TOXICOLOGY AND APPLIED PHARMACOLOGY **150,** 295–300 (1998)
ARTICLE NO. TO978363

Interspecies Scaling of Clearance and Volume of Distribution for Horse Antivenom F(ab′)$_2$

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Received April 22, 1997; accepted December 17, 1997

Interspecies Scaling of Clearance and Volume of Distribution for Horse Antivenom F(ab′)$_2$. Bazin-Redureau, M., Pepin, S., Hong, G., Debray, M., and Scherrmann, J. M. (1998). *Toxicol. Appl. Pharmacol.* **150, 295–300.**

F(ab′)$_2$ fragments are sometimes preferred to whole IgG for therapeutic or diagnostic uses. Preclinical pharmaceutical development studies are necessary before their use in humans. Here we propose an allometric approach among three mammalian species to predict F(ab′)$_2$ pharmacokinetic parameters in humans. Plasma disposition of horse antivenom F(ab′)$_2$ fragments labeled with iodine 125 was studied at a dose of 10 mg/kg iv in mice, rats, and rabbits. Using the allometric method, we demonstrate that the pharmacokinetic parameters that correlated with body weight were distribution volume (Vd_c (ml) = 0.125 $W^{0.87}$; Vd_{ss} (ml) = 0.251 $W^{0.87}$; Vd_β (ml) = 0.290 $W^{0.87}$, r^2 = 1), total clearance (Cl_{tot} (ml/h) = 0.049 $W^{0.53}$, r^2 = 0.99), and terminal half-life ($t½\beta$ (h) = 4.35 $W^{0.33}$). The F(ab′)$_2$ plasma concentration–time data plotted as a complex Dedrick relationship were superimposable. Using these allometric techniques, Vd_{ss}, Vd_β, Cl_{tot}, and $t½\beta$ were calculated as 4.12 liter, 4.78 liter, 19.07 ml/h, and 7.2 days, respectively, for a human subject of 70 kg body wt. Predicted human pharmacokinetic parameters were comparable for volume of distribution with the value reported by Hnatowich *et al.* **(***Cancer Res.* **47, 6111–6117, 1987): 3.5 liter. However, the clearance was six-fold lower than values given by Hnatowich** *et al.* **(130 ml/h) and Ho** *et al.* **(***Am. J. Trop. Med. Hyg.* **42, 260–266, 1990) (116.9 ml/h).**

Key Words: **F(ab′)$_2$ fragments; pharmacokinetics; interspecies scaling; clearance; volume of distribution.**

Antibodies may be administered in human therapy and for diagnosis as antibody fragments such as the 100 kDa F(ab′)$_2$ and the 50 kDa Fab. This technique has been applied over the last decade particularly for the treatment of poisoning due to toxins including small haptens such as digoxin or colchicine or larger toxins such as viper or scorpion venoms (Butler *et al.*, 1976; Scherrmann *et al.*, 1989; Sabouraud *et al.*, 1992). For their *in vivo* use, antibody fragments that have lost the Fc region have less risk of adverse effects and a greater volume of distribution and clearance than whole IgG because of their lower molecular weights. Whal (1983) suggested that in clinical practice, F(ab′)$_2$ is the best compromise between the rapidly cleared Fab and the slowly cleared IgG. For this reason, selection of ideal antibody size has rarely been based on pharmacokinetic criteria. However, knowledge of the volume of distribution and clearance could facilitate optimization of their administration in humans. Interscaling species could be helpful to extrapolate pharmacokinetic data of different compounds from animals to humans and especially for proteins (Mordenti *et al.*, 1991). In a recent study, we showed that primary Fab pharmacokinetic parameters could be reasonably estimated in humans using pharmacokinetic data from the mouse, rat, and rabbit (Grene-Lerouge *et al.*, 1996). In the allometric approach, the plasma drug kinetics observed in several animal species allow determination of species-specific pharmacokinetic parameters (volume of distribution, total body clearance), which can be scaled by the power equation $Y = aW^b$, where Y is a pharmacokinetic parameter, W is body weight, a is the allometric coefficient, and b is the allometric exponent (Boxenbaum, 1982). Moreover, by using the calculated power equations, the similarity of pharmacokinetics among species can be demonstrated by the collapse of pharmacokinetic profiles from different animal species into a unique species-independent profile, which introduces the term equivalent time (or pharmacokinetic time) (Dedrick *et al.*, 1970). In the present study, the feasibility of allometry was tested using ^{125}I-radiolabeled horse antivenom F(ab′)$_2$ in three animal species: mouse, rat, and rabbit. Finally, results are compared with some previously published data in F(ab′)$_2$-treated patients (Ho *et al.*, 1990; Hnatowich *et al.*, 1987).

