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Soy phosphatidylinositol containing nanoparticle prolongs hemostatic activity of B-domain deleted Factor VIII in Hemophilia A mice

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Abstract

Factor VIII (FVIII) replacement therapy in Hemophilia A (HA) is complicated by a short half-life and high incidence of inhibitory antibody response against the protein. Phosphatidylinositol (PI) containing lipidic nanoparticles have previously been shown to reduce the immunogenicity and prolong the half-life of full length FVIII. It has not been established whether this prolongation in half-life improves hemostatic efficacy and whether this approach could be extended to the B-domain deleted form of FVIII (BDD FVIII). In the current study, we evaluated the pharmacokinetics (PK), hemostatic efficacy and immunogenicity of BDD FVIII associated with PI nanoparticles (PI-BDD FVIII) in HA mice. Comparative human PK was predicted using an ‘informed scaling’ approach. PI-BDD FVIII showed a ~ 1.5-fold increase in terminal half-life compared to free BDD FVIII following i.v. bolus doses of 40 IU/kg. PI-BDD FVIII treated animals retained hemostatic efficacy longer than the free FVIII treated group in a tail vein transection model of hemostasis. PI association reduced the development of inhibitory and binding antibodies against BDD FVIII after a series of i.v. injections. The combined improvements in circulating half-life and hemostatic efficacy could significantly prolong the time above clinically established therapeutic thresholds of prophylactic FVIII replacement therapy in humans.

Keywords

Next-generation FVIII; B-domain deleted Factor VIII; protein delivery; lipids; Phosphatidylinositol nanoparticles; pharmacokinetics; hemostatic efficacy; simulations; inhibitor development

INTRODUCTION

Factor VIII (FVIII) is an essential blood protein that plays a central role in the coagulation cascade. Genetic deficiency or dysfunction of FVIII results in the bleeding disorder called Hemophilia A (HA). Recombinant human FVIII is currently used as the first line of therapy

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Supporting Information:

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in HA. In its native form, human FVIII is a large, multi-domain glycoprotein secreted as a heterodimer of a heavy (A1-A2-B) and light (A3-C1-C2) chain held together by a divalent metal cation¹. The main challenges of FVIII replacement therapy include a short circulating half-life of ~12 hr^{2,3} which necessitates frequent i.v. administration for this chronic therapy and also the development of activity abrogating inhibitory antibodies in nearly 30% of patients⁴. Several FVIII products, modified to increase plasma survival are in clinical development, but less focus has been directed towards designing strategies that can reduce immunogenicity⁵⁻⁷.

Our lab has previously reported a phosphatidylinositol (PI) containing lipidic nanoparticle for the delivery of full length FVIII⁸. This nanoparticle is highly efficient at loading FVIII and permits a deep penetration of the FVIII molecule into the lipid bilayer due to structural packing defects introduced by PI in phosphatidylcholine membranes⁹. The molecular topology of the PI-FVIII complex involves association of the C2 and A2 domains of FVIII⁸. These domains are involved in low density lipoprotein receptor-related protein (LRP) mediated cellular uptake of FVIII into endocytotic clearance pathways¹⁰⁻¹² and contain several immunodominant epitopes that can contribute to inhibitor development^{13,14}. We have shown that association of FVIII with PI nanoparticles not only extends the circulating half-life, but is also capable of mitigating immune responses against the protein^{8,15}.

The A and C domains of FVIII participate in extensive macromolecular interactions with von Willebrand factor (vWF), phospholipids as well as other clotting factors that are critical to the plasma survival and procoagulant activity of the protein^{11,16,17}. The heavily glycosylated B-domain is not essential for FVIII procoagulant activity¹⁸, is poorly conserved across species¹⁹, and is removed *in vivo* during enzymatic cleavage of FVIII to the active form, FVIIIa²⁰. Elimination of the B-domain from the recombinant protein vector increases cellular production²¹ and BDD FVIII is bioequivalent to the full length protein in humans²². Consequently, BDD FVIII products have found application in FVIII replacement therapy for HA. These products share the challenges of a short circulating half-life²³ and inhibitor development²⁴ with their full length counterparts.

