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Pharmacokinetics and allometric scaling of levormeloxifene, a selective oestrogen receptor modulator

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ABSTRACT: The pharmacokinetics of a new selective oestrogen receptor modulator levormeloxifene was investigated in mice, rats, cynomolgus monkeys and humans by compartmental pharmacokinetics. Levormeloxifene was administered as an oral solution in all studies. Allometric scaling was used to predict human pharmacokinetic parameters and the performance of the approach was evaluated. Mean values of clearance confounded by F(CL/F) were 0.073, 0.29, 3.18 and 2.41/h in mice, rats, monkeys and humans, respectively. Values of distribution volume at steady state confounded by F(V_{ss}/F) were 0.073 and 7.51 in mice and rats. In monkeys, values of the central volume F(V_c/F) and volume at steady state F(V_{ss}/F) were 28.9 and 57.91, respectively. In humans, values of V_c/F and V_{ss}/F were 106 and 5871, respectively. Predicted CL/F and V_{ss}/F showed a linear relationship when plotted vs *B*W on a log–log scale; for CL/F, r was 0.95–0.98 and for V_{ss}/F, r was 0.99. Using allometric scaling the predicted human V_{ss}/F deviated 3-fold from the experimentally determined values. Observed values of CL/F deviated 21–25 fold from the predicted, the latter depending on the scaling method. Confidence intervals for the predicted parameters showed major lack of precision for all the allometric scaling methods. Copyright © 2003 John Wiley & Sons, Ltd.

Key words: levormeloxifene; pharmacokinetic parameters; allometric scaling

Introduction

Toxicity and safety pharmacology studies must be performed before the onset of clinical trials; the goal of these studies is to predict drug actions in humans. The data obtained in these studies may not only be used to assay safety, but may also be used to extrapolate the pharmacokinetics of a compound from laboratory animals to humans. One method for extrapolation of pharmacokinetic data is allometric scaling. The method is based on the physiological similarities between mammals. The approach is empirical but is widely used to extrapolate from animals to humans; most often clearance (CL) and volume (*V*) are scaled by an exponential function of body weight (*B*W) [1,2]. When allometric scaling is applied to drugs that are extensively metabolised by the liver, over-estimation of human CL has been reported [3]. Correction factors such as brain weight (BrW) and maximum life span potential (MLP) have successfully improved the prediction of human CL for several drugs [4,5].

Levormeloxifene is a selective oestrogen receptor modulator shown to have the same beneficial effects as oestrogen replacement therapy (ERT) on bone turnover and serum cholesterol [6]. As an alternative to ERT levormeloxifene was under development for the prevention and treatment of postmenopausal osteoporosis.

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Plasma concentration data for levormeloxifene administered to mouse, rat and monkey were obtained as part of the toxicity studies; these data were subjected to pharmacokinetic analysis using compartmental methods. Allometric scaling methods were applied to examine the relationship of CL confounded by bioavailability (CL/F) and volume of distribution at steady state confounded by bioavailability (V_{ss}/F) to *B*W across species.

Levormeloxifene is mainly metabolised by the liver [7], thus the simple allometric scaling approach should not be expected to predict an accurate estimate of CL/F, but the inclusion of correction factors may improve the prediction.

Methods and Materials

In vivo experiments

Concentration–time data for compartmental modeling were obtained from the following toxicity studies: In a three-month toxicity study in CD-l mice, the pharmacokinetics were determined after administration of 0.2, 1, 5 and 25 mg/kg/day of levormeloxifene given once daily.

Levormeloxifene was administered perorally by gavage as a solution corresponding to a dosage volume of 10 ml/kg body weight. Blood samples (one per mouse) were taken from the retro-orbital sinus at 0, 0.5, 1, 2, 4, 6 and 24 h post dose on day 1 and after multiple doses on day 91, respectively. A total number of 336 mice were included in the study.

In a three-month toxicity study in CD rats, the pharmacokinetics were determined after administration of 0.025, 0.125, 0.625 and 3.125 mg/kg/day. Levormeloxifene was administered perorally by gavage as a solution corresponding to a dosage volume of 10 ml/kg body weight. On day 1, blood samples (one per rat) were taken from the ophthalmic plexus at 0, 1.5, 3, 5, 7, 24 h post dose. On day 91, after multiple doses the samples were collected at 0, 1.5, 3, 5, 7, 24, 36, 48, 60, 72, 84 and 96 h post dose. A total of 228 rats were included in the study. In *Cynomolgus* monkeys, the pharmacokinetics were determined after a single *iv* administration of 2.5 mg/kg through the caudal vein and after a single peroral by gavage administration of 5.0 mg/kg of levormeloxifene.

