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Full Length Article

Factor XIII deficiency enhances thrombin generation due to impaired fibrin polymerization – An effect corrected by Factor XIII replacement

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ABSTRACT

Introduction: Factor XIII (FXIII) cross-links fibrin, completing blood coagulation. Congenital FXIII deficiency is managed with plasma-derived FXIII (pdFXIII) or recombinant FXIII (rFXIII) concentrates.

Aim: As the mechanisms protecting patients with low FXIII levels (<5 IU/dL) from spontaneous bleeds remain unknown we assessed the interplay between thrombin generation (TG), fibrin formation and clot kinetics before and after FXIII administration in three patients with FXIII deficiency.

Methods: Patients received initially rFXIII (35 IU/kg, A-subunit) following with pdFXIII at 1250 IU or 2500 IU (12–30 IU/kg) monthly. TG (CAT), thromboelastometry (ROTEM), prothrombin fragments F1 + 2, fibrinogen and FXIII activity (FXIII:C) were measured at baseline and one-hour recovery.

Results: FXIII was at the target level of 20 ± 6 IU/dL at the 4-week trough. rFXIII corrected FXIII to 98 ± 15 and high-dose pdFXIII to a level of 90 ± 6 , whereas low-dose/half dose pdFXIII reached 45 ± 4 IU/dL. Although fibrinogen (Clauss Method) was normal, coagulation in FIBTEM was impaired, which FXIII administration tended to correct. CAT implied 1.6- to 1.9-fold enhanced TG, which FXIII administration normalized. Inhibition of fibrin polymerization by Gly-Pro-Arg-Pro peptide mimicked FXIII deficiency in CAT by enhancing TG both in control and FXIII recovery plasma. Antithrombin, α_2 -macroglobulin–thrombin complex and prothrombin were normal, whereas F1 + 2 were elevated compatible with *in vivo* TG.

Discussion: FXIII deficiency impairs fibrinogen function and fibrin formation simultaneously enhancing TG on the poorly polymerizing fibrin strands, when fibrin's antithrombin I-like function is absent. Our study suggests an inverse link between low FXIII levels and enhanced TG modifying structure-function relationship of fibrin to support hemostasis.

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1. Introduction

Active FXIII (FXIIIa) forms covalent bonds between fibrin α - and γ -chains. It also cross-links the inhibitors of fibrinolysis, α_2 -antiplasmin and thrombin-activatable fibrinolysis inhibitor (TAFI) to fibrin. This stabilizes the clot, rendering it resistant to plasmin degradation [1]. Overall, thrombin acts at multiple levels to catalyse fibrin formation by cleaving fibrinogen and activating FXIII to improve clot stability [2]. Increased thrombin concentrations result in the formation of stiffer, more tightly

packed clots where the thin fibrin fibres exhibit enhanced resistance to fibrinolysis [3].

Inherited FXIII deficiency, at the activity of FXIII:C < 5 IU/dL presents a high risk for spontaneous bleeds, and routine prophylaxis with FXIII concentrate is recommended. However, according to current expert opinion FXIII levels as low as 3–5 IU/dL protect some patients from serious haemorrhagic events, which represents an enigma [4–6]. Patients with congenital FXIII deficiency are successfully managed with prophylactic replacement therapy with FXIII concentrate having a long half-life of 9–14 days [4,7]. The standard prophylaxis with FXIII concentrate is administered every 4 weeks to maintain FXIII levels at around 20 IU/dL [8].

The mechanisms whereby patients with congenital FXIII deficiency are protected from bleeds, even at low levels of FXIII, require further investigation. Likewise, the changes in thrombin generation (TG) and

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fibrin structure contributed by the FXIII replacement are of interest. We investigated the interplay between TG capacity, fibrin formation and FXIII levels in plasma in three patients with FXIII A-subunit (FXIII-A) deficiency under repetitive occasions before (trough levels) and after replacement with FXIII.

