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Research Article

Interspecies Scaling of Clearance and Volume of Distribution Data for Five Therapeutic Proteins

Joyce Mordenti,[1,2] Sharon A. Chen,[1] Jerome A. Moore,[1] Bobbe L. Ferraiolo,[3] and James D. Green[4]

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The clearance and volume of distribution of five human proteins (recombinant CD4, CD4 immunoglobulin G, growth hormone, tissue-plasminogen activator, and relaxin) in humans and laboratory animals were analyzed as a function of body weight using allometric scaling techniques. These proteins cover a 16-fold range of molecular weight (6 to 98 kD), are produced by recombinant or synthetic methods, and may be cleared by different mechanisms. The analyses revealed that the clearance and volume data for each protein were satisfactorily described by an allometric equation ($Y = a\ W^b$). The allometric exponent (b) for clearance (ml/min) ranged from 0.65 to 0.84, the allometric exponent for the initial volume of distribution (ml) ranged from 0.83 to 1.05, and the allometric exponent for the volume of distribution at steady state (ml) ranged from 0.84 to 1.02. Exponent values from 0.6 to 0.8 for clearance and 0.8 to 1.0 for volumes are frequently cited for small molecules and are expected based on empirical interspecies relationships. When the preclinical data were analyzed separately, the preclinical allometric relationships were usually predictive of the human results. These findings indicate that the clearance and volume of distribution of select biomacromolecules follow well-defined, size-related physiologic relationships, and preclinical pharmacokinetic studies provide reasonable estimates of human disposition. Employing this methodology during the early phases of drug development may provide a more rational basis for dose selection in the clinical environment.

KEY WORDS: allometric equation; interspecies scaling; therapeutic proteins; pharmacokinetics; clearance; volume of distribution; soluble rCD4; CD4-IgG; growth hormone; tissue-plasminogen activator; relaxin.

INTRODUCTION

Physiological and anatomical parameters frequently scale across species as a function of body weight. The usual scaling methodology utilizes a power function (allometric equation) written as follows:

$$Y = a\ W^b \tag{1}$$

where Y is the parameter of interest, W is body weight, a is the allometric coefficient, and b is the allometric exponent. The power function can be linearized by taking its logarithm. The log-transformed allometric equation is written as follows:

$$\log Y = \log a + b \log W \tag{2}$$

where $\log a$ is the y-intercept and b is the slope. The allometric coefficient, a, is the value of the physiological variable (Y) at 1 unit of body weight, i.e., if W is in kilograms, then a is the value for a 1-kg animal. The sign and magnitude of the exponent indicate how the physiological variable is changing as a function of body weight (W). When $b < 0$, Y decreases as W increases; when $0 < b < 1$, Y increases as species get larger, but Y does not increase as rapidly as W; when $b = 1$, Y increases in direct proportion to increases in W; and when $b > 1$, Y increases faster than W.

This technique has been used successfully to describe a number of physiological and anatomical properties for birds, reptiles, fish, and mammals (1–3). In general, biological frequencies (e.g., heartbeats per minute) tend to have an exponent of −0.25, biological time periods (e.g., circulation time, maximum life-span potential) tend to have an exponent of 0.25, biological rates (e.g., clearance, physiologic flow rates, and metabolism) tend to have an exponent of 0.75, and volumes tend to have an exponent of 1.0. Body surface area has an exponent of 0.67.

Allometric scaling techniques have been used to extrapolate pharmacokinetic data for small organic molecules from laboratory animals to humans, and the results have appeared in several reviews (4–7). The allometric equations for the pharmacokinetic parameters tend to approximate the equations for corresponding physiologic variables, i.e., half-life (min) ~ $aW^{0.2}$ to $aW^{0.4}$, clearance (ml/min) ~ $aW^{0.6}$ to

[1] Department of Pharmacokinetics, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080.
[2] To whom correspondence should be addressed.
[3] Department of Drug Metabolism, R. W. Johnson Pharmaceutical Research Institute, Welsh and McKean Roads, Spring House, Pennsylvania 19477.
[4] Department of Safety Evaluation, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080.