The study included four monkeys, two of each sex and employed a crossover design with a washout period of 14 days. Blood samples were drawn from the femoral or a superficial vein at 0, 3, 5, 7, 12, 24, 30, 48, 72 h post dose.

In humans, the pharmacokinetics were determined after a single peroral administration of a solution containing 10, 30, 80, 160 and 320 mg levormeloxifene. The study included 24 healthy postmenopausal women aged 45–65 years inclusive. The women were fasted from 8 h before to 4 h after dose administration. Venous blood samples were collected by means of an indwelling cannula at 0, 1, 2, 4, 6, 9, 12, 24, 36, 48, 72, 120, 192, 264, 350, 456, 552 and 648 h post dose. For all species that received peroral gavage administration, levormeloxifene was formulated as follows: for each 10 ml suspension 12.5 mg of levormeloxifene was wet with 1 ml of glycerol and 1 ml of purified water. Four ml of 0.5% gelatine solution was added and using a 10% solution of methylhydroxypropylcelluose the final volume was adjusted to 10 ml. For intravenous administration levormeloxifene was formulated as follows: 500 mg of levormeloxifene was mixed with 7.536 g of hydroxy-proply β-cyclodextrine. A 5% isotonic glucose solution was added to produce a final volume of 400 ml and the mixture was placed in a warm water bath (70°C) and stirred until the substances were in solution.

Drug assay

In monkey, human and rat, plasma concentrations of levormeloxifene were determined by employing high-performance liquid chromatography and solid phase extraction [8,9]. The lower limit of quantification (LLOQ) for levormeloxifene was 1.5 ng/ml (human plasma), 5.2 ng/ml (monkey plasma) and 2.5 ng/ml (rat plasma). Concentrations of levormeloxifene in mouse plasma were determined using the same method as for rat plasma [9]; the LLOQ for levormeloxifene in mouse plasma was 2.5 ng/ml.

The determination of plasma protein binding of levormeloxifene in mouse, rat, monkey and human plasma was performed using an equilibrium dialysis technique [10].

Pharmacokinetics

Compartmental pharmacokinetic analyses were performed individually for each species. Mouse and rat data were analysed with simultaneous fitting of all dose levels, on both day 1 (single dose) and on day 91 (multiple dose). Monkey data were analysed with simultaneous fitting of *iv* and oral doses. Human data were analysed with simultaneous fitting of all dose levels. Pharmacokinetic calculations were performed using the non-linear least-squares regression analysis programme WinNonlin 2.1 software (Scientific Consulting Inc. Apex, NC, USA). Visual examination of the model-predicted plasma concentrations plotted against the observed plasma concentrations was used, together with F-tests, to ensure that the optimal model was chosen. Significance of differences was evaluated at the 5% level.

All data were weighted with a constant variance ($W_i = 1/\hat{c}_i$). The extent of oral bioavailability was estimated as an additional parameter in the model when analysing data obtained from monkeys. For a one-compartment model, the volume of the central compartment (V_c) is equal to V_{ss} whereas for two-compartment models, V_{ss} was determined by the following equation:

$$V_{ss} = V_c\left[1 + \frac{k_{12}}{k_{12}}\right]$$

where k_{12} and k_{21} are the rate constants for transfer of drug from the central compartment to the peripheral and from the peripheral to the central compartment respectively. CL was determined from $CL = k \times V_c$, where k is the elimination rate constant from the central compartment.

Allometric scaling

Allometric scaling was applied to V_{ss}/F and to CL/F. Three different models including (i) BW only, (ii) BW and BrW and (iii) BW and MLP were investigated for CL/F. For V_{ss}/F, only the simple allometric approach was used. The models are described by

$$CL = a \times BW^A$$

$$CL \times BrW = b \times BW^B$$

$$CL \times MLP = c \times BW^C$$

$$V_{ss} = d \times BW^D$$

where a, b, c and d are coefficients and A, B, C and D are exponents in the allometric equation.

