Prediction of Volume of Distribution Values in Humans for Neutral and Basic Drugs Using Physicochemical Measurements and Plasma Protein Binding Data

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We present a method for the prediction of volume of distribution in humans, for neutral and basic compounds. It is based on two experimentally determined physicochemical parameters, ElogD(7.4) and $f_{i(7.4)}$, the latter being the fraction of compound ionized at pH 7.4 and on the fraction of free drug in plasma (f_u). The fraction unbound in tissues (f_{ut}), determined via a regression analysis from 64 compounds using the parameters described, is then used to predict VD_{ss} via the Oie−Tozer equation. Accuracy of this method was determined using a test set of 14 compounds, and it was demonstrated that human VD_{ss} values could be predicted, on average, within or very close to 2-fold of the actual value. The present method is as accurate as reported methods based on animal pharmacokinetic data, using a similar set of compounds, and ranges between 1.62 and 2.20 as mean-fold error. This method has the advantage of being amenable to automation, and therefore fast throughput, it is compound and resources sparing, and it offers a rationale for the reduction of the use of animals in pharmacokinetic studies. A discussion of the potential errors that may be encountered, including errors in the determination of f_u, is offered, and the caveats about the use of computed vs experimentally determined log*D* and pK_a values are addressed.

Introduction

The successful design of new drugs requires that multiple properties be simultaneously optimized. In the past, drug design efforts were focused on the optimization of affinity and selectivity for the target enzyme or receptor and demonstration of efficacy in animal models of human disease. However, present drug design efforts must also optimize other properties such as the pharmacokinetic and metabolic profile. New drugs need to demonstrate adequate pharmacokinetic behavior, permitting convenient dosing regimens that result in high patient compliance and thus effective therapy. Such pharmacokinetic properties include a suitable half-life and, for orally administered compounds, adequate bioavailability (Figure 1), among others. Efforts in drug dispositional science over the past decade have resulted in the development of several methods and approaches, using in vitro and/or in vivo data, computational approaches, or all three, to the prediction of human pharmacokinetic parameters and other drug disposition properties.[1−3] Such data are of value in optimization of compound structure and selection of superior compounds for the drug development process.

To predict the half-life in human of a given compound, several experiments must be conducted. Half-life is a function of the clearance and apparent volume of distribution (Figure 1), each of which must be predicted and combined to predict half-life. Approaches to the prediction of human clearance typically involve the use of in vitro data obtained by using human-derived reagents.[1−4] While some human clearance prediction methods involve the use of animal pharmacokinetic data, marked interspecies differences in clearance rates and mechanisms for individual compounds reduce the confidence that such approaches are generally applicable. In contrast, the apparent volume of distribution of a compound is generally more related to the molecular properties of the compound, rather than interspecies differences in tissue distribution, and thus, this parameter is more successfully predicted using animal pharmacokinetic data.[4]

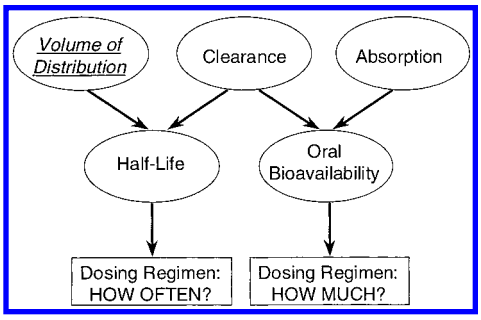

Figure 1. Relationship of volume of distribution to the prediction of human pharmacokinetics.

Volume of distribution is related to the extent of binding in tissues vs the extent of binding in plasma (the central compartment).[5] In general, for compounds that are equally bound to plasma proteins, a compound with a greater extent of tissue binding will have a greater volume of distribution. For compounds with equal tissue binding, a compound with a greater extent of binding to plasma proteins will have the smallest volume of distribution. (Tissue binding, as described in

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this report, represents a total composite of the multitude of low affinity binding interactions between a drug and various components of different tissues.) Thus, if measurements of overall tissue binding and plasma protein binding could be made, a volume of distribution could be estimated. However, while it is simple to measure the plasma protein binding using human plasma, measurement of tissue binding in humans is not possible. Previously described methods for the prediction of human volume of distribution have relied on the collection of animal pharmacokinetic data.[4]

In this paper, we describe a simple method whereby human volume of distribution can be reliably estimated for drugs that are strong or weak organic bases and organic compounds not ionizable in aqueous solution at pH 7.4, without requiring animal pharmacokinetic data. It should be emphasized that the application of such method(s) would provide a rationale for the reduction of the use of animals in pharmacokinetic studies, as well as a reduction in efforts on the part of the scientist(s) involved in conducting such studies. Furthermore, use of this approach to the prediction of human volume of distribution, by obviating the need for pharmacokinetic studies in animals, would reduce the quantity of material to be synthesized, from hundreds of milligrams to a few milligrams. To predict the volume of distribution for a new compound, three simple measurements are required: human plasma protein binding, experimental log*D* determined as previously described,[6] and pK_a. The method relies on a correlation derived between the unbound fraction in tissues, f_{ut}, for 64 basic and neutral drugs, calculated from human volume of distribution data and human plasma protein binding using the Oie−Tozer equation,[7] and a composite of physicochemical properties. Knowing ElogD, pK_a (transformed in the fraction ionized at pH 7.4 or $f_{i(7.4)}$), and f_u for the compound of interest, the predicted f_{ut} is calculated from the aforementioned correlation. This value is, in turn, combined with the fraction unbound in human plasma in the Oie−Tozer equation to predict the human volume of distribution. Although it is known that lipophilicity and fraction of (positive) charge are important for tissue binding,[8] and therefore VD_{ss}, and other authors have reported the use of similar parameters,[9−12] the present work, to the best of our knowledge, encompasses a much wider range of structures and parameters than previously reported.

