Brief Review

Factor XI as a Therapeutic Target

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Abstract—Factor XIa is a plasma serine protease that contributes to thrombin generation primarily through proteolytic activation of factor IX. Traditionally considered part of the intrinsic pathway of coagulation, several lines of evidence now suggest that factor XIa serves as an interface between the vitamin-K–dependent thrombin generation mechanism and the proinflammatory kallikrein–kinin system, allowing the 2 systems to influence each other. Work with animal models and results from epidemiological surveys of human populations support a role for factor XIa in thromboembolic disease. These data and the clinical observation that deficiency of factor XI, the zymogen of factor XIa, produces a relatively mild bleeding disorder suggest that drugs targeting factor XI or XIa could produce an antithrombotic effect while leaving hemostasis largely intact. Results of a recent trial comparing antisense-induced factor XI reduction to standard-dose low molecular–weight heparin as prophylaxis for venous thrombosis during knee replacement are encouraging in this regard. Here, we discuss recent findings on the biochemistry, physiology, and pathology of factor XI as they relate to thromboembolic disease. (Arterioscler Thromb Vasc Biol. 2016;36:1316-1322. DOI: 10.1161/ATVBAHA.116.306925.)

Key Words: factor XI  ■  factor XII  ■  hemorrhage  ■  thrombin  ■  thrombosis

Vitamin K antagonists such as warfarin have been mainstays of antithrombotic therapy for >50 years. Although these drugs demonstrate efficacy across a spectrum of clinical settings, they increase the risk of major bleeding, and frequent monitoring is required to maintain the drug effect within a narrow therapeutic window. In 2005, Hirsh et al described features of an ideal anticoagulant that included a high efficacy-to-safety index and a predictable dose response that obviates the need for laboratory monitoring. Direct oral anticoagulants (DOACs) that target thrombin or factor Xa are improvements on warfarin in these regards. They are at least as effective as warfarin for preventing stroke in patients with atrial fibrillation and treating venous thromboembolism (VTE) and are as effective as low molecular–weight heparin within a narrow therapeutic window. In 2005, Hirsh et al described features of an ideal anticoagulant that included a high efficacy-to-safety index and a predictable dose response that obviates the need for laboratory monitoring. Direct oral anticoagulants (DOACs) that target thrombin or factor Xa are improvements on warfarin in these regards. They are at least as effective as warfarin for preventing stroke in patients with atrial fibrillation and treating venous thromboembolism (VTE) and are as effective as low molecular–weight heparin within a narrow therapeutic window.

The Factor XI Molecule

FXI arose from a duplication of the gene for prekallikrein, the zymogen of α-kallikrein. Prekallikrein, factor XII (fXII), and high molecular–weight kininogen comprise the plasma kallikrein–kinin system (Figure 1). Among its functions, this system may contribute to the host response to infection by assembling on microorganisms, and generating inflammatory kinins and antimicrobial peptides. A similar process, contact activation, leads to coagulation when blood is exposed to artificial surfaces, such as medical devices used in extracorporeal blood oxygenation. During contact activation, reciprocal conversion of prekallikrein (PK) and fXII to α-kallikrein and factor XIIa (fXIIa) occurs on a surface (Figure 1). FXI, like its homolog PK, is activated by fXIIa and, similar to α-kallikrein, fXIIa has some capacity to activate fXII. However, fXI has features distinguishing it from PK that facilitate interactions with the thrombin generation mechanism (Figure 1). PK and fXI polypeptides each have 4 apple domains and a trypsin-like catalytic domain. The fXI apple 3 domain contains a factor IX-binding exosite not present on PK. The amino acid sequence adjacent to the fXI activation site (Arg369-Ile370) also differs from corresponding PK sequence, permitting fXI to be activated by thrombin, as well as by fXIIa. The combination of the premise that an antithrombotic effect can be achieved by targeting fXI without precipitating severe bleeding.