Linear regression was performed according to the following models:

$$\text{Log}(CL/F)_i = \text{Log}(a) + A\,\text{Log}(BW_i) + \varepsilon_i,\ i = 1,2,3$$

$$\text{Log}(CL/F \times BrW)_i = \text{Log}(b) + B\,\text{Log}(BW_i) + \varepsilon_i,\ i = 1,2,3$$

$$\text{Log}(CL/F \times MLP)_i = \text{Log}(c) + C\,\text{Log}(BW_i) + \varepsilon_i,\ i = 1,2,3$$

$$\text{Log}(V_{ss}/F)_i = \text{Log}(d) + D\,\text{Log}(BW_i) + \varepsilon_i,\ i = 1,2,3$$

where i is the number of species and ε is the residual error. The residual errors are assumed to be independent, and normally distributed with a mean 0 and variance σ^2. All observations were weighted equally. The allometric results were obtained using S-PLUS 2000 professional release 2 (MathSoft, Inc. MA, USA).

The 95% confidence intervals for the predicted values ($\hat{y}$) were calculated by

$$\hat{y} \pm t_{0.975,n-2} \cdot \sqrt{\text{Var}(\hat{y})} \text{ and}$$

$$\text{Var}(\hat{y}) = s^2\left(1 + \frac{1}{n} + \frac{(x - \bar{x})^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}\right)$$

where s^2 is the estimate of the variance on y, n is the number of observations (species), x is the maximum weight to be included in the confidence interval (70 kg for the human *B*W) and $\bar{x}$ is the mean of observed weights.

MLP was calculated according to the following: $MLP=185.4(BrW)^{0.636}(BW)^{-0.225}$ [11]. The weight and brain weight of the animals, measured at the end of each study, was used for the calculation of MLP; however, for the *Cynomolgus* monkey the brain weight set to 0.042 kg [12]. Predictions of human pharmacokinetic parameters were based on a 70 kg human with a brain weight of 1400 g [13].

Results

Observed plasma concentration–time courses and model predicted plasma concentration–time courses of levormeloxifene in mice, rats,

monkeys and humans are illustrated in Figures 1, 2, 3 and 4, respectively. Model predicted parameter estimates for mice, rats, monkeys and humans are listed in Table 1. In mice and rats, one-compartment models were fitted to the data. In the monkeys, a two-compartment model most adequately described the data. Thus, the model resulted in low coefficients of variation (CV) of pharmacokinetic parameter estimate; however, a high CV was seen for the absorption rate constant (k_a) due to lack of data points during the absorption phase of the drug. The bioavailability (f) was estimated to be 53% in monkeys, the only species where levormeloxifene was administered both intravenously and orally.

Among the animals, mice had the highest CL/F/g animal (2.4×10^{-3} l h^{-1} g^{-1}), the lowest value of CL/F/g was estimated in rats (1.2×10^{-3} l h^{-1} g^{-1}). In monkeys, CL/F/g. was estimated to 1.5×10^{-3} l h^{-1} g^{-1}. The V_{ss}/F was considerably higher than total body water volume for all animals indicating that levormeloxifene is highly bound to tissues. In humans, a two-compartment model provided the best fit to the data resulting in CV's of the estimated pharmacokinetic parameters being less than 12% (Table 1). Human CL/F was low 2.4 l/h (3.4×10^{-5} l h^{-1} g^{-1}) compared to the CL/F/g in the animals. V_{ss}/F was estimated as 5871 in humans indicating that levormeloxifene is extensively bound to tissues, as was also suggested by the animal data. The plasma protein binding was high (99.2–99.9%) and concentration independent in the range of 40–2000 ng/l. No significant differences were observed between species (9); the results are shown in Table 2.

The BW, BrW and MLP that were used for scaling are shown Table 3. The allometric scaling results are shown in Table 4 and in Figure 5 and 6. The correlation coefficient (r) indicated that CL/F in humans was best predicted using simple allometric scaling without correction factors ($r = 0.98$), although good correlations were also seen when BrW and MLP were included in the scaling ($r = 0.97$ and 0.95). However, comparison of predicted and the observed value of CL/F revealed that

