PREDICTION OF CLEARANCE IN HUMANS FROM *IN VITRO* HUMAN LIVER MICROSOMES AND ALLOMETRIC SCALING. A COMPARATIVE STUDY OF THE TWO APPROACHES*

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SUMMARY

The objective of this study was to evaluate whether the predicted clearance of a drug in humans from *in vitro* human liver microsomes was comparable with the predicted clearance in humans obtained by allometric scaling. Sixteen drugs were randomly selected from the literature and their hepatic clearances were predicted using human liver microsomes. For allometric scaling at least three animal species were used and three methods were utilized to generate allometric equations to predict the clearance in humans: (i) clearance vs body weight (simple allometry); (ii) product of the clearance and maximum life-span potential (MLP) vs body weight; and (iii) the product of clearance and brain weight vs body weight. The choice of one of the methods was based on the 'rule of exponents' as described by Mahmood and Balian /2,3/. The results of this study indicated that the

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use of human liver microsomes to predict hepatic clearance in humans may not provide reliable predictions. On the other hand, the prediction of clearance in humans using allometric scaling combined with the 'rule of exponents' can provide comparatively better prediction of clearance in humans.

KEY WORDS

allometric scaling, rule of exponents, *in vitro* scaling, clearance

INTRODUCTION

Allometry is based on the assumption that the relationships between anatomy and physiological functions are similar among mammalian species /1/. Over the years, allometry has become a useful tool for correlating pharmacokinetic parameters with body weight from different animal species. By establishing such a correlation, one can predict pharmacokinetic parameters in humans that can be useful during drug development.

Clearance (CL) is an important pharmacokinetic parameter and a lot of attention has been given to the prediction of clearance in humans from animal data. A review of the literature indicates that simple allometry is not adequate to predict clearance for every drug /2,3/. To improve the predictive performance of allometry for the prediction of clearance, several different approaches have been proposed, such as: (i) species weight and maximum life-span potential (MLP) /4/; (ii) two term power equation based on brain weight and body weight /5/; (iii) the product of CL × brain weight /2,3/; and (iv) normalized *in vivo* clearance by *in vitro* clearance vs body weight /6-8/. In a previous study, Mahmood and Balian /2,3/ evaluated the different circumstances under which one of the three different methods (simple allometry, CL × MLP, or CL × brain weight) is most suitable for the improved prediction of clearance in humans. They proposed the selection of one of these methods based on the exponents of the simple allometry: (i) if the exponent of simple allometry lies between 0.55 and 0.70, simple allometry will predict clearance more accurately than CL × MLP or CL × brain weight; (ii) if the exponent of simple allometry lies between 0.71 and 1.0, CL × MLP will predict clearance

better compared to simple allometry or CL × brain weight; and (iii) if the exponent of simple allometry is >1.0, the product of CL × brain weight is a more suitable approach to predict clearance in humans compared to the other two methods. This proposed 'rule of exponents' by Mahmood and Balian has helped a great deal in improving the predictive performance of allometry for clearance.

In recent years, attempts have been made to predict *in vivo* hepatic clearance of drugs in humans using human liver microsomes or hepatocytes /9,10/. Though some success in predicting clearance with reasonable accuracy for some drugs has been reported /10/, a comparative study in predicting clearance from this approach with *in vivo* interspecies scaling has not been thoroughly investigated. Obach *et al.* compared the two approaches in one study /7/, but interspecies scaling was done using only simple allometry (clearance vs body weight). The authors reported that the *in vitro* approach was slightly better than the *in vivo* interspecies scaling. The present study compares the prediction of clearance using human liver microsomes *in vitro* with *in vivo* interspecies scaling using Mahmood's and Balian's proposed 'rule of exponents' /2,3/.

METHODS

A literature search was conducted to obtain clearance data for a wide variety of drugs (n = 16) that have been studied in at least three species (mice, rat, rabbit, guinea-pig, monkey, or dog) following intravenous administration. These drugs were selected randomly and are extensively metabolized. The renal clearance of all but three drugs (quinidine = 18% of total clearance, dofetilide = 65% of total clearance, and citalopram =17% of total clearance) was negligible. The hepatic clearance of these three drugs was calculated by subtracting renal clearance from the total clearance.

