Targeting factor Xa and thrombin: impact on coagulation and beyond

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Summary
Great advances have been made in recent years in understanding the haemostatic system and the molecular and cellular basis of thrombus formation. Although directly targeting factor Xa or thrombin (factor IIa) for effective anticoagulation is now well established, evidence has emerged suggesting that factor Xa and thrombin are involved in other physiological and pathophysiological cellular processes, including inflammation. These non-haemostatic activities of factor Xa and thrombin are predominantly mediated via the activation of proteinase-activated receptors. Studies have indicated a potential role of coagulation proteins (including factor Xa and thrombin) in the progression of disease conditions such as atherothrombosis. Preclinical studies have provided evidence for the effects of direct factor Xa or direct thrombin inhibition beyond anticoagulation, including anti-inflammatory activities and atherosclerotic plaque stabilisation. In this article, the non-haemostatic activities of factor Xa and thrombin and the effects of direct inhibition of these coagulation factors on these activities are summarised. In addition, the potential roles of factor Xa and thrombin in atherosclerosis and atherothrombosis are explored and the cardiovascular profiles of rivaroxaban, apixaban and dabigatran etexilate observed in phase III clinical studies are discussed.

Keywords
Atherothrombosis, coagulation factors, proteinase-activated receptors (PAR), thrombin

Introduction
Normal haemostasis prevents excessive blood loss at the site of vascular injury and is accomplished through a network of systems, including procoagulant, anticoagulant and fibrinolytic proteins and platelets. Dysfunction of one or more of these systems can result in thrombosis or excessive haemorrhage. In recent years, great advances have been made in understanding the haemostatic system and the molecular and cellular basis of thrombus formation. It is now well recognised that the natural anticoagulant systems, particularly activated protein C (APC), exhibit additional beneficial effects, including anti-inflammation and endothelial barrier protection (1, 2). Factor Xa has a pivotal role within the coagulation cascade and is, therefore, a drug target for anticoagulant therapy. Both factor Xa and thrombin (factor IIa) have been investigated, and the development of oral, direct factor Xa and direct thrombin inhibitors has proven to be a translational success. The clinical efficacy of these agents has been demonstrated in a number of large, randomised, phase III clinical studies (3). These advances have provided new options for the management of thromboembolic disorders.

Cross-talk between coagulation and inflammation is now well recognised, including in atherosclerosis (4). In addition to their role in coagulation, factor Xa and thrombin are known to participate in other physiological and pathophysiological cellular activities, predominantly via activation of proteinase-activated receptors (PARs) (5, 6). Preclinical studies have demonstrated that targeted inhibition of factor Xa or thrombin exhibits effects beyond coagulation, such as anti-inflammation and potential influence on the progression of atherothrombosis (7–9), although supporting clinical data are currently lacking. After a brief overview of coagulation and its regulation by the natural anticoagulant systems, this article will summarise the interactions between coagulation and inflammation and the contributions of factor Xa and thrombin to the inflammatory activities. The potential roles of factor Xa and thrombin in atherosclerosis and atherothrombosis are also explored, together with recent preclinical data showing the non-haemostatic effects of direct factor Xa or direct thrombin inhibition. In addition, the cardiovascular profiles of rivaroxaban, apixaban and dabigatran etexilate observed in phase III clinical studies are also discussed.

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Overview of blood coagulation

Blood coagulation is initiated when the integrity of the endothelium is breached. Factor VIIa and tissue factor (TF) form the TF–factor VIIa complex, which activates factor X and factor IX. Factor Xa then converts prothrombin to thrombin. The small amount of thrombin produced amplifies coagulation by activating factor V, factor VIII and platelets. Factor VIIIa combines with factor IXa and forms the factor IXa–factor VIIIa–phospholipid complex – a major activator of factor X. Factor Xa binds to negatively charged phospholipid surfaces (e.g. activated platelets), and together with factor Va forms the prothrombinase complex – the central prothrombin activator that converts prothrombin to thrombin (▶Figure 1) (10). Thrombin has a central role in the clotting process, including converting soluble fibrinogen to fibrin, activating platelets and activating factor XIII to induce fibrin cross-linking (6). Blood-borne TF has been identified on microparticles derived from leukocytes and other cell types that are involved in the initiation of coagulation when vessel injury is limited to endothelial activation (11, 12).

