Integration of *in Vitro* Data into Allometric Scaling To Predict Hepatic Metabolic Clearance in Man: Application to 10 Extensively Metabolized Drugs

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Abstract □ In this study, we investigated rational and reliable methods of using animal data to predict in humans the clearance of drugs which are mainly eliminated through hepatic metabolism. For 10 extensively metabolized compounds, adjusting the *in vivo* clearance in the different animal species for the relative rates of metabolism *in vitro* dramatically improved the predictions of human clearance compared to the approach in which clearance is directly extrapolated using body weight. Using hepatocyte data to normalize the *in vivo* clearances led to lower median deviations between the observed and predicted clearances in man compared to the approach normalizing data with brain weight (30–40% vs 60–80%, respectively). In addition, the approach integrating *in vitro* data appeared to be superior with respect to the range of deviations: approximately 2-fold underestimation, in the worst case, was observed by using *in vitro* data, whereas normalizing data by brain weight led to up to 10-fold underestimation of clearance in man. In addition, the integration of *in vitro* data provides a more rational basis to predict the metabolic clearance in man and may be applicable to compounds undergoing phase I and phase II metabolism as well.

Introduction

Early knowledge of the human pharmacokinetics of new drug candidates is of major importance at several stages of the development process, for the selection of compounds as well as for the design of the first clinical protocol. Interspecies scaling is used to extrapolate pharmacokinetic parameters from animal to man, using physiologically based models or allometric procedures. Both approaches assume that pharmacokinetic parameters can be scaled according to fundamental physiological principles. Physiological models attempt to give a physiological meaning to the pharmacokinetic parameters. This approach has found only limited application in drug development since considerable time and efforts are required to develop, test, and implement such models.[1] In contrast, the allometric approach is more empirical but is simple and fast and utilizes data which are commonly available during preclinical drug development.

Conventional allometric scaling was shown to be predictive for the situation in man for renally excreted antibiotics[2−6] and proteins,[7,8] as well as drugs rapidly metabolized (where the elimination is dependent on liver blood flow[9]). However, for metabolized compounds characterized by low or intermediate hepatic extraction ratio, the elimination is strongly dependent on biochemical parameters such as intrinsic clearance and protein binding. For these compounds, allometric relationships usually fail to predict the human situation.[10] To improve the predictions of metabolic clearance in man for this class of compounds, correction factors such as brain weight or maximum life span have been proposed and used with some success.[10−13] Recently, increased accuracy in the prediction of metabolic clearance has been achieved, for compounds metabolized by cytochrome P450[14,15] as well as phase II reactions,[16] by combining *in vivo* and *in vitro* metabolic data in animals and humans. Furthermore, *in vitro* metabolic data provided a more rational basis for predicting the metabolic clearance in humans than the aforementioned empirical correction factors.

The aim of the present study was to further evaluate the approach combining *in vitro* and *in vivo* data to predict hepatic metabolic clearance in man with 10 metabolized compounds. In order to be representative of unknown compounds to be extrapolated, the test compounds were selected to represent a broad range of clearance, plasma protein binding and a variety of metabolic pathways in animals and man. The predictive value of correction factors such as brain weight or *in vitro* metabolic rates as well as the necessity to correct for interspecies differences in plasma protein binding was evaluated. In addition, the suitability of each laboratory animal species to predict the human situation was investigated in this study. The present investigation was carried out under the assumptions that metabolism is only by the liver. Furthermore, for some of the compounds the blood/plasma partition coefficient was assumed to be unity in the different species.

Material and Methods

Selection of Compounds—Pharmacokinetic and metabolic data for the 10 test compounds (antipyrine, bosentan, caffeine, mibefradil, midazolam, mofarotene, Ro 24-6173 (NMDA receptor antagonist), propranolol, theophylline, and tolcapone) were obtained from the literature and from Hoffmann La Roche. Publications were collected in which hepatic clearance and protein binding in animals and humans had been measured. For all compounds, the values of plasma clearance and free fraction in plasma were converted to the corresponding blood clearance and free fraction in blood using the reported blood/plasma partition coefficient in the different species. When binding to erythrocytes was not known (caffeine and theophylline), the blood/plasma partition coefficient was assumed to be unity.

For all compounds selected, liver was the main site of metabolism and *in vivo* pharmacokinetic data obtained after intravenous administration were available in at least three animal species (mice, rat, rabbit, monkey, pig, or dog). In the case of mofarotene, oral data were utilized. The bioavailability of this compound measured in mice, rats, and dogs ranged from 50 to 100%.[15]

***In Vitro* Data**—For all test compounds, *in vitro* metabolic data were generated from three or four batches of hepatocytes using different sets of livers. Hepatocytes were isolated from different animal species and humans.

Liver samples were obtained from male "Moro" mice (BRL, Fuellinsdorf, Switzerland), male albino "Roro" rats (BRL, Fuellinsdorf, Switzerland), male Burgundy rabbits (BRL), male marmoset monkeys (BRL), and male Swiss beagle dogs (BRL). Human liver specimens were collected at the Hautepierre Hospital (Strasbourg, France) from hepatic surgical resection, in accordance with the guidelines of the local Ethics Committee. The liver samples were obtained mainly from cancer patients (hepatic metastasis of colorectal cancers).

Rat and mouse hepatocytes were prepared from the *in situ* perfusion of a whole liver.[17] For rabbit, dog, pig, monkey, and man, a lobe or a lobe fraction was perfused.[18]

The isolation of hepatocytes involved a two-step perfusion of the liver tissue: first with a Ca^{2+}-free buffer containing EGTA and then

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with a buffer containing Ca^{2+} and collagenase (100−200 IU depending upon the species).

