Published in final edited form as:

Biopharm Drug Dispos. 2016 October ; 37(7): 409–420. doi:10.1002/bdd.2023.

Factor VIII associated with lipidic nanoparticles retains efficacy in the presence of anti-factor VIII antibodies in hemophilia A mice

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Abstract

The development of inhibitory antibodies against factor VIII (FVIII) is a major challenge in hemophilia A (HA) therapy. Such antibodies develop in nearly 30% of patients receiving replacement FVIII, abrogating therapeutic efficacy. This work evaluated whether B-domain deleted FVIII encapsulated in phosphatidylinositol containing lipid nanoparticles (PI-BDD FVIII) could serve as an efficacious FVIII replacement therapy in the presence of inhibitors. The HA mice were given clinically relevant doses of FVIII to develop inhibitors. The efficacy of free and PI-BDD FVIII was studied in inhibitor-positive HA mice using a tail clip assay. Mathematical modeling of these data was conducted to evaluate the hypothesis that lipid association sterically shields the protein from inhibitor binding. The immunization protocol resulted in a mean inhibitory titer level of 198 ± 52 BU/ml. Free BDD FVIII was ineffective at controlling blood loss in inhibitor-positive HA mice as early as 2 h post dose. In contrast, PI-BDD FVIII treated animals retained partial hemostatic efficacy as long as 18 h post dose. Mathematical modeling supports the hypotheses that a greater fraction of lipid-associated FVIII remains unbound to inhibitors and that PI-BDD FVIII has lower binding affinity to inhibitors than does the free protein. In addition, the modeling approaches extend current efforts to model the impact of immunogenicity on PK and the therapeutically meaningful endpoint of efficacy, thereby addressing an important knowledge gap, particularly in the FVIII scientific literature. Clinical translation of these findings could result in a significant improvement in the quality of care of inhibitor-positive HA patients.

Keywords

factor VIII; inhibitors; phosphatidylinositol nanoparticles; hemostatic efficacy

Introduction

Recombinant human factor VIII (FVIII) replacement is the first line of therapy in patients with hemophilia A (HA). However, one of the main challenges in HA therapy is the

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Conflict of Interest

The authors have no financial or non-financial conflicts of interest to declare.

development of anti-FVIII antibodies [1,2]. These antibodies can be binding antibodies [3], which upon binding to the protein could increase its apparent clearance, or neutralizing antibodies, which abrogate biological activity by binding to key functional epitopes [4]. While the impact of binding antibodies on FVIII clinical efficacy is poorly elucidated [5,6], inhibitors present a substantial clinical challenge [1]. Nearly 30% of patients receiving anti-FVIII therapy develop these anti-FVIII inhibitors [7,8], which greatly complicates the course of therapy [1] and increases the burden of therapeutic costs [9]. Therapy options for the management of acute bleeding episodes in inhibitor-positive patients range from treatment with high dose human FVIII (effective only in patients with low responding titers <5 BU/ml, which represents ~30% of inhibitor-positive patients [1]) and non-homologous FVIII (recombinant porcine FVIII is currently in development for congenital HA [10]), to bypass therapy with FEIBA or factor VIIa [11]. The hemostatic efficacy of bypass therapies is not considered equal to that of coagulation factor replacement by most physicians, and ~10–20% of inhibitor-positive patients are unresponsive to these therapies [1]. For long-term eradication of inhibitors, patients undergo immune tolerance induction or B-cell depletion therapy with rituximab, which can be expensive, challenging, and often unsuccessful [2].

In its native form, human FVIII is a large, multi-domain glycoprotein secreted as a heterodimer of a heavy (A1-A2-B) and a light (A3-C1-C2) chain held together by a divalent metal cation [12]. The C2 and A2 domains of FVIII contain several functional [13] as well as immunodominant T-cell epitopes that can contribute to inhibitor development [14,15]. Consequently, most inhibitors to FVIII are directed against the C2 and A2 domains for the protein [16,17]. Their mechanisms of action include sterically hindering the binding of FVIII to phospholipids [18] and vWF [19], interfering with the activation of FVIII by thrombin or FXa [20–22] (anti-C2 inhibitors), as well as prevention of FVIIIa-FIXa binding [23] (anti-A2 inhibitors).

