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Allometry of Factor VIII and informed scaling of next generation therapeutic proteins

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Abstract

Allometric scaling has been applied to the pharmacokinetics (PK) of factor VIII (FVIII), but published relationships are based on relatively small subsets of available data. Numerous next generation forms of FVIII are being developed (e.g. Fc fusion, PEGylated, and liposomal formulations) and traditional pharmacokinetic scaling of these products would not incorporate the wealth of existing knowledge for current FVIII therapy in humans. We conducted a meta-analysis and developed allometric relationships of FVIII from over 100 PK studies collected from literature. Normalized Wajima curves were used to relate mean FVIII profiles between species. An ‘informed scaling’ approach was derived for predicting first-in-human PK parameters and demonstrated with a case study for an Fc fusion FVIII. NCA values for FVIII PK were well described by the allometric equations $CL=6.59 \cdot W^{0.85}$ and $V_{ss}=65.0 \cdot W^{0.97}$. A subset of studies characterized by two compartment modeling showed strong linearity in scaling of total clearance and central volume, but more variability in distributional clearance and peripheral volume. Wajima curves for FVIII superimposed across species and the disposition of Fc fusion FVIII in humans was well predicted by ‘informed scaling.’ This approach might be generally applicable for predicting human PK of next generational therapeutics.

INTRODUCTION

Accurate prediction of pharmacokinetics (PK) for therapeutic agents from preclinical studies is a long-standing challenge in the pharmaceutical sciences. A traditional method for such predictions utilizes the principle of allometric scaling, wherein PK parameters are related to body weight on a log-log scale.¹ The success of this method stems from correlations between underlying physiological processes governing the PK of a drug, such as tissue volumes and flow rates, with body weight across species. Many modifications of the basic allometric relationship have been proposed in which scaled parameters are corrected for maximum lifespan, liver blood flow, brain weight, or other metrics.² The success of these methods ultimately depends on how closely the factors governing drug disposition relate to allometrically describable processes and the species included in the analysis.

Within the spectrum of small molecule drugs, those predominantly excreted into the urine tend to scale with higher accuracy than those eliminated primarily by metabolism, which can widely vary between species.² Protein therapeutics as a class tend to scale well across species based on simple allometry.³ Mahmood found that for 15 protein drugs representing a range of structures and therapeutic targets, clearance could be well predicted with simple allometry and improved by a correction for brain weight where the allometric exponent was greater than 1.⁴ Wang and colleagues observed similar success when applying allometric

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scaling to a larger set of 34 protein drugs.⁵ Allometric scaling has even demonstrated good predictive power for monoclonal antibodies displaying nonlinear pharmacokinetics in non-human primates, although it is unclear how generalizable such relationships may be to cases in which clinical dose levels result in relatively low drug exposures owing to target-mediated disposition.⁶

Much of the current focus in the biotherapeutic industry is on so called ‘next generation’ products engineered to improve one or more aspects of therapy. For factor VIII (FVIII), a blood coagulation factor deficient in hemophilia A, efforts to improve replacement therapy have included PEGylation,^{7–9} liposomal delivery,^{10,11} site directed mutagenesis,^{12,13} and Fc fusion strategies.^{14,15} The PK of FVIII has been shown to scale well with simple allometry, although previous efforts have incorporated a relatively limited number of the studies available in the literature and it is unclear how well the normal mice used in such efforts relate to the PK in animals and humans with HA.^{4,16,17} Allometric scaling can be used to predict the human PK of these next generation therapeutic agents from preclinical observations, but the accuracy of such predictions may be improved if informed by the wealth of information available for the unmodified protein. This may be accomplished through the use of physiologically based PK (PBPK) models, but the experiments to support such models are labor intensive and pose technical difficulties for large glycoproteins like FVIII that exhibit limited tissue distribution and are largely confined to the plasma volume.

Our goal was to conduct an extensive literature search for PK studies of FVIII in multiple species and develop comprehensive allometric relationships for PK parameters describing the disposition of the protein. For the subset of studies that provided concentration-time profiles or compartmental parameters to simulate them, the Wajima method was used to establish equivalence of normalized curves.¹⁸ We propose an ‘informed scaling’ method whereby assuming that an unmodified and a next generation therapeutic protein share the same interspecies relationships to body weight (reflected by the allometric exponent), PK parameters for the next generation product in humans can be predicted from preclinical observations and the known human parameters for the unmodified protein. Proof of concept is shown by predicting human concentration-time profiles for an Fc fusion FVIII product at multiple doses based upon preclinical animal studies and the established allometric relationships for FVIII.

