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Fibrinogen and Factor XIII: Newly-Recognized Roles in Venous Thrombosis Formation and Composition

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Abstract

Purpose of review—In spite of significant morbidity and mortality associated with venous thromboembolism, the underlying pathogenesis remains poorly understood.

Recent findings—Clues to operant pathogenic mechanisms are found in the unique morphology and composition of these thrombi, which have substantial red blood cell and fibrin content. Recent studies have revealed biochemical and biophysical mechanisms that dictate fibrin structure in venous thrombi and promote retention of red blood cells within the contracted clots. These mechanisms include newly-recognized contributions of fibrin network structure and factor XIII(a)-mediated fibrin crosslinking to venous thrombus composition, size, and stability.

Summary—Continued work to elucidate mechanisms by which fibrin(ogen), factor XIII, and red blood cells contribute to venous thrombus formation, structure, and stability may expose novel molecular targets and strategies for reducing thrombosis and thrombotic complications in certain at-risk patients.

Keywords

Venous thrombosis; fibrinogen; factor XIII; red blood cells

INTRODUCTION

Venous thrombosis (VT) with or without pulmonary embolism (PE), collectively venous thromboembolism (VTE), is a major public health concern. VTE affects 1–2 individuals per 1000 each year in the United States and in Europe.(1–3) VTE is associated with a high mortality rate, estimated between 10–30%. Deaths usually occur within the first 30 days of diagnosis and are typically associated with PE. Even in surviving patients, up to 50% develop long-term sequelae, including post-thrombotic syndrome or chronic thromboembolic pulmonary hypertension, which dramatically decrease quality-of-life.(1–3)

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CONFLICTS OF INTEREST

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All current antithrombotic drugs used to reduce primary or recurrent VTE are associated with an increased risk of bleeding. Moreover, a subset of patients experience thrombosis recurrence in spite of aggressive anticoagulation therapy. Thus, efforts to understand the pathophysiology of VTE and advance discoveries into novel therapeutic strategies are important.

Although cellular, molecular, and biophysical mechanisms leading to VTE remain poorly understood, recent studies have revealed specific roles for fibrinogen, and fibrin clot formation, structure, and crosslinking in the development and composition of venous thrombi. These findings extend our understanding of the mechanisms contributing to VTE, and suggest fibrin(ogen) and factor XIII (FXIII) are new potential therapeutic targets for reducing development of venous thrombi and accelerating their resolution. Herein, we review these discoveries and their implications for basic and translational research into the causes and treatment of VTE.

PATHOGENESIS OF VTE

The current conceptual model of events leading to VTE includes pathologic interactions between dysregulated blood coagulation pathways, vascular function, and blood flow (stasis). This combination of mechanisms is known as “Virchow’s Triad.” Briefly, VTE occurs in the veins and is thought to initiate in the pocket region behind venous valves. Within this region, non-laminar blood flow promotes endothelial cell activation. Activated endothelial cells express cell adhesion molecules, leading to the accumulation of leukocytes. Together, these activated leukocytes and endothelial cells initiate the coagulation cascade, which is propagated by blood flow- and red blood cell (RBC)-mediated accumulation of platelets within the venous valve pocket.(4) These processes produce a burst of thrombin, culminating in conversion of fibrinogen to fibrin, which traps resident proteins and blood cells within the growing and contracting thrombus. Cumulatively, this process results in the formation of an intravascular mass that occludes blood flow.

Venous thrombus composition

In contrast to arterial clots, which are primarily composed of aggregated platelets, venous thrombi are rich in fibrin and RBCs. Accordingly, when compared to relatively platelet-rich arterial “white” clots, venous thrombi are often referred to as “red clots.” Transmission electron microscopy reveals a remarkable venous thrombus ultrastructure in which RBCs lose their characteristic discoid shape and become distorted.(5) These compressed RBC shapes, termed “polyhedrocytes,” are indicative of substantial forces that occur within the thrombus during platelet-mediated clot contraction.(6) Polyhedrocytes often appear arranged among fibrin layers in a “brick-and-mortar” fashion within the thrombus (sometimes called “Lines of Zahn”), suggesting an ordered and cyclical process leads to venous thrombus growth within the vessel. The proximity and arrangement of RBCs and fibrin within the clot ultrastructure implies a mechanistic relationship that has only recently been identified.