MATERIALS AND METHODS

Chemicals and animals. Horse antivenom F(ab′)$_2$ was supplied by Pasteur-Mérieux Connaught (Marcy l'Etoile, France). Male New Zealand rabbits weighing from 2.6 to 3 kg were obtained from Charles River (Elbeuf, France). Male Swiss white mice weighing from 25 to 30 g and male Sprague–Dawley rats weighing from 200 to 240 g were from Iffa Credo (Lyon, France). All animals had free access to standard laboratory chow and tap water.

0041-008X/98 $25.00

Radioiodination of F(ab′)₂. $F(ab')_2$ fragments were labeled with [^{125}I]NaI using the Iodogen method (Fraker and Speck, 1978). One hundred micrograms of protein was incubated with 0.5 mCi of [^{125}I]NaI in Eppendorf tubes coated with 10 μg of Iodogent reagent for 5 min at room temperature. Free iodine was removed by chromatography on a PD-10 Sephadex G-25 column (Pharmacia, Uppsala, Sweden) equilibrated in phosphate-buffered saline. Precipitation of the iodinated proteins by trichloroacetic acid gave more than 95% of bound iodine. The specific activity was in the range of 1.5–2 μCi/μg. Purity of antibody preparations was also analyzed by SDS–PAGE.

Animal experiments. [^{125}I]-$F(ab')_2$ was injected at a dose of 10 mg/kg in rabbits, rats, and mice. Rabbits ($n = 5$) received a single intravenous (iv) bolus $F(ab')_2$ (0.33 ml/kg) via a marginal ear vein over 1 min. Blood (1 ml) was collected into heparinized tubes from the central artery of the contralateral ear at the following times: 0, 0.25, 0.5, 1, 2, 4, 8, and 24 h and daily for 10 days after injection. One day before experiments, rats were anesthetized with pentobarbital sodium (60 mg/ml, 50 mg/kg ip). Rectal temperature was continuously monitored by a rectal probe (Ellab Thermometer Model TE 3, Copenhagen, Denmark), and the femoral vein was cannulated with PE-10 tubing (Biotrol, Paris, France) for $F(ab')_2$ fragment administration. The following day, rats ($n = 6$) received a single bolus $F(ab')_2$ dose of 2.5 ml/kg. Blood samples (0.3 ml) were collected at 0, 0.25, 0.5, 1, 2, 4, 8, and 24 h and daily for 5 days after injection. Blood (0.3 ml) from a rat donor was infused via the femoral vein at the first seven blood samplings only to compensate for blood collected. $F(ab')_2$ (0.1 ml/10 g) was injected into the tail vein of mice. Blood (0.5 ml) was collected by cardiac puncture at each of the following times after injection: 0, 0.16, 0.33, 0.5, 1, 2, 4, 8, 24, 32, 48, and 72 h. Six mice were used at each sampling time. All blood samples were centrifuged at 4°C immediately after collection. An aliquot of plasma was counted for radioactivity before and after precipitation with 10% trichloroacetic acid (TCA), and correction was made for decay of the radioisotope. All plasma measurements were run in duplicate.

Pharmacokinetic data analysis. Plasma $F(ab')_2$ concentration–time data were analyzed using the computer program MK MODEL (Biosoft, United Kingdom) based on compartmental analysis. After iv administration of $F(ab')_2$, the data were best described by a biexponential curve defined by the following equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and B are intercept coefficients; α and β are first-order distribution and elimination rate constants, respectively; C is concentration; and t is time. A weighting of $1/C^2$ was applied to optimize the fitting. The distribution ($t_{1/2}\alpha$) and elimination ($t_{1/2}\beta$) half-lives were calculated as $0.693/\alpha$ and $0.693/\beta$, respectively. The area under the curve of plasma concentration versus time (AUC) was calculated according to the equation $A/\alpha + B/\beta$. Total body clearance (Cl_{tot}), central compartment volume (Vd_c), volume of distribution at steady state (Vd_{ss}), and volume of distribution (Vd_β) were obtained from standard formulas (Gibaldi and Perrier, 1982).