The reliability of the present method was tested using a previously described set of human volume of distribution data for proprietary compounds,[4] and the accuracy was compared to methods that require the collection of animal pharmacokinetic data (see Results and Discussion). Also, a discussion of whether the computed version of some of these parameters could take the place of experimental ones is offered.

Results and Discussion

VD_{ss}, as described in the Introduction section, is an essential parameter for the prediction of the half-life of a compound in vivo. Thus, the corresponding values for 64 basic and neutral drugs from clinical studies were collected in order to reach our goal of a predictive model that would not depend on any data requiring animal experimentation. The VD_{ss} and f_u (fraction unbound in plasma) data for the compounds used in the development of the model are reported in Table 1, together with relevant references.

The collection of a reasonably diverse data set, in terms of structures and range of data and especially when aimed at the derivation of a robust correlation, is not a trivial task, also considering the heterogeneity of literature sources. Such heterogeneity may be thought to be a consequence of the nearly impossible access to self-consistent clinical data for a wide range of structures, with the latter encompassing a wide range of independent variables to be studied and correlated with the property of interest. However, the pharmacokinetic data in the training set represent "real world" information and would, therefore, be inclusive of variability with regard to both interindividual variability and potential differences between healthy study subjects and patients, as well as experimental and interlaboratory variability.

In these correlation and prediction efforts, it should be kept in mind, however, that VD_{ss} is a composite parameter and that the fraction unbound in tissues (f_{ut}) would probably offer a better target for these quantitative structure pharmacokinetic relationships or QSPkRs. Other authors have reported the direct correlation of VD_{ss} with physicochemical parameters, but that work was either confined to a fairly small set of analogues [13] or based on the use of a small set of compounds, together with multiple linear regression approaches using quadratic and crossproduct terms, in addition to linear ones.[9] A positive correlation of VD_{ss} with log*D*(7.4) has also been shown for noncongeneric molecules.[8] However, a closer inspection of the plot presented reveals that basic compounds in a fairly narrow range of VD_{ss}, for instance between 1 and 2 L/kg, would encompass a range of 5 log*D* units. Similarly, a modest variation in log*D* around a "central" value, for instance, of 2 would result in a fairly wide range of VD_{ss} from 2 to 30 L/kg.

The Oie−Tozer equation,[7] shown below, relates with some species-dependent parameters the variables VD_{ss} and f_u to f_{ut}

$$VD_{ss} = V_P(1 + R_{E/I}) + f_u V_P(V_E/V_P - R_{E/I}) + \frac{V_R f_u}{f_{ut}} \quad (1)$$

The parameters V_P, V_E, and $R_{E/I}$ are taken to be the plasma and extracellular fluid volumes and the ratio of extravascular to intravascular proteins, respectively, with corresponding values in human of 0.0436 and 0.151 L/kg body weight for V_P and V_E and approximately 1.4 for the latter. It should also be mentioned that $R_{E/I}$ strictly takes into account only the distribution of albumin. V_R is defined as the physical volume into which the drug distributes minus the extracellular space, and its value is taken to be 0.380 L/kg body weight. Finally, f_u and f_{ut} are defined, respectively, as the fraction of drug unbound in plasma and as the ratio of the average equilibrium concentration of unbound drug over the average concentration of the drug in the space defined by V_R or as the fraction unbound in tissues.

A useful rearrangement of this equation[4] yields f_{ut} from the two other variables, using the set parameters described above. The rearranged equation is shown in the Experimental Section, together with the values of the relevant parameters.

Table 1. Pharmacokinetic Data for the 64 Compounds in the Training Set

compd	CAS no.	VD[a] (L/kg)	f_u[b]	f_{ut}[c]	ref[d]
acebutolol	37517-30-9	1.2	0.74	0.273	24
acetomidophenol	103-90-2	0.95	1	0.503	25
allopurinol	315-30-0	0.60	0.95	0.881	26
alprazolam	28981-97-7	0.72	0.29	0.187	27
alprenolol	13655-52-2	3.4	0.24	0.028	28
amiodarone	1951-25-3	66	0.0002	0.000 001	29
antipyrine	60-80-0	0.60	0.9	0.825	30
atropine	51-55-8	2.0	0.82	0.171	31
azelastine	58581-89-8	15	0.17	0.004	32
bromazepam	1812-30-2	0.91	0.3	0.146	33
caffeine	58-08-2	0.61	0.64	0.543	34
chloramphenicol	56-75-7	0.94	0.47	0.225	35
chlorpheniramine	132-22-9	3.2	0.3	0.037	36
chlorpromazine	50-53-3	21	0.03	0.001	37
cimetidine	51481-61-9	1.0	0.81	0.374	38
clonidine	4205-90-7	2.1	0.8	0.158	39
clozapine	5786-21-0	5.4	0.05	0.004	40
cocaine	50-36-2	2.0	0.09	0.018	41
colchicine	64-86-8	4.2	0.61	0.057	42
Δ^9-THC	1972-08-3	9.8	0.03	0.001	43, 44
desipramine	50-47-5	20	0.18	0.003	45
dexamethasone	50-02-2	0.8	0.32	0.182	46
diazepam	439-14-5	1.1	0.013	0.005	47
diltiazem	33286-22-5	3.1	0.22	0.028	48
diphenhydramine	58-73-1	4.5	0.22	0.019	49
ergotamine	113-15-5	2.7	0.02	0.003	50
estradiol	50-28-2	1.2	0.015	0.005	51
felodipine	72509-76-3	10	0.004	0.0001	52
fentanyl	990-73-8	4.0	0.16	0.016	53
flecainide	54143-55-4	4.9	0.39	0.031	54
fluconazole	86386-73-4	0.6	0.89	0.814	55
haloperidol	52-86-8	18	0.08	0.002	56
imipramine	50-49-7	18	0.1	0.002	45
itraconazole	84625-61-6	3.9	0.028	0.003	57
lidocaine	137-58-6	1.1	0.3	0.118	58
lorazepam	846-49-1	1.3	0.09	0.029	59
lormetazepam	848-75-9	6.8	0.12	0.007	60
metoclopramide	364-62-5	3.4	0.6	0.070	61
metoprolol	56392-17-7	4.2	0.89	0.084	62
metronidazole	443-48-1	0.74	0.89	0.609	63
mexiletine	31828-71-4	4.9	0.37	0.030	64
morphine	64-31-3	3.3	0.65	0.079	65
nefazodone	83366-66-9	0.51	0.009	0.008	66
nicotine	54-11-5	2.6	0.95	0.150	67
nifedipine	21829-25-4	0.78	0.04	0.023	68
nizatidine	76963-41-2	1.2	0.78	0.289	61
omeprazole	73590-58-6	0.34	0.05	0.082	69
paclitaxel	33069-62-4	2.4	0.03	0.005	70
pentoxifylline	6493-05-6	4.2	1	0.095	71
prednisolone	50-24-8	1.5	0.075	0.021	72
prednisone	53-03-2	0.97	0.25	0.113	72, 73
procainamide	614-39-1	1.9	0.84	0.186	74
propafenone	54063-53-5	3.6	0.05	0.005	75
propranolol	525-66-6	4.3	0.13	0.012	76
quinacrine	69-05-6	223	0.103	0.0002	77
quinidine	56-54-2	2.7	0.13	0.019	78
ranitidine	66357-35-5	1.3	0.85	0.289	79
risperidone	106266-06-2	1.1	0.11	0.042	80
sumatriptan	103628-46-2	0.65	0.82	0.661	81
tebufelone	112018-00-5	31	0.000 67	0.000 008	82
terbutaline	23031-32-5	1.8	0.8	0.187	83
tolterodine	124937-51-5	1.3	0.037	0.012	84
trazodone	19794-93-5	1.0	0.07	0.030	85
trimethoprim	738-70-5	1.6	0.63	0.166	86