Natural anticoagulant systems

Under physiological conditions, coagulation is tightly regulated by natural anticoagulants and the fibrinolytic system to ensure that thrombus formation is restricted to the site of vascular injury. The main natural anticoagulants are TF pathway inhibitor (TFPI), antithrombin and the protein C and protein S system (13).

TFPI is an important physiological inhibitor of factor Xa in the initial phase of blood coagulation, which acts by blocking the TF–factor VIIa–factor Xa complex (10), thus preventing the generation of both factor Xa and factor IXa. Antithrombin inhibits key coagulation enzymes such as thrombin and factor Xa. In the absence of heparin, antithrombin neutralises these coagulation enzymes in a slow, progressive manner, but its activity is increased dramatically in the presence of heparin (13).

The protein C system is a major natural anticoagulant pathway, which is activated when thrombin binds to thrombomodulin (TM; ▶Figure 2). Activation of protein C occurs primarily on the surface of the endothelium and activation is increased when protein C is bound to the endothelial cell protein C receptor (EPCR). The APC–EPCR complex must dissociate before APC can bind to protein S to form the protein S–APC complex responsible for the inactivation of factor Va and factor VIIIa. This decreases the formation of factor Xa–factor Va (the prothrombinase complex) and factor IXa–factor VIIIa (the intrinsic tenase complex), leading to the inhibition of coagulation (14). The binding of thrombin to TM decreases free thrombin levels, thus preventing thrombin from cleaving fibrinogen and activating platelets. In addition, thrombin within the thrombin–TM complex is more sensitive to inhibition by circulating antithrombin than free thrombin (6). APC can also stimulate fibrinolysis by forming a complex with plasminogen activator inhibitor 1 (PAI-1). The role of PAI-1 is to inhibit plasmin formation, leading to inhibition of fibrinolysis (or clot dissolution); thus, the inhibition of this inhibitor of fibrinolysis by APC further enhances anticoagulation (6).

![Figure 1: An overview of blood coagulation.](image-url)

F, factor; TF, tissue factor.
Impact of inflammation on coagulation and the anti-inflammatory activity of the protein C system

It is well known that inflammation and coagulation are closely linked. Inflammatory mediators can trigger coagulation activation by altering the procoagulant and/or the anticoagulant pathways. Inflammatory stimuli (e.g. tumour necrosis factor alpha (TNF-α)) can increase the expression of TF on the surface of monocytes (13). Furthermore, inflammation can downregulate natural anticoagulant pathways. For example, the protein C system is malfunctioning in patients with severe inflammation, including low plasma levels of protein C and protein S and downregulation of TM and EPCR (1, 13).

The protein C system has an important role in modulating inflammation. The anti-inflammatory vascular effects of APC include effects on endothelial cells and leukocytes. APC administration was shown to reduce the production of endotoxaemia-induced pro-inflammatory cytokines, such as interleukin (IL)-6, -8, -1β and TNF-α, and to downregulate vascular adhesion molecules (such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin), resulting in reductions in leukocyte adhesion and infiltration (2, 15). In addition to its anti-inflammatory activities, APC also has a role in maintaining the integrity of endothelial barrier function and inhibiting endothelial cell apoptosis (Figure 2) (16, 17). Early animal model studies showed that blocking the protein C pathway in septic baboons exacerbated the inflammatory responses (18); by contrast, infusion of APC ameliorated the systemic inflammatory responses induced by Escherichia coli (19). Subsequent studies have suggested that the APC system protectively modulates a variety of disease processes, including reperfusion-induced coronary injury (20), diabetic nephropathy (21) and stroke (22). These effects are believed to be attributed to the cytoprotective properties of the APC system and to be independent of its anticoagulant effect. Clinical studies have reported conflicting results on the efficacy of recombinant human APC in reducing mortality in patients with septic shock, although a recent meta-analysis using data from the past 10 years showed that its use was associated with significant reductions in hospital mortality (23). Similar to APC, TM has also been shown to exhibit anti-inflammatory activities via both APC-dependent and APC-independent mechanisms. Recombinant TM attenuated atherosclerosis in a mouse model by inhibiting thrombin-induced endothelial cell activation (24).

