

Role of Factor XIa and Plasma Kallikrein in Arterial and Venous Thrombosis

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Abstract

Cardiovascular disease, including stroke, myocardial infarction, and venous thromboembolism, is one of the leading causes of morbidity and mortality worldwide. Excessive coagulation may cause vascular occlusion in arteries and veins eventually leading to thrombotic diseases. Studies in recent years suggest that coagulation factors are involved in these pathological mechanisms. Factors XIa (FXIa), XIIa (FXIIa), and plasma kallikrein (PKa) of the contact system of coagulation appear to contribute to thrombosis while playing a limited role in hemostasis. Contact activation is initiated upon autoactivation of FXII on negatively charged surfaces. FXIIa activates plasma prekallikrein (PK) to PKa, which in turn activates FXII and initiates the kallikrein–kinin pathway. FXI is also activated by FXIIa, leading to activation of FIX and finally to thrombin formation, which in turn activates FXI in an amplification loop. Animal studies have shown that arterial and venous thrombosis can be reduced by the inhibition of FXI (a) or PKa. Furthermore, data from human studies suggest that these enzymes may be valuable targets to reduce thrombosis risk. In this review, we discuss the structure and function of FXI(a) and PK(a), their involvement in the development of venous and arterial thrombosis in animal models and human studies, and current therapeutic strategies.

Keywords

- ▶ factor XI
- ▶ plasma kallikrein
- ▶ thrombosis
- ▶ animal models
- ▶ clinical studies

Introduction

Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality worldwide. An estimated 422.7 million cases of CVD occurred in 2015, of which an estimated 17.9 million people died. Among CVDs, myocardial infarction (MI) and stroke have been the two major causes of CVD-related health loss worldwide.¹ While normal blood clotting is essential to stop bleeding at sites of vascular injury, excessive coagulation can result in vascular occlusions in arteries or veins eventually leading to thrombotic diseases.²

The coagulation cascade is based on a waterfall model, described by Macfarlane³ and Davie and Ratnoff⁴ in 1964, that involves the sequential activation of different zymogens

to active enzymes. Blood coagulation can be initiated by exposure of tissue factor (TF) to the blood stream or by activation of FXII through negatively charged surfaces, which can be either artificial (e.g., ellagic acid and kaolin) or of natural origin (e.g., polyphosphates and collagen).^{5,6} In the extrinsic pathway, complex formation of TF and FVIIa leads to activation of FX and FIX and subsequently to thrombin generation.⁷ The conversion of FXII to FXIIa is the primary step of the intrinsic coagulation pathway, which leads to subsequent activation of FXI, FIX, FX, and prothrombin. FXIIa also activates PK, thereby initiating the kallikrein–kinin pathway and amplifying the formation of FXIIa through the reciprocal activation of FXII by PKa.⁸ Thrombin converts fibrinogen to fibrin in the common pathway.

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Inhibition of coagulation factors is an obvious approach for antithrombotic therapies since excessive coagulation is a potential cause for thrombotic diseases. However, not all coagulation factors are equally suitable targets as some coagulation factors are essential for hemostasis and inhibition of these factors might increase the bleeding risk. For instance, FIX or FVIII-deficient patients suffer from spontaneous bleedings, whereas FXI-deficient individuals have none to a mild-bleeding phenotype.⁹ In this review, we discuss the role of FXI(a) and its homolog PK(a) in thrombosis, taking into account results from arterial and venous thrombosis animal models and reports from human studies and deficiencies.

Factor XI(a) Structure

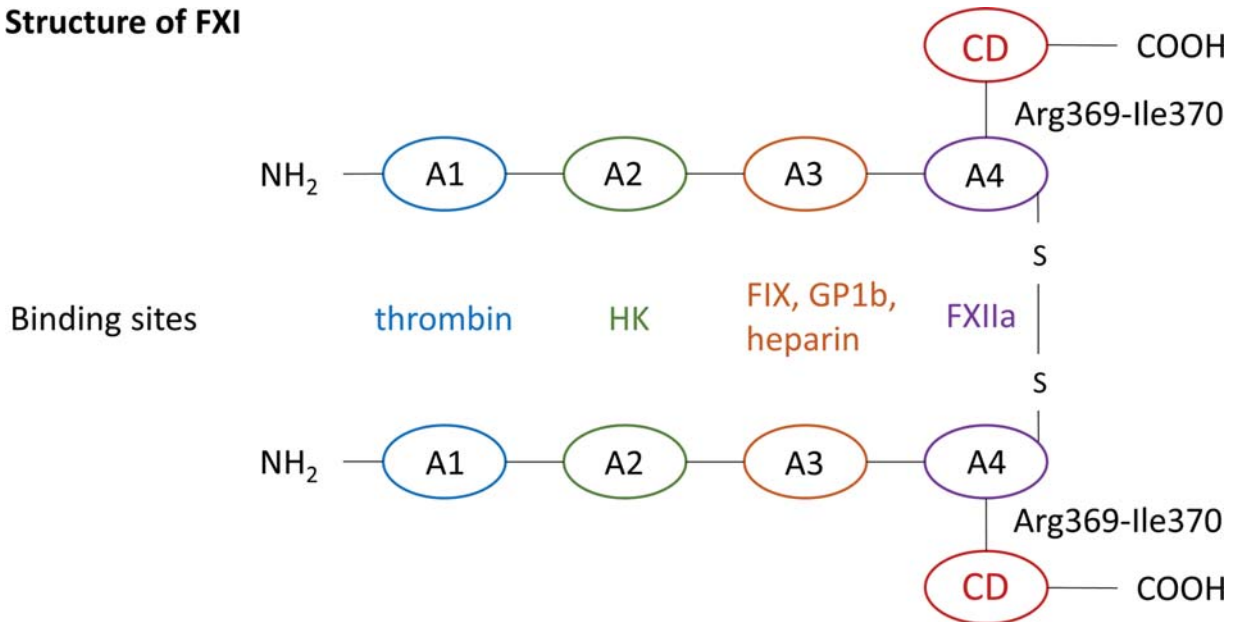
FXI is a 160 kDa zymogen that circulates in plasma at a concentration of 30 nM¹⁰ and is mostly bound in a noncovalent complex to high molecular weight kininogen (HK), a 120 kDa plasma protein possibly functioning as an adaptor for FXI to