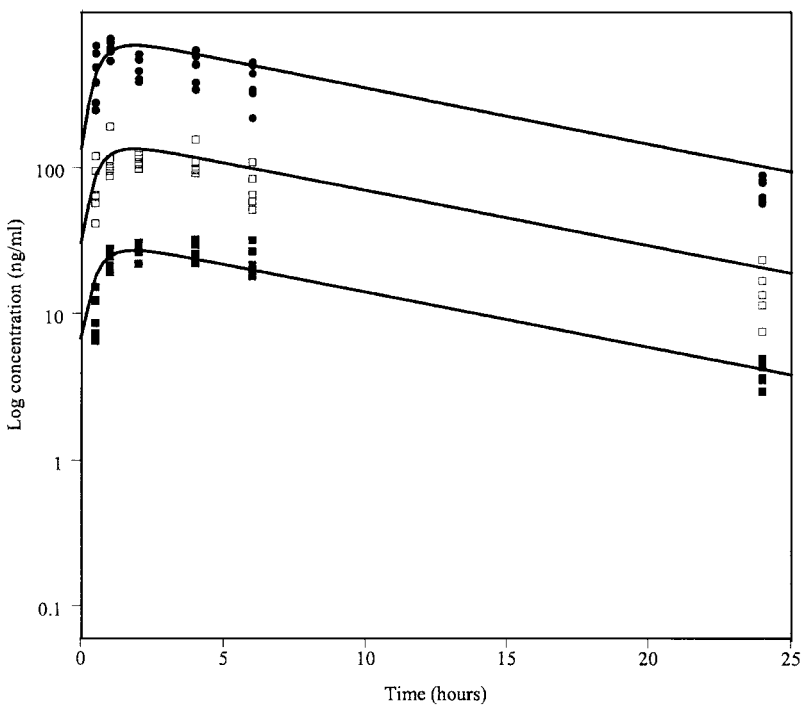

Figure 1. Semi-logarithmic plot of plasma concentrations–time courses of levormeloxifene mice after a single oral administration of 1(■), 5(□) and 25(●) mg/kg levormeloxifene, the lines represent the model predicted concentration

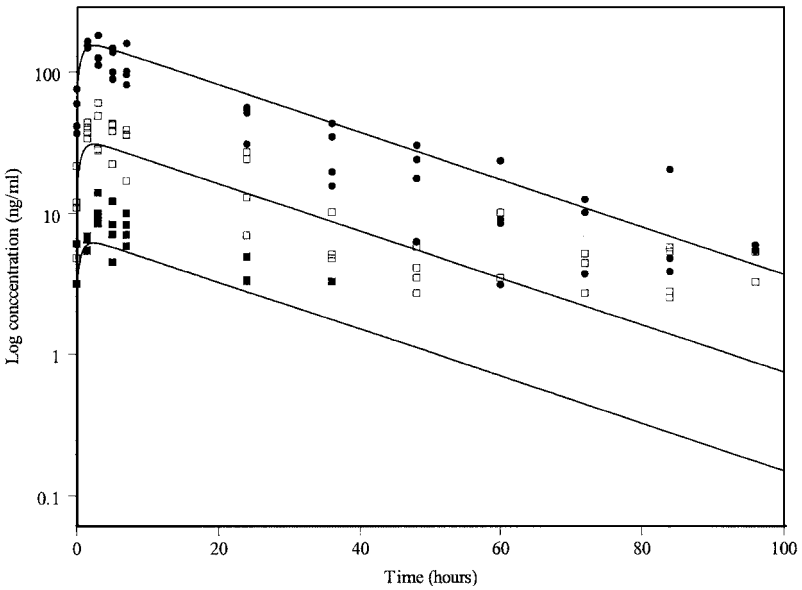

Figure 2. Semi-logarithmic plot of plasma concentrations-time courses of levormeloxifene in rats after multiple oral administrations of 0.125 (■), 0.625 (□) and 3.125 (●) mg/kg levormeloxifene on day 91, the lines represent the model predicted concentration

