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Review

Factors IX, XI, and XII: potential therapeutic targets for anticoagulant therapy in atherothrombosis

Vikrant Rai[1], Marcus W. Balters[2] and Devendra K. Agrawal[3,*]

[1]*Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE, 68178, USA*

[2]*Department of Surgery, Creighton University School of Medicine, Omaha, NE, 68178, USA*

[3]*Department of Translational Research, Western University of Health Sciences, Pomona, CA, 91766, USA*

*Correspondence: dagrawal@westernu.edu (Devendra K. Agrawal)

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Atherosclerosis is a leading cause of cardiovascular and neurological ischemic events. Plaque rupture leads to the exposure of highly thrombogenic material with blood and results in the activation of the coagulation cascade, thrombus formation, and embolic events. Although antiplatelets and anticoagulants are used to prevent thromboembolic episodes, bleeding episodes remain the major adverse effect. Decreased ischemic events have been reported while comparing oral rivaroxaban and apixaban with aspirin to improve the therapeutic outcome in several clinical trials, including Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome-Thrombolysis in Myocardial Infarction 51, Apixaban for Prevention of Acute Ischemic and Safety Events, and GEMINI-ACS-1 phase II clinical trials. However, there were bleeding episodes. Thus, there is an unmet need for better therapeutic strategies. Therefore, the current focus is to target Factors IX, XI, and XII to develop safer and efficient strategies. In this article, we critically reviewed and discussed the limitations of current therapies and the potential of targeting Factors IX, XI, and XII for anticoagulant therapy in atherothrombosis.

Keywords

Atherothrombosis; atherosclerotic plaque; plaque rupture; blood coagulation; factor IX, XI, and XII inhibition

1. Introduction

Atherothrombosis, a complex disease leading to myocardial infarction, ischemic heart disease, ischemic stroke, and peripheral vascular disease, is a major health care burden and a leading cause of mortality throughout the world including the United States (Bhatt et al., 2006). The atherosclerotic plaques contain highly thrombogenic material, including the platelet activator collagen and the procoagulant protein tissue factors (Mackman, 2014). Plaque rupture in an atherosclerotic artery leads to denudation of the arterial surface and exposure of the procoagulant material to the blood flow resulting in the formation of a thrombus and emboli. Various studies are going on to develop strategies to prevent plaque rupture and thromboembolic episodes by plaque stabilization or decreasing re-endothelialization after vessel injury (Gupta et al., 2016; Rai et al., 2016; Rao et al., 2016). Plaque rupture or vascular interventions to treat atherosclerotic arteries results in the injury to the endothelial surface of the artery. Tissue injury, as a natural defense mechanism, initiates activation of acute-phase reaction involving a combination of procoagulant and proinflammatory mechanisms to limit the tissue damage and restore hemostasis (de Jong et al., 2010). Injury to a blood vessel responds by the activation of coagulation cascade, comprises of intrinsic and extrinsic system, and results in the formation of platelet-rich fibrin plugs and ultimately thrombus formation. This is initiated by the activation of intrinsic system by the factors circulating in the blood, nucleic acids, and inorganic phosphates secreted after the injury and an extrinsic system activated by tissue factors (TFs) exposed on the vessel after an injury or surgery initiates the platelet-rich fibrin plug formation (Buller et al., 2015; Kenne and Renne, 2014). TFs are also secreted by activated neutrophils in association with web-like filamentous structures of decondensed chromatin called neutrophil extracellular traps (NETs) that are composed of DNA and histones. The NETs can activate both intrinsic and extrinsic pathways of the coagulation cascade (Badimon and Vilahur, 2015). Isolated nucleic acid binds to FXII and FXI to activate the coagulation pathway, however, this mechanism has recently been called into question which suggests that the original data showing this phenomenon were in fact due to silica contamination (Smith et al., 2017) (Fig. 1).