There is interest in targeting the plasma zymogen factor XI (fXI) and its protease form factor XIa (fXIIa) for prevention or treatment of thrombosis. FXI seems to contribute to VTE and ischemic stroke in humans and is required for formation of occlusive clots in animal thrombosis models. Because congenital absence of fXI is associated with a relatively mild bleeding disorder, it is anticipated that neutralizing fXI or fXII might produce a smaller defect in hemostasis than would warfarin or a DOAC. Results from a recent clinical trial involving patients undergoing knee replacement support the premise that an antithrombotic effect can be achieved by targeting fXI without precipitating severe bleeding. Here, we discuss recent findings on the biochemistry and physiology of fXI, the preclinical and clinical evidence supporting a role for this protein in thrombosis, and the mechanisms by which it may contribute to thromboembolism.
of PK-like and novel features permits fXI to promote thrombin generation through fXIa-dependent and fXIIa-independent processes (Figure 1).\(^4\)

**Factor XI in Hemostasis**

The phenotype associated with congenital fXI deficiency indicates that fXI has a role in limiting trauma-induced bleeding. In humans, severe deficiency (≤15% normal level) may exacerbate post-traumatic bleeding, particularly in areas with high fibrinolytic activity (urinary tract, nose, and mouth).\(^7\) Hemorrhage in other tissues is less frequent, and procedures such as appendectomy and cholecystectomy may be well tolerated without factor replacement.\(^7,14\) Because deficiencies of fXII, prekallikrein, or high molecular–weight kininogen are not associated with abnormal bleeding, fXI is probably activated by fXIIa-independent processes during hemostasis. In the model in Figure 1, fXI is activated by thrombin after the VIIa/tissue factor complex initiates coagulation, with fXIIa sustaining thrombin generation through factor IX activation.\(^15\) In addition to promoting fibrin formation, fXI-dependent thrombin generation may promote activation of TAFI (thrombin-activatable fibrinolysis inhibitor), a metalloproteinase that modifies fibrin by removing binding sites for fibrinolytic proteins, rendering it resistant to fibrinolytic degradation.\(^16\) Although severe fXI deficiency delays clot formation in surface-dependent assays such as the activated partial thromboplastin time, the magnitude of the abnormality correlates poorly with symptoms, and some patients with severe deficiency may not bleed abnormally, even with trauma. It is conceivable that some individuals have relatively robust factor VIIa/tissue factor activity or weak fibrinolytic activity that tips the balance in favor of clot stability, rendering fXIIa unnecessary. The clinical experience suggests that inhibitors targeting fXI/fXIIa would leave some patients more prone to trauma-induced bleeding. However, spontaneous soft tissue bleeding is not part of the phenotype of fXI deficiency, and such drugs would not be expected to precipitate severe bleeding as frequently as would warfarin or DOACs.

**Factor XI and Thrombosis in Humans**

Despite its modest role in hemostasis, there is substantial evidence supporting a role for fXI in thrombosis. Plasma fXI levels at the upper end of the normal range are linked to modest increases in VTE and ischemic stroke risk.\(^4,5\) The 10% of subjects with the highest fXI levels in the Leiden Thrombophilia Study had a 2-fold higher risk of VTE than the 90% of subjects with lowest fXI levels.\(^17\) This result is supported by data from the Longitudinal Investigation of Thromboembolism Etiology cohort,\(^18\) and the observation that fXI deficiency reduces the incidence of VTE.\(^19\) High fXI or fXIIa levels were associated...
with increased risk for ischemic stroke in several studies, including the Atherosclerosis Risk in Communities (ARIC) study\textsuperscript{20} and the Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study.\textsuperscript{21} Severe fXI deficiency reduces incidence of stroke.\textsuperscript{22} A role for fXI in myocardial infarction is less clear. FXI levels correlated with myocardial infarction risk in the Study of Myocardial Infarction Leiden study\textsuperscript{23} but not in the ARIC or RATIO studies,\textsuperscript{21,22} and the incidence of myocardial infarction in fXI-deficient people is similar to the expected incidence in age-matched controls.\textsuperscript{24} These data indicate that fXI participates in thrombosis in humans, but suggest that the contribution varies between vascular beds.