Removal of the bulky, negatively charged B-domain improves binding affinity to anionic lipids²⁵ which could result in improved liposomal encapsulation of BDD FVIII. In this study we investigated whether the therapeutic improvements conferred to full length FVIII by association with PI particles could be extended to BDD FVIII (PI-BDD FVIII). Comparative pharmacokinetic (PK) and relative immunogenicity studies were conducted in a mouse model of HA. We also conducted *in vivo* efficacy studies to address whether PI association can prolong retention of hemostatic efficacy. The results of these studies suggest that multi-functional PI containing lipidic nanoparticles have the potential to significantly improve FVIII replacement therapy in HA.

MATERIALS AND METHODS

Materials

BDD FVIII was expressed and purified in the lab of Dr. Philip Fay as previous described²⁶⁻²⁸. 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, Missouri, USA). FVIII chromogenic assay detection kits were purchased from Chromogenix (Chapel Hill, NC). Control plasmas and activated partial thromboplastin (aPTT) reagents were purchased from Precision Biologics (Dartmouth, Canada) and Tcoag (Parsippany, NJ) respectively. Monoclonal antibody ESH8 was purchased from American Diagnostica Inc. (Greenwich, CT). Alkaline phosphatase conjugates of goat anti-mouse IgG/IgM was obtained from Southern Biotechnology Associates, Inc. (Birmingham, AL). Buffer salts were purchased from Fisher Scientific.

Liposomal encapsulation studies

PI containing lipid nanoparticles (PI/DMPC/Cholesterol molar ratio 50:50:5) were prepared as previously described⁸. Encapsulation efficiency of BDD FVIII with the PI particle was determined with discontinuous dextran gradient centrifugation²⁹. Briefly, PI-BDD FVIII was loaded into the bottom layer of a 0%/10%/20% dextran gradient and subjected to ultracentrifugation at 190,000 X g for 30 minutes. FVIII activity of each layer was measured after centrifugation in duplicate with the aPTT assay and compared to a standard curve of known activity. Encapsulation efficiency was determined by comparing FVIII activity in the upper layers to the lowest layer.

Animals

An inbred colony of C57BL/6J 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 ~21.5 g.

Pharmacokinetic studies

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. Injections were prepared by dilution into HEPES buffer (20 mM HEPES, 300 mM NaCl, 5 mM CaCl₂, pH 7.0) for free protein or into tris buffer (20mM Tris, 150mM NaCl, pH 7.0) for lipid associated protein. Blood samples were collected up to 36 hr by cardiac puncture into acid citrate dextrose (ACD: 85 mM sodium citrate, 110mM D-glucose, 71 mM citric acid) at a 1:7 volume ratio. Plasma was immediately separated following collection by centrifugation at 5,000 × g for 5 minutes and stored at –80°C until analysis. BDD FVIII activity was measured with a two-stage chromogenic assay and concentrations were determined by comparison to a standard curve of known free protein activity.

Pharmacokinetic modeling and allometric scaling

Non-compartmental analysis (NCA) was performed on the resulting PK profiles using Phoenix WinNonlin v6.3 (Pharsight Corporation, Sunnyvale, CA) to compute basic PK parameters including area under the curve (AUC), half-life ($t_{1/2}$), clearance (CL), volume of distribution (V_{ss}), and mean residence time (MRT). Compartmental modeling was performed using various structural models to elucidate the disposition of free and PI-BDD FVIII. The models evaluated included one or two compartments with either linear or

Michaelis-Menten (MM) elimination. Previously generated multiple dose data for free BDD FVIII was used to determine MM parameters for the free protein. These parameter estimates and previously performed iterative model fitting with the full length protein were used to inform simultaneous fitting of the single dose free and PI-BDD FVIII data reported here. Model selection and evaluation of goodness of fit were guided by precision of the parameter estimates, objective measures like the Akaike information criteria and subjective measures like visual inspection of fitted profiles and residuals.