2. Current therapeutics

The contact of blood circulating factors with factor XII and TFs with factor VII initiates the activation of coagulation cascade (Kenne and Renne, 2014; Viles-Gonzalez et al., 2004; Viles-Gonzalez and Badimon, 2004). This results in the formation of activated FXII and FVII (FXIIa and FVIIa) leading to the development of thrombus and thromboembolic episodes. Antithrombotic drugs have been used to prevent such events. Since TF initiates the extrinsic pathway of coagulation, targeting TFs seems to be a promising strategy to prevent thrombus formation. Common antithrombotic drugs used to prevent thrombosis target the extrinsic (TF + VIIa) and common (Xa) pathway involved in coagulation required for hemostasis (Mackman, 2014) (Fig. 1). The cur-

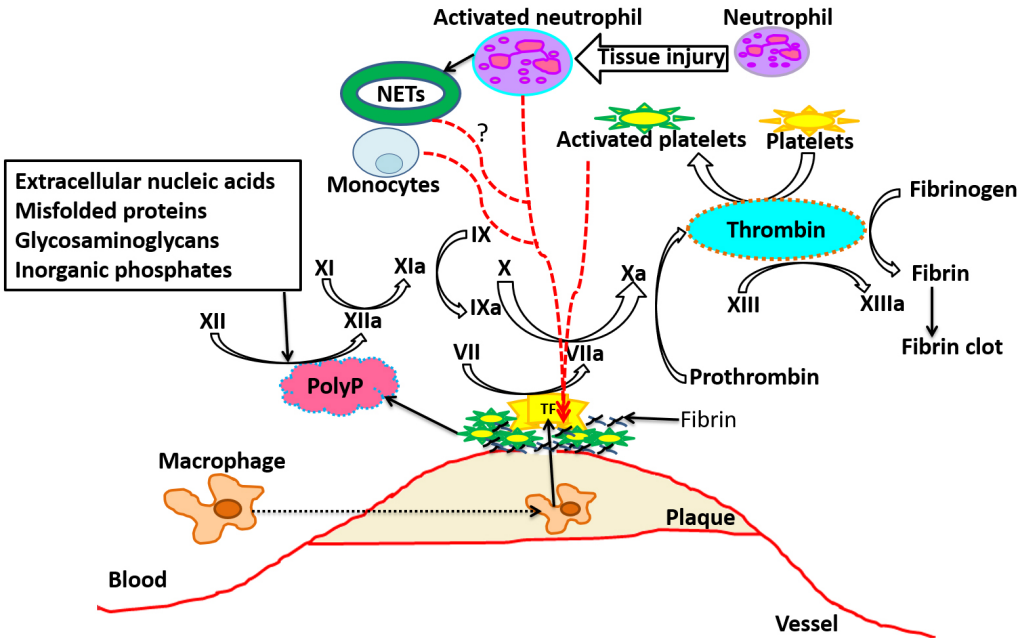

Figure 1. Schematic model for Atherothrombosis after plaque rupture. The plaque rupture brings in the thrombogenic material in contact with blood leading to the secretion of tissue factor (TF). This leads to the activation of factor (F) VII and FX followed by formation of thrombin, fibrin and platelet rich fibrin clot called as thrombus (extrinsic coagulation pathway). The activated platelets also secrete inorganic polymer polyphosphate (polyP) which activates FXII to FXIIa followed by activation of FXI, FIX, and FX leading to formation of thrombin, fibrin and thrombus (intrinsic coagulation pathway). TF secreted from circulating monocyte and plaque macrophages and neutrophil extracellular traps (NETs) from activated neutrophils also activate FXII.

rently used common antithrombotic agents include the specific TF inhibitors (TFPI-Tissue Factor Pathway Inhibitors), factor Xa inhibitors (direct inhibitor and indirect inhibitor requiring antithrombin III), and direct thrombin inhibitors (DTI) such as hirudin, bivalirudin, and melagatran (Viles-Gonzalez et al., 2004; Viles-Gonzalez and Badimon, 2004). However, inhibition of TFs does not appear to be a promising approach to prevent thromboembolic events, since TFs are critical to maintain hemostasis (Tatsumi and Mackman, 2015). Additionally, the use of these drugs is associated with increased bleeding events. The phase II and phase III trials of the oral anticoagulants, including dabigatran, apixaban, and darexaban, showed no reduction in ischemic events and were associated with increased bleeding events (Cohen and Iyer, 2014; Oldgren et al., 2011). However, TFs play a crucial role in the initiation of the coagulation cascade and platelet-rich fibrin plug formation resulting in ischemic events. Thus, inhibition of TF may attenuate the initiation and propagation of the coagulation cascade and reduce thrombin formation. Since thrombin induces TF-mRNA transcription, TF expression, and TF activity in the endothelial cells to render it procoagulant, inhibition of thrombin might be a potential option. Bivalirudin is a direct thrombin inhibitor and can prevent the deleterious effects of thrombin (Kenne et al., 2015). Furthermore, since thrombin activates FXI by the positive feedback, the addition of bivalirudin may attenuate the plasma level of FXIa. However, this hypothesis warrants further investigation (Fig. 2).