Interspecies scaling analysis. Allometric equations describing the relationship between body weight (W), total body clearance (Cl_{tot}), and the volumes of distribution (Vd_c, Vd_β, Vd_{ss}) were determined by fitting the variable parameter Y to the standard power equation:

$$Y = aW^b$$

where a is the allometric coefficient and b is the allometric exponent. The pharmacokinetic parameters Y and W were transformed logarithmically and fitted to the equation: $\mathrm{Log}Y = \mathrm{Log}a + b\,\mathrm{Log}W$ by linear regression analysis for clearance and by piecewise linear regression analysis for the volumes of distribution, since the three volumes must scale to a similar allometric exponent (Mordenti, 1985). Regression analysis and statistical characterization of the regression lines by the determination coefficient r^2 were carried out using Systat computer software (Systat, Inc., Evanston, IL). The terminal half-life, $t_{1/2}\beta = 0.63\ Vd_\beta/Cl_{tot}$, was scaled by introducing the power equations for clearance and Vd_β. An open two-compartment model with elimination only from the central compartment was assumed to be adequate to describe $F(ab')_2$ disposition, which corresponds to a biexponential decay. Substituting in the previous allometric equations for clearance and volumes of distribution into the central compartment equation gave a general equation in terms of interspecies constants (Boxenbaum and Ronfeld, 1983) whose graphical display is termed the complex Dedrick plot. Allometrically scaled plasma concentrations obtained in the three species were then plotted versus scaled time (apolysichron) to be superimposable with the predicted complex Dedrick plot. Finally, predicted $F(ab')_2$ plasma profiles for each species and for humans were obtained from the equation of the complex Dedrick plot by introducing the $F(ab')_2$ dose (D) and the weight of each species. Predicted values of pharmacokinetic parameters in humans were estimated using the determined allometric relationships for a body weight of 70 kg.

RESULTS

$F(ab')_2$ plasma concentration decreased biexponentially in mice, rats, and rabbits following a 10 mg/kg iv dose (Fig. 1). Table 1 summarizes the pharmacokinetic parameters for the three animal species. Results show that the smaller the animal species the higher the total body clearance, i.e., $F(ab')_2$ elimination was faster in mice than in rats and rabbits: 9.64, 4.63 ± 0.46 and 1.16 ± 0.08 ml/h/kg, respectively. Moreover, the higher the volume of distribution, the larger the animal species, but when divided by their respective body weight, volumes of

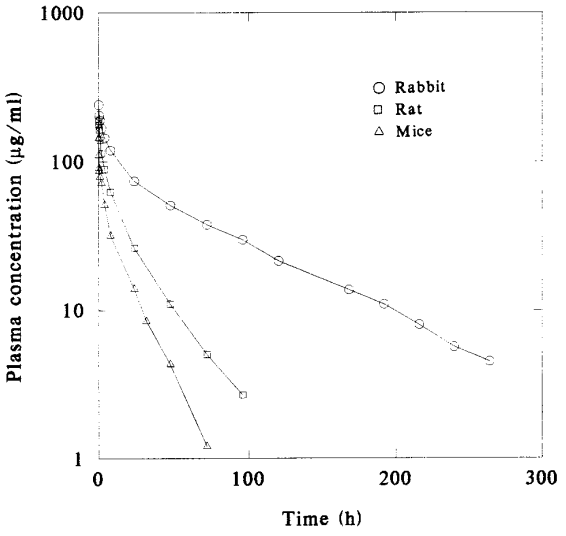

FIG. 1. Semilogarithmic plasma concentration–time profiles following administration of F(ab′)$_2$ (10 mg/kg iv) in different animal species.