MATERIALS AND METHODS

Data source

A literature search was performed for articles containing the keywords “factor VIII” and “pharmacokinetics.” Articles were considered for inclusion if they contained any concentration-time profiles, noncompartmental analysis (NCA), or PK parameters from compartmental fitting of FVIII dosed intravenously to animals or HA patients. The year 1985 was established as a cutoff since this period largely coincides with both the shift from FVIII cryoprecipitates prepared at local blood banks to commercially sourced high purity plasma derived or recombinant products, as well as the popularization of NCA methods for PK data analysis.¹⁹ Articles were excluded if they did not represent a robust source of information (e.g. a case study in a single patient), presented data in a nonstandard manner, or were a duplication of data already included in the analysis. Units were standardized to concentrations in IU/mL, volumes in mL, and clearances in mL/h. For papers expressing FVIII concentrations as % of normal, 100 % was assumed to represent 1 IU/mL in all studies.

For cases in which only partial parameters were presented, the missing information was computed from the standard noncompartmental equations for steady state volume of

distribution (V_{ss}), total clearance (CL), mean residence time (MRT), and area under the curve (AUC):

$$V_{ss}=CL \cdot MRT \quad (\text{Eq.1})$$

$$CL=\frac{Dose}{AUC} \quad (\text{Eq.2})$$

When available, V_{ss} was also calculated from the two compartmental parameters:

$$V_{ss}=V1+V2 \quad (\text{Eq.3})$$

With V1 and V2 representing the central and peripheral compartment volumes. Available concentration-time profiles were digitized with Engauge Digitizer v4.1 (digitizer.sourceforge.net) and subjected to NCA in Phoenix WinNonlin v6.3 (Pharsight Corporation, Sunnyvale, CA). NCA values were derived from digitized curves or from simulated profiles for papers in which compartmental, but no NCA parameters were given. The priority for the source of parameters was published > digitized > simulated. A body weight of 70 kg was assumed for all patient populations for which no age or weight was provided, or where the median age was 18 years. For studies in children where no weight was provided, mean weight from the CDC growth charts for the published mean or median age was used.²⁰ Mean body weight for animal species when not provided was assumed to be 22.5 g (mouse), 4.0 kg (monkey), 4.5 kg (rabbit), or 17.0 kg (dog). In studies where data were analyzed at multiple centers or by multiple activity assays, average reported values were used. Six unpublished PK studies of FVIII in HA mice conducted by our lab were also included.

Allometric scaling

Allometric scaling of NCA parameters CL, V_{ss} , MRT, and $t_{1/2}$ was conducted by fitting the standard allometric equation:

$$P=A \cdot W^b \quad (\text{Eq.4})$$

For a parameter of interest (P), allometric coefficient (A), body weight in kg (W), and allometric exponent (b). Linear regression and calculation of 95% prediction intervals were conducted in SigmaPlot 11.0 (Systat Software, Chicago, IL). The impact of data segregation on regression was examined for the following groups: normal versus hemophilia A models; activated partial thromboplastin time (APTT) versus chromogenic assay for determination of FVIII concentrations; use of full length recombinant, B-domain deleted (BDD) recombinant, or plasma derived FVIII products. All available data were pooled for the final analysis.

Compartmental modeling of Factor VIII

Most of the FVIII concentration-time curves displayed a biphasic profile with a small but distinct alpha phase. Large scale population PK models for FVIII have utilized a two compartment model and thus a similar approach for describing the literature data was applied.^{21,22} Parameters for CL, V1, CLd, and V2 were estimated from digitized data with WinNonlin. All profiles were fit independently. Studies were rejected from this portion of the analysis if fitting resulted in a CV% > 100 for any parameter estimate. Allometric scaling was conducted as above for each term. Mean parameters for each species were also

used to simulate a typical profile for an i.v. bolus of 50 IU/kg FVIII using the explicit equations:

$$C = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \quad (\text{Eq.5})$$

$$\alpha + \beta = \frac{CL}{V_1} + \frac{CLd}{V_1} + \frac{CLd}{V_2} \quad (\text{Eq.6})$$

$$\alpha \cdot \beta = \frac{CL}{V_1} \cdot \frac{CLd}{V_2} \quad (\text{Eq.7})$$

$$\alpha, \beta = \frac{(\alpha + \beta) \pm \sqrt{(\alpha + \beta)^2 - 4 \cdot \alpha \cdot \beta}}{2} \quad (\text{Eq.8})$$

$$A = \frac{\text{Dose} \cdot (\alpha - \frac{CLd}{V_2})}{V_1 \cdot (\alpha - \beta)} \quad (\text{Eq.9})$$

$$B = \frac{\text{Dose} \cdot (\frac{CLd}{V_2} - \beta)}{V_1 \cdot (\alpha - \beta)} \quad (\text{Eq.10})$$

Dedrick plots for FVIII

The Dedrick plot is an established method for predicting human PK profiles from individual animal studies based on conversion of chronological time to physiological time.¹

Applicability of the Dedrick plot method to describe the interspecies relationship of FVIII PK was assessed by attempting to predict the PK profile for a 50 IU/kg i.v. bolus of FVIII in a 70 kg human from each of HA mice, HA dogs, normal rabbits, or normal monkeys.