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Table I. Average Body Weight (W), Clearance (CL), and Volumes of Distribution (V_i, V_{ss}) for rCD4

Mammal	Number	Dose (μg/kg)	W (kg)	CL (ml/min)	V_i (ml)	V_{ss} (ml)
Rat	9	14	0.28	1.6	12.6	16.8
Rabbit	6	7	2.8	6.4	118	176
Rhesus Monkey	4	14	4.5	7.7	126	135
Human (44)	26	1–300	70.0	57	3640	4900

$aW^{0.8}$, and the volume of distribution (ml) ~ $aW^{0.8}$ to $aW^{1.0}$. If the pharmacokinetic parameters are normalized to body weight, the exponents must be adjusted accordingly: clearance [ml/(min * kg)] ~ $aW^{-0.3}$ and volume of distribution (ml/kg) ~ aW^{0}. These approximations are usually true for drugs or metabolites that are eliminated solely by physical processes, i.e., biliary, renal, or pulmonary excretion; they are applied cautiously, if at all, to compounds that are metabolized, that have nonlinear pharmacokinetics, or that exhibit saturable or species-specific binding.

The allometric equation for the turnover time for serum albumin ($5.68W^{0.296}$ days) demonstrates that endogenous proteins may follow these general guidelines (8). It is possible, however, that pharmacokinetic parameters for therapeutic human proteins derived from studies performed in laboratory animals will not be predictive of human disposition. Human proteins do not necessarily share sequence homology with proteins in other species; thus, they are recognized as foreign in laboratory animals and may activate immune-mediated clearance mechanisms. In addition, some proteins are extremely species specific in activity, and the effect of this specificity on organ clearance mechanisms is unknown.

The following analyses examine the relationship between two pharmacokinetic parameters (clearance and volume) and body weight for several therapeutic human proteins to determine whether these parameters can be described by allometric equations. Five proteins were evaluated: recombinant CD4 (rCD4), CD4-immunoglobulin G (CD4-IgG), recombinant human growth hormone (rhGH), recombinant tissue-plasminogen activator (rt-PA), and relaxin. These proteins cover a 16-fold range of molecular weight (6 to 98 kDa), are produced by recombinant or synthetic methods, and may be cleared by different mechanisms.

PROTEINS EVALUATED

Soluble rCD4. CD4 is a cell surface glycoprotein found primarily on a subset of mature peripheral T cells that recognizes antigens presented by class II MHC molecules (9,10); it is also the putative receptor for human immunodeficiency virus type 1 (HIV-1) (11). Recombinant CD4 is the soluble secreted form of human CD4 produced by Chinese hamster ovary (CHO) cells; it lacks the transmembrane and cytoplasmic sequences of CD4. It binds gp120 and blocks HIV-1 infection of T cells and monocytes *in vitro* (12,13). Recombinant CD4 has 368 amino acids and 3 intrachain disulfide bonds; it exists as a monomer with two carbohydrate attachment points (14,15). The pI is approximately 10. The theoretical molecular mass is 41 kDa; the apparent molecular mass is 50 kDa. The kidney is the primary organ of elimination.

CD4-IgG. Since interspecies scaling techniques suggested that rCD4 would have a rapid clearance and a short half-life in humans, development began on a second generation molecule that would have a more desirable, prolonged disposition profile. This research culminated in the development of the CD4 immunoadhesin (16). Several CD4 immunoadhesin molecules were produced and made available for *in vitro* and *in vivo* testing. One immunoadhesin, CD4-IgG, was selected for development based on favorable *in vitro* properties and a 25-fold reduction in clearance in rabbits compared to rCD4. This homodimer is a 98-kDa glycoprotein produced in CHO cells. It is composed of the two amino-terminal immunoglobulin-like domains of CD4 (residues 1–180) fused to the Fc domain (residues 216–441) of human IgG_1 (17). Not only does this molecule retain the gp120 binding capabilities of rCD4, but it displays the advantageous characteristics of an IgG antibody, including long serum half-life, placental transfer, and antibody-directed cell-mediated cytotoxicity (ADCC) against HIV-infected cells (18). Furthermore, there was a 25-fold reduction in CD4-IgG clearance in humans compared to rCD4, as suggested by preclinical studies.

Growth Hormone. Human growth hormone (hGH) is a nonglycosylated anterior pituitary hormone with 191 amino acids and two disulfide bonds. It has a theoretical molecular

Table II. Average Body Weight (W), Clearance (CL), and Volumes of Distribution (V_i, V_{ss}) for CD4-IgG

Mammal	Number	Dose (μg/kg)	W (kg)	CL (ml/min)	V_i (ml)	V_{ss} (ml)
Rat	9	140	0.333	0.0416	11.6	24.2
Rabbit	4	40	3.3	0.393	216.8	187
Cynomolgus monkey	4	140	5.6	0.252	171.4	255.9
Human[a]	2	1000	82	2.62	4100	6273

[a] T. Hodges, J. D. Allan, J. Kahn, and J. Groopman, personal communication.

