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The Effect of Blood Coagulation Factor XIII on Fibrin Clot Structure and Fibrinolysis

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Running title: Factor XIII Effects on Fibrin Clot

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Summary. Background: Factor XIII is a 320 kDa tetramer, comprising two enzymatic A-subunits and two carrier B-subunits (FXIII A_2B_2). Activated FXIII (FXIIIa) catalyses the formation of ε-(γ-glutamyl)lysyl covalent bonds between γ-γ, γ-α, and α-α chains of adjacent fibrin molecules and also cross-links the major plasmin inhibitor, α2-antiplasmin to fibrin. Objectives: We investigated the role of FXIII cross-linking of fibrin directly on clot morphology and its functional effect on clot formation and lysis, in the absence of α2-antiplasmin. Results and Conclusions: Our data show that the presence of FXIII during clot formation results in fibrin clots that have a significant 2.1 fold reduction in pore size, as determined by the Darcy Constant, Ks, and formed thinner fibres (74.7 nm ±1.5 nm) and higher density of fibres compared to those without FXIII (86.0 nm ±1.7 nm, $p<0.001$), as determined by scanning electron microscopy. Additionally, fibrinolysis showed a significant increase in the time to lysis for clots formed in the presence of FXIII in both static and flow systems. These data demonstrate that independent of α2-antiplasmin, FXIII activity plays a role in increasing the stability of the fibrin clot by altering its structure and increasing the resistance to fibrinolysis.

Key words: blood coagulation, electron microscopy, factor XIII, fibrin, fibrinolysis.

Introduction

Fibrinogen comprises duplicate sets of three polypeptide chains; Aα, Bβ and γ(1). In the final step of blood coagulation, thrombin cleaves fibrinopeptides A and B from fibrinogen, initiating polymerisation of fibrin monomers into fibres(1). Factor XIII comprises two enzymatic A-subunits and two carrier B-subunits (FXIII A_2B_2)(2;3). Thrombin cleaves the activation peptide from each A-subunit, and in the presence of calcium, the B-subunits dissociate and conformational changes in the A subunits result in exposure of the active site of FXIIIa(4;5). This FXIIIa catalyses formation of covalent bonds between γ-γ, γ-α, and α-α chains of adjacent fibrin molecules, stabilising the protofibrils(6). FXIIIa also cross-links α2-antiplasmin (α2-AP) to fibrin making the developing fibre more resistant to fibrinolysis(7).

Fibrin clot structure is determined by fibre thickness and distribution of the fibres and branch-points, and is an important determinant of vascular risk(8). The mechanism through which these structural changes are translated into enhanced vascular risk may partly be due to the association between the clot structure and its susceptibility to lysis. Due to the complex nature of the fibrinolytic process, it is difficult to determine the individual contribution of each factor. Recent work by Fraser et al. has highlighted the role of α2-AP in regulating fibrinolysis(9). The direct effects of FXIII cross-linking on fibrin structure and fibrinolysis is currently unknown. To further

examine this, our study investigated the role of FXIII cross-linking of fibrin on clot morphology and its functional effect on clot formation and lysis.

Material and Methods

Materials

Human fibrinogen, human glu-plasminogen, horse radish peroxidase conjugated (HRP) antibodies to factor XIII-A (SAF-13 HRP) and α2-antiplasmin (A2AP HRP) were purchased from Enzyme Research Laboratories (Swansea, UK). Tissue plasminogen activator (tPA) was from Technoclone (Vienna, Austria). Alexa Fluor® 488 conjugated human fibrinogen was purchased from Life Technologies Ltd (Paisley, UK). IF-1 monoclonal antibody was from Kamiya Biomedica, (Seattle, USA). Fibrogammin P was from CSL Behring (Haywards Heath, UK). Human thrombin was from Calbiochem, Merck KGaA (Darmstadt, Germany). The FXIII inhibitor (T101; Tetramethyl-2-[(2-oxopropyl)thio]imidazolium chloride) was purchased from Zedira GmBH (Germany), whilst the Ibidi µ-Slide were from Martinsried (Germany). The vinyl tubing used in the chandler loop experiments (inner diameter = 3 mm, external diameter = 4.2 mm, length = 33 cm) was from Portex (Kent, UK). All other reagents were purchased from Sigma-Aldrich Company Ltd (Gillingham, UK).