3. Limitations of the current therapy

Rivaroxaban is a factor X inhibitor and selectively inhibits FXa with rapid onset of action. The Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome-Thrombolysis in Myocardial Infarction 46 and 51 (ATLAS ACS 2-TIMI 46 and ATLAS ACS 2-TIMI 51) (Cavender et al., 2015; Crowther and Cuker, 2017; Mega et al., 2009, 2012) for oral rivaroxaban with dual antiplatelet therapy (acetylsalicylic acid (ASA) with a platelet P2Y12 inhibitor (adenosine diphosphate (ADP) receptor antagonist such as clopidogrel) in patients with ACS found decreased thromboembolism with significantly reduced risk of thromboembolic events and deaths due to cardiovascular events, myocardial infarction and stroke. The results of ATLAS ACS 2-TIMI 51 suggest that dual-pathway strategy using antiplatelet-anticoagulant combination can improve the clinical outcome. However, an increased risk of bleeding in a dose-dependent manner associated with the use of oral rivaroxaban might reduce the outcome (Cohen and Iyer, 2014; Mega et al., 2009, 2012). Further, the missing data in the ATLAS ACS 2-TIMI 51 trial (Krantz and Kaul, 2013) regarding the patients withdrawn from the trial, missing follow-up, non-availability of vital sign, etc. add up to the bias in the trial and warrant further studies. Due to the incidence of increased bleeding, clinical trials (Cavender et al., 2015; Povsic et al., 2016) with low-dose oral rivaroxaban as an adjunct therapy are currently ongoing.

The GEMINI-ACS-1 phase II study with low-dose rivaroxaban comparing dual antithrombotic therapy (rivaroxaban [2.5 mg twice daily] + P2Y12 inhibitor) with DAPT (aspirin [100 mg] +

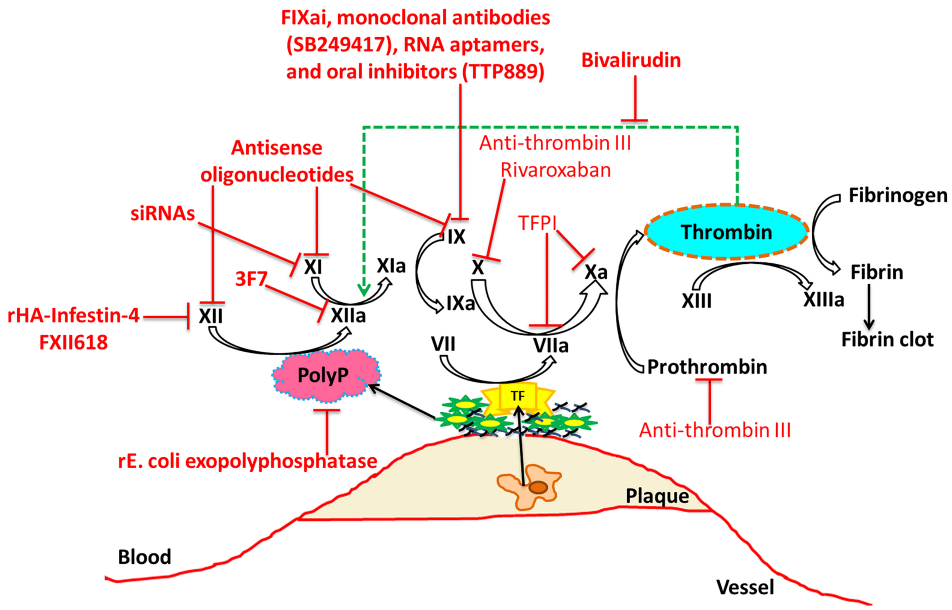

Figure 2. Potential targets in atherothrombosis. Factor IX, XI, and XII are the novel targets in atherothrombosis. NETs-neutrophil extracellular traps; polyP-inorganic polymer polyphosphate; TF-tissue factor; TFPI-tissue factor pathway inhibitors.