Macro-constants A, B, α , and β calculated from the average two compartment parameters for each species were used in a version of the Dedrick equation as described by Wajima and colleagues, and simplified here by dose normalization:¹⁸

$$C = A_{\text{animal}} \cdot \exp \left(-\alpha_{\text{animal}} \cdot \frac{BW_{\text{animal}}^{0.25}}{BW_{\text{man}}^{0.25}} \cdot t \right) + B_{\text{animal}} \cdot \exp \left(-\beta_{\text{animal}} \cdot \frac{BW_{\text{animal}}^{0.25}}{BW_{\text{man}}^{0.25}} \cdot t \right) \quad (\text{Eq.11})$$

Accuracy of the predicted curves was assessed visually.

Wajima normalized curves for FVIII

An extension of the Dedrick plot was proposed by Wajima and colleagues where PK profiles are expressed as concentrations normalized to the steady state concentration (C_{ss} , $C' = C/C_{ss}$) and time normalized to MRT ($t' = t/\text{MRT}$).¹⁸ The resulting equation for a biphasic system can be written as:

$$C' = \frac{A}{C_{ss}} \cdot e^{-\alpha \cdot \text{MRT} \cdot t'} + \frac{B}{C_{ss}} \cdot e^{-\beta \cdot \text{MRT} \cdot t'} \quad (\text{Eq.12})$$

This method assumes that PK profiles are similar between species and graphs of C' versus t' can be superimposed. The species-specific curves were converted to normalized concentration and time of form Eq. 12 using values for C_{ss} and MRT calculated as:

$$C_{ss} = \frac{Dose}{V_{ss}} \quad (\text{Eq.13})$$

$$MRT = \frac{V_{ss}}{CL} \quad (\text{Eq.14})$$

Where CL is the value from two compartment fitting and V_{ss} is described as in Eq. 3. Conversion of normalized curves in animals to predictions of human PK profiles were generated by correcting C' and t' for the human values for C_{ss} and MRT . Accuracy of the predicted curves was assessed visually.

'Informed scaling' of next generation predictions

Ideally, scaling next generation therapeutics should incorporate prior knowledge of the interspecies relationships of the unmodified products. We have developed an 'informed scaling' approach where the allometric exponent for a modified product, such as a PEGylated protein, is fixed to the same value observed for the unmodified protein. This results in simple relationships between the parameter (P) for an unmodified reference protein (ref) and a modified test protein (test) where each are governed by independent allometric equations sharing a common exponent:

$$P^{ref} = A \cdot W^b \quad (\text{Eq.15})$$

$$P^{test} = A' \cdot W^b \quad (\text{Eq.16})$$

For any given body weight (W), Eqs. 15 and 16 we can be rearranged as:

$$\frac{P^{test}}{P^{ref}} = \frac{A'}{A} \quad (\text{Eq.17})$$

The ratio of the test to reference parameters remains constant and independent of body weight. Thus when comparing parameters between animals and humans:

$$\frac{P_{animal}^{test}}{P_{animal}^{ref}} = \frac{P_{human}^{test}}{P_{human}^{ref}} \quad (\text{Eq.18})$$

and solving for P_{human}^{test} :

$$P_{human}^{test} = \left(\frac{P_{animal}^{test}}{P_{animal}^{ref}} \right) \cdot P_{human}^{ref} \quad (\text{Eq.19})$$

P_{human}^{ref} may come from direct observations in humans or it may be described allometrically to allow for prediction of the parameter for any species of a defined body weight:

$$P_{human}^{ref} = A \cdot W^b \quad (\text{Eq.20})$$

$$P_{human}^{test} = \left(\frac{P_{animal}^{test}}{P_{animal}^{ref}} \right) \cdot A \cdot W^b \quad (\text{Eq.21})$$

Thus in this ‘informed scaling’ approach, the parameter for a test product in humans may be described as a function of the observed parameter for the test and reference products in a preclinical species and the known value for the reference product in humans. Using parameters from different animal species will generate human estimates independently, but provided the assumption of a common exponent is valid, they will converge to a single value in humans. Combination of PK parameters, such as CL and Vss, predicted by equations 19 or 21 with normalized Wajima curves from preclinical studies can be used to simulate expected concentration-time profiles informed by the known PK of the reference product.

