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Nonclinical analysis of the safety, pharmacodynamics, and pharmacokinetics of plasma-derived human FXIII concentrate in animals

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Abstract

Factor XIII (FXIII) is a coagulation protein which plays a major role in hemostasis by covalently cross-linking fibrin molecules, thereby stabilizing the blood clot and increasing resistance to fibrinolysis. FXIII deficiency, either congenital or acquired, is associated with spontaneous bleeding, increased bleeding time, and poor wound healing. Purified plasma-derived human FXIII concentrate (pd hFXIII) has been available since 1993 for therapeutic use in congenital FXIII deficiency. This set of nonclinical investigations aimed to evaluate the pharmacodynamic effects and assess the safety profile of pd hFXIII. The efficacy and safety of pd hFXIII were evaluated by pharmacodynamic, pharmacokinetic, and toxicity studies in mice and rats, safety pharmacology studies in dogs, neoantigenicity study, local tolerance, and thrombogenicity tests in rabbits. Administration of pd hFXIII resulted in the correction of deficits in clot formation kinetics and strength as measured by thromboelastometry, and was not associated with thrombus formation up to 350 IU/kg in FXIII knockout mice. There was no production of neoantigens resulting from the viral elimination manufacturing steps detected, and no adverse reactions were observed in toxicity studies with single doses up to 3550 IU/kg in mice and 1420 IU/kg in rats; nor from repeat doses of 350 IU/kg in rats. In addition, local tolerance tests revealed a good tolerability profile in rabbits. Overall, this data showed that pd hFXIII was well tolerated and pharmacodynamically active in preclinical animal models, supporting pd hFXIII as a therapy for FXIII deficiency.

Abbreviations

AUC, area under curve; CFT, clot formation time; Cmax, maximum concentration; CT, clotting time; ELISA, enzyme-linked immunosorbent assay; FFP, fresh frozen plasma; FXIII-A_2, Factor XIII A subunits; FXIII, AgHuman Factor XIII Antigen; FXIII-B_2, Factor XIII B subunits; FXIII, Factor XIII; i.a., intraarterial; i.v., intravenous; IU, international units; KO, knockout; LI60, lysis index at 60 min; p.v., paravenous; pd hFXIII, plasma-derived human FXIII; PLT, platelet count; s.c., subcutaneous; TEG-MA, thromboelastography maximum amplitude.

Introduction

Coagulation factor XIII (FXIII) is a plasma proenzyme composed of four proteins, two A subunits (FXIII-A_2) and two B subunits (FXIII-B_2), which plays a major role in the clotting cascade (Muszbek et al. 1996, 2011). FXIII is converted by calcium and thrombin into the active enzyme FXIIIa, which covalently links fibrin molecules to each other, and other molecules to fibrin (Muszbek et al. 1996, 2011). In this way, the loose fibrin polymer is converted into a highly organized structure with increased tensile strength, which is firmly anchored to the site of the wound and is resistant to fibrinolysis (Anwar and Miloszewski 1999). Other important roles of FXIII

include the promotion of angiogenesis during wound healing and the maintenance of pregnancy (Hsieh and Nugent 2008; Muszbek et al. 2011).

Congenital FXIII deficiency is a rare bleeding disorder with an estimated frequency of one in every 1–5 million live births (Hsieh and Nugent 2008; Lusher et al. 2010; Levy and Greenberg 2013; Odame et al. 2014). Two types of FXIII deficiency have been described: FXIII-A deficiency due to either a reduction in FXIII-A synthesis (Type I) or function (Type II); and FXIII-B deficiency, which is much less common (<5% of reported FXIII deficiency cases) and tends to be associated with milder bleeding symptoms (Hsieh and Nugent 2008; Levy and Greenberg 2013; Odame et al. 2014). Recent studies indicate that FXIII-B mutations may make up a greater proportion of the milder, heterozygous FXIII deficiency cases, with mutation screening of 200 patients referred for genetic analysis due to reduced FXIII activity identifying 23 novel missense mutations (16 in *FXIII-A* and 7 in *FXIII-B*) associated with heterozygous FXIII deficiency (Biswas et al. 2014). Acquired FXIII deficiency can arise as a result of disorders resulting in overconsumption or reduced synthesis of FXIII or through antibody development against FXIII (Levy and Greenberg 2013). FXIII deficiency is associated with a high risk of potentially life-threatening intracranial hemorrhage, poor wound healing, spontaneous abortion, and a tendency toward spontaneous and severe bleeding after trauma and surgery (Nugent 2012; Levy and Greenberg 2013; Odame et al. 2014).

