetic data vs. actual values measured after intravenous administration. A, Method C2a. B, Method C2b. C, Method C2c. D, Method C2d. Dashed lines represent lines of unity, and the area between the solid lines represents an area within 2-fold error. The identity of outlier compounds are indicated.

The predictions of CL by allometric scaling methods C3a, C3b, C3c and C3d are presented in figure 4, A–D, respectively. The most predictive allometric method was C3c (allometric scaling of CL/f_u without MLP consideration), in which nine of 13 predictions (69%; fig. 4C) were within 2-fold of

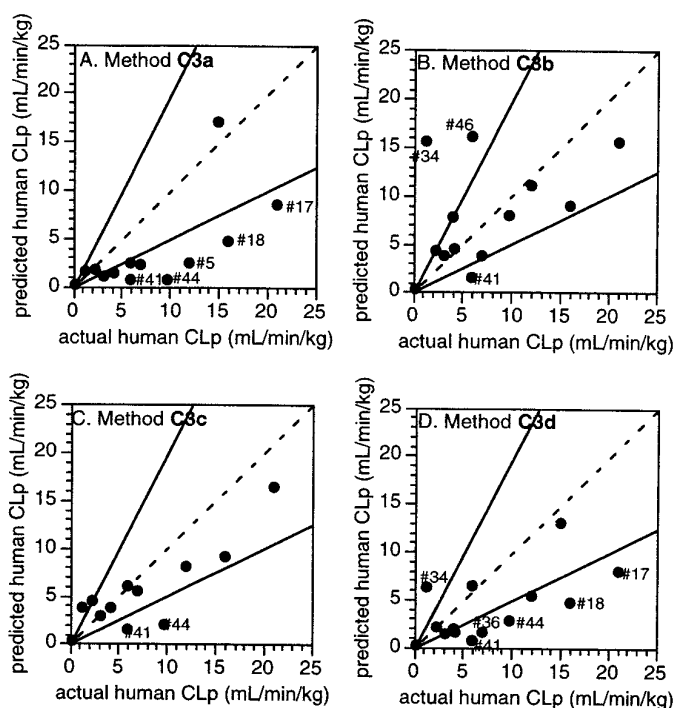


Fig. 4. Plots of human CL values predicted from allometric scaling vs. actual values measured after intravenous administration. A, Method C3a. B, Method C3b. C, Method C3c. D, Method C3d. Dashed lines represent lines of unity, and the area between the solid lines represents an area within 2-fold error. The identity of outlier compounds is indicated.

actual, and the geometric mean prediction accuracy was 1.79 (table 4). When allometric scaling was done without inclusion of protein binding and MLP considerations (method C3b), nine of 14 compounds (64%; fig. 4B) were predicted to have CL within 2-fold of actual and the mean geometric prediction value was 1.91. Inclusion of MLP considerations in methods C3a and C3d resulted in poor predictions of CL such that the geometric mean values were 2.67 and 3.36, respectively. Predictions of CL were within 2-fold of actual for three of 13 compounds (23%; fig. 4A) using method C3a and three of 14 (21%; fig. 4D) compounds using method C3d. No readily apparent trend could be discerned among the outlier compounds (*i.e.*, those identified in figure 3 for which allometric methods did not predict CL).

Predictions of human $t_{1/2}$. Combination of each of the four volume of distribution methods with the 12 CL prediction methods resulted in a total of 48 $t_{1/2}$ prediction methods. A histogram of success rates for each of these prediction methods is given in figure 5, and the mean accuracy values are listed in table 5. Mean accuracies for the various methods ranged from 2.13 (T_{V3aC2a} and T_{V3aC2c}) to 8.25 (T_{V3aC2d}). In general, the more accurate CL prediction methods yielded more accurate $t_{1/2}$ prediction methods. *In vitro* CL methods that disregarded the impact of protein binding generally yielded $t_{1/2}$ prediction methods with mean accuracies between 2- and 3-fold of actual $t_{1/2}$ values, whereas those that included protein binding were generally inaccurate. Allometric CL prediction methods, when combined with any volume of distribution prediction methods, gave $t_{1/2}$ predictions that were less accurate than *in vitro* methods that disregarded protein binding but more accurate than *in vitro* methods that