P2Y12 inhibitor) showed that low-dose rivaroxaban has a similar risk of significant bleeding (Gurbel and Tantry, 2017; Huynh, 2017; Ohman et al., 2017). Further, triple therapy using rivaroxaban or apixaban or dabigatran with ASA and clopidogrel (P2Y12 inhibitor) resulted in increased episodes of bleeding (Sorensen and Gislason, 2014). Similarly, Apixaban for Prevention of Acute Ischemic and Safety Events (APPRAISE-2) trial for oral factor X inhibitor apixaban found no significant reduction in the ischemic events, but there was increased dose-dependent bleeding when apixaban was added with antiplatelet therapy in ACS patients (Alexander et al., 2009, 2011). The use of rivaroxaban reduced death due to cardiovascular events but was associated with increased rates of major bleeding and intracranial hemorrhage. Similarly, the phase III APPRAISE-2 trial was terminated prematurely due to major bleeding events (Alexander et al., 2011). The association of bleeding episodes in these trials at a low-dose or in combination warrants larger, adequately powered clinical trials with more convincing evidence for efficacy and safety to find better therapeutic targets and anticoagulant therapies without risk of bleeding episodes.

4. Factor IX, XI, and XII: Novel targets for anticoagulation therapy

The modest therapeutic benefits, narrow clinical application, and increased bleeding risk of current therapeutic agents stipulate the need to develop better strategies without increasing bleeding events. To increase the safety, there is a growing interest to develop a safer anticoagulant by targeting factor IX (FIX), factor XI (FXI), and factor XII (FXII, Hageman factor) (Eikelboom et al., 2010; Muller et al., 2011). The interest to target the TFs is because studies using mice and clinical studies in patients with hereditary deficiencies reported the importance of these factors in thrombosis with a minor role in hemostasis. Patients with deficiency in FIX, FXII and FXI generally do not bleed spontaneously (bleeding is associated with severe deficiency, injury or surgery) and tend to have relatively mild bleeding tendencies. Since FIX, FXII, and FXI play a crucial role in thrombus formation than in normal hemostasis, pharmacological inhibition of FIX, FXII, and FXI may be a potential possibility of anticoagulation therapies with minimal or no bleeding risk (Buller et al., 2015; Eikelboom et al., 2010; Kenne et al., 2015; Kenne and Renne, 2014; Morrissey, 2013; Muller et al., 2011; Nickel et al., 2017; van Montfoort et al., 2013). Thus, inhibition of FIXa, FXIa, or FXIIa would relatively be a safer target and seems to be a promising approach. However, studies are needed to compare and analyze the results of IXa, XIa, and XIIa inhibition.

The essential role of FXII- and XIIa-mediated activation of FXI in tissue factor-induced thrombosis and pulmonary embolization has been documented in animal models (Cheng et al., 2010; Gruber and Hanson, 2003; Kenne and Renne, 2014; Nickel et al., 2017). However, the role of factor XIIa in human remains controversial (Key, 2014; Nickel et al., 2017). An *in-vitro* model of atherothrombosis using human atheromatous plaque material suggested no role of XIIa in thrombus formation (Reininger et al., 2010). But, increased plasma levels of FXIIa have been associated with increased risk of cardiovascular events (Nickel et al., 2017). After an injury, binding of TFs with FXII zymogen result in the serine protease-activated FXII (FXIIa). Activated FXII (FXIIa) has an advantage over FXII that, among the inhibitors of FXII and FXIIa, those having a higher affinity for inhibiting FXIIa than FXII can be dosed at a significantly lower plasma concentration compared to FXII (Kenne and Renne, 2014). Activation of FXII (FXIIa) results in successive activation of coagulation factor XI (FXI), FIX (FIXa), and FX (FXa) and generation of thrombin. FXI could also interact with and inhibit the activity of TFPI which inhibits FXa and FVIIa-TF complex (Fig. 1 and 2). These results suggest that FXIa not only activates FIX in intrinsic pathway but may also attribute to the promotion of the extrinsic pathway through TFPI inactivation (Puy et al., 2015). However, when the

tissue factor is inhibited by TFPI, thrombus formation and normal hemostasis can be maintained by platelet-localized feedback activation of factor XI by a higher concentration of thrombin (Walsh, 2003; Wielders et al., 2004). But it has also been reported that the feedback activation of factor XI by thrombin does not occur in plasma (Pedicord et al., 2007).