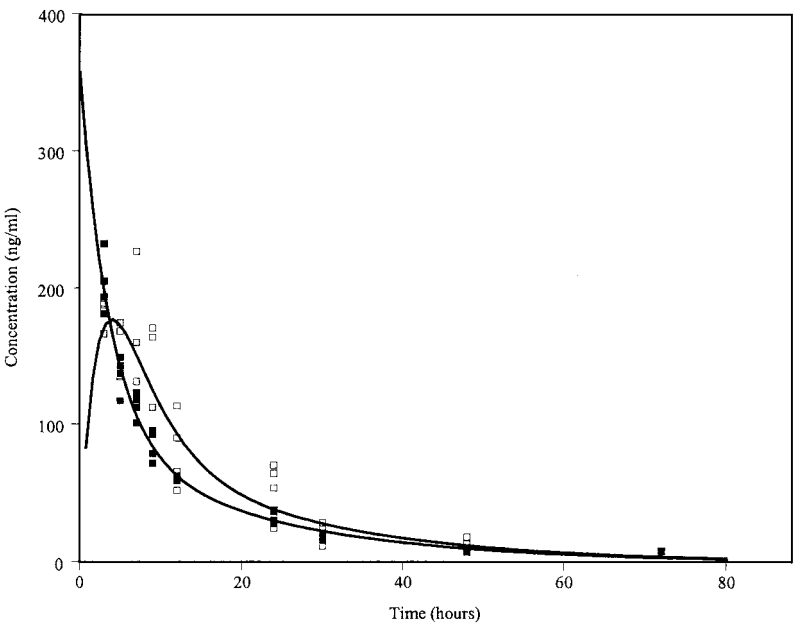

Figure 3. Plot of plasma concentrations–time courses of levormeloxifene in monkeys after *iv* administration of 2.5(■) mg and oral administration of 5.0(□) mg levormeloxifene, the lines represent the model predicted concentration

the predicted CL/F was substantially higher than the observed (Table 4). The observed pharmacokinetic parameter values in humans were contained in the confidence intervals for predicted values, however these were large and covered all expected values, clearly indicating high variability in the data and uncertainty in the prediction.

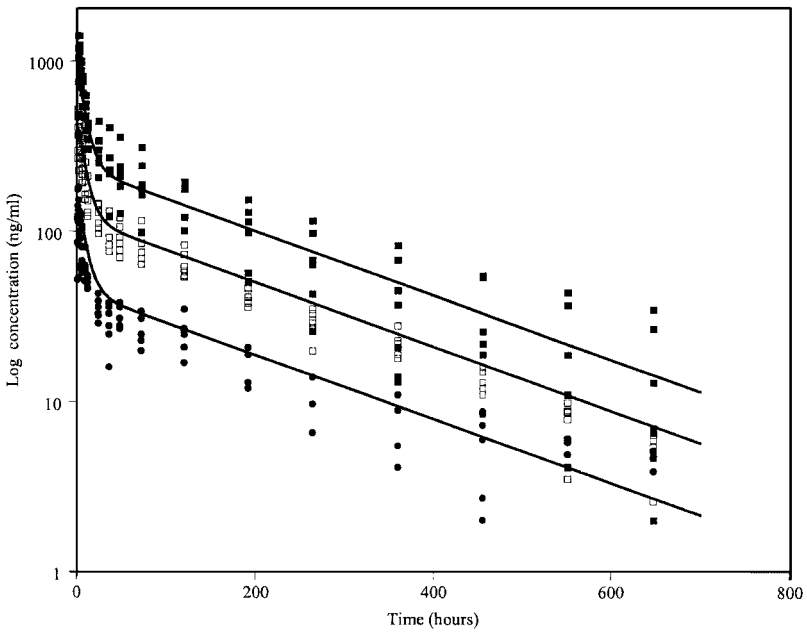

Figure 4. Semi-logarithmic plot of plasma concentrations-time courses of levormeloxifene in humans after single oral administration of 30 (■), 80 (□) and 160 (●) mg levormeloxifene, the lines represent the model predicted concentration

Table 1. Pharmacokinetic parameters for levormeloxifene in mouse, rat, monkey and human, the figures in parenthesis gives the coefficient of variation (%)

Parameter	Mouse	Rat	Monkey	Human
V_c/F (l)	0.95 (3.5)	7.51 (5.0)	28.9 (17.0)	106 (6.6)
V_{ss}/F (l)	0.95 (3.5)	7.51 (5.0)	57.9	587
k_a (h^{-1})	1.71 (9.2)	1.30 (59.2)	0.33 (19.7)	0.93 (7.0)
k_e (h^{-1})	0.086 (4.7)	0.038 (7.6)	0.11 (15.7)	0.023 (6.4)
k_{12} (h^{-1})	Not calculated	Not calculated	0.084 (34.7)	0.20 (11.8)
k_{21} (h^{-1})	Not calculated	Not calculated	0.083 (32.2)	0.044 (7.0)
CL/F ($1 \times h^{-1}$)	0.073	0.29	3.18	2.4
CL/F/g animal ($h^{-1} \times g$)	2.4×10^{-3}	1.2×10^{-3}	1.3×10^{-3}	3.4×10^{-5}
F	Not measured	Not measured	0.53 (5.97)	Not measured