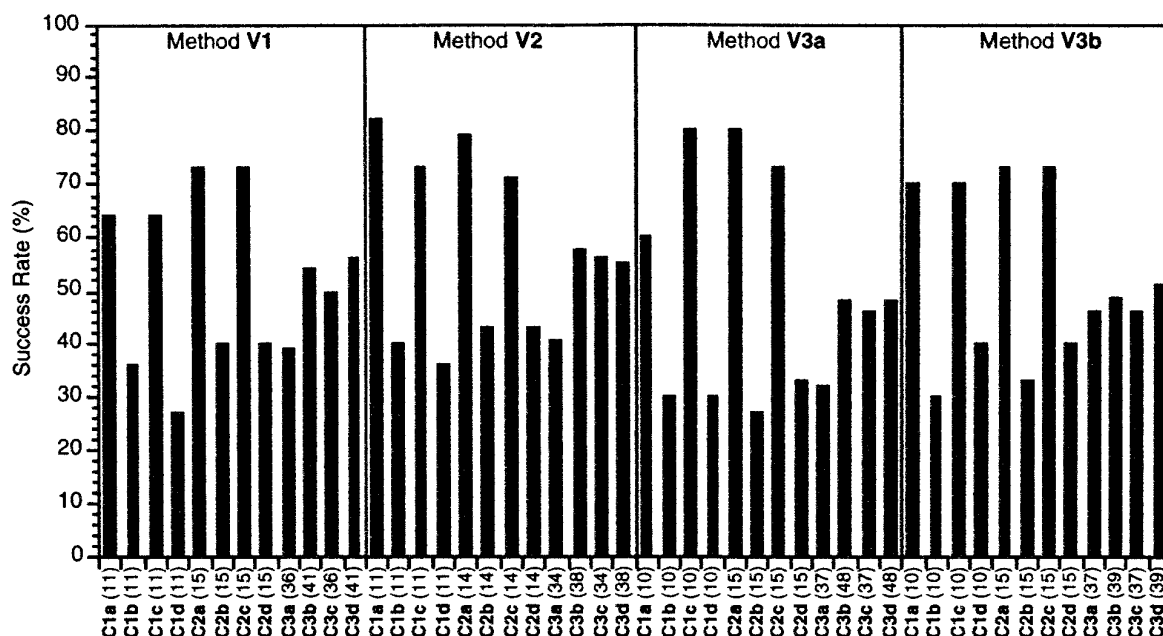


Fig. 5. Histogram of success rates for human $t_{1/2}$ predictions obtained by combining CL and volume of distribution predictions. Success was assessed by placing a compound into an appropriate $t_{1/2}$ zone of 0 to 4, 4 to 12, 12 to 48 or >48 hr.

TABLE 5

Accuracy of $t_{1/2}$ prediction methods derived by combination of clearance and volume of distribution predictions

Method	Average-fold error ($t_{1/2}(\text{predicted})/t_{1/2}(\text{actual})$)			
	V1	V2	V3a	V3b
C1a	2.55 (11)	2.29 (11)	2.82 (10)	2.23 (10)
C1b	6.74 (11)	7.41 (11)	7.54 (10)	4.94 (10)
C1c	2.73 (11)	2.37 (11)	3.05 (10)	2.50 (10)
C1d	7.37 (11)	7.31 (11)	8.22 (10)	5.22 (10)
C2a	2.83 (15)	2.38 (14)	2.13 (15)	2.52 (15)
C2b	5.19 (15)	4.95 (14)	8.02 (15)	4.43 (15)
C2c	3.26 (15)	2.66 (14)	2.13 (15)	2.94 (15)
C2d	5.44 (15)	4.92 (14)	8.25 (15)	4.50 (15)
C3a	3.47 (36)	3.72 (34)	5.15 (37)	3.42 (37)
C3b	4.06 (41)	4.28 (38)	3.51 (48)	4.46 (39)
C3c	3.35 (36)	3.44 (34)	4.39 (37)	3.74 (37)
C3d	3.18 (41)	3.27 (38)	4.38 (48)	3.40 (39)