[a] VD_{ss} data from iv clinical studies. See Experimental Section for further details. [b] Experimentally determined fraction unbound in human plasma, from literature or in-house data. [c] Calculated via a rearranged form of the Oie−Tozer equation. See Experimental Section. [d] References for the clinical iv VD_{ss} data reported.

Armed with a reasonably large data set and the values of f_u and f_{ut} transformed into their respective logarithm,[9] we set out to establish a correlation with lipophilicity plus the fraction of the drug ionized at pH 7.4. Our aim was to find a model that could ultimately be used to predict VD_{ss} in the vicinity of a factor of 2, on average, since we would consider this value as a good approximation for the prediction of VD_{ss} in humans.

It should be emphasized that only systemic doses offer a legitimate basis for calculating VD_{ss} from concentration vs time data, and a great deal of caution has to be exercised in evaluating the data. Additionally, potential metabolic or analytical problems may limit the reliability of the data and should be considered. We aimed at a correlation using only human volume of distribution data, and that was a further limiting factor in trying to expand the data set with reliable data. Testa et al. have recently reviewed several QSPkRs and have discussed the overinterpretation of data and faulty statistics encountered in the analysis of some of these correla-

tions.[14] In our opinion, the deconvolution of VD_{ss} into a less "composite" parameter is very useful and perhaps necessary. Fraction unbound in tissues (f_{ut}) is still a composite parameter, and it does not separate the binding to specific tissues, which may be of different relative importance for different drugs and therapeutic areas, including possible toxic effects due to accumulation in tissues. However, in terms of equilibrium distribution and passive diffusion through cellular and subcellular membranes, adhesion to membranes and organelles,[15] and sequestration into specific organelles (e.g., lysosomes), f_{ut} is a better target for QSPkRs than VD_{ss}. Additionally, our approach makes the broad assumption that tissue partitioning is a function only of relative affinities of molecules for tissue components vs plasma components and the total binding capacity of these components: the potential for uptake or efflux of molecules via active processes is not accounted for.

The f_{ut} data, presented in Table 1, were calculated as described in the Experimental Section, via a rearranged form of the Oie−Tozer equation (eq 1). It was assumed that when only a value in liters was reported, the average weight of the subjects in the study was 70 kg. This is also in keeping with the estimates of V_P and V_E.

In parallel with the efforts aimed at deriving a good and predictive relationship on the basis of experimentally determined parameters, efforts were devoted to the exploration of computed parameters. Several surface area, charge, and volume parameters were computed, largely via in-house software, but no robust and predictive correlation was observed, at least at the level of accuracy we aimed to reach. The computed parameters included several polar and nonpolar surface area terms, as well as computed H-bond donor and acceptor terms.

Computed parameters are, of course, attractive given the general ease of their calculation and the obvious advantages of virtual screening. Unfortunately, when dealing with complex druglike structures, especially when capable of conformational changes, they may not possess the necessary ruggedness. Therefore, the prediction of fairly complex pharmacokinetic aspects, on the basis of computed parameters, remains a significant challenge. Our efforts are continuing in this area, to examine the scope and limitations of computed parameters in predicting VD_{ss}, and the findings will be reported in due course.

Experimental parameters that can be generated in a medium to high throughput (HT) fashion are, in our opinion, to be preferred, at least at present, over computed ones, and they represent an important advance with respect to approaches requiring animal pharmacokinetic studies.[4,16] Our recently published method[6] was used for the generation of ElogD(7.4) values, and we relied on literature or in-house data for pK_a values to calculate the fraction ionized at pH 7.4 [$f_{i(7.4)}$]. Medium and HT pK_a methods, which can be used for this purpose and are based on readily available instrumentation, have been described,[17,18] and the current state of HT physicochemical profiling has been recently reviewed by Kerns.[19] The physicochemical data for the compounds in the training set are reported in Table 2.

