CLINICAL INVESTIGATION

Recombinant factor XIII (rFXIII): a novel treatment of congenital factor XIII deficiency

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Factor XIII (FXIII) is composed of two catalytic A subunits and two carrier B subunits. Following activation by thrombin FXIII becomes plasma transglutaminase, which cross-links the γ-glutamyl-ε-lysine residues of fibrin chains stabilizing fibrin clot. Congenital deficiency of factor XIII results in a severe life-long hemorrhagic disorder, abnormal wound healing in about 30% of patients and recurrent abortions. Most of the FXIII deficiency patients have mutations in the F13A gene. Only few mutations in F13B gene have been published. Plasma-derived concentrate of factor XIII used to be the treatment of choice. Recently, recombinant FXIII concentrate has been developed and tested in multinational clinical studies. This new product appears to be safe and appropriate for life-long prophylactic treatment of patients with FXIII A deficiency.

Keywords: congenital FXIII deficiency • recombinant FXIII concentrate

Factor XIII molecular structure & function

Factor XIII (FXIII) is part of the family of protransglutaminases and plays a crucial role in the final step of clot formation. It circulates in plasma at an average concentration of 22 μg/ml and has a half-life of 9–14 days [1].

Plasma FXIII molecule comprise an Mr ~340,000 heterotetramer composed of two A and two B noncovalently linked subunits (FXIII-A_2B_2). The A subunit (FXIII-A_2, Mr ~82,000) is synthesized in megakaryocytes and packed into platelets during their formation. An intracellular form (cFXIII-A_2) has also been identified in monocytes, tissue macrophages/histiocytes, liver, chondrocytes and osteoblasts [2–6]. The B subunit (FXIII-B_2, Mr ~76,500) is synthesized in the liver and secreted as a dimer into plasma [7]. The A subunit contains the catalytic domain, and the B subunit serves as the carrier and regulatory protein. Their assembly probably occurs in the circulation and is functionally essential, because in the absence of the B subunit, the A subunit is spontaneously activated and cleared. Recently, Katona *et al.* [8] determined that the affinity between the A and B subunits is substantially higher than previously thought, confirming that 99% of plasma FXIII-A_2 is in complex with FXIII-B2.

FXIII is activated by thrombin in the presence of calcium in the final stage of the blood coagulation cascade. Thrombin hydrolyzes the R37-G38 bond in each A subunit, releasing a 37-amino acid activation peptide at the N terminus (A'); calcium (Ca^{++}) induces conformational changes that lead to dissociation of the B subunits. Both steps are enhanced by fibrin polymers [9]. As a result, the enzymatically active Cys314 residue on the truncated A dimer (G38-FXIII-A_2*) is exposed, allowing for cross-linking the peptide-bound glutamine residue (γ-glutamyl) and adjacent peptide-bound lysine residue (-lysine) of the fibrin α and γ chains. This creates an insoluble fibrin network, preventing clot degradation [10]. FXIII also cross-links antiplasmin to fibrin to strengthen the clot [11].

X-ray crystallography studies have provided further insight into the structure of FXIII and its relationship to other cysteine proteinases. Researchers showed that the A subunit may be further divided into four sequential domains, namely, β-sandwich

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(Glu43 – Phe184), catalytic core (Asn185 – Arg515), barrel 1 (Ser516 – Thr628) and barrel 2 (Ile629 – Arg727). Cys314 is located in a catalytic triad with His373 and Asp396, forming one hydrogen bond with His373, while the other nitrogen atom forms a hydrogen bond with Asp396 [12].

The noncatalytic FXIII-B subunit regulates the calcium-dependent activation of FXIII and stabilizes the A subunit [13] by protecting it from proteolysis, thereby prolonging its half-life [14]. It is composed of ten homologous consensus or 'sushi' repeats [15], each approximately 60 amino acids long and containing four disulfide bonds, Cys1–4 (with Cys1 linked to Cys3 and Cys2 linked to Cys4).

The gene for FXIII-A is located on chromosome 6p24-p25 [16,17]. It spans 177 kb and contains 15 exons. Exon I comprises a 5′ noncoding region; exon II encodes the activation peptide (amino acids 1–37) [17]. The gene for FXIII-B has been localized to chromosome 1q31-q32.1 [18]. It spans 28 kb and contains 12 exons. Exon I encodes the leader sequence; exons II-XI each encode a single 'sush' repeat [18].