Numbers in parentheses indicate the number of predictions made that could be compared with authentic human *in vivo* data.

included protein binding. Compared with CL and volume of distribution prediction methods alone, the combination of these two parameters for $t_{1/2}$ prediction methods yielded generally less accurate predictions.

When assessed by success rate criteria, the best volume and CL combinations were those that included *in vitro* metabolic rate data but disregarded protein binding in the prediction of CL (e.g., T_{V2C1a} , T_{V2C2a} , T_{V3aC1c} , T_{V3aC2a} ; fig. 5). Such methods yielded success rates in the 70% to 80% range. Methods that combined allometric volume and CL prediction methods were generally successful 50% of the time (e.g., T_{V3bC3d}). Allometric CL prediction methods appeared to be somewhat improved when combined with method V2; in this case, success rates exceeded 50%.

Human $t_{1/2}$ values were also predicted directly from animal $t_{1/2}$ data. In figure 6, plots of monkey, dog and rat $t_{1/2}$ vs. human $t_{1/2}$ are presented for both in-house data and data from the scientific literature. The data were subjected to $1/x$ -weighted linear regression, and the functions obtained

were used with individual animal $t_{1/2}$ data to calculate a predicted human $t_{1/2}$. (Functions are listed in the caption of fig. 6.) When subjected to the success criteria from equation 22, average -fold errors were 1.94, 2.19 and 1.79 using monkey (T1), dog (T2) and rat (T3) data, respectively. Success rates for prediction of appropriate dosing regimen using this procedure were 87%, 72% and 83% for the monkey (T1), dog (T2) and rat (T3) methods, respectively.

Predictions of human oral bioavailability. Each of the 12 CL prediction methods were used in predictions of oral bioavailability, assuming that all compounds were 100% absorbed. Success rates for the prediction methods are given in figure 7, with values ranging from 60% to 100% success. Allometric CL methods predicted oral bioavailability in the proper zone (i.e., unacceptable, intermediate, or satisfactory, see Methods) 71% to 93% of the time. *In vitro* metabolism prediction methods were comparably accurate.

Discussion

This retrospective analysis was successful in identifying methods that were generally applicable for the prediction of human pharmacokinetic parameters using preclinical pharmacokinetic and *in vitro* drug metabolism data. The methods examined represent a wide array of techniques, but certainly are not an exhaustive list of all possibilities. Some of these methods were taken directly from the literature (e.g., V3b, C2b), and some were developed as variations of literature methods (e.g., C2a); some involved customizing ideas and equations from the literature for prediction purposes (e.g., V1), whereas others were newly developed and described for the first time here (e.g., V2, C1a).

To our knowledge, methods by which human pharmacokinetic parameters can be accurately (e.g., within 10% of actual values) predicted for a wide range of compounds do not presently exist. In general, many of the prediction methods that were tested in this report yielded an adequate level of accu-