The cell viability, assessed by erythrosin B exclusion, was greater than 80%. Fresh cells were seeded onto collagen-coated dishes at the density of 1×10^6 viable cells (or 1.5×10^6 cells for human hepatocytes) covered in 2 mL of culture medium (William's E supplemented with penicillin/streptomycin (0.5%, v/v), insulin (10^{-7} M), fetal calf serum (10%, v/v), hydrocortisone (10^{-6} M), and 0.4 mM glutamine). After attachment of the cells, each compound was added at concentrations ranging from 0.1 to 100 μM in 1 mL of culture medium. Incubations were for up to 72 h for slowly metabolized compounds (e.g. caffeine, antypirine), whereas ca. 2 h incubations were sufficient for rapidly metabolized compounds (e.g. propranolol). The concentrations incubated were below the K_m values reported in the literature for each compound. At the end of the incubation, the medium (ca. 1 mL) was collected into Eppendorf vials. Intracellular sample was obtained by scraping the cell monolayer into 1 mL of acetonitrile (or methanol):water (50:50). The unchanged compounds were analyzed in the medium and cell lysate by sensitive and specific HPLC−UV (or fluorimetric) methods. For all compounds, the analytical methods had sensitivity below 1% of the initial concentration incubated with a CV of less than 20% for intraday comparisons.

Data Analysis—*In Vitro Experiments: Intrinsic Clearance (CL_{int})*—CL_{int} was calculated from the ratio of the initial amount of compound in the incubation medium and the corresponding AUC values.[19] For all compounds, total (bound + unbound) concentrations were measured in the incubation medium at six different time points and used for the calculation of CL_{int} The area under the concentration in the incubation medium versus time curve (AUC) was calculated using the linear trapezoidal rule. The area was extrapolated to infinite time by adding C_t/β to the AUC, where C_t is the predicted concentration at the last sampling time and β is the slope of the terminal phase of the log concentration−time curve, determined by linear regression of the last three or four data points. The extrapolated part of the AUC was usually less than 10% of the AUC, except for very slowly metabolized compounds in man (e.g. antipyrine), where extrapolations up to 50% were obtained. CL_{int} determined in the linear range of the kinetics were used in this study. In each species, CL_{int} was normalized to 1 million hepatocytes.

Allometric Scaling—For allometric scaling, *in vivo* clearances (CL, blood clearance of total (bound + unbound) drug; CL_u, blood clearance of unbound drug; CL_{int}, intrinsic metabolic clearance) of the compounds in animal species were correlated with their corresponding mean body weights (B), using allometric equations of the form clearance = aB^x. The values of the allometric coefficients (a) and exponents (x) were estimated by linear least squares regression of the log transformed allometric equations (log clearance = log a + x log B).

CL_u and CL_{int} were calculated in each animal species and in humans according to the following equations

$$CL_u = \frac{CL}{f_u}$$

$$CL_{int} = \frac{(CL)Q}{f_u(Q - CL)}$$

where f_u is the unbound fraction of the drug in blood and Q is the liver blood flow (90, 60, 60, 58, 43, and 20 mL/min/kg for mouse, marmoset, rat, rabbit, dog, and man, respectively (Hoffmann La Roche, data on file)).

When *in vitro* metabolic rates were used as a correction factor, the *in vivo* clearance in each animal species was normalized by the ratio of the *in vitro* clearance values; for example, $CL_{rat(in\ vivo)}(CL_{human(hepatocytes)}/CL_{rat(hepatocytes)})$. These normalized values were then extrapolated using allometric scaling.

The clearances predicted for humans were compared with the values determined by conventional allometric scaling and also with the values obtained by normalizing clearance (CL, CL_u, CL_{int}) in the different animal species for brain weight (BRW), which has been suggested as an empirical correction factor for metabolized compounds.[13] In this case the product (clearance x BRW) was scaled from animal to man.[11]

In addition, the hepatic metabolic clearance in man was calculated from each individual species by combining *in vivo* and *in vitro* data, according to the following equation:

$$CL_{man} = CL_{animal}\frac{CL_{human(hepatocytes)}}{CL_{animal(hepatocytes)}}\left(\frac{B_{human}}{B_{animal}}\right)^x$$

where x is the mean allometric exponent of the allometric equations

$$CL_{animal}\frac{CL_{human(hepatocytes)}}{CL_{animal(hepatocytes)}} = aB^x$$

describing the relationship between CL normalized with *in vitro* data and body weight for the 10 test compounds. The mean allometric exponent used for these calculations was 0.86.

Statistical Analysis—Statistical differences between the different approaches were determined using ANOVA or Student's t-test for paired samples.

Table 1—*In Vivo*, *in Vitro* (Hepatocytes) Clearances and Free Fractions in Animals and Man for the 10 Compounds Investigated