There is an increasing body of evidence to support the multiple functions of FXIII beyond its role in hemostasis. These functions include: angiogenesis through downregulation of thrombospondin1 (TSP-1) [19]; bacterial immobilization through cross-linking of bacterial surface proteins to fibrin within a plasma clot [20]; wound healing through cross-linking of extracellular matrix proteins and enhancement of monocyte and fibroblast proliferation, migration [21] and phagocytosis [22]. Although at present, the main indication for FXIII treatment is in FXIII deficiency, its administration in a series of clinical conditions including bacterial infections and wound healing may provide a potential clinical benefit.

Furthermore, researchers have identified several other substrates for activated FXIII including factor V, plasminogen activator inhibitor-2, collagen, thrombospondin, von Willebrand factor, vinculin, vitronectin, fibronectin, actin, myosin and lipoprotein(a) [23].

Congenital FXIII deficiency

Congenital FXIII deficiency is a rare autosomal recessive bleeding disorder. It was first described 54 years ago in a Swiss boy with bleeding diathesis and impaired wound healing [24] and has since been reported in more than 500 patients [25].

Most congenital FXIII deficiency is caused by FXIII-A subunit deficiency; this occurs at a frequency of approximately 1 in 2 million [26]. Congenital deficiency of the FXIII-B subunit, which has been reported in only 11 patients, is a rare cause of clinically significant FXIII deficiency [27]. Among the mutations causing FXIII-A deficiency, 50% are missense mutations and the rest are deletions/insertions, splice site and nonsense mutations.

Clinical manifestations include umbilical stump bleeding during the first few days of life, postoperative bleeding and intracranial hemorrhage – more frequent in FXIII deficiency than in other inherited bleeding disorders – in addition to ecchymoses, hematomas and prolonged bleeding following trauma [28]. Bleeding into the joint spaces or muscles also occurs, but less often than in hemophilia. About 30% of FXIII-deficient patients have delayed wound healing [25]. Habitual pregnancy loss has been reported in affected women and FXIIIA-deficient rats, perhaps due to intrauterine bleeding [29]. The reported impaired formation of the cytotrophoblastic shell, perhaps due to deficient fibrin/fibronectin cross-linking at the implantation site, may lead to placental detachment [30].

Diagnosis of FXIII deficiency

In patients with suspected FXIII deficiency, the usual coagulation tests (prothrombin time, activated partial thromboplastin time, thrombin time) are normal, so clinicians need to test directly for plasma FXIII-A and FXIII-B levels. In patients deficient in FXIII-A, this subunit is absent or low in plasma and platelets. In patients deficient in FXIII-B, the FXIII-A level is decreased by 5–40% in plasma and normal in platelets [31].

Traditionally, screening for FXIII deficiency was performed by incubating the patient's plasma with thrombin and calcium and testing the formed clot for increased solubility in 5 M urea, diluted monochloroacetic acid or acetic acid. However, this approach is feasible only in cases of severe FXIII deficiency (<0.5–2% activity) and can be false positive in patients with fibrinogen deficiency. Furthermore, solubility tests are poorly standardized, and their sensitivity depends on the features and concentrations of the solubilizing agent as well as the concentration of fibrinogen. They are not recommended for diagnosis of Factor XIII deficiency. TEG is not standardized and is not recommended as well. Therefore, the solubility tests are being largely replaced by quantitative photometric FXIII activity assays that measure the incorporation of fluorescent or radioactive amines by proteins [32]. Yet this method too has an important disadvantage, because the commercial assays are inaccurate at FXIII activity levels between 0 and 10% of normal, potentially leading to misdiagnosis of severe deficiency. To counter this problem, researchers recommend to subtract a plasma blank [33]. For this reason, more sensitive photometric assays with a detection limit of greater than 0.6% are being developed (Reanal-Ker, Buda-

pest, Hungary), in addition to specific ELISA tests of FXIII-A, FXIII-B and FXIII-A_2B_2 antigen levels [34].

Treatment of FXIII deficiency

To prevent the severe bleeding associated with congenital FXIII deficiency, affected patients require monthly replacement therapy. Prophylactic treatment has yielded highly satisfactory results owing to the long half-life of FXIII (9–14 days) and the need for only small quantities (~5%) for effective hemostasis. Although fresh-frozen plasma and cryoprecipitates are inexpensive sources of FXIII and were used in the past for replacement therapy [35,36], they pose a risk of blood-borne infections and allergic reactions. Furthermore, their potency and pharmacokinetics are unknown.