Case study in ‘informed scaling’ with an Fc fusion FVIII

Our ‘informed scaling’ method was applied to predict the human PK of an Fc fusion and reference FVIII product as a case study. Concentration-time profiles and the corresponding NCA parameters were available for studies of Fc FVIII and a reference FVIII product in HA mice, normal mice, HA dogs, and HA humans.^{23,24} Predictions of human profiles for Fc FVIII were generated in four steps:

1. Validation of predictions for reference FVIII:

The allometric relationships for NCA derived CL and Vss of FVIII were used to predict typical human values for a 70 kg individual. MRT and C_{ss} for doses of 25 and 65 IU/kg were calculated as per equations 13 and 14. The normalized Wajima curve for FVIII in humans developed from the literature meta-analysis was used to simulated profiles in which $t = \text{MRT} \cdot t'$ and $C = C_{ss} \cdot C'$ for each dose. Profiles were visually compared to those observed for the reference FVIII product in the respective clinical study.¹⁴

2. Construction of a normalized Wajima curve for Fc FVIII:

Observed concentrations following administration of Fc FVIII to HA mice, normal mice, and HA dogs were normalized to C' and t' by the published C_{ss} and MRT values for each curve.¹⁵ Points from all species were plotted together to evaluate superposition and fit by a biphasic regression line (Eq. 5) using WinNonlin.

3. Prediction of human CL and Vss for Fc FVIII:

The ‘informed scaling’ approach was used to predict human CL and Vss for Fc FVIII as in (Eq. 21). Allometrically derived values of CL and Vss for reference FVIII in a 70 kg human were used as above and corrected for observed parameters for reference and Fc FVIII in mice (average values for normal and HA) or in HA dogs.

4. Simulation of human concentration-time profiles for Fc FVIII:

Human CL and Vss values predicted from mice and HA dogs were used to calculate MRT and C_{ss} for 25 and 65 IU/kg doses of Fc FVIII. The normalized Wajima curve for Fc FVIII was corrected for these values in which $t = \text{MRT} \cdot t'$ and $C = C_{ss} \cdot C'$ for each dose. The profiles were visually compared to the observed concentrations for Fc FVIII collected in a clinical trial.¹⁴

RESULTS

Compiled dataset

The complete set of collected data is summarized in Table 1. A total of 60 publications were included representing mice, dogs, and humans with HA as well as normal mice, rats, rabbits, and monkeys.^{7–11,14–16,25–76} A summary of the NCA derived PK parameters CL, Vss, MRT, and half-life ($t_{1/2}$) are presented in Table 2. Doses in humans were heavily weighted towards the median of 50 IU/kg, with only three studies using doses in excess of 55 IU/kg. Clinically, FVIII is typically provided in lyophilized vials that may contain anywhere from 250 to 4000 IU (Advate.com). As a result patients are frequently dosed to the nearest whole vial resulting in some measure of inter-individual variance in the actual dose received. Where reported, the mean CV% for the administered dose was 10%. In general, doses of FVIII studied in normal models tended to be larger than those utilized in hemophilia models as necessitated to overcome interference from endogenous FVIII.

The inconsistent use of the one- or two-stage assays for the quantification of FVIII has been the source of analytical complication.⁷⁷ Even within the same assay, use of recombinant or plasma standards can lead to different interpretations of data.⁵⁴ Since the early 90s the two-stage chromogenic assay has steadily increased in popularity and of the included studies conducted since 2000, analysis via APTT and chromogenic assays were nearly evenly split. The chromogenic assay appears to be strongly preferred in studies involving mice or BDD FVIII where it accounted for 80 and 89% of the analyses respectively.

Allometric Scaling

Interspecies scaling across all collected data revealed CL and Vss to be highly correlated with body weight (Fig. 1). None of the segregation categories resulted in significant changes to the regression and thus all available data were pooled. Observed allometric exponents for CL and Vss of 0.85 and 0.97 show good agreement with the traditional expected values of 0.75 and 1.0. MRT and $t_{1/2}$ showed lower correlation as might be expected from the hybrid nature of these parameters; more advanced modeling and simulation methods should be used when trying to predict such values. Previous allometric scaling efforts for FVIII have not included more than three studies, and although the source of the middle species has varied among rats, mild HA dogs and severe HA dogs, the same mouse and human data have been reported.^{4,17,78} Those prior studies found the allometric exponents of 0.6–0.7 for clearance and approximately 0.85 for steady state volume. The results presented here do not represent a radical departure from previous relationships, but the comparative size of the data set used in their generation should serve to increase confidence in their accuracy.