drug	species	B (kg)	f_u (blood)	CL *in vivo* (blood) mL/min/kg	CL *in vitro*[b] μL/min/10^6 cells	ref
Antipyrine	Rat	0.23	1	7.0	0.36	24
	Rabbit	2.93	1	8.9	1.1	24
	Dog	14.2	1	4.9	1.0	24
	Man	70.0	1	0.46	0.092	24
Bosentan	Mouse	0.04	0.01	46.0	1.2	14
	Marmoset	0.41	0.06	45.0	4.0	14
	Rat	0.27	0.02	55.0	1.3	14
	Rabbit	2.50	0.04	100	4.3	14
	Dog	13.9	0.03	1.9	0.22	14
	Man	70.0	0.02	3.7	0.22	14
Caffeine	Rat	0.28	0.90	13.0	0.58	25
	Rabbit	2.42	0.87	6.3	0.32	25
	Dog	12.9	0.90	2.6	0.21	*a*
	Man	68.8	0.96	2.0	0.13	25
Mibefradil	Rat	0.25	0.02	94.0	5.4	*a*
	Marmoset	0.30	0.08	74.0	5.5	*a*
	Rabbit	2.50	0.14	64.0	16.0	*a*
	Dog	16.5	0.08	36.0	6.2	*a*
	Man	76.0	0.01	7.0	0.92	*a*
Midazolam	Rat	0.25	0.04	130	110	26
	Rabbit	3.50	0.18	19.0	20.0	*a*
	Dog	16.6	0.04	48.0	33.0	27
	Pig	23.2	0.09	41.0	19.0	28
	Man	72.5	0.04	11.0	9.3	29
Mofarotene	Mouse	0.04	0.001	27.0	1.1	15
	Rat	0.30	0.001	16.0	2.3	15
	Dog	12.8	0.001	5.8	1.0	15
	Man	70.0	0.001	11.0	2.0	15
Ro 24-6173	Rat	0.25	0.13	110	39.0	*a*
	Rabbit	3.60	0.10	39.0	27.0	*a*
	Dog	17.0	0.11	35.0	13.0	*a*
	Man	70.0	0.10	12.0	2.9	*a*
Propranolol	Rat	0.25	0.08	92.0	51.0	30
	Rabbit	3.60	0.05	180	56.0	31
	Dog	17.0	0.03	34.0	19.0	30
	Man	70.0	0.07	13.0	4.2	30
Theophylline	Rat	0.25	0.40	2.2	0.18	32
	Rabbit	3.60	0.58	2.1	0.24	32
	Dog	17.0	0.58	1.5	0.15	32
	Man	70.0	0.58	0.61	0.11	32
Tolcapone	Rat	0.25	0.001	15.0	2.6	16
	Rabbit	2.50	0.001	17.0	4.4	16
	Dog	16.5	0.001	3.3	1.4	16
	Man	76.0	0.001	2.7	1.2	16

[a] Hoffmann La Roche, data on file. [b] Median value, n = 3–4 batches of hepatocytes).

Results

Table 1 lists all *in vivo* and *in vitro* data available in the different animal species and in humans which were used for the purposes discussed above. The test compounds represented a broad range of clearance. For example, antipyrine, caffeine, theophylline, and tolcapone are characterized by a low clearance in animals and humans. By contrast, propranolol and Ro 24-6173 exhibit an intermediate to high clearance (at least 60% of the liver blood flow) in all animal species and humans. Compounds exhibiting large interspecies differences in clearance were also included in this evaluation. Bosentan, for example, is characterized by a high clearance, close to the liver blood flow in rabbits. Intermediate to high clearance values were observed in mice, marmosets, and rats, while dogs exhibited a low blood clearance which represented less than 5% of the corresponding liver blood flow. The protein binding of the test compounds ranged from 0 (e.g. antipyrine) to 99.9% (e.g. tolcapone). Some compounds (mibefradil, bosentan, midazolam, and propranolol) exhibited large interspecies differences in free fraction in blood. For example, the free fraction of mibefradil in blood was around 1% in humans and 14% in rabbits.

The allometric equations obtained for the 10 test compounds and the corresponding correlation coefficients (r^2) are provided in Table 2. Figures 1 and 2 illustrate the log−log relationship between clearance (CL, CL × BRW, and CL normalized with *in vitro* data on hepatocytes) and body weight for antipyrine and midazolam, respectively. The clearances predicted in man according to the different methods are provided in Table 2 for the 10 test compounds. The deviations between the predicted and observed clearance values in man are presented graphically in Figures 3−6 for the different approaches evaluated.

The mean allometric exponent values for clearance (CL, CL_u, and CL_{int}) (mL/min) with or without correction for *in vitro* data ranged from 0.7 to 0.9. This is in agreement with the exponent values of 0.6−0.8 frequently cited in the literature for clearance.[2] The mean exponent value (ca. 1.6) for clearance normalized with BRW compares also favorably with literature values of 1.6−1.9.[11]

Correcting clearance with *in vitro* metabolic rates significantly improved the predictions of clearance in man ($p < 0.05$), in comparison to the approach extrapolating clearance directly or after correction with BRW. The median deviations ((obs − pred)/obs)) between the observed and predicted clearances were (no correction/BRW correction/hepatocyte correction): −87.2/63.4/34.7, 5.5/77.7/41.0, and 18.9/77.8/35.3 %, respectively for CL, CL_u, and CL_{int}. A clear tendency to overpredict the clearance in man was observed when CL was directly extrapolated using body weight. This is in agreement with the fact that humans tend to have lower metabolic rates than animal species.[9] By contrast, correcting clearance (CL, CL_u, and CL_{int}) with *in vitro* metabolic rates or brain weight tended to underpredict the clearance in man. Also, the range of deviations obtained when hepatocyte data were used to normalize *in vivo* clearances in animals was much smaller than with the two other approaches. Using *in vitro* data, a 2.1-fold underestimation of the clearance of Ro 24-6173 in man was the maximum deviation observed, whereas approximately 10-fold overestimation or underestimation were obtained for antipyrine and mofarotene when clearance was extrapolated directly or after BRW correction, respectively.

