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Nonlinear pharmacokinetics of Factor VIII and its phosphatidylinositol lipidic complex in hemophilia A mice

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Abstract

Factor VIII (FVIII) is an important cofactor in the blood coagulation cascade and its deficiency or dysfunction causes Hemophilia A (HA), a bleeding disorder. Replacement with recombinant FVIII is limited by a short half-life and the development of inhibitory antibodies. We have developed a phosphatidylinositol (PI) containing lipid nanoparticle that, when associated with FVIII, reduces immunogenicity and prolongs circulation of the therapeutic protein in HA mice. Here, we conducted a multiple dose level pharmacokinetic (PK) study of human free FVIII and its FVIII-PI complex over a clinically relevant range of doses (20, 40, and 200 IU/kg) in HA mice to investigate linearity of the PK and to determine if reduced catabolism of FVIII following association with PI particles, previously only observed in the terminal phase following 400 IU/kg, could be extendable over a range of doses. Our findings suggest that the disposition of FVIII is best characterized by a two-compartment model with saturable Michaelis-Menten elimination. Spontaneous complexation of FVIII with PI particles significantly increases plasma survival of the protein at 20 and 40 IU/kg doses. Human simulations at 40 IU/kg project an increase in terminal half-life and time to reach a minimum therapeutic threshold of 0.01 IU/mL of 5.4 h and 40 h respectively compared to free FVIII. Formulation with PI containing lipid particles may represent a viable delivery strategy for improving FVIII therapy.

INTRODUCTION

Hemophilia A (HA) is a bleeding disorder caused by the deficiency or dysfunction of Factor VIII (FVIII), a blood clotting protein. Replacement with recombinant or plasma-derived FVIII is the first line of therapy for Hemophilia A, but a half-life of only 10–14 h in humans necessitates prophylactic infusions up to 3–4 times a week in patients with severe HA [1, 2]. Aided in part by frequent administration, FVIII is highly immunogenic with up to 30% of patients developing an inhibitory antibody response against the protein [3]. A phospholipid FVIII preparation with a prolonged half-life would reduce the required frequency of administration, improving both quality of life and patient compliance, as well as potentially reducing the incidence of immunogenic reactions [1, 4–6].

Our lab developed a phosphatidylinositol (PI) containing lipidic particle to improve the delivery of FVIII. Preclinical studies of FVIII associated with PI particles (FVIII-PI) were conducted in a murine HA model based on a C57BL/6J mouse with targeted deletions in exon 16 FVIII gene [7]. Compared to free protein, FVIII-PI reduced development of

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CONFLICT OF INTEREST

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inhibitory antibodies after both SC and IV administration through a combination of passive protein shielding and active modulation of regulatory cytokines[8, 9]. HA mice are also widely used to study the preclinical pharmacokinetics (PK) of FVIII[10–16]. FVIII-PI prolonged plasma survival compared to the free protein in these mice after a 400 IU/kg dose, but extension of the terminal half-life only became evident after plasma concentrations had fallen below approximately 1 IU/mL[8]. Similar trends were observed in both normal and HA mice for an Fc-fusion FVIII; plasma concentrations overlapped at early time points between the Fc- and unmodified-FVIII products, but improvements in circulating half-life were evident at concentrations under 1 IU/mL. These trends may be indicative of a previously unreported nonlinear clearance pathway for FVIII in mice and raise important questions as to whether the PK benefits previously observed for our PI formulation is capable of providing similar benefits over a range of doses and if this murine model can be used to accurately predict the PK of FVIII products in humans.