Depletion of FXIII from Fibrinogen

To generate fibrinogen that was free from FXIII activity we employed a modification of an ammonium sulphate precipitation method previously described(10). Fibrinogen was also FXIII-depleted using an affinity chromatography method using the calcium dependent IF-1 monoclonal antibody as previously described(11). For the following described methods, the fibrinogen source employed was dependent on the requirement for each assay. IF-1 purification produces comparative small quantities of fibrinogen depleted of FXIII compared with ammonium sulphate precipitation, therefore the ammonium sulphate precipitation method was preferentially used in assays requiring larger volumes (e.g. chandler loop). The purity of both preparations was examined by SDS-PAGE and western blot analysis for α2-antiplasmin. Depletion of FXIII activity was confirmed using the pentylamine incorporation assay(10;12).

Albumin Depletion of Fibrogammin

FXIII was purified from Fibrogammin P using a Sepharose 6B gel filtration column on a Biocad sprint automated chromatography system to remove the contaminating albumin and glucose(13).

Turbidity

Fibrin polymerization was monitored in the presence and absence of FXIII in a microtiter plate turbidity assay, as previously described(14). Clots were formed in triplicate using final concentrations of 1.5 mg/ml IF-1 purified fibrinogen, 0.125 U/ml human thrombin, and 5 mM $CaCl_2$ in the presence or absence of 11 µg/ml FXIII. Increase of turbidity was monitored every 12 seconds at 340 nm in a multiwell plate reader (ELx808, Bio-tek) for 60 minutes at 37°C. For the dimethyl sulfoxide (DMSO) and T101 inhibitor studies 0, 1.0, 2.5 and 5% DMSO and a range of

concentrations of T101 with 1% DMSO (final concentration) was added to 1.5 mg/ml IF-1 purified fibrinogen prior to activation with thrombin and calcium.

Permeation

Clots were formed in triplicate with 1.5 mg/ml ammonium sulphate purified fibrinogen, 1 U/ml human thrombin, and 5 mM $CaCl_2$ in TBS, in the presence or absence of 11 µg/ml FXIII, as previously described (15;16).

Chandler Loop

Lysis rates of fibrin clots formed in the presence and absence of FXIII were measured using a modification of a method previously described(17) using a Chandler loop system. Clots were formed in triplicate using final concentrations of 1.5 mg/ml ammonium sulphate purified fibrinogen (containing 5% Alexa Fluor® 488 conjugated human fibrinogen), 0.125 U/ml human thrombin, 33 µg/ml human plasminogen, 100 ng/ml tissue plasminogen activator (tPA) and 5 mM $CaCl_2$, in the presence or absence of 11 µg/ml FXIII. Each reaction mix was placed in vinyl tubing and the open ends were joined using a short sleeve of larger tubing to form a Chandler loop with a diameter of 10.5 cm. Loops were attached to a horizontal axle and rotated at 30 r.p.m. 5 µl samples of the supernatant were taken every 10 min for 80 minutes at room temperature. Each sample was mixed with 95 µl of TBS and the released fluorescence was measured in a Varioskan Flash fluorescence plate reader (Thermo Fisher Scientific) at excitation of 494 nm and emission of 519 nm.

Confocal Microscopy

Fibrinolysis rates of fibrin clots in the presence or absence of FXIII were measured using confocal microscopy as previously described(18). Clots were formed in triplicate using final concentrations of 1 mg/ml ammonium sulphate purified fibrinogen, 0.1 U/ml human thrombin, and 5 mM $CaCl_2$, 5% Alexa Fluor® 488 fibrinogen in the presence or absence of 11 µg/ml FXIII, in uncoated Ibidi µ-Slide for 2 hours at room temperature in a humidity chamber. Lysis was initiated by addition of 280 µg/ml plasminogen and 1 µg/ml tPA . The clot was visualised under low magnification every 20 seconds using a Leica TCS SP-2 laser scanning 1072 confocal microscope (Leica Microsystems; Heidelberg, Germany) and the time taken for the lysis front to migrate from a fixed point was measured. Lysis front velocity was determined and used to calculate the mean overall fibrinolysis rate in µm/second. For the DMSO studies the solvent and inhibitor were added to normal pool and FXIII depleted plasma at the concentrations indicated prior to activation of clot formation with 0.5 U/ml thrombin and 10mM calcium.