Soy phosphatidylinositol (PI)-containing lipid nanoparticles have been shown previously to prolong plasma survival and hemostatic efficacy as well as to decrease the immunogenicity of B-domain deleted FVIII (BDD FVIII) in HA mice [24]. Lipidic BDD FVIII is also more potent than the free form of the protein in HA mice [24]. These lipid particles are highly efficient at loading FVIII and permit a deep penetration of the FVIII molecule into the lipid bilayer because of structural packing defects introduced by PI in phosphatidylcholine membranes [25]. The molecular topology of the PI-FVIII complex involves association of the C2 and A2 domains of FVIII [26]. Therefore, a significant surface area of FVIII is sterically shielded by the PI particle, which could prevent binding of inhibitors to putative epitopes in the C2 and A2 domains. Previously, sandwich ELISA-based *in vitro* studies were conducted which support the hypothesis that the PI particle competitively reduces antibody binding to FVIII [26]. Phosphatidylinositol associated FVIII could consequently retain efficacy in the presence of inhibitors against the protein. Lipidic FVIII will circulate associated with the lipid particle under steady-state physiological conditions, be available on demand in the presence of a bleeding stimulus, and will be utilized rapidly at the site of tissue damage prior to neutralization.

In this study the comparative efficacy of free and PI-associated B-domain deleted FVIII (PI-BDD FVIII) was assessed in HA mice having circulating inhibitors against FVIII. The BDD

form of FVIII is likely to be more effective than full length FVIII in this strategy, since removal of the bulky, negatively charged B-domain decreases electrostatic repulsion between the protein and the anionic phospholipid membrane surface. This allows BDD FVIII to bind with greater affinity [27] and to penetrate deeper into the particle than full length FVIII, permitting more effective shielding of the A2 and C2 domains from anti-FVIII antibodies. The HA mice were immunized with a clinically relevant dose of FVIII to develop anti-FVIII antibodies. The relative efficacy of free and PI- BDD FVIII was then evaluated in these mice using a tail clip assay. Mathematical modeling was conducted to describe the efficacy profiles of free and lipid-associated BDD FVIII in the presence of anti-FVIII antibodies. The data indicate that PI-BDD FVIII retains hemostatic efficacy in the presence of inhibitory antibodies longer than does free BDD FVIII. Mathematical modeling suggests that this finding could be attributable to two factors. First, PI association could hinder the binding of antibodies to the protein, and second, a lower concentration of lipidic protein that is unbound to antibodies could be sufficient to retain hemostatic activity because of its greater potency.

Materials and Methods

Materials

The BDD FVIII was expressed and purified in the laboratory of Dr Philip Fay as described previously [28–30]. The specific activity of the protein was 6.5 IU/ μ g. Dimyristoylphosphatidylcholine (DMPC) and soybean PI were purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Cholesterol was purchased from Sigma-Aldrich (St Louis, MI, USA). Two-stage chromogenic assay kits for FVIII activity were purchased from Chromogenix (Chapel Hill, NC). Murine anti-FVIII monoclonal antibody ESH-8 was purchased from American Diagnostica (Stamford, CT). Horseradish peroxidase conjugated anti-mouse IgG antibody and 3,3',5,5'-tetramethylbenzidine were purchased from Sigma-Aldrich (St Louis, MO). Control plasmas and activated partial thromboplastin time (aPTT) reagents were purchased from Precision Biologics (Dartmouth, Canada) and Tcoag (Parsippany, NJ). Buffer salts were purchased from Fisher Scientific.

Animals

An inbred colony of C57BL/6 J mice with a targeted deletion at exon 16 of the FVIII gene is maintained on site in accordance with the Institutional Animal Care and Use Committee of the University at Buffalo, SUNY. Study mice were ~12 weeks old and weighed ~24 g.