Discussion

This study revealed that the pharmacokinetics of levormeloxifene were best described by two-compartment models in monkeys and humans and by one-compartment models in mice and rats. This may be due to lack of sufficient data points in the two latter species rather than a real difference in pharmacokinetics. If plasma samples had been collected in the interval between l0–15 h in mouse and rat, two-comparmental models may have fitted the data significantly better. A more thorough investigation of the disposition of levormeloxifene in mouse and rat is necessary in order to explain this difference. Data were therefore also analysed by standard non-compartmental methods to see if differences in compartmental models might have introduced bias. However, pharmacokinetic parameter estimates in each species were similar using both approaches. The scaling of V_{ss}/F resulted in approximately 3-fold overestimation; the allometric exponent (0.96) was close to the commonly reported value of 1 [14]. V_{ss}/F was the

Table 2. Plasma protein binding (expressed as a percentage) for levormeloxifene in mouse, rat, monkey and human

Plasma concentration of Levormeloxifene (ng/ml)	Mouse	Rat	Monkey	Human
2000	99.8 ± 0.0	99.8 ± 0.0	99.2 ± 0.2	99.8 ± 0.1
200	99.7 ± 0.3	99.8 ± 0.0	Not measured	99.5 ± 0.7
40	99.8 ± 0.2	99.9 ± 0.0	99.6 ± 0.2	99.9 ± 0.1

Table 3. Mean BW, BrW and MLP for the involved species

Factor	Mouse	Rat	Monkey	Human
BW (g)	30.2	246	2158	70000
BrW (g)	0.5	2.1	42.4	1400
MLP (years)	3.2	4.6	21	88

pharmacokinetic parameter that performed best in the scaling, although the prediction was far from optimal. The use of correction factors such as MLP and BrW have been reported to improve the allometric prediction of human CL/F especially for drugs that are characterised by a low hepatic extraction ratio [3]. It has been proposed that when the exponent of the simple allometric equation lies between 0.71 and 1.0 then correction by MLP will improve the prediction [15]. Since the exponent of the simple allometric equation in this study was 0.89 and since levormeloxifene is metabolised mainly in the liver, the use of such correction factors should theoretically improve the prediction of the human CL/F. In the case with levormeloxifene, incorporation of both MLP and BrW resulted only in small improvements in the prediction of human CL/F. With the simple approach, the predicted value of the human CL/F deviated from the observed by 25-fold, and the deviation was 21 and 23-fold, respectively, when BrW and MLP were used as correction factors. The extent of bioavailability was studied in monkeys only; if *iv* data had been available from all the species it would have been possible to estimate F for all species, and hence perform alometric scaling on V_{ss} and CL, taking into account a species dependent F. In humans CL/F/g (3.4×10^{-5} l h^{-1} g^{-1}) was low in comparison with monkeys (1.3×10^{-3} l h^{-1} g^{-1}), this may result in an increased human bioavailability compared to monkeys (F = 53%), as low first pass metabolism is associated with low CL.

The correlation coefficient (r) was high for all three methods, 0.99 for V_{ss}/F and 0.95–0.98 for CL/F, but r is a poor indicator of the precision of the prediction. The confidence interval for CL/F clearly shows, that the simple approach with and without correction factors lacks precision. Confidence intervals are seldom used in studies of allometric scaling. Most likely when used in allometric scaling, they will show a lack of predictability, mainly due to the lack of species with *B*W close to humans. The lack of oral bioavailability for mouse, rat and humans and a less than perfect model fit to plasma concentrations vs time data due to lack of later data points clearly limits the discussion. However these

Table 4. The parameters of the allometric calculations, the standard error (SE) is given in parenthesis, the correlation (r) for each equation, the allometric predicted parameter estimates, their confidence intervals and the observed human parameter values are given