bind to negatively charged surfaces.^{11,12} FXI is mainly synthesized in hepatocytes¹⁰ and regulated by the transcription factor hepatocyte nuclear factor-4 α .¹³ Recently, FXI pre-mRNA could be identified in platelets by Zucker et al. They showed that FXI pre-mRNA is spliced into mature mRNA upon platelet activation by either thrombin or adenosine diphosphate. The resulting protein has similar properties as plasmatic FXI when analyzed by western blot or an activity assay.¹⁴

The structure of FXI is fundamentally different from other coagulation factors since it forms a dimeric structure and unlike vitamin K-dependent coagulation factors lacks a Gla domain (\rightarrow Fig. 1). Papagrigoriou et al provided a crystal structure of full-length FXI purified from human plasma.¹⁵ FXI consists of two identical subunits of 607 amino acids, which are linked to each other via an interchain disulfide bond at Cys321. In addition, hydrophobic interactions and salt bridges stabilize this dimeric structure.¹⁵

The activation of the zymogen monomers occurs through cleavage of the Arg369–Ile370 bond (\rightarrow Fig. 1), generating a

Structure of FXI



Structure of PK

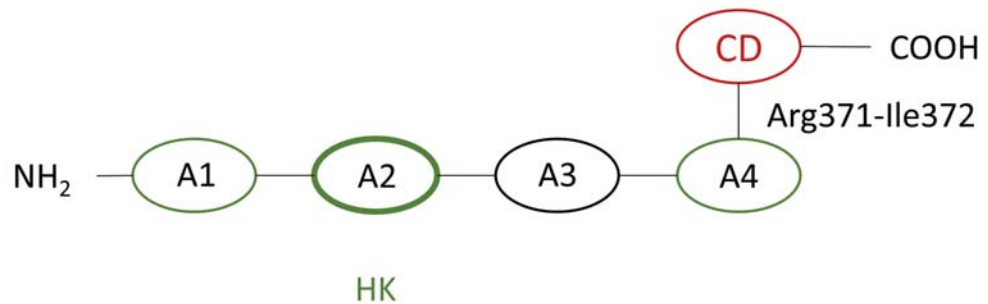


Fig. 1 Structure of human factor XI and plasma prekallikrein. The domain structure of both zymogens comprises four tandem repeats called apples domains. The catalytically active serine protease domain (catalytic domain) is situated at the C-terminus. The activation of FXI and PK occurs through cleavage of the Arg369–Ile370 and the Arg371–Ile372 bond, respectively. While FXI comprises two identical subunits linked to each other via the A4-domains, PK does not form a dimeric structure. The apple domains contain binding sites for various proteins and enzymes. CD, catalytic domain; FXI, factor XI; PK, prekallikrein.

47 kDa heavy chain and a 33 kDa light chain linked by a disulfide bond.¹⁶

Each monomer comprises four tandem repeats of 90 to 91 amino acids each. These so-called apple domains A1 to A4 at the N-terminus of the heavy chain contain seven antiparallel β -strands and an α -helix. The serine protease domain in the light chain is situated at the C-terminus and connected to the apple 4 domain.^{15,17} This linkage generates a so-called “cup and saucer” conformation with the catalytic domain located on the disk-like arrangement of the apple domains.¹⁵ Each apple domain contains binding sites for various proteins and enzymes (►Fig. 1). Thrombin and HK interact with FXI via the A1 and A2 domains,^{18,19} respectively, whereas the A3 domain has binding sites for FIX,²⁰ the platelet receptor GPIb,²¹ and heparin.²² FXIIa seems to bind to the A4 domain of FXI, which also carries the linkage domain for dimerization.^{23,24}

Factor XI Activation and Function

FXI is mainly activated via two enzymes, namely FXIIa²⁵ in the contact activation pathway and thrombin^{26,27} as part of

an amplification loop (►Fig. 2). Upon vessel injury, sub-endothelial TF is exposed to the peripheral blood stream. Together with FVIIa, it activates FX to FXa and FIX to FIXa. In complex with the cofactor FVa, FXa converts prothrombin to thrombin,⁷ which is considered the key enzyme at the end of the coagulation cascade triggering various (patho) physiological pathways, including fibrin formation and stability, platelet activation, and initiating inflammatory processes. Thrombin is inactivated via antithrombin,²⁸ the TF–FVIIa–FXa complex by tissue factor pathway inhibitor (TFPI),⁷ quickly. Larger amounts of thrombin are generated during thrombus stabilization and propagation in positive feedback loops including amongst others (FV and FVIII) the activation of FXI by thrombin.²⁸