Nonlinear PK is commonly associated with a dose-dependent saturation of absorption, distribution, metabolism, or excretion producing concentration-time profiles that lack dose proportionality. Saturation of disposition mechanisms can both prolong the circulation of a drug, such as saturation of clearance, or accelerate its removal, as when carrier binding is saturated[17]. FVIII is typically administered clinically as a short intravenous infusion in the dose range of 25–50 IU/kg yielding a C_{max} approaching normal endogenous concentrations[1, 18]. However, many preclinical studies of FVIII in mice have been conducted at significantly higher doses, up to 400 IU/kg[8, 19]. High concentrations following such supra-therapeutic doses may be sufficient to saturate elimination pathways and reduce the apparent rate of clearance. Concavity observed in the terminal elimination phase of FVIII in HA mice has suggested a possible Michaelis-Menten (MM) type nonlinear elimination[8, 16]. Studies of drug products at large, saturating concentrations may not be reflective of the expected human PK where therapeutic doses are effectively linear. Non-compartmental analysis (NCA) is routinely applied for FVIII data analysis[4], but many of the methods used to calculate PK parameters are not appropriate under nonlinear conditions[20]. Sparse sampling and application of monoexponential PK models can also fail to accurately capture the concentration-time profiles[12, 21]. Thus, it is important when studying FVIII to characterize the behavior within the clinically relevant concentration range, sample intensely enough to thoroughly characterize the PK profile, administer at least three dose levels to discern the presence of nonlinearity, and apply modeling methods appropriate to the system.

It was hypothesized that the PK of FVIII is nonlinear resulting from a saturable elimination process and a multiple dose level PK study (20, 40, and 200 IU/kg) of FVIII in HA mice with robust sample collection was conducted. The PK of FVIII-PI was investigated at the same dose levels to determine if the lipid based formulation could prolong the plasma survival of FVIII over a range of clinically relevant doses in an HA mouse model. Modeling and simulation were used to characterize the observed PK in mice and predict human disposition of the lipid associated FVIII product.

MATERIALS AND METHODS

Materials

Albumin-free recombinant full-length FVIII obtained from Baxter International Inc. (Deerfield, IL) was used for the studies. Dimyristoylphosphatidylcholine (DMPC) and soybean Phosphatidylinositol (PI) were purchased from Avanti Polar Lipids (Alabaster, AL). Cholesterol was purchased either from Avanti Polar Lipids or from Sigma-Aldrich (St Louis, MO). Coamatic FVIII kits were purchased from DiaPharma Group (West Chester,

OH) and used to determine FVIII activity in plasma samples. Buffer salts were obtained from Sigma-Aldrich (St Louis, MO).

Preparation of FVIII-PI complex

PI lipid vesicles were prepared as previously described[8]. Briefly, a thin lipid film consisting of DMPC:PI:cholesterol (50:50:5 molar ratio) was dried and rehydrated with tris buffer (25 mM tris, 150 mM NaCl, pH=7.0). Particles were extruded through two polycarbonate membranes of 80 nm pore size and sterile-filtered. Phospholipid concentration was determined by inorganic phosphorous assay[22]. Expected particle size of ~100–120 nm was confirmed with a Nicomp Model CW 380 particle size analyzer (Particle Sizing Systems, Santa Barbara, CA) and FVIII incubated with vesicles for 30 minutes at 37°C in a molar ratio of 1:10,000 for all experiments. All preparations were prepared using pyrogen free water and sterile filtered prior to use.

Animals

A colony was established from breeding pairs of HA mice (C57BL/6J) with a targeted deletion in exon 16 of the FVIII gene provided by Dr. Kazazian and Dr. Sarkar from University of Pennsylvania, PA[7]. Male HA mice (20–25 g) 8–12 weeks old were used for PK studies. All animal handling was performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University at Buffalo, SUNY.

Pharmacokinetic studies of FVIII and FVIII-PI

Animals received either free FVIII or FVIII-PI complex at doses of 20, 40, or 200 IU/kg as a single IV bolus injection via the penile vein (n=3 mice/time point). Injections were prepared by dilution in tris buffer with (free FVIII) or without (FVIII-PI) 5 mM CaCl₂. Blood samples were collected by cardiac puncture into a 7:1 (v/v) ratio of acid citrate dextrose (ACD: 85 mM sodium citrate, 110 mM D-glucose, 71 mM citric acid) at 0.08, 0.5, 1, 2, 4, 8, 16, 24, 30, 36, 42 and 48 hr post injection or until FVIII was no longer detected, and rapidly cooled. Plasma samples were separated by centrifugation at 5000x g for 5 minutes and stored at –80°C until analysis. Results of the 200 IU/kg dose of free FVIII were previously reported elsewhere [23].