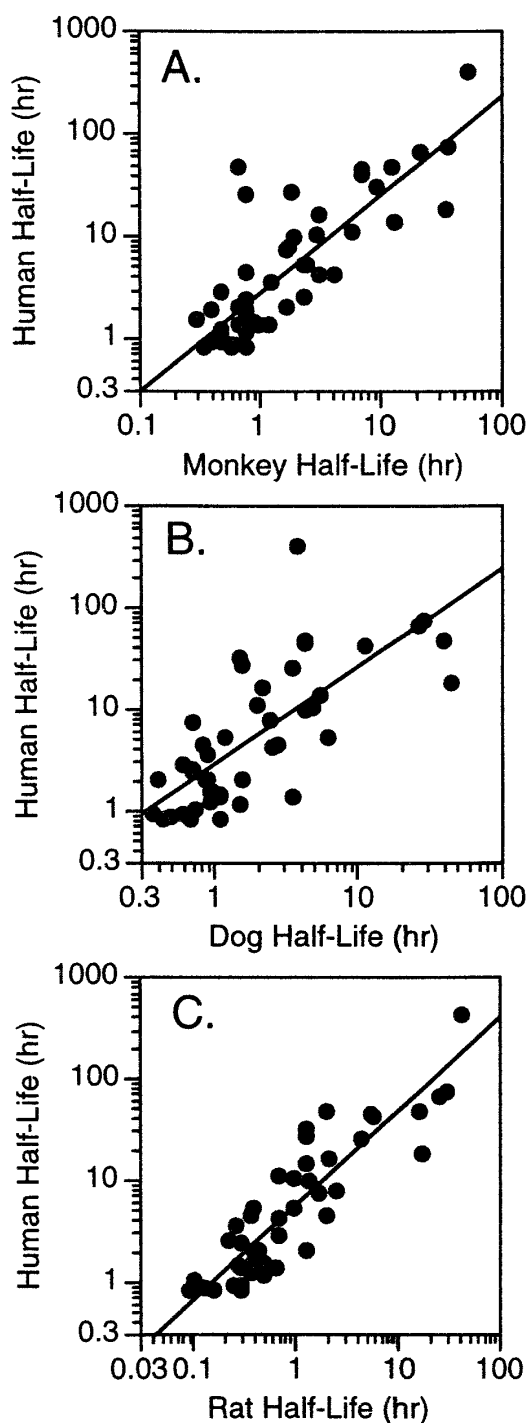


Fig. 6. Plots of human $t_{1/2}$ vs. monkey (A), dog (B) or rat (C) $t_{1/2}$. Success rates for human $t_{1/2}$ predictions obtained by correlation with animal $t_{1/2}$ data were 87%, 72% and 83% for monkey, dog and rat, respectively. Success criteria were as described in figure 5. The functions were obtained from $1/x$ weighting. The weighting was done due to a single outlier point with an extremely high $t_{1/2}$ (compound 30), which gave poorer correlations when done without weighting: $\log_{10} t_{1/2}(\text{human}) = 0.938 \cdot \log_{10} t_{1/2}(\text{monkey}) + 0.451$ ($r^2 = 0.668$); $\log_{10} t_{1/2}(\text{human}) = 0.934 \cdot \log_{10} t_{1/2}(\text{dog}) + 0.433$ ($r^2 = 0.540$); $\log_{10} t_{1/2}(\text{human}) = 0.906 \cdot \log_{10} t_{1/2}(\text{rat}) + 0.723$ ($r^2 = 0.793$). The compounds used in this analysis were 1, 2, 7, 8, 11, 12, 17, 18, 22, 26, 27, 28, 29, 30, 31, 32, 33, isotretinoin, bisoprolol, FCE22101, carumonam, meropenem, abecarnil, CP-65,207, cefepime, aztreonam, isosorbide dinitrate, cefmetazole, amphotericin B, ciprofloxacin, norfloxacin, acivicin, furosemide, AL01576, AL01567, ceftazidime, panipenem, betamipron, cefotetan, cefoperazone, moxolactam, cefpiramide, ceftizoxime, nicardipine, propranolol and cefazolin.