Factor Xa and thrombin – activities beyond coagulation

The available data suggest that coagulation proteases (such as factor Xa and thrombin) are involved in non-haemostatic cellular...
activities, including inflammation. The bidirectional interaction of coagulation and inflammation is well recognised and the PARs form the molecular connection between coagulation and inflammation (4, 25). Four PARs (PAR-1–4) have been identified in humans and have been found to be expressed on the membranes of platelets and several cell types, including endothelial cells, leukocytes and smooth muscle cells (26). PAR-1, -3 and -4 are thrombin receptors that are activated rapidly by thrombin, and PAR-1 can also be activated by the TF–factor VIIa complex and factor Xa. PAR-2 can be activated by factor Xa or the TF–factor VIIa complex, but not by thrombin (▶ Figure 3) (6, 25). However, thrombin-cleaved PAR-1 has been shown to donate its tethered ligand to transactivate PAR-2 and form heterodimers (27).

In addition to coagulation, factor Xa has been shown to be involved in a variety of pathophysiological conditions, including inflammation, vascular remodelling and tissue fibrosis (5, 28, 29). Factor Xa exhibits pro-inflammatory activity in vitro, shown by the induction of IL-6, IL-8 and monocyte chemotactic protein 1 expression in endothelial cells and leukocytes, thereby contributing to the inflammatory process (5, 28, 29). Most of these activities of factor Xa are mediated via PAR-1 and PAR-2; when factor Xa forms a complex with TF–factor VIIa, these signalling pathways become more efficient (5, 30).

Thrombin is a multifunctional molecule and its non-haemostatic activities have been well documented. PAR-1 is responsible for mediating most of the pro-inflammatory as well as the profibrotic effects of thrombin (6). Thrombin-induced pro-inflammatory activities include the upregulation of leukocyte adhesion molecules and P-selectin onto platelets and endothelial cells and the stimulation of pro-inflammatory cytokines (e.g. IL-6 and IL-8 by monocytes) and chemokine production (6). Thrombin also potentiates the effects of vascular endothelial growth factor; clot-bound thrombin, which is protected from inactivation by antithrombin, contributes to angiogenesis and tissue repair at the sites of injury. By stimulating the release of histamine and serotonin, thrombin contributes to the increased vascular permeability, oedema and swelling that are associated with inflammation (6). Furthermore, the APC–EPCR complex cleaves PAR-1, leading to generation of sphingosine 1 phosphate (S1P) and induction of S1P receptor-1 activation and signalling, which is believed to mediate, at least in part, the cytoprotective effect of APC (31, 32). Although inhibiting thrombin activity may potentially be anti-inflammatory, it is not known whether long-term suppression of the APC system (e.g. by thrombin inhibition) would have any adverse consequences.

Atherothrombosis and the role of factor Xa, thrombin and platelets

It is now well recognised that inflammation is an important contributor to the pathogenesis of coronary artery disease. Atherosclerosis is characterised by the formation of atherosclerotic plaques, with low-density lipoproteins and other atherogenic lipoproteins entering the intima of the arterial wall and initiating an inflammatory response. Dysregulation of endothelial cells forms the basis for the initial development of atherosclerosis (4). Increased expression of adhesion molecules and release of pro-inflammatory cytokines by activated endothelial cells promotes recruitment of blood cells. The inflammatory state is maintained by the recruitment of T cells and other cells that secrete the macrophage-activating cytokine interferon-γ. Sustained inflammation decreases plaque stability, which prompts plaque rupture, leading to the onset of acute coronary syndrome (ACS) (33, 34). The levels

![Figure 3: Schematic representation of coagulation factor-mediated activation of PARs and cell types in which each PAR is expressed. Four PARs (PAR-1–4) have been found to be expressed on the membranes of platelets and/or several cell types. Thrombin (factor IIIa) activates PAR-1, -3 and -4 (but not PAR-2). Factor Xa and TF–factor VIIa complex can each activate both PAR-1 and PAR-2, F, factor; PAR, proteinase-activated receptor; SMC, smooth muscle cell; TF, tissue factor.](https://www.thrombosis-online.com/content/111/4/628.html)
of C-reactive protein and IL-6, as well as other inflammatory markers, have been shown to be elevated in patients with unstable angina and myocardial infarction (MI) (35).