Table III. Average Body Weight (W), Clearance (CL), and Volumes of Distribution (V_i, V_{ss}) for rhGH

Mammal	Number	Dose (μg/kg)	W (kg)	CL (ml/min)	V_i (ml)	V_{ss} (ml)
Mouse	19	100	0.016	0.32	2.26	3.8
Rat	5	125	0.127	2.03	11.88	15.7
Cynomolgus monkey (48)	12	124	3.8	14.7	199	314
Human	18	20	73.7	152.4	2432	4149

Table IV. Average Body Weight (W), Clearance (CL), and Volumes of Distribution (V_i, V_{ss}) for rt-PA

Mammal	Number	Dose (μg/kg)	W (kg)	CL (ml/min)	V_i (ml)	V_{ss} (ml)
Mouse[a]	9	100–125	0.025	0.68	3.4	13.6
Hamster (49)	3	250	0.09	2.8	12	20.6[b]
Rat	7	300	0.234	6.15	16.1	29.5
Rabbit (38)	8	1000	2.5	27.3	113.8	NR[c]
Rhesus monkey (50)	11	1000	3.1	28.9	415.4	NR
Dog[d] (51)	3	45	21.5	330.0	2331	NR
Human[e] (52)	12	250–500	76.0	620.0	4450	8050

[a] Median data from three mice receiving 125 μg/kg (CL = 0.105 ml/min, V_{ss} = 508 ml/kg, V_i not reported) (53) and six mice receiving 100 μg/kg (CL = 1.25 ml/min, V_i = 137 ml/kg, V_{ss} = 580 ml/kg).
[b] Calculated as dose$[A/\hat{a}^2 + B/\beta^2]$/AUC^2 from data in report.
[c] NR = Not reported.
[d] Anesthetized.
[e] Median data from six volunteers receiving 250 μg/kg (CL = 640 ml/min, V_i = 4.6 L, V_{ss} = 8.1 L) and six volunteers receiving 500 μg/kg (CL = 600 ml/min, V_i = 4.3 L, V_{ss} = 8.0 L).

Table V. Average Body Weight (W), Clearance (CL), and Volumes of Distribution (V_i, V_{ss}) for Recombinant and Synthetic Relaxin

Mammal	Number	Dose (μg/kg)	W (kg)	CL (ml/min)	V_i (ml)	V_{ss} (ml)
Mouse[a] (54)	6[b]	88	0.021	0.33	4.7	14.1
Rat[a]	6	100	0.27	1.59	19.4	79.4
Rabbit	6	100	3.1	18.3	177	756
Rhesus monkey[c]	11	100	5.35	19.2	379	2,655
Human	25	10	62.4	175	4,870	17,350

[a] Synthetic relaxin.
[b] Six mice sacrificed per time point; 606 total.
[c] Median data from six monkeys (5.5 kg) receiving 100 μg/kg synthetic relaxin (CL = 17.1 ml/min, V_i = 352 ml, V_{ss} = 1710 ml) and five monkeys (5.2 kg) receiving 100 μg/kg recombinant relaxin (CL = 21.3 ml/min, V_i = 406 ml, V_{ss} = 3600 ml).

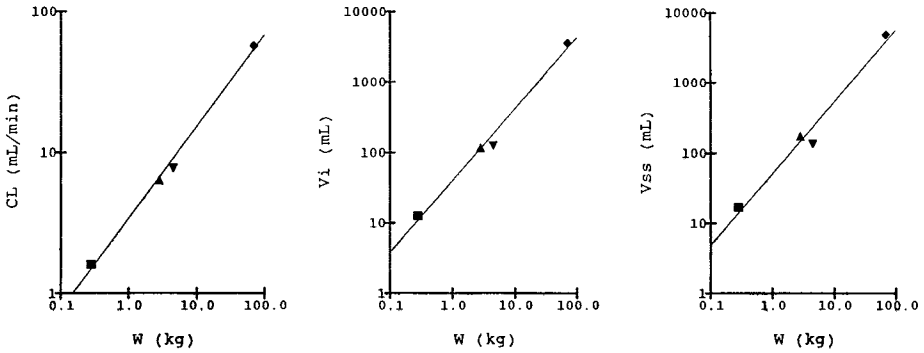

Fig. 1. Log–log plots of clearance (CL), initial volume (V_i), and volume of distribution at steady state (V_{ss}) versus species body weight (W) for rCD4. Data are from Table I, and the solid lines are the allometric relationships from Tables VI–VIII. Filled square, rat; filled triangle, rabbit; filled inverted triangle, rhesus monkey; filled diamond, human.

