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Coagulation factor XIII: a multifunctional transglutaminase with clinical potential in a range of conditions

Gerhard Dickneite[1]; Heiko Herwald[2]; Wolfgang Korte[3]; Yannick Allanore[4]; Christopher P. Denton[5]; Marco Matucci Cerinic[6]

[1]Preclinical Research & Development, CSL Behring GmbH, Marburg, Germany;

[2]Department of Clinical Sciences, Lund University, Lund, Sweden;

[3]Centre for Laboratory Medicine and Haemostasis and Haemophilia Centre, St Gallen, Switzerland;

[4]Department of Rheumatology A, Paris Descartes University, APHP, Hôpital Cochin, Paris, France;

[5]Centre for Rheumatology, UCL Medical School, Royal Free Campus, London, UK;

[6]Department of Experimental and Clinical Medicine, Careggi Hospital (AOUC), University of Florence, Florence, Italy

Correspondence to: Prof. Dr Gerhard Dickneite, Preclinical R&D, CSL Behring, PO Box 1230, 35002 Marburg, Germany

Tel.: +49 6421 392306

Fax: +49 6421 394663

E-mail: gerhard.dickneite@cslbehring.com

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Conflicts of interest

G. Dickneite is an employee of CSL Behring GmbH and owns CSL Behring stock. CSL Behring GmbH (Marburg, Germany) and Hansa Medical AB (Lund, Sweden) have filed a patent application on FXIII. G. Dickneite and H. Herwald are listed as inventors. W. Korte has received research support and speaker fees from CSL Behring and Novo Nordisk, and holds and has filed patents on point-of-care tests for FXIII, as well as preoperative identification of patients at risk for intraoperative bleeding complications. Y. Allanore has received honoraria from CSL Behring, Actelion, Bayer, Sanofi, Roche and Pfizer. C Denton has received research grants from Actelion and consulted for Actelion, Pfizer, GSK, Roche, CSL Behring, Sanofi and Merck.

Summary

Coagulation factor XIII (FXIII), a plasma transglutaminase, is best known as the final enzyme in the coagulation cascade, where it is responsible for cross-linking of fibrin. However, a growing body of evidence has demonstrated that FXIII targets a wide range of additional substrates that have important roles in health and disease. These include antifibrinolytic proteins, with cross-linking of α_2-antiplasmin to fibrin, and potentially fibrinogen, being the principal mechanism(s) whereby plasmin-mediated clot degradation is minimised. FXIII also acts on endothelial cell VEGFR-2 and $\alpha_v\beta_3$ integrin, which ultimately leads to downregulation of the antiangiogenic protein thrombospondin-1, promoting angiogenesis and neovascularisation. Under infectious disease conditions, FXIII cross-links bacterial surface proteins to fibrinogen, resulting in immobilisation and killing, while during wound healing, FXIII induces cross-linking of the provisional matrix. The latter process has been shown to influence the interaction of leukocytes with the provisional extracellular matrix and promote wound healing. Through these actions, there are good rationales for evaluating the therapeutic potential of FXIII in diseases in which tissue repair is dysregulated or perturbed, including systemic sclerosis (scleroderma), invasive bacterial infections, and tissue repair, for instance healing of venous leg ulcers or myocardial injuries. Adequate levels of FXIII are also required in patients undergoing surgery to prevent or treat perioperative bleeding, and its augmentation in patients with/at risk for perioperative bleeding may also have potential clinical benefit. While there are preclinical and/or clinical data to support the use of FXIII in a range of settings, further clinical evaluation in these underexplored applications is warranted.
(Word count: 250 words; limit 250 words)

Keywords

Coagulation, factor XIII, infection, scleroderma, wound healing

Introduction

Factor XIII (FXIII) is the final enzyme in the coagulation cascade and it plays a key role in maintaining the functional integrity of fibrin clots. This coagulation factor circulates in plasma as a protransglutaminase that consists of two catalytic A subunits and two carrier/protective B subunits (1, 2). The A subunits contain the catalytic component, activation peptide and the substrate-recognition regions of FXIII; they are found bound to B subunits in plasma or alone as intracellular homodimers (2, 3).

FXIII is activated by thrombin (factor IIa) to form a transglutaminase that catalyses covalent bond formation between the γ-carboxyamine group of a glutamine residue and the ε-amino group of a lysine residue. During clot formation, stability is achieved through covalent cross-linking of fibrin γ- and α-chains and covalent binding of antifibrinolytic proteins to fibrin (2, 4). The critical function of FXIII in haemostasis is reflected in the broad spectrum of bleeding complications that is observed in patients with congenital FXIII deficiency, a rare autosomal recessive trait (5). Patients often suffer from umbilical stump bleeding, subcutaneous bleeding, muscle haemorrhage, postoperative haemorrhage, and potentially fatal intracranial haemorrhage, the latter occurring in approximately one-third (34%) of patients (5). Among rare bleeding disorders, FXIII deficiency is associated with the highest proportion of severe bleeding episodes, underscoring the importance of this coagulation factor in normal haemostasis (6). Another clinical feature in patients with congenital FXIII deficiency is poor and slow wound healing (7). FXIII deficiency is not identified by standard coagulation testing, and the lack of a readily available and accurate test for the condition hampers diagnosis, particularly in less severe cases (4). FXIII concentrate (human) is recommended as prophylactic treatment for patients with congenital FXIII deficiency (8).

While the 'classic' substrates for FXIII are the non-cross-linked fibrin polymers and, potentially, fibrinogen (9), other biologically important molecules have been shown to be substrates as well (10). Antifibrinolytic proteins such as α_2-antiplasmin, thrombin-activatable fibrinolysis inhibitor (TAFI) and α_2-macroglobulin are also incorporated into the clot, with cross-linking of α_2-antiplasmin to fibrin being the principal mechanism whereby plasmin-mediated degradation of the fibrin clot is minimised (10). Furthermore, macromolecular components of plasma and the extracellular matrix, e.g. fibronectin and vitronectin are also cross-linked to fibrin, influencing the behaviour of fibroblasts, neutrophils and leukocytes through integrin signalling properties (10).

An increasing body of evidence indicates that FXIII is a multifunctional protein that, beyond a role in haemostasis, plays an important part in a wide range of other physiological and pathological

processes, including tissue repair and wound healing. The aim of this review article is to provide an overview of these diverse functions of FXIII and to highlight the potential for clinical use of FXIII replacement therapy in a range of conditions beyond congenital FXIII deficiency.