Many coagulation proteins are detectable in atherosclerotic plaques and may be involved in various processes, including inflammation, angiogenesis and cell proliferation (36, 37). Studies in animals have shown a correlation between hypercoagulability and increased atherosclerosis (36). Recent evidence suggests that TF and coagulation proteases, such as factor Xa and thrombin, have an important role in the progression of atherosclerotic plaques, in addition to participating in thrombus formation after plaque rupture (Figure 4) (37). The activities of TF, factor Xa, thrombin and factor XIIa were found to be significantly higher in early atherosclerotic lesions than in stable advanced lesions, indicating an enhanced procoagulant state in the early stage of plaque development (37). TF–factor VIIa-induced signalling via PAR-2 is believed to be involved in several proatherogenic processes, including inflammation, monocyte chemotaxis, vascular remodelling and angiogenesis. Factor Xa contributes to atherosclerosis via the activation of PAR-1 and PAR-2 signalling, leading to the production of pro-inflammatory cytokines and the expression of cell adhesion molecules (4). In addition, factor Xa-mediated PAR activation or factor Xa-induced TF expression in vascular endothelial cells and smooth muscle cells can lead to abnormal cell proliferation and extracellular matrix accumulation (25). The contribution of thrombin to atherothrombosis includes not only fibrin formation and platelet activation, but also the induction of expression of pro-inflammatory cytokines and chemokines in monocytes and its proliferative effects on endothelial cells and vascular smooth muscle cells (4, 6). Platelets have a prominent role in atherothrombosis upon plaque rupture and also as pro-inflammatory mediators that are directly involved in the early development of atherosclerotic lesions. Upon adherence to a compromised vascular endothelial surface, platelets secrete pro-inflammatory mediators such as cytokines, chemokines, growth factors, adhesion molecules and coagulation factors. Binding of platelets to circulating monocytes and neutrophils further supports the activation, adhesion and transmigration of these cells, which contribute to plaque formation and progression (4). Collectively, the persistent inflammatory environment within the arterial wall, supported in part by coagulation-mediated activities, may maintain local thrombin generation, contributing to thrombus formation within the plaques and ultimately leading to plaque instability and rupture and the onset of ACS.

**Effects of direct factor Xa and thrombin inhibition beyond anticoagulation**

Factor Xa and thrombin are indispensable components of the coagulation cascade and targeted inhibition of factor Xa or thrombin has proven to be an effective means of anticoagulation therapy (3). Inhibition of factor Xa or thrombin has effects other than anticoagulation. It was demonstrated that factor Xa inhibition could...
block some of the pro-inflammatory processes, such as the expression of pro-inflammatory cytokines (38) and limit restenosis after balloon angioplasty (39). More recent studies showed that rivaroxaban – an oral, direct factor Xa inhibitor – concentration-dependently inhibited the procoagulant activity of activated monocytes and macrophages and the secretion of inflammatory chemokines by activated monocytes and THP-1 cells, possibly via blocking the generation of thrombin and consequently, thrombin-induced PAR-1 signalling (40). In primary human umbilical endothelial cells, rivaroxaban prevented thrombin generation, leading to the downregulation of thrombin-mediated pro-inflammatory cytokine expression to the same extent as PAR-1 antagonist and the direct thrombin inhibitor dabigatran (7). Moreover, data from a study in apolipoprotein E-deficient mice with established atherosclerotic lesions suggest that treatment with rivaroxaban may increase the stability of atherosclerotic plaques in this model (8). In the same study, rivaroxaban reduced the expression of pro-inflammatory mediators in thoracic aortas, including IL-6, TNF-α and monocyte chemotactic protein 1 (8). Further evidence has emerged in a recent study that examined biologically relevant plasma proteins in Japanese patients with non-valvular chronic atrial fibrillation (AF). Compared with warfarin, treatment with rivaroxaban for 24 weeks was associated with a significant increase in TM levels and a trend towards a reduction in matrix metalloproteinase-9 levels (41). The precise mechanism for the observed increase in TM levels is not clear, but because inflammation downregulates TM, it is possible that the potential anti-inflammatory property of rivotaxaban may have indirectly contributed to the increase in TM levels. The increase in TM levels could be an advantage because TM has also been shown to exhibit anti-inflammatory activities via both APC-dependent and APC-independent mechanisms (42). A reduced TM expression has been shown on endothelium overlying atherosclerotic plaques in coronary arteries in patients (43), and studies in animal models indicate that TM downregulation correlates with increases in thrombotic complications (44).