Compartmental modeling of Factor VIII

The compiled results of fitted and published two compartment parameters for FVIII are summarized by species in Table 3. Strong linear correlation was observed for the allometric relationship of CL and V1 (Fig. 2). The allometry of CL was similar whether determined by NCA or compartmental fitting. V1 scaled with an exponent of 0.99 and closely mimicked the expected plasma volume. Greater variability was observed for CLd and V2. FVIII is a very large protein of ~280 kDa that shows little tissue distribution and is confined largely to the plasma, but rapid clearance of high molecular weight species by the reticuloendothelial system (RES) can lead to an apparent biphasic PK profile.⁷⁹ The presence or absence of this distinct early alpha phase can vary between products, but between individual patients as well.⁵³ The lack of body weight dependence for distributional clearance in a large scale population model of a single FVIII product suggests a large portion of the distribution may be independent of allometrically defined processes.²¹

Dedrick plots

Representative concentration-time profiles for a 50 IU/kg dose across species show a range of profiles (Fig. 3a). The alpha phase is readily pronounced in some species like monkey, but more subdued in others such as rabbit. The resulting terminal half-life also varies from around 14 h in humans down to 5 h for mice. Attempts to predict the human profile made from each of the species based on the Dedrick plot method result in considerable inter-species variance in the predictions (Fig. 3b). Preclinical species selection would in turn have a significant impact on the end prediction with no clear strategy to reconcile the differences. The poor predictive power of this method for FVIII, combined with the inability to incorporate any of the information for the established allometric relationships, make this approach less than ideal for scaling up next generation therapeutics.

Wajima curves

The method of Wajima is a robust technique for simulating concentration-time profiles from a reference normalized curve, and predictions of CL and Vss.¹⁸ In the original publication this was used to predict human PK for several antibiotics from observed profiles in preclinical animal models and human CL and Vss calculated from a mixed allometric scaling/QSPKR approach, but values of CL and Vss can come from any method. Here we adapt the technique to show that it can be used to successfully predict known PK profiles of FVIII and extend it to provide predictions for next generation protein therapeutics. The foundation of the Wajima method relies upon the ability of PK curves from multiple species to superimpose when converted to normalized concentration (C') and time (t') after division by observed C_{ss} and MRT. When normalized Wajima curves were generated from the average parameters in humans, dog, rabbit, monkey, and mouse, the resulting profiles were superimposable (Fig. 3c). This high degree of overlap and the ability to generate profiles based on predictions of MRT and C_{ss} (derived from CL and Vss) make Wajima curves a suitable method for adaption to scaling up next generation therapeutics.

'Informed scaling' case study

To demonstrate applicability of the 'informed scaling' approach, we applied the method to predict the human PK of FVIII and an Fc FVIII product from preclinical animal studies and validated the predictions against published clinical data. The standard allometric relationships describing FVIII (Fig. 1) were used to calculate an expected CL and Vss of 4010 mL and 244 mL/h for a 70 kg human. These correspond to an MRT of 16.4 h and C_{ss} of 0.44 and 1.14 IU/mL for doses of 25 and 65 IU/kg. C' and t' of the normalized Wajima curve from Fig. 3c were multiplied by these estimates of C_{ss} and MRT to simulate human concentration-time profiles of FVIII. Concentrations of rFVIII in the patient population observed by Powell and colleagues following intravenous administration of 25 and 65 IU/kg doses showed close agreement with the predicted profiles (Fig. 4a).²⁴ There was some systematic under prediction, particularly at later time points, but these concentrations approach the lower therapeutic limit for FVIII (0.01 IU/mL) and may be of limited clinical relevance.^{22,80}

Collected concentration-time data for Fc FVIII dosed to HA mice, normal mice, and HA dogs were converted to C' and t' following division by the published C_{ss} and MRT values for each respective curve. These normalized values were plotted together and fit by a biphasic regression line (Fig. 4b). Normalized observations from the different animal models were superimposable, indicating that the Wajima method is suitable for describing the observed interspecies relationship. Macro-constants describing the curve were estimated with good precision: $A=0.794$ (14.2%), $B=0.537$ (10.5%), $\alpha=3.69$ (22.1%), $\beta=0.750$ (4.4%) (CV%). It is interesting to note the close agreement between the normalized curves for

rFVIII and Fc FVIII, though this approach makes no explicit assumptions about their uniformity.