There are several options available for treatment of patients with FXIII deficiency. Cryoprecipitate or fresh frozen plasma (FFP) contain FXIII; however, these treatments carry a persistent risk of transmission of blood-borne infections (Nugent 2006; Odame et al. 2014) or increased risk of transfusion-related lung injury (Murad et al. 2010). A highly purified, pasteurized plasma-derived FXIII concentrate (pd hFXIII), which has been available in Europe since 1993 (provided under the trade names Fibrogammin P, Corifact™ in the US and Canada, or Cluvot in several EU countries; CSL Behring GmbH, Marburg, Germany), may also be used for FXIII replacement. Additionally, a recombinant FXIII subunit-A molecule expressed in *Saccharomyces cerevisiae* is available (rFXIII-A; catridecagog, Novo Nordisk A/S, Copenhagen, Denmark), and has been evaluated for replacement therapy in congenital FXIII deficiency (Inbal et al. 2012). Several studies have demonstrated the efficacy and safety of both prophylactic and on-demand treatment with pd hFXIII for congenital FXIII deficiency caused by mutation in the A domain as well as the B domain, with reductions in the incidence of both spontaneous and postoperative bleeding (Lusher et al. 2010; Dreyfus et al. 2011; Nugent 2012; Ashley et al. 2015).

The current set of data describes a comprehensive nonclinical analysis of pd hFXIII investigating the safety profile of FXIII concentrate as a treatment for FXIII deficiency. These studies represent historical data and were undertaken as part of the clinical development of Fibrogammin.

Materials and Methods

All studies described were carried out in line with international guidelines applicable to the nonclinical safety assessment of human plasma-derived products, and were approved by local governmental ethics committee. Relevant international guidelines for animal care and treatment were complied with.

The choice of animal species used for these studies (i.e., dogs, mice, rabbits, and rats) was supported by the pharmacological activity of human FXIII in these species (van Giezen et al. 1993; Karges et al. 1994; Ogawa et al. 1995; Dickneite et al. 2002; Lauer et al. 2002; Inbal et al. 2005). Coagulation factors are highly conserved between species and human FXIII as used in the present studies has been shown to be activated by its target animal serine protease, thrombin (Karges et al. 1994). An overview of the studies conducted together with animal specifications and dose ranges of pd hFXIII (CSL Behring GmbH, Marburg, Germany) tested is shown in Table 1.

Male and female animals were included in the safety pharmacology study in beagle dogs and in the single-dose toxicity studies in mice and rats, in order to comply with regulatory requirements. An in-house computer program was used in order to randomly assign animals to treatment groups; animals were then given a unique identification number and identified by color marks. For the pharmacodynamic and pharmacokinetic studies, the doses of pd hFXIII used correspond to a standard human dose of 40 IU/kg every 4 weeks (prophylaxis). The standard human dose is as recommended in the product insert for Fibrogammin, and has been supported by pharmacokinetic studies in humans which demonstrated this regimen maintained through FXIII activity at or above 10% of normal (Nugent et al. 2015). In order to accurately evaluate the toxicity of pd hFXIII, multiple doses of the standard human dose were used to investigate worse case scenarios for the safety pharmacology study and all toxicity studies.

Pharmacodynamic and pharmacokinetic studies

The pharmacodynamic effects of pd hFXIII were evaluated using thromboelastometry (ROTEM) measurements in whole blood from FXIII knockout (KO) and C57Bl/

Table 1. Characteristics of the animals used and the doses of plasma-derived human factor XIII concentrate administered in each of the studies.

Study	Species/strain/sex	Human FXIII concentrate
Pharmacodynamic and pharmacokinetic studies		
1	Mouse/FXIII KO/f and m or C57Bl/6J:7/f	20 IU/kg–i.v.
2	Rat/CD/f and m	100 IU/kg– i.v.
Safety pharmacology		
3	Dog/Beagle/f and m	10/35/70 IU/kg–i.v.
Single- and repeat-dose toxicity		
4	Mouse/NMRI/f and m	710/1775/3550 IU/kg–i.v.
5	Rat/Wistar/f and m	71/710/1420 IU/kg–i.v.
6	Rat/Wistar/f and m	35/100/350 IU/kg/day–i.v.
Neoantigenicity		
7	Rabbit/NZW/f	2.5 mg–s.c.
Local tolerance		
8	Rabbit/CHB/f and m	284 IU/4 mL–i.v. or i.a. 7.1 IU/0.1 mL–p.v.
Thrombogenicity		
9	Rabbit/NZW/f	35/100/350 IU/kg–i.v.

f, female; FXIII, Factor XIII; IU, international units; i.a., intraarterial; i.v., intravenous; KO, knockout; m, male; p.v., paravenous; s.c., subcutaneous.