distribution were comparable (165.35, 116.66 ± 10.64, and 94.38 ± 7.06 ml/kg for Vd_{ss} and 187.85, 144.90 ± 13.42, and 102.97 ± 7.95 ml/kg for Vd_β in mice, rats, and rabbits, respectively). The logarithm of pharmacokinetic parameters fitted the logarithm of the animal weights linearly (Fig. 2), according to the following equations:

$$Cl_{tot}\,(\text{ml/h}) = 0.043\,W^{0.53} \quad (r^2 = 0.99)$$

$$Vd_c\,(\text{ml}) = 0.125\,W^{0.87} \quad (r^2 = 1.00)$$

$$Vd_{ss}\,(ml) = 0.251\,W^{0.87} \quad (r^2 = 1.00)$$

$$Vd_\beta\,(\text{ml}) = 0.290\,W^{0.87} \quad (r^2 = 1.00)$$

$$t^{1}\!/_{2}\beta\,(\text{h}) = 4.35\,W^{0.33}$$

The allometric parameters for volumes of distribution and clearance were substituted in the Boxenbaum equation and gave a good fit for the allometrically scaled data across all species to a biexponential equation (Fig. 3)

$$y = 12.35e^{-0.1163x} + 7.20e^{-0.0167x}$$

where

$$x = tW^{-0.337} \quad \text{and} \quad y = C/DW^{0.871}$$

Using these equations, predicted F(ab′)$_2$ plasma kinetics for each species and for a 70-kg human were plotted for the 10 mg/kg of F(ab′)$_2$ dose and the body weight of each species (Fig. 4). The predicted values of Cl_{tot}, Vd_c, Vd_β, and $t^{1}\!/_{2}\beta$ for a 70-kg human were, 19.07 ml/h, 2.07 liters, 4.78 liters, and 7.2 days, respectively.

TABLE 1

Pharmacokinetic Parameters of Total [^{125}I]-F(ab′)$_2$ Fragments in Different Animal Species Following iv Administration

	Number	Weight (g)	A (μg/ml)	α (h^{-1})	$t^{1}\!/_{2}\alpha$ (h)	B (μg/ml)	β (h^{-1})	$t^{1}\!/_{2}\beta$ (h)	AUC (0→∞) (μg/ml · h)	Vd_c (ml)	Vd_{ss} (ml)	Vd_β (ml)	Cl_{tot} (ml/h)
Mice	5/per point	28	69.50	0.49	1.42	46.00	0.05	13.70	1053.00	2.42	4.63	5.26	0.27
Rats	6	216	123.70 ± 13.80	0.26 ± 0.03	2.70 ± 0.30	53.80 ± 3.10	0.03 ± 0.001	21.70 ± 0.60	2167.00 ± 170.00	12.20 ± 1.20	25.20 ± 2.30	31.30 ± 2.90	1.00 ± 0.10
Rabbits	5	2690	127.60 ± 17.80	0.17 ± 0.05	4.3 ± 1.20	88.90 ± 6.90	0.01 ± 0.001	61.40 ± 7.00	8611.00 ± 791.50	125.30 ± 14.10	253.90 ± 19.00	277.00 ± 21.40	3.14 ± 0.23

Note. Parameters are means ± SD.

