Interspecies Scaling of Bosentan, A New Endothelin Receptor Antagonist and Integration of *in Vitro* Data into Allometric Scaling

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Purpose. The goal of this study was to find a rational and reliable method of using animal data to predict the clearance of metabolised drugs in humans.

Methods. One such approach is to use in vitro liver models (e.g. hepatocytes and microsomes) to determine the relative capacities of the various animal species and humans to metabolise the test compound. These data can then be combined with the in vivo clearances in animals, to calculate the in vivo clearance in humans using allometric scaling techniques. In this study, this approach was evaluated with a new endothelin receptor antagonist, bosentan, which is eliminated mainly through metabolism and is characterized by very large interspecies differences in clearance. Therefore, this compound provided a stringent test of our new extrapolation method for allometric scaling.

Results. The results obtained with bosentan showed that adjusting the in vivo clearance in the different animal species for the relative rates of metabolism in vitro gave a far better prediction of human clearance than an empirical correcting factor (brain weight).

Conclusions. This approach provided a more rational basis for predicting the clearance of metabolised compounds in humans.

KEY WORDS: allometric scaling; interspecies scaling; pharmacokinetics; clearance; in vitro models; bosentan.

INTRODUCTION

Interspecies scaling can be used to extrapolate pharmacokinetic parameters between species, through the application of physiologically based models or by empirical allometric procedures. Allometric techniques have been applied successfully to predict human pharmacokinetics of renally excreted compounds like β-lactam antibiotics (1) and metabolised drugs that are highly cleared by the liver, where the elimination is dependent on liver blood flow (2). Similarly for low extraction compounds whose metabolic elimination is independent of liver blood flow, allometric scaling can adequately predict the clearance between different animal species; however the oxidative clearance calculated for man is often higher than the observed value (3). This observation has been ascribed to a lower oxidative metabolic activity in man, relative to the animal species (2).

To improve the predictions for this class of compounds, allometric correction factors such as brain weight or maximum life span have been used with some success (4,5). Various hypothesis to rationalize these empirical corrections have been proposed (2), but no proven explanation is available. Furthermore, it has been shown that these empirical factors do not always improve the predictions for humans, particularly when the capacity to metabolise a drug is similar in animals and man (6). We have, therefore, endeavored to find more rational and reliable methods of using animal data to predict the clearance of metabolised drugs in humans. One such approach is to use in vitro liver models (e.g., hepatocytes and microsomes) to determine the relative capacities of the various animal species and humans to metabolise the test compound. These data can then be combined with the in vivo clearances in animals, to calculate the in vivo clearance in humans using allometric scaling techniques. This approach has been evaluated with new endothelin receptor antagonist, bosentan, which is eliminated mainly through liver metabolism and is characterized by very large interspecies differences in clearance.

MATERIALS AND METHODS

In Vitro Experiments

Microsomes. Liver microsomes were prepared according to a modified method of Von Bahr (7).

Hepatocytes. Mouse and rat hepatocytes were prepared from the in situ perfusion of the whole liver (8). For the other species, a lobe or a lobe fraction was perfused (9). The metabolic turnover was measured at various time points for up to 72 hrs during the incubation with animal and human hepatocytes in primary culture. At each sampling time, the extracellular medium (ca. 1 ml) was combined with the intracellular medium obtained by scraping the cell monolayer with 1 ml methanol.

In Vivo Experiments

Single doses of bosentan were administered intravenously, as a bolus injection, to: mice [male albino "Moro"], rats [male albino "Roro"], rabbits [New Zealand white male], marmoset monkeys and dogs [Swiss male beagle] at 2 mg/kg (except for the dogs : 1 mg/kg). All the test animals were from BRL Füllinsdorf (Switzerland). Blood samples were collected over a 48 hr period post dosing.

Bosentan was determined in the in vitro incubation medium and in plasma using a reversed phase HPLC - UV method (H. Eggers, data on file, F. Hoffmann La Roche Ltd). The lower limit of quantification of the assay was 20-25 ng/ml. The inter-assay precision as well as the inaccuracy were always below 10% in the concentration range 50 to 5000 ng/mL.

