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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, α2-macroblobulin–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.

1. Introduction

Active FXIII (FXIIIa) forms covalent bonds between fibrin α- and γ-chains. It also cross-links the inhibitors of fibrinolysis, α2-antiplasmin and thrombin-activable 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-A_2B_2), 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 2000*g* for 10 min, and re-centrifuged at 10,000*g* 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-A_2 (35 IU/kg), or the low (12 IU/kg) or high (30 IU/kg) dose of pdFXIII-A_2B_2. Prothrombin fragments F1 + 2 (enzyme immunoassay Enzygnost F1 + 2, monoclonal, Siemens Healthcare Diagnostics, Marburg, Germany), fibrinogen (modified Clauss method, Multifibren 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 × time), peak (maximum thrombin concentration, nM) time to peak (ttPeak, min) and α2-macroglubulin-thrombin complex (α2MT, nM).