**Factor XI and Thrombosis—Animal Models**

Mice lacking coagulation factors have been compared for resistance to thrombosis by a variety of techniques.\textsuperscript{4,6} Our data for ferric chloride (FeCl\textsubscript{3})-induced carotid artery occlusion are summarized in Figure 2. Factor IX-deficient mice have a significant bleeding disorder (hemophilia B), and high FeCl\textsubscript{3} concentrations are required to induce thrombosis in these animals.\textsuperscript{25} Mice lacking fXI or fXII do not have obvious hemostatic abnormalities. Despite this, they are at least as resistant to FeCl\textsubscript{3}-induced thrombosis as factor IX-deficient mice,\textsuperscript{25,26} showing that a bleeding phenotype is not a prerequisite for resistance to thrombus formation. Mice lacking PK or high molecular–weight kininogen are also resistant to FeCl\textsubscript{3}-induced thrombosis,\textsuperscript{27} suggesting that a contact activation-like process drives thrombosis in mice.

In a primate model, fXI and fXII contributed to thrombosis, but in contrast to mice, fXI inhibition had a greater effect than fXII inhibition.\textsuperscript{26,28–30} Perhaps this reflects stronger thrombin-mediated fXI activation in primates. When tissue factor–coated or collagen-coated vascular grafts are inserted into arteriovenous shunts in baboons, platelets and fibrin deposit within the coated graft segment, followed by clot extension downstream into the uncoated graft. Polyclonal anti-fXI IgG\textsuperscript{28} or a monoclonal IgG that blocks fXIa activation of factor IX (O1A6)\textsuperscript{29} reduces platelet and fibrin accumulation within coated graft portions and blocks downstream growth. Monoclonal IgG inhibiting fXI activation by fXIIa (14E11/3G3)\textsuperscript{30} or fXII activation (15H8)\textsuperscript{31} has little effect on platelet accumulation, and a modest effect on fibrin deposition, in coated portions of grafts (Figure 3) but do limit clot extension. The results support a larger role for fXI than fXII in the primate model, in agreement with data indicating that fXI makes a greater contribution to VTE and stroke than does fXII in humans.\textsuperscript{3}

In the mouse and primate models, lack of fXI activity interferes with thrombus growth. Although this could reflect a decreased rate of clot formation, increased clot breakdown may also be a factor. Fibrinolytic degradation of plasma thrombi introduced into the jugular veins of rabbits was \( \approx 2 \) fold greater in the presence of neutralizing anti-fXI antibody than in controls,\textsuperscript{31} consistent with in vitro results demonstrating increased clot sensitivity to fibrinolysis in the absence of fXI.\textsuperscript{32} A role for fXI in enhanced clot resistance to fibrinolysis is consistent with the observation that fXI-deficient individuals bleed most frequently from tissues with high intrinsic fibrinolytic activity.