Immunization protocol and assays

To mimic the development of antibodies in HA patients, the animals were immunized intravenously with four weekly doses of 1 IU (0.2 μ g)/injection full length FVIII [31]. The animals ($n = 5$) were killed at week 6 and plasma was collected for analyses of total and inhibitory antibody concentrations. Total (binding + inhibitory) anti-FVIII antibody concentrations were determined using an ELISA as described previously [32]; however, the assay was modified to use the murine monoclonal anti-FVIII antibody ESH-8 [33] as a standard and antibody levels are reported as μ g/ml ESH-8 equivalents. Inhibitory anti-FVIII antibody titers were quantified with the Bethesda assay [26].

Pharmacokinetic (PK) studies

Pharmacokinetic studies with free and PI-BDD FVIII conducted in antibody-negative mice have been reported previously [24]. In that study, male HA mice ($n = 3-6$ mice/time point) received a single i.v. bolus injection of 40 IU/kg free or PI-BDD FVIII via the penile vein. Citrate anti-coagulated plasma samples were collected up to 36 h post dose, and the BDD FVIII activity was measured with a two-stage chromogenic assay. Where FVIII plasma concentrations are expressed in $\mu\text{g/ml}$, activity levels measured in IU/ml were converted to antigen concentrations using the specific activity of the protein.

Interference from the circulating anti-FVIII antibodies in FVIII assays makes acquiring pharmacokinetic data in antibody-positive animals infeasible. Our attempts to do so using several conventional assays such as ELISA, aPTT, the two-stage chromogenic assay and the thrombin generation assay were unsuccessful.

Hemostatic efficacy studies

The comparative hemostatic efficacy of free and PI-BDD FVIII was assessed in antibody-positive HA mice with a tail clip assay as described previously [24]. Briefly, mice ($n = 3-6$ /time point/group; greater n at later time points) were placed on a heating pad under isoflurane anesthesia. The mice received a single 40 IU/kg i.v. dose of free or PI-BDD FVIII via penile vein injection. At fixed time points up to 18 h post-injection, the tails were transected at 6 mm from the distal tip with a sterile blade. Following transection, the tail was immediately immersed into 12 ml of normal saline maintained at 37 °C and bleeding was observed for 30 min. The volume of blood loss was determined gravimetrically. Between injection and scheduled bleeding time points, the mice were recovered from anesthesia, and group-housed (2–3 per cage) with *AD libitum* access to food and water. Mice were examined to ensure no visible bleeding episodes occurred between FVIII administration and initiation of the tail clip assay. Throughout the bleeding interval the mice were maintained under isoflurane anesthesia. The pharmacodynamics (PD) of free and PI-BDD FVIII in antibody-negative HA mice were assessed in an identical manner and reported previously [24].

PK-PD modeling

Theoretical—FVIII plasma concentrations cannot be measured reliably in antibody-positive animals due to assay limitations. In the absence of such data, appropriate models were applied to estimate PK in these animals and antibody-unbound concentrations were used to drive the PD model. The PK profile of BDD FVIII in antibody-negative HA mice is well described by a one-compartment model with Michaelis–Menten (MM) elimination as reported previously [24]. It was proposed that this non-linearity in FVIII PK in HA mice is due to saturable clearance of the protein via its primary clearance receptor, low-density lipoprotein receptor-related protein (LRP) [34].

All PD data from inhibitor-positive and negative animals treated with free and PI-BDD FVIII were modeled simultaneously. A simple direct effect model is adequate to describe the PD of this protein. In both the applied modeling approaches, the PD model parameters EC_{50} , $E_{\max}$ and γ (defined below) are common for data obtained from antibody-negative and

antibody-positive animals. This is because the intrinsic properties of free (antibody-unbound) FVIII and the underlying system do not change in the presence of antibodies, and it is assumed that the antibody-bound FVIII has negligible pharmacological activity. Treatment-specific EC_{50} values were used for free and PI-BDD FVIII to account for the differences in potency of the two protein forms. The first modeling approach uses a fixed structural PK model and parameter values obtained from modeling of data from antibody-negative animals:

$$\frac{dC_p}{dt} = - \left(\frac{V_{\max} C_p}{K_m + C_p} \right) \left(\frac{1}{V} \right) \quad (1)$$