Taking into account interspecies differences in protein binding to extrapolate unbound clearance or intrinsic clearance from animal to man tended to improve the prediction of clearance in man. Surprisingly, this was no longer true when *in vitro* clearances on hepatocytes were used to normalize the *in vivo* clearances in animals (Figures 3 and 4). In this case,

Table 2—Observed and Predicted Blood Clearances (mL/min/kg) in Man According to the Various Approaches Evaluated and Corresponding Allometric Equations of the Form[a] $Clearance = aB^x$

Compounds	CL *in vivo* Observed	Total Clearance			Unbound Clearance			Intrinsic Clearance		
		CL	CL × BRW	CL × Wh[b]	CL_u	CL_u × BRW	CL_u × Wh	CL_{int}	CL_{int} × BRW	CL_{int} × Wh
Antipyrine	0.46	$7.13B^{0.93}$ $r^2 = 0.98$	$0.05B^{1.84}$ $r^2 = 0.99$	$1.09B^{0.66}$ $r^2 = 0.99$	$7.13B^{0.93}$ $r^2 = 0.98$	$0.05B^{1.84}$ $r^2 = 0.99$	$1.09B^{0.66}$ $r^2 = 0.99$	$8.18B^{0.93}$ $r^2 = 0.98$	$0.05B^{1.84}$ $r^2 = 0.99$	$1.25B^{0.66}$ $r^2 = 0.99$
		5.3	1.2	0.25	5.3	1.2	0.25	4.6	1.2	0.29
Bosentan	3.7	$24.9B^{0.58}$ $r^2 = 0.53$	$0.21B^{1.46}$ $r^2 = 0.86$	$4.29B^{0.80}$ $r^2 = 0.93$	$899B^{0.39}$ $r^2 = 0.39$	$7.47B^{1.27}$ $r^2 = 0.90$	$155B^{0.62}$ $r^2 = 0.66$			
		4.2	1.0	1.8	1.4	0.35	0.64			
Caffeine	2.0	$7.95B^{0.59}$ $r^2 = 0.99$	$0.05B^{1.58}$ $r^2 = 0.99$	$2.52B^{0.86}$ $r^2 = 0.99$	$8.93B^{0.59}$ $r^2 = 0.98$	$0.05B^{1.58}$ $r^2 = 0.99$	$2.83B^{0.86}$ $r^2 = 0.99$	$10.6B^{0.54}$ $r^2 = 0.99$	$0.07B^{1.53}$ $r^2 = 0.99$	$3.36B^{0.81}$ $r^2 = 0.99$
		1.4	0.40	1.4	1.5	0.43	1.5	1.4	0.41	1.3
Mibefradil	7.0	$63.6B^{0.90}$ $r^2 = 0.99$	$0.617B^{1.62}$ $r^2 = 0.97$	$15B^{0.82}$ $r^2 = 0.96$	$1135B^{0.65}$ $r^2 = 0.73$	$10.95B^{1.37}$ $r^2 = 0.97$	$145.8B^{0.57}$ $r^2 = 0.53$	$3783B^{0.60}$ $r^2 = 0.62$	$36.3B^{1.31}$ $r^2 = 0.97$	$483.3B^{0.51}$ $r^2 = 0.46$
		42.0	6.3	3.78	1.3	0.18	0.12	3.0	0.48	0.30
Midazolam	11.0	$64.7B^{0.78}$ $r^2 = 0.87$	$0.37B^{1.64}$ $r^2 = 0.95$	$10.7B^{1.11}$ $r^2 = 0.99$	$1070B^{0.68}$ $r^2 = 0.53$	$6.04B^{1.54}$ $r^2 = 0.83$	$177B^{1.01}$ $r^2 = 0.85$			
		25.0	4.1	17.0	9.4	1.5	6.5			
Mofarotene	11.0	$11.6B^{0.73}$ $r^2 = 0.99$	$0.08B^{1.68}$ $r^2 = 0.99$	$17.1B^{0.76}$ $r^2 = 0.97$	$11585B^{0.73}$ $r^2 = 0.99$	$78B^{1.68}$ $r^2 = 0.99$	$17070B^{0.76}$ $r^2 = 0.97$	$14865B^{0.70}$ $r^2 = 0.99$	$100B^{1.64}$ $r^2 = 0.99$	$21903B^{0.73}$ $r^2 = 0.97$
		3.7	1.0	6.3	3.7	1.0	6.3	3.4	1.0	5.1
RO 24-6173	12.0	$68.1B^{0.72}$ $r^2 = 0.99$	$0.38B^{1.61}$ $r^2 = 0.99$	$6.6B^{0.96}$ $r^2 = 0.97$	$580B^{0.77}$ $r^2 = 0.99$	$3.28B^{1.65}$ $r^2 = 0.99$	$56.1B^{1.00}$ $r^2 = 0.99$			
		21.0	3.6	5.6	22.0	3.7	5.8			
Propranolol	13.0	$97.2B^{0.82}$ $r^2 = 0.85$	$0.55B^{1.70}$ $r^2 = 0.98$	$9.08B^{1.02}$ $r^2 = 0.98$	$1598B^{1.01}$ $r^2 = 0.91$	$9.03B^{1.90}$ $r^2 = 0.99$	$149B^{1.22}$ $r^2 = 0.99$			
		45.0	7.7	10.0	110	20.0	26.0			
Theophylline	0.61	$2.04B^{0.92}$ $r^2 = 0.99$	$0.01B^{1.80}$ $r^2 = 0.99$	$1.17B^{0.95}$ $r^2 = 0.99$	$4.33B^{0.82}$ $r^2 = 0.99$	$0.02B^{1.71}$ $r^2 = 0.99$	$2.50B^{0.85}$ $r^2 = 0.99$	$4.50B^{0.82}$ $r^2 = 0.99$	$0.03B^{1.71}$ $r^2 = 0.99$	$2.59B^{0.85}$ $r^2 = 0.99$
		1.4	0.25	0.93	1.2	0.21	0.77	1.2	0.21	0.77
Tolcapone	2.7	$12.5B^{0.65}$ $r^2 = 0.86$	$0.08B^{1.55}$ $r^2 = 0.97$	$5.35B^{0.78}$ $r^2 = 0.99$	$12468B^{0.65}$ $r^2 = 0.86$	$77.9B^{1.55}$ $r^2 = 0.97$	$5347B^{0.78}$ $r^2 = 0.99$	$16472B^{0.60}$ $r^2 = 0.78$	$103B^{1.51}$ $r^2 = 0.96$	$7065B^{0.74}$ $r^2 = 0.99$
		2.7	0.61	2.1	2.7	0.61	2.09	2.5	0.64	2.0

[a] The values of the allometric coefficients (a) and exponents (x) were estimated by linear least squares regression of the log-transformed allometric equations (log clearance = log a + x log B); r is the correlation coefficient. [b] Wh = $(CL_{human(hepatocytes)}/CL_{animal(hepatocytes)})$.