Simulations were conducted to predict PK profiles of free and PI-BDD FVIII in humans after a 50 IU/kg dose using a previously reported 'informed scaling' approach³⁰. The 'informed scaling' approach utilizes relative changes caused by PI in mice and the known behavior of the free protein in humans to predict human PK of PI-BDD FVIII. This method combines allometry based scaling of PK parameters with normalized Wajima curves³¹ from preclinical species to generate human concentration-time profiles. The suitability of this method to scale PK of modified FVIII products has been previously demonstrated^{30,32}. Briefly, parameter estimates of Vss and CL in mice were obtained by fitting of a 1 compartment, linear clearance model to PK data. MRT and C_{ss} were calculated as MRT=Vss/CL and C_{ss}=Dose/Vss. Wajima curves for free and PI-BDD FVIII in mice were generated by normalizing time and concentrations as t'=t/MRT and C'=C/C_{ss}. Estimates for Vss and CL of the free protein in humans were obtained from literature^{18,33}. Predictions of Vss and CL of PI-BDD FVIII in humans were generated using the following equation³⁰:

$$P_{Human}^{PI-BDDFVIII} = \frac{P_{Mouse}^{PI-BDDFVIII}}{P_{Mouse}^{FreeBDDFVIII}} \cdot P_{Human}^{FreeBDDFVIII}$$

with P as the parameter of interest. Concentration time profiles for free and PI-BDD FVIII were obtained by multiplying t' and C' of the normalized Wajima curves with human parameters of MRT and C_{ss} of the respective protein form.

Hemostatic efficacy studies

Hemostatic efficacy was assessed in HA mice with a tail vein transection assay. FVIII-naïve male mice were placed on a heating pad under isoflurane anesthesia. Mice (n=3–11/time point/group; higher n at later time points) received 40 IU/kg free or PI-BDD FVIII via a penile vein injection. At fixed time points up to 36 hr post-injection, 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 observed for 30 min. Volume of blood loss was determined gravimetrically.

Immunogenicity studies

Relative immunogenicity of free and PI-BDD FVIII was studied in HA mice. These mice are an appropriate model to study relative immunogenicity owing to a FVIII immune response that is qualitatively similar to humans^{34–36} and to high sequence homology (84–93%) in the conserved regions of murine and human FVIII¹⁹. Naïve male HA mice (n=8/group) were challenged with 4 weekly i.v. injections of either formulation at a dose of 1 µg/injection. Animals were sacrificed at week 6 and plasma was collected. Total anti-FVIII

titers were determined using an ELISA as previously described³⁷. Inhibitory anti-FVIII titers were quantified with the Bethesda assay.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (La Jolla, CA). Volume of blood loss over time as a function of FVIII formulation was compared by two-way ANOVA. Comparison of total and inhibitory titers was made using unpaired two-sample t-tests. P-values <0.05 were considered statistically significant.

RESULTS

Encapsulation efficiency of BDD FVIII with PI particle

The association efficiency of BDD FVIII with PI nanoparticles was determined using discontinuous dextran gradient centrifugation. Association of BDD FVIII with PI particles was found to be highly efficient, with $84.3 \pm 10.6\%$ of recovered protein found in association with lipid particles. This encapsulation efficiency is higher than that observed with full length FVIII ($72.1 \pm 9.1\%$)⁸.

Pharmacokinetic studies

To investigate whether association with the PI particle prolongs plasma survival of BDD FVIII, comparative PK studies were conducted at the clinically relevant dose of 40 IU/kg. The PK profiles (Fig. 1) indicate that association of BDD FVIII with the PI particle results in marked PK improvements. Parameters calculated using non-compartmental analyses are summarized in Table 1. Initial recovery or C_{max} was higher for free BDD FVIII compared to PI-BDD FVIII. This is likely due to the fact that FVIII associated deep within the lipid particle may not be released fast enough to contribute to conversion of the chromogenic substrate and is thus not accounted for in the assay. Volumes of distribution of both free and PI-BDD FVIII were close to the physiological plasma volume in mice (~50 mL/kg). However, clearance of PI-BDD FVIII was reduced by ~20% and the terminal half-life was increased ~1.5-fold compared with free FVIII.

The final compartmental model that best described the data comprised a 1 compartment model with MM elimination. Previously conducted dose ranging studies with the free protein showed an increase in clearance at lower doses; this data was used to estimate V_{max} and K_m of the free protein (supplementary data). Fitting of the single dose data presented here utilized a common V_{max} and treatment specific K_m values. Final parameter estimates from the compartmental model are listed in Table 2 and final model fitting to the data is shown in Fig. 1. Compared to the free protein, PI-BDD FVIII demonstrated a greater K_m value. The PK data establishes that association with the PI particle improves the plasma survival of BDD FVIII in HA mice.