Field Emission Scanning Electron Microscopy

Clots were formed with 1 mg/ml IF-1 purified fibrinogen, 0.1 U/ml thrombin, 2.5 mM $CaCl_2$, in the presence or absence of 6.7 µg/ml FXIII. The clots were allowed to form in small perforated plastic vessels in a moist chamber for 2 hours, washed with sodium cacodylate buffer and then fixed overnight in 2% glutaraldehyde solution. Clots were recovered, washed with sodium cacodylate buffer and further processed by dehydration using a stepwise acetone gradient, critical point drying, and sputter coated with gold-palladium(11;15;19). Clots were photographed in 10 different areas using a FEI Quanta 200 FEGSEM scanning electron microscope. (FEI; Hillsboro, USA). Fibre diameter was measured with the computer image analysis software package ImageJ (National Institute of Health, USA). Fibre density was determined by counting the number of fibres crossing an arbitrary line of fixed length drawn through a single optical section. Three lines were drawn per image and this was repeated in four different micrographs per sample.

Statistical Analysis

All statistical analyses were carried out using PASW 18.0, Release Version 18.0.0 (SPSS Inc, Chicago). Data are expressed as mean and standard error of the mean (SEM). The independent samples t-test was used and p values <0.05 were considered statistically significant.

Results

FXIII influences fibrin clot structure

Two methods have been employed to produce fibrinogen depleted of any FXIII. Both these methods have been characterised to show no contaminating FXIII activity(10) and shown to be depleted of α2-antiplasmin (Figure 1). The rate of fibrin polymerisation was slightly increased in the presence of FXIII (Figure 2A). However, the presence of FXIII decreased the maximum absorbance compared to control (- FXIII 0.68 ±0.01 OD; + FXIII 0.54 ±0.01 OD). The Darcy constant (Ks) represents the pore size of fibrin clots was measured using the permeation technique. Fibrin clots formed in the presence of FXIII showed a significant 2.1 fold reduction in pore size compared to clots formed in the absence of FXIII, indicating a direct effect of FXIII on the structure of the fibrin network (Figure 2B, n=5). Structural analysis of clots formed in the presence and absence of FXIII by scanning electron microscopy (SEM) demonstrated a significant difference in fibre thickness. In the presence of FXIII, clots formed significantly thinner fibres (74.7 nm ±1.5 nm), compared to those without FXIII (86.0 nm ±1.7 nm, 20 measurements of 4 images, $p<0.001$). In addition, clots formed in the presence of FXIII had a higher density of fibres (6.06 ±0.9 fibres/μm) compared to those without FXIII (5.40 ±0.1 fibres/μm, 3 measurements of 4 images $p<0.001$; Figure 3). Data are expressed as mean ±SEM.

Clot Lysis is delayed in the presence of FXIII

Clot lysis was investigated using a static confocal microscopy method and under flow in the Chandler loop. Confocal images supported the SEM images showing a denser clot in the presence of FXIII compared to clots formed in the absence of FXIII (Figure 4A). After the addition of tPA and plasminogen to the clot front we observed a significant increase in the time to fibrinolysis for clots formed in the presence of FXIII, as would be expected for the stabilisation of protofibrils by FXIII activity (n=10, $p< 0.05$; Figure 4B).

The effect of FXIII on fibrinolysis in a flow system also demonstrated slower and reduced fibrinolysis in the presence of FXIII over an 80 minute time period (Figure 4C, n=15). As a control to ensure the amount of FITC-labelled fibrinogen was consistent between experiments, the clots were incubated for 1200 minutes to ensure complete clot lysis. The amount of FITC fibrin release measured in the presence and absence FXIII showed comparable levels, as expected.