Allometric scaling

The allometric equation for clearance was generated using at least three animal species (human data were not included in the scaling) by the following three methods, and the predicted values were compared with the reported observed values in humans.

Method I

Clearance of each compound was plotted against body weight on a log-log scale and the following allometric equation was used to predict clearance in humans:

$$CL = a\,(W)^b \qquad [1]$$

where W is body weight and a and b are the coefficient and exponent of the allometric equation, respectively.

Method II

The observed clearance values in the different animal species were multiplied by their respective maximum life-span potential (MLP) and were plotted as a function of body weight on a log-log scale. From the allometric equation, the product clearance × MLP was estimated in man and the result was then divided by the MLP in man (8.18×10^5 h) to predict the clearance in humans:

$$CL = a\,(W)^b / 8.18 \times 10^5 \qquad [2]$$

The maximum life-span potential (MLP) in years was calculated from the following equation as described by Sacher /11/:

$$MLP\ (yr) = 185.4\,(BW)^{0.636}\,(W)^{-0.225} \qquad [3]$$

where both brain weight (BW) and body weight (W) are in kilograms.

Method III

Clearance of each animal was multiplied by the brain weight of the species and the product was plotted as a function of body weight on a log-log scale. The allometric equation was then used to predict the clearance in man using the brain weight (1.53 kg) of humans.

$$CL = a\,(W)^b / 1.53 \qquad [4]$$

The choice of one of the above-mentioned methods was based on Mahmood's and Balian's rule of exponents /2,3/ as described above.

***In vitro* method**

The enzyme kinetic parameters K_m and V_{max} measured in liver microsomal incubations were used to calculate intrinsic clearance (ml/min/mg protein):

$$CL'_{int} = \frac{K_m}{V_{max}} \quad [5]$$

where K_m is the Michaelis-Menten constant and V_{max} is the maximal rate of metabolism for the metabolic reaction.

The intrinsic clearance in a 70 kg human was calculated as follows:

$CL_{int} = CL'_{int} \times 52.5$ mg of protein per g liver weight x 1800 g liver.

Both the well-stirred and parallel tube models of hepatic clearance were used to predict clearance in humans according to the following equations:

Well-stirred:

$$CL_h = \frac{Q * CL_{int}}{Q + CL_{int}} \quad [6]$$

Parallel tube:

$$CL_h = Q\,(1 - e^{\frac{-CL_{int}}{Q}}) \quad [7]$$

where Q is hepatic blood flow.

Hepatic blood flow of 1500 ml/min or plasma flow of 825 ml/min was used to estimate hepatic clearance in humans.

Statistical analysis

The precision of the predicted values was measured by calculating the root mean square error (RMSE) according to the following equations:

$$\text{Mean Square Error (MSE)} = \frac{\Sigma_{i=1}\,(\text{predicted} - \text{observed})^2}{n} \quad [8]$$

$$RMSE = (MSE)^{1/2} \quad [9]$$

RMSE was expressed as the percent of the observed mean as follows:

$$\text{Percent RMSE} = \frac{(\text{RMSE})*100}{\text{observed mean}} \quad [10]$$

RESULTS

A good correlation (r = >0.9) between body weight and clearance was observed for all studied drugs. Tables 1 and 2 summarize the exponents of allometry and the predicted clearances of the studied drugs using simple allometry, the rule of exponents and the *in vitro* approach using human liver microsomes.

