Inhibition of Factor XIa as a New Approach to Anticoagulation

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Abstract—The dose-limiting issue with available anticoagulant therapies is bleeding. Is there an approach that could provide antithrombotic protection with reduced bleeding? One hypothesis is that targeting proteases upstream from the common pathway provides a reduction in thrombin sufficient to impede occlusive thrombosis yet allows enough thrombin generation to support hemostasis. The impairment of intrinsic coagulation by selective inhibition of factor XI (FXI) leaves the extrinsic and common pathways of coagulation intact, making FXI a drug target. This concept is supported by the observation that human deficiency in FXI results in a mild bleeding disorder compared with other coagulation factor deficiencies, and that elevated levels of FXI are a risk factor for thromboembolic disease. Moreover, FXI knockout mice have reduced thrombosis with little effect on hemostasis. The results from genetic models have been supported by studies using neutralizing antibodies, peptide inhibitors, and small-molecule inhibitors. These agents impede thrombosis without affecting bleeding time in a variety of experimental animals, including primates. Together, these data strongly support FXIa inhibition as a viable method to increase the ratio of benefit to risk in an antithrombotic drug. (Arterioscler Thromb Vasc Biol. 2010;30:388-392.)

Key Words: intrinsic pathway ■ coagulation ■ factor XI ■ thrombosis

The Need for Improved Anticoagulant Therapy

Hemostasis is an adaptive process that maintains blood in a fluid state and preserves vasculature integrity. Thrombosis is a maladaptive process of vascular occlusion and remains a primary cause of cardiovascular morbidity and mortality. Antithrombotic therapy is effective for the prevention and treatment of thromboembolic disease. However, the established oral anticoagulant warfarin has numerous limitations, including lack of reversibility, a steep dose response, food and multiple drug-drug interactions, need for monitoring, and a narrow therapeutic index. The availability of newer oral anticoagulants, such as direct and selective inhibitors of factor Xa (FXa) and thrombin, has overcome many of these liabilities; however, dose-dependent bleeding continues to be observed.1,2 An overlap of antithrombotic benefit and a disruption of hemostasis are not unexpected given that both processes involve interactions between the vessel wall, platelets, blood coagulation, and fibrinolysis. Improving the risk to benefit ratio remains a viable goal for antithrombotic drug discovery. This requires selecting a molecular target that defines a difference between hemostasis and thrombosis. Selecting such a target derives from the detailed study of human physiology and animal models. A good example is the inhibition of blood coagulation FXIa as a novel mechanism for preventing and treating thromboembolic diseases.

Role of FXIa in Blood Coagulation

Blood coagulation is the coordinated activation of plasma proteases, their cofactors, and platelets. The end product is the protease thrombin, which cleaves fibrinogen to generate a fibrin clot. This cascade has been classically divided into the intrinsic (contact-activated), extrinsic (tissue factor [TF]–activated), and common (prothrombin and thrombin production) pathways, as recently reviewed3–5 and shown in the Figure. The most important physiological activator of blood coagulation is TF, which is expressed in the vessel wall, and, under pathological conditions, in circulating monocytes and microparticles. The TF-FVIIa complex catalyzes the formation of FXa, which in turn cleaves prothrombin to generate thrombin. The activation of the intrinsic cascade remains an area of active research. In the classic model, FXIa, formed by contact activation (collagen, RNA, and polyphosphates6), catalyzes the formation of FXa, which in turn cleaves prothrombin to generate thrombin. The activation of the intrinsic cascade remains an area of active research. In the classic model, FXIIa, formed by contact activation (collagen, RNA, and polyphosphates6), catalyzes the formation of FXIa, which leads to the sequential activations of FIX and FX. However, thrombin and TF-FVIIa also activate intrinsic coagulation proteases, resulting in pathways that are closely linked to optimize thrombin generation.

Platelets play an important role in the final common pathway of blood coagulation by providing a surface for the
formation of FXa (tenase complex) and thrombin (prothrombinase complex). Platelets are also activated by thrombin, and they may support the formation of FXIa. The overlapping activation of coagulation and platelets thereby supports thrombosis and hemostasis, while locally regulated fibrinolysis helps resolve both processes.