6J:7 mice (Study 1). The pd hFXIII was intravenously (i.v.) administered via single injection into the lateral tail vein (20 IU/kg). Whole blood was then withdrawn in 10% v/v sodium citrate by puncturing the Vena cava under deep anesthesia one hour after treatment. In general, the clotting curve is characterized by the clot firmness over time using main thromboelastography parameters like clotting time (CT – represents the time between start of the test and achievement of 2 mm amplitude), clot formation time (CFT – the time between achievement of 2 mm amplitude and achievement of 20 mm amplitude), maximum clot firmness (MCF – constitutes the maximum clot firmness during the measurement), alpha angle (the tangent of the clotting curve which crosses the 2 mm amplitude), and lysis index at 60 min (LI60 – the relation between the amplitude to MCF at 60 min after CT, calculated by amplitude divided by the MCF at CT 60 min and multiplied by 100). Coagulation was activated by the extrinsic pathway using tissue factor containing EXTEM reagent (Tem International GmbH, München, Germany).

The pharmacokinetic profile of pd hFXIII was determined in rats (Study 2). Eight male and eight female rats per group received a single i.v. injection of 100 IU/kg of pd hFXIII into the lateral tail vein. Blood samples (with sodium citrate as the anticoagulant) were drawn at 10 min, and 2 h, 8 h, and 24 h after administration. Human Factor XIII Antigen (FXIII:Ag) concentration was determined using a validated, commercially available ELISA technique (Biozol Diagnostica Vertrieb GmbH, Eching, Germany) with a lower limit of quantification of 0.0625 IU/mL and a variation coefficient of 5%.

Safety pharmacology

One study investigated safety pharmacological parameters in beagle dogs (Study 3; $n = 2$, 1 female and 1 male). Before the start of the treatment procedure the dogs were narcotized with Nembutal (0.5 ml/kg i.v.) and catheters for injection were placed into the vena jugularis. The dogs received three successive doses of pd hFXIII (10, 35, and 70 IU/kg body weight) by i.v. injection into a jugular vein, at 5 min intervals. Following the treatment procedure the animals were kept under observation for 1 h; during the observation period circulatory, respiratory, coagulation/fibrinolysis (by thromboelastography [TEG]), hematological and clinical chemical parameters were examined. Samples for hematology (plasma), coagulation/fibrinolysis (whole blood), and clinical chemistry (serum) were collected at the following observation times: before treatment; after first, second, and third treatments; and 15, 30, and 60 min after the third treatment.

Single- and repeat-dose toxicity

Three studies evaluated the toxicity of pd hFXIII. Single-dose toxicity studies were performed in mice (Study 4; $n = 10$, 5 male and 5 female per group) and rats (Study 5; $n = 10$, 5 male and 5 female per group). The pd hFXIII was i.v. administered (slow bolus) at escalating dose levels (mice: 710, 1775, 3550 IU/kg; rats: 71, 710, 1420 IU/kg). Administration of isotonic saline served as a negative control. Toxicological parameters were monitored, including survival, clinical signs, body weight and gross pathology data. Clinical observations were performed on day 1 (day of treatment) at 15 min and 1 h post dosing, and daily thereafter for the next 14 days. All animals were weighed on study days 1, 2, 8, and 15, and were sacrificed on day 15 with gross necropsy examinations.

A 14-day i.v. toxicity study was performed in Wistar rats (Study 6; $n = 20$, 10 male and 10 female per group). The pd hFXIII was administered via tail vein injection at dose levels of 35, 100 and 350 IU/kg/day as a single daily dose; administration of isotonic saline served as a negative control. Half of the animals per sex and treatment group were sacrificed after 5 days of treatment (interim sacrifice) and the remaining animals were sacrificed after 14 days of treatment. The animals were then necropsied and examined post-mortem, and histological examination was performed on organs and tissues. In addition, three rats per sex and group were used for toxicokinetic

evaluation. Clinical signs, food consumption, and body weight were recorded periodically during acclimatization, treatment, and observation period. Ophthalmoscopic examination was performed during acclimatization, on day 5 and during week 2. On days 6 and 15 blood samples were withdrawn for hematology and clinical chemical analysis, and urine samples were collected for urinalysis.

Neoantigenicity

The potential for neoepitope formation as a result of virus filtration step during the manufacturing process of pd hFXIII was investigated in one neoantigenicity study (Study 7) in rabbits. Three female rabbits were subcutaneously sensitized to pd hFXIII with doses of 2.5 mg (approx. 6 IU/kg) on days 0, 14, and 28, and the resultant serum was used for evaluation of neoantigenicity in rabbits using a previously described western blot method (Ronneberger 1986; Beyerle et al. 2014). Briefly, protein A purification of the IgG fractions was performed and serum eluates were used for western blot analysis in the presence and absence of Fibrogammin as blocking reagent.

Local tolerance

Local tolerance of pd hFXIII was investigated in rabbits using a previously described method (Study 8) (Beyerle et al. 2014), based on published recommendations (Jochims et al. 2003). The animals received pd hFXIII at doses of 284 IU/4 mL (124 IU/kg) for i.v. and intra-arterial (i.a.) or 7.1 IU/0.1 mL (3 IU/kg) for paravenous (p.v.) injection into the ear. Isotonic saline served as a negative control injected into the opposite ear of the same animal. Clinical observations and macroscopic evaluation were performed during the observation period of 72 h, after necropsy macroscopic and histopathological investigations as well as clinical observations were conducted.