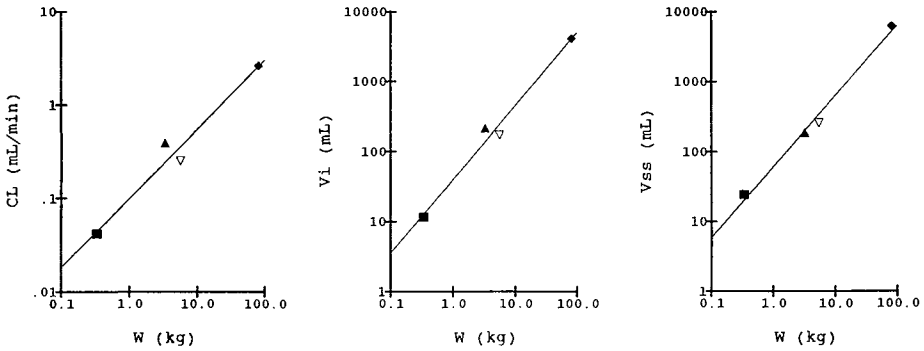

Fig. 2. Log–log plots of clearance (CL), initial volume (V_i), and volume of distribution at steady state (V_{ss}) versus species body weight (W) for CD4-IgG. Data are from Table II, and the solid lines are the allometric relationships from Tables VI–VIII. Filled square, rat; filled triangle, rabbit; open inverted triangle, cynomolgus monkey; filled diamond, human.

mass of 22.1 kDa and an apparent molecular mass of approximately 20 kDa. Recombinant human growth hormone (rhGH), produced in *Escherichia coli*, has the same structure as natural pituitary hGH and is used to treat short stature related to growth hormone deficiency. The primary mechanism of hGH elimination has been reported to be glomerular filtration, reabsorption in the proximal tubule, and catabolism by renal cells (19). A specific GH binding protein has been identified in several species, including humans (20,21). Approximately 50% of the circulating hGH is bound, forming a 70- to 90-kDa complex; when bound in this complex, the clearance and volume of distribution of hGH are reduced (22,23).

Tissue-Plasminogen Activator. Recombinant tissue-plasminogen activator (rt-PA, Activase) is used for thrombolytic therapy of acute myocardial infarction by promoting rapid reperfusion of occluded coronary arteries (24–27). Recombinant t-PA is a glycoprotein produced by CHO cells (28) and contains approximately 8% carbohydrates consisting of N-linked oligosaccharides of both the high-mannose and the complex types (29). It is a monomer that exists as a mixture of one-chain (80%) and two-chain (20%) forms, with the two chains attached by disulfide bonds (30–32). The isoelectric point of rt-PA is heterogeneous, with most of the components falling within the range of pI 6–8. The heterogeneity of rt-PA is due to the carbohydrate content of the molecules that are glycosylated at either two or three sites (33). The theoretical molecular mass is 59 kDa, and the apparent molecular mass are 63 kDa (unreduced, diglycosylated) and 65 kDa (unreduced, triglycosylated), respectively (30). The liver has been identified as the organ of elimination from tissue distribution studies (34), isolated perfused liver studies (35), and hepatocyte binding studies (36). Experiments with deletion mutants of t-PA have demonstrated that the rapid clearance recognition sites reside on the growth factor domain, the kringle 1 domain, or both (37). The removal of rt-PA from the circulation *in vivo* depends significantly upon the nature of the oligosaccharide structures linked to asparagine at amino acid 117 of kringle 1 (38). It is not known whether one or multiple receptors are involved in the clearance of rt-PA.