Melagatran – an direct thrombin inhibitor – was shown to reduce the progression of atherosclerosis in apolipoprotein E-knockout mice and to promote plaque stability by inhibiting pro-inflammatory transcription factors and the synthesis of matrix metalloproteinases (45). A more recent study with dabigatran has also found that thrombin inhibition impaired the formation and size of atherosclerotic plaques in an apolipoprotein E-deficient mouse model, in addition to preventing disease progression and associated stenosis (9, 46). Although preclinical data seem to indicate a direct protective effect of factor Xa or thrombin inhibition on endothelial function and the progression of atherosclerosis, clear clinical evidence is still lacking.

### Cardiovascular profiles of oral, direct factor Xa and direct thrombin inhibitors in clinical studies

Rivaroxaban, apixaban (another direct factor Xa inhibitor) and dabigatran have all demonstrated potential for the management of thromboembolic disorders. Data from phase III studies suggest that rivaroxaban may have a favourable cardiovascular profile. In a phase III study assessing rivaroxaban for the prevention of stroke in patients with AF, compared with dose-adjusted warfarin, a numerically lower incidence of MI was observed in patients treated with rivaroxaban (47). Dabigatran has been reported to be associated with a higher risk of MI or acute coronary events compared with warfarin in some of the phase III studies, such as those investigating stroke prevention in patients with AF (48, 49) and treatment of venous thromboembolism (50). A meta-analysis of seven trials of dabigatran showed that dabigatran was associated with a significantly higher risk of MI or acute coronary events when tested against different controls, including warfarin and enoxaparin (51). However, further analysis of data from the RE-LY study of

![Table 1: Cardiovascular profiles of rivaroxaban, apixaban and dabigatran in phase III clinical studies of stroke prevention in patients with atrial fibrillation and of treatment of venous thromboembolism.](image)

<table>
<thead>
<tr>
<th>Stroke prevention in patients with AF</th>
<th>Treatment of VTE</th>
</tr>
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<tbody>
<tr>
<td><strong>ROCKET AF (47)</strong>&lt;br&gt;Incidence of MI</td>
<td>Rivaroxaban 0.9%&lt;br&gt;Warfarin 1.1%&lt;br&gt;</td>
</tr>
<tr>
<td><strong>RE-LY</strong>&lt;br&gt;(first publication) (62)&lt;br&gt;Incidence of MI (Further analysis) (52)</td>
<td>Dabigatran 110 mg 0.72%&lt;br&gt;150 mg 0.74%&lt;br&gt;Warfarin 0.53%&lt;br&gt;</td>
</tr>
<tr>
<td><strong>ARISTOTLE</strong>&lt;br&gt;Incidence of MI (65)</td>
<td>Apixaban 0.53%&lt;br&gt;Warfarin 0.61%&lt;br&gt;</td>
</tr>
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*SOC, standard of care = subcutaneous enoxaparin followed by either warfarin or acenocoumarol. ACS, acute coronary syndrome; AF, atrial fibrillation; MI, myocardial infarction; PE, pulmonary embolism.*
dabigatran showed that the increase in the occurrence of MI with both doses of dabigatran did not reach statistical significance (52). The findings of a more recent publication of real-world data from a prospective nationwide cohort study from a Danish registry showed that the incidence of MI and mortality were lower in patients receiving dabigatran than those receiving warfarin (53). The incidences of MI or acute coronary events in some of the phase III studies of rivaroxaban, dabigatran and apixaban are shown in Table 1.