The initial condition for Equation 1 is:

$$C_p(0) = \frac{\text{Dose}}{V}$$

A fraction-unbound (f_u) term was included in the PD model to account for the fact that only the free fraction of the drug (unbound from antibodies) is able to exert pharmacological action:

$$E = E_0 \left(1 - \frac{E_{\max} (f_u C_p)^\gamma}{EC_{50}^\gamma + (f_u C_p)^\gamma} \right) \quad (2)$$

with C_p as the plasma FVIII activity, V is the volume of distribution, $V_{\max}$ and K_m are Michaelis Menten elimination parameters, E_0 is the volume of blood loss in the absence of treatment, $E_{\max}$ is the maximum response, EC_{50} is the concentration of FVIII at which 50% of blood loss is abated, and γ is the Hill coefficient. Model parameters and their sources when fixed are presented in Table 1. Although the assumption that free FVIII concentrations in antibody-positive subjects at any time are a proportional fraction of corresponding concentrations in antibody-negative subjects might not be mechanistically valid in all instances, this model is a reasonable basic approach to account for the impact of anti-drug antibody development on PD. The second approach involves estimating BDD FVIII PK in the presence of antibodies using the quasi-equilibrium or rapid binding approximation of the target mediated drug disposition (TMDD) model [35]. The model structure is presented in Figure 1. The TMDD model is appropriate to describe drug disposition when a significant proportion of the administered drug is bound with high affinity to a pharmacological target or other entity, such that this interaction is reflected in the PK properties of the drug [36]. The rapid binding approximation of the TMDD model is reasonable for this system because the binding of FVIII with anti-FVIII antibodies and dissociation of the complex are much faster than the rest of the system processes. It is assumed that there is no significant turnover of the antibody since the duration of the study is very short relative to the typical lifetime of these antibodies. Under these conditions, the system can be described by Equations 3a, 5 and 6 (adapted from [35,37]). Model parameters, and their sources when fixed, are presented in

Table 1. Plasma BDD FVIII concentrations were modeled in $\mu\text{g/ml}$ and total antibody concentrations were in $\mu\text{g/ml}$ EHS-8 equivalents. When k_{deg} is not included, the total antibody concentration (Ab_{tot}) remains constant and was fixed to the value measured in immunized animals at week 6 as described previously (Ab_{observed}).

$$\frac{dFVIII_{\text{tot}}}{dt} = - \left(\frac{V_{\text{max}} FVIII}{K_m + FVIII} \right) \left(\frac{1}{V} \right) \quad (3a)$$

$$\frac{dFVIII_{\text{tot}}}{dt} = - \left[\left(\frac{V_{\text{max}}}{K_m + FVIII} \right) \left(\frac{1}{V} \right) - k_{\text{deg}} \right] \quad (3b)$$

$$FVIII - k_{\text{deg}} FVIII_{\text{tot}}$$

$$\frac{dAb_{\text{tot}}}{dt} = - k_{\text{deg}} (FVIII_{\text{tot}} - FVIII) \quad (4)$$

$$FVIII = \frac{1}{2} \left[(FVIII_{\text{tot}} - Ab_{\text{tot}} - K_D) + \sqrt{(FVIII_{\text{tot}} - Ab_{\text{tot}} - K_D)^2 + 4K_D FVIII_{\text{tot}}} \right] \quad (5)$$

$$E = E_0 \left(1 - \frac{E_{\text{max}} FVIII^\gamma}{EC_{50}^\gamma + FVIII^\gamma} \right) \quad (6)$$

The initial conditions for Equation (3) and (4) are:

$$FVIII_{\text{tot}}(0) = \frac{\text{Dose}}{V}$$

$$Ab_{\text{tot}}(0) = Ab_{\text{observed}}$$

with $FVIII$ as the antibody-unbound BDD FVIII plasma concentration, $FVIII_{\text{tot}}$ is the total FVIII plasma concentration (antibody-unbound + antibody-bound, i.e. $FVIII_{\text{tot}} = FVIII + Ab.FVIII$), V_{max} and K_m are MM elimination parameters, V is the apparent volume of distribution, Ab_{tot} is the total antibody concentration (free + FVIII-bound i.e. $Ab_{\text{tot}} = Ab + Ab.FVIII$), K_D is the equilibrium dissociation constant, and k_{deg} is the first-order elimination rate constant of the complex. The parameters in Equation 6 are the same as for Equation 2.