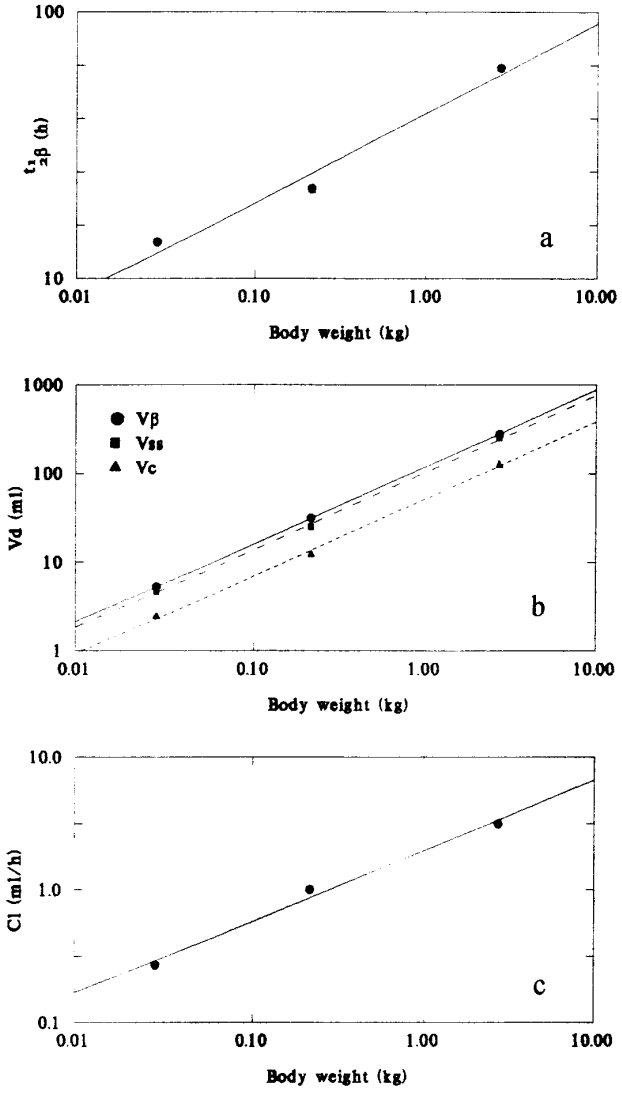

FIG. 2. Allometric relationship between (a) elimination half-life ($t½\beta$), (b) volume of distribution (Vd_c, Vd_{ss}, Vd_β), (c) total body clearance (Cl_{tot}), and species body weight for $F(ab')_2$.

DISCUSSION

A new generation of horse $F(ab')_2$ directed against several toxins from viper and scorpion venoms was recently proposed for treatment of envenomation (Pépin-Covatta *et al.,* 1996). Their clinical application has until now been mainly based on experience of their efficacy and little pharmacokinetic information was available. Since clinical Phase I studies are impossible for ethical reasons, it is important to know whether allometric concepts can predict human $F(ab')_2$ pharmacokinetics using data from several animal species. For this purpose, $F(ab')_2$ pharmacokinetics were investigated in three animal species: mice, rats, and rabbits, and allometric methods were used to investigate possible relationships between physiological variables such as time and body weight and pharmacokinetic parameters. The interspecies scaling of renally excreted small compounds has been reported to be highly predictive. In contrast, when clearance is mainly via oxidative metabolism, interspecies scaling is less predictive (Boxenbaum, 1980). The question is whether interspecies scaling is also dependent on the clearance mechanisms of macromolecules. Successful allometric applications for renally eliminated macromolecules have been shown for 50 kDa Fab (Grene-Lerouge *et al.,* 1996) and 20 kDa interferon-α (Lavé *et al.,* 1995). Moreover, Mordenti *et al.* (1991) reported successful interspecies scaling of five therapeutic macromolecules whose molecular weight covered a 16-fold range (6–98 kDa), some of which were elimi-

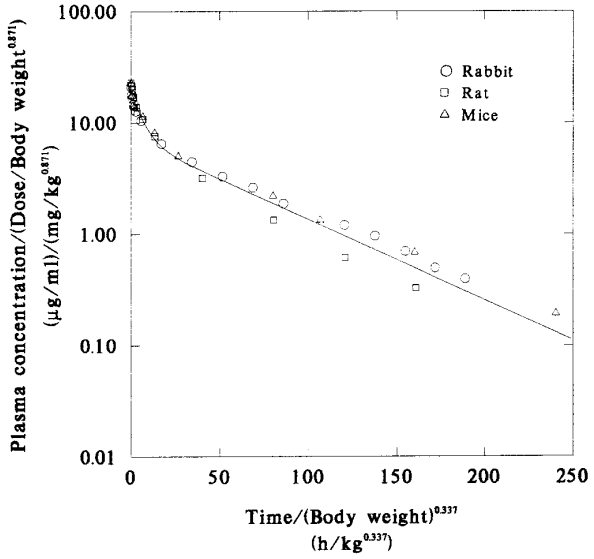

FIG. 3. Complex Dedrick plot of biexponential $F(ab')_2$ concentration–time curves in mouse, rat, and rabbit.