Relaxin. This protein has been proposed for use in pregnant women at or near term to increase cervical ripening, i.e., the thinning and softening of the cervix that are necessary to accommodate the passage of the fetus during delivery. While the influence of relaxin on cervical softening appears to be a general phenomenon among mammalian species (39), the mechanism of action is unknown. Relaxin is produced by solid-phase synthesis (40) or recombinant DNA methodology. It is composed of two chains containing two

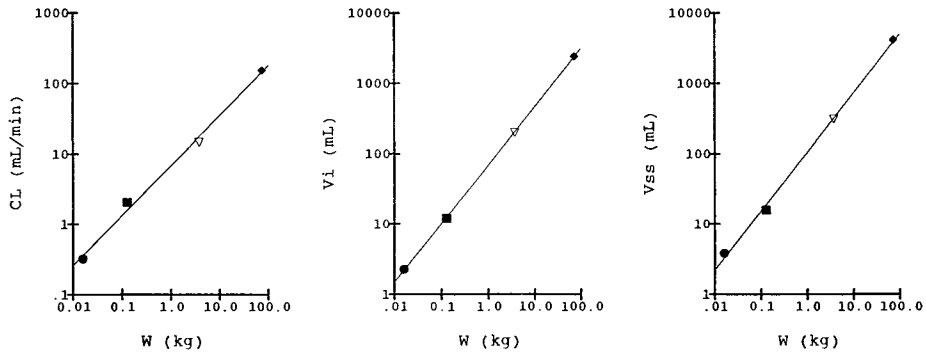

Fig. 3. Log–log plots of clearance (CL), initial volume (V_i), and volume of distribution at steady state (V_{ss}) versus species body weight (W) for rhGH. Data are from Table III, and the solid lines are the allometric relationships from Tables VI–VIII. Filled circle, mouse; filled square, rat; open inverted triangle, cynomolgus monkey; filled diamond, human.

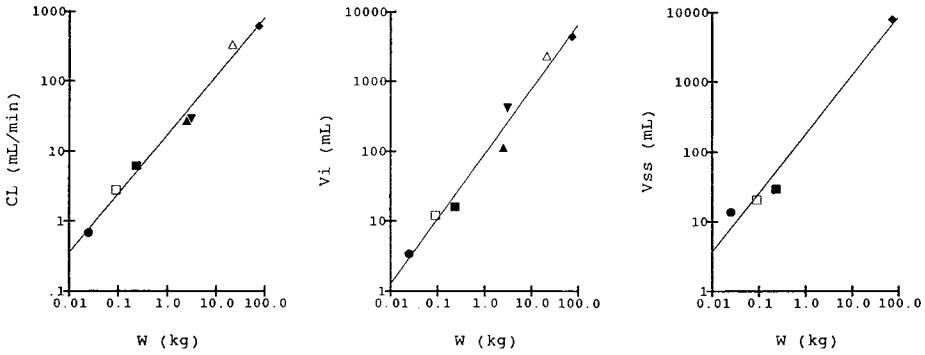

Fig. 4. Log–log plots of clearance (CL), initial volume (V_i), and volume of distribution at steady state (V_{ss}) versus species body weight (W) for rt-PA. Data are from Table IV, and the solid lines are the allometric relationships from Tables VI–VIII. Filled circle, mouse; open square, hamster; filled square, rat; filled triangle, rabbit; filled inverted triangle, rhesus monkey; open triangle, dog; filled diamond, human.

interchain and one intrachain disulfide bonds: the A-chain has 24 amino acids, and the B-chain has 29 amino acids. It has a theoretical molecular mass of 6.0 kDa, has a pI of 9–10, and is not glycosylated. The mechanism of elimination of relaxin is unknown, although the kidneys and liver have been implicated.

METHODS

Plasma or serum clearance (CL), initial volume of distribution (V_i), volume of distribution at steady state (V_{ss}), and body weight (W) for the five proteins appear in Tables I through V. References are provided for published data; otherwise, the data are on file at Genentech, Inc. Only data where the pharmacokinetics appeared linear with dose were selected from these data bases.

Equation (2) was fitted to log-transformed data using unweighted linear least-squares regression analysis. When more than one clearance or volume of distribution value was available for a species, the median values were used in the linear regression to avoid biasing the data for any particular species. Actual reported animal weights were employed whenever possible; otherwise, weight estimates were based on the age of the animals (as reported by the investigator) or standard adult weights. Statistical significance of the regression was tested using the standard t test ($t_{0.975}$, $N - 2$ degrees of freedom).