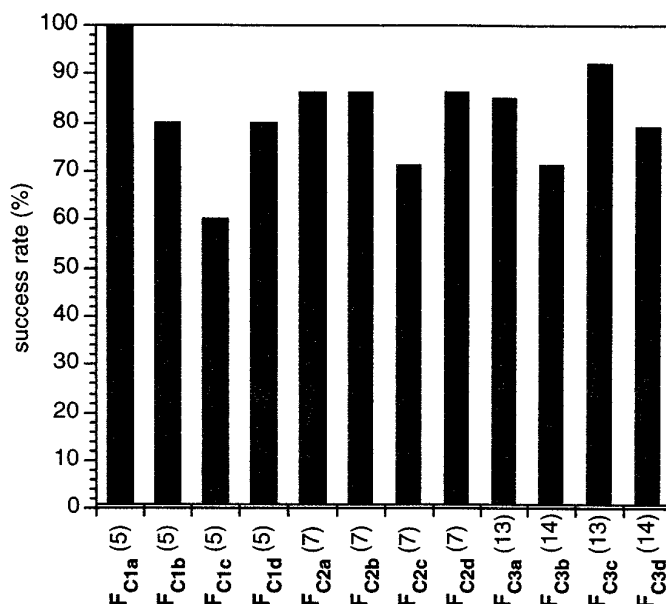


Fig. 7. Histogram of success rates for human oral bioavailability predictions obtained from human CL predictions. Success was assessed by placing compounds into one of three appropriate oral bioavailability zones: <10%, 10% to 30% and >30%.

racy when success criteria were applied that represent suitable decision-making metrics in the drug discovery process. For example, it is our contention that a goal to be strived for in predicting human $t_{1/2}$ should be prediction of a dosing regimen and not a precise $t_{1/2}$ value. Similarly, it may be unnecessary to predict a precise value for human oral bioavailability but rather to answer the question of whether a given compound will have satisfactory or unsatisfactory oral bioavailability. There are numerous reports in the literature of a particular method accurately predicting the pharmacokinetics of an individual compound, but the purpose of our analysis was to identify methods that would be most broadly applicable in the prediction of human pharmacokinetics. Literature reports typically describe only successes, and it is expected that failed attempts at the prediction of human pharmacokinetics are neither reported nor published. The purpose of this work was to compare various prediction methods in as objective a fashion as was possible. We relied on in-house data for this purpose, rather than relying on the scientific literature for accounts of successful prediction methods. However, in many cases, the absence of authentic human CL, volume of distribution and oral bioavailability data for compounds used in this analysis confounded our efforts to obtain a large number of data points for some methods. This paucity is due to a lack of human intravenous pharmacokinetic data. The data pool is rich in human $t_{1/2}$ data obtained after oral administration, and therefore predictions of CL and VD could be assessed indirectly when combined to generate $t_{1/2}$ predictions.

Methods of predicting volume of distribution were, for the most part, highly successful. Our intention was not to predict this parameter *per se* but rather to use these predicted values in combination with CL projections for predicting human $t_{1/2}$, which is a more meaningful parameter with decision-making impact on the drug discovery and development processes. The importance of plasma protein binding should be emphasized in the successful prediction of volume of distribution. Of

the four methods of predicting volume of distribution described here, three contained protein-binding data as an essential element. These three represented the most successful methods, whereas the method in which protein binding was disregarded was substantially less successful. Method V2 represents a novel, simple method to accurately predict human volume of distribution. The only experiments needed for this method are determinations of protein binding in dog and human plasma (or serum) and intravenous pharmacokinetics in dog. A similar method using rat data (VD and f_u) was examined (data not shown) but did not appear to approach the accuracy of the dog method and was not further pursued.

The other two volume of distribution prediction methods that appeared to have similar accuracy to method V2 were methods V1 and V3b. Both of these methods require more data than method V2 but do not appear to offer any advantages with regard to general accuracy of predictions. Method V1 is a more elaborate version of V2 (or V2 is a simplified version of V1) using the same principles regarding the relationship between plasma protein binding and volume of distribution. It was derived from an expression first described by Oie and Tozer (1979) relating plasma protein binding, tissue binding and various compartmental volumes. The equation was rearranged to calculate the free fraction in tissues (f_{ut}) of preclinical species; these values were then averaged as an estimate of tissue binding in humans, and this average f_{ut} value was used in conjunction with human plasma protein binding to compute the volume of distribution. Thus, the experiments needed to generate the minimum data required for this method include intravenous pharmacokinetics in at least two preclinical species and plasma protein binding in these species and humans. Method V3b required the same data as method V1 and was similarly predictive as this method. Clearly, allometric scaling of volume of distribution required correction to free volume of distribution (method V3b) because method V3a, which did not incorporate protein binding data, was substantially less successful than V3b.