Data Analysis

In vivo *Kinetics : Systemic Plasma Clearance.* Total systemic clearance (CL) was calculated using the following relationship: CL = Dose/AUC. The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal rule extrapolated to time infinity. The

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Table I. Bosentan: in Vitro Intrinsic Clearance Data on Liver Microsomes and Hepatocytes, and Corresponding in Vivo Systemic Plasma Clearance Values

Species	Body weight (kg)	CL_{int} liver microsomes[a] (mL/min/mg prot)	CL_{int} hepatocytes[a] (mL/h/10^6 cells)	In vivo systemic plasma clearance (mL/min)
Mouse	0.035	0.032 (0.029–0.035)	0.070 (0.062–0.095)	1.1[b]
Marmoset	0.410	N.A.	0.238 (0.192–0.257)	12.7 (6.0–17.4)
Rat	0.273	0.040 (0.032–0.054)	0.080 (0.070–0.185)	10.4 (9.8–11.2)
Rabbit	2.5	0.062 (0.052–0.073)	0.260 (0.185–0.335)	180 (135–225)
Dog	13.9	0.021 (0.014–0.027)	0.011 (0.009–0.013)	18.1 (11.1–25.0)
Human	70	0.019 (0.011–0.022)	0.013 (0.012–0.015)	

N.A.: not available.
[a] n = 3 independent experiments (median value and ranges).
[b] 2–3 animals/sampling time were used.

area was extrapolated to time infinity 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 plasma concentration-time curve, determined by linear regression of the last three or four data points.

In vitro *Experiments : Intrinsic Clearance (CL_{int})* For liver microsomes, the CL_{int} was estimated from the ratio of the maximum velocity (Vm expressed in nmole/min/g liver) and the Michaelis-Menten affinity constant (Km). For hepatocytes, the CL_{int} was calculated from the ratio of the initial amount of bosentan in the incubation medium and the corresponding AUC values, calculated as described under "In vivo kinetics."

Allometric Scaling

For allometric scaling, the clearance for bosentan in animals was correlated with the mean body weights (B), using allometric equations of the form $CL = aB^x$. The values of the allometric coefficients (a) and exponents (x) were estimated by linear least squares regression of the log transformed allometric equations ($\log CL = \log a + x \log B$).

To determine whether the inclusion of in vitro metabolic data improved the extrapolation to man, the in vivo clearance in each animal species was normalized by the ratio of the in vitro clearance values, for example : CLrat (in vivo) x (CLhuman (hepatocytes) / CLrat (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 normalising clearance in the different animal species for brain weight (BrWt), which has been suggested as an empirical correction factor for oxidized compounds (2). The allometric equations obtained from the animal data by these different methods were used to calculate clearance values for a 70 kg man, then these were compared with the in vivo clearance found in humans.

RESULTS

The in vitro intrinsic clearance values for mouse, rat, marmoset, rabbit, dog and human microsomes and hepatocytes, and the clearances in vivo in the corresponding animal species are indicated in Table I.

Large interspecies differences were observed in the plasma clearance of bosentan in vivo. A high systemic plasma clearance, close to the liver blood flow (LBF) was found in rabbits. Intermediate to high clearance values were observed in mice, marmosets and rats, while dogs exhibited a low systemic plasma clearance which represented less than 5 % of the corresponding liver blood flow. The comparisons of plasma clearance with liver blood flow were made assuming a blood plasma ratio of unity in the different species. The findings with liver microsomes and hepatocytes were consistent with the in vivo kinetic data : the rate of metabolism of bosentan increased from dog to mouse/rat and rabbit. The metabolism of bosentan by human liver microsomes and human hepatocytes was slow, and comparable to the dog.

Table II shows the results of the linear least squares fitting of log CL (with and without corrections) versus log B data in the animals. The clearance value corresponding to a 70 kg man was then estimated, using the allometric equations obtained from the animal data.

The direct regression of LogCL vs LogB (Fig. 1) showed a poor correlation coefficient ($r^2 = 0.525$) which did

Table II. Interspecies Scaling of the Clearance for Bosentan in Animals and Predicted Values in Man

Pharmacokinetic parameters	Allometric equation	Correlation coefficient (r^2)	Data in man estimated[a]
CL [mL/min]	$17.2B^{0.576}$	0.525	196
CL^b,BrWt[c] [mL/min]	$0.15B^{1.436}$	0.895	44.1
CL^b,$R_{microsomes}$ [mL/min]	$8.3B^{0.614}$	0.725	126
CL^b,$R_{hepatocytes}$ [mL/min]	$3.6B^{0.783}$	0.976	100

[a] Data predicted by substituting 70 kg for B in the appropriate allometric equation.
[b] $R_{hepatocytes\ or\ microsomes}$ = CL_{int} in vitro in humans/CL_{int} in vitro in animals.
[c] For the purpose of this calculation, brain weight was arbitrarily considered to be unitless.
Observed clearance in man = 140 mL/min (mean, n = 6), (C. Weber, data on file, F. Hoffmann La Roche Ltd).