Normalized Wajima curves generated from the fitting of a 1 compartment, linear clearance model to PK data in mice were used to simulate human PK profiles of free and PI-BDD FVIII after a dose of 50 IU/kg (Fig. 2). The simulations indicate that association with PI

nanoparticles is expected to prolong circulating half-life of BDD FVIII from 11.8 hr to 18 hr in humans.

Hemostatic efficacy studies

Next, we investigated whether the improvement in plasma survival of BDD FVIII translates to prolonged hemostatic efficacy. An *in vivo* murine tail vein transection model of acute bleeding was used to study comparative hemostatic efficacy. The profiles (Fig. 3) indicate that immediately post i.v. bolus administration, both free and PI-BDD FVIII restore normal clotting function and the volume of blood loss was similar to normal C57BL/6J mice. Free BDD FVIII displayed a sharp decline in clotting efficacy and bleeding was uninhibited by treatment 20 hr after dosing. In contrast, the return to baseline blood loss was far more gradual in PI-BDD FVIII treated animals and partial hemostatic efficacy was retained in these animals as long as 28 hr post dose. Mean ($\pm$ standard error of mean) volume of blood lost by PI-BDD FVIII treated animals was much less than that lost by free FVIII treated animals at 20 (234 ± 27.3 v/s 413 ± 86.8 μ L), 24 (174 ± 48.8 v/s 328 ± 71.7 μ L) and 28 hr (217 ± 97.1 v/s 332 ± 39.9 μ L). Due to high variability in the bleeding response, which is an inherent challenge of the tail vein transection model, these differences were not statistically significant. However, in repeated evaluations of comparative efficacy at 24 hr post dose, including a study where the investigator was blinded to the treatment groups, the mean volume of blood lost by PI-BDD FVIII treated animals was consistently lower than free BDD FVIII treated animals and lower than baseline blood loss by naïve HA mice, suggesting that PI associated FVIII does in fact retain hemostatic efficacy longer. It is also particularly noteworthy that an overlay of the PK and pharmacodynamic (PD) plots for free and lipidic protein (Fig. 4 a and b) showed that bleeding was uninhibited by free FVIII treatment below 0.1 IU/mL; for PI-BDD FVIII this apparent efficacy threshold was found to be lower ~ 0.01 IU/mL. *Ex vivo* assays of plasma FVIII activity incompletely assess concentration-effect relationships in the coagulation network owing to the complexity of molecular signaling pathways involved³⁸. For instance, in the chromogenic assay used in our PK studies, free and PI-BDD FVIII standards of equal FVIII activity promoted comparable Factor Xa mediated conversion of the chromogenic substrate. However, the differences in therapeutic efficacy at equivalent concentrations became apparent in an *in vivo* assay such as tail vein transection in the presence of physiologically relevant, global hemostasis machinery.

Immunogenicity studies

To evaluate the relative immunogenicity of free and lipidic BDD FVIII, a series of i.v. injections were administered to HA mice. Mean ($\pm$ standard deviation) inhibitory (122.7 ± 93.1 v/s 259.7 ± 182.7 BU/mL) and total (422.5 ± 254.3 v/s 721.9 ± 361.3 arbitrary titer units) titers were lower for the PI-BDD FVIII treatment group compared to the free FVIII treatment group (Figs. 5a and b). In addition, PI-BDD FVIII treated animals had inhibitory titers (8/8) and total titers (7/8) less than the respective means of the free treatment group. Despite a clear trend towards decreased immunogenicity of lipidic FVIII, statistical significance could not be established due to a single animal in the free treatment group that was a complete non-responder to the protein.

DISCUSSION

The need for a long acting, less immunogenic form of FVIII is well recognized. We have previously established the ability of PI containing lipid nanoparticles to improve the plasma survival and decrease the immunogenicity of full length FVIII⁸. In this work, we evaluated whether lipid association could prolong hemostatic efficacy and whether this strategy could be extended to BDD FVIII. PK, PD and immunogenicity studies with free and PI associated BDD FVIII were conducted in HA mice. PK modeling and allometry were used to gain insights and to evaluate the scalability of these benefits to humans.

A higher percentage of BDD FVIII compared to the full length protein associated with the PI nanoparticle. Removal of the bulky, negatively charged B-domain decreases coulombic repulsion between the protein and the anionic phospholipid membrane surface. Combined with packing defects caused by PI in the membrane, this allows BDD FVIII to penetrate much deeper into the particle providing improved *in vivo* stability and masking the domains from binding to clearance receptors.