Target Validation
The utility of FXIa as a therapeutic target has been revealed by in vitro models of coagulation, human physiology, and animal models of thrombosis and hemostasis. The animal models have included mice deficient in FXI and in vivo studies conducted with FXI or FXIa inhibitors or mechanisms that reduce FXI or FXIa formation. Each of these lines of evidence is reviewed in the subsequent sections.

FXIa Inhibition Assessed In Vitro
FXIa enhances both the formation and the stability of clots in vitro. FXIa amplifies thrombin generation when coagulation is initiated by low levels of TF or thrombin.7 The activation of coagulation by high levels of TF in the prothrombin time (PT) is not affected by FXIa inhibition, whereas contact activation in the activated partial thromboplastin time (aPTT) is affected. In this manner, FXIa inhibition limits the amplification of thrombin formation by the intrinsic cascade, while exerting a limited effect on the initiation of coagulation by TF.

FXI-dependent amplification of thrombin formation also leads to activation of the thrombin-activatable fibrinolysis inhibitor, which renders clots less sensitive to fibrinolysis.8 In this manner, an FXIa inhibitor might indirectly enhance clot dissolution.

FXI in Human Physiology
Bleeding Disorder of Hemophilia C
The strongest human data supporting FXIa as a therapeutic target address its relative safety. A deficiency in FXI (hemophilia C) results in a more benign bleeding phenotype compared with a deficiency in either FVIII (hemophilia A) or FIX (hemophilia B), as described in recent reviews.9–11 Hemophilia A and B are recessive X-linked bleeding disorders and show a strong correlation between coagulant level and the severity of bleeding, which can be life threatening and disabling. Blood loss tends to be spontaneous and occurs frequently in joints and muscles, although hematuria, skin bruising, and postoperative bleeding are also observed.

Unlike hemophilia A and B, hemophilia C is present in both sexes. Most cases involve Ashkenazi Jews12 and result from either of 2 mutations in the FXI gene, an E117X and an F283L substitution. However, greater than 180 mutations have been described in lesser frequency. FXI deficiency also arises from acquired inhibitors that neutralize its activity; in these cases, patients may not respond well to FXI replacement therapy. Bleeding in those with hemophilia C is rarely spontaneous; it tends to occur in response to surgery or trauma, and especially in tissues that are prone to fibrinolysis (the urinary track or oral cavity). There is often a poor correlation between bleeding and coagulant level; however, a prolonged aPTT often supports the diagnosis of a deficiency. Severe FXI deficiency in women does not necessarily affect pregnancy or delivery, even though excessive bleeding during menstruation may be observed.

Bleeding associated with FXI deficiency is typically corrected by FXI replacement therapy with recombinant clotting factor. Fibrinolysis inhibitors and desmopressin13 or recombinant FVIIa14 may provide hemostatic support, although these are not FXI-selective treatments.

Severe FXI deficiency as an inherited coagulation disorder is rare in the general population (approximately 1:106), except as previously mentioned for Ashkenazi Jews (1:450). The deficiency has also been repeatedly described in Holstein cows15,16; as in humans, the bleeding phenotype is highly variable.

Unlike other clotting factors, hemostasis is not impaired with severe FXII deficiency, even during major surgical procedures.17 Bleeding related to severe coagulation factor deficiency is tolerable for FXIII,18 less tolerable for FV,19 and severe for FX.20 Near-complete reductions in FVII21 and FII22 are not observed in humans, suggesting that their absence is not physiologically tolerated.

FXI in Thromboembolic Disease
Although the bleeding phenotype of human FXI deficiency has been well characterized, the potential cardiovascular benefit associated with the phenotype is less well understood. Data from limited studies do not support FXIa deficiency as providing protection against myocardial ischemia,23 but they do suggest benefit in ischemic stroke.24 These studies are hampered by the relative infrequency of and narrow patient population associated with FXI deficiency and the lack of clinical outcome evaluation. Evidence is stronger for FXI as a biomarker, with increased levels reported as a risk factor for venous thromboembolism25–27 and myocardial ischemia or stroke.27,28 It remains unclear whether elevated FXI levels are causal for or just correlated with cardiovascular risk. Data supporting FXI as an antithrombotic target have been best documented in animal studies, the first of which described the targeted deletion of FXI in mice.29
Genetic Manipulation of FXI in Mice

FXI-null mice are healthy, and their reproduction follows the expected mendelian ratios without impaired fecundity. In contrast, targeted deletion of coagulation factors in the common pathway (FX, FII, and FV) and extrinsic pathway (FVII and TF) is lethal prenatally or perinatally in mice as a result of bleeding in most cases.