In a supplemental allometric analysis, the preclinical data from Tables I through V were reanalyzed without the human data. The resultant allometric equations were used to predict CL, V_i, and V_{ss} in humans by substituting in the weight of the patients or volunteers. This analysis was performed to determine if the preclinical data alone would have predicted the human results. The predictions for rCD4 and CD4-IgG that are provided in this report reflect how the preclinical data were actually analyzed and interpreted for our regulatory submission.

RESULTS

The log–log plots of CL, V_i, and V_{ss} versus W for each protein are displayed in Figs. 1–5. The allometric equations, the correlation coefficients (r^2), and the corresponding t value for each regression are provided in Tables VI (CL), VII (V_i), and VIII (V_{ss}). The log-transformed data tend to fall on a straight-line ($r^2 > 0.96$), and the null hypothesis was rejected in every case. The preclinical pharmacokinetic data predicted the observed human pharmacokinetic parameters reasonably well (Table IX).

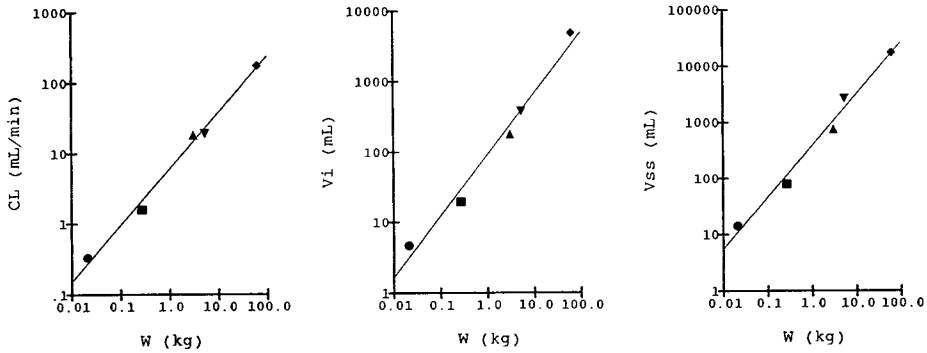

Fig. 5. Log–log plots of clearance (CL), initial volume (V_i), and volume of distribution at steady state (V_{ss}) versus species body weight (W) for relaxin. Data are from Table V, and the solid lines are the allometric relationships from Tables VI–VIII. Filled circle, mouse; filled square, rat; filled triangle, rabbit; filled inverted triangle, rhesus monkey; filled diamond, human.

Table VI. Allometric Equations for Clearance (CL)

Protein	CL (ml/min)	r^2	t value[a]	Degrees of freedom
rCD4	$3.4W^{0.65}$	0.99	19.32	2
CD4-IgG	$0.1W^{0.74}$	0.96	6.79	2
rhGH	$6.8W^{0.71}$	0.99	19.11	2
rt-PA	$17.0W^{0.84}$	0.99	18.62	5
Relaxin	$6.0W^{0.80}$	0.99	19.06	3

[a] H_0: slope (b) = 0; $t_{0.975}$ (2 df) = 4.303, $t_{0.975}$ (3 df) = 3.182, $t_{0.975}$ (5 df) = 2.571. When $t < t_{0.975}$, accept H_0; that is, the parameters do not depend on weight.

DISCUSSION

The CL, V_i, and V_{ss} for these five proteins are well described by an allometric relationship, and animal data predict human data reasonably well. The allometric exponent (b) for clearance (ml/min) ranged from 0.65 to 0.84. The allometric exponent for the volumes of distribution (ml) ranged from 0.83 to 1.05. Exponent values from 0.6 to 0.8 and 0.8 to 1.0 are frequently cited for the clearance and volume of distribution of small molecules, respectively, and are expected based on empirical interspecies relationships. In a recent publication, rt-PA clearance data from infusion studies conducted in rats, rabbits, marmosets, dogs, and humans were scaled allometrically (41). The reported value for clearance ($18.9W^{0.824}$ ml/min) was similar to our findings ($17W^{0.84}$ ml/min).