On the whole, methods of predicting human CL were less accurate than those for predicting volume of distribution. However, some appeared to be adequate for combination with predictions of volume of distribution for subsequent predictions of $t_{1/2}$ (see below). The recent increase in availability, characterization and utility of human reagents, such as human liver microsomes, has added another dimension to the prediction of human pharmacokinetics by allowing for prediction of human metabolic CL from *in vitro* metabolism studies. The use of *in vitro* hepatic microsomal intrinsic CL data to predict systemic CL carries several assumptions and caveats: (1) metabolic CL is the primary CL mechanism (*i.e.*, $CL_m > CL_{renal} + CL_{biliary} + CL_{other}$), (2) the liver is the major CL organ, (3) oxidative microsomal metabolism is the predominant route of metabolism (compared with nonmicrosomal metabolism and conjugative metabolism) and (4) metabolic rates and enzyme activities measured *in vitro* are truly reflective of those that occur in intact systems *in vivo*. Two types of *in vitro* methods were examined: one type that used simple *in vitro* degradation rate data (1a–d) and one type that used the more elaborate enzyme kinetic data (2a–d). In both cases, human hepatic intrinsic CL values were calculated from *in vitro* data in human liver microsomes. The *in vitro* $t_{1/2}$ method of determining intrinsic CL carried several

caveats. First, the experiment must be conducted at a substrate (drug) concentration below the apparent K_M value (which is not known *a priori* to conducting this type of analysis). In the data we present, substrate concentrations were typically 1 μ M. Second, no significant enzyme inactivation can occur during the incubation period for an accurate determination. For cytochrome P450-catalyzed reactions, enzyme inactivation due to the concomitant formation of reactive reduced oxygen species (*e.g.*, H_2O_2) is typically observed. Finally, the reaction cannot approach equilibrium (which is not a problem for cytochrome P450-catalyzed reactions). Despite these caveats, the predictions of human CL obtained using *in vitro* $t_{1/2}$ data were fairly comparable to those made using the more extensive enzyme kinetic data (V_{max}/K_{Mapp}).

Four variations were applied for *in vitro* $t_{1/2}$ and enzyme kinetic approaches using two different models of hepatic extraction (well-stirred and parallel tube) with or without inclusion of plasma protein-binding data. The inclusion of protein-binding data is traditionally a cornerstone of these models (Pang and Rowland, 1977; Wilkinson and Shand, 1975); however, in our analysis, disregarding this factor yielded superior predictions of CL (*e.g.*, C1a *vs.* C1b, C1c *vs.* C1d, C2a *vs.* C2b, C2c *vs.* C2d). This was primarily due to highly protein bound compounds for which CL was severely underpredicted when the very low values (<0.1) for free fraction in plasma were included. Interestingly, most of these compounds were lipophilic amines. Our current working hypothesis is that these compounds were also highly bound to the liver microsomes used in *in vitro* incubations, leading to underestimates of free intrinsic CL (Obach, 1996). If binding to microsomes and binding to plasma proteins are equivalent, the unbound fraction terms will cancel and equations 13 and 14 will degenerate to equations 16 and 17, respectively. Experiments are under way to address this. Geometric means of predicted CL/actual CL were <2 for each of methods C1a, C1c, C2a and C2c, suggesting that CL will be predicted within 2-fold of actual values. The low number of data points (six to eight) preclude conclusions regarding the anticipated success of prospective application of these methods at this time.