Factor VIII Activity and Modeling

Activity of FVIII in plasma samples was determined by a two-stage chromogenic assay according to the manufacturer's instructions. Concentration-time profiles were compared between free FVIII and FVIII-PI at each dose level by two-way ANOVA. Non-compartmental analysis (NCA) was utilized to compute basic pharmacokinetic parameters including total clearance (CL), half-life (t_{1/2}), and area under the curve (AUC). NCA was conducted using Phoenix WinNonlin v6.3 (Pharsight, Sunnyvale, CA). As each point represents a separate animal, systemic exposures were compared using the Bailer-Satterwaite method by enabling the sparse sampling option in WinNonlin[24]. The ratio of AUC_t for free FVIII and FVIII-PI for each dose was calculated as:

$$\% \text{ Change in } AUC_t = \frac{AUC_{t, \text{FVIII-PI}} - AUC_{t, \text{free FVIII}}}{AUC_{t, \text{free FVIII}}} \cdot 100\% \quad \text{Eq. (1)}$$

Compartmental modeling was conducted using Adapt 5 (BMSR, University of Southern California, CA) with the maximum likelihood estimator and linear variance model. Various structural models were evaluated to describe the disposition of free FVIII and FVIII-PI in

HA mice. Models included one- and two-compartment models with either MM elimination, linear elimination, or a combination thereof. Data for free FVIII and FVIII-PI at all doses were modeled simultaneously. Iterative model fitting was used to identify the smallest set of unique parameters required to capture the changes in disposition caused by association with PI. Model selection and evaluation of the goodness of fit were guided by precision of the parameter estimates, objective measures such as Akaike information criterion, F-test, and subjective measures including visual predictive checks.

The final model was a two-compartmental model with MM elimination defined by the following differential equations:

$$\frac{dC_1}{dt} = -\frac{CL_{obs}}{V_1} \cdot C_1 + \frac{CL_d}{V_1} (C_2 - C_1); C(0) = \frac{Dose}{V_1} \quad \text{Eq. (2)}$$

$$\frac{dC_2}{dt} = \frac{CL_d}{V_2} (C_1 - C_2); C_t(0) = 0 \quad \text{Eq. (3)}$$

$$CL_{obs} = \frac{v_{max}}{k_m + C_1} \quad \text{Eq. (4)}$$

C_1 and C_2 represent the concentration of FVIII in the central and peripheral compartments, and V_1 and V_2 represent the apparent volumes of distribution for the central and peripheral compartments. Distributional clearance is defined by CL_d . Observed clearance (CL_{obs}) is a function of C_1 , maximum elimination rate (v_{max}), and the MM constant (k_m). Beginning with a hypothesis of no difference between profiles of free FVIII and FVIII-PI, individual parameters were replaced with unique estimates for each formulation and significance of the improved fitting was evaluated with the F-test.

Scaling PK of FVIII and FVIII-PI

Simulations were conducted to predict the behavior of a 40 IU/kg dose of FVIII and FVIII-PI in humans using an ‘informed scaling’ approach that combines allometric based scaling of PK parameters with the use of normalized Wajima curves to simulate concentration-time profiles [25, 26]. Parameters for V_{ss} and CL in mice were calculated from the fitted model as $V_{ss} = V_1 + V_2$ and $CL = Dose/AUC$, the appropriate methods for a two-compartmental system with MM elimination [27]. This scaling method assumes that free FVIII and FVIII-PI share a common allometric exponent for CL and also for V_{ss} . Thus predicted parameter values in humans can be calculated according to the equation:

$$P_{human}^{FVIII-PI} = \left(\frac{P_{mice}^{FVIII-PI}}{P_{mice}^{Free FVIII}} \right) \cdot P_{human}^{Free FVIII} \quad \text{Eq. (5)}$$