Activated FXIa also plays an important role in the activation of FIX (FIXa) (Gailani et al., 2014). Factor IXa generated by factor XIa on the platelet surface involves the intrinsic pathway of coagulation and along with FVIII (FIXa/VIIIa complex) aids in the recruitment of FX to the platelet surface. The activation of FX by FIXa/VIIIa complex is nearly 50-times more efficient compared to extrinsic pathway activation by FVIIa/TF (Lawson and Mann, 1991). Decreased atherothrombosis without an increase in bleeding by inhibiting factor XIIa and reduction in factor XI with factor XI antisense oligonucleotides in acutely ruptured atherosclerotic carotid arteries in a mouse model suggest the significance of using these factors for better therapeutic strategy (Kuijpers et al., 2014; van Montfoort et al., 2014). These studies suggest the crucial role of FIX, XI, and FXII in the coagulation cascade and the therapeutic potential of targeting these factors for better therapeutics. Small molecules, including active site blockers or allosteric modulators, monoclonal antibodies that block activation or activity, RNA aptamers, oral inhibitors targeting FIX, FXI, and FXII, and antisense oligonucleotide that reduce the hepatic synthesis of the clotting proteins targeting FXI and FXII (Howard et al., 2007; Weitz and Fredenburgh, 2017), are the most common strategies inhibiting these factors to reduce thromboembolism (Fig. 2).

4.1 Factor IX inhibition

The important role of FIXa in activating FX and fibrin clot formation signify the role of FIX in coagulation. FIX has a unique ability to diffuse capably from TF-bearing cells to platelets and links the initiation and propagation phases of the coagulation reaction. Inhibiting FIX seems to be a promising target to decrease thromboembolic events by discontinuing the coagulation cascade (Howard et al., 2007). The plasma factors antithrombin III, nexin-2/amyloid β protein precursor, neutrophil elastase, and protein Z-dependent protease inhibitor inactivates FIX, albeit inefficiently compared to FXa and thrombin inactivation. Howard et al. (2007) reviewed the role, mechanism of action, preclinical data, and clinical trials of FIX active site blockers (FIXai), monoclonal antibodies (SB249417), RNA aptamers, and oral inhibitors (TTP889), and concluded that these clinical trials have not followed the preclinical data. FIXai had no clinical trial, no safety profile was reported for monoclonal antibodies, RNA aptamers were under phase IB and IC clinical trial, and results of FIXIT trial for oral inhibitors were not published (Howard et al., 2007). Sullenger et al. (2012) studied the mechanistic aspect of RNA aptamer in a late-stage clinical development and found that binding of aptamers to factor IXa results in prolonged human plasma clotting time and activation of FX regardless of the presence of factor VIIIa but does not completely block synthetic substrate cleavage and only slow down the rate of cleavage. The inadequacy of the results in these studies warrants more research and clinical trials to develop therapeutic strategies targeting FIX for anticoagulation without risk of bleeding episodes.

4.2 Factor XI inhibition

FXI plays a crucial role in coagulation, thromboembolism, and peripheral vascular disease mediated by venous thrombus growth in an endothelial denudated vessel and/or blood stasis. Promotion of the platelet aggregation and fibrin formation at low shear stress by the interaction of FXI and thrombin signify the role of FXI in thromboembolism (Takahashi et al., 2010). The reduction in the thrombus formation in a denuded vessel with anti-FXI antibody indicates FXI to be a promising target in the coagulation cascade to prevent thromboembolic events (Lowenberg et al., 2010; Takahashi et al., 2010). Many studies have demonstrated the reduced thrombus formation without increasing the risk of bleeding with an antisense oligonucleotide (ASO) along with the increased number of fluorescent platelets shed from the thrombus without reducing the bleeding time to lower factor XI. These findings suggest the therapeutic potential of blocking FXI (Crosby et al., 2013; van Montfoort et al., 2014; Zhang et al., 2010). However, the slow onset of ASO-mediated knockdown factor XI levels was the potential disadvantage. In a murine thrombosis model (van Montfoort et al., 2013), prevention of the cessation of the blood flow without inducing a bleeding tendency with inhibitory anti-human FXI antibodies FXI-175 and FXI-203 suggest the importance of blocking FXI as a therapeutic strategy for thromboembolism (Fig. 2).