Predicted human values specific for the CL and Vss of Fc FVIII were generated using an 'informed scaling' method. Allometric predictions of CL and Vss for rFVIII were corrected for observations of rFVIII and Fc FVIII in mice (average of normal and HA) and HA dogs as expressed by (Eq. 22). Predictions from both mice and HA dogs generated identical CL and Vss values, supporting the assumption of common scaling exponents between the normal and Fc fusion form (Table 4). Estimates of human CL and Vss were within 34% and 27% of the clinically observed geometric mean for the 25 IU/kg dose or 10% and 4% for the 65 IU/kg dose.¹⁴ The 25 IU/kg dose also demonstrated CL and Vss values lower than the allometric predictions for rFVIII, thus the larger deviations in the predictions may be a function of the individuals in this limited sample (n=6). The 'informed scaling' parameter estimates of CL and Vss for Fc FVIII correspond to a MRT of 28.4 h and C_{ss} values of 0.32 and 0.84 IU/mL for the 25 and 65 IU/kg doses.

The normalized Wajima curve for Fc FVIII was corrected for predicted C_{ss} and MRT values to simulate expected profiles for the two administered doses in humans and compared with the clinical data (Fig. 4c). Overall, systematic under prediction is observed for both dose levels, but within the realm of *de novo* simulations. The profiles can be further improved by substituting the values of CL and Vss for rFVIII observed in the patient group in place of the allometric values (Eq. 19), but such information would be unavailable *a priori*.

DISCUSSION

Therapeutic proteins represent an ever expanding segment of the pharmaceutical spectrum. As unique as these macromolecules are from small molecule drugs in many respects, they share some of the same issues in others. Historical concerns about dissolution rates and metabolic profiles have been replaced by complications of aggregation and target mediated disposition. With many of the patents on the first generation biotherapeutics expiring, there is a mounting push for so called next generation protein drugs. Through PEGylation, hyperglycosylation, advanced delivery systems, or other modification strategies the goal is to improve some aspect of the therapy (for review of delivery approaches see⁸¹). Often that desired endpoint is improvement of PK profiles and early, accurate predictions of human disposition are critical for guiding strategic development of candidates.

FVIII has been extensively studied for decades. Yet despite the wealth of information available, allometric scaling in the literature has been revisited sparingly since the initial effort by Mordenti and colleagues.^{4,16,17} Of particular concern is whether the disposition of FVIII at 8-times the therapeutic human dose in a normal mouse was an appropriate preclinical model. Beyond predictions for humans, robust allometric relationships could also aid the design of PK studies in newly developed rat and sheep hemophilia models.^{82,83} The literature meta-analysis produced over 100 PK studies in a wide range of species and is by far the largest single collection of published FVIII PK data. Allometric scaling of these studies showed a strong linear relationship for CL and Vss. Segregation of the dataset for comparison of disease state, assay type, or FVIII product did not significantly impact the calculated allometric parameters. Similar scaling success was observed for the subset of data characterized by a two compartment model, although the distributional component proved largely variable owing to the unconventional nature of the FVIII alpha phase.⁷⁹ Simulations of human PK profiles from allometrically scaled two compartment parameters were highly sensitive to changes in the more variable CL and V₂ terms (data not shown), but the Wajima method provided better superposition of PK profiles from all species.¹⁸

The traditional principles of allometry date back nearly a century.⁸⁴ The simple power law equation can be used to relate any number of physiological processes to size. It is this universality that makes the technique applicable to scaling the PK parameters of a wide variety of compounds.² The utility of allometric scaling for proteins has been shown many times,^{3,4,6,17,85} but traditional methods would treat the scaling of a next generation therapeutic independently from any established relationship for the unmodified product. Although the two are distinct entities, knowing the PK behavior for the existing biological agent can address a large extent of the uncertainty surrounding first-in-human clinical trials for the new agent. In most circumstances, modifications such as PEGylation are used to interfere with clearance by reducing affinity to a clearance receptor, but similar mechanisms may be responsible for clearing both the PEGylated and unmodified products. Assuming that PK parameters for both of these products can be related to body weight by a common allometric exponent is an extension of that basic principle and considering the alternatives of choosing a 'typical' value or extrapolating a regression line from a limited sample of preclinical models, 'informed scaling' may be one of the more conservative approaches. A caveat to this approach is when PEGylation or similar covalent modification is employed to prevent glomerular filtration for some small renally cleared peptides, significantly altering their disposition.⁸⁶ In such cases, the de novo assumption of a common exponent between products may not be appropriate.

As a consequence of assuming a common exponent, the ratio of parameters for an unmodified and modified product is expected to be constant, regardless of whether or not the respective allometric relationships have been defined. The advantage of using allometrically defined parameters in humans (Eq. 21) over an observed value (Eq. 19) is that the body weight for any intermediate species may be substituted. Thus, this technique can be used not only to predict human parameters, but also to plan a preclinical study in subsequent animal models—such as designing a PK study in an intermediate species after completing a study in rodents. If the end goal is only prediction of human PK, use of observed human parameters for the unmodified reference or those derived from population models for a specific patient subset would prove equally valid.²¹ Our 'informed scaling' approach also remedies the problems of under- or over-prediction of parameters in individual species that can complicate traditional allometric scaling—the ratio of the parameters between the two products becomes more important than their absolute values. For an Fc fusion FVIII, fixing the exponent to the value for unmodified FVIII was validated when the same estimates of the human parameters were generated from both mice and dogs.