Early studies demonstrated that FXI-null mice were protected against oxidative iron chloride (FeCl₃)-induced carotid artery thrombosis, and that infusion of human FXI reversed this protection. This established a cause-effect relationship between FXI deficiency and reduced thrombosis. Impaired thrombosis was subsequently observed in the vena cava and mesenteric arterioles of FXI-null mice in response to FeCl₃ injury. In comparing FIX- with FXI-null mice, similar efficacy was observed in FeCl₃-associated carotid artery thrombosis; tail bleeding times were prolonged to a greater extent with FIX compared with FXI deletion. In fact, there was no detectable effect on bleeding time in FXI-null mice, even when comparing aspirin-treated FXI-null mice with wild-type mice. FXII-null mice are also protected in FeCl₃-associated arterial thrombosis, with no demonstrable effect on bleeding time. The bleeding profile in null mice is fairly consistent with human deficiencies in FXI and FXII.

The involvement of FXI in murine thrombosis is not limited to FeCl₃ injury models. Thrombus formation in FXI-null mice was also reduced in response to laser injury of arterioles in the cremaster muscle. These experiments involving continuous monitoring of thrombosis revealed reduced thrombus stability in the knockout mice along with decreased deposition of fibrin and especially platelets. FXI deletion was also effective in preserving carotid artery blood flow in response to compression injury.

Genetically modified mice provide the opportunity to examine combined deficiencies in more than 1 coagulation component. Activated protein C impairs coagulation by inactivating FVa and FVIIIa. Deletion of protein C in mice leads to fibrin deposition in multiple tissues and perinatal lethality. Mice deficient in both protein C and FXI were partially protected in that they did not die until much later in life. These data further validate FXI as a therapeutic target in a model in which fibrin formation is activated in a physiological manner.

Overall, studies with FXI-null mice are consistent with the tolerable hemostatic defect of hemophilia C. They also strongly support FXI as a therapeutic target for preventing occlusive thrombosis in response to severe vessel injury. The question of whether efficacy could be obtained with a more proportionate and incomplete inhibition of FXI or FXIa requires the evaluation of selective pharmacological tools.

Experimental FXIa Inhibitors

Most inhibitor approaches have directly targeted FXI or FXIa with an antibody, a peptide inhibitor, or a small-molecule inhibitor. The disruption of FXI production or FXIa activation offers another approach.

Inhibiting FXI Activation or Production

To the extent that thrombin derived from TF-FVIIa contributes to FXI activation, indirect FXIa inhibition could be obtained, disrupting these downstream proteases. The inhibition of FXIIa could also affect FXI activation; however, testing this approach has thus far been limited to FXII-deficient mice. Potent FXIa inhibition (inhibition constant, 0.067 nmol/L) was observed with an insect-derived serine protease inhibitor, but this protein was only 10-fold selective over FXa.

The inhibition of FXI biosynthesis was obtained with an antisense oligonucleotide, which produced near-complete inhibition of hepatic FXI mRNA. Oligonucleotide treatment strongly inhibited FeCl₃ venous thrombosis without affecting tail bleeding time. Another direct approach is the use of FXI neutralizing antibodies, which would allow preexisting FXIa to remain active.

Neutralizing Antibodies

FXI neutralizing antibodies were evaluated in a nonterminal baboon model in which a graft of varying materials was inserted in an arteriovenous shunt to induce a nonocclusive thrombus. The first study used a polyclonal antibody administered at a dose that produced a 3.2-fold aPTT prolongation without affecting the PT. Heparin at a dose that increased the aPTT by 3.8-fold was tested as a reference agent. Both the FXI antibody and heparin elicited strong and comparable inhibition of platelet accumulation on grafts constructed of dacron, polytetrafluoroethylene, and polytetrafluoroethylene coated with TF. The initial accumulation of platelets was affected to a lesser extent by these treatments. Template bleeding times were unaffected by FXI antibody, but were increased 1.4-fold by heparin. Subsequent baboon studies used an FXI monoclonal antibody that maximally inhibited plasma FXI activity. This antibody partially limited thrombus accumulation on collagen-coated grafts. It also preserved flow in smaller-diameter grafts that were prone to occlusive thrombosis; this effect was not observed with aspirin.