Equation 2 shows the correlation we obtained, via a regression analysis, using the data described above, which encompass neutral, weakly, and strongly basic compounds, the latter being positively charged at pH 7.4. The equation and the statistics were derived directly from a multiple linear regression, but they were also checked via principal component analysis. This approach was taken to examine the potential impact of collinearity between ElogD and logf_u data, to ensure the statistical quality and numerical stability of the equation. Indeed, the principal component regression analysis showed that all of the three principal components derived from the three variables are statistically significant, confirming the validity and stability of eq 2. More details on the data and procedure are offered in the Experimental Section

$$\log f_{ut} = -0.0389(\pm 0.1012) - 0.1739(\pm 0.0628)\mathrm{ElogD} - 0.8324(\pm 0.1205) f_{i(7.4)} + 1.0400(\pm 0.1376)\log f_u \quad (2)$$

where $N = 64$; $R^2 = 0.8839$; rmse = 0.3998; $Q^2 = 0.8639$; $F_{3,60} = 152.25$; and p-value < 0.0001.

The statistical outcome is very good, in particular when considering the often wide error margin for clinical and biological data in general and the heterogeneity of the data. It would probably be futile to expect a better correlation and a smaller error on the basis of the above considerations. Furthermore, the signs of the coefficients are physically reasonable and show, for instance, that an increase in lipophilicity, expressed by ElogD, determines a decrease in the fraction unbound in tissues, and so does a change in the electrical state of the drug, when the fraction of cation increases. The increase in the cationic fraction would likely translate into binding to anionic cellular and tissue components represented largely by membrane phospholipids. The fraction unbound in plasma, instead, shows a positive correlation with the fraction unbound in tissues. An increase in free fraction in plasma would thus yield an increase in unbound fraction in tissues, which is reasonable considering the presence of extravascular proteins in interstitial fluids and in cells and organelle membranes. The large amount of proteins present in the extravascular compartment may also contribute to explain the magnitude of the coefficient for logf_u, as compared to ElogD for instance, where the range of data is similar and spans a single digit range. Once the fraction unbound in tissues (f_{ut}) is calculated from eq 2, the value is used to calculate VD_{ss} via the Oie−Tozer equation (eq 1). Figures 2 and 3 show the plots of the predicted vs observed logf_{ut} and the predicted vs observed VD_{ss} (L/kg) for the compounds in the training set, respectively.

We have also examined, in addition to the introduction of computed parameters in the equation, the use of quadratic and interaction terms, with particular attention to ElogD and $f_{i(7.4)}$. While adding or substituting a quadratic ElogD term in eq 2 does not yield a significant improvement, the use of a quadratic $f_{i(7.4)}$ term in place of the first-order term does yield a slight improvement in the statistics of eq 2. It is possible that a further expansion of the data set and/or further refinement of the data used in the present study may bring about a clearer differentiation between linear and quadratic response surfaces. However, the quality of the

Table 2. Physicochemical Data for the 64 Compounds in the Training Set

compd	CAS no.	ElogD[a]	$f_{i(7.4)}$[b]	pK_a[c]	clogD[d]	c$f_{i(7.4)}$[e]	cpK_a[f]	ref[g]
acebutolol	37517-30-9	−0.39	0.995	9.67	0.89	0.981	9.11	87
acetamidophenol	103-90-2	0.38	0	n/a	0.34	0	n/a	
allopurinol	315-30-0	−0.1	0	n/a	−0.54	0	n/a	
alprazolam	28981-97-7	2.16	0	n/a	2.5	0	n/a	
alprenolol	13655-52-2	0.62	0.992	9.51	1.13	0.983	9.17	*h*
amiodarone	1951-25-3	5.95	0.955	8.73	6.64	0.989	9.37	88
antipyrine	60-80-0	0.34	0	n/a	0.27	0	n/a	
atropine	51-55-8	−0.16	0.996	9.84	−0.94	0.997	9.98	*h*
azelastine	58581-89-8	1.93	0.993	9.54	1.96	0.983	9.16	*h*
bromazepam	1812-30-2	1.38	0	n/a	2.41	0	n/a	
caffeine	58-08-2	−0.01	0	n/a	−0.08	0	n/a	
chloramphenicol	56-75-7	1.55	0	n/a	1.02	0	n/a	
chlorpheniramine	132-22-9	1.56	0.986	9.26	1.48	0.988	9.33	*h*
chlorpromazine	50-53-3	3.2	0.986	9.24	3.36	0.991	9.43	89
cimetidine	51481-61-9	0.4	0.271	6.97	0.17	0.173	6.72	*g*
clonidine	4205-90-7	0.29	0.817	8.05	0.84	0.671	7.71	90
clozapine	5786-21-0	3.38	0.629	7.63	3.44	0.078	6.33	*h*
cocaine	50-36-2	0.48	0.952	8.7	1.51	0.974	8.97	91
colchicine	64-86-8	0.9	0	n/a	1.03	0	n/a	
Δ^9-THC	1972-08-3	6.8	0	n/a	7.64	0	n/a	
desipramine	50-47-5	1.3	0.999	10.23	1.23	0.999	10.4	*h*
dexamethasone	50-02-2	2.03	0	n/a	2.06	0	n/a	
diazepam	439-14-5	2.98	0	n/a	3.86	0	n/a	
diltiazem	33286-22-5	2	0.82	8.06	3.02	0.970	8.91	*h*
diphenhydramine	58-73-1	1.38	0.98	9.1	2.29	0.958	8.76	*h*
ergotamine	113-15-5	4.3	0.074	6.3	2.85	0.624	7.62	92
estradiol	50-28-2	3.9	0	n/a	4.13	0	n/a	
felodipine	72509-76-3	4.52	0	n/a	4.92	0	n/a	
fentanyl	990-73-8	2.39	0.915	8.43	2.27	0.979	9.07	93
flecainide	54143-55-4	0.49	0.988	9.3	0.72	0.999	10.39	94
fluconazole	86386-73-4	0.66	0	n/a	0.3	0	n/a	
haloperidol	52-86-8	2.46	0.947	8.65	3.16	0.876	8.25	*h*
imipramine	50-49-7	1.97	0.992	9.51	2.41	0.992	9.49	*h*
itraconazole	84625-61-6	5.79	0	n/a	3.23	0.089	6.39	
lidocaine	137-58-6	1.29	0.776	7.94	1.2	0.931	8.53	89
lorazepam	846-49-1	2.8	0	n/a	2.48	0	n/a	
lormetazepam	848-75-9	2.77	0	n/a	3.27	0	n/a	
metoclopramide	364-62-5	0.73	0.988	9.33	0.18	0.994	9.62	*h*
metoprolol	56392-17-7	−0.62	0.995	9.7	0.03	0.984	9.18	87
metronidazole	443-48-1	0.12	0	n/a	−0.02	0	n/a	
mexiletine	31828-71-4	0.23	0.981	9.11	0.96	0.938	8.58	*h*
morphine	64-61-3	0.32	0.858	8.18	0.46	0.846	8.14	89
nefazodone	83366-66-9	4.83	0.197	6.79	3.35	0.183	6.75	*j*
nicotine	54-11-5	0.23	0.834	8.1	0.02	0.799	8.00	*h*
nifedipine	21829-25-4	2.84	0	n/a	3.05	0	n/a	
nizatidine	76963-41-2	0.06	0.134	6.59	0.97	0.448	7.31	*h*
omeprazole	73590-58-6	2	0	n/a	1.79	0	n/a	
paclitaxel	33069-62-4	4.45	0	n/a	7.24	0	n/a	
pentoxifylline	6493-05-6	0.24	0	n/a	0.37	0	n/a	
prednisolone	50-24-8	1.6	0	n/a	1.69	0	n/a	
prednisone	53-03-2	1.22	0	n/a	1.56	0	n/a	
procainamide	614-39-1	−0.57	0.986	9.24	−1.14	0.997	9.86	95
propafenone	54063-53-5	1.49	0.987	9.27	2.75	0.988	9.31	*h*
propranolol	525-66-6	0.93	0.991	9.45	1.36	0.983	9.15	87
quinacrine	69-05-6	1.1	1.664	10.2 7.73	1.88	1.343	10.48 7.12	96
quinidine	56-54-2	1.51	0.817	8.05	1.72	0.982	9.13	97
ranitidine	66357-35-5	−0.5	0.922	8.47	0.24	0.909	8.4	*h*
risperidone	106266-06-2	1.59	0.764	7.91	2.22	0.764	7.91	*l*
sumatriptan	103628-46-2	−0.4	0.992	9.5	−1.38	0.992	9.49	98
tebufelone	112018-00-5	5.56	0	n/a	5.83	0	n/a	
terbutaline	23031-32-5	−1.49	0.952	8.7	−1.31	0.984	9.19	88
tolterodine	124937-51-5	1.04	0.997	9.87	2.95	0.999	10.6	99
trazodone	19794-93-5	2.97	0.197	6.79	1.6	0.134	6.59	*h*
trimethoprim	738-70-5	0.61	0.319	7.07	0.52	0.466	7.34	*h*