Plasma PK profiles following i.v. bolus doses of both free and PI-BDD FVIII were characterized by a mono-exponential decline of plasma FVIII activity. The pronounced alpha phase, or rapid initial decline, we have previously observed with full length FVIII is not observed with BDD FVIII. This is consistent with the notion that this alpha phase is caused by rapid removal of high molecular weight species containing heavily glycosylated and heterogeneous B-domains³⁹ and is not true peripheral or non-specific distribution. Non-compartmental analyses indicated that PI association decreases the clearance and prolongs plasma survival of BDD FVIII.

Compartmental model fitting was performed to gain further insight into the underlying factors controlling the disposition of free and PI-BDD FVIII. Beginning with the assumption of no difference between free and PI-BDD FVIII, various structural models were fit to the data. The final structural model comprised one distribution compartment and MM elimination. A single Vmax and unique Km values were utilized to describe the MM elimination for free and PI-BDD FVIII. Under MM formalism, Km is an estimate of the affinity of a ligand for a given receptor and Vmax is dependent on receptor capacity. Compared to the free protein, PI-BDD FVIII demonstrated a higher Km. We have previously shown that binding to the PI particle involves the A2 and C2 domains of the FVIII molecule. Epitopes that mediate binding of FVIII to the primary clearance receptor LRP have been mapped to these domains^{40–42}. Mechanistically, this increase in Km is consistent with our hypothesis that steric shielding of these domains by the PI particle reduces the interaction of FVIII with LRP. The common Vmax used is consistent with the assumption that the LRP receptor pool available for clearance of both forms of the protein will be the same.

The best fit of a 1 compartment, linear clearance model to PK data from HA mice was used to simulate comparative human PK. This was necessitated by the fact that BDD FVIII PK is non-linear in mice but no evidence of such non-linearity has been observed in humans¹⁸. A 1 compartment linear clearance model does not characterize mouse data well; terminal

concentrations are over-predicted for both free and PI forms. In contrast, human PK of BDD FVIII is well described by this model. Since our objective was to predict human PK and our method of scaling (which utilizes a ratio of free and PI FVIII parameters in mice instead of the actual value of the parameter) corrects for the over prediction of mouse data, the use of this model was considered acceptable. The simulated profile for free FVIII and predicted half-life of 11.8 hr showed excellent agreement with clinically observed PK of free BDD FVIII (Fig. 2). The predicted half-life of PI-BDD FVIII is 18 hr. These simulations therefore project a ~1.5-fold improvement in plasma half-life of PI associated BDD FVIII in humans. In mice the asialoglycoprotein receptor (ASGPR) is capable of interacting with the B-domain of FVIII. Co-administration of full length FVIII with an ASGPR antagonist resulted in an increase in the terminal half-life of > 50%⁴³. We have also observed a longer circulation time for the BDD FVIII in HA mice than for the full length protein. It is possible that PK benefits of lipid association in mice stem from the fact that the ASGPR plays a greater role in clearance of free full length FVIII in mice than in humans. Lipidic delivery of BDD FVIII could therefore be more 'scalable' to humans than full length FVIII.

PD studies in HA mice indicated that lipid association prolongs hemostatic efficacy. Based on the combined results of PK and PD studies, the prolongation in hemostatic efficacy can be attributed to two factors. First, PI association prolongs plasma survival of FVIII (Fig. 1) so the time required for plasma concentrations to decrease towards a minimum therapeutic threshold is longer for PI-BDD FVIII than for the free protein. Second and more striking is the fact that PI association lowers the threshold protein concentration required for efficacious clotting. We hypothesize that this improved activity of PI-BDD FVIII could be due to stabilization of the activated FVIII hetero-trimer upon association with the lipid particle. FVIIIa is the most hemostatically active form of FVIII⁴⁴. However, FVIIIa has a very short half-life⁴⁵; FVIIIa not bound to a phospholipid surface (presented *in vivo* by activated platelets) is rapidly cleared. We have previously shown that PI particles act as a vWF mimetic and protect the free fraction of FVIII not otherwise bound to vWF⁴⁶. Activation decreases binding affinity to vWF but improves lipid binding affinity of FVIII²⁵. FVIIIa bound to PI nanoparticles remains associated with this phospholipid surface which could decrease its clearance and improve efficacy.