Modeling platform—Model fitting was performed using Phoenix WinNonlin v6.3 (Pharsight Corporation, Sunnyvale, CA). Model selection and evaluation of the goodness of fit were guided by precision of the parameter estimates, objective measures such as the Akaike information criteria, and visual inspection of fitted profiles and residuals.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (La Jolla, CA). The volume of blood loss over time in inhibitor-positive animals as a function of FVIII formulation was compared by two-way ANOVA. Values of $p < 0.05$ were considered statistically significant.

Results

Development of anti-FVIII antibodies

The immunization protocol resulted in mean ($\pm$ SEM) inhibitory titer levels of 198 ± 52 BU/ml as measured by the standard Bethesda assay. To express total antibody levels, $\mu\text{g/ml}$ ESH-8 equivalents were used, instead of the typically reported quasi-quantitative endpoint titer values (which are affected by both the avidity and concentration of antibodies [38]). ESH-8 is a standard antibody used frequently as a model inhibitor to FVIII and binds to FVIII with very high affinity ($K_D = 0.5$ nM) [33]. When reported in this manner, the total antibody concentrations in these animals were 152 ± 27 $\mu\text{g/ml}$ ESH-8 equivalents. This approach circumvents the key technological limitations that currently prevent the quantification of absolute concentrations and binding affinities of endogenous polyclonal anti-FVIII antibodies.

Hemostatic efficacy in antibody-negative and positive HA mice

Baseline blood loss in untreated antibody-negative and positive HA mice was comparable (320 ± 49 μl vs 359 ± 40 μl). In antibody-positive animals that were treated with the free protein, BDD FVIII was ineffective at arresting blood loss as early as 2 h post dose (Figure 2). For reasons that are not yet understood, partial clotting efficacy was temporarily restored in these animals at 6 h post dose. At all other time points studied, blood loss in these animals was comparable to untreated, antibody-positive HA mice. However, in antibody-positive animals treated with PI-BDD FVIII, partial hemostatic efficacy was retained up to 18 h post dose (Figure 2). Because of the high inherent variability in the bleeding response [39], statistical significance could not be established.

The efficacy vs time profile of free and PI-BDD FVIII in antibody-negative HA mice has been discussed in detail previously [24]. To summarize, immediately post dose, both free and PI-BDD FVIII restored normal clotting function (comparable to normal C57BL6/J mice), followed by a subsequent decline in clotting efficacy over time. Bleeding was uninhibited by treatment 20 h after dosing in free BDD FVIII treated animals. However, partial hemostatic efficacy was retained in PI-BDD FVIII treated animals as long as 28 h post dose. The PK-PD data in antibody-negative animals also suggested that the PI-BDD FVIII is more potent than the free form of the protein, i.e. lower concentrations of PI-BDD FVIII suffice to maintain hemostatic activity. It is hypothesized that this increased potency

of PI-BDD FVIII is due to the stabilization of the activated FVIII hetero-trimer upon association with the lipid particle [24].

Mathematical modeling

Two mathematical models were applied to compare the PD profiles of free and PI-BDD FVIII in these animals. The first approach utilizes a f_u term in the PD model to account for the fact that only the antibody-unbound fraction of the protein retains physiological activity. The fit of this model to the data is presented in Figure 3 and the parameter estimates are presented in Table 2. The model describes the data reasonably well and supports the hypothesis that a higher fraction of the lipidic protein remains unbound from the inhibitors.

The second approach applies a TMDD-based model to estimate PK in the presence of antibodies and uses the antibody-unbound plasma concentrations of FVIII as described by this model, to drive the PD model. Model-estimated plasma concentrations, the fit of this model to the PD data, and the corresponding parameter estimates are presented in Figure 4, Figure 5 and Table 2. As with the fraction-unbound model, this approach describes the data well. It also suggests that PI association decreases the binding affinity of BDD FVIII to antibodies, which in turn results in a higher antibody-unbound fraction and the observed physiological activity. Because the endogenous antibody concentrations were measured in $\mu\text{g/ml}$ ESH-8 equivalents, the K_D value estimated by model fitting is arbitrary.