[a] As described in ref 6. [b] Fraction ionized at pH 7.4 calculated from experimental pK_a values. [c] Experimental pK_a values. For compounds having only a single pK_a value and a value <5, the notation "not applicable" is used. [d] logD(7.4) value calculated via ACDLabs logD module. Batch Mode, UNIX platform, version 4.5. [e] Fraction ionized at pH 7.4 from calculated pK_a. [f] pK_a values calculated via ACDLabs pK_a module. Batch Mode, UNIX platform, version 4.5. [g] References for experimental pK_a data reported. [h] Potentiometric determination, as described in the Experimental Section. [j] Taken to be identical to trazodone. [l] Computed value, see footnote *f*.

prediction for a set of proprietary compounds, which we are describing below, did not improve and we did not consider interaction or quadratic terms further.

As a test of this approach, we predicted the VD_{ss} for 14 proprietary compounds, structurally diverse and not included in the training set, and compared the value with a 2-fold error margin, as reported in previously published work.[4] Table 3 shows the comparative VD_{ss} data predicted by this approach and derived from clinical studies. A mean error very close to 2-fold was achieved, as shown in Table 4, without the use of a "f_u filter" (see below), while when applying such a filter the average prediction error was well within this limit. There were some large outliers, however, for which we

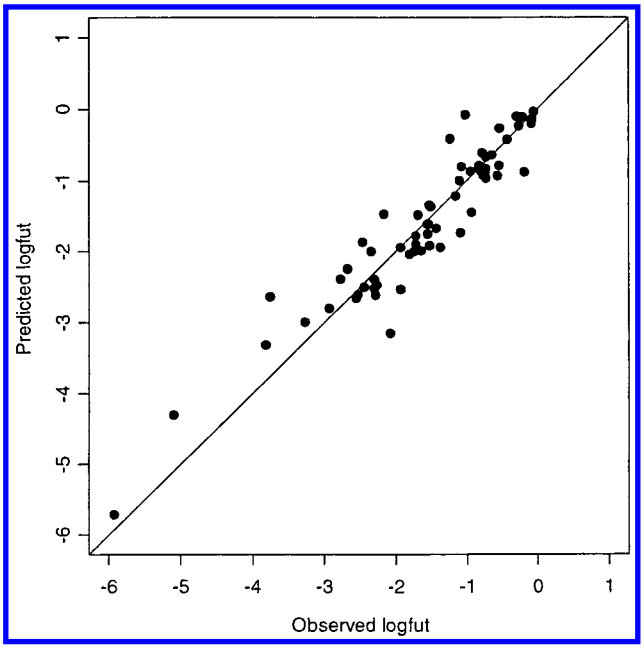

Figure 2. Plot of predicted f_{ut} vs observed f_{ut} for the 64 compounds in the training set.