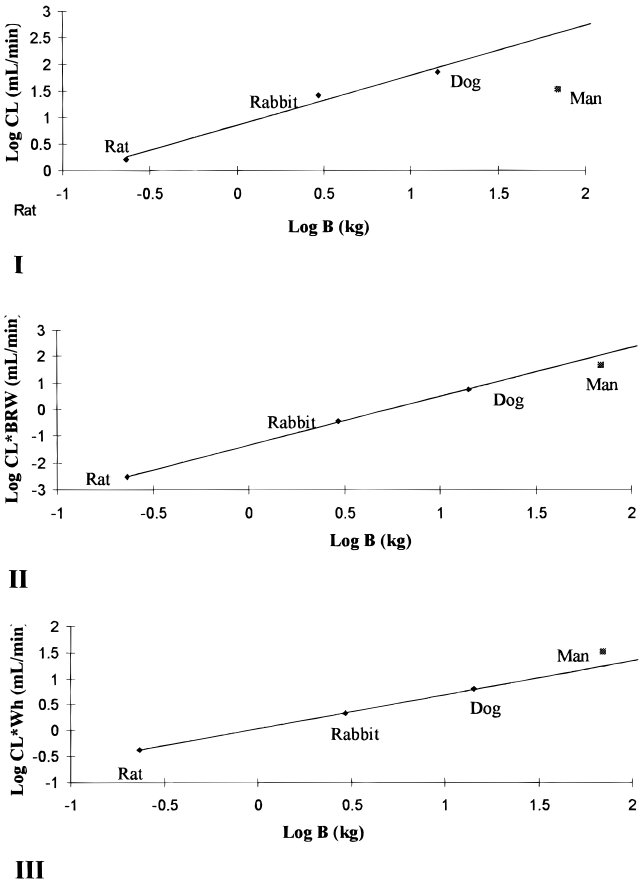

Figure 1—Antipyrine: allometric scaling of clearance (I), clearance normalized with brain weight (II), and clearance normalized with hepatocyte data (III). The square symbols for man are *in vivo* data points based on actual data. Wh = ($CL_{human(hepatocytes)}/CL_{animal(hepatocytes)}$).

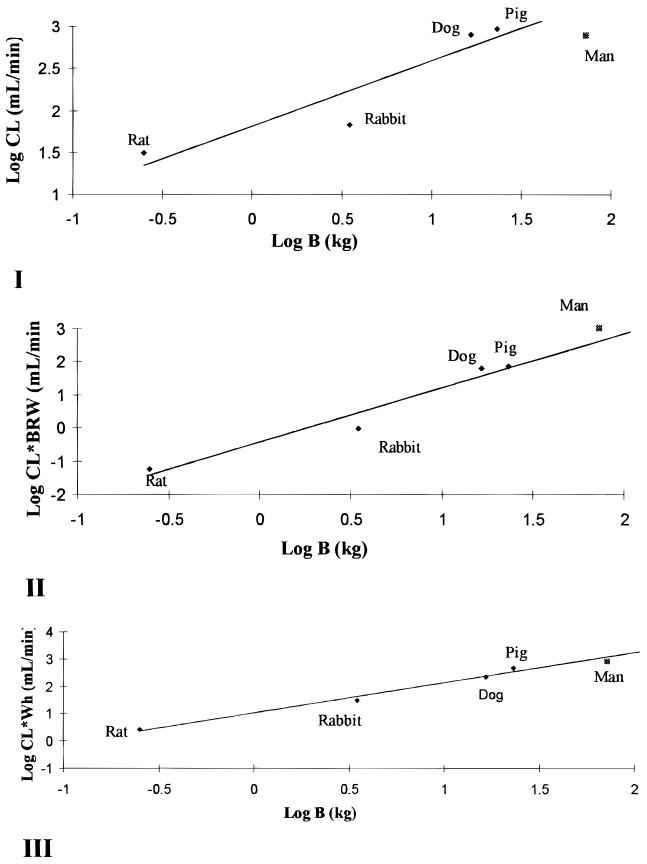

Figure 2—Midazolam: allometric scaling of clearance (I), clearance normalized with brain weight (II), and clearance normalized with hepatocyte data (III). The square symbols for man are *in vivo* data points based on actual data. Wh = ($CL_{human(hepatocytes)}/CL_{animal(hepatocytes)}$).

the predictions obtained with CL tended to be slightly better than with CL_u and CL_{int}. The median deviations observed were 34.7/41.0/35.3%, respectively, for CL, CL_u and CL_{int}.