Besides the activation by thrombin, FXIIa converts FXI to FXIa in the contact activation pathway. Here, the autoactivation of FXII on negatively charged surfaces, which can be either artificial (e.g., ellagic acid and kaolin) or of natural origin (e.g., polyphosphates, collagen, and nucleic acids),^{5,6,29} represents the initial step and results in sequential activation of FXI, followed by FIX, FX, and prothrombin.⁸

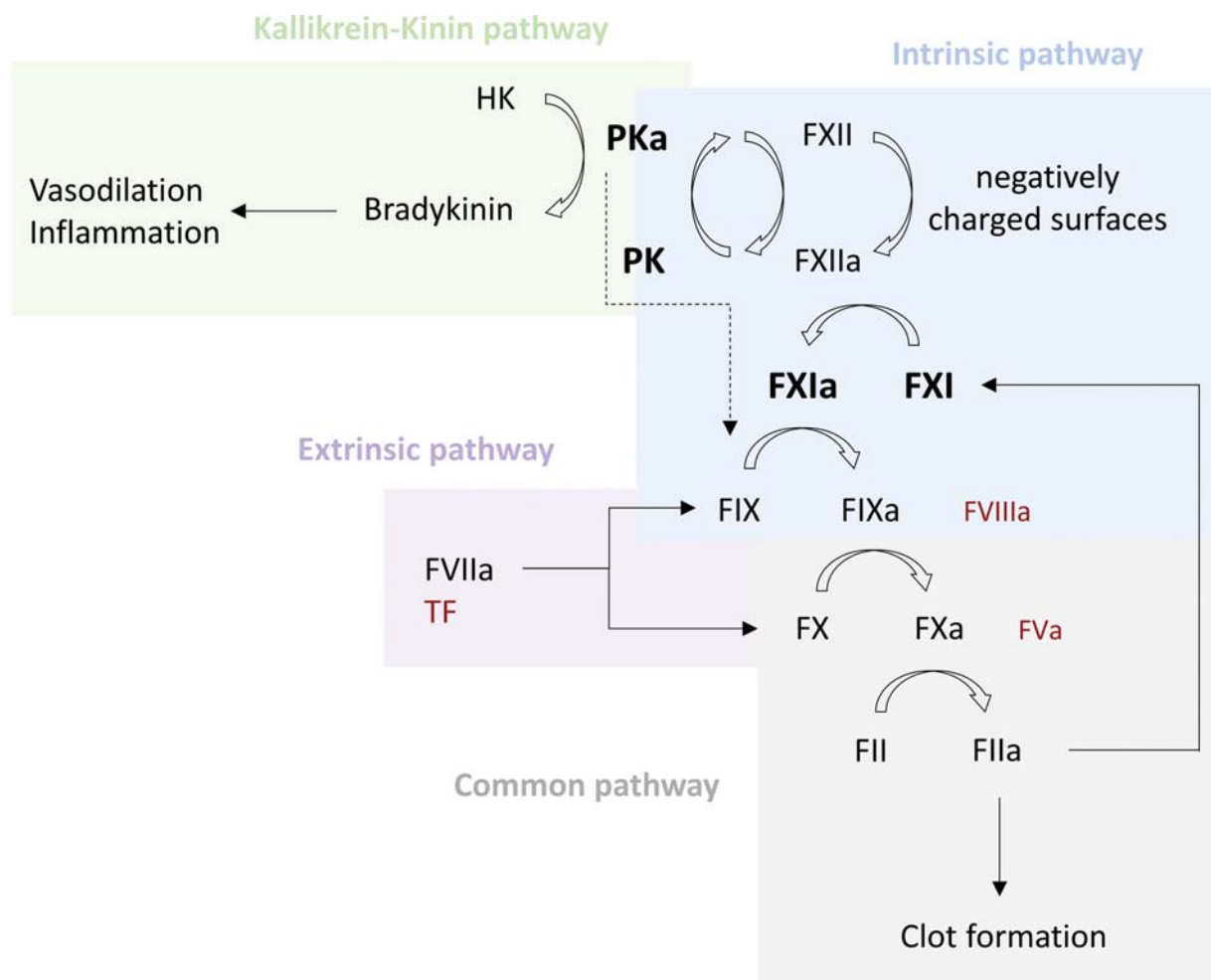


Fig. 2 Coagulation cascade. The coagulation cascade is based on a waterfall model that involves the sequential activation of different zymogens to active enzymes. The conversion of FXII to FXIIa is the primary step of the intrinsic coagulation pathway, leading to subsequent activation of FXI, FIX, FX, and prothrombin. FXIIa also activates PK, which reciprocally activates FXII, initiates the kallikrein–kinin pathway and, in the absence of FXI, activates FIX (dashed line). The FVIIa–TF complex of the extrinsic pathway activates FIX and FX, leading to thrombin generation. Zymogens are indicated in roman numerals and their activated forms end with an a. Cofactors within the coagulation cascade are shown in red. FXI, factor XI; PK, prekallikrein; TF, tissue factor.

In summary, FXI activation plays a critical role in both, the propagation phase of thrombin generation and during contact activation.