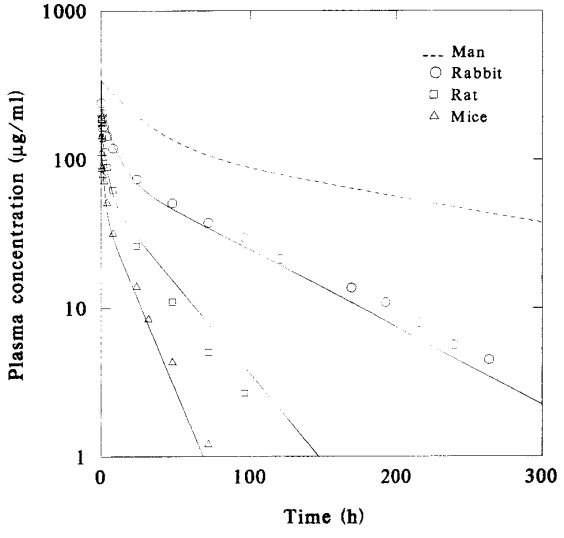

FIG. 4. Predicted $F(ab')_2$ plasma kinetics for mouse, rat, rabbit, and a 70-kg human after a 10 mg/kg $F(ab')_2$ iv bolus dose.

nated only renally, e.g., the recombinant soluble CD4 (50 kDa), or only by the liver, e.g., the tissue plasminogen activator (63 kDa). Because of their molecular weight of 100 kDa, F(ab′)$_2$ are cleared by extrarenal mechanisms, in contrast to Fab fragments, which are renally excreted, and the percentage of intact Fab excreted in urine is 8% in dog (Keyler *et al.*, 1991), 15% in rat (Arend and Silverblatt, 1975), and 57% in humans (Schaumann *et al.*, 1986).

Our data demonstrate that Cl_{tot}, *Vd*, and $t½\beta$ are well described by an allometric relationship. Allometric exponents for Cl_{tot} (0.53), *Vd* (0.87) and $t½\beta$ (0.33) approximated exponent values frequently cited and ranged from 0.6 to 0.8, 0.8 to 1.0, and 0.2 to 0.4, respectively. Moreover, the use of transformed plasma concentration–time data showed the superimposability of F(ab′)$_2$ plasma concentration–time data for the three species (Fig. 3).