Prevention of postoperative venous thromboembolism in patients undergoing elective primary unilateral total knee arthroplasty with second-generation antisense oligonucleotide FXI-ASO suggest its safety and effectiveness for the first time in a human without any risk of bleeding (Buller et al., 2015). The antisense oligonucleotide FXI-ASO binds to the mRNA of factor XI in hepatocytes, the primary site of factor XI formation, leading to a reduced downstream plasma level (Cuker, 2015) (Fig. 2). siRNAs are other nucleotides which can be used to decrease thromboembolic episodes as they target the specific mRNA and change the target gene. Inhibition of the coagulation factors in liver can be achieved by these siRNAs. Recently Heestermans and van Vlijmen (2017) have reviewed various ongoing preclinical and clinical trials in zebrafish, mammals, and human with a conclusion that oligonucleotide-based silencing of coagulation factors to treat thrombosis warrant studies focused on the safety and efficacy of oligonucleotides (Fig. 2).

4.3 Factor XII inhibition

The role of FXII and FXIIa in atherothrombosis and inflammation has been documented in various studies. Since inflammation does play a role in the pathogenesis of plaque formation and plaque rupture resulting in thrombus formation, inhibiting FXII/FXIIa might abrogate the ongoing inflammation and thrombus formation (Kenne and Renne, 2014; Nickel et al., 2017). The findings of a substantially less infarcted brain without an increase in infarct-associated hemorrhage in FXII-deficient and FXII inhibitor-treated mice after transient middle cerebral artery occlusion suggest FXII be dispensable for hemostasis but instrumental in fibrin formation. This effect was due to impaired pathological fibrin formation after FXII inhibition without increased bleeding (Kleinschnitz et al., 2006). Additionally, minimized trauma-induced microvascular thrombus formation and ischemic injury with factor XII inhibitor rHA-Infestin-4 in mice signify the thromboprotective effect of FXII inhibition (Hopp et al., 2016)

Table 1. Clinical Trials for anticoagulation.

Trials	Therapy	Subjects	Outcome
Dabigatran vs. placebo Randomized double-blind, phase II trial (Oldgren et al., 2011)	Dabigatran 50 mg (n = 369), 75 mg (n = 368), 110 mg (n = 406), 150 mg (n = 347), or placebo (n = 371).	1861	Dabigatran was associated with a dose-dependent increase in bleeding events Significantly reduced coagulation activity in patients with a recent myocardial infarction.
ATLAS ACS 2-TIMI 46 phase II trial (Mega et al., 2009)	Aspirin only (n = 761) or aspirin + thienopyridine (n = 2730) and placebo or rivaroxaban (5-20 mg) given once daily to both groups.	3491	Increases bleeding in a dose-dependent manner. Might reduce the deaths from cardiovascular cause or stroke.
ATLAS ACS 2-TIMI 51 Phase III (Cavender et al., 2015)	2.5 mg or 5 mg of rivaroxaban or placebo twice daily	15,526	Rivaroxaban reduced the risk of death from cardiovascular causes, myocardial infarction, or stroke. Rivaroxaban increased the risk of major bleeding and intracranial hemorrhage but not the risk of fatal bleeding.
GEMINI-ACS-1 phase II (Ohman et al., 2017; Povsic et al., 2016)	Rivaroxaban [2.5 mg twice daily] + P2Y12 inhibitor with dual pathway antithrombotic therapy (aspirin [100 mg] + P2Y12 inhibitor)	3037	Low-dose rivaroxaban with a P2Y12 inhibitor had similar risk of clinically significant bleeding as aspirin and a P2Y12 inhibitor.
APPRAISE phase II trial (Alexander et al., 2009)	Placebo (n = 611) or 1 of 4 doses of apixaban: 2.5 mg twice daily (n = 317), 10 mg once daily (n = 318), 10 mg twice daily (n = 248), or 20 mg once daily (n = 221)	1715	A dose-related increase in bleeding Reduction in ischemic events with the addition of apixaban to antiplatelet therapy

ATLAS-ACS-TIMI; Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome-Thrombolysis in Myocardial Infarction, APPRAISE; Apixaban for Prevention of Acute Ischemic and Safety Events.