where P is the parameter of interest for free FVIII or FVIII-PI in humans and mice [26]. V_{ss} and CL in humans were mean values for all collected literature profiles described by a two-compartment model: $CL = 218 \text{ mL/h}$ and $V_{ss} = 4230 \text{ mL}$ for a body weight of 70.2 kg [26]. At lower doses the PK of FVIII in mice approaches a first-order linear system, thus MRT ($=V_{ss}/CL$) and C_{ss} ($=Dose/V_{ss}$) values were calculated for 40 IU/kg doses from observed values of CL and V_{ss} (free FVIII mice, FVIII-PI mice, free FVIII humans) or from predicted values (FVIII-PI humans). Wajima curves for FVIII in mice were generated by normalization of time and concentrations as $t' = t/MRT$ and $C' = C/C_{ss}$ [25]. A simulated concentration-time profile for a 40 IU/kg dose of FVIII-PI in humans was generated by multiplying t' and C' of the normalized curves with the projected human values of MRT and

Css for FVIII-PI. The simulated profile was compared to a representative human profile for 40 IU/kg of free FVIII[26].

To evaluate the impact of nonlinearity in mice on the superposition of normalized Wajima curves across a range of doses, IV bolus profiles were simulated for 4, 10, 40, 100, 400, and 1000 IU/kg doses of FVIII-PI with the two compartment MM model. Simulated curves were normalized to the corresponding C_{ss} (=Dose/V_{ss}) and MRT (=V_{ss}/CL), and evaluated for superposition.

RESULTS

Concentration-time profiles of FVIII and FVIII-PI

Plasma activity profiles obtained in HA mice following IV injection of free FVIII were characterized by distinct alpha- and beta- phases (Fig. 1). Following the 200 IU/kg dose there was a period of slower elimination between 4–16 hours before reaching the first order elimination phase at 24 hours. FVIII is confined to the plasma volume due to its large size, but rapid removal of high molecular weight species can lead to a pronounced alpha-phase of apparent distribution[28].

Calculated NCA parameters are summarized in Table 1. Total clearance of free FVIII decreases with increasing dose, which together with pronounced concavity in the profile of 200 IU/kg, implies a saturable nonlinear elimination pathway for human FVIII in this mouse model. Formulation with PI particles was found to have a significant effect on the resulting profiles by two-way ANOVA at 20 IU/kg ($p<0.01$) and 40 IU/kg ($p<0.05$), but not 200 IU/kg ($p>0.05$) (Fig 1). Application of compartmental modeling was utilized to identify a single set of parameters capable of describing clearance across all doses and provide more insightful interpretation of the underlying processes.

Pharmacokinetic Modeling

Simultaneous model fitting was applied to both the free FVIII and FVIII-PI concentration-time data. The final model contained two-compartments and a MM nonlinear elimination mechanism. Model fitting was statistically improved by allowing formulation specific parameters for k_m ($p<0.001$), V_2 ($p<0.05$), and CL_d ($p<0.05$). Unique values of V_1 and V_{max} for each formulation did not statistically improve fitting ($p>0.05$). In the final model, separate values of k_m , CL_d, and V_2 were fit for free FVIII ($k_{m, free}$, CL_{d, free}, $V_{t, free}$) and FVIII-PI ($k_{m, pi}$, CL_{d, pi}, $V_{2, pi}$), but both formulations shared common estimates for V_1 and V_{max} . The model captured the range of data well (Fig. 1) and all parameters were estimated with good precision (Table 2).

To more directly compare estimates of the MM function for free FVIII and FVIII-PI, CL_{obs} was simulated as a function of concentration (Fig. 2). Clearance decreases with increasing concentration. Both free FVIII and FVIII-PI share the same V_{max} and the difference between clearance of free FVIII and FVIII-PI is relatively small at large concentrations where $C \gg k_m$ and $CL_{obs} \approx V_{max}/C$. However as $C \ll k_m$ then $CL_{obs} \approx V_{max}/k_m$ and benefits of association of FVIII with the PI particle are evident by up to a two-fold reduction of clearance.