Perioperative bleeding

Adequate levels of FXIII are required in patients undergoing surgery to prevent or manage perioperative bleeding. In a study of 226 consecutive patients who underwent elective surgery, parameters relating to the final steps of coagulation were compared in those with unexplained intraoperative bleeding (defined according to prespecified clinical criteria) *versus* those with no such bleeding (11). Patients included in the study underwent different types of surgery in the departments of general surgery, neurosurgery, orthopaedics and urology. In total, 8.8% of the cohort developed "unexplained" (at the time) bleeding and, compared with "non-bleeders", these patients had significantly lower levels of FXIII activity during and after surgery. These findings were paralleled by significantly lower availability of FXIII per unit thrombin generated and early loss of clot firmness of the whole blood clot, as assessed by the thromboelastometry (ROTEM) NATEM test, at all time points for "bleeders" *versus* "non-bleeders". Of note, reduced clot firmness was detected at a time point when increased blood loss was not yet apparent, leading to the suggestion that early administration of FXIII may be beneficial in patients at risk of perioperative bleeding. In fact, this approach was subsequently evaluated prospectively in a randomised, placebo-controlled trial and found to be beneficial (see below) (12).

Inadequate levels of FXIII activity have been associated with an increased risk of postoperative haemorrhage. In a retrospective study of 1264 patients who underwent intracranial surgery, postoperative FXIII activity levels were measured in patients in whom coagulopathy was suspected despite normal results from standard coagulation monitoring (13). Of 34 such patients, 8 were found to have FXIII deficiency postoperatively, and all of these patients suffered a major postoperative haemorrhage. In contrast, only three of the 26 patients with suspected coagulopathy and normal FXIII activity levels experienced a postoperative haemorrhage, a difference that was statistically significant ($p<0.00001$). A likely explanation for these phenomena is the fact that reduction of available FXIII activity is associated with reduced clot firmness as well as a decreased resistance of the clot to fibrinolysis (14, 15).

Clinical trial data

While these retrospectively collected clinical or *in vitro* data do not demonstrate causality between low FXIII activity levels and perioperative haemorrhage, this putative relationship is supported by data from randomised, placebo-controlled clinical trials of FXIII supplementation. In a proof-of-concept trial in elective gastrointestinal cancer surgery, loss of maximum clot firmness was significantly attenuated when FXIII concentrate (30 international unit [IU]/kg)[a] was administered early during surgery (p=0.004 *versus* placebo, **Figure 1A**) in patients at risk (12). Significant reductions in the secondary endpoints of blood loss (**Figure 1B**) and fibrinogen consumption (**Figure 1C**) were also observed following FXIII concentrate administration (12). In a randomised, placebo-controlled study of FXIII concentrate (1250 or 2500 IU) in 75 patients undergoing coronary surgery, postoperative blood loss and blood product transfusion were significantly reduced in those with a post-administration FXIII level of ≥70% *versus* a level <70% (16). Although these trials have demonstrated positive outcomes of using FXIII concentrate to reduce postoperative blood loss, the study by Korte *et al*. was a small single centre study and did not evaluate blood loss as a primary endpoint (12), while the study by Godje *et al*. only evaluated FXIII concentrate in all patients undergoing coronary surgery, rather than in those with low FXIII levels (16). Therefore, further trials are needed to fully evaluate the potential of FXIII concentrate in these patients and others at risk of perioperative bleeding.

Case reports

A clinical utility of FXIII supplementation to manage bleeding in patients undergoing surgery has also been suggested by a number of case reports. For example, a patient with clopidogrel-related refractory bleeding following coronary artery bypass graft surgery was reported to have benefitted from the use of FXIII concentrate plus fibrinogen concentrate, after the administration of packed red blood cells, fresh frozen plasma, platelet concentrate, high-dose aprotinin, desmopressin, and recombinant activated factor VII had failed to stop the bleeding (17).

In another patient requiring major abdominal intervention for liver rupture, addition of FXIII concentrate for the correction of repeatedly occurring low preoperative FXIII activity (50%) pre-, intra- and perioperatively led to normalisation of FXIII activity and to the interruption of secondary bleeding because of low FXIII activity; this case provides evidence and insight into the fact that the half-life of FXIII is dramatically shortened during a state of high consumption. The subsequent postoperative course was uneventful in this patient (18).

In the same article, the authors describe a third patient who received a massive transfusion during emergency surgical treatment for multiple traumatic injuries. The resulting FXIII deficiency was

treated by administration of FXIII concentrate. Additional FXIII concentrate administration was used to address the ensuing generalised inflammation, sepsis and disseminated intravascular coagulation with bleeding, guided by timely measurements of FXIII activity (18).

All three cases demonstrate that measurement of FXIII activity in the perioperative setting is of utmost importance in the identification of patients that can benefit from FXIII concentrate therapy; this is especially important in light of the strong evidence that FXIII replacement therapy in patients undergoing cardiac surgery is not beneficial if their FXIII activity is normal to begin with (19).

In rare instances, FXIII deficiency is not related to a consumption process but occurs due to an inhibitor. In a recent case report by Hayashi *et al*. (20) a 75-year-old patient who presented with an enlarged subcutaneous haematoma following tooth extraction, despite no previous history of bleeding diathesis or familial bleeding, was described. FXIII activity was markedly decreased (<3%), while dot-blotting revealed the presence of immunoglobulin bound to FXIII-A subunit; evaluation of fibrin cross-linking revealed delayed formation of fibrin γ-chain dimers and α-chain polymers. The bleeding tendency in this patient was controlled by infusion of FXIII concentrate combined with immunosuppressive therapy (oral prednisolone) (20).

Management guidelines

The potential of FXIII for the management of perioperative bleeding is reflected in recent clinical practice guidelines. The guidelines established by the Society of Thoracic Surgeons Blood Conservation Task Force mention the use of blood derivatives, including FXIII, as an area of major revision (21). In addition, recent guidelines from the European Society of Anaesthesiology (ESA) on the management of severe perioperative bleeding note: "*in cases of ongoing or diffuse bleeding and low clot strength despite adequate fibrinogen concentrations, it is likely that FXIII activity is critically reduced. In cases of significant FXIII deficiency (i.e. <60% activity), we suggest that FXIII concentrate (30 IU/kg) can be administered*" (22).

Thus, there is evidence that acquired FXIII deficiency in the perioperative setting represents an interesting and probably under-recognised target to improve perioperative outcome. Clearly, immediate identification of such patients would be an important step forward. However, this needs close collaboration with the hospital laboratory, as FXIII assays with short turnaround times are not yet readily available in many hospital laboratories.

Angiogenesis and the role of thrombospondin

There are multiple lines of evidence to suggest that the impaired wound healing seen in patients with congenital FXIII deficiency stems, at least in part, from the proangiogenic properties of FXIII. More than a decade ago, it was reported that endothelial cells adhere to activated FXIII (FXIIIa) in an integrin-dependent manner (23, 24). In addition, both FXIII and FXIIIa were shown to mediate the interaction of platelets with endothelial cells by bridging between endothelial $\alpha_v\beta_3$ and platelet glycoprotein IIb/IIIa integrins (24). Using a range of *in vitro* assays, Dardik *et al.* (25) demonstrated that FXIIIa dose-dependently enhanced endothelial tube formation, increased endothelial cell migration and the rate of thymidine incorporation, and decreased the number of apoptotic cells. After treatment of endothelial cells with FXIIIa, there was downregulation of thrombospondin-1 (TSP-1), as reflected in almost complete disappearance of TSP-1 mRNA and a marked reduction in TSP-1 protein levels (25). The antiangiogenic properties of TSP-1 suggest the potential significance of this observation for FXIIIa-mediated promotion of angiogenesis.