Thrombogenicity

The thrombogenicity of pd hFXIII was investigated by a modified Wessler test (Giles et al. 1980). Briefly, at the start of the experimental work, rabbits (n = 3 females per group) were anesthetized with sodium pentobarbital (31 mg/kg, 0.5 mL/kg) by i.v. injection into the marginal vein of the ear (Study 9). Animals were then treated with pd hFXIII at single doses of 35, 100 or 350 IU/kg; isotonic saline served as a negative control. The duration of administration depended on the total administration volume (fixed concentration of 72 IU/mL, perfused at a rate of approximately 1 mL/10 sec). The detailed methodology to produce and evaluate the stasis and venous thrombus formation has previously been described (Dickneite et al. 2009).

Statistical analysis

Pharmacodynamic and pharmacokinetic studies

Pharmacodynamic and pharmacokinetic variables were estimated by using descriptive statistics and two-sided t-test (for pharmacodynamics study) and Welch's t-test for two samples with unequal variances (pharmacokinetic).

Results

Pharmacodynamic and pharmacokinetic studies

When assessing the pharmacodynamic efficacy of pd hFXIII (Study 1), pd hFXIII effectively corrected the animals' hemostatic deficit assessed by measurement of thromboelastographic parameters (Fig. 1 A–E). After 60 min, pd hFXIII was able to correct clotting time (Fig. 1A), CFT (Fig. 1B), MCF (Fig. 1C), the corresponding alpha angle (Fig. 1D) and the LI60 back to control levels seen in C57Bl/6J wild-type mice. Statistical significance was shown for CFT, MCF, alpha angle and LI60 compared to FXIII KO treated with isotonic saline. A pharmacokinetic study in rats (Study 2) was performed (Fig. 2). The in vivo recovery was calculated on the assumption of a total plasma volume of 40 mL/kg in rats. With a maximum concentration (Cmax) of 1.535 $\pm$ 0.167 IU/mL the in vivo recovery was approximately 60% and the area under the curve ($AUC_{0-24\ h}$) 11.2 $\pm$ 1.27 (h*IU/mL).

Safety pharmacology

The administration of pd hFXIII was well tolerated in beagle dogs (Study 3). Some small alterations were observed in circulation and respiration. Hematology evaluation demonstrated a decrease in platelet counts following the second injection, which became more evident after the third treatment (Fig. 3A). FXIII activity was moderately elevated following the end of treatment (Fig. 3B). TEG maximum amplitude was moderately narrowed following the second and third injection (Fig. 3C). Serum lactate concentration was moderately lowered after the third injection and became more evident toward the end of the observation period (Fig. 3D). Due to low number of animals per group there were no statistically significant effect of treatment on the parameters evaluated.

