Factor XI as target for antithrombotic therapy

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Chapter 2

Anticoagulation beyond direct thrombin and factor Xa inhibitors: indications for targeting the intrinsic pathway?

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J.C.M. Meijers
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2013
Chapter 2

Abstract

Antithrombotic drugs like vitamin K antagonists and heparin have been the gold standard for the treatment and prevention of thromboembolic disease for many years. Unfortunately, there are several disadvantages of these antithrombotic drugs: they are accompanied by serious bleeding problems, it is necessary to monitor the therapeutic window and there are various interactions with food and other drugs. This has led to the development of new oral anticoagulants, specifically inhibiting either thrombin or factor Xa. In terms of effectiveness, these drugs are comparable to the currently available anticoagulants, however they are still associated with issues such as bleeding, reversal of the drug and complicated laboratory monitoring. Vitamin K antagonists, heparin, direct thrombin and factor Xa inhibitors have in common that they target key proteins of the hemostatic system. In an attempt to overcome these difficulties we investigated whether the intrinsic coagulation factors (VIII, IX, XI, XII, prekallikrein and high-molecular weight kininogen) are superior targets for anticoagulation. We analyzed epidemiological data concerning thrombosis and bleeding in patients deficient in one of the intrinsic pathway proteins. Furthermore, we discuss several thrombotic models in intrinsic coagulation factor-deficient animals. The combined results suggest that intrinsic coagulation factors could be suitable targets for anticoagulant drugs.
Introduction

In the early 1920’s, an unexplained outbreak of cattle disease emerged in the United States and Canada. These animals suffered from a bleeding disorder, varying from bleeding to death after a small invasive procedure to spontaneous lethal bleeding. It took over twenty years before the responsible agent was discovered and characterized, and in 1948 warfarin, a vitamin K-antagonist, was registered as a rodenticide. In the early 1950’s, clinical studies began with warfarin as a therapeutic agent for the treatment of thrombosis. To date, vitamin K-antagonists are still the gold standard for the treatment of venous thrombosis and the prevention of stroke in patients with non-valvular atrial fibrillation(1). The reason for this success is because of the strong efficacy of these drugs in the prevention and treatment of venous and arterial thrombosis. Unfortunately, this effectiveness comes with a downside; warfarin has many side effects of which bleeding is the most pronounced(2). On a yearly basis, an estimated 0.5% of patients on vitamin K-antagonists suffer from major bleeding for which medical treatment is necessary and even 0.25% of the patients die because of bleeding(3). Therefore, the search for new anticoagulants is ongoing; the ideal drug has a similar or superior effectiveness as compared to warfarin, but with reduced and preferably without the bleeding side effects. Recently, several new anticoagulants have been registered for the prevention and treatment of thrombo-embolic disease(4). Both thrombin (dabigatran) and factor Xa inhibitors (rivaroxaban, apixaban, edoxaban) have been extensively tested in several large randomized trials (5-9). Despite a similar or even superior effectiveness compared to vitamin K-antagonists, these drugs demonstrate different limitations. First, bleeding is still an important problem in these studies with no major improvement in safety(10). Second, there is no routine, widely available diagnostic test, which can safely monitor the therapeutic window of all novel anticoagulants (11;12). Finally, there is no reversal agent available for all these anticoagulants, which is particularly undesirable in trauma patients(13). Therefore the search for new anticoagulants will continue. This review investigates whether there is a role for inhibiting the proteins of the intrinsic pathway of coagulation in achieving this goal.

Characteristics of an ideal anticoagulant drug

In 2005, Hirsh et al. proposed the characteristics of an ideal anticoagulant, what conditions it must meet and what should be improved with respect to the current drugs(14). Of these, the most important is a high efficacy-to-safety index, meaning effective in the prevention of thrombo-embolic disease without potentially life threatening side effects. Another important characteristic is a predictable dose response of the therapeutic agent, which would enable dosing without the need for laboratory control. Other issues are administration (either parenteral and/or oral), a rapid onset of action, the availability...
of a safe antidote, freedom from non-anticoagulant side-effects and minimal interaction with other drugs.