2. Methods

2.1. Study design

Our study was approved by the ethics committee of Helsinki University Hospital, and informed consent was obtained in accordance with the Declaration of Helsinki. Three adult patients receiving recombinant FXIII (rFXIII) (NovoThirteen, Novo Nordisk A/S, Copenhagen, Denmark) replacement therapy for their congenital FXIII-A deficiency for at least three years as a part of the Novo Nordisk mentor™ 2 trial were enrolled. During this trial 35 IU/kg rFXIII concentrate was infused intravenously (IV) every 28 ± 2 days in 2008–2014. After the trial, the patients switched back to their previous prophylactic replacement with plasma-derived FXIII (pdFXIII) concentrate (Fibrogammin, CLS Behring, UK) (pdFXIII-A₂B₂), as rFXIII is not marketed in Finland. Standard doses of pdFXIII were administered at 1250 IU or 2500 IU (12–15 IU/kg and 24–30 IU/kg, respectively) IV every 28 ± 4 days.

2.2. Patient demographics

Patient 1 is a 51-year old physically active woman without regular medication other than FXIII replacement therapy (weight 81 kg, height 183 cm).

Patient 2 is a 58-year old man without regular medication other than FXIII replacement therapy (weight 103 kg and height 182 cm). Hepatitis C was eradicated in 2005.

Patient 3 is a 56-year old woman (weight 102 kg, height 170 cm) with medication for hypertension (hydrochlorothiazide and valsartan) and prevention of epilepsy (oxcarbazepine). She suffered an intracranial haemorrhage (ICH) in 1988, and has recovered except for the tendency of epilepsy. She had continued irregular FXIII substitution therapy prior to entering the mentor™ 2 trial. Hepatitis C was eradicated in 2006. Patient 2 and 3 are siblings.

2.3. Sample collection

Anticoagulated (109 mM sodium citrate) blood samples were collected from the patients with a single venous puncture before and 1 h after FXIII concentrate administration. The washout from prior FXIII replacement was 28 ± 4 days. Platelet poor plasma (PPP) was collected by centrifugation at 2000g for 10 min, and re-centrifuged at 10,000g for 10 min. PPP was stored in aliquots at -40°C .

Normal PPP (nPPP) (1 batch) was pooled in-house (Helsinki University Hospital Research Institute, Helsinki, Finland) from 11 healthy donors, collected after informed consent, and used as normal control in calibrated automated thrombogram (CAT).

Unless otherwise stated, coagulation markers, rotational thromboelastometry (ROTEM) and CAT were determined from samples collected both at baseline and one-hour recovery for each FXIII concentrate and dosing.

2.4. Coagulation markers

Baseline FXIII activities (Berichrom chromogenic FXIII, Dade Behring, Marburg, Germany) were measured during the Novo Nordisk mentor™ 2 trial, and up to 17 successive determinations for each patient were averaged to obtain the trough FXIII level. For the one-hour recovery FXIII was measured once after each IV infusion of the FXIII concentrate: namely rFXIII-A₂ (35 IU/kg), or the low (12 IU/kg) or high (30 IU/kg) dose of pdFXIII-A₂B₂. Prothrombin fragments F1 + 2 (enzyme

immunoassay Enzygnost F1 + 2, monoclonal, Siemens Healthcare Diagnostics, Marburg, Germany), fibrinogen (modified Clauss method, Multifibrin U, Siemens) and D-dimer (immunoturbidometric Tinaquant, Roche Diagnostics, Mannheim, Germany) were measured. Baseline values for fibrinogen and D-dimer were examined eight times. Reference ranges for F1 + 2, fibrinogen and D-dimer were 69–229 pM, 1.7–4.0 g/L and ≤ 0.5 mg/L, respectively.