This activation does not happen in a single step but through the formation of an intermediate with one activated subunit ($\frac{1}{2}$ -FXIa). It can be distinguished from fully activated FXIa on sodium dodecyl sulphate-polyacrylamide gel electrophoresis under reducing conditions.³⁰ The natural substrate for FXIa is FIX, a 57 kDa single-chain glycoprotein that binds to the A3 domain of FXIa, but does not interact with the zymogen FXI since the binding site on A3 for FIX is only exposed after a conformational change upon FXI activation.³¹ FIX seems to interact with FXIa via its Gla domain.³¹

In vitro, data of several groups suggest that FXIa might activate other proteins in the coagulation cascade as well, including FV, FVIII, and FX. The sites cleaved by FXIa on FV and FVIII appear to be similar to those observed during their activation by thrombin, which is still viewed as the main activator of both cofactors.³² Matafonov et al described that FX can be activated by FXIa, but to a lesser extent than the main FXIa substrate FIX. While FIX activation via FXIa is calcium-dependent, FX and FV activation are apparently not.³³ It is also reported that TFPI, an inhibitor of the TF/FVIIa/FXa complex, can be inactivated by FXIa through proteolytic cleavage of TFPI between the Kunitz 1 and 2 domains.³⁴ Because lack of functional TPFI may lead to prolonged activity of the TF/FVIIa/FXa complex, this may have an impact on the thrombin concentration in the early amplification phase.

Recent data suggest a role for FXI as a molecular linker of coagulation and inflammation. Ge et al demonstrated that prochemerin (chem163S) can be cleaved in plasma by contact phase-activated FXIa to a partly active intermediate, which is subsequently completely activated by plasma carboxypeptidases to chemerin, an adipokine and chemo-attractant. The formation of the intermediates via FXIa could be enhanced by the addition of phospholipids to plasma or in the presence of platelets.³⁵ The interaction of FXI and the GPIIb receptor on platelets and its linkage to inflammation and coagulation has also been investigated in previous studies, in which an impact of thrombin-activated FXI bound to platelets in arterial hypertension was demonstrated.³⁶

In vivo, data show the contribution of FXIa to intrinsic coagulation. Infusion of purified FXIa into chimpanzees resulted in activation of the factors IX, X, and prothrombin demonstrating that FXIa is an important factor in the coagulation cascade.³⁷ In addition, it has also been shown that the inhibition of FXIa caused an increase of fibrinolysis in a thrombosis model in rabbits due to decreased activation of TAFI by thrombin possibly contributing to the mild-bleeding phenotype in some FXI-deficient individuals.³⁸ Recently, Mohammed et al also provided insights in the role of FXIa acting downstream of FIX *in vivo* by showing that the infusion of FXIa or increasing the plasmatic FXI level up to 200% reduced the bleeding time in a saphenous vein bleeding model in FIX^{-/-} mice indicating an involvement of FXI

pathways independent of FIX.³⁹ Further investigations are needed to determine the role of the possible alternative pathways in hemostasis or thrombosis.

FXIa is inhibited by binding of several proteins. *In vivo*, C1 inhibitor is the dominant inhibitor (68% of FXIa in complex with the inhibitor), followed by α -2 antiplasmin (13%), α 1-antitrypsin (α 1-AT, 10%) and antithrombin III (9%).⁴⁰ Almost all FXIa-inhibitor complexes had a half-life of 95 to 104 minutes, while the half-life of the FXIa-a1AT complexes was 349 minutes.⁴⁰ Recently, it was demonstrated that FXIa can also form a complex with endothelial plasminogen activator inhibitor-1 (PAI-1), thereby inhibiting FXIa activity. These complexes were also observed in a baboon model of *Staphylococcus aureus*, suggesting that the complex formation with PAI-1 leads to clearance of FXIa.⁴¹

Prekallikrein(Kallikrein) Structure

PK is the 85 to 88 kDa precursor of the serine protease PKa and circulates in plasma at a concentration of approximately 580 nM. It is mainly expressed in hepatocytes.⁴² However, mRNA of PK was also found in nonhepatic cells, such as endothelial cells, fibroblasts, leukocytes, and in extrahepatic tissues. PK expressed in nonhepatic cells and tissues might contribute to local actions, but its physiological role is not completely understood.^{43,44} The amino acid sequence of PK is 58% identical to that of FXI. The zymogen contains, such as FXI, the characteristic four apple domains that are comprised of 90 to 91 amino acids (\rightarrow Fig. 1) each, but there is a major structural difference between both zymogens. While FXI comprises two identical subunits linked to each other, PK only has one subunit and does not form a dimeric structure.^{42,45} PK is mostly bound to HK in a noncovalent complex.⁴⁶ HK facilitates the activation of PK by FXIIa and on the other hand, serves as a natural substrate for PKa, which cleaves HK to liberate the potent pro-inflammatory peptide bradykinin (BK).^{12,42} The complex with HK also allows PK to interact with cells such as endothelial cells,⁴⁷ platelets,⁴⁸ and neutrophils.⁴⁹ The HK binding sites in PK are situated in the domains A1, A2, and A4, whereby the A2 domain seems to be most important for complex formation with the D6H domain of HK (\rightarrow Fig. 1). The A3 domain is, however, not involved.⁵⁰

PK is activated by cleavage of the Arg371-Ile372 bond (\rightarrow Fig. 1). The active enzyme PKa consists of a heavy chain (371 residues) and a light chain (248 residues) that are connected to each other via a disulfide bond.⁴² A recent study provided a full-length crystal structure for PKa.⁵¹ Compared to the dimeric FXI structure, there is a conformational difference in the apple 4 domain that may explain the monomeric structure of PKa. There is also a large conformational difference in the A3 domain and a 180 degrees rearrangement of the apple domains relative to the protease domain compared with the FXI structure. This indicates that a conformational change occurs upon activation of PK.⁵¹ However, this still needs to be confirmed by comparison with a crystal structure of PK.