This finding has major implications in FVIII prophylactic therapy. The paradigm of HA clinical management is shifting increasingly towards prophylactic v/s on-demand replacement therapy⁴⁷. This was due to the observation that patients with moderate HA (FVIII: 0.01–0.05 IU/mL) bleed less often than those with severe HA (<0.01IU/mL)⁴⁸. Clinical evidence shows that maintaining trough concentrations 0.01 IU/mL decreases spontaneous bleeding and associated morbidity. However the current prophylactic dosing regimen, together with significant inter-patient variability in FVIII PK, makes sustaining the desired trough concentration challenging in a sizeable proportion of patients⁴⁹. Lipid association prolongs time before FVIII plasma concentrations decrease to a chosen threshold. Also, since hemostatic efficacy is retained at lower concentrations, the efficacy threshold itself could be lowered from 0.01 IU/mL. The simulated human PK profiles presented in Fig. 6 illustrate the implications of these findings. Based on these simulations, free BDD FVIII concentrations are expected to fall below the clinically established efficacy

threshold of 0.01IU/mL within three days. This is in good agreement with current clinical practice; FVIII is administered once every 2–3 days⁴⁹. In contrast, PI-BDD FVIII should remain efficacious for 4.5 days if only the prolongation in plasma survival is considered. In addition to this benefit, since the lipid bound form is more potent, if it is assumed the 10-fold lowering in efficacy threshold observed in HA mice translates to humans, PI-BDD FVIII is projected to retain efficacy for 7 days. If a more conservative improvement in efficacy of only 2-fold is assumed, PI-BDD FVIII will retain efficacy for 5.5 days. Consequently, PI-BDD FVIII could potentially be administered once weekly instead of thrice weekly, as is currently necessary with free FVIII. Successful translation of these findings to the clinic would therefore greatly improve prophylactic HA therapy.

It is appropriate to acknowledge here that several factors could affect the translatability of this data to humans. Due to the intrinsic variability of the bleeding response, the difference in efficacy between free and PI-BDD FVIII observed in these studies did not reach statistical significance. In addition, the tail vein transection assay is not a strict representative model of prophylaxis. This assay replicates physiological and pharmacodynamic events in the presence of aggressive venous assault rather than spontaneous bleeding. The spontaneous bleeding phenotype, which is very common in humans with HA, is rarely observed in HA mice^{50,51}. Comparative prophylactic efficacy evaluation of free and PI-BDD FVIII in higher species such as HA dogs, which exhibit frequent spontaneous bleeding⁵⁰ is therefore necessary to establish the improvement in prophylactic efficacy offered by PI-BDD FVIII.

The development of an immune response to FVIII in the HA patient population is a major concern. Up to 30% of patients develop some level of inhibitory response towards FVIII, complicating the management of bleeding episodes. Using a murine model of HA, we showed that PI-BDD FVIII is less immunogenic than free BDD FVIII at equivalent doses. The dose of 1ug/injection used in these studies has previously been shown to elicit a robust anti-FVIII antibody response against full length FVIII⁸. PI-mediated decrease in immune response could be due to multiple underlying mechanisms. Binding to PI nanoparticles and deep penetration of the protein into the lipid membrane results in shielding of immunodominant epitopes from the immune system, leading to immunological ignorance. PI nanoparticles also interfere with processing of FVIII by antigen presenting dendritic cells. Uptake of PI complexed FVIII by dendritic cells results in lowered expression of the T-cell costimulatory signal CD40 and increased production of the regulatory cytokines TGF- β and IL-10¹⁵. Whereas the decrease in immunogenicity at equivalent doses is beneficial in itself, decreased antigen load over chronic dosing, due to less frequent administration might afford further advantages.

CONCLUSIONS

Association of BDD FVIII with PI containing lipidic nanoparticles prolongs plasma survival of BDD FVIII and also improves its hemostatic efficacy. In prophylactic FVIII therapy, the decrease in dosing frequency from thrice to once weekly with lipidic FVIII along with the lowered immunogenicity could greatly enhance patient quality of life as well as decrease the burden of therapeutic cost. Lipidic FVIII therefore has the potential to significantly improve the quality of care in Hemophilia A.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

FVIII	Factor VIII
HA	Hemophilia A
PK	pharmacokinetic
PD	pharmacodynamic
PI	phosphatidylinositol
LRP	low density lipoprotein receptor-related protein
vWF	von Willebrand Factor
DMPC	dimyristoylphosphatidylcholine
aPTT	activated partial thromboplastin time
NCA	non-compartmental analysis
AUC	area under the curve
t_{1/2}	half-life
CL	clearance
V_{ss}	volume of distribution
MRT	mean residence time
MM	Michaelis-Menten

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Figure 1. Pharmacokinetics of free and PI-BDD FVIII

PK profiles of free and PI-BDD FVIII in HA mice after an i.v. bolus dose of 40 IU/kg.