(Fig. 2). The results of these studies along with others (Kenne et al., 2015) support the notion that FXII inhibition might be safe and a selective thromboprotective strategy.

To date, several classes of inhibitors of FXII and FXIIa including antibodies, biological inhibitors, recombinant proteins, synthetic peptides, and antisense oligonucleotides (ASO) along with their mode of action, concurrent side-effects and the animal models in which they have been tested have been discussed (Kenne and Renne, 2014; Nickel et al., 2017). However, there is a lack of studies in humans. The weak binding affinity and/or poor selectivity of the synthetic FXII inhibitors instigated the development of peptide macrocycle to inhibit FXIIa (FXII618) with a higher inhibition constant and selectivity, selectively blocking the initiation of the intrinsic coagulation pathway without affecting the extrinsic pathway (Baeriswyl et al., 2015). This inhibitor also led to disentangling the intrinsic and extrinsic pathways and differentially examining the extrinsic pathway of coagulation. Both factor VII and XII contribute to atherothrombosis. The initial thrombus formation is facilitated by tissue factor-FVIIa pathway, and FXIIa mediates the thrombus stability (Kuijpers et al., 2014). Recently, the use of an active-site inhibited factor VIIa (FVIIai) to inhibit the factor VIIa resulted in a reduced early thrombosis formation (0.5 minutes after plaque rupture) without affecting stability (Kuijpers et al., 2014). There was no effect on the initial formation of the thrombus, however, the size of the thrombus at a later time (2 and 10 minutes after plaque rupture) and the stability was reduced with the use of corn trypsin inhibitor or r-HA-infestin-4 to inhibit factor XIIa (Fig. 2).

Efficient inhibition of the fibrin deposition and thrombus formation by plasma protease FXIIa-neutralizing antibody, 3F7, by binding specifically to the enzymatic pocket of FXIIa in an extracorporeal membrane oxygenation (ECMO) system similar to heparin but without treatment-associated increase in hemorrhage signifies thromboprotective properties of 3F7 (Larsson et al., 2014). Furthermore, inhibition of the thrombus formation without impairing the hemostasis indicates FXII as a potential target for the prevention of atherothrombosis. The use of ECMO simulating the clinical settings signifies the meaningful importance of this study to use in clinics. However, the prevention of the contact-induced FXIIa formation, thrombus formation in mice and rabbits and coagulation in-vitro with 3F7 have also been documented (Larsson et al., 2014; Schmaier, 2014; Worm et al., 2015) (Fig. 2). FXII activation is also mediated by inorganic polymer polyphosphate (polyP), which is stored in platelets and secreted on platelet activation (Nickel et al., 2017). Reduced fibrin accumulation and attenuated thrombus formation without increased risk of bleeding by targeting polyP (Labberton et al., 2016) with recombinant *Escherichia coli* exopolyphosphatase to abolish the procoagulant platelet activity in a factor XII-dependent manner suggest the role of polyP in thrombus formation as well as targeting polyP as a potential thromboprotective strategy (Fig. 2).

Taken together, targeting the FIX, FXI, and FXII appear to be a promising approach to decrease the atherothrombosis and thromboembolic events. However, in-depth knowledge of the proteins and other factor involved in the process of thrombus formation is crucial to design a safer and efficient therapeutic strategy by targeting these novel sites. Clinical trials and research work are the cornerstones to understand the underlying mechanism of proteins and interaction with other factors. The ongoing clinical trial might be useful in this aspect (Table 1 and 2).

Table 2. Ongoing clinical trials related to atherothrombosis.