Prekallikrein Activation and Function

The plasma contact activation system comprises a group of proteins including FXII, PK, FXI, and HK, which are activated upon binding of FXII to negatively charged surfaces. FXII undergoes a conformational change during binding, causing the protein to autoactivate to FXIIa. Besides activating FXI, as discussed before, FXIIa also cleaves PK to form the enzyme PKa (→Fig. 2). PKa in turn activates FXII, thereby amplifying the initiation of the FXIIa-mediated intrinsic coagulation cascade.

PK can also be activated to PKa independently of FXII. Prolylcarboxypeptidase expressed by endothelial cells for instance was found to activate PK when bound to cells^{52,53} and also heat shock protein 90 has been considered as an activator of PK, possibly by enhancing its autoactivation.⁵⁴

Recently, we demonstrated that PKa contributes to coagulation in a FXI-independent manner. In the absence of FXI, activation of FXII on ellagic acid or long-chain polyphosphates led to thrombin generation in human plasma and in a mouse model via FIX activation by PKa.⁵⁵ PKa is also involved in inflammatory processes mostly via release of the pro-inflammatory peptide BK out of HK. By binding to its G-protein coupled B1 or B2 receptors, BK causes vasodilation and increased vascular permeability (→Fig. 2).⁵⁶ Besides its role in coagulation and inflammation, PKa acts on the fibrinolytic system by activating pro-urokinase-type plasminogen activator (pro-uPA) to uPA and on the renin-angiotensin system by activating prorenin.^{57,58} In addition, the complement pathway can be initiated through cleavage of the central complement component C3 by PKa.⁵⁹

Given the fact that PKa is involved in so many pathways, it is not surprising that this protease is associated with various diseases. Hereditary angioedema is in many cases primarily a consequence of a C1 inhibitor deficiency, which results in hyperactivity of the kallikrein-kinin signaling pathway.⁶⁰ In

recent studies, it has been shown that the events in HAE patients can be reduced in number and severity by the inhibition of PKa.^{61,62}

In addition, increased PKa activity could be detected in diabetes patients⁶³ and the protease appears to play a role in the development of diabetic retinopathy.⁶⁴

Recent data suggest a role for PKa in processes inducing neuroinflammation. Göbel et al demonstrated that PK levels were increased in the central nervous system lesions of multiple sclerosis patients and that PKa might amplify leukocyte trafficking by modulating the blood-brain barrier in a PAR2-dependent manner.⁶⁵

Furthermore, it has been shown that PKa potentiates adenosine diphosphate-initiated platelet activation in a PAR1-dependent manner.⁶⁶

PKa in plasma is mainly inhibited by endogenous C1 inhibitor (52%) and α 2-macroglobulin (48%).⁶⁷

Animal Models to Study Thrombosis and Bleeding

Both arterial and venous thrombosis can be studied in various animal models. Knockout animal models are widely used to investigate the contribution of coagulation factors to thrombosis risk (→Table 1). *F11*^{-/-} mice, first described by Gailani et al,⁶⁸ are viable and have a normal reproductive capacity. Although the activated partial thromboplastin time is significantly prolonged, the bleeding times of *F11*^{-/-} mice are comparable to wild-type mice and they do not exhibit spontaneous bleedings.⁶⁸

The role of FXI in arterial thrombus formation was initially investigated by Rosen et al using *F11*^{-/-} mice in a ferric chloride (FeCl₃)-induced carotid artery injury model.⁶⁹ Compared with wild-type mice, thrombus formation was markedly reduced in *F11*^{-/-} mice and infusion of human FXI led to vessel occlusion times similar to control animals, indicating

Table 1 Contribution of factor XI and prekallikrein to thrombosis in animal models

	Arterial thrombosis model	Literature	Venous thrombosis model	Literature
<i>F11</i> ^{-/-}	↓ FeCl ₃ induced thrombus formation (mouse)	69, 70	↓ FeCl ₃ induced thrombus formation (mouse)	72
<i>K1kb1</i> ^{-/-}	↓ FeCl ₃ induced thrombus formation (mouse) ↓ Middle cerebral artery occlusion induced intracerebral thrombosis (mouse)	75,76,77	↓ FeCl ₃ induced thrombus formation (mouse)	75
FXI antibody	↓ Thrombus formation in a vascular graft occlusion model (baboon)	73	↓ FeCl ₃ induced thrombus formation (mouse)	105
FXI ASO	↓ Thrombus formation in a vascular graft occlusion model (baboon)	74	↓ FeCl ₃ induced thrombus formation (mouse) ↓ FeCl ₃ induced mesenteric vein thrombosis (mouse) ↓ Stenosis induced thrombosis (mouse)	108
PK ASO	↓ FeCl ₃ induced mesenteric arterial thrombosis (mouse)	79	↓ FeCl ₃ induced thrombus formation (mouse) ↓ Stenosis induced thrombosis (mouse)	79

Abbreviations: ASO, antisense oligonucleotide; FXI, factor XI; PK, prekallikrein.