**Factor XI and Inflammation—Animal Models**

Although the contributions of fXI to coagulation have received considerable study, data from mouse models suggest that fXI also influences inflammatory processes in a manner that impacts blood vessel biology.\textsuperscript{6} Absence of protein C (PC), the zymogen of a protease that regulates coagulation and inflammation, results in perinatal death with intravascular thrombus formation and tissue inflammation prominently featured. FXI deficiency reduces the severity of this phenotype, with some PC\textsuperscript{−/−}/fXI\textsuperscript{−/−} mice living to adulthood.\textsuperscript{33} FXI deficiency reduces ischemia–reperfusion injury after temporary middle cerebral artery occlusion.\textsuperscript{34} Although reduced fibrin deposition may have a role in the tissue-sparing effect, alteration of the inflammatory response could be involved. Shnerb Ganor et al\textsuperscript{35} reported that fXI deficiency reduces atherosclerotic plaque growth and plaque infiltration by macrophages in Apolipoprotein E-deficient mice. van Montfoort et al\textsuperscript{36} also noted reduced macrophage infiltration and an absence of neutrophils in arteries in ApoE-null mice after knockdown of fXI expression. FXI-deficient mice have a survival advantage.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Ferric chloride (FeCl\textsubscript{3})-induced carotid artery occlusion. Carotid artery occlusion was induced in wild-type (WT) C57Bl/6 mice, and in mice lacking factor IX (fIX\textsuperscript{−/−}), factor XI (fXI\textsuperscript{−/−}), fXII (fXII\textsuperscript{−/−}), prekallikrein (PK\textsuperscript{−/−}), or high molecular–weight kininogen (HK\textsuperscript{−/−}) with varying concentrations of FeCl\textsubscript{3} as indicated at the bottom of the graph. The percent of animals with patent arteries 30 minutes after FeCl\textsubscript{3} exposure is shown (\( n=10 \) for each bar). HK\textsuperscript{−/−} mice are homozygous null for deletions of the Kng1 gene. Mice, unlike humans, have 2 kininogen genes (Kng1 and Kng2). Kng1 is thought to be responsible for most, if not all, of the HK in plasma. Image derived from data in references 25–27.
and puncture are reduced in fXI-deficient mice.13,37 The mechanism in markers of systemic inflammation after cecal ligation and puncture.13,37 Although initial studies pointed to reduced consumptive coagulation in fXI-deficient animals, we noted that a coagulopathy is not a consistent feature after cecal ligation and puncture. In the absence of a coagulopathy, the early cytokine response and neutrophil migration in vitro, and its absence could alter neutrophil behavior during inflammatory processes.37,38

**Therapeutic Targeting of Factors XI and Xla**

Several therapeutic strategies to target fXI and fXla are under development.3,39 Antisense oligonucleotide (ASO) knockdown of fXI expression has undergone phase II testing. 2′-Methoxymethyl DNA ASOs are avidly taken up by hepatocytes and bind to mRNAs through complementary base pairing, followed by RNase H–dependent degradation of the ASO–mRNA complex.40 The anti-fXI ASO IONIS-FXIrx (formerly ISIS-416858) was compared with standard-dose enoxaparin for prevention of VTE in patients undergoing knee replacement.8 IONIS-FXIrx was given in 200 or 300 mg doses at specific intervals for 36 days before surgery. On the day of surgery, average plasma fXI levels were 38% and 20% of normal in patients on 200 or 300 mg ASO, respectively. There were few symptomatic clots in any treatment group in this study. Venography performed 8 to 12 days post surgery detected lower extremity thrombi in 30% of patients on enoxaparin, 27% on 200 mg ASO, but only 4% on 300 mg ASO. Interestingly, thrombi were not only rarer but also smaller in the 300 mg ASO group, suggesting that thrombus growth is compromised when fXI is reduced below a threshold. Alternatively, because venography was performed more than a week after surgery, the results could reflect greater fibrinolytic degradation of clot formed intraoperatively in the absence of fXI. Although clinically relevant bleeding was not statistically different in ASO- and enoxaparin-treated patients, the study was not powered to show a difference in bleeding. It is worth noting that patients started ASO treatment 5 weeks before surgery and were under the full drug effect during surgery.8 Despite this, abnormal intraoperative hemostasis was not observed, and postoperative bleeding was rare, even with fXI levels <10% of normal.

Because of the slow onset of action, ASO therapy cannot be applied in situations where an antithrombotic effect is
required rapidly. Anti-fXI antibodies have shown promise in preclinical studies, and an antibody specifically targeting fXIIa is entering clinical evaluation. Antibody-based therapies are highly specific and provide rapid onset of action. Their half-lives make them better suited for long-term inhibition than for situations requiring brief treatment. Small molecule active site inhibitors of fXIIa are efficacious in rodent and rabbit thrombosis models, and some are entering phase I testing. Most have relatively short half-lives, facilitating dose adjustment and more rapid dissipation of effect after discontinuation. Specificity is an issue with these agents, as the fXIIa active site is structurally similar to those of other proteases. Some anti-fXIa compounds demonstrate activity toward the fXIIa homolog α-kallikrein. This might be beneficial, because it may produce anti-inflammatory and antithrombotic effects distinct from those attributable to fXIIa inhibition.