In both the fraction-unbound and TMDD-based models, modeling the PD data with treatment-specific EC_{50} values yields a lower EC_{50} for PI-BDD FVIII than for the free protein. This is in keeping with the finding that PI associated FVIII is more potent than the free protein [24].

Discussion

The management of hemophilia in patients having inhibitors against FVIII is a serious clinical challenge. There is a large unmet medical need for FVIII therapies that retain efficacy in the patient population that has circulating anti-FVIII antibodies. BDD FVIII encapsulated in lipid nanoparticles that sterically shield the protein from circulating antibodies could be a strategy successful for retaining efficacy in this patient population. In order to evaluate this, the comparative efficacy of free and PI-BDD FVIII was studied in HA mice having circulating anti-FVIII inhibitors.

All HA mice treated with human FVIII developed antibody responses to human FVIII. The HA mouse model is a complete genetic knockout model. Consequently these mice have no central tolerance to the FVIII protein. Additionally, despite high sequence homology (84–93%) in the conserved regions of murine and human FVIII, human FVIII presents as an exogenous antigen to the murine immune system. This results in the higher anti-FVIII antibody incidence and magnitude in mice relative to humans. However, these mice are an appropriate model to study the relative immunogenicity of various FVIII forms owing to a FVIII immune response that is qualitatively similar to humans [40] and to the high sequence homology in the conserved regions of murine and human FVIII [41].

The results of this study indicate that PI-BDD FVIII retains efficacy longer than the free protein in the presence of inhibitors against FVIII. We hypothesize that this is because PI-association interferes with inhibitor binding. Upon administration of free FVIII to an inhibitor-positive animal, vWF and inhibitors compete for binding. Even if the association rate constant of the inhibitor is lower and FVIII initially binds to vWF, the dissociation rate of FVIII from vWF is high (studies on human FVIII–vWF binding suggest that half the FVIII bound to vWF dissociates from it within 7–13 min [42]). Upon dissociation from vWF, FVIII is exposed to nearly irreversible binding to inhibitors [42]. Lipid-bound FVIII will redistribute between vWF, inhibitors, lipid particle and free protein *in vivo*. Once redistribution to vWF occurs, this fraction will behave like free FVIII. The fraction of FVIII that stays deeply embedded within the lipid particle remains shielded from inhibitor binding and retains efficacy under bleeding conditions. The greater potency of the lipidic protein could also contribute to retention of efficacy in the presence of inhibitors, by amplifying the impact of even small differences in antibody-unbound concentration.

Mathematical modeling was applied to evaluate these hypotheses because information on antibody-unbound concentrations of FVIII is difficult to acquire experimentally in antibody-positive animals. The fraction-unbound model described the data reasonably well, and indicates that a higher fraction of PI-BDD FVIII remains unbound to inhibitors. The relatively high CV% around the estimate of f_u for free BDD FVIII is likely attributable to a complete absence of clotting activity in the presence of very high circulating antibody levels. f_u could be estimated with more confidence if similar studies could be conducted in animals with lower circulating antibodies or upon administration of a FVIII formulation that retains partial activity in the presence of anti-FVIII antibodies such as PI-BDD FVIII. Although it is a sound basic approach to account for the effect of immunogenicity on PD, this model is less mechanistic than the TMDD-based model.

TMDD-based model estimated plasma PK profiles of free and PI-BDD FVIII in antibody-negative and positive HA mice are presented in Figure 4. As expected, the unbound concentrations of BDD FVIII in antibody-positive animals are much lower than the corresponding concentrations in antibody-negative mice. However, antibody-unbound concentrations of PI-BDD FVIII are higher than the corresponding free BDD FVIII concentrations in antibody-positive mice. Similar to the fraction-unbound model, the TMDD-based model describes the data reasonably well. The EC_{50} values estimated (as antigen concentration) by this model agree well with the EC_{50} values estimated (as FVIII plasma activity) by the fraction-unbound model. The high CV% of the K_D estimate for free BDD FVIII is likely attributable to the same reasons that resulted in a high CV% of the f_u estimate. As mentioned previously, the absolute value of the estimated K_D is arbitrary and has no direct physiological relevance. However, it retains utility in comparing the relative binding affinities of native protein and protein forms modified to resist binding to anti-drug antibodies, as was our goal here.