that FXI contributes to arterial thrombus formation.⁶⁹ This was confirmed by a comparative study in which the effects of FIX and FXI deficiencies on arterial thrombosis were investigated in a FeCl₃-induced carotid artery injury model in mice using different concentrations of FeCl₃.⁷⁰ While the vessels in wild-type mice exposed to 3.5% FeCl₃ occluded within 10 minutes, *F9*^{-/-} and *F11*^{-/-} mice were fully protected from occlusion at 5% FeCl₃ and partially protected at 7.5% FeCl₃. Remarkably, the bleeding time in *F11*^{-/-} mice was similar to that of wild-type mice, whereas it was significantly prolonged in *F9*^{-/-} mice.⁷⁰ Interestingly, a recent study showed that FXI, but not FIX deficiency in mice with low levels of TF is associated with increased blood pool size in the placenta, suggesting a tissue-specific role for FXI in mice.⁷¹ Postnatal survival of low TF mice was however dependent on FIX levels.⁷¹ Similar antithrombotic effects of FXI deficiency could be shown in a FeCl₃-induced vena cava thrombosis mouse model.⁷²

Likewise, the role of FXI in development of thrombosis has been studied in higher species. A human FXI monoclonal antibody was used to prevent vascular graft occlusion and to reduce thrombus formation in a primate thrombosis model.⁷³ In addition, FXI ASO inhibited thrombus formation without increasing bleeding risk in a primate model.⁷⁴

To determine the role of PK in the development of thrombosis, Bird et al used PK-deficient mice showing normal blood pressure and heart rate.⁷⁵ Compared with wild-type mice, PK-deficient mice were completely protected from occlusion in a 3.5% FeCl₃-induced carotid artery injury model and partially protected at 5% FeCl₃, indicating that PK contributes to arterial thrombosis. In addition, thrombus weight in a venous thrombosis model induced by 3.5% FeCl₃ was significantly reduced in PK-deficient mice compared with wild-type mice. In contrast to *F11*^{-/-} mice, PK-deficient mice had a slightly increased tail bleeding time, but a similar renal bleeding time compared to wild-type mice.⁷⁵ The antithrombotic effect of PK deficiency in arterial thrombosis was also demonstrated in a FeCl₃-induced carotid artery injury mouse model by Kokoye et al.⁷⁶ Given the fact that PKa is an important activator of FXII and vice versa, it is not surprising that FXII-deficient mice were also protected from carotid artery occlusion.⁷⁶

Since PK is not only involved in contact activation, but also induces the kinin-pathway, Göb et al investigated whether PKa contributes to thromboinflammation. In transient and permanent models of ischemic stroke PK-deficient mice had reduced intracerebral thrombosis and improved cerebral blood flow compared with wild-type mice, while infarct-associated hemorrhage was not increased.⁷⁷ A similar outcome could be observed using a PK-specific antibody.⁷⁷

In addition, there is evidence that PK also contributes to arterial thrombosis independently of FXII. It has been shown that PK deficiency in mice resulted in increased generation of prostacyclin leading to reduced vascular TF expression.⁷⁸ Selective depletion of PK by ASO was also found to be thrombo-protective in arterial and venous thrombosis mouse models without increasing the bleeding risk.⁷⁹ However, contradictory results exist indicating a pro-thrombotic

effect in PK-deficient mice, for instance when prolyl carboxypeptidase as activator of PK is depleted simultaneously.⁸⁰

The results of these animal studies (► **Table 1**) strongly support the hypothesis that both, FXI and PK, play a role in the development of thrombosis and that the zymogens or their activated forms could be suitable drug targets to reduce the risk of thrombosis. Whether their respective impact might differ quantitatively remains to be elucidated.

Results from Human Studies and Deficiencies

FXI deficiency in humans is a rare disorder and was first described in 1953 by Rosenthal et al.⁸¹ This disorder, inherited as an autosomal recessive trait, is more frequently observed in Ashkenazi Jews. In contrast to FIX or FVIII deficiency, FXI deficiency, which is defined by levels below 20 IU/dL (severe deficiency), is associated with a mild-bleeding phenotype with very rare spontaneous bleedings. Bleeding in FXI-deficient subjects is more likely to occur following trauma or surgery, especially if tissues with high-fibrinolytic activity are affected.⁹ The bleeding tendency is, different from FIX or FVIII deficiency, not correlated with the FXI level.^{9,82} Therefore, other hemostatic abnormalities including low levels of von Willebrand factor have been suggested to contribute to the bleeding risk in FXI-deficient subjects.⁸³

There are several studies that examine the role of FXI in thrombosis in humans. High levels of FXI within the general population have been identified as risk factor for ischemic stroke⁸⁴ and deep vein thrombosis (DVT).⁸⁵ In addition, results from the risk of arterial thrombosis in relation to oral contraceptives case-control study showed an association between high levels of FXIa-C1 and FXIa-AT inhibitor complexes and ischemic stroke in young women.⁸⁶ A study with 115 severe FXI-deficient subjects demonstrated that FXI deficiency might be protective against ischemic stroke.⁸⁷ Furthermore, the incidence of DVT is reduced in FXI-deficient individuals compared to the incidence in individuals with normal activity. In the study of Salomon et al, no cases of DVT could be reported in 219 individuals with severe FXI deficiency.⁸⁸ A lower risk for DVT in FXI-deficient subjects could be confirmed by a comparative study, in which a protective effect of FXI deficiency against cardiovascular events (composite of MI, stroke, and transient ischemic attack) was observed.⁸⁹ A recent study investigating the association between FXI and thrombosis risk in a cohort of patients with a first unprovoked venous thromboembolism demonstrated that lower levels of FXI reduced the risk of recurrent venous thrombosis.⁹⁰ Taken together, the results from these epidemiology studies strongly support an association between FXI and ischemic stroke and DVT, respectively, in humans. The contribution of FXI to MI is less clear. Some data suggest that individuals with FXI deficiency are not protected from MI,⁹¹ while another study observed an association between FXI and MI.⁹² In addition, Minnema et al found a significant increase of FXI activity in patients with MI demonstrated by detection of FXIa-C1 inhibitor complexes in 24% of the patients with an acute MI (AMI).⁹³ Other studies