Plasma samples were collected till 36 hr post dose and FVIII activity in recovered samples was measured using a two-stage chromogenic assay. Mean $\pm$ standard deviation of observed data is presented. The solid and dashed lines represent the fit of a 1 compartment model with MM elimination to free and PI-BDD FVIII data respectively. PI association prolongs plasma survival of BDD FVIII. PI-BDD FVIII had greater K_m value, consistent with the hypothesis of lower binding affinity to the primary clearance receptor LRP. * No FVIII activity was detected in the 36hr samples from free BDD FVIII treated animals.

Figure 2. Projected human PK of free and PI-BDD FVIII

Projected human PK profiles of free (solid) and PI-BDD FVIII (dashed) after a 50 IU/kg dose. Profiles were obtained by correcting the normalized Wajima curves with human PK parameters predicted using an 'informed scaling' approach. The observed disposition of free BDD FVIII in humans was obtained from literature and is shown for reference (intermittent line).

Figure 3. Hemostatic efficacy of free and PI-BDD FVIII

Efficacy of free and PI-BDD FVIII in HA mice after an i.v. bolus dose of 40 IU/kg. Tail vein transection assay was performed on FVIII treated mice till 36 hr post dose. Mean $\pm$ standard error of volume of blood loss at 0.5, 20, 24 and 28 hr by free and PI-BDD FVIII treated animals is presented. FVIII treatment corrected bleeding phenotype to normal levels immediately post dose. There was a return to baseline over time which was more gradual in PI-BDD FVIII treated animals. Volume of blood lost by PI-BDD FVIII treated animals at 20, 24 and 28 hr was notably less than free BDD FVIII treated animals but statistical significance could not be established.

Figure 4.

a- Overlay of PK and PD profiles

4a- PK and PD profiles after free BDD FVIII treatment. Bleeding was uninhibited by free BDD FVIII levels below 0.1IU/mL.

b- Overlay of PK and PD profiles

4b- PK and PD profiles after PI-BDD FVIII treatment. Partial hemostatic efficacy was retained till FVIII levels fall below 0.01IU/mL.

Figure 5.

a – Immunogenicity of free and PI-BDD FVIII

5a- Inhibitory titers were determined by Bethesda assay in HA mice treated intravenously for 4 weeks with either free or PI-BDD FVIII (n=8/group).

Immunogenicity of free and PI-BDD FVIII

5b-Total titers in these animals were determined using an ELISA. Mean and standard deviation are shown. PI-BDD FVIII showed a strong trend towards being less immunogenic but the difference was not statistically significant.

Figure 6. Projected clinical benefits of PI-BDD FVIII

Simulated clinical PK profiles and projected impact of PI association on prophylactic BDD FVIII administration. Free BDD FVIII is currently administered thrice weekly in order to maintain trough plasma concentrations of 0.01 IU/mL. PI-BDD FVIII has a longer circulating half-life and retains hemostatic efficacy at lower concentrations (10-fold lower efficacy threshold was observed in mice). PI-BDD FVIII can therefore be administered every 5–7 days.

Table 1

NCA parameter estimates

	C _{max} (IU/mL)	AUC _{0-∞} (h × IU/mL)	$\overline{CL}$ (mL/h × kg)	V _{ss} (mL/kg)	MRT (h)	T _{1/2} (h)
Free BDD FVIII	1.29	8.67	4.52	35.5	7.29	5.24
PI-BDD FVIII	0.77	11.1	3.55	38.2	10.2	7.89

Table 2

PK model parameter estimates

	V (CV%) (mL/kg)	V_{max} (fixed) (IU/h × kg)	Km (CV%) (IU/mL × kg)
Free BDD FVIII	45.1 (11.6)	4.13	0.478 (8.89)
PI-BDD FVIII	47.0 (8.67)		0.664 (8.26)