DMSO and the FXIII inhibitor, T101 independently modify fibrin clot structure

A commonly used compound solvent (vehicle), DMSO, was added to fibrin clots at increasing concentrations to investigate its direct effects on clot morphology. Turbidity analysis showed a 4.6 fold decrease in maximum absorbance with 5% (v/v) DMSO (Figure 5A, n=3). The turbidity data was corroborated using confocal microscopy using normal pool plasma which demonstrated a dose dependent increase in clot density with increasing DMSO concentrations (Figure 5B).

The compound, T101 has been used in many studies to inhibit the activity of FXIII but, as far as the authors are aware, has not been investigated for its direct effect on fibrin clots. To determine its effect on clot structure independently of FXIII inhibition, clot formation was followed by turbidity analysis. Turbidity analysis showed a non-significant dose-dependent trend of increase in maximum absorbance with increasing T101 concentrations in the absence of FXIII (Figure 6; $p=0.06$). However, as would be expected, in the presence of FXIII a similar trend was observed that was statistically significant (Figure 6; $p<0.05$).

Discussion

There is growing evidence to suggest that fibrin clot morphology plays an important role in arterial and venous thrombosis(8). Several studies in the past few years have demonstrated that patients with thrombosis show a propensity to form clots that are denser, have smaller pores and increased resistance to fibrinolysis(18;20-23). Taken together, these data indicate that fibrin clot structures with increased fibre branching and resistance to fibrinolysis are a risk factor for thrombosis, although robust evidence from longitudinal studies is lacking. Fibrin clot formation is a dynamic process, and re-arrangements of fibrin fibres have been shown to occur several minutes after the visible gel has been formed(24). Previous studies of a genetic variant of FXIII that changes the FXIII activation rate by thrombin (Val34Leu) have indicated that cross-linking by FXIIIa does not merely stabilise an existing fibrin network structure, but that FXIIIa is able to influence the dynamics of fibrin formation and therefore clot structure(15;25). However, the specific effects of FXIII on clot structure have hitherto not been comprehensively investigated. We therefore addressed this question by studying fibrin formation in a purified system in the absence and presence of FXIII, and demonstrated that FXIII has a direct effect both on clot structure and fibrinolysis, in the absence of α2-antiplasmin. These data are important for our understanding of the role of FXIII in fibrin clot formation and stability and its role in haemostasis and thrombosis.

Our study shows that cross-linking by FXIIIa reduces average fibrin fibre diameter, increases fibre density in the clot and increases resistance of the fibrin to fibrinolysis. The effect of FXIII on fibre diameter and fibre density was studied using scanning electron microscopy of fibrin made from fibrinogen that was depleted of FXIII in the presence and absence of purified human FXIII. Consistent with the EM data, fibrin polymerisation studies using turbidity analysis showed a lower maximum absorbency indicative of thinner fibres. We also studied fibrin pore-structure by permeation, which showed reduced pore-size in clots cross-linked by FXIIIa. Laser scanning confocal microscopy showed that in fully hydrated state, cross-linked clots showed denser structure, and we showed that cross-linking by FXIIIa prolonged fibrinolysis under static and flow conditions. A previous study by Ryan et al. examined fibrin clot structure in the presence and absence of FXIII inhibitor T101 which indicated that transglutaminase dependent cross-linking decreased fibre diameter and increased fibre length as analysed by scanning electron microscopy(26), largely in agreement with our findings. However, the study by Ryan et al. was entirely based on the use of an inhibitor against FXIIIa (T101) which also targets other transglutaminases. Furthermore our data suggests that the T101 compound itself may modulate fibrin clot structure causing an increase in turbidity maximum absorbance, in the absence of FXIII. We also found effects of the solvent DMSO, which is often used to dissolve small molecule compounds (including T101) on fibrin clot structure. Therefore, caution should be taken to use appropriate controls in the presence of DMSO when observing small molecule effects on clot structure.

We have investigated the effects of FXIII on fibrin clot structure using fibrinogen preparations that were free of FXIII activity(10) with and without the addition of purified FXIII using a wide array of complementary methods. These studies unequivocally and consistently showed significant effects of FXIIIa on the fibrin clot, rendering its structure more prothrombotic and resistant to fibrinolysis.