In vivo models also provide evidence for the proangiogenic effects of FXIIIa. In a neonatal heterotopic mouse heart allograft model, injection of thrombin-activated FXIII into the grafted tissue produced significantly more new vessels and significantly higher contractile performance compared with saline-injected graft tissue (26). In FXIII-knockout mice, the formation of new vessels into a subcutaneously injected Matrigel™ plug (a mixture that resembles the complex extracellular environment found in many tissues) was significantly decreased compared with control mice. This effect could be almost completely reversed by the addition of FXIIIa to the gel (26). In rats, addition of FXIII concentrate to a tibial defect filled with a biodegradable hydroxyapatite implant has been shown to stimulate in-growth of microvessels into the biomaterial. In contrast, in defects filled with hydroxyapatite alone, formation of capillaries was limited to the host bone–hydroxyapatite surface (27).

The inhibitory effect of FXIIIa on TSP-1 expression observed in *in vitro* and *in vivo* studies provides an insight into the mechanism by which this coagulation factor promotes angiogenesis. TSP-1 is one of the best characterised antiangiogenic factors (28) and has been shown to induce endothelial cell apoptosis and inhibit neovascularisation in sponge implants in mice with severe combined immunodeficiency. This is mediated through increased expression of the proapoptotic protein Bax, decreased expression of the antiapoptotic protein Bcl-2, and activation of the caspase cell-death pathway through processing of caspase-3 into smaller proapoptotic forms (29).

The importance of TSP-1 in angiogenesis is demonstrated by additional animal studies. In transgenic mice, targeted overexpression of TSP-1 in the skin was associated with potent inhibition of cutaneous tissue repair, granulation tissue formation and wound angiogenesis (30). The observation that TSP-1 was not associated with major alterations in cutaneous vascular architecture or microvascular permeability suggests that chronic overexpression of TSP-1 in the skin preferentially affects wound-healing-associated angiogenesis rather than the angiogenesis associated with normal development and skin homeostasis. In a rabbit model of corneal neovascularisation, FXIIIa administration resulted in blood-vessel formation that was visible 48 hours after injection, whereas no such effect was observed following injection of saline into control eyes. Immunohistochemical analysis revealed that neovascularisation was accompanied by almost complete disappearance of TSP-1 in the cornea (25).

Molecular studies in human umbilical vein endothelial cells (HUVECs) have elucidated some of the processes whereby the proangiogenic effect of FXIIIa is mediated (31). Co-immunoprecipitation analysis demonstrated that FXIIIa cross-links $\alpha_v\beta_3$ integrin with vascular endothelial growth factor receptor 2 (VEGFR-2) and enhances non-covalent binding between these two receptors. Furthermore, FXIIIa was found to induce tyrosine phosphorylation of VEGFR-2 on both the cross-linked and non-covalent $\alpha_v\beta_3$ integrin–VEGFR-2 complexes. The effects of FXIIIa on complex formation and on tyrosine phosphorylation of VEGFR-2 were abolished by iodoacetamide treatment of FXIIIa. This treatment renders FXIII inactive, thus demonstrating that these effects were dependent on transglutaminase activity. Using quantitative real-time PCR analysis, FXIIIa was shown to cause upregulation of the transcription factors c-Jun and Egr-1. FXIIIa treatment of HUVECs resulted in binding of the transcription factor Wilm's tumour-1 (WT-1) but not Egr-1 to the TSP-1 promoter sequence, consistent with the hypothesis that WT-1 is involved in downregulation of TSP-1 expression.

The above evidence suggests an important role for FXIII in angiogenesis, and a model integrating these and related findings has been described (**Figure 2**) (32). Key elements of this model are the FXIII-mediated cross-linking of endothelial cell VEGFR-2 and $\alpha_v\beta_3$ integrin, and the downstream effects of this interaction, which lead to downregulation of TSP-1, ultimately promoting angiogenesis and neovascularisation. This proangiogenic activity may, in part, be responsible for the role of FXIII in tissue repair and remodelling, indicating that FXIII supplementation may have therapeutic potential in some types of wound healing.

Systemic sclerosis (scleroderma)

Systemic sclerosis (SSc), also known as scleroderma, is a severe autoimmune connective tissue disease that is characterised by early involvement of the microcirculation and immune system, fibroblast activation and increased extracellular matrix component production (33). The disease process results in diffuse arteriolo−capillary damage and tissue fibrosis. Potentially fatal involvement of major organs such as kidney and lung is common in advanced stages of the disease.

Owing to the proangiogenic effect of FXIII, there is potential for the use of FXIII treatment in patients with systemic sclerosis, who have a complex perturbation of connective tissue repair, fibrosis and altered vascular function. Many facets of the disease could potentially be modulated by FXIII. Indeed, small studies have shown some indications of benefit of FXIII treatment in this patient population.

Pathophysiological findings in systemic sclerosis

Upregulation of TSP-1 has been demonstrated in a number of studies of systemic sclerosis in the skin. This includes increased gene expression with upregulated mRNA (34, 35) and also increased protein expression detected by immunostaining and analysis of skin and explanted dermal fibroblast cultures (36). In recent studies, levels of TSP-1 mRNA have been correlated with the severity of skin fibrosis assessed by the modified Rodnan skin score (37). Strong upregulation of TSP-1 has been documented in affected and unaffected skin of patients with early diffuse systemic sclerosis (36). In addition, FXIIIa has been shown to inhibit collagen deposition *in vitro* (38), providing a further rationale for the potential use of FXIII in the setting of systemic sclerosis. Moreover, a genetically determined mouse model of systemic sclerosis, in which transforming growth factor-β (TGF-β) signalling is increased in fibroblasts, demonstrated high levels of TSP-1 expression, and this was the most upregulated transcript in skin fibroblasts of this mouse model in microarray analysis (39). This is important because TSP-1 is implicated as a key activator of latent TGF-β ligand and germ line deletion of TSP-1 replicates very closely the phenotype of TGF-β1 knockout in mice (40). A study using the fibroblast populated collagen lattices model of matrix contraction showed that TSP-1 is a key mediator of enhanced contractility of lesional fibroblasts in systemic sclerosis, indicating that strategies that interfere with TSP-1 might be viable antifibrotic therapies (34).