Other studies with neutralizing antibodies have been conducted in rabbits. A monoclonal antibody against FXI or FXIa reduced thrombus formation in balloon-injured iliac arteries. There was no effect on PT or platelet aggregation, whereas the aPTT was prolonged 2.4-fold. In another in vivo study, TF-activated clots containing radiolabeled fibrin, and either a control or a polyclonal FXI antibody, were injected into jugular vein segments. The FXI antibody increased clot lysis whether it was incorporated into the clot or administered systemically.

Peptide and Small-Molecule Inhibitors

A series of potent ketoarginine-based peptidomimetics was reported by Daiichi Sankyo Co. These are irreversible inhibitors of FXIa and form a covalent bond to the catalytic serine of the enzyme. The most potent compound produced 50% inhibition of FXIa at 6 nmol/L (IC₅₀), doubled the aPTT at 2.4 μmol/L, and showed good selectivity over FVIIa, FXa, and thrombin. Intravenous infusion of this compound was efficacious in a rat model of venous thrombosis. Its antithrombotic activity was comparable to that of heparin. A pyridyl analog with an FXI IC₅₀ of 12 nmol/L was evaluated in a rat mesenteric bleeding model; at a 4-fold efficacious dose, it did not alter bleeding time. Modifications to reduce
the peptidic character and molecular weight of these inhibitors led to compounds with reduced FXIa affinity; however, the results of in vivo experiments with these compounds were not reported.44

Clavatadine A, a natural product isolated from a marine sponge, was also reported to be an irreversible inhibitor of FXIa, with an IC50 of 1300 nmol/L.45 This compound had no effect on FIXa at a 170-fold greater concentration. In vivo data for clavatadine were not described in this study.

Several small-molecule inhibitors have also been reported. A racemic boronate was reported to have an FXIa IC50 of 1400 nmol/L, with 30- and 8-fold selectivity over FXa and thrombin, respectively.46 A potent time-dependent irreversible inhibitor of FXIa with an IC50 of 2.8 nmol/L, and that doubled the aPTT at 2.2 μmol/L, was reported by Bristol-Myers Squibb, Pennington, NJ.47 The efficacy in FeCl2/H9262 doubled the aPTT at 2.2

Potential Issues
Selective disruption of intrinsic coagulation is mostly untested in clinical medicine and may exert physiological effects beyond thrombosis and hemostasis. For example, the phenotype observed in a cross of mice with plasminogen and FXI deletions suggests a role for FXIa in tissue inflammation.48 Although we have drawn much from genetic models, these may be problematic aside from species differences. Lifelong FXI deficiency may differ from pharmacological disruption of FXI function by triggering physiological adaptation. FXI deficiency in heterozygous humans can result in increased bleeding; therefore, the target may not be devoid of a safety issue. It is also obvious that preclinical models contain, at best, certain components of human disease, making it difficult to predict the extent of FXI or FXIa inhibition required for clinical efficacy. The therapeutic targeting of FXIa has progressed to early-phase clinical studies.49,50 In contrast, the testing of FXI or FXIa inhibition is in the preclinical stage.

Summary and Conclusions
Therapies targeting FXI disrupt intrinsic coagulation without affecting either the extrinsic (TF) or common (FXa or thrombin) pathways of blood coagulation. The hypothesis that this approach could inhibit thrombosis while preserving hemostasis is supported by several lines of evidence. In humans, FXI deficiency is a tolerable bleeding disorder (hemophilia C), and elevated levels of FXIa are a risk factor for thrombotic disease and may predispose to ischemic stroke. FXI-null mice have reduced thrombosis without impaired hemostasis. Studies with inhibitors of FXI or FXIa have shown antithrombotic efficacy with minimal effects on bleeding time in rats, rabbits, and primates. Thus, the blocking of FXI function is a promising path to making agents having an increased ratio of efficacy to bleeding available to patients.

Disclosures
All authors are employed by Bristol-Myers Squibb Company.

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