The exponents of the simple allometry ranged from 0.428 to 1.300. The results of this study, as observed previously /2,3/, indicate that simple allometry is not adequate for the prediction of clearance for all drugs. Incorporation of MLP or brain weight based on the exponents of simple allometry as suggested by Mahmood and Balian vastly improved the prediction of clearance. When simple allometry was used, the precision of the prediction of clearance in humans was poor. When all 16 drugs were included in the analysis the RMSE was 476% of the mean observed value. This high error was mainly due to the fact that the prediction error for tacrolimus was very high. When tacrolimus was excluded from the analysis, the RMSE was 156%. When the rule of exponents was used, there was a significant improvement in the predicted clearances of the studied drugs (n = 16) as observed by the precision of the prediction. The RMSE was 32.6% of the mean observed value.

When human microsomes were used for the *in vivo* prediction of clearance, two values for Q were used in equations [6] and [7] (blood flow of 1500 ml/min or 825 ml/min plasma flow). The predicted clearance values using plasma flow of 825 ml/min was superior than the predicted clearances using blood flow (1500 ml/min). This was not surprising as the reported observed hepatic clearances with the exception of tacrolimus were plasma clearances. The RMSE for well-stirred or parallel tube model was as follows:

Well-stirred model:

when Q = 1500 ml/min: 104.5%
when Q = 825 ml/min: 70.7%

TABLE 1

Comparison of the predicted clearances using simple allometry, the rule of exponents and the *in vitro* approach using human liver microsomes (Q = 825 ml/min)

Drug	Exponent	r	Obs CL	Pred CL (SA)	Pred CL (RE)	Pred CL (WS)	Pred CL (PT)	References
Diazepam	0.737	0.939	26	860	466	326	395	/10,15/
Warfarin	1.126	0.928	4	44	8	61	64	/10,16/
Quinidine	0.805	0.960	270 [a]	1392	285	363	449	/10,17-19/
Propafenone	0.827	0.991	616	1809	550	784	825	/20-22/
Sildenafil	0.680	1.000	420	523	523	314	379	/23,24/
Metoprolol	0.428	0.962	1050	826	826	439	560	/25-28/
Tolcapone	0.650	0.970	118	113	113	100	106	/6/
Theophylline	0.657	0.954	45	42	42	4	4	/10,29/
Dofetilide	0.530	0.969	105 [a]	210	210	42	43	/10,30/
Citalopram	0.724	0.994	290 [a]	969	395	306	367	/31,32/
Propranolol	0.674	0.918	850	716	716	742	825	/33,34/
Cyclophosphamide	0.863	0.989	200	627	264	398	500	/4,35/
Ethinylestradiol	0.716	0.974	378	799	449	742	825	/36,37/
Troglitazone	0.824	0.993	172	382	180	376	468	/38,39/
Tirilazad	0.693	0.928	580	503	503	802	825	/40-42/
Tacrolimus *	1.300	0.957	2100	10560	2300	1191	1468	/43-47/

SA = simple allometry; RE = rule of exponent; WS = well-stirred; PT = parallel tube; [a] hepatic clearance = (total CL - renal CL);
* Blood clearance (Q = 1500 ml/min).

TABLE 2

Comparison of the predicted clearances using simple allometry, the rule of exponents and the *in vitro* approach using human liver microsomes (Q = 1500 ml/min)

Drug	Exponent	r	Obs CL	Pred CL (SA)	Pred CL (RE)	CL (well-stirred)	CL (parallel tube)
Diazepam	0.737	0.939	26	860	466	396	452
Warfarin	1.126	0.928	4	44	8	63	65
Quinidine	0.805	0.960	270 [a]	1392	285	453	526
Propafenone	0.827	0.991	616	1809	550	1369	1500
Sildenafil	0.680	1.000	420	523	523	379	431
Metoprolol	0.428	0.962	1050	826	826	577	697
Tolcapone	0.650	0.970	118	113	113	105	109
Theophylline	0.657	0.954	45	42	42	4	4
Dofetilide	0.530	0.969	105 [a]	210	210	43	44
Citalopram	0.724	0.994	290 [a]	969	395	367	415
Propranolol	0.674	0.918	850	716	716	1245	1489
Cyclophosphamide	0.863	0.989	200	627	264	508	601
Ethinylestradiol	0.716	0.974	378	799	449	1246	1489
Troglitazone	0.824	0.993	172	382	180	473	554
Tirilazad	0.693	0.928	580	503	503	1427	1500
Tacrolimus*	1.300	0.957	2100	10560	2300	1191	1468

SA = simple allometry; RE = rule of exponents; [a] hepatic clearance = (total CL - renal CL); * Blood clearance (Q = 1500 ml/min).