Clearance of protein therapeutics has been shown to scale with exponents between 0.5–1.3,^{5,17} but monoclonal antibodies and Fc fusion proteins have scaled closer to 0.85 and 0.8.^{5,87} Although a common exponent between unmodified and modified proteins seems justifiable, it is possible that the success of our case study results from rFVIII and Fc FVIII coincidentally scaling to a similar, but physiologically unrelated exponent. Although many of the next generation products are currently in clinical trials and available data are limited, erythropoietin (EPO) and darbapoietin, a hyperglycosylated EPO, lend at least one more example in support of our approach. Darbapoietin showed about a 70% reduction in clearance compared to EPO in all of rats, dogs, and humans.^{88,89} Fitting of an allometric equation demonstrated an exponent of 0.84 for darbapoietin, analogous with that of EPO at 0.86. This limited sample is not to say the common exponent will unequivocally be the case with all modified proteins, but there is precedent to expect it. Fortunately, routine PK studies in two or three preclinical animals should be sufficient to discern whether the 'informed scaling' approach is appropriate for a therapeutic agent as reflected by a constant ratio of the PK parameters for the modified and unmodified products among species. If the observed ratio is inconsistent, then alternate methods interspecies scaling should be pursued.

CONCLUSION

Strong allometric relationships were found for the CL and Vss of FVIII. By assuming a common allometric exponent between unmodified and next generation protein products, an 'informed scaling' approach was developed for predicting the PK of improved therapeutic agents that may be generally applicable for biologics. Where the normalization of concentrations to C_{ss} and time to MRT superimposes curves between species, the Wajima method is suitable for extending these scaled parameters to simulated concentration-time profiles. This appears to represent a useful strategy in the prediction of human PK informed by prior knowledge for a reference product.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

FVIII	Factor VIII
PK	pharmacokinetics
PBPK	physiologically based PK
NCA	noncompartmental analysis
APTT	activated partial thromboplastin time
BDD	b-domain deleted
CL	clearance
V1	central volume
CL_d	distributional clearance
V2	peripheral volume
i.v	intravenous
C_{ss}	steady state concentration
MRT	mean residence time
RES	reticuloendothelial system
EPO	erythropoietin

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Figure 1. Allometric scaling of NCA derived PK parameters for FVIII

Linear regression was performed to fit an allometric equation to clearance (CL), steady state volume (Vss), mean residence time (MRT), and half-life ($t_{1/2}$). Each dot represents a separate PK study and studies with multiple doses or formulation are reflected as multiple dots. Solid black lines represent the lines of allometry and dashed red lines are the 95% prediction intervals. Square of the correlation coefficients and final scaling equations are shown.

Figure 2. Allometric scaling of two compartment PK parameters for FVIII

Linear regression was performed to fit an allometric regression line to clearance (CL), central volume (V1), distributional clearance (CLd), and peripheral volume (V2). Each dot represents a separate PK study and studies with multiple doses or formulations are reflected as multiple dots. Solid black lines represent the lines of allometry and dashed red lines are the 95% prediction intervals. Square of the correlation coefficients and final scaling equations are shown.

Figure 3. Relating PK profiles of FVIII across species

(3a) Representative profiles for the PK of FVIII in different species normalized to a 50 IU/kg dose. Curves are shown for HA human (black), HA dog (red), normal rabbit (green), normal monkey (gold), and HA mouse (blue) profiles. (3b) Predictions of a 50 IU/kg dose of FVIII in humans based on the Dedrick plot method and segregated by predictive species. The solid line represents the expected HA human (black) profile as compared to predictions from HA dog (red), normal rabbit (green), normal monkey (gold), and HA mouse (blue). (3c) Normalized Wajima curves for FVIII across species. Mean parameters in each species were used to simulate concentration-time curves that were converted to normalized time (t') and normalized concentrations (C') based on the observed C_{ss} and MRT in each species. Normalized curves are shown in HA human (black), HA dog (red), normal rabbit (green), normal monkey (yellow), and HA mouse (blue).