Proteins of the intrinsic pathway and clot formation
Coagulation occurs when the plasma protease activated factor VII comes into contact, and subsequently forms a complex, with tissue factor (TF). The TF/factor VIIa-complex can activate factor X and activated factor X can convert prothrombin into thrombin. Thrombin, a key contributor in coagulation, in turn converts fibrinogen into fibrin. In addition to this direct factor Xa generation, the TF/factor VIIa-complex can also indirectly activate factor X. The indirect route of factor X activation goes via the activation of factor IX. Factor IXa in the presence of cofactor factor VIIIa can activate factor X, thereby forming an amplification loop. For this sequence of events, TF must come into contact with blood, for instance upon injury or inflammation. Additionally, a TF independent pathway has evolved in vertebrates. Coagulation factor XII can be activated on charged surfaces (for instance polyphosphate, RNA) by a process called contact activation (Figure 1). Following autoactivation, factor XIIa can activate factor XI, eventually leading to the formation of thrombin, via factor IX, with its cofactor factor VIIIa, and factor X as described above. Deficiency in one of the factors involved in this pathway results in a variety of bleeding disorders. For example Hemophilia A (factor VIII) or Hemophilia B (factor IX) result in severe and often spontaneous bleeding. In contrast to factor VIII or factor IX deficiencies congenital factor XI deficiency, also known as Hemophilia C, typically causes only mild and injury-induced bleeding, where factor XII deficient patients do not have a bleeding tendency at all. These observations suggest that contact activation is not essential for normal haemostasis in vivo.

Two other proteins are important to mention regarding contact activation, namely high molecular weight kininogen (HK) and prekallikrein (PK). Patients deficient in either protein do not exhibit a bleeding phenotype, despite a prolonged aPTT clotting time. HK forms a noncovalent complex with factor XI, which is necessary for the binding of factor XI to negatively charged surfaces and for its activation to factor Xla by factor XIIa; HK serves as a nonenzymatic cofactor in this reaction. Prekallikrein also circulates in complex with HK and is the precursor of kallikrein (Kal), a serine protease that can liberate kinins, but can also cleave factor XII to generate additional factor XIIa.

Inhibitors of factor VIII
The first factor VIII null mice were developed in 1995(15). Interestingly, these mice appear to have a mild phenotype compared to their human counterparts(15) and especially the lack of spontaneous bleeding and joint bleeding is worth mentioning. Apparently, factor VIII is more important for normal hemostasis in man than in mice. A study by Singh et al. also points to this direction and shows the antithrombotic effect of a human monoclonal
antibody that inhibits factor VIII in a murine vena cava thrombosis model (16) (Table 1). This antibody successfully inhibited thrombosis without causing an overt bleeding tendency. The antibody was modified in order to achieve only partial factor VIII inhibition and tested in baboons, both in venous and arterial thrombosis, with similar results(17). These results were the set up for several clinical trials, in one of which the antibody (TB-402) reduced the rate of venous thromboembolism (VTE) in patients undergoing total knee replacement when compared to enoxaparin, with a similar bleeding incidence(18). A phase 2b study with TB-402 was completed in August 2012, but the results have not been published yet. Furthermore, several dosing, safety and reversal studies have been performed investigating the safety and efficacy of this drug(19;20). Several small molecule inhibitors directed against the C2 domain of factor VIII have been developed, however, no in vivo studies have been performed(21).

**Fig.1** The contact system is a direct link between inflammation and coagulation. Activation of factor XII induces factor XI activation and this will ultimately lead to thrombin formation. Factor XII can be activated via negatively charged substances like polyphosphates, RNA and neutrophil extracellular traps (NETs). Activated factor XII can also cleave plasma prekallikrein (PK) into kallikrein (Kal), which on its turn releases several vasoactive substances like bradykinin. At the same time, plasma kallikrein produces additional factor XIIa. Prekallikrein and factor XI are both bound to high molecular weight kininogen (HK) in plasma. Indicated alongside of the coagulation factors are the various types of inhibitor (antisense oligonucleotides, antibodies, small molecule inhibitors and naturally occurring inhibitors) as described in this review.
Table 1 Inhibitors of the intrinsic pathway and their respective stages of development

<table>
<thead>
<tr>
<th>Targeted coagulation factor</th>
<th>Pre-clinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Comments</th>
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<tr>
<td>Factor VIII</td>
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<td>C-type lectin like protein (Crataeva tapia) [24]</td>
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<td>Small molecule inhibitors [22]</td>
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<td>Factor IX</td>
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<td>Factor XIi</td>
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<td>Phase 3 planned for 2013</td>
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<td>Safety trials are planned</td>
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<td>Factor XII antisense</td>
<td>62</td>
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<td>Not specific for factor XIIa</td>
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<tr>
<td>Pro-Phe-Arg-chloromethylketone</td>
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<td>Not specific for factor XIIa</td>
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<td>rHA-infestin-4</td>
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<td>Ir-CPI</td>
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<td>Not specific for factor XIIa</td>
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<td>High molecular weight kininogen</td>
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<td>Produces a prothrombotic state, not specific for prekallikrein.</td>
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<td>No specific inhibitors</td>
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<td>Prekallikrein antisense</td>
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<td>Produces a prothrombotic state, not specific for prekallikrein.</td>
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<tr>
<td>Pro-Phe-Arg-chloromethyl ketone</td>
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<td></td>
<td>Produces a prothrombotic state, not specific for prekallikrein.</td>
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<tr>
<td>Soy bean trypsin inhibitor</td>
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<td>Produces a prothrombotic state, not specific for prekallikrein.</td>
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<td>PSK1570</td>
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Inhibitors of factor IX