It is widely reported that anti-drug antibody binding creates an additional clearance pathway for the protein therapeutic. Immune complexes are thought to be cleared primarily by components of the mononuclear phagocytic system via Fc-receptor-dependent and independent pathways [38]. Therefore, we attempted to include a k_{deg} term in this model

(Eq. 3b and 4) that represents a first-order endocytosis and degradation rate constant of the *Ab.FVIII* complex. k_{deg} was included both as an estimated parameter and as a constant derived from the literature [43]. However, inclusion of this term led to the model being overparametrized with the available data. Estimation or inclusion of k_{deg} would be improved in cases in which plasma concentrations of the *Ab.FVIII* complex over time can be reliably determined. As it stands, in the current TMDD-based model, the increase in the apparent clearance of the protein is attributable to the nearly irreversible binding [42] of antibodies.

Based on these findings, we propose that BDD FVIII associated with PI containing lipid nanoparticles could be a potential therapeutic option for HA patients with pre-existing inhibitors against FVIII. The retention of hemostatic efficacy in patients will likely depend upon several factors. Binding affinities of the inhibitors to FVIII relative to the lipid-FVIII binding affinity, and circulating levels of high affinity antibodies, will determine the extent of redistribution and neutralization of the lipid-bound fraction. The mechanism of action of the inhibitor is another important consideration, because the impact of inhibitors that act once the tenase complex has been assembled will likely not be mitigated by the lipid particle [44]. Future efforts with the lipidic delivery strategy will focus on modifying the lipid membrane composition such that a higher fraction of FVIII remains encapsulated within the particle and on prolonging the survival of the particle itself in circulation. Future studies with a focus on translation to the clinic will involve evaluation of free and lipidic FVIII in higher animal models such as inhibitor positive HA dogs, which are a well-recognized model of the human disease [45,46].

Two PK-PD modeling approaches were adapted to characterize the impact of anti-drug antibody development on the PD of recombinant human BDD FVIII. Because of the impact of these antibodies on the course of therapy, several approaches to establish tolerance towards FVIII, or reverse established antibody responses, are under investigation [47]. Therapeutic modalities that retain efficacy in the presence of anti-FVIII antibodies are also under development. Models such as those presented here, in particular the TMDD-based model, enable the characterization and prediction of expected efficacy profiles at different antibody concentrations. These models could therefore aid the development of tolerance induction strategies by predicting FVIII efficacy profiles in subjects as antibody levels decrease over the course of therapy. They could also be used to evaluate the comparative efficacy of modified FVIII products in the presence of anti-FVIII antibodies and to predict the efficacy of a given treatment in subjects having different circulating anti-FVIII antibody levels. Both of these approaches facilitate this characterization in situations where collecting reliable PK data is challenging due to assay limitations.

Conclusion

In summary it was demonstrated that BDD FVIII encapsulated in PI containing lipid nanoparticles retains efficacy in the presence of circulating inhibitors in an HA mouse model. Two mathematical modeling approaches were applied to characterize the resulting PD profiles, which lend support to our hypothesis of hindered binding interactions between the antibodies and lipid encapsulated protein. Successful translation to the clinic would lead

to a significant improvement in the quality of care available to inhibitor-positive HA patients.

Acknowledgments

This work was supported by a grant from the National Institutes of Health (R01 HL-70227) to Dr Balu-Iyer. We are grateful to Drs Wakabayashi and Fay at the University of Rochester, School of Medicine for providing us with the BDD FVIII protein that was used in these studies. We also wish to thank Dr W. Kryzanski for scientific advice on model development. Lastly, the authors thank the Pharmaceutical Sciences Instrumentation facility, University at Buffalo, State University of New York, for use of the shared microplate reader.

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Figure 1.

Rapid binding approximation of the TMDD model with no antibody turnover was used to model the PK of BDD FVIII in antibody-positive HA mice. Adapted from [35,37]. *FVIII* is free BDD FVIII plasma concentration, *Ab* is the free antibody concentration, *Ab.FVIII* is the antibody-BDD FVIII complex and K_D is the equilibrium dissociation constant. Michaelis–Menten (MM) elimination is described by the parameters V_{max} and K_m . k_{deg} is the first-order elimination rate constant of the complex

Figure 2.