An alternative, heat-treated, plasma-derived FXIII concentrate (Fibrogammin® P, CSL Behring, GmbH, Marburg, Germany) has been available in Europe since 1993 and was approved by the US FDA in 2011 under the name Corifact™. The concentrate contains the plasmatic heterotetrameric form of FXIII (FXIII-A_2B_2) and is therefore suitable for the treatment of both FXIII-A and FXIII-B deficiency.

Early reports found Fibrogammin P/Corifact to be effective at doses of 10–20 U/kg, administered intravenously every 4–6 weeks [35]. During pregnancy, more frequent injections were necessary to prevent pregnancy loss [36]. Two small retrospective studies reported that in patients treated with Fibrogammin P, the mean number of spontaneous bleeds decreased considerably, from 2.5 events in the year before treatment onset to 0.2 events during the first year of treatment [37], and from 4.2 ± 1.5/year before treatment to 0.2 ± 0.2/year during regular treatment for 10–19 years [38], with no cases of life-threatening (including intracerebral) hemorrhage [38]. These results were supported by a long-term prospective study based on retrospective data [39]. More recently Nugent [40] conducted a prospective multicenter, open-label study in which 41 patients with congenital FXIII deficiency were treated with 40 IU/kg Fibrogammin P/Corifact every 28 days for 52 weeks. A total of five spontaneous bleeding episodes not requiring treatment occurred, reaching the mean spontaneous bleeding rate of 0.3 bleeds per patient per year. Nine of 41 patients reported trauma-associated bleeding episodes. All over, the concentrate was well tolerated and an efficacious level of FXIII activity has been achieved in all patients.

Recombinant FXIII concentrate

It is noteworthy that the virucidal procedure that is part of the manufacturing process of Fibrogammin P/Corifact does not eliminate the risk of transmission of nonlipid-enveloped pathogens, such as parvovirus B19 and hepatitis A. It also does not destroy prions, and there is currently no screening test for blood donors for the prion that causes Creutzfeldt-Jakob disease. Therefore, given that affected patients require life-long treatment purified recombinant FXIII concentrate has a substantial advantage with regard to patients' safety. The USA price for NovoThirteen (called Tretten in the USA) is 13.3 US$/IU. The USA price for Corifact is 8.6$/IU [41].

A new recombinant FXIII (rFXIII) concentrate comprising A2 homodimers (rFXIII-A2) has been developed. This concentrate was originally developed by ZymoGenetics, Inc., and later transferred to Novo Nordisk (NovoThirteen®/Tretten®, Novo Nordisk A/S, Copenhagen, Denmark) for treatment of FXIIIA deficiency patients. It has been manufactured in *Saccharomyces cerevesiae* (yeast) and contains no human/mammalian products. It is licensed in European Union countries, Canada and recently in the USA, for use in patients with congenital FXIII-A subunit deficiency [42,43]. Following intravenous injection rFXIII-A_2 homodimers associate in plasma with the endogenous FXIII-B_2 to form the stable heterotetramer FXIII-A_2B_2.

The rFXIII concentrate has been tested to date for safety, pharmacokinetics and immunogenicity in eight clinical studies (Table 1). Good results were reported in randomized, placebo-controlled single-dose [44,45] and multidose [46] trials in otherwise healthy volunteers [44,46] and patients after cardiac surgery [45]. In a 2006 Phase I trial, Lovejoy *et al.* [47] showed that rFXIII had a similar half-life to native FXIII and was appropriate for monthly prophylactic administration. Others reported that rFXIII was associated with similar estimated half-lives of FXIII-A_2 and FXIII-A_2B_2, in addition to similar FXIII activity, to those documented for plasma-derived FXIII-containing products [48].

These findings were supported by a recent multinational, open-label, single-arm, multi-dose, Phase III trial (Mentor™1) of once monthly rFXIII treatment in 41 patients aged ≥6 years [49]. No spontaneous treatment-requiring bleeds or intracranial hemorrhage occurred during the trial. The only treatment-requiring bleeds were observed in four out of 41 patients, all of them due to trauma. The age-adjusted rate of treatment requiring bleeds was 0.048 bleeds per patient per year, which is markedly lower than the treatment-requiring bleeds in patients on regular Fibrogammin-P replacement therapy – 0.2 to 0.3 bleeds per patient per year [37–38,40], and the 2.91 treatment-requiring bleeds per year found in patients receiving on-demand treatment in retrospectively collected data.