The following routine coagulation assays were used to exclude other factor abnormalities: activated partial thromboplastin time (APTT, Actin FSL®, Siemens), thrombin time (TT) (BC Thrombin reagent, Siemens), prothrombin time (PT, Nycotest PT® with the Owren buffer, Axis-Shield PoC As, Oslo, Norway), antithrombin activities (AT, a chromogenic assay, Berichrom Antithrombin III), von Willebrand factor antigen (VWF:Ag with VWFag Latex Reagent) ristocetin cofactor activity (VWF:RCo with Berichrom von Willebrand Reagent) (Siemens), FII activity assay (Dade, Innovin and FII Deficient Plasma) (Siemens), FVII (FVII:C) (Dade, Innovin and FVII Deficient Plasma), FVIII (FVIII:C) (one-stage clotting assay, Pathromtin SL and FVIII Deficient Plasma) (Siemens) and FIX activity (one-stage clotting assay, Pathromtin SL and FIX Deficient Plasma). All factor levels were analysed with the BCS XP analyzer (Siemens), and the reagents were from Siemens. The reference values for APTT were 23–33 s, TT 17–25 s, AT 84–108%, VWF:Ag 50–169 IU/dL, and VWF:RCo 44–183 IU/dL. The reference ranges used were; FII:C 68–144 IU/dL, FVII:C 76–170 IU/dL, PT 70–130%, FVIII:C 52–148 IU/dL, FIX:C 67–135 IU/dL and FXIII:C 76–156 IU/dL, respectively.

2.5. ROTEM

Thromboelastic measurements were performed with a ROTEM device (TEM International GmbH, Munich, Germany) at 37°C in citrated whole blood according to the manufacturer's instructions. Single shot reagents were applied in INTEM, EXTEM and FIBTEM assays. The INTEM assay is activated by ellagic acid initiating the intrinsic pathway of coagulation. The EXTEM and FIBTEM assays are activated by tissue factor (TF) triggering the extrinsic pathway of coagulation. In the FIBTEM assay the contribution of platelets has been eliminated by cytochalasin D. Clotting time (CT, s), clot formation time (CFT, s), clot formation after 10 min (A10, mm), maximum clot firmness (MCF, mm) and maximum lysis (ML, %) were measured. The reference values for INTEM were: CT 100–240 s, CFT 30–110 s, A10 44–66 mm, MCF 50–72 mm and ML 0–15%. The reference values for EXTEM were: CT 38–79 s, CFT 34–159 s, A10 43–65 mm, MCF 50–72 mm and ML 0–15% and for FIBTEM: A10 7–23 mm and MCF 9–25 mm. Further details about thromboelastometry are described elsewhere [9].

2.6. Thrombin generation assay

TG was measured with CAT (Labscan, Thermo Fisher, Helsinki, Finland) assay using the Thrombinoscope software (Thrombinoscope, Maastricht, The Netherlands) and reagents (Diagnostica Stago, Asnières sur Seine Cedex, France), in the absence of corn trypsin inhibitor according to the method by Hemker et al. [10]. TG was analysed in round bottom 96-microtiter plates (Diagnostica Stago). Patient PPP or nPPP, (80 μL), was supplemented with either an inner method (standard Thrombin Calibrator®) or a trigger targeted (20 μL) to specific TF or TF and phospholipid (PL) concentrations: 1 pM TF and 4 μM PL (PPP-Reagent Low), 5 pM TF and 4 μM PL (PPP-Reagent). TG was initiated by adding the FluCA® reagent (20 μL). The CAT parameters included lag time (time to initiation of thrombin formation, min), endogenous thrombin potential (ETP; the area under the curve; nM thrombin $\times$ time), peak (maximum thrombin concentration, nM) time to peak (ttPeak, min) and $\alpha 2$ -macroglobulin-thrombin complex ($\alpha 2\text{MT}$, nM).