FIBRIN FORMATION AND FUNCTION

During coagulation, fibrin is generated by thrombin-mediated proteolytic cleavage of fibrinogen. This reaction releases N-terminal fibrinopeptides from the fibrinogen Aα- and Bβ-chains, exposes “knobs” at the N-termini of the α- and β- chains, and permits assembly of half-staggered fibrin monomers into fibrin protofibrils. Subsequent lateral aggregation and branching of protofibrils results in the formation of an insoluble 3-dimensional fibrin network. The molecular mechanisms governing this process have been extensively reviewed.(7–10) Abnormalities in circulating fibrinogen level and/or function, including congenital and acquired hypofibrinogenemia, afibrinogenemia, and dysfibrinogenemia, are associated with increased risk of bleeding and/or thrombosis.(11, 12)

Thrombin also proteolytically converts the (pro)transglutaminase FXIII to activated FXIII (FXIIIa). In contrast to other coagulation enzymes that proteolytically cleave their substrates, FXIIIa catalyzes the formation of new covalent bonds. These bonds are generated between lysyl residues in an amine donor molecule and glutamyl residues in an amine acceptor molecule, producing novel ε-N-[γ-glutamyl]-lysyl crosslinks. The primary substrate for FXIIIa is fibrin, where it crosslinks γ-chains and α-chains into γ-γ dimers and high molecular weight α-α and γ-α polymers. FXIIIa also crosslinks anti-fibrinolytic proteins (e.g., α_2-antiplasmin) to the fibrin network. Studies have demonstrated essential contributions of these crosslinks to fibrin clot mechanical stability and resistance to fibrinolysis.(13)

Several findings are particularly noteworthy with regard to fibrin network formation, crosslinking, and function. First, the concentration of thrombin present during fibrin formation profoundly influences not only the rate of fibrinogen conversion to fibrin, but also the structure and function of the resulting fibrin network. Low thrombin concentrations produce thick fibrin fibers arranged in coarse, permeable networks with large pores, and these networks are relatively susceptible to fibrinolysis. In contrast, high thrombin concentrations produce thin fibrin fibers arranged in less permeable, dense networks with small pores, and these networks are relatively resistant to fibrinolysis.(7–10) Second, although FXIIIa crosslinking of fibrin has little-to-no influence on fibrin network density, it increases fibrin fiber compaction and stiffness by promoting protofibril coupling within individual fibers.(14–18) Fiber compaction also decreases the size of pores within individual fibers, with potentially important effects on diffusion of molecules like tissue-type plasminogen activator (tPA) through fibers and consequently, on a clot’s resistance to fibrinolysis. Thus, fibrin clot structural properties are not only interesting from an observational standpoint, but also appear to have direct functional implications for hemostasis and thrombosis.

FIBRIN FORMATION, STRUCTURE, AND FUNCTION IN VT

Abnormal ex vivo fibrin clot structure and VT

Several studies have documented abnormal fibrin formation, structure, and stability in patients with a history of VTE.(19) Compared to healthy controls, VTE patients generally have lower plasma clot permeability, lower clot compaction, and prolonged clot lysis times.

Similar, though blunted, findings have also been observed in apparently healthy relatives of VTE patients, suggesting shared genetic or environmental causes of the abnormal clot structure.(20) Moreover, following discontinuation of anticoagulation after first DVT, faster formation of less permeable fibrin networks and prolonged clot lysis time is associated with increased risk of DVT recurrence (odds ratio 15.8; confidence interval 7.5–33.5).(21) Abnormal clot properties, including reduced plasma clot permeability and susceptibility to fibrinolysis are also associated with increased risk of developing long-term sequelae, including chronic thromboembolic pulmonary hypertension, post-thrombotic syndrome, and persistent venous obstruction.(22–24) Notably, although these observations are of platelet-poor plasma clots formed ex vivo, their association with VTE risk suggests these clot properties recapitulate aspects of venous thrombus (dys)function in vivo. Thus, abnormal fibrin clot structure, permeability, and resistance to fibrinolysis are not only powerful potential biomarkers for thromboembolic disease, but may also reveal fundamental aspects of the pathophysiology underlying intravascular clot formation and impact.