Scaling PK of FVIII and FVIII-PI

Human simulations were performed with the clinically relevant dose of 40 IU/kg. Parameters for CL and V_{ss} were obtained from the fitted model for FVIII-PI in mice (CL=8.03 mL/h/kg, V_{ss} = 102 mL/kg) and the informed scaling method was used to predict parameters for FVIII-PI in humans (CL=1.61 mL/h/kg, V_{ss}=48.0 mL/kg). A normalized

Wajima curve was generated from the FVIII-PI 40 IU/kg concentration-time profile in mice and combined with projected human parameters to simulate the expected disposition of FVIII-PI in humans (Fig. 3). The PK of 40 IU/kg free FVIII in humans from a literature meta-analysis is shown for reference ($CL=3.11$ mL/h/kg, $V_{ss}=60.3$ mL/kg) [26]. FVIII-PI is projected to substantially improve plasma survival of FVIII, offering a 37% increase in half-life over the free protein (14.8 v 20.2 h) and extending the time to reach a 0.01 IU/mL threshold from 85 to 125 h. A prerequisite for the Wajima method is the superposition of PK profiles between doses and species in the normalized domains. To evaluate superposition of Wajima curves for escalating doses of FVIII-PI in our mouse model, PK simulations were conducted for doses of 4, 10, 40, 100, 400, and 1000 IU/kg (Fig. 4a). Inflection of the profiles is evident around the k_m of 1.72 IU/mL as expected. Profiles were normalized to the C' and t' domain by the respective C_{ss} and MRT values (Fig. 4b). Doses of up to 40 IU/kg are superimposable, but begin to diverge at 100 IU/kg with greater deviations at larger doses. The high degree of overlap at doses ≤ 40 IU/kg suggests that the Wajima method is still applicable for interspecies scaling of FVIII-PI profiles despite the underlying nonlinearity within this range.

DISCUSSION

The series of dose ranging studies presented here demonstrate that the PK of FVIII in mice is nonlinear. This phenomenon has not been previously reported in the literature as most previous studies have not utilized a suitable range of doses to properly assess linearity. Of five PK studies available in HA mice utilizing a dose of FVIII > 150 IU/kg, two displayed marked concavity in the terminal elimination profiles [8, 16], whereas the others were sampled too sparsely [12, 13] or for an insufficient length of time to properly characterize the curves [11]. Many development efforts are underway to engineer a FVIII product for improved therapy with extension of half-life as a primary goal, but failure to account for nonlinearity in the disposition of FVIII may lead to a misinterpretation of clinical prospects—our own FVIII-PI increased overall AUC_t by $<5\%$ over free FVIII in previous studies at 400 IU/kg [29], but demonstrated increasing improvements when the dose was reduced up to a 173% increase following 20 IU/kg. For similar reasons, association with PI particles in the present study failed to show a statistical effect at 200 IU/kg, but was significant at lower doses.

Non-compartmental analysis is routinely applied in the evaluation of FVIII PK [1]. Although a useful tool for analyzing PK without specification of a specific structural model, it assumes the underlying system is both linear and stationary. Under nonlinear conditions, calculated summary parameters are termed “apparent or total” as true values are condition-dependent and will change over a range of concentrations and/or times [20]. These NCA parameters are not without utility—systematic deviations in clearance or dose normalized AUC can be an important diagnostic in discerning nonlinearity and observations following low doses can provide useful initial estimates for more comprehensive modeling efforts—but appropriate compartmental models are important in understanding the nature of nonlinear behavior.

The typical interpretation of MM elimination is such that V_{max} denotes the capacity of the system and k_m reflects the affinity for a clearance mechanism. PI inhibits interaction of FVIII with elimination pathways thus reducing affinity ($\uparrow k_m$), but does not affect capacity. If concentrations are sufficiently large, observed clearance for free FVIII and FVIII-PI will converge to V_{max}/C . At 10 IU/mL, the initial concentration observed in our previous study of FVIII-PI in HA mice following a 400 IU/kg dose, the current model projects little less than 8% difference in CL_{obs} between free and lipid associated FVIII [8]. As a result, free FVIII and FVIII-PI profiles overlapped until 24 hours. A similar phenomenon is observed in the

current studies for 200 IU/kg where free FVIII and FVIII-PI overlap until 6 hours, but 20 and 40 IU/kg exhibited pronounced separation throughout the entire time course. Thus when studied at too large of a dose, the true impact of beneficial protein modification strategies may be underestimated and nonlinearity becomes an important consideration in experimental design.