2.7. Fibrin polymerization inhibitor, peptide, Gly-Pro-Arg-Pro

The Gly-Pro-Arg-Pro peptide (GPRP) was obtained from Stago. GPRP inhibits fibrinogen polymerization by direct binding to the polymerization sites and modifying the glutamine residues both in the α - and γ -chains of fibrinogen [11]. According to previously studied functional GPRP concentrations of 0.1–0.3 mM in human plasma [12] we spiked nPPP with increasing final concentrations of GPRP at 0.2, 0.6, 0.8, 1.0 and 1.4 mM to assess turbidity changes in fibrinogen assessed with Clauss method. The effect of dilution was controlled with buffer. Control nPPP had fibrinogen levels of at 3.1 g/L, and the lowest GPRP concentration (0.2 mM) did not modify it. In contrast, the higher concentrations (0.6, 0.8, 1.0 and 1.4 mM) dose-dependently impaired fibrinogen clotting activity to 3.0, 2.8, 2.0 and 1.3 g/L, respectively. GPRP-spiked PPP was pre-incubated for 120 s at room temperature before the CAT assay in the presence of 1 pM TF and 4 μ M PL (PPP-Reagent Low), as described earlier.

2.8. Statistical analysis

Group comparisons were performed by Mann–Whitney test using Prism 6.0d, 2013 by GraphPad Software, Inc. Differences were considered significant with p -values ≤ 0.05 .

3. Results

3.1. FXIII

In all patients the trough and one-hour recovery were similar and reproducible with respect to the FXIII regimen (Table 1). Stable prophylactic treatment with a successful FXIII target level (>20 IU/dL) [8] was reached, and no bleeds were observed. The low-dose (1250 IU, 12–15 IU/kg) of pdFXIII failed to normalize FXIII (mean $\pm$ SD 45 ± 4 IU/dL for all patients), whereas both the high-dose (2500 IU, 24–30 IU/kg) of pdFXIII and the rFXIII dose (35 IU/kg A-subunit) corrected FXIII:C (90 ± 6 IU/dL and 98 ± 15 IU/dL, respectively) (Table 1).

3.2. Prothrombin fragments F1 + 2 and conventional coagulation markers

Ex vivo TG measured with F1 + 2 exceeded the reference range (69–229 pM) in all patients at baseline and remained above normal levels at recovery in patients 1 and 2 (Table 2). As for patient 3, F1 + 2 at recovery was 219 ± 33 pM. Patient 1 had 1.9-fold ($p < 0.0001$) higher levels of F1 + 2 than patients 2 and 3 (Table 2). Fibrinogen levels were mainly within references (patient 1; 2.6 ± 0.3 g/L, patient 2; 2.5 ± 0.2 g/L), being higher in patient 3 (4.1 ± 0.5 g/L, 1.6-fold higher than in others, $p < 0.0001$) with the history of ICH, hypertension and epilepsy medication. Neither FXIII treatment modality influenced fibrinogen levels. All patients had normal baseline and one-hour recovery D-dimer levels under each FXIII regimen. Conventional coagulation markers APTT, TT, PT, VWF:Ag, VWF:RCO, AT, FII:C, FVII:C, FVIII:C and FIX:C were all within reference ranges, excluding other coagulation factor abnormalities. Haemoglobin and platelet counts were normal in all patients (data not shown).

Table 1

Coagulation activity of FXIII, FXIII:C (76–156%) with Berichrom assay at baseline (BL) and one-hour recovery. BL is FXIII trough level $\pm$ SD ($n = 17$). One-hour measured once with each replacement regimen. (pd-L = plasma-derived FXIII low; pd-H = pdFXIII high; rec = recombinant FXIII).

	BL $\pm$ SD	pd-L	pd-H	rec
Patient 1	16 $\pm$ 3	44	90	81
Patient 2	24 $\pm$ 4	41	84	103
Patient 3	15 $\pm$ 3	49	95	110

Table 2

Prothrombin fragments (F1 + 2) at baseline and one-hour recovery after administration of pdFXIII and rFXIII. (BL, Baseline ($n = 3$); pd-L, pdFXIII low; pd-H, pdFXIII high; rec rFXIII).