In predictions of human CL, two of the four allometric scaling methods yielded accurate predictions (C3b and C3c). Interestingly, inclusion of corrections for MLP were less accurate than corresponding methods that lacked this correction. Of the compounds examined in this analysis using allometric scaling, most are cleared *via* hepatic oxidative metabolism. Interspecies differences in intrinsic abilities to metabolize compounds (Lin, 1995) can confound allometric scaling. Despite this, allometry generally provided good predictions of human CL. Method C3c, allometric scaling of CL that included corrections for interspecies differences in plasma protein binding but did not correct for MLP, was the most successful of the allometric methods yielding predictions of human CL that were typically within 2-fold of actual values. It should be further noted that the knowledge of regression coefficients of allometric relationships for each compound was not used. We did not discard compounds for which such a regression value was low; all data were included. In a prospective manner, allometric methods of predicting human CL would likely not be used for compounds demonstrating poor allometric relationships. Furthermore, observation of outlier species on allometric plots would sug-

gest that such data be removed from the relationship, whereas in our analysis all data points were included, regardless of how well each of the species “lined-up” in allometric plots. Furthermore, compounds for which data were available in only two preclinical species were still included in the assessment of allometric scaling. In a pure sense, allometry should only be used when data from at least three preclinical species are available (Mahmood and Balian, 1995); however, in the drug discovery process, one is frequently faced with situations in which such extensive data are not available. In our efforts to apply these methods as they could be applied in the “real world” situations of drug discovery (as opposed to having all the data truly needed for the best predictions), assessments of the predictive abilities of allometric scaling (and other methods as well) in this report represent an underestimate of predictive use. Application of drug metabolism/pharmacokinetic insight to the process of prospectively predicting the human pharmacokinetics of any individual compounds would lead to improvements in the accuracy of predictions.

The predictions of human CL and volume of distribution, although interesting and useful in their own right, represent a means to predicting human $t_{1/2}$, a parameter that is better understood by nonpharmacokineticist colleagues in the drug discovery and development field (e.g., medicinal chemists, pharmacologists, clinicians). Furthermore, the $t_{1/2}$, along with knowledge of the therapeutic index and pharmacokinetic/pharmacodynamic relationships, dictates the dosing frequency. A frequently asked question in the preclinical drug discovery process is, “Will compound X be a once-per-day drug?” Thus, an ability to predict dosing regimen by predicting human $t_{1/2}$ will provide tremendous value to drug discovery efforts in the compound selection process. To this end, we targeted prediction of half-lives not as absolute values but rather as an ability to place compounds into appropriate dosing regimen zones. These zones were preset before the analysis at 0 to 4, 4 to 12, 12 to 48 and >48 hr, which, in cases of “average” therapeutic index and straightforward relationships between PK and PD, approximately correlate to dosing regimens of three times a day or more often, twice a day, once a day and potentially less than once a day, respectively. Furthermore, so as not to restrict predictions to absolute cutoff values for success (e.g., not to classify a prediction of 3.8 hr a failure when the actual $t_{1/2}$ was 4.5 hr), a 2-fold accuracy criterion was overlaid on the dosing regimen success criterion. In this work, we described two types of approaches to predicting human $t_{1/2}$: a combination of CL and distribution volume predictions and a direct correlation of animal and human $t_{1/2}$ values.

In combining CL and volume of distribution predictions to predict $t_{1/2}$, all methods were combined, again to remain unbiased and comprehensive in the assessment of methods. Thus, methods that were unsuccessful in the prediction of CL and volume were not excluded from being examined in combinations to predict $t_{1/2}$. Also, methods were “mixed”; for example, an allometric (*in vivo*) volume prediction method was combined with an *in vitro* CL prediction method. As might be expected, methods that were more successful in predicting the independent parameters of CL and volume were generally more successful in combination in predicting $t_{1/2}$. The best combination methods yielded success rates of 70% to 80%. In light of the cost of drug development, the