In order to evaluate the predictive value of rat, rabbit, and dog for the situation in man, the expected clearance in man was calculated from each individual species by combining *in vivo* and *in vitro* data. These clearances predicted in man are provided in Table 3 for the ten test compounds in comparison to the values observed in man. The deviations between the predicted and observed clearances for the ten test compounds are illustrated in Figure 6. All three species appeared to be equally good predictors for the clearance in man. The median deviations observed, 25.6 for rat, 16.0 for rabbit, and 28.0% for dog, were very close to the median deviation obtained when using the allometric approach combining several species (34.7%). A tendency to systematically underpredict the clearance in man was observed with rabbit and dog. By examining the range of deviations obtained from the different species, some outliers could be identified when clearance was predicted from rat and rabbit: in the case of Ro 24-6173, predictions from rat and rabbit underestimated the observed value of clearance in man by a factor of 3−4. For the dog, an approximately 2-fold underestimation of the clearance of propranolol and Ro 24-6173 in man was the maximum deviation observed.

Discussion

In this study, we evaluated several methods of using animal data to predict in humans the clearance of drugs which are mainly eliminated through hepatic metabolism. One such approach is to use *in vitro* liver models (e.g. hepatocytes) to determine the relative capacities of the various animal species and humans to metabolize drugs. These data can then be combined with the *in vivo* data in animals, to derive the *in vivo* clearance in humans using allometric scaling techniques. Such an approach has already been illustrated with selected compounds metabolized by cytochrome P450[14,15] and/or by phase II reactions.[16]

The present study further validates this approach with a total of 10 compounds that are metabolized in the liver and represent a variety of metabolic pathways and a broad range of hepatic metabolic clearance and plasma protein binding in animals and man. *In vitro* data were generated in primary hepatocytes, since they possess a complete complement of drug-metabolizing enzymes and have been shown to provide intrinsic clearances more representative of the *in vivo* situation than other *in vitro* models such as liver microsomes or liver slices.[20−22]

The allometric approach performed with at least three animal species and integrating *in vitro* data from animal and human hepatocytes dramatically improved the predictions of human clearance compared to the approach in which clearance is directly extrapolated using body weight. Using hepatocyte data to normalize the *in vivo* clearances led to median deviations between the observed and predicted clearances in man of approximately 20−40%. Furthermore, the approach integrating *in vitro* data appeared to be superior with respect to the range of deviations: in the worst case, the clearance was underestimated by 2.1-fold when correcting for *in vitro* data (e.g. Ro 24-6173: 5.6 mL/min/kg predicted versus 12 mL/

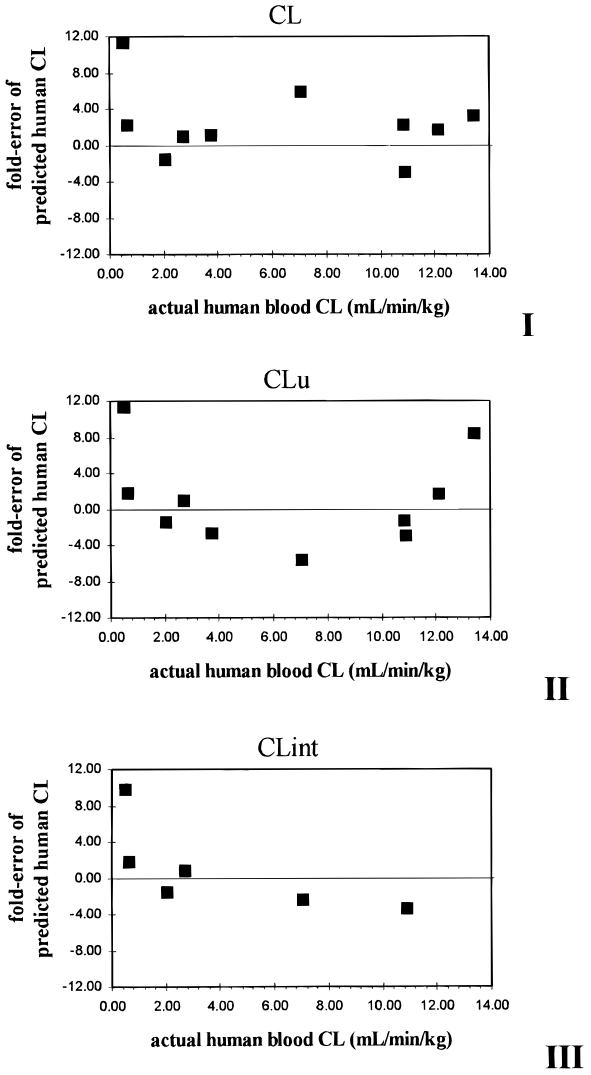

Figure 3—Fold-error of predicted human clearance using allometric scaling of clearance. (I, CL; II, CL_u; III, CL_{int}).