	BL $\pm$ SD	pd-L	pd-H	rec
Patient 1	490 $\pm$ 90	452	421	610
Patient 2	300 $\pm$ 83	253	237	372
Patient 3	239 $\pm$ 18	194	257	207

3.3. ROTEM

INTEM and EXTEM modestly detected FXIII deficiency and response to treatment, in contrast to FIBTEM, which was uniformly attenuated, especially at the trough FXIII level. Results in FIBTEM accorded with both FXIII and fibrinogen levels. Both patients 1 and 2 had baseline FIBTEM MCF below the reference range, and in patient 3 the baseline MCF was within the low reference range. Fibrin clot without the platelet contribution (FIBTEM) was strengthened by both pdFXIII and rFXIII (Table 3). The increase in MCF caused by FXIII, the Δ FIBTEM MCF, was moderate for patients 1 and 2. Patient 3, with the elevated fibrinogen levels, doubled Δ FIBTEM MCF (Table 3). FIBTEM A10 was a good estimate of MCF in all patients (data not shown). Patients 1 and 2 had a slightly prolonged baseline INTEM CFT, whereas Patient 3 had a normal CFT. PdFXIII and rFXIII similarly shortened INTEM CFT in all, identical to the EXTEM CFT (Table 3). In intrinsic (INTEM) and extrinsic (EXTEM) pathway activation, CT, MCF, A10 and ML were all within references at baseline, and remained unmodified after FXIII replacement (data not shown).

3.4. Thrombin generation assay in plasma

The CAT results obtained with the patient PPP were compared with nPPP. The baseline peak TG in the patient plasma before FXIII replacement therapy was surprisingly 1.8-fold (patient 1), 1.6-fold (patient 2) and 1.8-fold (patient 3) ($p < 0.0001$) greater compared with nPPP (TF 1 pM). For patients 2 and 3, both pdFXIII and rFXIII normalized TG. As for patient 1, only the high-dose pdFXIII (30 IU/kg) nearly normalized (1.3-fold) TG in comparison with nPPP (Fig. 1). None of the FXIII replacements affected lag time, ETP, ttPeak and α 2MT, all being similar to nPPP. As prothrombin, α 2MT and AT were all normal, the enhanced TG referred to accelerated prothrombin conversion.

Moreover, as expected, TG was markedly enhanced at 5 pM TF as compared with 1 pM TF. Increased TG at 5 pM TF paralleled reduced lag time and both increased peak height and area under the curve (ETP) (data not shown). However, at the higher 5 pM TF concentration, CAT lost its sensitivity for the significant differences between baseline, one-hour recovery and nPPP. Overall, compared with nPPP in baseline FXIII-A subunit deficient plasma TG in CAT was uniformly increased at 1 pM TF (Fig. 1).

3.5. Gly-Pro-Arg-Pro (GPRP) and thrombin generation in CAT assay

To investigate the enhanced TG in FXIII-deficient plasma we interfered with fibrin polymerization *in vitro* both in nPPP and the patient

Table 3

Results of rotational thromboelastometry (ROTEM). BL, Baseline ($n = 3$) at trough FXIII:C level; Δ ($n = 3$), increase or decrease ($-$) caused by all three FXIII regimen.

	INTEM CFT (30–110 s)			EXTEM CFT (34–159 s)		FIBTEM MCF (9–23 mm)	
	BL $\pm$ SD	Δ $\pm$ SD		BL $\pm$ SD	Δ $\pm$ SD	BL $\pm$ SD	Δ $\pm$ SD
Patient 1	117 $\pm$ 15	−36 $\pm$ 8		177 $\pm$ 32	−42 $\pm$ 17	5 $\pm$ 2	3 $\pm$ 1
Patient 2	125 $\pm$ 16	−29 $\pm$ 6		183 $\pm$ 12	−45 $\pm$ 11	5 $\pm$ 1	3 $\pm$ 2
Patient 3	72 $\pm$ 12	−19 $\pm$ 6		108 $\pm$ 2	−34 $\pm$ 6	11 $\pm$ 2	6 $\pm$ 0

Fig. 1. A–C. Thrombin generation (TG) in platelet poor plasma (PPP) in calibrated automated thrombogram (CAT) at tissue factor 1 pM concentration. Panels A, B and C illustrate plasma-derived FXIII (pdFXIII) (12–15 IU/kg), pdFXIII (24–30 IU/kg) and recombinant FXIII (rFXIII) (35 IU/kg), respectively. All patients had enhanced TG at the trough (light grey line) compared with pooled normal platelet poor plasma from healthy donors (red line). The higher dose pdFXIII (24–30 IU/kg) normalized TG in all patients (one hour recovery: black line) (Fig. 1B).