**Conclusions, Conjecture, and Future Considerations**

FXI was identified as a plasma constituent missing in patients with abnormal surface-dependent clotting and a mild bleeding disorder. As part of the intrinsic pathway, the protein provides a link between contact activation and factor IX, driving thrombin generation in activated partial thromboplastin time assays. Subsequent work identified fXIIa-independent mechanisms for fXI activation, explaining the phenotypic differences between fXI and fXII deficiencies. Genomic studies have shed additional light on the relationship between fXI and the traditional coagulation cascade.

The advantage of strategies targeting fXI compared with current therapies likely lies in the area of safety. Preclinical and epidemiological data indicate that fXI/fXIa inhibitors will not promote bleeding after injury to the same extent as warfarin or DOACs, or as short-term prophylaxis after procedures where even moderate bleeding needs to be avoided (eg, neurosurgery). Inhibitors of fXI/fXII should also be considered in situations where blood is exposed to artificial surfaces (dialysis, extracorporeal membrane oxygenation, cardiopulmonary bypass, and artificial heart valves). The recent ASO trial provided proof of concept that targeting fXI can produce a useful therapeutic effect in patients at risk of thrombosis. Future work will be directed at determining whether these promising results are relevant to other clinical settings. It seems prudent to first test fXIIa inhibitors for prophylaxis, such as in primary or secondary prevention of VTE or prevention of stroke in patients with atrial fibrillation. At this point, we do not have preclinical data to indicate that fXI/fXIIa inhibition will be useful in treating active thrombosis. fXIa inhibition may be useful in patients who are poor candidates for warfarin or DOACs, or as short-term prophylaxis after procedures where even moderate bleeding needs to be avoided (eg, neurosurgery). Inhibitors of fXI/fXIIa should also be considered in situations where blood is exposed to artificial surfaces (dialysis, extracorporeal membrane oxygenation, cardiopulmonary bypass, and artificial heart valves). The advantage of strategies targeting fXI compared with current therapies likely lies in the area of safety. Preclinical and epidemiological data indicate that fXI/fXIIa inhibitors will not promote bleeding after injury to the same extent as warfarin or DOACs, or as short-term prophylaxis after procedures where even moderate bleeding needs to be avoided (eg, neurosurgery). Inhibitors of fXI/fXII should also be considered in situations where blood is exposed to artificial surfaces (dialysis, extracorporeal membrane oxygenation, cardiopulmonary bypass, and artificial heart valves).
are uncertain. Still, brief treatment with FXII inhibitors may be effective for procedures in which blood is exposed to artificial surfaces.11 If inhibitors of FXI/FXIIa and FXII/FXIIa prove to be effective in humans, their safety profiles should broaden the spectrum of clinical situations in which antithrombotic therapy can be applied.

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References

**Highlights**

- The plasma protease factor Xla contributes to thrombin generation by activating factor IX. Traditionally considered part of the intrinsic pathway of coagulation, factor Xla may function as an interface between thrombin generation and the proinflammatory kallikrein–kinin system.
- Data from animal models and epidemiological surveys indicate that factor XI contributes to thromboembolic disease.
- Therapeutic strategies designed to neutralize factor Xla (antibodies, RNA aptamers, and small molecule active site inhibitors) or its zymogen form factor XI (antisense oligonucleotides and antibodies) are being developed, with the goal of testing them in prevention or treatment of thromboembolic disease.
- Congenital factor XI deficiency is associated with a mild-to-moderate bleeding disorder, and it is anticipated that therapies targeting this protein will be associated with a relatively low risk of serious bleeding compared with currently available anticoagulants.
- Results of a recent trial of antisense oligonucleotide-mediated factor XI reduction for venous thrombosis prophylaxis in knee replacement surgery indicate that factor XI is an important contributor to thrombosis in this setting and that targeting factor XI can produce a useful therapeutic effect.
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