FXIII cross-links several proteins to the fibrin clot, including α2-antiplasmin(7;27). Using purified systems, we found that FXIIIa directly influences fibrin clot structure, independently of other proteins. While these proteins may contribute to the modulation of fibrin clot structure and function, our data provide evidence that the cross-linking of fibrin itself by FXIIIa influences the structure and function of the clot and reduced fibrinolysis rates. A recent study by Fraser et al. indicated that the resistance of a cross-linked fibrin clot to fibrinolysis was exclusively due to the incorporation of α2-antiplasmin into the fibrin α-chain by FXIIIa(9), however our data indicate that FXIII cross-linking of fibrin also plays a role in prolonging fibrinolysis. The differences between these studies may be explained by different methodologies; (i) our assays were performed

in a purified system free from endogenous FXIII activity, whereas Fraser et al. used either FXIII depleted plasma or the addition of a transglutaminase inhibitor; (ii) in our study we modified the Chandler loop protocol from fibrinolysis post-thrombi formation in a static system to the more physiological relevant addition of fibrinolytic agents before clot formation and measuring fibrinolysis under flow conditions. We postulate that our experimental approach enables greater sensitivity to fibrinolysis, therefore, we conclude that FXIII cross-linking in the absence of α2-antiplasmin does prolong fibrinolysis by altering the clot structure. This conclusion is further substantiated as previous studies have shown that clots with thin fibres and smaller pores are more resistant to fibrinolysis than clots consisting of larger pores and thicker fibres(28). It has also been suggested that this may be due to decreased access of the fibrinolytic proteins to clots with lower permeability(29). In addition, other studies have shown that clots consisting of thinner fibres that are more densely packed show reduced binding of tPA and plasminogen leading to reduced conversion rates of plasminogen to plasmin(18;30;31). An elegant review by Muszbek et al. discussed the importance of the time scale of the two events: α2-antiplasmin incorporation occurs at an early stage of clot formation whilst cross-linking of α–chains is a much slower process(32). However, γ-γ chain cross-linking is likely to occur in tandem with α2-antiplasmin incorporation and hence the role of γ-γ chain cross-linking with respect to fibrinolysis remains to be established. Therefore, whilst we agree that cross-linking of α2-antiplasmin plays the most significant role with respect to resistance to fibrinolysis in the physiological setting, our data show that FXIII cross-linking of fibrin does modulate clot structure and rate of fibrinolysis. Further studies on this effect in plasma, whole blood and in vivo systems would help delineate the role of cross-linking of α2-antiplasmin compared with the direct modulation of fibrin structure by FXIII.

A recent study by Carlisle et al. showed that cross-linking preferentially strengthened branch-point structures in fibrin, suggesting a potential role for FXIIIa in branch-point formation(33). Covalent cross-linking of adjacent fibrin αC-region by FXIIIa may help to increase the ordered structure underpinning αC-αC interactions(34), thereby reducing space between laterally aggregated protofibrils and reducing average fibre diameter. Additionally, fibrin fibres twist around each other and covalent cross-linking of the γ-chains may reduce the amount of stretch outer protofibrils are able to undergo, limiting fibre growth to a smaller diameter in which the thermodynamic energy involved in the stretch of the twisting protofibrils balance that of the molecular interaction forces(35). Further studies are required to elucidate the molecular mechanisms involved in the modulation of fibrin clot structure by FXIIIa.

Our findings that cross-linking by FXIIIa influences the structure of the fibrin clot have major implications for our understanding of the final stages of blood clot formation. Our data show that rather than stabilising an already existing fibrin clot structure, FXIIIa is able to influence fibrin formation, leading to the formation of clots with thinner fibrin fibres and increased fibre density. Furthermore, our data show that cross-linking by FXIIIa directly slows down fibrinolysis of the clot, through alterations in fibrin structure, independently of other plasma proteins. These data therefore present a novel physiological process regulating fibrin clot structure and stability. Future studies elucidating the molecular mechanisms underpinning these effects may provide new targets for therapeutics to treat disorders of haemostasis and thrombosis.