PPP at one-hour recovery, e.g. under normal FXIII activity. GPRP peptide dose-dependently heightened the thrombin peak in nPPP, with 1.1-, 2.0- and 2.3-fold higher values obtained at 0.2, 0.6 and 0.8 mM GPRP, respectively, compared with buffer-treated nPPP (Fig. 2A). ETP was unmodified by 0.2 mM GPRP, whereas the higher concentrations of GPRP enhanced also ETP (up to 1.4-fold). Recovery plasma samples, having 90 IU/dL of FXIII activity (Table 1) after the high-dose of pdFXIII, were studied for the effect of GPRP. GPRP at a concentration of 0.2 mM enhanced the peak of TG 1.1–1.8-fold compared with buffer-treated one-hour recovery plasma. GPRP at 0.6 mM augmented the peak of TG 1.6–3.2-fold, while 0.8 mM GPRP yielded again even higher peak with a 1.9–3.2-fold increases in TG compared with the one-hour recovery plasma without GPRP (Fig. 2B–D). ETP was also dose-dependently enhanced by 0.2 mM (1.1–1.4-fold), 0.6 mM (1.2–1.6-fold) and 0.8 mM (1.3–1.7-fold) of GPRP.

Overall, TG was enhanced in FXIII-deficient PPP and GPRP-modified normal plasma, the finding associating with enhanced prothrombin conversion and inhibited fibrin polymerization (functional antithrombin I).

4. Discussion

Our aim was to study the global haemostatic effect of FXIII in plasma, in the presence and absence of platelets, as well as in whole blood. Administration of high dose pdFXIII and rFXIII resulted in equal recovery, while low dose pdFXIII reached half the response. Whether FXIII

contained both the A and B domains or exclusively the A domain did not seem to impact in the results of any coagulation assay studied. The main observation was increased circulating prothrombin fragments and compatibly the enhanced thrombin formation in CAT (with low TF) under low trough FXIII levels (20 IU/dL) in congenital FXIII deficiency patients using regular FXIII replacement therapy. Previously Ghosh et al. [13] have presented among several other severe coagulation factor deficiencies, that deficient FXIII associates with reduced ETP. However, when observing their individual peak TG, the finding is in line with our results. By implementing GPRP in the assay we modified fibrin structure and attenuated the antithrombin I action of polymerizing fibrin. Subsequently, GPRP enhanced TG in plasma both at normalized FXIII levels by r/pdFXIII recovery and in normal plasma – similar to the TG at the low (20 IU/dL) FXIII trough levels. These findings support the previous data on increased free thrombin in the defibrinated plasma, while polymerization acts as antithrombin I [14]. Also, a more recent study suggest that the presence of fibrin(ogen) both diminishes the velocity of thrombin degradation and reinforces prothrombin conversion [15]. As determined by thromboelastometry, FXIII replacement partially corrected the defect in fibrin clot formation, emphasizing the role of blood cells, mainly platelets and red blood cells, in the fibrin network [16–19]. Despite the altered fibrin behaviour, fibrinolysis could not be detected by ROTEM nor by circulating D-dimer.

Viscoelastic tests, such as ROTEM, are established as global coagulation assays which, unlike the coagulation screens in routine laboratory, are able to detect FXIII deficiency [20]. As reported by others, FIBTEM in

Fig. 2. A–B. Calibrated automated thrombogram (CAT) and Gly-Pro-Arg-Pro (GPRP) in normal platelet poor plasma (nPPP). A illustrates the effect of GPRP on pooled nPPP from healthy donors. The light grey continuous line represents untreated nPPP. GPRP inhibits fibrin polymerization and enhances thrombin generation. B, C and D show a thrombogram, obtained from patients 1, 2 and 3 at recovery after high dose plasma-derived FXIII (24–30 IU/kg) supplemented with GPRP. The light grey continuous line represents untreated nPPP and patient plasma at recovery. The black stratified line shows GPRP 0.2 mM. The dark grey dots represent GPRP 0.6 mM and the black continuous line illustrates GPRP 0.8 mM.