The only safety concern was the development of transient, low-titer, non-neutralizing anti-rFXIII antibodies in four patients. However, the presence of the antibod-

ies was not associated with treatment-requiring bleeds, changes in FXIII pharmacokinetics, allergic reactions or specific genotype, and levels ultimately dropped below the LOD in all patients, despite continued treatment with rFXIII or other FXIII-containing products. Taken together, this study demonstrated that rFXIII as monthly replacement therapy is efficacious and safe for prophylactic treatment in patients with congenital FXIII-A subunit deficiency [49].

The pharmacokinetics (PK) and safety of rFXIII in younger children (1 to <6 years) with congenital FXIII deficiency has been assessed in the Mentor™4 trial, and compared extrapolated PK parameters with the Mentor1 trial [50]. PK properties were similar in all the children, with FXIII activity maintained above the lower target level (0.1 IU/ml). rFXIII half-life was 15.1 days (range: 10–25). PK parameters of rFXIII were similar in patients from both trials. No safety findings of clinical concern were observed.

Two safety extension clinical trials Mentor™2 (extension of Mentor1 in patients aged ≥ 6 years) and Mentor™5 (extension of Mentor4 in pediatric patients aged ≤ 6 years) (Table 1) are currently ongoing. Interim results of the Mentor2 and Mentor5 recently presented at the World Federation of Hemophilia Congress in Melbourne, Australia [51] show that in Mentor2 trial, the mean annual rate of bleeds requiring treatment was 0.056 bleeds per patient per year while in Mentor5 trial no treatment requiring bleed was reported. No safety issues were identified during 8.7 cumulative follow-up, especially there were no thromboembolic events, allergic reactions or development of anti-rFXIII antibodies [51]. In addition, the pharmacokinetics of rFXIII was investigated in 54 patients aged 1–60 years. The geometric mean peak of rFXIII activity ranged from 0.67 to 0.88 IU/ml, while that of half-life ranged from 11.6 to 15 days [51]. The results of these interim analyses demonstrate that PK does not differ between adult and pediatric patients and further validate the absence of safety concerns in all age groups.

Overall, the results suggest that bleeding symptoms of FXIII deficiency can be prevented or controlled by life-long monthly prophylactic treatment with rFXIII concentrate, making it possible for patients of all ages to enjoy a normal and active life.

Conclusion

In future, every patient diagnosed with congenital FXIII deficiency is expected to be regularly prophylactically treated with FXIII concentrate throughout life. Although the main indication for FXIII concentrate is still FXIII deficiency, the additional functions of FXIII suggest

Table 1. Clinical studies with recombinant FXIII.

Trial ID	Title	Doses	Number	Ref.
UKHV-1	Randomized, placebo-controlled single-dose, double-blind study of the safety and pharmacokinetics of rFXIII in healthy volunteers	0, 2, 5, 10, 25, 50 U/kg	50 healthy subjects	[44]
112C01	Randomized, placebo-controlled, double-blind, multidose study of the safety and pharmacokinetics of rFXIII administration in healthy volunteers	0, 10, 25 U/kg daily for 5 days	24 healthy subjects	[46]
CD1.3 Phase I trial	Escalating-dose study of the safety and pharmacokinetics of rFXIII in patients with congenital FXIIIA deficiency	2, 6, 20, 50, 75 U/kg	11 patients	[47]
F13CARD-1660	Randomized, double-blind, placebo-controlled, dose-escalation study of the safety and pharmacokinetics of rFXIII in patients after cardiac surgery	12, 30, 60, 89, 119 IU/kg equivalent to 10, 25, 50, 75, 100 IU/kg	50 patients	[45]
F13CD-1725 Mentor™1 Phase III trial	Multicenter, multinational, open-label, single-arm, multidosing trial of rFXIII in patients with congenital FXIIIA deficiency	35 IU/kg	41 patients	[49]
F13CD-3760 Mentor™4 Phase IIIb	Mentor4 trial of pharmacokinetics of rFXIII in children with congenital FXIIA deficiency	35 IU/kg	Six patients	[50]
F13CD-3720 Mentor™2	Ongoing extension trial of Mentor1	35 IU/kg	23 patients	[51]; ongoing
F13CD-3835 Mentor™5	Ongoing extension trial of Mentor4	35 IU/kg	Six patients	[51]; ongoing

that it may be beneficial in the future in other clinical conditions such as angiogenesis and wound healing.

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The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

- Monthly dosing with recombinant FXIII concentrate provides substantial protection against breakthrough bleeds in both adult and pediatric patients.
- The pharmacokinetic profile of recombinant FXIII does not differ between adults and children with congenital FXIII A subunit deficiency.
- No safety concerns are associated with recombinant FXIII treatment at any age.

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