Criterion of similarity for therapeutic proteins can be derived from allometric equations for pharmacokinetic parameters and related physiological variables. For example, rt-PA is cleared primarily by the liver. An invariant, dimensionless relationship is formed between rt-PA plasma clearance ($CL_{rt\text{-}PA} = 17W^{0.84}$ ml/min) and hepatic blood flow ($Q = 55.4W^{0.894}$ ml/min (42)) as follows:

$$CL_{rt\text{-}PA}/Q = (17W^{0.84})/(55.4W^{0.894}) \sim 0.31 \quad (3)$$

thus, rt-PA plasma clearance is approximately 30% of hepatic blood flow for all mammalian species studied, assuming that the residual mass, $W^{-0.054}$, approximates unity. If rt-PA blood clearance had been used in Eq. (3), the ratio would have yielded the extraction ratio. Also, since both rt-PA clearance and liver weight [$L = 0.037W^{0.849}$ kg (42)] are related to W through the simple allometric relationship

Table VII. Allometric Equations for Initial Volume of Distribution (V_i)

Protein	V_i (ml)	r^2	t value[a]	Degrees of freedom
rCD4	$40W^{1.02}$	0.99	12.65	2
CD4-IgG	$40W^{1.05}$	0.98	10.25	2
rhGH	$68W^{0.83}$	1.00	145.10	2
rt-PA	$91W^{0.93}$	0.98	14.95	5
Relaxin	$91W^{0.87}$	0.98	12.20	3

[a] H_0: slope (b) = 0; $t_{0.975}$ (2 df) = 4.303, $t_{0.975}$ (3 df) = 3.182, $t_{0.975}$ (5 df) = 2.571. When $t < t_{0.975}$, accept H_0; that is, the parameters do not depend on weight.

Table VIII. Allometric Equations for Volume of Distribution at Steady State (V_{ss})

Protein	V_{ss} (ml)	r^2	t value[a]	Degrees of freedom
rCD4	$51W^{1.02}$	0.97	8.57	2
CD4-IgG	$60W^{1.01}$	0.99	13.62	2
rhGH	$105W^{0.84}$	1.00	32.21	2
rt-PA[b]	$178W^{0.84}$	0.98	8.97	2
Relaxin	$380W^{0.92}$	0.98	13.91	3

[a] H_0: slope (b) = 0; $t_{0.975}$ (2 df) = 4.303, $t_{0.975}$ (3 df) = 3.182. When $t < t_{0.975}$, accept H_0; that is, the parameters do not depend on weight.

[b] V_{ss} values for rt-PA based on data from mouse, rat, hamster, and human.

expressed in Eq. (1), they may be related to each other accordingly.

$$CL_{rt\text{-}PA} = 17(L/0.037)^{0.84/0.849} = 443L^{0.989} \quad (4)$$

If the exponent is considered to be unity, the relationship simplifies to $CL_{rt\text{-}PA} \sim 440$ ml/(min*kg liver weight). Thus it appears that in all mammalian species investigated, rt-PA plasma clearance is approximately equal to 440 ml/(min * kg liver weight).

The CL, V_i, and V_{ss} predictions for rCD4 and CD4-IgG in humans assisted with the selection of Phase I doses and subsequent successful prediction of peak and steady-state serum concentrations (43). The data from monkeys were not used in the prospective predictions of V_i and V_{ss} for rCD4 (Table IX); the volume predictions for CD4-IgG in humans were based on the results of clinical trials with rCD4 (44). These approaches were utilized because the monkey data (very small V_i and V_{ss}) appeared to have too great an influence on the slope of the regression lines. For rCD4, the human V_i and V_{ss} predictions were unrealistically small when the monkey data were included (i.e., 1612 and 1845 ml, respectively); when the monkey data were omitted, the V_i and V_{ss} predictions were 2650 and 4725 ml, respectively, and the allometric exponents for V_i and V_{ss} were approximately 1.0. For CD4-IgG, the V_i and V_{ss} in humans were assumed to be similar to the V_i and V_{ss} of rCD4 observed in Phase I studies, corrected for patient body weight. This assumption was employed because the V_i and V_{ss} values for rCD4 and CD4-IgG in all animal species were similar, with the monkey data being consistently low. The V_{ss} prediction for rt-PA (Table IX) is 37 times smaller than the observed value. This prediction was based on data from mouse, rat, and hamster only, since V_{ss} was not reported in the other species. The exponent in the resultant allometric equation is the first indication that the analysis is questionable (i.e., $V_{ss} = 48W^{0.35}$ ml). In addition, the predicted V_{ss} in humans, 219 ml, is physiologically unreasonable. It appears that the allometric equation is overly influenced by the V_{ss} data being tightly clustered in a narrow weight range from species that were more than 300 times smaller than humans. These problems are usually overcome by using data from three or more species over a 50-fold or greater weight range, although rCD4 and CD4-IgG required additional considerations. The above examples illustrate how allometric extrapolations are af-