Clinical trial data

Several preliminary studies have demonstrated a possible positive effect of FXIII concentrate treatment in patients with systemic sclerosis (**Table 1**) (41-48). These studies differ with respect to

patient profiles, FXIII concentrate regimens, duration of treatment, and outcome measures reported. However, promising trends were apparent with respect to skin sclerosis and improvement of arthralgia, pulmonary function, and Raynaud's phenomenon (41-48). Common limiting features of these studies were the relatively small sample sizes and, with the exception of the randomised, placebo-controlled trial by Guillevin and coworkers (43), the lack of a comparator group.

Despite these initial promising results, the potential benefits of FXIII treatment in systemic sclerosis have not been fully evaluated clinically, possibly owing to the strict regulation of the use of FXIII following the human immunodeficiency virus (HIV) epidemic (49). However, the availability of FXIII replacement therapies with improved safety profiles may now allow the potential benefits of FXIII treatment in this setting to be more fully investigated. Results from studies demonstrating the effect of FXIII on TSP-1 and angiogenesis (25, 26, 31) have generated a renewed interest in the potential therapeutic use of FXIII concentrate in systemic sclerosis. Nevertheless, as clinical data are so far only available from preliminary studies with small patient populations, appropriately designed and powered studies are needed to further investigate the use of FXIII concentrate in this regard.

Bacterial infections and wound healing

Bacterial infections

There is convincing evidence that FXIII plays an important role in host defence against invasive bacteria (50-52). In normal plasma, the induction of coagulation by *Streptococcus pyogenes* results in immobilisation of bacterial cells within the clot, with transmission electron microscopy revealing that the cells are cross-linked to fibrin fibres at multiple interaction sites (53). This bacterial entrapment within the plasma clot was shown to be FXIII-dependent; cross-linking was not observed when FXIII-deficient plasma was used. Immunostaining with a gold-labelled antibody against N-ε-γ-glutamyl-lysine elucidated further the nature of the interaction, revealing covalent interaction between one terminal of streptococcal M1 surface protein and a globular domain of fibrinogen (**Figure 3**) (53).

In a model of streptococcal skin infection, wild-type mice developed signs of tissue damage and severe pathological inflammation at the site of infection. When bacterial load was determined in blood, spleen and liver in these animals, colonisation was found to be significantly decreased by FXIII concentrate injection compared with non-treated controls (data for blood presented in **Figure 4**) (53). Although the effects of FXIII concentrate treatment on FXIII-deficient mice were not investigated in this study, results from patient biopsies provided further evidence of a role for FXIII in the immobilisation of streptococcal bacteria. FXIII-dependent bacterial killing and cross-linking of

streptococcal M1 protein to fibrin networks was detected in tissue biopsy material from patients with streptococcal necrotising fasciitis (53), suggesting that the mechanisms observed in mice also take place in clinical situations.

Additional studies have shown that FXIII also cross-links fibrin to surface proteins from other bacterial species such as *Escherichia coli* and *Staphylococcus aureus* (54). In the case of *S. aureus*, there is evidence that von Willebrand Factor binding protein (vWbp) secreted by colonising cells activates FXIII in a non-proteolytic manner and recruits fibronectin to staphylococcal clots (55). This has been interpreted as evidence of a mechanism whereby *S. aureus* modulates host immune responses to infection. The observation of analogous cross-linking of bacterial surface proteins from *S. aureus* and *E. coli* indicates that such cross-linking is an immune defence mechanism against multiple bacterial species and suggests that FXIII administration may have potential benefit in patients with invasive infections such as necrotising fasciitis.

Patients with sepsis have been shown to have lower FXIII-A and FXIII cross-linking activity levels than healthy individuals (56) and, in an experimental sepsis model in rats, FXIII was shown to protect mucosal capillary perfusion against endotoxin-induced impairment (57). However, other data have suggested that FXIII may also have negative effects in sepsis, i.e. increasing the risk of intravascular thrombosis (58).

The involvement of FXIII in innate immunity extends to proteins of the complement system. Functional proteomics data show that complement C3, the central component of the complement system, is a novel component of plasma clots and has an antifibrinolytic action (59). Other data show that complement C3 is a substrate for FXIIIa, and that incorporation of this protein into clots occurs by covalent cross-linking by FXIIIa (60, 61). In a further demonstration of the interaction between FXIII and the complement system, it has been shown that mannan-binding lectin-associated serine protease-1 (MASP-1) of the complement lectin pathway activates FXIII and is involved in fibrin clot formation (62).

FXIII also interacts with cells of the immune system (reviewed by Bagoly *et al*.) (63), being activated by human neutrophil elastase and downregulated by granulocyte proteases. Furthermore, FXIIIa enhances monocyte proliferation and migration, and inhibits monocyte apoptosis (64). Although some investigators have suggested that monocytes and macrophages are a source of plasma FXIIIa, arising from a non-classical secretory pathway (63, 65), this has not been shown unequivocally.

Within the context of infectious disease conditions, FXIII has been implicated in the entrapment of various bacteria, and has been shown to interact with components of the immune system. This

aspect of the physiological role of FXIII certainly warrants more examination, as does any potential benefit for FXIII supplementation for the treatment of bacterial infections.

Wound healing

Impaired wound healing was noted in the original description of a patient with congenital FXIII deficiency (7), and subsequent reports have documented that up to 29% of those with severe congenital FXIII deficiency exhibit poor wound healing (5, 66, 67). The multiple FXIII-dependent processes that are involved in wound healing are depicted in **Figure 5** (52).

A key role for FXIII in wound healing has been demonstrated in FXIII-deficient transgenic mice, where healing of an excisional wound was markedly delayed compared with healing in normal mice or in $FXIII^{-/-}$ mice receiving FXIII supplementation (68). Impaired healing was accompanied by cellular and tissue defects, and these were also corrected by FXIII supplementation. A role for FXIII in wound healing has also been demonstrated in a rat burn model in which FXIII deficiency was induced by treatment with CCl_4 (which caused hepatic injury) (69), and in a bone-healing model in sheep (70). Another relevant mechanism for altered wound healing in FXIII deficiency is through interaction with TSP-1 and TGF-β activation as discussed above in relation to the pathogenesis of systemic sclerosis.