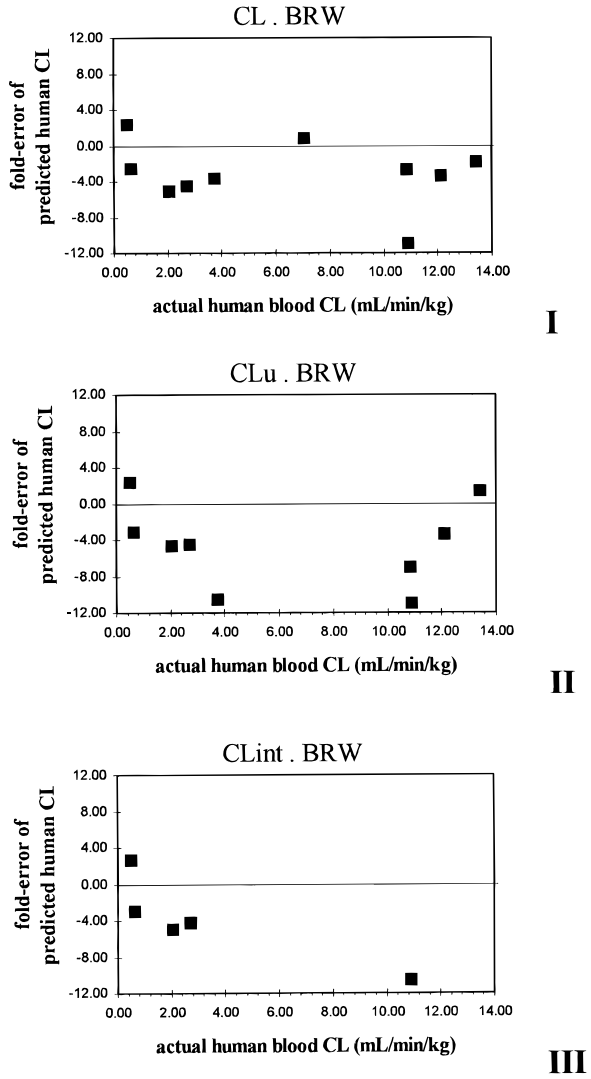

Figure 4—Fold-error of predicted human clearance using allometric scaling of clearance with correction for BRW. (I, CL; II, CL_u; III, CL_{int}). Remark: the clearance of mibefradil in man was underpredicted by 40- and 15-fold, using allometric scaling of CL_u and CL_{int}, respectively. Due to these large deviations, mibefradil was not plotted on this graph.

min/kg observed), whereas up to 10-fold underestimations were obtained when using brain weight as a correction factor (e.g. mofarotene).

In agreement with previous findings,[23] taking into account interspecies differences in protein binding to correlate unbound clearance in various animal species tended to improve the prediction of clearance in man. However, when *in vitro* data were used to normalize clearance, better predictions were obtained with *total*, rather than with unbound or intrinsic clearances. This was observed despite a broad range of plasma protein binding (from ca. 0 to 99.9% bound) and in some instances large interspecies differences in the free fractions in blood. Such results are surprising but could be partially explained by some degree of similarity between the *in vivo* binding to plasma proteins and the *in vitro* binding to the hepatocytes. In theory, the correction factor of unbound *in vitro* intrinsic clearances to normalize the corresponding unbound *in vivo* intrinsic clearances should be considered as the method of choice. However, considering the difficulty of estimating accurately the unbound intrinsic clearance (the proper hepatic model, an accurate measurement of the hepatic blood flow, and a clear understanding of the role of erythrocyte and protein binding in hepatic clearance are needed), the extrapolations performed with this parameter should be interpreted cautiously.

Compared to the allometric approach using empirical correction factors (e.g. brain weight), the integration of *in vitro* data provides a more rational basis for predicting metabolic clearance in man. Also, this approach may be applicable to compounds undergoing phase I as well as phase II metabolism, in contrast to the empirical approach which was proposed for compounds undergoing exclusively oxidative metabolism. For the majority of test compounds, the liver is likely to be the main site of metabolism. It is, therefore, reasonable to assume that interspecies differences in the *in vivo* clearances will be reflected by their rates of metabolism *in vitro*. Furthermore, it is of interest to note that if the *in vivo* interspecies differences are entirely reproduced *in vitro*, the allometric extrapolation of clearance normalized with the relative *in vitro* metabolic rates is expected to be parallel to that of the liver size. This is demonstrated using the following approximation: ignoring the impact of liver blood flow and protein binding, the clearance can be assumed to be proportional to liver size (LS) and *in vivo* liver enzyme activity ($EA_{in\ vivo}$).

The clearance can therefore be expressed as $CL = (LS)(EA_{in\ vivo})$. Correcting the *in vivo* clearance in each animal

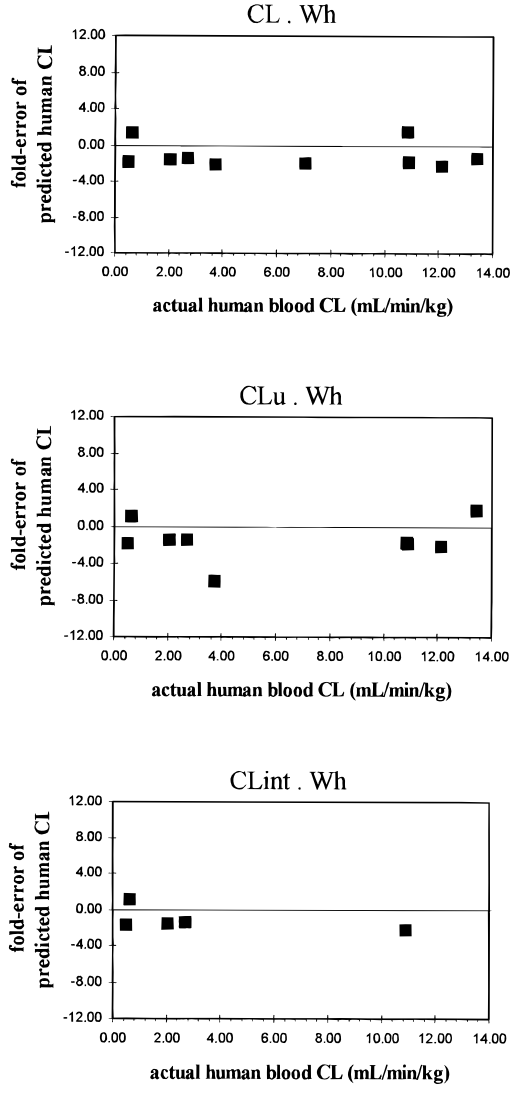

Figure 5—Fold-error of predicted human clearance using allometric scaling of clearance with correction for *in vitro* clearance. (I, CL; II, CL_u; III, CL_{int}). Wh = ($CL_{human(hepatocytes)}/CL_{animal(hepatocytes)}$). Remark: the clearance of mibefradil in man was underpredicted by 60- and 23-fold, using allometric scaling of CL_u and CL_{int}, respectively. Due to these large deviations, mibefradil was not plotted on this graph.

species (CL_{animal}) by our *in vitro* correction factor, we obtain

$$CL_{animal} = (LS_{animal})(EA_{in\ vivo\ animal})\left(\frac{EA_{in\ vitro\ human}}{EA_{in\ vitro\ animal}}\right)$$

Since *in vitro* and *in vivo* enzyme activities correlate, the ratio $EA_{in\ vivo\ human}/EA_{in\ vitro\ animal}$ will tend to be constant.