Table 2 Contribution of factor XI and prekallikrein to thrombosis risk in humans

	Findings		Literature	
High levels of FXI	↑ Ischemic stroke ↑ Deep vein thrombosis		84, 85	
Lower levels of FXI	↓ Recurrent venous thrombosis		90	
FXI deficiency	↓ Ischemic stroke ↓ Deep vein thrombosis		87, 88, 89	
FXI	Association with MI	No association with MI	92, 93, 94, 95	91
High PKa/PK levels	↑ Ischemic stroke in young women ↑ Incidence of arterial vascular disease → VTE risk in general population		86, 100, 101	
PK deficiency	→ Thrombosis Associated with cardiovascular disease		98, 99	

Abbreviations: ASO, antisense oligonucleotide; FXI, factor XI; MI, myocardial infarction; PK, prekallikrein; PKa, plasma kallikrein; VTE, venous thromboembolism.

also showed an increase in FXI activation during the acute phase in AMI patients, either by detection of FXIa-C1 complex levels or by a thrombin generation-based FXIa assay.^{94,95} These studies show that FXI plays an important role in thrombosis and might be a valuable drug target to prevent thrombosis (►Table 2).

While FXI deficiency has been described relatively well in literature, there are fewer studies on PK deficiency in humans (►Table 2). PK deficiency, also known as “Fletcher trait”, was first described in a family by Hathaway et al back in 1965.⁹⁶ At least 80 reported cases of severe PK deficiency (levels below 15% of normal), inherited as an autosomal recessive trait, can be found in literature. However, most cases of PK deficiency may be undetected since it is clinically asymptomatic and not associated with an increased bleeding tendency, although individuals with this disorder exhibit a prolonged aPTT.⁹⁷ The role of PK as risk factor for thrombosis in humans is therefore difficult to estimate. In 2011, Girolami et al hypothesized that deficiency of one of the contact system proteins may not protect against thrombosis⁹⁸ and in 2018, it was stated that PK deficiency often seems to be associated with CVDs.⁹⁹ The RATIO case-control study provides data suggesting that increased PKa levels is associated with ischemic stroke in young women. In this study, it was demonstrated that high levels of PKa-C1 inhibitor complexes led to a fourfold increase in ischemic stroke.⁸⁶ The association of increased PK levels with a higher incidence of arterial vascular disease such as MI has also been shown in another study.¹⁰⁰ However, there is also evidence that PK levels cannot be associated with a higher venous thromboembolism risk in the general population.¹⁰¹ Overall, more human studies have to be carried out to determine if PKa may be associated with thrombosis risk.

Therapeutic Strategies

The development of direct oral anticoagulants (DOACs) has improved the treatment of patients in need for anticoagulation, which was previously dominated by vitamin K antagonists as the only available oral drug class. In contrast to warfarin, no

routine monitoring is required.¹⁰² However, the general risk of bleeding remains, with a focus on gastrointestinal bleeding¹⁰² and other types of mucosa-related bleeds.

There are several therapeutic approaches to target FXI by inhibiting antibodies, its synthesis by antisense oligonucleotides (ASO), or FXIa by antibodies and small molecule inhibitors (summarized in ►Table 3).

Antibodies are characterized by a rapid onset of action, which depends on the site of application (intravenous vs. subcutaneous) and usually have a long half-life, requiring follow-up treatment only after weeks or months. First human data on safety, pharmacodynamics, and pharmacokinetics of antibodies directed against the active site of FXIa were derived from a phase 1 study. Compared with controls, the aPTT was prolonged and FXIa activity was reduced in cohorts receiving the anti-FXIa antibody osocimab (BAY 1213790).¹⁰³ Administration of MAA868, an antibody against FXI and FXIa, also diminished FXIa activity and increased aPTT in a phase 1 study¹⁰⁴ and is currently investigated in a phase 2 trial (NCT04213807). Anti-FXI antibodies reduced thrombus formation in a primate model⁷³ and decreased thrombus size in FXI-deficient mice administered with human FXI.¹⁰⁵

ASOs bind to the RNA of the target protein, thereby preventing its expression and finally lowering its plasma concentration.¹⁰⁶ Since the impact on FXI levels via synthesis inhibition by FXI ASO is rather slow, this approach is not feasible as standalone therapy for early secondary prevention after an event or when urgent protection is needed but might be more suitable for primary prevention or during elective procedures. A FXI-directed ASO (IONIS 416858) has been tested in patients undergoing total knee arthroplasty in a phase 2 trial, in which ASO treated patients were compared to patients receiving enoxaparin instead. Lowering FXI levels with 300 mg ASO reduced the incidence of venous thromboembolism after surgery and the size of the clots without increasing the bleeding risk.¹⁰⁷ These data confirmed the results obtained from studies in primates⁷⁴ and mice,¹⁰⁸ in which thrombus formation was reduced without increasing the bleeding risk.