Figure 4. Simulated PK profiles for FVIII and Fc FVIII

(4a) Observed concentrations following 25 (closed circles) and 65 (open circles) IU/kg doses of FVIII in humans in the study by Powell et al.²⁴ The Wajima method was used to generate predictions for the 25 (solid line) and 65 (dashed line) IU/kg doses using CL and Vss values predicted from the allometric relationships for a 70 kg individual. (4b) Observed concentrations for Fc FVIII given to HA mice (closed circles), normal mice (open circles), and HA dogs (closed triangles) following a 125 IU/kg dose.²⁴ Values are expressed as normalized time (t') and normalized concentration (C') based on the MRT and C_{ss} values observed for each treatment. The fitted regression line for Fc FVIII is shown (solid line) with the normalized Wajima curve for rFVIII (dotted line) as reference. (4c) Observed concentrations following 25 (closed circles) and 65 (open circles) IU/kg doses of Fc FVIII in humans.²⁴ The Wajima method was used to generate predictions for the 25 (solid line) and 65 (dashed line) IU/kg doses using allometrically derived CL and Vss values for a 70 kg individual and corrected for preclinical observations with the 'informed scaling' approach.

Table 1

Summary of FVIII PK studies.

	Hemophilia Models	Normal Models
Year range	1985–2012	1996–2012
Number of infusion sets	Total = 107 Published = 101 Unpublished = 6	Total = 7 Published = 7 Unpublished = 0
Number of publications	58	5
FVIII formulation	BDD rFVIII = 23 Cryoprecipitate = 2 Full length rFVIII = 39 Plasma derived = 40 Mixture = 3	BDD rFVIII = 2 Full length rFVIII = 5
Assay type	APTT = 55 Chromogenic = 35 Both = 5 Unspecified = 12	APTT = 0 Chromogenic = 5 ELISA = 2
Species	Human = 80 Dog = 8 Mouse = 19	Monkey = 2 Rabbit = 1 Rat = 1 Mice = 3
Dose range IU/kg (median)	Human: [15,100] (50) Dog: [50,500] (125) Mouse: [10,400] (125)	Monkey: [100,317] (n=2) Rabbit: 100 Rat: 400 Mice: [125,400] (222)

Table 2

Summary of FVIII NCA parameters.

Species	Disease Condition	N=	Dose (IU/kg)	BW (kg)	CL (mL/h)	V _{ss} (mL)	MRT (h)	Half-life (h)
Human	HA	80	44.9 (12.9)	68.1 (12.5)	231 (63)	3950 (1040)	18.1 (4.5)	13.0 (2.0)
Mouse	HA	19	152 (119)	0.0225	0.312 (0.306)	1.65 (0.65)	7.3 (3.1)	4.7 (2.1)
Dog	HA	8	212 (178)	17.3 (0.8)	101 (33)	1220 (360)	12.1 (2.0)	9.7 (2.3)
Mouse	Normal	3	249 (139)	0.0229 (0.0005)	0.377 (0.254)	2.13 (1.23)	6.2 (1.3)	4.6 (0.8)
Rat	Normal	1	400.	0.253	4.10	31.6	7.7	5.5
Monkey	Normal	2	209	4.00	26.7	298	12.2	9.0
Rabbit	Normal	1	100.	4.50	38.3	336	8.8	6.7

Data presented as mean (standard deviation)

Table 3

Summary of FVIII two compartment parameters.

Species	Disease Condition	N=	Dose (IU/kg)	BW (kg)	CL (mL/h)	V1 (mL)	CLd (mL/h)	V2 (mL)
Human	HA	35	47.8 (10.6)	70.2 (11.5)	218 (44)	3160 (590)	178 (111)	1070 (540)
Mouse	HA	3	127 (133)	0.0225	0.274 (0.029)	1.30 (0.72)	0.565 (0.373)	0.777 (0.222)
Dog	HA	4	111 (13)	17.6 (1.1)	96.7 (28.9)	871 (245)	47.4 (43.1)	202 (63)
Monkey	Normal	2	209	4.0	25.0	159	45.0	132
Rabbit	Normal	1	100	4.5	38.4	270	18.0	76.5

Data presented as mean (standard deviation)

Table 4

Predicted PK parameters for Fc FVIII in humans.

Species	Dose (IU/kg)	rFVIII Observed		Fc FVIII Observed		Fc FVIII Predicted for Humans	
		V _{ss} (mL)	CL (mL/h)	V _{ss} (mL)	CL (mL/h)	V _{ss} (mL)	CL (mL/h)
Mice*	125	1.13	0.155	1.53	0.088	5440	191
HA Dogs	117/125**	1090	111	1470	86.7	5410	191
HA Humans	25	3830	223	4060	150	--	--
HA Humans	65	4520	284	4940	183	--	--

* Average of normal and HA

** FVIII dose 117 IU/kg for rFVIII, 125 IU/kg for Fc FVIII