Equation	Allometric coefficient	Allometric exponent	r	Predicted PK parameter in humans		Observed PK parameter in humans
				Mean	Confidence interval	
$Cl = a \times BW^A$	$a = 3.0 \times 10^{-3}$ (0.34)	$A = 0.89$ (0.13)	0.98	591/h	[3×10^{-3} 1×10^{6}]	2.41/h
$Cl \times BrW = b \times BW^B$	$b = 3.5 \times 10^{-5}$ (0.86)	$B = 1.9$ (0.34)	0.97	511/h	[4×10^{-10} 6×10^{10}]	2.41/h
$Cl \times MLP = c \times BW^C$	$c = 1.7 \times 10^{-3}$ (0.73)	$C = 1.3$ (0.29)	0.95	541/h	[3×10^{-8} 1×10^{11}]	2.41/h
$V_{ss} = d \times BW^D$	$d = 3.6 \times 10^{-2}$ (0.03)	$D = 0.96$ (0.01)	0.99	16601	[645 4365]	5871

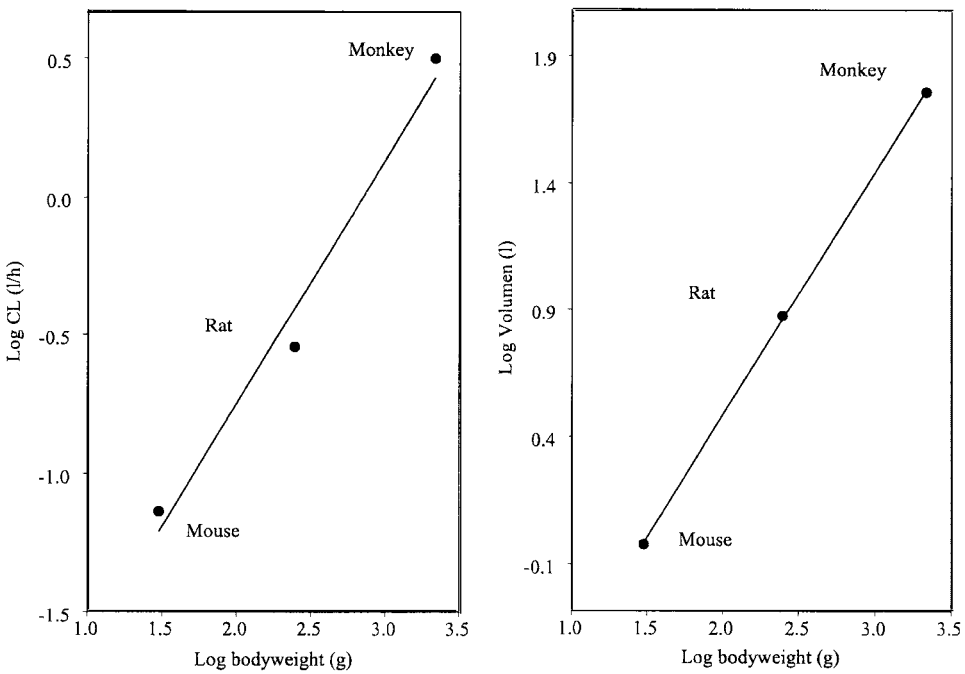

Figure 5. Allometric scaling plots for CL and volume

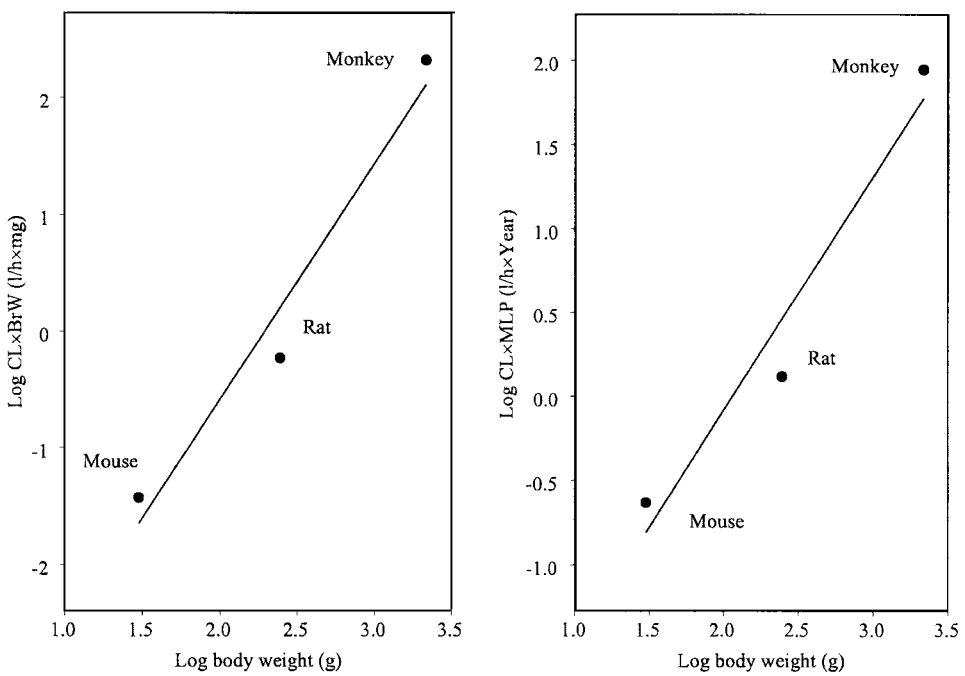

Figure 6. Allometric scaling plots for CL × MLP and CL × BrW

results indicate that caution is warranted, when allometric scaling is used to extrapolate pharmacokinetic parameters from animals to humans.

References

1. Mahmood I, Balian JD. The pharmacokinetic principles behind scaling from preclinical results to phase I protocols. *Clin Pharmacokinet* 1999; **36**(1): 1–11.
2. Lave T, Coassolo P, Reigner B. Prediction of hepatic metabolic clearance based on interspecies allometric scaling techniques and in vitro-in vivo correlations. *Clin Pharmacokinet* 1999; **36**(3): 211–231.
3. Lave T, Dupin S, Schmitt C, Chou RC, Jaeck D, Coassolo P. Integration of in vitro data into allometric scaling to predict hepatic metabolic clearance in man: application to 10 extensively metabolized drugs. *J Pharm Sci* 1997; **86**(5): 584–590.
4. Boxenbaum H. Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: extrapolation of data to benzodiazepines and phenytoin. *J Pharmcokinet Biopharm* 1980; **2**: 165–176.
5. Mahmood I, Balian JD. Interspecies scaling: predicting pharmacokinetic parameters of antiepileptic drugs in humans from animals with special emphasis on clearance. *J Pharm Sci* 1996; **85**(4): 411–414.