Extrapolation to human pharmacokinetics gave Vd_c, Vd_β, Cl_{tot}, and $t½\beta$ of 2.07 liters, 4.78 liters, 19.07 ml/h, and 7.2 days, respectively, for a 70-kg body wt human. The estimated Vd_c approximated the mean human plasma volume (2.8 liters). This estimated Vd_c was comparable to the mean Vd_c value of 2.5 liters (2.4–2.6 liters) reported for mouse F(ab′)$_2$ by Hnatowich *et al.* (1987) but was lower than the mean Vd_c value of horse F(ab′)$_2$ of 5.8 liters (2.7–6.7 liters) reported in envenomed patients by Ho *et al.* (1990). Similarly, estimated Vd_β (4.78 liters) was of the same order as the Vd_{ss} (3.5 $\pm$ 1.5 liters) described by Hnatowich *et al.* (1987) whereas Ho *et al.* (1990) found a higher Vd_β of 16.3 liters (12.4–27 liters). Our estimated total clearance (19.07 ml/h) was six-fold lower than the mean clearance reported by Hnatowich *et al.* (1987) (130 $\pm$ 50 ml/h) and by Ho *et al.* (1990) (range 63.7–177.8 ml/h). Moreover, our estimated elimination half-life was longer, 7.2 days compared to about 4 days (3.3–5.5 days for Ho *et al.* (1990) and 1.12 $\pm$ 0.3 days for Hnatowich *et al.* [1987]). However, these discrepancies can perhaps be explained by the fact that the studies of Ho *et al.* (1990) and Hnatowich *et al.* (1987) were conducted in envenomed and cancer patients, respectively. It is thus possible that the presence of specific antigens would modify F(ab′)$_2$ clearance because of the formation of the antigen–antibody complex. Moreover, the experiment of Ho *et al.* used a short sampling protocol of 100 h, resulting in higher total clearance and volume of distribution and shorter $t½\beta$ values. Because of the high molecular weight of F(ab′)$_2$ predicted Vd_c and Vd_β reflect the extremely low diffusibility of this compound through the capillary wall. The volume of distribution is quite similar to that of whole IgG antibody and far lower than that of Fab, which can attain the volume of the extracellular water space (Bazin-Redureau *et al.*, 1997). Despite our reservations concerning the clearance values reported by Hnatowich *et al.* (1987) and Ho *et al.* (1990), several questions might be raised. The allometric clearance exponent (0.53) and volume of distribution exponent (0.87) were below the generally reported range, i.e., 0.6–0.8 for clearance and 1.0 for volume of distribution. By using ''standard'' scaling exponents of 0.75 and 1.0 for the clearance and volume of distribution, respectively, predicted clearance rose from 19.1 to 47.7 ml/h and Vd_β from 4.8 to 8.3 liters for a 70-kg human. This twofold increase in the predicted values of clearance and volume of distribution do not markedly change our initial conclusion on the disagreement between our clearance values and those of Hnatowich *et al.* (1987) and Ho *et al.* (1990). Factors contributing to the uncertainty of allometric scaling are frequently evoked. For example, the effect of scaling interferon-αA with different sets of data abstracted from various publications, including different analytical drug assays and protocols, has already been discussed (Lavé *et al.*, 1995). In the present study, the same F(ab′)$_2$ preparation was simultaneously investigated in three animal species using the same assay, thus limiting the influence of interassay variability. However, several uncertainties remain. First, the use of three small species could perhaps be considered insufficient for an accurate prediction in humans, more especially for molecules that are not eliminated renally. However, the allometric concept has been reported to be applicable using only three species if body weights span more than the minimum 50-fold range required in interspecies scaling (Mordenti *et al.*, 1991), which is the case in our study. Second, our use of [^{125}I]-labeling for investigating F(ab′)$_2$ pharmacokinetics could lead to dissociation of iodine from the protein over time, which would overestimate protein levels and result in lower clearance. To control for this, in our experiments, TCA precipitation was applied to each plasma sample to determine the free iodine content. This never exceeded 10% of the total radioactivity in any experiment and validated our measurement of radioactivity bound to F(ab′)$_2$. Moreover, in previous studies we also used an immunoradiometric assay to measure the F(ab′)$_2$ plasma concentration in rabbits after iv administration of unlabeled F(ab′)$_2$ (Pepin-Covatta *et al.*, 1996). Pharmacokinetic parameters from the immunoradiometric assay were comparable with those obtained with [^{125}I]-F(ab′)$_2$ i.e., a biexponential decay of plasma F(ab′)$_2$. The specificity of F(ab′)$_2$ measurement following [^{125}I]-F(ab′)$_2$ infusion has also been confirmed by Bazin-Redureau *et al.* (1997) who reported that plasma analysis by SDS–PAGE and autoradiography did not reveal the presence of lower- or higher-molecular-weight products following iv administration of murine F(ab′)$_2$ in rats. Third, interanimal variability was not considered in mice because they were subjected to serial euthanasia whereas rats and rabbits received serial sampling of individuals. Finally, the application of allometric methods to proteins could be disturbed by another parameter not observed with drugs, i.e., the species origin of the protein vs the host species relationship. For example, when administered to humans, homologous human IgGs are characterized by a terminal half-life of about 20 days, whereas that of murine monoclonal IgGs is only about 2 days (Callagan *et al.*, 1993). Protein origin and recipient species-related factors multiply the risk of variability in the pharmacokinetic parameters, especially for systemic clearance. The higher clearance of

mouse $F(ab')_2$ in man observed by Hnatowich *et al.* compared to our predicted clearance of horse $F(ab')_2$ might be due to this species difference in protein and host.

In conclusion, it appears that $F(ab')_2$ clearance and volume of distribution follow a size- and time-related physiological relationship. Moreover, the prediction of $F(ab')_2$ pharmacokinetics in humans shows that these fragments distribute like IgG but are cleared at a faster rate. Finally, our hypothesis that $F(ab')_2$ species origin may play a major role in determining pharmacokinetic parameters could mean that the allometric method would need to be applied to all $F(ab')_2$ species to predict the corresponding human pharmacokinetics.

ACKNOWLEDGMENT

We thank Pasteur-Mérieux Connaught for their gift of horse antivenom $F(ab')_2$.

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