Table 3 Pharmacological agents targeting factor XI(a) or plasma kallikrein in clinical trials

	Mode of action	Site of action	Reported clinical trial	Literature
BAY 1213790	Antibody	Adjacent to the FXIa active site	Phase 2	115 NCT03276143 NCT0378368
MAA868	Antibody	Catalytic domain of FXI and FXIa	Phase 2	104 NCT04213807
IONIS 416858	Antisense	FXI mRNA	Phase 2	107
BMS-962212	Small molecule	FXIa inhibitor	Phase 1	109
ONO-7684	Small molecule	FXIa inhibitor	Phase 1	NCT03919890
BMS-986177	Small molecule	FXIa inhibitor	Phase 2	NCT03891524 NCT03766581
BAY 2433334	Small molecule	FXIa inhibitor	Phase 2	NCT04218266
Ecallantide	Recombinant protein	PKa inhibitor	Registered for HAE	114
Lanadelumab	Antibody	PKa active site	Registered for HAE	62
BCX7353	Small molecule	PKa inhibitor	Phase 3	61 NCT03485911

Abbreviations: FXI, factor XI; HAE, hereditary angioedema; PKa, plasma kallikrein.

Synthetic small molecules can be administered either parenterally or orally and are suitable for situations, in which a rapid antithrombotic effect is required. First data on safety, pharmacodynamics, and pharmacokinetics of a small molecule inhibitor (BMS-962212) was reported in a phase 1 study. Intravenous administration of the inhibitor to healthy individuals resulted in prolongation of the aPTT and reduction of FXI activity.¹⁰⁹ Several oral FXIa inhibitors are reported to be at different development stages; while ONO-7684 is reported to be in phase 1 (NCT03919890),¹¹⁰ BMS-986177 (NCT03766581, NCT03891524),^{111,112} and BAY 2433334 (NCT04218266)¹¹³ are currently investigated in phase 2 studies.

The data on PKa as a therapeutic approach in thrombotic diseases are rather limited compared to FXI. There are some preclinical data on the effect of ASO specific to PKa. The efficacy of ASO was investigated in a mouse model showing that selective depletion of PKa is thrombo-protective in arterial and venous thrombosis.⁷⁹ Ecallantide, a peptidic inhibitor of PKa,¹¹⁴ and lanadelumab (DX-2930) are registered for HAE, while active site small molecules inhibitors of PKa (e.g. BCX7353) demonstrated that this approach might be effective in patients with HAE.^{61,62} However, these inhibitors were not tested for their antithrombotic effect in clinical studies.

The main advantage of FXI/FXIa inhibition over currently used antithrombotic strategies is most likely in the area of safety. Animal studies^{68,70} and recent clinical trials^{107,115} indicate that inhibition of FXIa or lower FXI levels reduce the risk of venous thrombosis without major impairment of hemostasis. In the previously conducted FOXTROT trial, the highest dose of osocimab was superior to enoxaparin in reduction of asymptomatic DVT. In this phase 2 study, a very low number of relevant bleeding events was found in all groups.¹¹⁵ Thus, further safety assessment of osocimab will have to be conducted in future studies.

Inhibition of PKa may also be a safe antithrombotic strategy since PK-deficient animals did not show any impairment in

hemostasis as compared with WT animals.⁷⁵ While limited data on the antithrombotic effects of PKa inhibitors exist, the additional anti-inflammatory properties render these inhibitors a very interesting approach for the treatment of thromboinflammatory diseases.⁷⁷

Conclusion

Since CVD is still one of the most common causes of morbidity and mortality worldwide, it remains important to develop new antithrombotic therapies. However, antithrombotic efficacy is consistently linked to bleeding. Based on risk associations in epidemiologic studies, both with venous and arterial thromboembolism, recent years spurred an interest in targeting FXI(a) to reduce the risk of thrombosis. This development has now proceeded toward extensive proof-of-concept clinical testing as demonstrated by several FXI(a) inhibitors entering or completing phase 2 trials in preventing venous thrombosis. There is potential to expand toward other indications, including prevention of ischemic stroke, MI, or prevention of clotting in extracorporeal devices including extracorporeal membrane oxygenation as these artificial surfaces may lead to contact activation. The premise of FXI(a) inhibitors as effective and safe anticoagulants still needs to be established for a broader range of indications.

The contribution of the contact system protein PK to the development of thrombosis is not quite as clear. Although results from animal studies indicate that depletion of PK decreases thrombosis risk, there is only limited and somewhat contradictory clinical data of human studies showing possible protective effects in humans. More clinical studies in populations at risk of thrombosis, in PK-deficient individuals or with compounds registered or developed for HAE are necessary to investigate the contribution of PK(a) and its inhibition to thrombosis, its prevention and the impact on bleeding risk. Considering the role of the kallikrein-kinin pathway in

inflammatory responses, targeting PKa might be a valuable approach in reducing thromboinflammation.

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None.

Conflict of Interest

S.H. is an employee of Bayer AG, Germany. M.V., H.T.C. and H.M.H.S. have nothing to disclose. H.T.C. was a fellow of the Gutenberg Research Foundation and is adjunct professor at the Center for Thrombosis and Hemostasis, Gutenberg University, Mainz, Germany. H.M.H.S. and H.T.C. receive support from the Netherlands Heart Foundation: CVON2014-09, reappraisal of atrial fibrillation: interaction between hypercoagulability, electrical remodeling, and vascular destabilization in the progression of atrial fibrillation (RACE V).

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