Table IX. Comparison of the Predicted and Observed Clearance (CL) and Volumes of Distribution (V_i, V_{ss}) Estimates for Each Protein in Humans[a]

Protein	Clearance (ml/min)		V_i (ml)		V_{ss} (ml)	
	Predicted	Observed	Predicted	Observed	Predicted	Observed
rCD4[b]	40	57[c]	2,650	3,640	4,725	4,900
rCD4-IgG[b]	2.6	2.62	4,264	4,100	5,740	6,273
rhGH	126	152.4	2,243	2,432	3,256	4,149
rt-PA	646	620	5,814	4,450	219[d]	8,050
Relaxin	148	175	2,069	4,870	15,354	17,350

[a] Weight of humans taken from Tables I–V, respectively. Although single values appear for each prediction in this report, ranges are usually included in our regulatory submissions. These ranges are obtained by allowing the predicted parameters to fluctuate from ½- to 2-fold, as is customarily observed clinically.

[b] Predictions for rCD4 and CD4-IgG were taken from our regulatory submission and adjusted for patient weight in Phase I studies. Monkey data omitted for V_i and V_{ss} predictions; see Discussion.

[c] Value from Ref. 44. Average clearance in nine patients receiving 1–30 μg/kg/day by continuous infusion was 42.5 ml/min based on observed steady-state serum concentrations (55).

[d] V_{ss} predicted value for rt-PA based on limited data from mouse, rat, and hamster; see Discussion.

fected by experimental interpretation, random and systematic analytical errors, and species' weight range. Moreover, scientific judgment and common sense must be emphasized when interpreting the results of allometric analyses.

The allometric equations for the clearance and volumes of distribution of each protein were different; therefore, unique mechanisms of distribution and elimination may exist that affect these relationships. Species specificity and immune-mediated clearance mechanisms have already been cited as potential concerns when scaling preclinical data to humans. Compounds such as murine monoclonal antibodies may present special scaling challenges, because some species (mouse, rat) may not mount an immune response, while other species may eventually clear the compound via antibody formation. Thus, different and/or nonconstant clearance mechanisms are active and may not relate to species weight. Additional factors that influence the clearance and volumes of distribution of proteins include size, charge, and structure; presence of sialic acid; binding to serum proteins; proteolysis; and relative abundance of tissue clearance receptors, such as carbohydrate receptors in the liver. For example, there exists at least three immunologically different groups of t-PA inhibitors in blood (45). Rabbits have less endothelial cell type PA inhibitor (PAI-1) than humans (46). A comparison of rat, dog, and human blood demonstrated that the inhibitor activity was similar in dog and human and greater in rat (47). The pharmacokinetic studies used for our allometric scaling exercise were performed using concentrations 100 times above the concentrations of these inhibitors; however, these inhibitors are present, and they differ from species to species in their ability to complex human rt-PA. It is not known at this time how the t-PA inhibitors affect the allometric scaling of rt-PA clearance.

CONCLUSIONS

The interspecies scaling guidelines for rCD4, CD4-IgG, rhGH, rt-PA, and relaxin appear to be the same as the guidelines for many small molecules, i.e., (a) small animals tend to eliminate proteins more rapidly than humans; (b) volumes of distribution tend to be proportional to weight across species; (c) pharmacokinetic scaling is sometimes accomplished with allometric equations; (d) the allometric exponents for the pharmacokinetic parameters tend to approximate the exponents for corresponding physiologic variables; and (e) when few species are available or when all the species are clustered in a narrow weight range, data from individual species may have a significant impact on the linear regression.

Clearly, more work in this area is necessary before we will be able to appreciate fully the similarities and differences underlying the distribution and elimination of therapeutic human proteins in different species, particularly after repetitive exposures. From our series of evaluations, it appears that the clearance and volumes of distribution of select biomacromolecules follow well-defined, size-related physiologic relationships and that preclinical pharmacokinetic data can be predictive of human pharmacokinetics.

In situations where interspecies scaling principles apply, preclinical disposition studies can form the basis of relating doses used in preclinical efficacy models and toxicology studies to potentially safe and effective regimens in initial clinical studies. In addition, this information provides the foundation upon which concentration-controlled clinical studies can be conducted.

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