Both platelet aggregation and activation of coagulation contribute to arterial thrombosis in ACS, which provides the rationale for adding antiplatelet therapy to antithrombotic therapy to reduce further the risk of cardiovascular events (4). A number of studies have assessed oral anticoagulant therapy in addition to antithrombotic therapy and have shown an improvement in cardiovascular outcomes; however, this improvement was accompanied by an increase in major bleeding (54). Rivaroxaban, apixaban and dabigatran have also been investigated in clinical studies for secondary prevention in patients with ACS (55–57). In the phase III ATLAS ACS 2 TIMI 51 study, co-administration of low doses of rivaroxaban (2.5 mg or 5.0 mg twice daily) with standard antithrombotic therapy (acylsalicylic acid alone or acetylsalicylic acid plus clopidogrel or ticlopidine) significantly reduced the relative risk of the primary efficacy endpoint (cardiovascular death, MI or stroke) compared with antithrombotic therapy alone. Moreover, the lower dose of rivaroxaban (2.5 mg) co-administered with antithrombotic therapy demonstrated a significant relative risk reduction (34%) in cardiovascular mortality. The efficacy benefit was achieved without a significant increase in fatal bleeding, although rates of major bleeding and intracranial hemorrhage were higher in the rivaroxaban group (55). By contrast, studies investigating apixaban (phase III, APPRAISE-2) and dabigatran (phase II, RE-DEEM) in addition to antithrombotic therapy in patients with ACS have shown increases in major bleeding events but without a clear efficacy benefit (56, 57). Although there is a clear rationale for anticoagulant therapy plus antithrombotic therapy in patients with ACS based on the roles of coagulation proteins and platelets, this has not proven to be a translational success in the clinical studies with apixaban and dabigatran. The doses used in these studies may be one of the contributing factors for the observed outcomes.

**Discussion and conclusions**

Factor Xa and thrombin are viable targets for anticoagulation therapy, as confirmed by data from phase III clinical studies investigating direct factor Xa or direct thrombin inhibitors (3). In addition, both factor Xa and thrombin are also involved in other physiological and pathophysiological cellular processes. A growing evidence base challenges the traditional view that factor Xa has limited activities outside coagulation compared with thrombin. Similar to thrombin, factor Xa is involved in other cellular activities including inflammation, cell proliferation and tissue remodelling. Given the role of pro-inflammatory activities in the development of atherothrombosis (e.g. destabilisation of atherosclerotic plaques), the anti-inflammatory effect of direct factor Xa inhibition (e.g. as shown with rivaroxaban) may be of particular benefit in arterial thrombosis.

It has been argued that factor Xa may be a preferred target to thrombin, because of its upstream position in the coagulation cascade. Inhibition of factor Xa prevents thrombin generation, but at the same time it may allow the functions of existing thrombin to continue, such as activating protein C. Preliminary data from *in vitro* studies showed that factor Xa inhibition with rivaroxaban did not have a direct effect on the APC system in contrast with the direct thrombin inhibitor melagatran (58). Similar findings were reported in a recent study comparing melagatran with another direct factor Xa inhibitor, edoxaban (59). This is further supported by the recent clinical study showing that compared with warfarin, treatment with rivaroxaban for 24 weeks increased TM levels in patients with AF (41). Whether long-term administration of direct thrombin inhibitors would have a negative impact on the TM–APC system is currently unknown. Similarly, there is no clear explanation at present for the observed apparent difference in cardiovascular profiles of rivaroxaban and dabigatran in some of the phase III clinical studies. One possible mechanism could be that, upon rupture of plaques, high levels of thrombin are generated locally and therapeutic doses of direct thrombin inhibitors might be insufficient to block the high procoagulant activity of these thrombin molecules and prevent rapid clot formation. Conversely, direct factor Xa inhibitors suppress thrombin generation, resulting in a lower level of local thrombin at the rupture site. In conclusion, great advances have been made in understanding the coagulation system and its interactions with other physiological and pathophysiological processes, but much remains to be elucidated, such as the long-term impact of direct factor Xa and thrombin inhibition beyond the haemostatic system.

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**Conflicts of interest**

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