Clinical trial	Aim	Status
CONTACT NCT02785718	To investigate the influence of the proteins of the contact activation system on thrombus formation in human blood in a flow and static model	Recruiting patients
Thrombus Formation Under Different Flow-conditions NCT01114074	To study the effects of the proteins of the contact activation system on platelet mediated thrombus formation, embolization and degradation on collagen in a perfusion flow model.	Recruiting patients
ATIS-NVAF NCT03062319	To evaluate the efficacy and safety of mono-drug therapy with oral anticoagulant compared to combination therapy with antiplatelet drug, in ischemic stroke patients with non-valvular atrial fibrillation and atherothrombosis.	Not yet open for participant recruitment.
CHARISMA trial NCT00050817	To assess the efficacy and safety of clopidogrel 75 mg once-daily by comparison with a placebo in preventing cardiovascular morbidity/mortality.	This study has been completed but results have not been posted https://clinicaltrials.gov
Triple versus dual antiplatelet therapy NCT00404716	To evaluat the safety and efficacy of triple antiplatelet regimen of aspirin, clopidogrel and cilostazol compared with dual antiplatelet regimen of aspirin and clopidogrel in patients with acute coronary syndrome undergoing successful coronary artery stenting.	This study has been completed but results have not been posted https://clinicaltrials.gov
Trial for efficacy and safety of rivaroxaban prophylaxis compared with placebo in ambulatory cancer patients at high risk for venous thromboembolism; phase III NCT02555878	To demonstrate that rivaroxaban is superior to placebo for reducing the risk of the primary composite outcome	Currently recruiting participants

ADRIE; Antiplatelet Drug Resistances and Ischemic Events, ATIS-NVAF; Optimal Antithrombotic Therapy in Ischemic Stroke Patients with Non-Valvular Atrial Fibrillation and Atherothrombosis, CONTACT; The Proteins of the Contact Activation System, CHARISMA; Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance, NAPS; Nattokinase Atherothrombotic Prevention Study.

5. On-going clinical trials for anticoagulation therapy

The focus of the ongoing clinical trials is to understand the interaction between various proteins and circulating factors playing a role in atherothrombosis and anticoagulation. Ongoing clinical trial assessing the influence of the proteins of the contact activation system on thrombus formation in human blood in a flow and static model (NCT02785718, FXI and FXII); embolization and degradation of collagen in a perfusion flow model (NCT01114074, FXI and FXII); randomized multicenter trial to evaluate the efficacy and safety of mono-drug therapy with oral anticoagulant compared to combination therapy with antiplatelet drug (NCT03062319) will surely help in our in-depth understanding in this arena to develop better therapeutics. Further, Antiplatelet Drug Resistances and Ischemic Events (ADRIE) trial (NCT00501423); Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) trial (NCT00050817); triple versus dual antiplatelet therapy in patients undergoing coronary stent implantation (NCT00404716); Nattokinase Atherothrombotic Prevention Study (NAPS) trial (NCT02080520); and trial for efficacy and safety of rivaroxaban prophylaxis compared with placebo in ambulatory cancer patients at high risk for venous thromboembolism (NCT02555878) [https://clinicaltrials.gov/] might help in advancing our knowledge on the complex process of anticoagulation in atherothrombosis and development of more efficient therapeutics (Table 2).

6. Conclusion

Endothelial injury or plaque rupture initiates the coagulation cascade with the release of coagulation factors that are involved in the activation of intrinsic and extrinsic pathways resulting in thromboembolic events. The association of bleeding episodes with the current therapeutic strategies warrants the need of a safe and specific anticoagulant. Targeting the TFs including FIX, FXI, and FXII might be an alternative therapeutic strategy because these factors take part in thrombus formation but play a minor role in hemostasis. Further, deficiency of these factors is not associated with spontaneous bleeding. Studies from the inhibition of FIX, FXI, and FXII have provided promising results but are associated with bleeding episodes. Thus, future in-depth studies are required to exclude potential limitations and to increase the safety and efficacy.

Abbreviations

ACS: Acute Coronary Syndrome; APPRAISE-2: Apixaban for Prevention of Acute Ischemic and Safety Events; ASA: Acetylsalicylic acid; ASO: Antisense oligonucleotide; ATLAS ACS 2-TIMI 51: Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome-

Thrombolysis in Myocardial Infarction 51; DNA: Deoxyribose nucleic acid; NETs: Neutrophil extracellular traps; TFPI: Tissue Factor Pathway Inhibitors; TFs: Tissue factor (s).

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Conflict of Interest

The authors declare no competing interests.

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