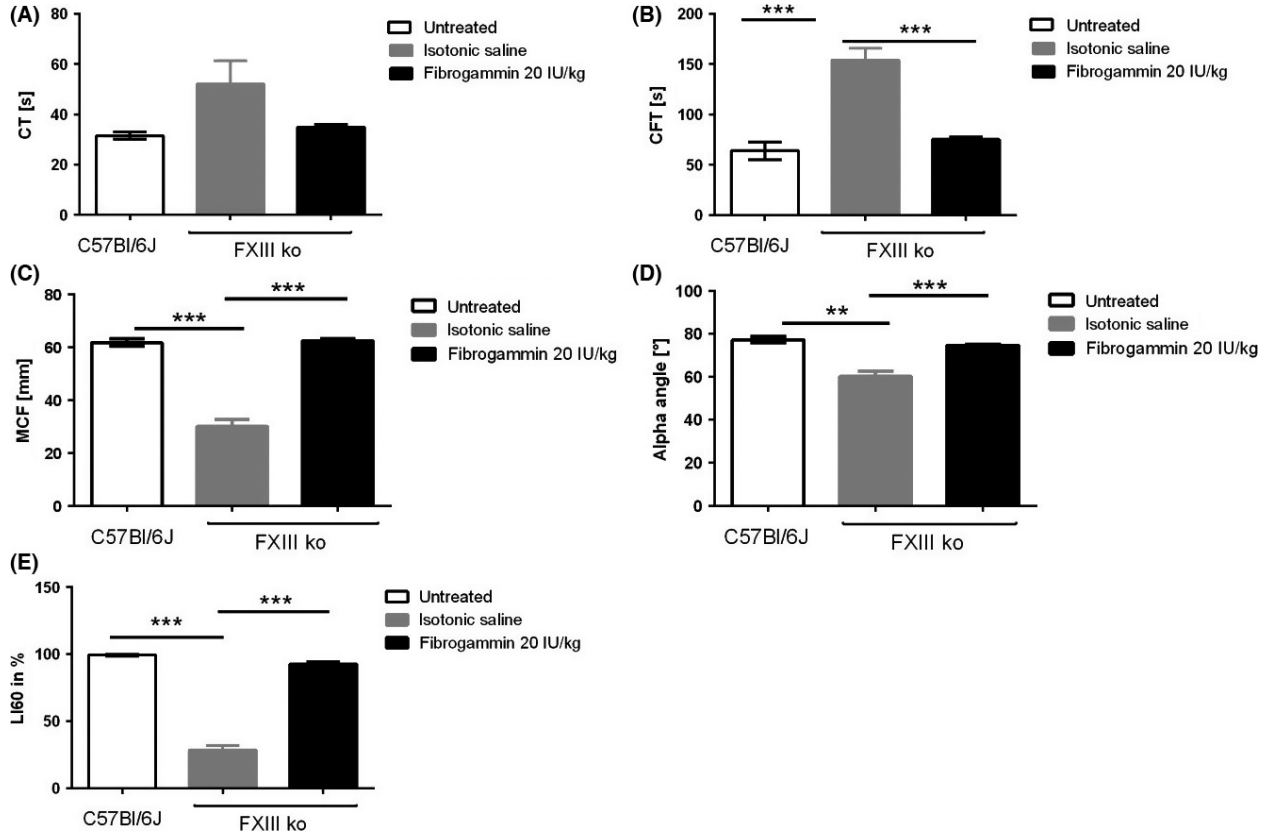

Figure 1. Alterations in the thromboelastographic parameters (A) clotting time (CT), (B) clot formation time (CFT), (C) maximal clot firmness (MCF), (D) the alpha angle, and (E) lysis index at 60 min (LI60) in FXIII knockout mice following administration of 20 IU/kg plasma-derived FXIII concentrate (Fibrogammin) (Study 1); values are expressed as mean ±SEM; one-way ANOVA by applying Dunnett's multiple comparisons test was performed using a confidence interval of 95%; *P*-values are shown for statistical significance with $^{**}P < 0.01$; $^{***}P < 0.001$. CFT, clot formation time; CT, clotting time; KO, knockout; LI60, lysis index at 60 min; MCF, maximal clot firmness.

Single- and repeat-dose toxicity

The single-dose studies in mice and rats (Studies 4 and 5) demonstrated that i.v. administration of dose levels up to 89-fold (3550 IU/kg body weight) and 36-fold (1420 IU/kg body weight) higher than the highest intended clinical single dose (40 IU/kg body weight), respectively, were well tolerated without any adverse reactions. Criteria evaluated for general toxicological effect included survival, clinical signs, body weight and gross pathology data.

Repeated administrations to rats for a period of up to 2 weeks of pd hFXIII at dose levels up to 350 IU/kg per day (i.e., 18-fold above the highest recommended clinical repeated dose of 20 IU/kg per day), showed no evidence of systemic toxicity (Study 6). All animals survived the scheduled observation period of 2 weeks following substance administration. Microscopical findings were restricted to the injection site, and included thrombosis, vasculitis, perivasculitis, and fibrosis. A higher incidence and/or mean severity was seen in the FXIII-treated groups compared with controls, which could be related to the application procedure. Toxicokinetic evaluation of human FXIII:Ag concentration was carried out (Fig. 4). FXIII:Ag concentration was statistically significantly increased in all animals treated with pd hFXIII with the highest FXIII:Ag concentration seen 14 days after treatment in the high dose (350 IU/kg) group (Fig. 4). Following i.v. administration of 35 IU/kg Fibrogammin, FXIII:Ag concentration was between 20 and 30 mIU/mL, at 100 IU/kg Fibrogammin the FXIII:Ag plasma concentration ranged between 50 and 150 mIU/mL, and at 350 IU/kg Fibrogammin the FXIII:Ag concentration was detected to be between 900 and 3300 mIU/mL. This compares with published studies in human subjects, in which median FXIII activity increased to 1110 mIU/mL following i.v. administration of 30 IU/kg (Korte et al. 2009) and a peak FXIII:Ag concentration of 1045 mIU/mL was reached in patients receiving i.v. 40 IU/kg every 4 weeks (Ashley et al. 2015). The FXIII recovery in these studies was higher than 100%

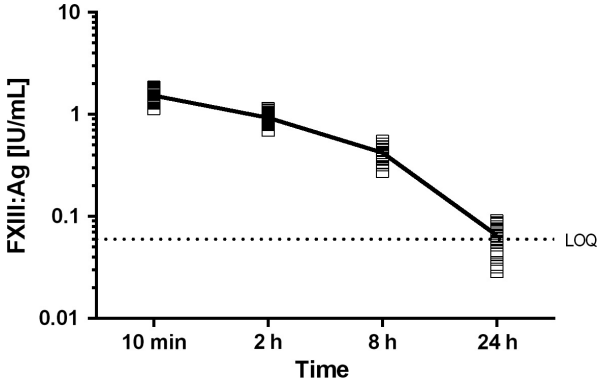

Figure 2. Pharmacokinetic evaluation of human FXIII:Ag concentration in rats following i.v. injection of 100 IU/kg human plasma-derived FXIII concentrate (Study 2); results for male and female animals have been combined due to minimal variation between the sexes and for ease of presentation. Limit of quantification (LOQ) is given as a dotted line.