The PK of FVIII in humans may exhibit either a monoexponential or biphasic disposition, which can vary widely between different individuals and FVIII products[2]. Although the alpha phase is not a true peripheral distribution and is believed to be the rapid clearance of high molecular weight species by the reticulo-endothelial system (RES), population approaches have modeled the disposition of FVIII in HA patients using a two-compartment model with linear elimination [30–32]. Dose-escalation studies of FVIII conducted in HA patients are sparse, but neither a B-domain deleted FVIII (BDD-FVIII) administered at 25, 50, and 100 IU/kg or a full length FVIII at 15, 30 and 50 IU/kg showed any obvious trend in NCA derived parameters that would suggest nonlinear elimination [33, 34].

Compared to the free protein, FVIII-PI demonstrated a reduction in the clearance (k_m) and distribution (V_2 , CLd) components of the system. PI shields a substantial portion of the protein structure within the particle or intercalated in the lipid-bilayer[8]. Mechanistically, the observed changes in k_m following association of with PI are consistent with reduced interaction of FVIII with low density lipoprotein receptor-related protein 1 (LRP1), the predominant clearance pathway of FVIII [35]. We hypothesize that reduction in the apparent distribution also results from PI particle-mediated shielding of the protein from rapid initial clearance, whereas the estimate of V_1 is close to the physiological plasma volume in mice (~50 mL/kg)[36] and unaffected by lipid association.

Over 95% of FVIII in plasma circulates as a high affinity complex (K_d ~0.32 nM) with the von Willebrand Factor (vWF) carrier protein [35, 37] which is essential for proper survival and function of the protein [38, 39]. Association with PI particles led to a more pronounced reduction in catabolism at lower doses and given the overlapping of binding sites in the C2 domain [8, 38], binding of FVIII to vWF and PI may be competitive. *In vitro* studies of FVIII-PI demonstrated a rapid release of ~60% of the FVIII cargo upon dilution in plasma [8] and an accelerated clearance of FVIII-PI in the absence of vWF would suggest that interactions between FVIII-PI and vWF are still important [23]. The K_d for binding between human vWF and FVIII is 0.32 nM, approximately 0.2 IU/mL for full length FVIII with a specific activity of 2.5 IU/ μ g [37]. FVIII is effective in preventing spontaneous bleeding above the therapeutic threshold of 0.01 IU/mL [40] thus between 0.2–0.01 IU/mL there is potentially significant disassociation from vWF where protection from binding to PI particles may prove advantageous.

Prediction of human PK for FVIII-PI from the nonlinear murine model is a unique situation. Allometric scaling of PK for linear to linear as well as nonlinear to nonlinear systems has been described, but to transition between the two presents a more difficult problem [41]. Further, while FVIII-PI is untested in humans the free protein has been well characterized across species. The ‘informed scaling’ approach estimates human parameters by incorporating both the relative changes caused by PI in mice and known behavior of the free protein in humans. Parameters obtained in this method may be utilized with the Wajima method to simulate concentration-time profiles [25, 26].

The original Wajima method assumed the system is linear; a concession necessary to ensure the superposition of normalized Wajima curves within a species [25]. However, administration of sufficiently low doses to a nonlinear system approaches a linear disposition and the Wajima method is still applicable in such cases. Thus it is only when the

nonlinearity is such that normalized curves for a given dose fail to overlap with the lower pseudo-linear doses within a species that the method is expected to become inaccurate. Extrapolation of simulated 40 IU/kg human profiles for free FVIII and FVIII-PI suggest that liposomal formulation may be capable of extending the time required to reach the 0.01 IU/mL threshold by 40 hours, thus potentially reducing the required dosing frequency in such therapeutic regimens. Given the observed nonlinearity in mice at doses greater than 40 IU/kg, these methods are insufficient to speculate how the FVIII-PI particle might behave at larger doses in humans.

The current manuscript demonstrates strong clinical prospects for FVIII-PI, but the unexplained failure of reduced clearance in HA mice for a PEGylated phosphatidylserine based liposome FVIII formulation to translate to humans does raise some concern about the clinical relevance of this protection [6, 12]. FVIII is sequestered within PI particles [8] and differs morphologically from those PEGylated liposomes in which FVIII was bound to the surface, but validation of similar PK improvements in an additional animal species is recommended before initiation of human trials. Comparative binding studies of FVIII, human vWF, and PI particles *in vitro* may also be warranted. Studies of FVIII and FVIII-PI in HA dogs are currently ongoing to help clarify the issues of interspecies scaling for this novel formulation.