6. Alexandersen P, Riis BJ, Stakkestad JA, Delmas PD, Christiansen C. Efficacy of levormeloxifene in the prevention of postmenopausal bone loss and on the lipid profile compared to low dose hormone replacement therapy. *J Clin Endocrinol Metab* 2001; **86**(2): 755–760.
7. Mountfield RJ, Panduro AM, Wassmann O, Thompson M, John B, van der MN. Metabolism of levormeloxifene, a selective oestrogen receptor modulator, in the Sprague–Dawley rat, Cynomolgus monkey and postmenopausal woman. *Xenobiotica* 2000; **30**(2): 201–217.
8. Kiehr B, Christensen MS. Assay for levormeloxifene, a selective oestrogen receptor modulator, in human and monkey plasma employing high-performance liquid chromatography and solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 1999; **729**(1–2): 315–322.
9. Mountfield RJ, Kiehr B, John AB. Metabolism, disposition, excretion, and pharmacokinetics of levormeloxifene, a selective estrogen receptor modulator, in the rat. *Drug Metab Dispos* 2000; **28**: 503–513.
10. Kiehr B, Plum A, Larsen PS, Jansen J. Plasma protein binding - an iterative approach of performing equilibrium dialysis. *Exp Toxico1 Pathol* 1999; **2**: 114–115.
11. Sacher GA. The Life span of animals, Relation of life span to brain weight and body weight in mammals. Little brown and Co; Boston, 1959.
12. Boxenbaum and Fertig. Scaling of antipyrine intrinsic clearance of unbound drug in 15 mammalian species. *Eur J Drug Metab Pharmacokinet* 1984; **9**: 177–183.
13. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993; **10**(7): 1093–1095.
14. Mahmood I. Allometric issues in drug development. *J Pharm Sci* 1999; **88**(11): 1101–1106.
15. Mahmood I, Balian JD. Interspecies scaling: predicting clearance of drugs in humans. Three different approaches. *Xenobiotica* 1996; **26**(9): 887–895.