Parallel tube model:

when Q = 1500 ml/min: 114.1%
when Q = 825 ml/min: 63.2%.

The predicted clearance was also assessed for individual drugs. It was assumed that the prediction of clearance is successful if the error between predicted and observed clearance is 30% or less. The 30% cut-off point was based on the assumption that 30% difference between observed and predicted clearance for most of the drugs will probably not produce toxicity or sub-therapeutic levels. Since the objective of allometric scaling is to select a safe dose for the first administration to humans, 30% difference between predicted and observed clearance was considered appropriate. Based on this analysis the following numbers of drugs were found within this limit:

Allometry

Simple allometry: 6 drugs
Rule of exponents: 11 drugs

In vitro *approach:*

Well-stirred model:

when Q = 1500 ml/min: 3 drugs
when Q = 825 ml/min: 4 drugs

Parallel tube model:

when Q = 1500 ml/min: 4 drugs
when Q = 825 ml/min: 5 drugs.

The percent error (difference between observed and predicted clearances) for different approaches is summarized in Table 3.

Based on the current analysis, it appears that allometric scaling using the rule of exponents predicts clearance of drugs in humans with much more accuracy than the *in vitro* microsomal approach. It should be noted, however, that the *in vitro* approach was better than using only simple allometry. The well-stirred and the parallel tube models were comparable in their predictive performance of clearance in humans.

TABLE 3

Percent error in prediction of clearance using simple allometry, the rule of exponents and the *in vitro* approach using human liver microsomes

Drug	SA	RE	Well-stirred		Parallel tube	
			Q = 1500	Q = 825	Q = 1500	Q = 825
Diazepam	3208	1692	1423	1154	1639	1419
Warfarin	1000	100	1485	1425	1519	1500
Quinidine	416	6	68	34	95	66
Propafenone	194	-11	122	27	143	34
Sildenafil	25	25	-10	-25	3	-10
Metoprolol	-21	-21	-45	-58	-34	-47
Tolcapone	-4	-4	-11	-15	-7	-10
Theophylline	-7	-7	-92	-91	-92	-91
Dofetilide	100	100	-59	-60	-58	-59
Citalopram	234	36	27	6	43	27
Propranolol	-16	-16	47	-13	75	-3
Cyclophosphamide	214	32	154	99	200	150
Ethinylestradiol	111	19	230	96	294	118
Troglitazone	122	5	175	119	222	172
Tirilazad	-13	-13	146	38	159	42
Tacrolimus	403	10	-43	NA	-30	NA

SA = simple allometry; RE = rule of exponents; Q in ml/min; NA = not applicable (blood clearance).
Percent error = (observed-predicted)*100/observed.

DISCUSSION

Over the years allometric scaling has become a useful tool for the selection of a first time dose to be administered to humans /12/. The selection of such a dose may be based on the prediction of pharmacokinetic parameters such as clearance, volume of distribution and half-life. The knowledge of clearance is especially important during drug discovery or screening processes, since drugs which are eliminated quickly may have a low bioavailability and may not be suitable for further investigation. Clearance can also play an important role for the selection of the first dose in humans (as inverse of clearance indicates the total exposure [AUC] of a drug). Therefore over the years a lot of attention has been focused in order to improve the performance of allometry to predict clearance.