Comparative hemostatic efficacy of free and PI-BDD FVIII in antibody-positive HA mice after an i.v. bolus dose of 40 IU/kg. Animals were immunized with four weekly 1 IU injections of FVIII to mimic clinical antibody development. At week 6, a tail clip assay was performed on FVIII-treated mice at various points after dosing. Error bars represent SEM of observed data. Free BDD FVIII is ineffective at controlling blood loss as early as 2 h post dose, whereas PI-BDD FVIII retains partial hemostatic efficacy as long as 18 h post dose. Statistical significance could not be established

Figure 3.

Fit of the fraction-unbound model to efficacy data collected from antibody-positive and negative HA mice after an i.v. bolus dose of 40 IU/kg free BDD FVIII (a) or PI-BDD FVIII (b). Symbols represent mean and error bars represent SEM of observed data (OBS). Lines represent the fit of the fraction-unbound model to the data (PRED)

Figure 4. Plasma concentrations of antibody-unbound free and PI-BDD FVIII concentrations in antibody-positive and negative HA mice estimated by the TMDD-based model

Figure 5.

Fit of the TMDD-based model to efficacy data collected from antibody-positive and negative HA mice after an i.v. bolus dose of 40 IU/kg free BDD FVIII (a) or PI-BDD FVIII (b). Symbols represent mean and error bars represent SEM of observed data (OBS). Lines represent the fit of the TMDD-based model to the data (PRED)

Description of model parameters

Table 1

Category	Parameter	Description	Value (if fixed)			Source
			Free BDD FVIII	PI-BDD FVIII	FVIII	
Fraction-unbound model	V (ml /kg)	Volume of distribution	45.1	47.0		Shetty <i>et al.</i> [24]
	$V_{\max}$ (IU/h. kg)	Maximum elimination rate	4.13			
	K_m (IU/ml)	FVIII concentration at which elimination is 50% of $V_{\max}$	0.48	0.66		
	E_0 (ml)	Volume of blood loss in the absence of treatment	320 (antibody-negative) 359 (antibody-positive)			Observed
	$E_{\max}$	Maximum response	1.0			Model estimation
	f_u		Estimated	Estimated		-
TMDD-based model	EC_{50} (IU/ml)	Concentration of FVIII at which 50% of blood loss is abated	Estimated	Estimated		-
	γ	Hill coefficient	1.3			Model estimation
	V (ml/kg)	Volume of distribution	42.1	44.1		Estimated using PK data in $\mu\text{g/ml}$ in a manner identical to Shetty <i>et al.</i> [24]
	$V_{\max}$ ($\mu\text{g/h.kg}$)	Maximum elimination rate	0.59			
	K_m ($\mu\text{g/ml}$)	FVIII concentration at which elimination is 50% of $V_{\max}$	0.067	0.096		
	Ab_{tot} ($\mu\text{g/ml}$) ESH-8 equivalents)	Free + FVIII bound i.e. $Ab_{tot} = Ab + Ab \cdot FVIII$	152			Observed
	K_D ($\mu\text{g/ml}$)	Equilibrium dissociation constant	Estimated	Estimated		-
	E_0 (ml)	Volume of blood loss in the absence of treatment	320 (antibody-negative) 359 (antibody-positive)			Observed
	$E_{\max}$	Maximum response	1.0			Model estimation
	EC_{50} ($\mu\text{g/ml}$)	Concentration of FVIII at which 50% of blood loss is abated	Estimated	Estimated		
	γ	Hill coefficient	1.3			Model estimation

Table 2

Model parameter estimates

Category	Parameter	Estimate (CV%)	
		Free BDD FVIII	PI-BDD FVIII
Fraction-unbound model	f_u	0.12 (199)	0.28 (55.4)
	EC_{50} (IU/ml)	0.61 (52.6)	0.18 (40.7)
TMDD-based model	K_D ($\mu\text{g/ml}$)	25.9 (160)	32.1 (76.5)
	EC_{50} ($\mu\text{g/ml}$)	0.10 (52.5)	0.03 (40.3)