Plasma hypercoagulability, abnormal clot properties, and VT

Given the close relationship between thrombin generation and fibrin formation and crosslinking, it is perhaps not surprising that abnormal levels and/or function of proteins that regulate thrombin generation or activity are associated with both formation of abnormal clots and increased VTE risk. These abnormalities include elevated levels of factor VIII, prothrombin, or fibrinogen, presence of the factor V_{Leiden} mutation, or reduced levels of antithrombin or proteins C or S.(2) For example, presence of the G20210A mutation is associated with elevated levels of circulating prothrombin and increased risk of DVT.(25, 26) During coagulation, elevated prothrombin enhances thrombin generation, with substantial effects on the fibrin network: formation of dense fibrin networks composed of thin fibrin fibers, and decreased clot permeability.(27, 28) Hyperprothrombinemic mice have increased fibrin deposition in thrombi, and increased thrombus weight.(29) Intriguingly, venous thrombi isolated from hyperprothrombinemic mice also have increased RBC content (MM Aleman & AS Wolberg, unpublished observation), suggesting fibrin network properties also influence the cellular content of venous thrombi formed in vivo.

Fibrin network density and FXIIIa-mediated fibrin crosslinking promote RBC retention in VT

Following the observation that thrombi isolated from hyperprothrombinemic mice showed increased RBC content, a second major clue to understanding the structure and composition of venous thrombi arose during experiments to analyze fibrin(ogen) function during VT. Using both genetic and pharmacologic approaches, we discovered that FXIII promotes RBC retention in venous thrombi.(30) Clots generated from whole blood isolated from *F13a*$^{-/-}$ mice demonstrate dramatic loss of RBCs during platelet-mediated clot contraction, resulting in the formation of abnormally small clots. Using an inferior vena cava ligation model of VT, we demonstrated that this mechanism also occurs in vivo.(30, 31) Using plasma from a patient with FXIII-deficiency and transglutaminase inhibitors in normal whole blood, we translated this observation to human blood, and showed a dose-dependent effect of FXIII on RBC retention.(30, 32) These findings are reminiscent of observations of “excessive RBC fallout” previously noted during contraction of whole blood clots in a family of FXIII-

deficient patients.(33) Collectively, these observations expose a previously unrecognized role for FXIII activity during clot formation and VT.

Work to interrogate the operant mechanism by which fibrin(ogen) and FXIII promote RBC retention in thrombi has revealed at least two independent mechanisms. First, experiments to examine the contribution of fibrin network density showed that RBCs are effectively "trapped" by the fibrin network, and that clots with increased fibrin network density trap higher numbers of RBCs.(32) This observation may provide a mechanism linking plasma hypercoagulability and the formation of dense fibrin networks to increased thrombosis risk. For example, elevated levels of FVIII and prothrombin that enhance thrombin generation, fibrin deposition, and network density(27, 29, 34) may generate fibrin networks that trap higher numbers of RBCs in clots during clot contraction and produce larger thrombi.

Notably, however, since FXIII has little-to-no effect on fibrin network density(14, 15), the effects of FXIII on RBC retention in clots appear to be independent of fibrin network density. Experiments with recombinant fibrinogen variants capable of undergoing either α-chain or γ-chain crosslinking but not both, as well as the detection of crosslinked fibrin species in clots treated with crosslinking inhibitors, associated the effect of FXIII activity on RBC retention with the formation of high molecular weight fibrin species containing crosslinked α-chains.(32) This observation invokes intriguing findings that crosslinking – and particularly α-chain crosslinking – has significant functional consequences for fibrin biophysical and biomechanical properties.(14–18, 35–38) Crosslinking increases fibrin fiber elastic modulus (stiffness) and elasticity (3.75- and 1.2-fold, respectively), and decreases fiber extensibility (1.2-fold). Notably, α-chain crosslinking, even in the absence of γ-chain crosslinking, recapitulates a substantial portion of these effects, including a 2.5-and 1.5-fold increase in fiber stiffness and elasticity, respectively.(38) Given the considerable forces generated by contracting platelets(39), these studies suggest fibrin crosslinking enables the fibrin network to resist deformation and retain RBCs in clots during clot contraction. Thus, these observations may provide a (patho)physiologically-relevant rationale for the remarkable biophysical characteristics of fibrin in hemostasis and thrombosis.