Clinical trial data in leg-ulcer patients

An early study in patients with leg ulcers noted decreased FXIII activity in patients with either post-phlebitic syndromes, post-phlebitic ulcers, varicose leg ulcer or mixed leg ulcers compared with those without venous disease (71). Subsequent clinical evaluations of topical treatment of venous leg ulcers with FXIII have shown promising results in small case studies and trials (72-75). In a randomised, double-blind, placebo-controlled trial in 30 patients with chronic venous leg ulcers, topical application of FXIII concentrate was shown to produce a significant reduction in ulcer size compared with placebo, and this was accompanied by a significant reduction in fibrinolytic activity in tissue samples (75). In another placebo-controlled trial of 24 patients, a significantly greater daily reduction in ulcer size was observed in FXIII concentrate-treated patients with small leg ulcers ($<1000\ mm^2$) relative to those with larger ulcers ($>1000\ mm^2$) ($p<0.015$), although there was no significant difference between treated patients and controls (74). Interestingly, genotype/phenotype studies in humans have shown that the *FXIII* V34L polymorphism affects chronic venous leg ulcer size, healing time and response to superficial venous surgery, with healing time after superficial venous surgery increased in *FXIII* 34V homozygotes and ulcer size inversely related to the number of *FXIII* 34L alleles (76). This is biologically plausible because the *FXIII* 34L variant is associated with a faster rate of transglutaminase activation compared with the *FXIII* 34V variant (77, 78), an effect that

could be predicted to strengthen the extracellular matrix, and increase fibroblast migration/proliferation, and angiogenesis (earlier reports of increased transglutaminase activity (79) may have been observed due to incomplete thrombin activation but were later disproven (78)).

These studies indicate that FXIII may play an important role in the healing of leg ulcers. However, they do have some limiting features, such as the low patient numbers and, while two of the studies were placebo-controlled (74, 75), future larger, controlled clinical trials are required to determine the therapeutic potential of FXIII administration for wound healing of venous leg ulcers.

Wound healing after myocardial infarction

Experimental evidence from transgenic mice indicates that FXIII has a role in wound healing after myocardial infarction (MI) (80). Evaluation of myocardial repair in FXIII-deficient mice demonstrated that $FXIII^{-/-}$ and $FXIII^{+/-}$ mice died from left ventricular rupture within 5 days after experimental MI, whereas $FXIII^{-/-}$ animals that received FXIII replacement therapy had a normal rate of survival. Molecular imaging detected significantly higher FXIII activity in wild-type mice and supplemented $FXIII^{-/-}$ mice compared with untreated $FXIII^{-/-}$ animals. Migration of neutrophils into the infarct region was significantly reduced in $FXIII^{-/-}$ mice compared with wild-type animals, and this reduction could be partially corrected in $FXIII^{-/-}$ animals by administration of FXIII. *In vivo* imaging showed that post-MI left ventricular dilatation was significantly attenuated in FXIII-treated mice compared with control animals post MI (81). An imbalance in extracellular matrix turnover (upregulation of metalloproteinase-9 expression/downregulation of collagen expression) was observed in $FXIII^{-/-}$ mice after MI. This may potentially explain the cardiac rupture that was consistently observed in these transgenic animals (80).

These animal findings appear to have clinical relevance as case studies indicate that patients presenting with acute myocardial rupture following MI are found to have significantly reduced levels of FXIII relative to controls with uncomplicated MI (81, 82). In one patient with cardiac rupture who underwent valve replacement because of mitral valve insufficiency secondary to a papillary-muscle rupture, postoperative healing was delayed until FXIII supplementation was implemented (82). Together with the results from studies in mice (80), these case studies suggest that FXIII is implicated in tissue repair following MI (81, 82), and FXIII supplementation may have a positive effect on the rate of wound healing in this clinical setting; however, robust clinical data in are lacking and larger prospective studies are required.

Future perspectives

In addition to its presence in plasma, FXIII-A can also be found in various cells including platelets, monocytes, macrophages, chondrocytes, osteoblasts and osteoclasts (as reviewed by Muszbeck 2011 (83)). There is evidence to support a role for cellular FXIII-A in several processes, including proliferation and migration of monocytes and fibroblasts (64), preadipocyte proliferation and energy metabolism (84), promotion of fibronectin matrices (85, 86) and osteoblast matrix secretion and deposition (87). This suggests that cellular FXIII-A is involved in a range of physiological and pathological functions in addition to those for plasma FXIII discussed within this review, and that further research into the potential role of FXIII in arthritis progression, cartilage repair, bone formation and adipogenesis may be warranted. As the mechanism of activation and functional roles of plasma and cellular FXIII may be different, the use of current FXIII replacement therapies to correct defective cellular FXIII-A function also remains to be evaluated.

Summary and conclusions

FXIII concentrate (human) has supplanted cryoprecipitate and fresh frozen plasma for the treatment of patients with congenital FXIII deficiency because the latter products carry a risk for transmissible disease, and plasma transfusion may induce transfusion-related acute lung injury (88). Indeed, FXIII concentrate (human) is recommended in management guidelines for treatment of congenital FXIII deficiency (8). Replacing FXIII in these patients restores the integrity of the last steps of the coagulation pathway, leading to restoration of adequate fibrin clot structure and strength as well as normal resistance of the clot and its substrate, fibrinogen, to the fibrinolytic system. While a recombinant FXIII-2A product (NovoThirteen®/Tretten®, NovoNordisk A/S, Copenhagen, Denmark) is licensed in European Union countries, Canada, and most recently in the USA, for use in patients with congenital FXIII A-subunit deficiency , this product is not licensed for patients who have a deficiency in the FXIII B-subunit (89, 90).

FXIII deficiency may also be acquired, and can be associated with severe perioperative bleeding in major surgical interventions such as in tumour, heart or orthopaedic surgery, as well as large wound surfaces such as burns, or chronic concomitant inflammatory diseases. Despite promising data from initial randomised, placebo-controlled trials (12, 16), additional data from appropriately designed clinical trials are required to understand fully the benefits of FXIII administration for patients at risk of or suffering from perioperative bleeding. In case reports in which FXIII concentrate was used to

manage perioperative bleeding, the results of routine coagulation tests were normal (18, 20), emphasising the need to use FXIII-specific tests in patients with unexplained perioperative bleeding.

There is an increasing body of evidence to support the view that FXIII also plays a key role in a range of physiological functions. Beyond a role in haemostasis, these functions include: 1) angiogenesis, through signalling that involves downregulation of TSP-1; 2) bacterial immobilisation, through cross-linking of bacterial surface proteins to fibrin within a plasma clot; 3) wound healing, through cross-linking of integrin components of plasma and extracellular matrix, and enhancement of cell adhesion.

While administration of FXIII concentrate may provide clinical benefits in the disease contexts described in this review, there are certain conditions in which FXIII-A may have unwanted effects. For example, fibrin accumulation in synovial joints is thought to play a part in pathogenic pathways leading to rheumatoid arthritis (91, 92). In these cases, the current FXIII replacement therapies available (FXIII concentrate and recombinant FXIII-A) may be detrimental rather than beneficial to patients.

In conclusion, clinical studies have demonstrated that FXIII administration may provide clinical benefits in a range of underexplored indications, including perioperative bleeding, systemic sclerosis, invasive bacterial infections such as necrotising fasciitis, and wound healing. These studies have generally been underpowered to allow definitive conclusions to be made, but the promising results to date support, the case for further clinical evaluation of FXIII administration in appropriately designed and powered studies in these clinical settings.