its baseline MCF was reduced despite normal fibrinogen values, but somewhat increased as a response to FXIII replacement [21]. Notably though, despite normalized FXIII and normal fibrinogen levels, FIBTEM MCF remained reduced. Since FIBTEM is measured in whole blood without platelets, this finding underlines the importance of cytoplasmic FXIII-A stored in platelets, which upon its release, stabilizes FXIII-depleted thrombi against fibrinolysis [22,23]. Compared with nPPP, TG was enhanced at trough FXIII level, while FXIII replacement therapy normalized TG. Moreover, *in vivo* TG, based on the levels of circulating prothrombin fragments, was high at baseline and after FXIII replacement. These findings refer to the role of fibrin structure in regulating thrombin activity [14].

Thrombin cleaves N-terminal peptides from the fibrinogen A α and B β chains leading to the spontaneous formation and thickening of fibrin protofibrils, followed by their lateral aggregation [24–26]. High thrombin concentrations form stiffer, tightly packed clots with thin fibrin fibres [3]. Furthermore, fibrinogen stimulates prothrombin conversion thereby increasing thrombin formation. TG is the net result of prothrombin conversion and thrombin inactivation. High thrombin concentrations can be caused by an increase of prothrombin, by overactive prothrombin conversion or by decreased thrombin breakdown. Major thrombin inactivators in plasma are antithrombin and α 2-macroglobulin. Also, minor serpins, such as C1 inhibitor and α 1-antitrypsin, inhibit thrombin irreversibly [27]. In our study prothrombin, antithrombin and α 2-macroglobulin-thrombin complex levels were all normal, indicating that enhanced TG associates with overactive thrombin conversion. However, the possible role of other regulators of thrombin cannot be excluded. The enhanced TG is supported by the action of altered fibrin caused by FXIII deficiency, alike the effect of GPRP. GPRP, by inhibiting fibrin protofibril lateral aggregation, promoted TG both in normal plasma and one-hour recovery patient plasma. Our novel results suggest that enhanced TG due to inhibited polymerization alter fibrin structure and provide protection against bleeds even at low

levels of FXIII. Our study suggests also, that when interpreting TG in CAT, fibrin polymerization defect needs to be included.

In patients with congenital FXIII deficiency, FXIII above 5 IU/dL may offer sufficient protection against spontaneous bleeds, although maintaining FXIII between 5 and 20 IU/dL during FXIII replacement therapy is recommended [5]. The relationship between low FXIII and bleeding tendency is not straightforward. FXIII replacement therapy reduced TG in PPP, but partly restored the fibrinogen function. Our data propose a possible explanation to this phenomenon; FXIII deficiency impairs fibrinogen function and fibrin formation, but simultaneously enhances TG on the poorly polymerizing fibrin strands, when fibrin's antithrombin I-like function is absent. In conclusion, our study suggests an inverse link between low FXIII levels and enhanced TG, which seems to modify structure–function relationship of fibrin to support hemostasis.

Author contribution

Design of the study: Riitta Lassila. Execution of the study: Hanna Pitkänen, Annukka Jouppila, Marja Lemponen, Minna Ilmakunnas, Jouni Ahonen. Analysis of the data: Hanna Pitkänen, Riitta Lassila. Writing of the manuscript: Hanna Pitkänen, Annukka Jouppila, Minna Ilmakunnas, Jouni Ahonen, Riitta Lassila.

Disclosures

Riitta Lassila is a member of Nordic Advisory Board of CLS Behring and the National Coordinator for mentor™ program by NovoNordisk in Finland. Riitta Lassila's responsibilities in CLS Behring or NovoNordisk played no role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the report for publication. All other authors stated that they have no competing interests.

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