Plasma FXIII promotes RBC retention in VT

FXIII is present in both plasma and cellular forms. Plasma FXIII is composed of 2 catalytic A subunits (FXIII-A_2) and 2 non-catalytic B subunits (FXIII-B_2) arranged as a tightly-associated heterotetramer (FXIII-A_2B_2).(40) FXIII-A_2B_2 circulates in plasma bound to fibrinogen at residues γ-chain 390–396 and possibly also to α-chain residue E396.(30, 41, 42) During coagulation, thrombin rapidly cleaves N-terminal peptides from the FXIII A-subunits. Calcium then induces a conformational change in FXIII, which promotes release of the FXIII-B subunits and yields fully activated FXIII-A_2* (FXIIIa). Cellular FXIII is a homodimer consisting only of the catalytic A subunits (FXIII-A_2). FXIII-A_2 is found in the cytoplasm of bone marrow-derived cells, including megakaryocytes, monocytes, macrophages, osteoblasts, and platelets. Cellular FXIII-A_2 can be activated non-enzymatically by elevated intracellular calcium concentrations that occur during platelet activation.(43) Following platelet activation, FXIII-A_2* is exposed on the platelet surface(44); however, cellular mechanisms leading to its externalization in platelets and

other cells are unknown. Although some studies have suggested platelet FXIII is essential for platelet contraction(45), we and others have detected only moderate or no influence of platelet FXIII on platelet contractile events.(31, 46, 47)

Since both plasma and platelet FXIII possess catalytic A subunits, both are capable of catalyzing fibrin crosslinking. Mitchell et al detected platelet FXIII-mediated crosslinking in vitro, but effects were only apparent when levels of plasma FXIII were less than 10% of normal levels.(44) Interestingly, however, plasma FXIII, but not platelet FXIII, promotes RBC retention in contracted clots.(31) This function is associated with an apparent need for fibrin α-chain crosslinking *prior to* initiation of platelet-mediated clot contraction.(31) Since plasma FXIII circulates bound to fibrin, it is rapidly converted to FXIIIa in concert with fibrin formation and can fulfill this temporal requirement for fibrin crosslinking.(41) In contrast, slower exposure of platelet FXIII likely happens *after* clot contraction – too late to mechanically stabilize fibrin fibers and enable them to retain RBCs during clot contraction.

FXIII as a therapeutic target

Given the roles of FXIII(a) in determining fibrin network permeability, resistance to fibrinolysis and mechanical disruption, and thrombus composition and size, it is intriguing to consider that FXIII(a) and fibrin crosslinking may be therapeutic targets for reducing VTE. This approach may offer certain advantages over anticoagulants that reduce plasma concentrations of vitamin K-dependent proteins (vitamin K antagonists) or directly target enzymes within the coagulation cascade (factor Xa, thrombin - the so-called “direct oral anticoagulants” or DOACs). Although each of these anticoagulants significantly reduce the incidence of VT, all are associated with increased bleeding risk.(2) Notably, all of these antithrombotics target molecules that lead to thrombin generation and therefore, reduce fibrin formation. A strategy that permits fibrin formation in situations necessitating hemostasis, but reduces the stability of thrombotic networks might mitigate this risk. In this regard, targeting FXIII(a) and/or fibrin crosslinking might offer advantages not realized with other anticoagulants. Unlike vitamin K antagonists or DOACs, a FXIII(a) inhibitor would not be expected to interfere with thrombin generation or fibrin deposition (Figure). Indeed, genetic reduction of FXIII in mice does not alter thrombin generation in plasma or prolong the time to hemostatic clot formation in saphenous vein puncture or tail transection bleeding models.(31) These findings are consistent with clinical observations indicating that although patients with <4% FXIIIa activity have a significantly increased risk of bleeding, individuals with ≥30% FXIIIa activity, including those with acquired FXIII deficiency, are usually asymptomatic.(48) Thus, FXIII(a) antagonists may enjoy a wide therapeutic range and could be used alone or in concert with conventional anticoagulants. Moreover, association of FXIII impact on thrombus RBC retention and size with plasma, but not platelet, FXIII(31) may facilitate development of antagonists that would only need to inhibit the plasma compartment. Strategies that target plasma FXIII but leave platelet FXIII intact may allow sufficient residual activity to prevent major bleeds associated with antithrombotics that target hemostatic proteins.

CONCLUSION and FUTURE DIRECTIONS

Collectively, these studies have identified newly-recognized mechanisms by which fibrin, FXIII activation, and fibrin crosslinking promote RBC retention in thrombi and mediate venous thrombus size. These discoveries have enhanced our understanding of VTE pathogenesis, but also revealed knowledge gaps that must be filled to advance these findings into clinical applications.