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Addendum: E.L.H and A.L.C. performed the experiments, assisted in study design, analysed data and wrote the manuscript. C.D. performed the electron microscopy and reviewed the manuscript. M.A and P.J.G. assisted study design and reviewed the manuscript. R.A.S.A. supervised the work,

assisted in study design and data analysis, and wrote the manuscript; H.P. provided overall supervision, was responsible for study design and data analyses, and wrote the manuscript.

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Reference List

(1) Doolittle RF. Fibrinogen and Fibrin. In: Bloom A, Forbes C, Thomas D, Tuddenham E, editors. Haemostasis and Thrombosis.Edinburgh: Churchill Livingstone; 1994. p. 491-513.

(2) Schroeder V, Kohler HP. New developments in the area of factor XIII. J Thromb Haemost 2013 Feb;11(2):234-44.

(3) Muszbek L, Bereczky Z, Bagoly Z, Komaromi I, Katona E. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. Physiol Rev 2011 Jul;91(3):931-72.

(4) Takagi T, Doolittle RF. Amino acid sequence studies on factor XIII and the peptide released during its activation by thrombin. Biochemistry 1974 Feb 12;13(4):750-6.

(5) Lorand L, Gray AJ, Brown K, Credo RB, Curtis CG, Domanik RA, Stenberg P. Dissociation of the subunit structure of fibrin stabilizing factor during activation of the zymogen. Biochem Biophys Res Commun 1974 Feb 27;56(4):914-22.

(6) Lorand L. Factor XIII: structure, activation, and interactions with fibrinogen and fibrin. Ann N Y Acad Sci 2001;936:291-311.

(7) Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. J Clin Invest 1980 Feb;65(2):290-7.

(8) Undas A, Ariens RA. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. Arterioscler Thromb Vasc Biol 2011 Dec;31(12):e88-e99.

(9) Fraser SR, Booth NA, Mutch NJ. The antifibrinolytic function of factor XIII is exclusively expressed through alpha(2)-antiplasmin cross-linking. Blood 2011 Jun 9;117(23):6371-4.

(10) Smith EL, Cardinali B, Ping L, Ariens RA, Philippou H. Elimination of coagulation factor XIII from fibrinogen preparations. J Thromb Haemost 2013 May;11(5):993-5.

(11) Ajjan R, Lim BC, Standeven KF, Harrand R, Dolling S, Phoenix F, Greaves R, Abou-Saleh RH, Connell S, Smith DA, Weisel JW, Grant PJ, Ariens RA. Common variation in the C-terminal region of the fibrinogen beta-chain: effects

on fibrin structure, fibrinolysis and clot rigidity. Blood 2008 Jan 15;111(2):643-50.

(12) Philippou H, Rance J, Myles T, Hall SW, Ariens RA, Grant PJ, Leung L, Lane DA. Roles of low specificity and cofactor interaction sites on thrombin during factor XIII activation. Competition for cofactor sites on thrombin determines its fate. J Biol Chem 2003 Aug 22;278(34):32020-6.

(13) Standeven KF, Carter AM, Grant PJ, Weisel JW, Chernysh I, Masova L, Lord ST, Ariens RA. Functional analysis of fibrin {gamma}-chain cross-linking by activated factor XIII: determination of a cross-linking pattern that maximizes clot stiffness. Blood 2007 Aug 1;110(3):902-7.

(14) Collet JP, Moen JL, Veklich YI, Gorkun OV, Lord ST, Montalescot G, Weisel JW. The alphaC domains of fibrinogen affect the structure of the fibrin clot, its physical properties, and its susceptibility to fibrinolysis. Blood 2005 Dec 1;106(12):3824-30.

(15) Ariens RA, Philippou H, Nagaswami C, Weisel JW, Lane DA, Grant PJ. The factor XIII V34L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. Blood 2000 Aug 1;96(3):988-95.