Footnote

[a]To maintain consistency, and as international units (IU) and units (U) are equivalent, all FXIII concentrate doses used in the studies described within this review are denoted in IU (or IU/kg), although some data were originally reported in the cited articles using U (or U/kg).

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Legends to tables and figures

Table 1: Overview of studies of FXIII concentrate treatment in patients with systemic sclerosis. BID, "*bis in die*" (twice a day); IU, international unit; QD "*quaque die*" (once daily).

Figure 1. Effect of FXIII concentrate administration on clot parameters and blood loss in patients undergoing elective gastrointestinal surgery. A, change in maximum clot firmness of the whole blood clot assessed by the thromboelastometric NATEM test; B, median overall blood loss; C, change in fibrinogen content. Administration of FXIII concentrate 30 IU/kg or matching placebo (albumin) was started 15 minutes after the beginning of surgery. Bar chart created from data reported by Korte *et al.* (12).

Figure 2. Proposed mechanism of FXIII-induced angiogenesis (32). FXIII binds to endothelial-cell $\alpha_v\beta_3$ integrin, resulting in interaction between $\alpha_v\beta_3$ and vascular endothelial growth factor receptor-2 (VEGFR-2), and autophosphorylation (P) and activation of VEGFR-2. Activation of VEGFR-2 results in phosphorylation and activation of ERK, which promotes endothelial cell survival. Activated VEGFR-2 also results in increased endothelial cell proliferation, through phosphorylation of ERK and upregulation of Egr-1. Finally, activation of VEGFR-2 leads to upregulation of c-Jun, and downregulation of thrombospondin-1 (TSP-1) via the transcription factor Wilm's tumour-1 (WT-1), resulting in increased cell proliferation, migration and survival.

Figure 3. Electron micrographic visualisation of FXIII-mediated cross-linking between streptococcal M1 protein and fibrinogen within a plasma clot (53). A, before FXIII-mediated cross-linking; B, after FXIII-mediated cross-linking; C, cross-linking detected by immunostaining fibrinogen–M1 protein complex with gold-labelled antibody against N-ε-γ-glutamyl-lysine. Panels D–F show schematic drawings of the fibrinogen (grey) and M1 protein (black) in panels A–C, respectively. Scale bars in panels C and F indicate 25 nm.

Figure 4. Effect of FXIII concentrate treatment on bacterial dissemination in wild-type mice infected with subcutaneous *Streptococcus pyogenes* (53). Treated mice received an injection of FXIII concentrate (human) 3 hours after infection. At 24 hours after infection, mice were killed and bacterial loads in the blood were determined. Data are the mean and individual values for 10 mice per group; *$p<0.05$.

Figure 5. FXIII-dependent processes in wound healing (52). Plasma FXIII plays a role in wound healing through a number of mechanisms: A, Plasma FXIII enhances aggregation of platelets to the

endothelium at the site of injury; B, FXIIIa promotes the cross-linking of fibrin, thus increasing the integrity and tensile strength of the clot. FXIIIa also mediates incorporation of pathogens, structural macromolecules and fibrinolysis inhibitors into the fibrin clot; C, FXIIIa-induced cross-linking of the provisional matrix enables the infiltration of leukocytes, which interact with the matrix via integrins; D, Cross-linked macromolecules facilitate the invasion of fibroblasts and endothelial cells into the wound, enabling collagen deposition and angiogenesis. FXIII mediates the formation of a complex between $\alpha_v\beta_3$ integrin and VEGFR-2 on endothelial cells. This complex formation results in the activation of both receptors and the upregulation of downstream angiogenic signalling pathways (shown in **Figure 2**). FXIIIa, activated FXIII; IL-8, interleukin-8; PAI-2, plasminogen activator inhibitor-2; PDGF, platelet-derived growth factor; TAFI, thrombin-activatable fibrinolysis inhibitor; TGF-β, transforming growth factor-β; VEGFR-2, vascular endothelial growth factor receptor 2; vWF, von Willebrand factor.

Table 1.

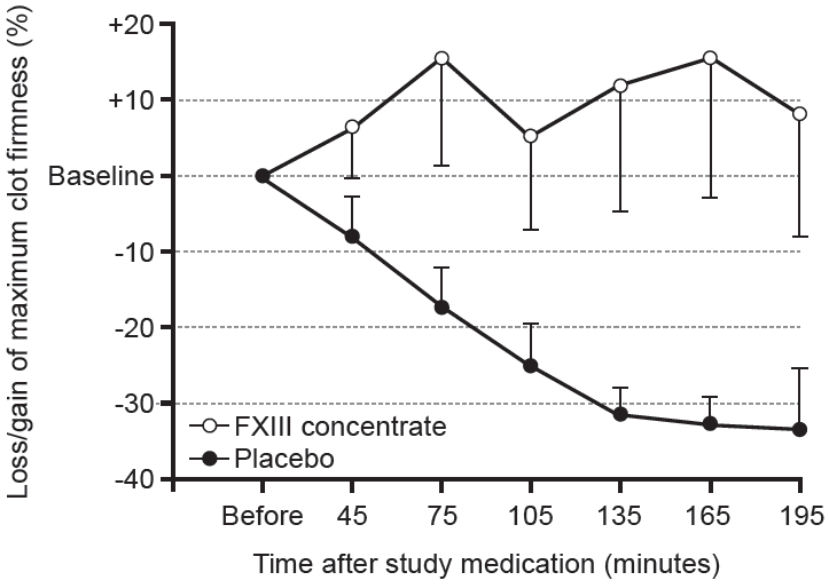
Study	N	Treatment regimen	Main findings
Thivolet *et al*. (1975)(48) and Delbarre et al. (1981)(41)	20	FXIII concentrate 250 IU BID for 2 weeks to 6 months	• Definite beneficial effect in 7 patients and a more moderate effect in 5 patients • Improvement of arthralgia in 6 of 20 patients • Improvement in Raynaud's phenomenon in 9 of 16 patients • Normalisation of alveolar–capillary diffusion in 2 of 5 patients • No effect on oesophageal abnormalities in 4 patients who were evaluated radiologically, despite some improvement in dysphagia
Guillevin *et al*. (1982, 1985, 1985)(43-45)	25	FXIII concentrate 250 IU BID or placebo for 3 weeks (randomised, double-blind, crossover design)	• Cutaneous improvement leading to improvement in functional index • Improvement of arthralgia in 10 of 25 patients
Euller-Ziegler *et al*. (1984)(42)	10	FXIII concentrate 1500 IU every 21 days for 6 to 37 months	• Amelioration of cutaneous symptoms in 6 of 10 patients • Improvement of arthralgia in 9 of 10 patients • Vital capacity improved from an initial low value in 3 of 8 patients, but further diminished in 4 patients
Maekawa *et al*. (1987)(46)	2	FXIII concentrate 480 IU QD for 3 weeks	• Improvement in lower oesophageal sphincter pressure in both patients; correlation with clinical improvement not reported