CONCLUSION

Human FVIII appears to exhibit nonlinear pharmacokinetics in HA mice as characterized by a two-compartmental model with MM elimination. Association of FVIII with PI particles prolonged the circulation of FVIII in mice over a range of doses and simulation of expected human PK predict up to a 37% increase in half-life. FVIII-PI may represent a viable clinical strategy to prolonging the half-life and reducing the immunogenicity of this therapeutic protein.

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ABBREVIATIONS

FVIII	Factor VIII
HA	hemophilia A
PI	phosphatidylinositol
MM	Michaelis-Menten
NCA	non-compartmental analysis
DMPC	dimyristoylphosphatidylcholine
CL	clearance
t_{1/2}	half-life
AUC	area under the curve
LRP1	low density lipoprotein receptor-related protein 1
BDD	B-domain deleted

vWF

von Willebrand Factor

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Figure 1. Pharmacokinetics profiles of FVIII and FVIII-PI in HA mice

Plasma concentration-time profiles of following IV administration of free FVIII at 200 (closed circles), 40 (closed triangles), or 20 (closed squares) IU/kg or FVIII-PI at 200 (open circles), 40 (open triangles), or 20 (open squares) to HA mice. Model fitting is shown for free FVIII (solid lines) and FVIII-PI (dashed lines). Saturation of clearance was apparent following a 200 IU/kg dose and association with PI prolonged the terminal half-life of FVIII at all studied doses.

Figure 2. Concentration dependence of FVIII clearance

To understand the impact of changing concentrations on the observed clearance of FVIII, CL_{obs} was plotted as a function of concentration for free FVIII (black) and FVIII-PI (red). Clearance for both formulations converges at higher concentrations, but association with PI particles reduced clearance by up to two-fold at low concentrations.

Figure 3. Projected human PK of FVIII and FVIII-PI

Human simulations for 40 IU/kg doses of FVIII-PI (black) are shown along with the typical concentration-time profile for free FVIII in humans (red). A substantial increase in half-life is predicted following association with PI containing particles.

Figure 4. Simulated Wajima curves of FVIII and FVIII-PI

4a. Concentration-time profiles of FVIII-PI were simulated in HA mice for doses of 4 (orange), 10 (gold), 40 (green), 100 (blue), 400 (red), and 1000 (black) IU/kg. Those simulated profiles were normalized to C' ($=C/C_{ss}$) and t' ($=t/MRT$) in **4b**.

Table 1

Non-compartmental PK Parameters for FVIII and FVIII-PI in mice

Formulation	Dose (IU/kg)	Terminal $t_{1/2}$ (h)	AUCt (IU·h/mL)	CL (mL/h/kg)	% Change in AUCt versus free FVIII
Free FVIII	20	0.5	0.242±0.017	65.0	--
	40	3.5	2.93±0.18	13.1	--
	200	2.9	20.3±2.5	9.82	--
FVIII-PI	20	0.6	0.667±0.131	29.7	173
	40	5.4	6.02±0.58	6.33	105
	200	7.3	30.1±2.49	6.43	48.3

Table 2

Model fitted PK parameters for FVIII and FVIII-PI in mice

Parameter	Formulation	Units	Estimate	CV%
V_1	Both	mL/kg	42.4	10.8
$V_{\max}$		IU/h/kg	16.0	14.5
$k_{m,\text{free}}$	Free FVIII	IU/mL	0.819	24.8
$V_{2,\text{free}}$		mL/kg	59.8	13.3
$CLd_{,\text{free}}$		mL/h/kg	78.9	33.1
$k_{m,\text{pi}}$	FVIII-PI	IU/mL	1.72	21.5
$V_{2,\text{pi}}$		mL/kg	38.9	17.6
$CLd_{,\text{pi}}$		mL/h/kg	29.8	42.2