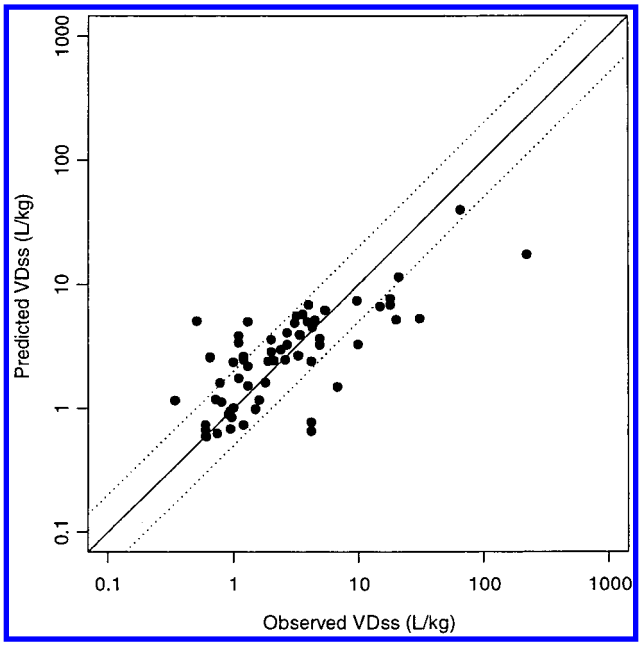

Figure 3. Plot of predicted VD_{ss} vs observed VD_{ss} for the 64 compounds in the training set. The dotted lines represent the 2-fold error limits.

have no reasonable explanation, and those "errors" may stem from the participation of influx/efflux mechanisms and/or selective uptake of the compound by specific tissues, as opposed to a purely equilibrium (diffusion) distribution. Plots of predicted vs observed f_{ut} or VD_{ss} values for the compounds in the test set are shown by Figures 4 and 5, respectively.

The accurate determination of f_u is, of course, of great importance in any pharmacokinetic profiling, and great care should be exercised in its generation, especially in the case of highly bound compounds. In fact, plasma represents only a small fraction of the total body mass (~4%).[20] However, f_u determination has important consequences for VD_{ss}. Changes in unbound fraction in plasma (whether real or due to incorrect determination)

Table 3. Physicochemical and Pharmacokinetic Parameters for the Test Set Compounds

no.	ElogD	pK_a	$f_{i(7.4)}$[a]	f_u[b]	f_{ut}[c]	obsvd VD_{ss}[d] (L/kg)	pred VD_{ss}[e] (L/kg)	accuracy[f]
1	0.78	6.99	0.28	0.12	0.04	0.7	1.2	Y
2	4.26	7.2	0.387	0.001	0.000[g]	1.5	6.4	N
3	0.66	7.26	0.42	0.6	0.19	1.5	1.4	Y
4	0.97	9.09	0.98	0.19	0.02	6.6	4.4	Y
5	−0.1	8.98	0.974	0.6	0.09	5.5	2.8	Y
6	2.85	7.24	0.409	0.01	0.001	1	3.5	N
7	0.53	1.76	0	0.89	0.66	0.7	0.7	Y
8	1.51	8.66	0.948	0.02	0.001	15.1	5.6	N
9	0.7	7.13	0.349	0.43	0.15	1.5	1.3	Y
10	−0.5	8.2	0.863	0.02	0.004	9	2.2	N
11	0.83	8.03	0.81	0.36	0.05	2.8	3.0	Y
12	1.38	9.82	0.996	0.12	0.01	2.1	5.4	N
13	2.17	9.09	0.98	0.03	0.001	21	7.6	N
14	2.56	6.8	0.2	0.04	0.01	1.5	2.1	Y

[a] Fraction ionized at pH 7.4. [b] Fraction unbound in human plasma. [c] Fraction unbound in tissues (f_{ut}) predicted from eq 2. [d] Experimental VD_{ss} value from iv clinical studies. [e] Calculated VD_{ss} value from the predicted f_{ut} data in this table, using the Oie−Tozer equation. [f] Prediction accuracy: Y = value within 2-fold of experimental value. No f_u filter was used (see text). [g] Actual value 0.000 06.

can cause a large change in volume of distribution but only a relatively small change in drug concentration in tissue. At any rate, determination of the amount of drug bound to plasma proteins is amenable to automation in a 96 well format,[21,22] and that contributes to the ease and speed of these efforts, which is one of the goals of the present work. It would be useful, of course, to run these determinations at or near therapeutic drug/protein ratios, but this appears to be more the exception than the rule.

As mentioned above, the coefficient for the fraction unbound in plasma, expressed as logf_u, is the largest one in eq 2, while this parameter is of the same magnitude of ElogD and not too dissimilar in magnitude from f_i. The f_u value ranges between 1 (acetaminophen) and approximately 0.0002 (amiodarone). Thus, our concern over the potential errors in determination of very small fractions of unbound drug in plasma, with f_u values = 0.02 or logf_u = −1.7, prompted us to examine a "filter" for the prediction of VD_{ss} for a drug that would have a very small f_u. That is, if the experimentally determined value for f_u is lower than 0.02, then any prediction of volume of distribution using this approach should be interpreted with caution, although it may not necessarily be inaccurate.

Because it is possible to calculate, specifically, log*D* and pK_a values, we also tested the hypothesis that computed parameters in eq 2 may yield an adequate prediction of VD_{ss}. Therefore, three other equations were generated (termed eqs 3−5) where, in turn, a computed log*D* (clog*D*), a computed F_i [$f_{i(7.4)}$], or both parameters would take the place of the experimentally determined counterparts.