attrition rate of new chemical entities in clinical development and the extent to which inadequate pharmacokinetics contributes to this attrition rate, such a $t_{1/2}$ prediction success rate would result in an effective strategy for compound selection. An additional consideration in the prediction of human $t_{1/2}$ is the fact that the $t_{1/2}$ values for compounds exhibiting multiphasic plasma concentration-*vs.*-time profiles (i.e., multicompartmental kinetic models) will be underpredicted using combinations of CL and VD predictions. In the data set used, there were no compounds that exhibited such a behavior. In the case of multiphasic behavior, the parameter VD_{β} should probably be used in the prediction of terminal phase $t_{1/2}$. However, this should also be done cautiously because long multiphasic terminal phase $t_{1/2}$ values seldom have an impact on dosing regimens, and the underlying purpose of predicting human $t_{1/2}$ is primarily to assess potential dosing regimens.

In addition to testing the ability of preclinical pharmacokinetic data to predict human pharmacokinetics by the use of allometric scaling, evaluations were conducted to determine the ability to predict human pharmacokinetics from animal data using simple correlations of pharmacokinetic parameters. Although previous reports have retrospectively described the predictability of human $t_{1/2}$ and volume of distribution from rat pharmacokinetics using simple correlations, that research did not compare the success rates of these correlations between the species commonly used in preclinical evaluations (Bachmann *et al.*, 1996). In the present research, a population of compounds was identified from both in-house and literature sources for which there was intravenously derived $t_{1/2}$ data for rat, dog and monkey and $t_{1/2}$ data from either intravenous or oral studies in humans. The current analysis was limited to evaluation of $t_{1/2}$ predictions to take advantage of the relatively large amounts of human oral pharmacokinetic data compared to intravenous data. For this set of 46 compounds, the success rates for prediction of dosing regimen ranged from 72% for the dog to 87% for the monkey. Thus, for the prediction of human $t_{1/2}$, there potentially exist relatively simple animal/human correlation methods based on preclinical intravenous pharmacokinetic data, which have success rates approaching or exceeding those for more complex techniques involving *in vitro* metabolism data or pharmacokinetic data from multiple species. Because this evaluation was retrospective in nature, using existing preclinical and clinical data, future research will focus on testing the usefulness of these correlations to prospectively predict human $t_{1/2}$ from preclinical data.

Predictions of oral bioavailability were generally successful for the small number of compounds for which *in vivo* data were available. This success was despite the fact that only hepatic microsomal metabolism was considered as a limitation in these methods. Clearly, this undervalues the potential impact of limitations of absorption and first-pass metabolism mediated by the intestinal mucosa. However, our objective was to place a compound into an oral bioavailability category as dictated by drug development decision making criteria and not to predict precise values. It is anticipated that *in vitro* methods (e.g., Caco-2 cells) by which fraction absorbed values can be quantitatively predicted will be available in the near future, and these can be incorporated into more refined oral bioavailability predictions. In addition, recent reports have shown that intestinal metabolism can have a pro-

nounced first-pass effect on oral bioavailability, especially for CYP3A substrates (Thummel *et al.*, 1996; Wu *et al.*, 1995). Thus, future methods to accurately predict of oral bioavailability may involve the prediction of intestinal first-pass effects as well.

The population of compounds used in this analysis represented all Pfizer compounds to enter Phase I clinical trials from 1981 through 1994. Over this time period, there were substantially more human pharmacokinetic data generated after oral administration than after intravenous administration. For this reason, the current data set available to assess prediction techniques for parameters requiring intravenous administration (volume of distribution, plasma CL and absolute bioavailability) was smaller than that available for techniques to predict parameters that can be adequately determined after oral administration ($t_{1/2}$).

In conclusion, several methods using preclinical pharmacokinetic and *in vitro* human metabolism data have been found to be useful in the prediction of human pharmacokinetic parameters. Future extensions of this research will focus on increasing the population of compounds with human CL, bioavailability and $t_{1/2}$ predictions derived from *in vitro* metabolism data. In addition, future research will include assessments of the usefulness of *in vitro* data to predict oral CL, thereby reducing the need for human intravenous pharmacokinetic data to determine the usefulness of these *in vitro* techniques.

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