(16) Woodhead JL, Nagaswami C, Matsuda M, Arocha-Pinango CL, Weisel JW. The ultrastructure of fibrinogen Caracas II molecules, fibers, and clots. J Biol Chem 1996 Mar 1;271(9):4946-53.

(17) Mutch NJ, Moir E, Robbie LA, Berry SH, Bennett B, Booth NA. Localization and identification of thrombin and plasminogen activator activities in model human thrombi by in situ zymography. Thromb Haemost 2002 Dec;88(6):996-1002.

(18) Dunn EJ, Philippou H, Ariens RA, Grant PJ. Molecular mechanisms involved in the resistance of fibrin to clot lysis by plasmin in subjects with type 2 diabetes mellitus. Diabetologia 2006 May;49(5):1071-80.

(19) Weisel JW, Veklich Y, Collet JP, Francis CW. Structural studies of fibrinolysis by electron and light microscopy. Thromb Haemost 1999 Aug;82(2):277-82.

(20) Scott DJ, Prasad P, Philippou H, Rashid ST, Sohrabi S, Whalley D, Kordowicz A, Tang Q, West RM, Johnson A Woods J, Ajjan RA, Ariens RA. Clot architecture is altered in abdominal aortic aneurysms and correlates with aneurysm size. Arterioscler Thromb Vasc Biol 2011 Dec;31(12):3004-10.

(21) Undas A, Nowakowski T, Ciesla-Dul M, Sadowski J. Abnormal plasma fibrin clot characteristics are associated with worse clinical outcome in patients with peripheral arterial disease and thromboangiitis obliterans. Atherosclerosis 2011 Apr;215(2):481-6.

(22) Rooth E, Wallen NH, Blomback M, He S. Decreased fibrin network permeability and impaired fibrinolysis in the acute and convalescent phase of ischemic stroke. Thromb Res 2011 Jan;127(1):51-6.

(23) Collet JP, Allali Y, Lesty C, Tanguy ML, Silvain J, Ankri A, Blanchet B, Dumaine R, Gianetti J, Payot L, Weisel JW, Montalescot G. Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherothrombosis. Arterioscler Thromb Vasc Biol 2006 Nov;26(11):2567-73.

(24) Chernysh IN, Weisel JW. Dynamic imaging of fibrin network formation correlated with other measures of polymerization. Blood 2008 May 15;111(10):4854-61.

(25) Lim BC, Ariens RA, Carter AM, Weisel JW, Grant PJ. Genetic regulation of fibrin structure and function: complex gene-environment interactions may modulate vascular risk. Lancet 2003 Apr 26;361(9367):1424-31.

(26) Ryan EA, Mockros LF, Weisel JW, Lorand L. Structural origins of fibrin clot rheology. Biophys J 1999 Nov;77(5):2813-26.

(27) Kimura S, Aoki N. Cross-linking site in fibrinogen for alpha 2-plasmin inhibitor. J Biol Chem 1986 Nov 25;261(33):15591-5.

(28) Collet JP, Park D, Lesty C, Soria J, Soria C, Montalescot G, Weisel JW. Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy. Arterioscler Thromb Vasc Biol 2000 May;20(5):1354-61.

(29) Sakharov DV, Rijken DC. The effect of flow on lysis of plasma clots in a plasma environment. Thromb Haemost 2000 Mar;83(3):469-74.

(30) Mutch NJ, Engel R, Uitte de WS, Philippou H, Ariens RA. Polyphosphate modifies the fibrin network and down-regulates fibrinolysis by attenuating binding of tPA and plasminogen to fibrin. Blood 2010 May 13;115(19):3980-8.

(31) Longstaff C, Thelwell C, Williams SC, Silva MM, Szabo L, Kolev K. The interplay between tissue plasminogen activator domains and fibrin structures in the regulation of fibrinolysis: kinetic and microscopic studies. Blood 2011 Jan 13;117(2):661-8.

(32) Muszbek L, Bagoly Z, Bereczky Z, Katona E. The involvement of blood coagulation factor XIII in fibrinolysis and thrombosis. Cardiovasc Hematol Agents Med Chem 2008 Jul;6(3):190-205.