(105% for Ashley et al. and 150% for Korte et al.) compared to our animal study where the in vivo recovery was approximately 60%.

Neoantigenicity

Neoantigenicity study based on Western blot analysis following immunization of rabbits (Study 7) confirmed the absence of new antigenic determinants following introduction of an additional virus filtration step (data not shown).

Local tolerance

Local tolerance of pd hFXIII was evaluated in rabbits by the intended i.v. route, or via i.a. or p.v. administration, intended to represent possible accidental administration in the clinic (Study 8). None of the injection routes led

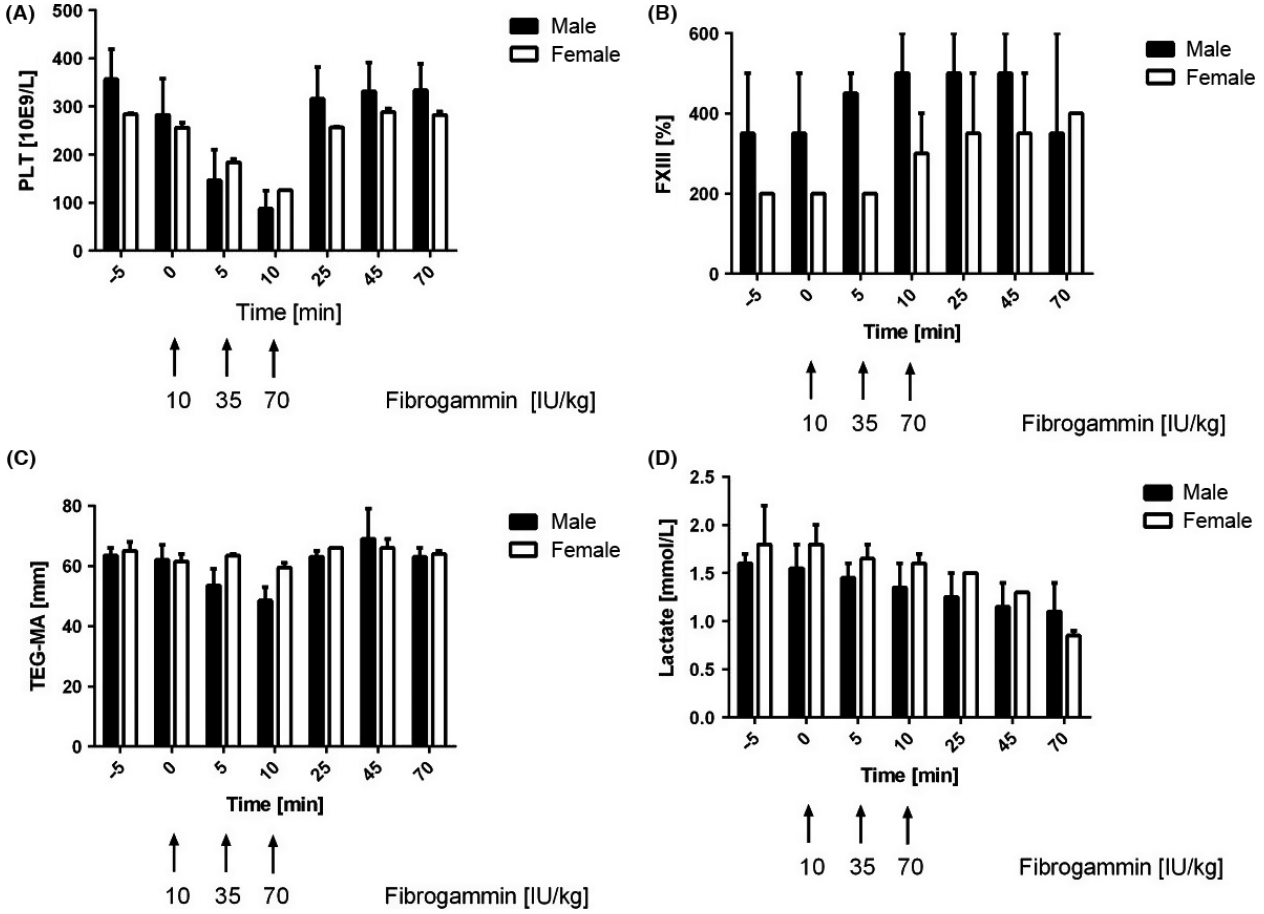

Figure 3. Alterations in the hematological and coagulation parameters (A) platelet count (PLT), (B) FXIII activity [% of the norm] in comparison to standard human plasma, (C) thromboelastography maximum amplitude (TEG-MA) and (D) serum lactate level following successive dosing with Fibrogammin in dogs (Study 3). Statistical analysis revealed no statistically significant difference due to very low numbers of animals. FXIII, Factor XIII; PLT, platelet count; TEG-MA, thromboelastography maximum amplitude.