Therefore, we get

$$CL_{animal} = (constant)(LS_{animal})(EA_{in\ vitro\ human})$$

Since ($EA_{in\ vitro\ human}$) is used to correct the *in vivo* clearance in each animal species, we get, $CL_{animal} = (constant)(LS_{animal})$. This indicates that, through normalization of *in vivo* clearance with the relative rates of *in vitro* metabolism, the allometric scaling performed should be parallel to allometry of liver size. This is consistent with the exponents of 0.86 obtained for allometric scaling of clearance normalized with *in vitro* data, which is very close to the value reported for the allometric exponent for liver weight (0.85).

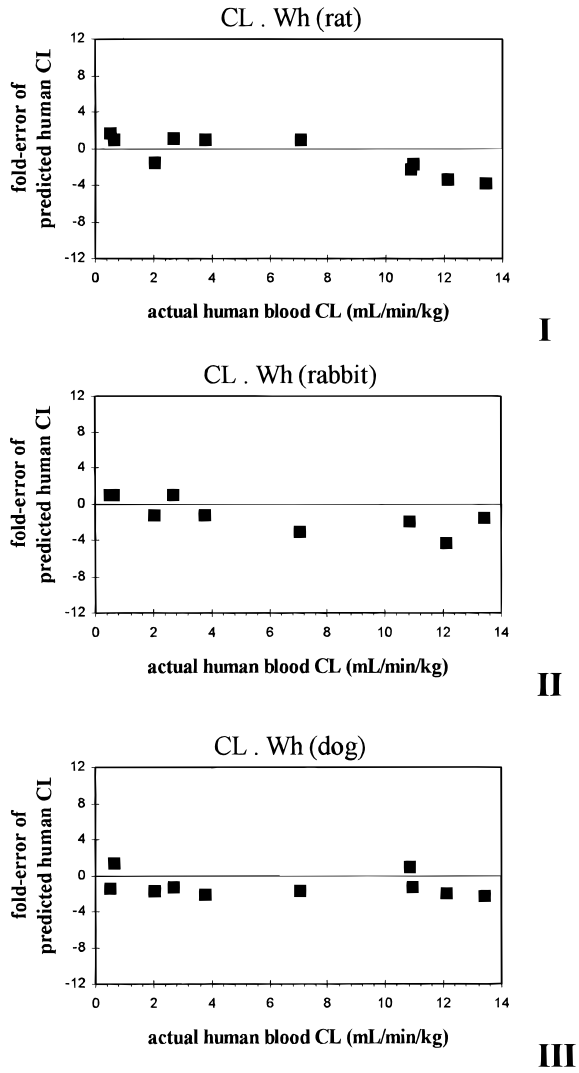

Figure 6—Fold-error of predicted human clearance using allometric scaling of clearance (CL) with correction for *in vitro* clearance. Extrapolation from each animal species (I, rat; II, rabbit; III, dog) to man. Wh = ($CL_{human(hepatocytes)}/CL_{animal(hepatocytes)}$).

Table 3—Values of Clearance Observed and Predicted in Man from Individual Species (Rat, Rabbit, and Dog)

	CL in Man (mL/min/kg)				
		Predicted from			
Compounds	Observed	Rat	Rabbit	Dog	All Species Combined
Antipyrine	0.46	0.82	0.48	0.36	0.25
Bosentan	3.7	4.2	3.3	1.8	1.8
Caffeine	2.0	1.3	1.6	1.3	1.4
Mibefradil	7.0	7.2	2.3	4.3	3.7
Midazolam	11.0	4.9	5.9	11.0	17.0
Mofarotene	11.0	6.7	ND	8.7	6.3
Ro 24-6173	12.0	3.7	2.8	6.2	5.6
Propranolol	13.0	3.5	9.0	6.4	10.0
Theophylline	0.61	0.61	0.65	0.89	0.93
Tolcapone	2.7	3.2	2.9	2.3	2.1

Using the above allometric exponent of clearance normalized with *in vitro* data, the suitability of each animal species (rat, rabbit, or dog) to predict the metabolic clearance in man was investigated. All three species appeared to be equally good predictors of the clearance in man. However, the dog was a suitable species to undertake allometric scaling for all ten compounds, in contrast to rat and rabbit for which a few

outliers, namely propranolol and Ro 24-6173, were observed. It can be considered, therefore, that allometric scaling using only *in vivo* and *in vitro* data in the dog and *in vitro* data in man was as predictive as the allometric approach combining *in vivo* and *in vitro* data in at least three animal species and *in vitro* data in man. Such information can be of considerable value to facilitate and to improve the efficiency of drug development.

In conclusion, combining *in vivo* and *in vitro* metabolic data in animals and humans into allometric scaling is a valuable approach to predict the hepatic metabolic clearance in humans from preclinical data. This approach, validated with test compounds selected to represent a broad range of clearance, plasma protein binding, and a variety of metabolic pathways in animals and man, may be applicable to a variety of compounds to be extrapolated. However, the influence of factors such as protein binding on the approach integrating *in vitro* data remains to be elucidated.

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