(33) Carlisle CR, Sparks EA, Der LC, Guthold M. Strength and failure of fibrin fiber branchpoints. J Thromb Haemost 2010 May;8(5):1135-8.

(34) Tsurupa G, Hantgan RR, Burton RA, Pechik I, Tjandra N, Medved L. Structure, stability, and interaction of the fibrin(ogen) alphaC-domains. Biochemistry 2009 Dec 29;48(51):12191-201.

(35) Weisel JW, Nagaswami C, Makowski L. Twisting of fibrin fibers limits their radial growth. Proc Natl Acad Sci U S A 1987 Dec;84(24):8991-5.

FIGURE LEGENDS

Figure 1. Fibrinogen purified by IF-1 affinity chromatography and ammonium sulphate precipitation are depleted of α2-antiplasmin contamination. Western blot analysis of the two fibrinogen preparations using an anti-α2-antiplasmin antibody.

Figure 2. Factor XIII plays a role in modulating clot structure. Fibrinogen was clotted in the absence (grey line) and presence (black line) of FXIII. Turbidity measurements demonstrated a reduced maximum absorbance in the presence of FXIII (A; +FXIII = 0.54 ±0.01 OD, -FXIII = 0.68 ±0.01 OD). The coefficient of permeability (Darcy constant, Ks) represents average pore size of the fibrin structure by measuring the flow rate of a buffer through a fibrin clot. The experiments are based on 3 clots in the presence or absence of FXIII. Mean Ks ±SEM presented. Clots formed in the presence of FXIII have a significantly lower Ks (B; +FXIII = 1.52 ±0.20 $x10^{-9}$ cm^2, -FXIII = 3.22 ±0.44 $x10^{-9}$ cm^2 *$p<0.05$).

Figure 3. FXIII induced changes in fibrin clot ultra-structure. Clots were formed by incubating fibrinogen and thrombin in the presence or absence of FXIII. Clots were visualised in 10 different areas using the FEI Quanta 200 FEGSEM electron microscope. Four different magnifications are presented (A). Fibre thickness was analysed using ImageJ and mean ±SEM are represented. Clots formed in the presence of FXIII had significantly thinner fibres (B; +FXIII = 74.7 ±1.5 nm, -FXIII = 86 ±1.7 nm *$p<0.001$) but significantly more fibres/μm (C; determined from 3 different cross-sections of each image; +FXIII = 6.06 ±0.9 fibres/μm, -FXIII = 5.40 ±0.1 fibres/μm *$p<0.001$)

Figure 4. The presence of FXIII delays fibrinolysis. Laser scanning confocal microscopy of fibrin clots formed using fibrinogen containing 5% (v/v) FITC fibrinogen in the presence of FXIII demonstrated a denser structure (A). Fibrinolysis of clots was initiated by the addition of plasminogen and tPA. The time to lysis was significantly delayed in fibrin clots formed in the presence of FXIII (B; +FXIII = 1830 ±97.1 secs, -FXIII = 1573 ±61.7 secs $p<0.05$). Clot formation using fibrinogen containing 5% (v/v) FITC fibrinogen in the presence and absence of FXIII with simultaneous fibrinolysis (plasminogen and tPA) was performed under flow using the Chandler loop (C). Fibrinolysis was determined by measuring the release of fluorescence (FITC fibrin) from the fibrin clot. Fibrinolysis was slower in clots formed in the presence of FXIII. Mean ±SEM presented.

Figure 5. DMSO independently alters clot structure. Ammonium sulphate purified fibrinogen was clotted with increasing DMSO concentrations. Turbidity measurements demonstrated a dose dependent reduction in maximum absorbance with increasing DMSO concentration (A). Laser scanning confocal microscopy of normal pool plasma clots demonstrated a dose dependent increase in clot density with increasing concentrations of DMSO (B).

Figure 6. The FXIII inhibitor (T101) may modify clot structure, independently of FXIII. Turbidity of fibrin clot formation in presence and absence of FXIII with increasing concentrations of T101 inhibitor [1% (v/v) DMSO final concentration] demonstrated a trend of dose dependent increase in maximum absorbance with increasing T101 concentration independent of FXIII ($p=0.06$).