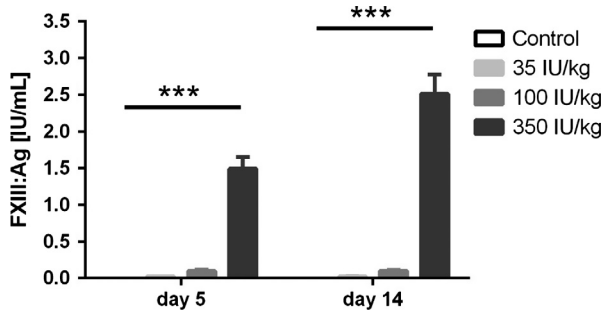

Figure 4. Toxicokinetic evaluation of human FXIII:Ag concentration in Wistar rats following administration of Fibrogammin (Study 6); values are expressed as mean ±SEM; one-way ANOVA by applying Dunnett's multiple comparisons test was performed using a confidence interval of 95%; *P*-values are shown for statistical significance with ***$P < 0.001$.

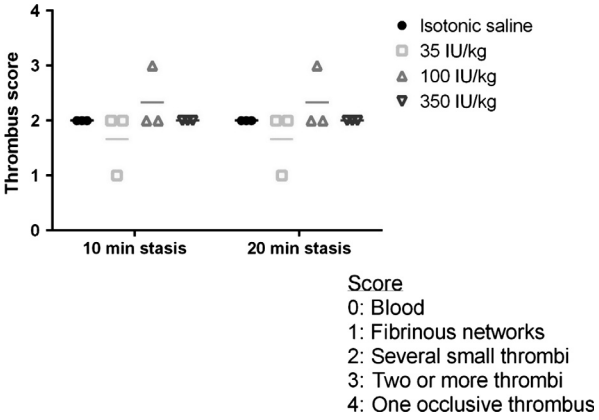

Figure 5. Thrombosis score in rabbits receiving Fibrogammin or isotonic saline (Study 10). Horizontal lines indicate median values. No statistical significance was detected by using descriptive statistical methods.

to clinical or histopathological changes at the injection site (data not shown). Therefore, pd hFXIII was regarded as well tolerated in rabbits.

Thrombogenicity

The modified Wessler test was used to investigate the thrombogenic activity after i.v. administration of pd hFXIII to rabbits (Study 9). Several small thrombi were detected in all groups and therefore these results were considered to be related to the treatment procedure. It was concluded that single i.v. administration of doses up to 350 IU/kg pd hFXIII showed no thrombogenic activity under the conditions of this study (Fig. 5).

Discussion

The nonclinical investigations presented here demonstrate the safety of human plasma-derived FXIII concentrate in different animal models. We found that the administration of pd hFXIII was well tolerated, with only small alterations in circulation and respiration observed in dogs over an observation period of 60 min; these effects were considered to be consequences from the narcosis state. No toxic effects were observed in either single- or repeat-dose toxicity studies, despite the administration of doses considerably higher than clinical recommendations (up to 3550 IU/kg in mice and 1420 IU/kg in rats) suggesting a large safety margin of 36- to 89-fold of the highest intended clinical single dose (40 IU/kg body weight). Additionally, a local tolerance study demonstrated no clinical or histopathological changes at the injection site following i.v., i.a. or p.v. administration. Thrombogenicity tests in rabbits showed no thrombogenic potential for pd hFXIII at doses as high as 350 IU/kg, which represents nearly 9-fold higher doses compared with the maximum clinical dose of 40 IU/kg for prophylactic treatment. It should be noted that higher dosing has been reported, for example, doses of 60–80 IU/kg have been administered to neonates; however, this dosing was not observed to trigger thrombotic events, and is still considerably lower than the dose of 350 IU/kg administered in our study (Naderi et al. 2014). When considering on-demand use during surgery, in a worst case scenario a second maximum clinical dose of 40 IU/kg may be administered, resulting in a total dose of 80 IU/kg over 8 days, while the recommended daily dosing for the treatment of hemorrhagic diathesis in acquired FXIII deficiency is a maximum of 20 IU/kg. Taking into account that the in vivo recovery was approximately 60% in our animal studies, the safety margins were reduced down to 21- to 53-fold for the single-dose toxicity studies and down to fivefold for the repeated dose toxicity and thrombogenicity study, but they were still considered as adequate to assess the safety and toxicity profile of pd hFXIII in the animal studies.