A lot of interest in using *in vitro* data in allometric scaling has been developed and a comprehensive review was published by Houston on this topic /13/. Lave *et al.* /6/ predicted hepatic clearance of ten extensively metabolized drugs in man by incorporating *in vitro* data into allometric scaling. These authors concluded that integrating the *in vitro* data with the allometric approach improved the prediction of clearance in humans as compared to the approach of simple allometry or the product of clearance and brain weight. The authors, however, assumed that clearance of all drugs can be predicted either by simple allometry or by the product of clearance and brain weight. Since this assumption is incorrect, the data of Lave *et al.* /6/ were reanalyzed by Mahmood /8/ and the results indicated that the normalization of clearance by MLP (as required based on the exponents) could have produced the same results as observed by the *in vitro* approach. Furthermore, based on the exponents of simple allometry, it was found that the product of clearance and brain weight was not a suitable approach for the prediction of clearance for these drugs.

There have been attempts to predict clearance of drugs in humans using human liver microsomes or hepatocytes with some degree of success. Iwatsubo *et al.* /10/ reviewed literature data to demonstrate that one can successfully predict clearance of some drugs in humans using *in vitro* human microsomal data. The authors, however, also noted large differences in the predicted and observed clearances for many drugs and proposed reasons for such large differences. In a separate study, Obach *et al.* /7/ compared allometric scaling and the *in*

vitro microsomal approach and concluded that an *in vitro* approach was slightly better than allometric scaling (only a simple allometric approach was used). Since simple allometry is not suitable for the prediction of clearance for all drugs, the present study compares the *in vitro* microsomal approach with allometric scaling when clearance was predicted based on the rule of exponents.

The results of this analysis indicated that the *in vitro* approach produced a larger error in prediction of clearance for most of the drugs considered compared to the rule of exponents. On the other hand, simple allometry was less accurate in predicting clearance compared to the *in vitro* approach. This is a clear indication that simple allometry is not appropriate to predict clearance for all drugs; therefore, incorporation of MLP or brain weight based on the exponents of simple allometry is essential to obtain comparatively accurate prediction of clearance of drugs in humans. Both the RMSE and the 30% difference between observed and predicted values indicated the superiority of allometric scaling combined with the rule of exponents over the *in vitro* approach in predicting clearances of drugs in humans.

In vitro data can provide important qualitative insights into the metabolism of xenobiotics without the need for *in vivo* exposure but the limitations of *in vitro* systems cannot be ignored. One major concern is the concentration used in an *in vitro* system to generate V_{max} which may not be relevant to the *in vivo* concentrations. The importance of the selection of concentration in *in vitro* metabolic studies can be demonstrated by the *in vitro* metabolic study of diazepam. The metabolism of diazepam is regulated by a high- and a low-affinity component. The intrinsic clearance (sum of both low- and high-affinity) of diazepam is 0.019 ml/min/mg protein at the concentration of 2 μM and 0.004 ml/min/mg protein at he concentration of 200 μM diazepam /14/. Thus, these two different intrinsic clearances will have an impact on the prediction of hepatic clearance. In reality, for prediction purposes, it is very difficult to assess the relevant concentration to be used in an *in vitro* study which may be close to the *in vivo* concentration. Furthermore, *in vitro* data may be misleading if the clearance of a drug is dependent on liver blood flow. In this report, propranalol, a highly extracted drug, was predicted with a fair degree of accuracy, but this may not be the case for other highly extracted drugs.

Based on this study, it is suggested that for the estimation of hepatic clearance from equations [6] or [7], one should use plasma flow rate rather than blood flow rate if the reported clearance in humans is anticipated to be plasma clearance.

In short, it appears from this study that a more accurate prediction of clearance in humans can be obtained from allometric scaling using the rule of exponents as compared to *in vitro* microsomal data. Furthermore, it was shown previously that *a priori* knowledge of cytochrome P450 isozymes involved in drug metabolism may not be of help in predicting clearance in humans from animal data /48/. The present study was made based on a limited number of drugs (n = 16), thus extensive work is needed in this direction before one can clearly establish the advantages and accuracy of an *in vitro* approach in predicting clearance of drugs over other existing methods. However, it should be kept in mind that an *in vitro* approach in allometric scaling is one of many suggested approaches to improve the prediction of clearance, and should be used with caution in conjunction with other methods.

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