In Table 4, we present the results of our testing for the four equations described, including or excluding the "$f_u \leq 0.02$ filter" and the corresponding prediction statistics for the 14 proprietary compounds reported in Table 3. Also, the coefficients for the parameters are shown. The mean fold prediction error is very close to 2, with eight out of 14 compounds within this limit, without the application of the f_u filter, and it should also

Table 4. Statistical Data and Comparison of Accuracy of Four Predictive Equations[a]

	eq 2[b]	eq 3	eq 4	eq 5
LogD	experimental	computed	experimental	computed
$f_{i(7.4)}$	experimental	experimental	computed	computed
		Training Set		
intercept	−0.0389	−0.0722	−0.0763	−0.0907
ElogD or clogD coeff	−0.1739	−0.1434	−0.1503	−0.1448
$f_{i(7.4)}$or c$f_{i(7.4)}$ coeff	−0.8324	−0.75	−0.7666	−0.7149
f_u coeff	1.0400	1.0815	1.079	1.0728
N	64	64	64	64
R^2	0.8839	0.8840	0.8679	0.8717
rmse	0.3998	0.3997	0.4265	0.4203
Q^2	0.8639	0.8632	0.8473	0.8507
F statistics	152.25	152.34	131.36	135.86
mean fold error	2.26	2.25	2.52	2.47
		Test Set (All Compounds)		
prediction accuracy[c]	8 of 14	10 of 14	6 of 14	9 of 14
mean fold error	2.20	2.13	2.73	2.37
		Test Set (Compounds with $f_u > 0.02$)		
prediction accuracy[c]	8 of 10	9 of 10	6 of 10	8 of 10
mean fold error	1.62	1.47	1.99	1.65

[a] All coefficients are significant unless otherwise noted. [b] See text. [c] Fraction of compounds predicted to have a VD_{ss} value within a 2-fold error from the experimental value.

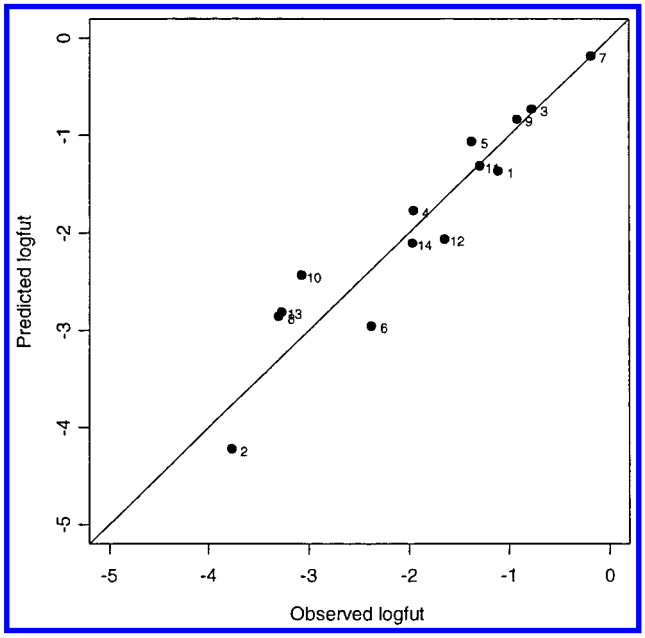

Figure 4. Plot of predicted f_{ut} vs observed f_{ut} for the 14 compounds in the test set.

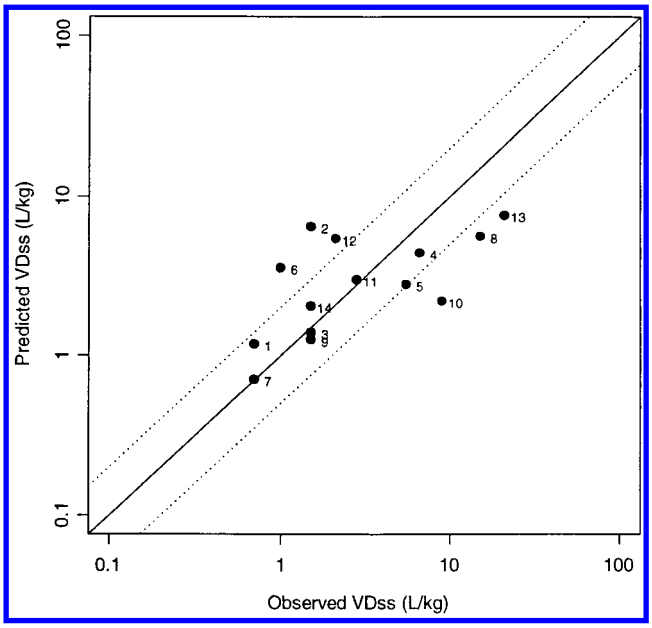

Figure 5. Plot of predicted VD_{ss} vs observed VD_{ss} for the 14 compounds in the test set. The dotted lines represent the 2-fold error limits.

be noted that four of the outliers have indeed f_u values ≤ 0.02 and that the next outlier (compound **13**) is also very close to that f_u limit. On the other hand, compound **12**, with a f_u value 6-fold larger than the limit set by the filter, should still be considered an outlier, according to the VD_{ss} prediction limit we set. However, the predicted VD_{ss} value is only slightly above the 2-fold mean error we considered acceptable and may still be useful. In considering these results, it should be further emphasized that the present method offers a much higher throughput, together with a drastic reduction of compound and resources, with particular regard to the use of animals. In fact, the mean fold error obtained using this method can be directly compared to the mean fold error of methods that require the collection of animal pharmacokinetic data, since a nearly identical set of test compounds was used.[4] The previously reported methods that utilized animal pharmacokinetic data had mean fold errors ranging from 1.56 to 2.78, while the mean fold error for the present method is 2.20. When only compounds with $f_u > 0.02$ were considered, a mean fold error of 1.62 was observed (Table 4). While comparably accurate, the present method has the advantages of obviating the need for animal pharmacokinetic experiments and requiring only minimal amounts of test compound for experimental data collection (ElogD, f_u, and pK_a). These aspects make this method more suitable for data collection in an early drug discovery setting in which hundreds to thousands of compounds must be examined.