Marzano *et al*. (1995)(47)	12	FXIII concentrate 250 IU BID on alternate days for 10 doses, followed by 250 IU BID every 10 days for 6 months	• Marked improvement in skin sclerosis in 9 of 12 patients • Improvement of arthralgia in 7 of 11 patients • Improvement in passive joint mobility for at least one major joint in all 12 patients • Improvement in Raynaud's phenomenon in 3 of 8 patients • Improvement in vital capacity and diffusion capacity for carbon dioxide in 2 of 7 patients • No improvement in dysphagia

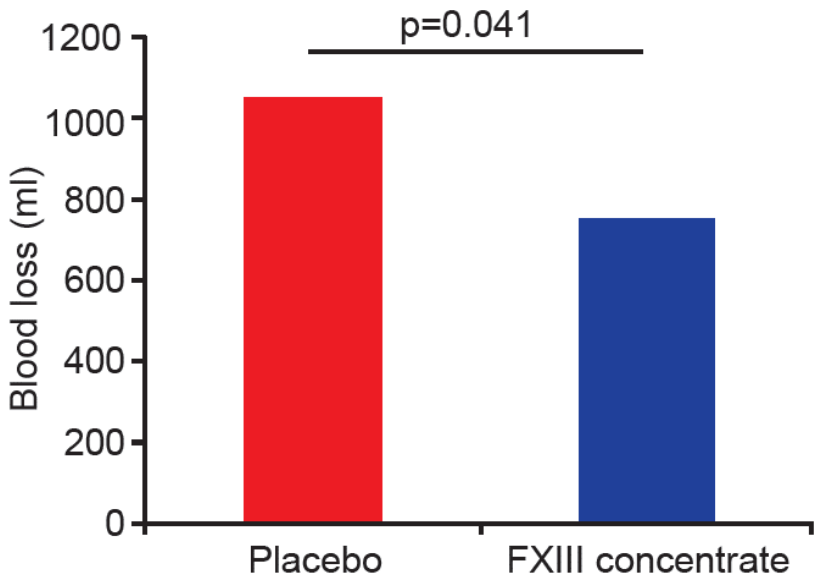
Figures

Figure 1.

A



B



C

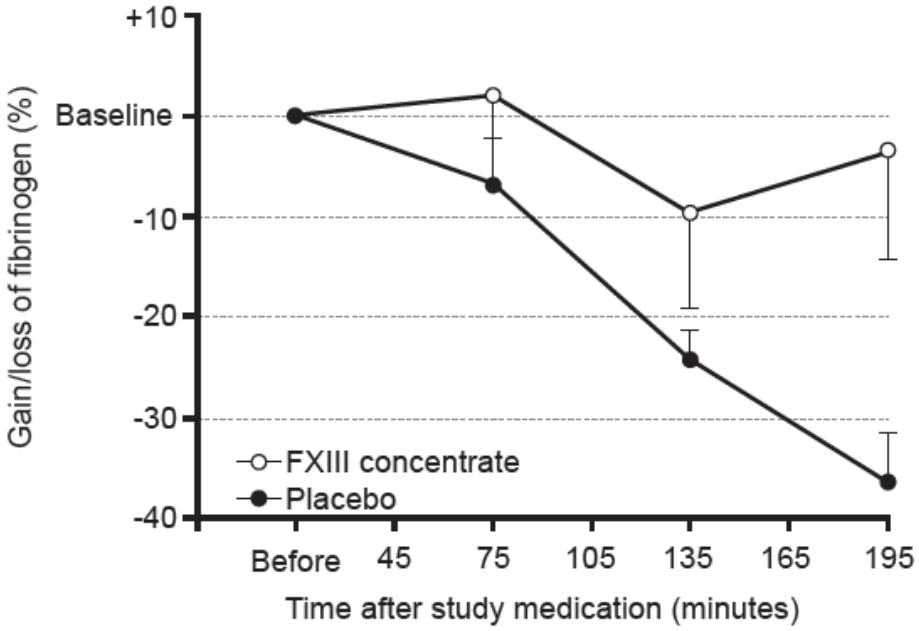

+10
Baseline
-10
-20
-30
-40
Gain/loss of fibrinogen (%)
FXIII concentrate
Placebo
Before
45
75
105
135
165
195
Time after study medication (minutes)

Figure 2.

Figure 2

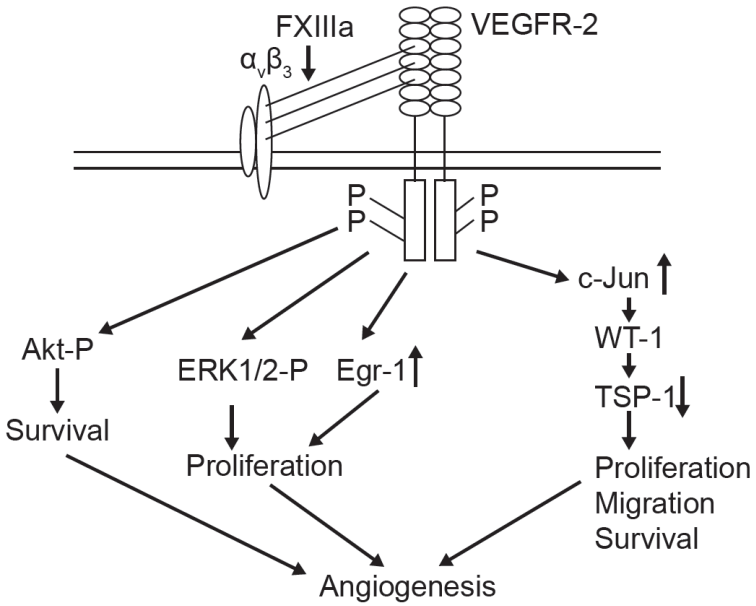


Figure 3.