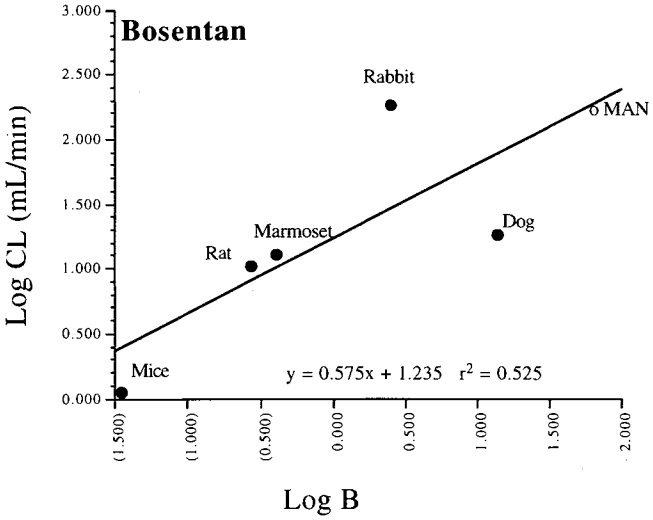

Fig. 1. Allometric scaling of clearance.

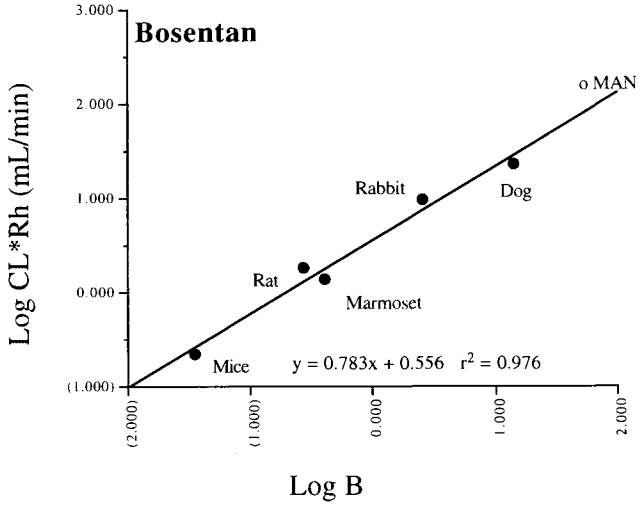

Fig. 3. Allometric scaling of clearance normalized with data obtained with hepatocytes.

not allow a reasonable prospective prediction in human. The correlation was improved (r^2 = 0.895) after normalizing for brain weight (Fig. 2). However, using this correction factor, the human clearance was underestimated by approximately four fold. By contrast, using the relative in vitro metabolic rates to normalize the in vivo clearances significantly improved the predictions for human. Also the slopes of the regression lines (0.783 and 0.638 for the hepatocyte and microsomal data; Figs. 3 and 4, respectively) were very close to clearance exponent values previously reported for small organic molecules (10). Furthermore, the predicted values of clearance in man (1.8 and 1.4 mL/min/kg, respectively, for the microsomal and hepatocyte data) were very close to the value observed in humans (2.0 mL/min/kg). However, the correlation coefficient obtained with the data from hepatocytes (r^2 = 0.976; Fig. 3) was much higher than the corresponding value obtained using the microsomal data (r^2 = 0.725; Fig. 4).

DISCUSSION

A number of reports have described the use of interspecies scaling to predict the human pharmacokinetics of different drugs. In general, it has been shown that simple allometric scaling of clearance works best for renally excreted compounds, where the elimination occurs through physical processes. Simple allometric scaling is also successful for metabolized compounds with high extraction ratios, probably because the blood clearance is mainly dependent on the hepatic blood flow, which itself scales allometrically (2). For metabolized compounds with low extraction ratios, simple allometric scaling does not predict the human situation adequately, and empirical factors such as maximum life span or brain weight are necessary to obtain acceptable predictions in man. However, these corrections do not always provide acceptable predictions for low clearance compounds in humans, for example, in the case of mofarotene (6). In this

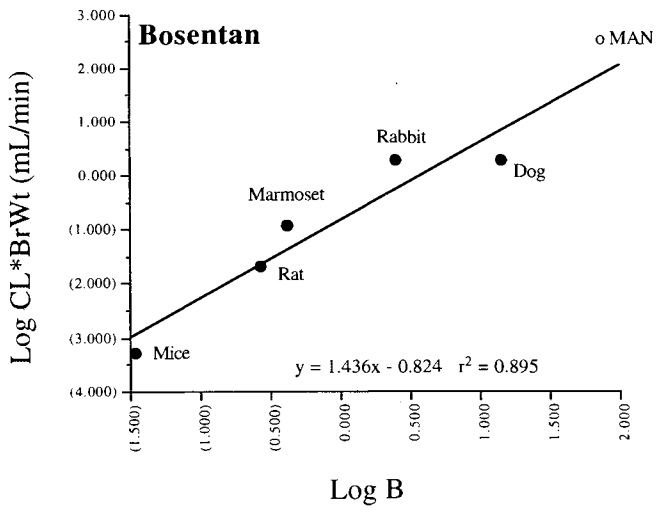

Fig. 2. Allometric scaling of clearance normalized by brain weight.