In considering the application of computed parameters, the statistical quality of eqs 3−5 (Table 4) may seem attractive and lead to the conclusion that some of the experimental efforts needed to determine pK_a and ElogD may not be necessary. However, it should be kept in mind that the average error for the prediction of log$D$ or pK_a is likely to be significantly higher for newly

synthesized or experimental compounds than it is for well-characterized, or even marketed, compounds. This is, in part, a consequence of the fact that most "known" or "commercial" classes of drugs are likely to be widely represented in the training set of any given software. As an example, the rmse for the experimental vs computed pK_a values for the compounds in our training set, and amenable to ionization in the physiological range, was found to be 0.52. However, the corresponding rmse for the pK_a values for the 14 compounds in the test set was 1.27. The same trend was observed for log*D* calculations, when comparing the performance of calculations on larger proprietary sets of data to commercial compounds and to the present test set (data not shown).[23] These computed values might be useful for a "preliminary" or "bin" prediction, but they still would require an experimentally determined f_u value under the present model; therefore, they would require the availability of the actual compound being examined. The user should then be mindful of the error propagation risks, when data from computational models are in turn used to model physicochemical and/or pharmacokinetic end points.[14]

Conclusion

We have presented a facile and accurate predictive model, which we believe offers a good approach to the prediction of VD_{ss} in man and which does not require animal pharmacokinetic data. This approach should find applicability in a drug discovery setting to predict human VD_{ss}, a parameter necessary for prediction of $t_{1/2}$.

On average, the accuracy of the predicted values was within or very close to a 2-fold error for the actual values in the test data set. This method offers the advantages of not relying upon animal pharmacokinetic data and only requiring three fairly routine and automated determinations: ElogD, pK_a, and f_u. It may be difficult to expect a better performance without an even more refined and accurate data set of noncongeneric molecules, given the errors inherent in these studies. Efforts at refining the model, through the addition of clinical, structural, and physicochemical data, are being pursued in our laboratories. Also, the exploration of a fully computational model, which may be useful at virtual or at otherwise very early screening stages, is being pursued.

The important question of whether a better prediction may be achieved when dealing with classes of analogues in the context of pharmacokinetic optimization within a class of compounds is also being addressed, and the findings will be reported in due course.

Experimental Section

Materials and Methods. Most of the drugs were purchased directly from commercial sources (Aldrich, Fluka, ICN, RBI, Sigma, Tocris) and were used as received in all cases. In several cases, they were available through our Materials Management Group as either proprietary compounds or samples extracted from commercial formulations. The ElogD data were determined using our recently published method,[6] which is based on a linear regression of capacity factors (as logk') obtained from polycratic reversed-phase high-performance liquid chromatography (RP-HPLC) determinations and extrapolated to 0% of organic solvent. Its ruggedness and similarity to the balance of forces present in classical "two phase" systems have been discussed in detail in the original work. The pK_a data were either taken from the literature or available in-house from potentiometric or UV spectrometric determinations, or in several instances, they were obtained from potentiometric determinations performed by pIon Inc., Woburn, MA, on either commercial or proprietary samples. When more than one source was available, the pK_a data were averaged. The $f_{i(7.4)}$ value was then determined using the pK_a data. The computed clogD and cpK_a data were calculated using software from ACDLabs (ACDLabs, Toronto, Canada, version 4.5), and the respective $f_{i(7.4)}$ values were calculated from the latter data.

Volume of Distribution and Plasma Protein Binding Data. Volume of distribution data and plasma protein binding data for the 64 compounds constituting the training set were obtained from the scientific literature. The f_u data for tebufelone and quinacrine were determined in-house using equilibrium dialysis. The VD_{ss} data, in either set, were taken from literature or in-house clinical trials reports, using only data from studies in which a systemic dose was administered, as accurate measurement of volume of distribution requires that the entire dose is completely available to the systemic circulation. In a few cases, VD data for the compounds used for the calculation of f_{ut} had been reported as VD_β values, rather than VD_{ss}. In the cases when only a volume of distribution in liters was reported, an average of 70 kg for each study subject was assumed. The literature data used for the correlation are listed in Table 1.

Calculation of Fraction Unbound in Tissues. Literature data for VD_{ss} and f_u were used in the following rearragement of the Oie−Tozer equation.[4]

$$f_{ut} = \frac{V_R f_u}{[VD_{ss} - V_P - (f_u V_E)] - [(1 - f_u) R_{E/I} V_P]}$$

In this equation, f_{ut} is the fraction unbound in tissues, f_u is the fraction unbound in plasma, VD_{ss} is the steady state volume of distribution, and $R_{E/I}$ refers to the ratio of binding proteins in extracellular fluid vs plasma (1.4). V_P, V_E, and V_R refer to the volumes of plasma, extracellular fluid, and remainder fluid with values of 0.0436, 0.151, and 0.380 L/kg body weight, respectively, in human. In general, the use of logarithmic values is the most common mean of data transformation, and Veng-Pedersen[9] has discussed means of data transformation, to linearize the response and stabilize the variance points, in some detail. Therefore, we applied this transformation to the f_{ut} and f_u values. The original form of the Oie−Tozer equation (eq 1) was used to calculate the VD_{ss} for the compounds in the test set, knowing their calculated f_{ut} (from eq 2) and experimental f_u.

Statistical Analysis. The statistical analysis was performed using S-PLUS 2000 (MathSoft, Inc.) and JMP, version 3.2.6 (SAS Institute Inc.). Ordinary least-squares method was used to fit the regression model for predicting f_{ut} and generating eqs 2−5. All of the predictor variables in the equation are statistically significant. We also examined the correlation between the predictor variables and noticed that the sample correlation coefficient between ElogD and logf_u was −0.8607. We subsequently performed principal component regression analysis and observed that all three principal components derived from the three variables were statistically significant. This indicates that all three predictor variables contribute significantly in predicting logf_{ut}. We would have obtained the same regression equation by principal component regression analysis. In addition, when the removal of the $f_{i(7.4)}$ term was considered, we obtained an equation with lower R^2 and Q^2 values, or 0.7916 and 0.7708, respectively, further confirming the significance of this term.

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