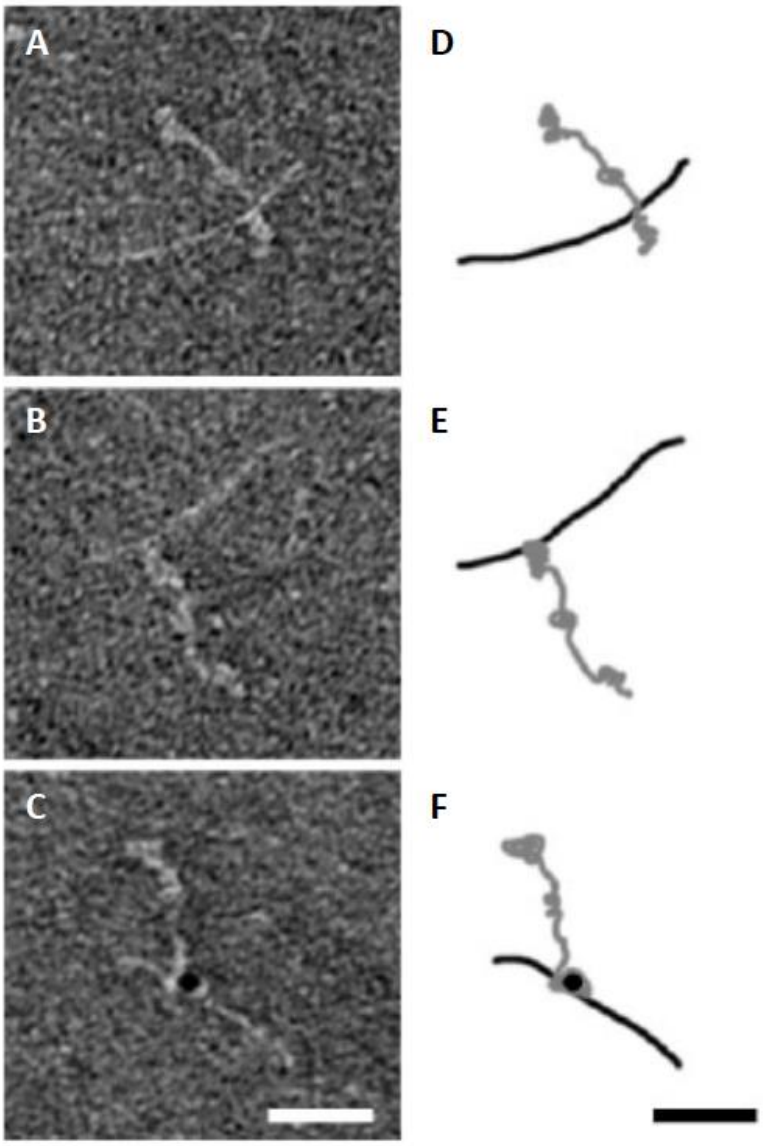


Figure 4.

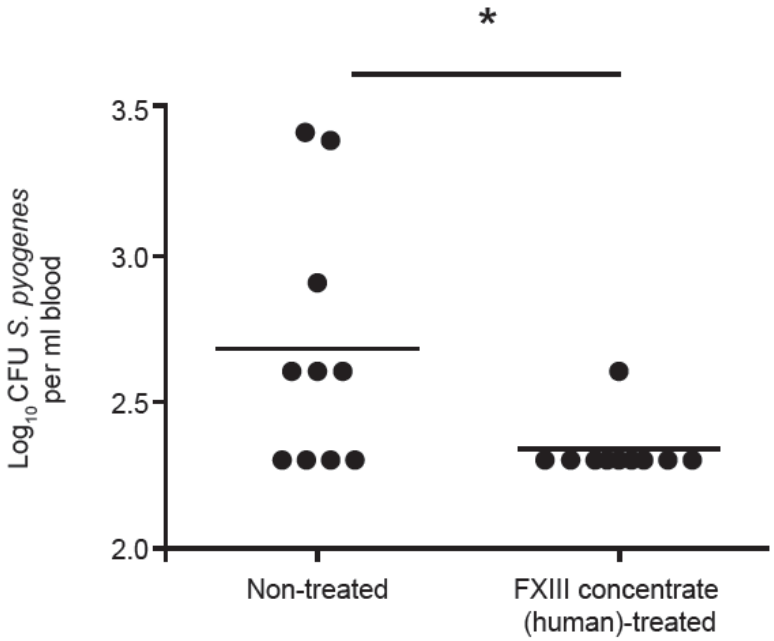
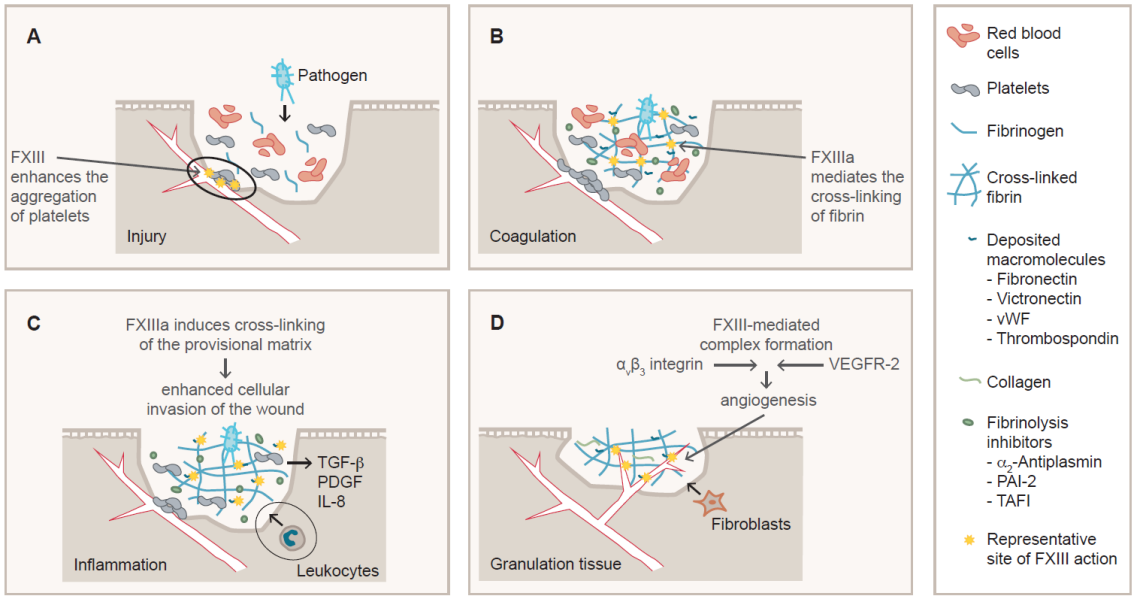

*
3.5
3.0
2.5
2.0
Log10 CFU S. pyogenes per ml blood
Non-treated
FXIII concentrate (human)-treated

Figure 5.


A
Pathogen
FXIII enhances the aggregation of platelets
Injury
B
FXIIIa mediates the cross-linking of fibrin
Coagulation
C
FXIIIa induces cross-linking of the provisional matrix
enhanced cellular invasion of the wound
TGF-β
PDGF
IL-8
Inflammation
Leukocytes
D
FXIII-mediated complex formation
αVβ3 integrin
VEGFR-2
angiogenesis
Fibroblasts
Granulation tissue
Red blood cells
Platelets
Fibrinogen
Cross-linked fibrin
Deposited macromolecules
- Fibronectin
- Victronectin
- vWF
- Thrombospondin
Collagen
Fibrinolysis inhibitors
- α2-Antiplasmin
- PAI-2
- TAFI
Representative site of FXIII action