First, fibrin(ogen) has pleiotropic roles in hemostasis and thrombosis, including functions that influence inflammation, host defense, and thrombus resolution. Continued studies are necessary to understand these functions and identify antagonists with minimal effects on homeostatic effects of fibrinogen and fibrin deposition. Second, RBCs have biochemical and biophysical properties that may influence their incorporation into thrombi and their subsequent effects on thrombus stability and resolution. For example, RBCs with abnormal deformability as in sickle cell disease or spherocytosis may lead to increased retention in thrombi and therefore, result in formation of larger, more occlusive thrombi.(49) RBCs can also induce platelet accumulation and activation within clots in the presence of flow(4), and RBC compaction within contracted thrombi can decrease thrombus permeability and accessibility of fibrinolytic enzymes.(6) Thus, the relative impact of altering RBC presence in thrombi warrants careful evaluation. Third, identification of polyhedrocytes within venous thrombi demonstrates the presence and function of activated platelets within thrombi. Surprisingly, however, the contribution of platelets to VTE is poorly understood. Platelet antagonists that target platelet contraction may have important effects on thrombus development and function. Fourth, currently available transglutaminase inhibitors lack specificity for FXIII and have short half-lives not amenable to prophylactic dosing strategies. Moreover, the FXIII active site is structurally superficial, complicating efforts to design specific antagonists to this molecule. Advanced screens and sophisticated medicinal chemistry will be essential to identify and develop compounds with the necessary pharmacologic properties for in vivo use. Finally, it is clear that many biologic functions of FXIII have not been thoroughly elucidated. In addition to coagulation, FXIII may have roles in arthritis(50), adipogenesis(51), and cardiac repair(52, 53). It will be important to understand the impact of FXIII inhibition on these situations before the benefit of a FXIIIa antagonist can be realized in the clinic.

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KEY POINTS

- The fibrin(ogen)-factor XIII-red blood cell axis mediates venous thrombus formation and composition.
- Thrombin generation promotes venous thrombosis, in part, by altering fibrin network structure.
- Fibrin network density and factor XIII influence red blood cell retention in venous thrombi through independent mechanisms.
- Plasma, but not platelet, factor XIII promotes fibrin α-chain crosslinking and red blood cell retention in clots.
- Fibrin and factor XIII(a)-mediated crosslinking may be therapeutic targets for reducing venous thromboembolism.

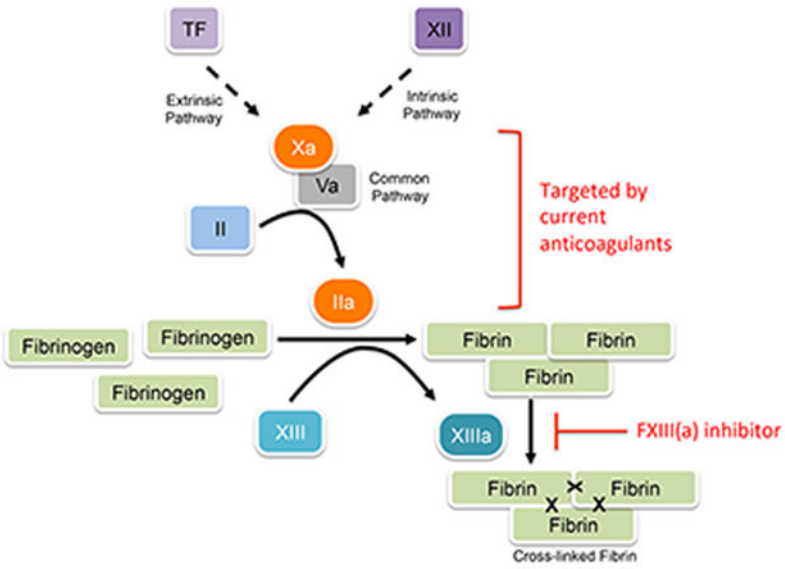

Figure. Coagulation cascade illustrating different points of intervention of current anticoagulants and a potential factor XIII/fibrin crosslinking inhibitor.

Current anticoagulants target enzymes leading to thrombin generation and therefore, interfere with fibrin formation. Blocking factor XIII(a) activity would permit normal fibrin formation, but reduce fibrin stability, red blood cell retention in thrombi, and thrombus size. In this way, factor XIII(a) inhibition would be mechanistically different from existing approaches. TF, tissue factor.