In all, these results indicate a favorable safety profile of pd hFXIII, a conclusion supported by a trial evaluating pharmacokinetics and safety of FXIII concentrate in humans with congenital factor XIII deficiency (Nugent et al. 2015). In this 12-week study, patients ($n = 14$) received prophylactic infusions of FXIII concentrate at a dose of 40 IU/kg on days 0, 28, and 56. Although two patients experienced possible treatment-related adverse events (elevated thrombin-antithrombin complex levels and elevated prothrombin in one patient, and elevated fibrin D-dimer levels in the second patient), no reports of thromboembolism, viral transmission, bleeding events, or treatment-related hypersensitivity were received. Nevertheless, the presented preclinical data would add value to the safety and toxicology profile of pd hFXIII and would support the development of recombinant FXIII concentrate.

In addition, in our pharmacodynamic study administration of Fibrogammin restored thromboelastometric parameters in FXIII KO mice to levels equivalent to those observed in wild-type mice within one hour. This confirms the findings of previous studies, which have shown that treatment of FXIII-A-deficient mice with pd hFXIII restored FXIII plasma activity, bleeding time (tail tip), and clot stabilization (as measured by thromboelastography) to normal or near-normal levels, demonstrating that human FXIII can functionally replace FXIII in mice (Lauer et al. 2002). Animal models have demonstrated biological activity of FXIII across a number of mammalian species, confirming the role of FXIII in clot formation/strength and wound healing. FXIII-deficient mice display delayed reepithelization and wound healing (Inbal et al. 2005), as well as delayed arrestment of tail-tip bleeding and impaired clot stabilization (Lauer et al. 2002). Similarly, reducing FXIII activity in rats via carbon tetrachloride-induced hepatic injury delays the healing of burns induced on the back skin (Ogawa et al. 1995). In this model, a significant negative correlation was observed between plasma FXIII activity and wound healing, and elevating FXIII activity by administration of FXIII concentrate significantly shortened the delay in wound healing. The role of FXIII in wound healing is further confirmed by the finding that the addition of FXIII concentrate to fibrin sealants improved fibrin cross-linking and clot strength; fibrin sealants are used in surgical practice to produce a firm, stable blood clot, and thereby reduce blood loss, attach cut tissues together, and support wound healing (Dickneite et al. 2002). It has also been shown that disseminated intravascular coagulation-induced organ damage is reduced in FXIII-A depleted rabbits in an animal model of severe inflammatory conditions, which the authors attribute to the reduced clot stability resulting from loss of FXIII activity (Lee et al. 2001). Additionally, congenital FXIII deficiency has been observed in dogs, resulting in episodes of excessive bleeding associated with trauma or surgical procedures (Kong et al. 2014).

A number of retrospective and prospective studies have demonstrated the clinical efficacy and safety of pd hFXIII for the treatment of congenital FXIII deficiency patients (Lusher et al. 2010; Dreyfus et al. 2011; Nugent 2012; Ashley et al. 2015; Janbain et al. 2015; Nugent et al. 2015), and this is an approved indication for the treatment. However, FXIII has also been shown to be effective for reducing bleeding in acquired FXIII deficiency, for example, reducing bleeding and transfusion requirements, and improving clot firmness during surgery (Godje et al. 2006; Korte et al. 2009). Preclinical animal studies have also been carried out using the recombinant human FXIII, rFXIII-A, in adult cynomolgus monkeys (Ponce et al. 2005). This study demonstrated that rFXIII-A was well tolerated with no observed toxicological effect with repeated intravenous doses up to 1136 IU/kg, and these data were used to support clinical dosing of 2–50 IU/kg in healthy and congenital FXIII deficiency patients. Subsequently, a phase III trial of rFXIII-A in 41 patients with FXIII-A subunit deficiency indicated that rFXIII-A is safe and effective in preventing bleeding episodes (Inbal et al. 2012).

Caution is required when translating these results from animals to humans due to the preclinical nature of the studies performed. It should also be noted that the low number of animals included may limit the relevance of these studies to humans. However, nonclinical studies of this type are necessary in animals in order to obtain the necessary safety information required to support study in human trials. While all studies were conducted in pharmacologically relevant species, an additional limitation is that most data reported here were derived from healthy, nonbleeding animals, and so may not accurately represent the clinical situation of FXIII deficiency.

Conclusion

This set of nonclinical studies support the efficacy and favorable safety profile of pd hFXIII. The data presented indicate that pd hFXIII is well tolerated and capable of correcting clot formation in FXIII-deficient models, supporting the conclusion that pd hFXIII can be considered a useful tool for the treatment of FXIII deficiency.

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Disclosures

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