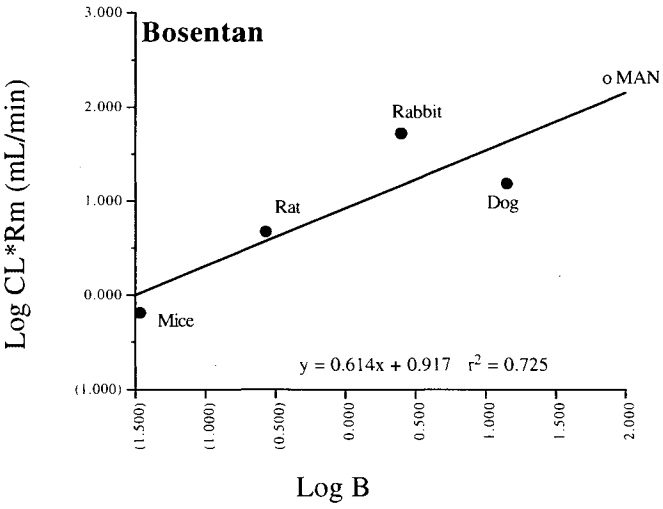

Fig. 4. Allometric scaling of clearance normalized with data obtained with microsomes.

case, the integration of in vitro metabolic data into allometric scaling substantially improved the predictions in humans.

In contrast, to the low clearance drug mofarotene, bosentan is characterized by large interspecies differences in its rate of elimination. Depending on the species, bosentan can exhibit a high, a low or an intermediate extraction ratio. Therefore, this compound provides a stringent test of our new extrapolation method for allometric scaling.

The results obtained with bosentan showed that adjusting the in vivo clearance in the different animal species for the relative rates of metabolism in vitro gave a far better prediction of human clearance than an empirical correcting factor (brain weight). Furthermore, if the in vivo interspecies differences are totally reproduced in vitro, the extrapolation of clearance normalized with the relative in vitro metabolic rates must be parallel to that of the liver size. This is consistent with the exponents (0.783 and 0.638 for the hepatocyte and microsomal data; Figs. 3 and 4, respectively) found in this study.

For most test compounds the liver is likely to be the main site of metabolism, and it is therefore reasonable to assume that interspecies differences in the in vivo clearances will be reflected by the rates of metabolism in vitro. In practice, since the intrinsic clearance (CL_{int}) in vitro is used to normalize the corresponding clearance in vivo, the unbound intrinsic clearance, which has to be regarded as the parameter of choice to reflect the organism ability to metabolize drugs (5), should be used for the allometric scaling, rather than the more hybrid metabolic clearance based on the total (bound + unbound) plasma concentrations. This requires determination of the unbound fraction in plasma of the different species. While these data are available for bosentan, they could not reliably be used in the present study because of the very large intra-species variations in the free fractions (e.g. fu varied from 1.4 to 5.2 % in dog and 1.9 to 4.3 % in human). Furthermore, for high extraction drugs the oral clearance can provide a better estimate of the intrinsic clearance, since the intravenous clearance is limited by the liver blood flow. However, the estimation based on oral clearance makes the basic assumption that the drug is completely absorbed from the gastrointestinal tract, which is not true for bosentan.

Despite all of these limitations, the total plasma clearance corrected for relative rates of in vitro metabolism gave a successful extrapolation for bosentan. This can be explained by the intermediate to high clearance values observed in mice, marmoset, rabbits and rats which are only slightly influenced by changes in protein binding. Furthermore, the protein binding in the dog and man (the two species which have the lowest systemic clearances) are very similar. Thus the interspecies differences observed in clearance are mainly due to differences in liver enzyme activities, and these were reproduced in our in vitro liver models.

In our study hepatocytes gave a better correlation than the microsomal data. Also, the hepatocytes were more representative of the interspecies differences in the rate of metabolism in vivo. This varied about 50 fold in the different animal species, whereas the corresponding ratios for the in vitro data on hepatocytes and liver microsomes were ca. 12 and 3-4 fold, respectively. The difference between the two in vitro systems might be related to the absence of phase II metabolism on liver microsomes, which could result in enzyme inhibition due to the accumulation of the oxidative metabolites (11).

To predict the in vivo clearance from in vitro data, the in vitro intrinsic clearance can be converted to the corresponding in vivo clearance by using scaling factors and pharmacokinetic liver models (e.g. well stirred model) ; most of the examples illustrating this approach are dealing with rat data (11-13). There are only few studies where the in vivo metabolic clearance of a drug in humans was directly predicted from in vitro data (14,15). This more direct approach is currently under evaluation in our laboratory by testing retrospectively for several compounds the accuracy of the predictions in man from data on human hepatocytes.

In conclusion, by using the relative rates of metabolism in vitro to normalise the in vivo data for allometric scaling, the systemic plasma clearance for bosentan in man was predicted successfully. This approach provides a rational basis for extrapolating the metabolic clearance in humans from data obtained in animal species. Moreover, compared to the conventional correction factors (e.g. brain weight), the integration of in vitro metabolic data gave a more accurate prediction of the human pharmacokinetics. Such information is important for designing the protocol for the first study in humans. Bosentan is the second case in which this new procedure has been applied successfully, and its validity will be confirmed with additional examples in the near future.

REFERENCES

1. Y. Sawada, M. Hanano, Y. Sugiyama, and T. Iga . Prediction of the disposition of beta-lactam antibiotics in humans from pharmacokinetic parameters in animals. *J. Pharmacokinet. Biopharm.* 12:241-61 (1984).
2. B. Boxenbaum, and R. W. D'Souza. Interspecies Pharmacokinetic Scaling, Biological Design and Neoteny. In B. Testa (eds.), *Advances in Drug Research,* Academic Press Limited, London, 1990, pp. 139-196.
3. P. J. McNamara. Interspecies scaling in pharmacokinetics. In P. G. Welling, F. L. S. Tse and S. V. Dighe (eds.), *Pharmaceutical bioequivalence,* Marcel Dekker, New York, 1991, pp. 267-300.
4. D. B. Campbell. Can allometric interspecies scaling be used to predict human kinetics. *Drug Inf. J.* 28:235-245 (1994).
5. H. Boxenbaum, and J. B. Fertig . Scaling of antipyrine intrinsic clearance of unbound drug in 15 mammalian species. *Eur. J. Drug Metab. Pharmacokinet.* 9:177-83 (1984).
6. T. Lave, A. H. Schmitt-Hoffmann, P. Coassolo, G. Ubeaud, B. Vallès, B. Ba, R. Brandt, and R. C. Chou . A new extrapolation method from animal to man ; application to a metabolized compound, mofarotene. *Life Sci.* 56:473-478 (1995).
7. C. Von Bahr, C.-G. Groth, H. Jansson, G. Lundgren, M. Lind, and H. Glaumann . Drug metabolism in human liver in vitro : Establishment of a human liver bank. *Clin. Pharm. Ther.* 27:711-725 (1980).
8. P. O. Seglen . Preparation of isolated rat liver cells. *Meth. Cell Biol.* 13:29-83 (1976).
9. T. Seddon, I. Michelle, and R. J. Chenery . Comparative drug metabolism of diazepam in hepatocytes isolated from man, rat, monkey and dog. *Biochem. Pharmacol.* 38:1657-1665 (1989).
10. J. Mordenti . Man versus beast: pharmacokinetic scaling in mammals. *J. Pharm. Sci.* 75:1028-40 (1986).

11. J. B. Houston . Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. *Biochem. Pharmacol.* 47:1469-1479 (1994).
12. K. S. Pang, P. Kong, J. A. Terrell, and R. E. Billings . Metabolism of acetaminophen and phenacetin by isolated rat hepatocytes. A system in which the spatial organization inherent in the liver is disrupted. *Drug Metab. Dispos.* 13:42-50 (1985).
13. A. Rane, G. R. Wilkinson, and D. G. Shand . Prediction of hepatic extraction ratio from in vitro measurement of intrinsic clearance. *J. Pharmacol. Exp. Ther.* 200:420-4 (1977).
14. B. A. Hoener . Predicting the hepatic clearance of xenobiotics in humans from in vitro data. *Biopharm. Drug Dispos.* 15:295-304 (1994).
15. C. Bäärnhielm, H. Dählback, and I. Skanberg . In vivo pharmacokinetics of felodipine predicted from in vitro studies in rat, dog and man. *Acta Pharm. Toxicol.* 59:113-22 (1986).