Factor IX knockout mice were first described in 1997(22) and these mice demonstrated an almost identical phenotype as severe hemophilia B patients(22). In 2011, a study was published regarding the inhibitory effects of a C-type lectin-like protein derived from a plant (Crataeva tapia)(23). C-type lectin-like proteins belong to a family of calcium-dependent non-enzymatic proteins that can be found in the venom of various animals. They are functionally linked to several biological processes, such as adhesion, endocytosis and pathogen neutralization. A number of C-type lectin-like proteins are involved in haemostasis by inhibiting or activating specific platelet membrane receptors or blood coagulation factors, especially factors IX and X (24). In the presence of calcium ions, these proteins bind to the γ-carboxyglutamic acid (Gla) domain of these coagulation factors, thereby inhibiting activity. This particular plant derived drug showed an anticoagulant effect against all proteins of the intrinsic pathway in vitro, but no (pre)clinical data has been obtained. Furthermore, there is a group of natural occurring factor IX binding proteins, derived from insects, bats and snakes, but few preclinical or clinical studies have been performed(25). Nitrophorin 2, derived from the saliva of the blood sucking insect rhodnius prolixus, proved to be a long lasting inhibitor of arterial thrombosis in rats, without overt bleeding(26). Another way of factor IX inhibition is by means of active site blocked antagonists. Incubation with glutamyl-glycyl-arginyl-chloromethylketone produces a protein without functional capacity, making it a competitive inhibitor (factor IXai). This protein has been extensively studied in different animal models for arterial thrombosis, revealing similar effectiveness as heparin without a marked effect on hemostasis(25). No clinical trials with factor IXai have been performed. Several monoclonal antibodies against the Gla domain of factor IX have been developed. Encouraging results from different animal models led to the clinical testing of one of them (SB 249417) in 2002(25). Despite the fact that no safety concerns were mentioned, no further actions have been undertaken. Two factor IX inhibitors have undergone clinical testing. The small molecule inhibitor TTP889 did not reduce the risk of venous thromboembolism (VTE) in patients undergoing hip fracture surgery in a phase 2 study(27). The aptamer based factor IXa inhibitor RB006 (pegnivacogin) and its active control agent RB007, were investigated in a phase 2b study in 800 patients with acute coronary syndrome (RADAR substudy)(28). This dose selection and safety study paved the way for active comparator phase 3 clinical trials, of which the first is planned in 2012 (website RegadoBiosciences: www.regadobio.com).
Incidence of venous and arterial thrombosis in patients deficient in factor VIII and factor IX

As mentioned before, patients with a factor VIII or factor IX deficiency suffer from a pronounced bleeding tendency. However, this does not mean that these patients are protected against venous and arterial thrombosis. For instance, a venous catheter in hemophiliacs is frequently complicated by catheter thrombosis, indicating that even a severe clotting deficiency can be reversed by a mechanical device stimulating the vascular endothelium (29;30). Furthermore, up to 10% of hemophilic patients undergoing major orthopedic surgery develop (subclinical) venous thrombosis (31). But even spontaneous venous thrombosis occurs in patients with hemophilia A and B. In 2006, Girolami et al. reviewed all reported cases, establishing thrombosis in 12 patients with hemophilia A and 15 patients with hemophilia B, diagnosed with deep vein thrombosis (10 distal, 8 pulmonary embolism) and superficial thrombosis (32). Most cases were provoked by treatment of patients with recombinant factor VIIa (rFVIIa) or surgery in combination with prothrombin complex concentrates (PCC). These observations indicate that even patients with a severe form of hemophilia can develop thrombosis. However, spontaneous, unprovoked venous thrombosis is extremely rare (32). Similar reviews were conducted regarding the incidence of myocardial infarction and other arterial occlusions in hemophilia A and B patients, also by Girolami et al. (33;34). 36 patients with myocardial infarction and 6 patients with cerebrovascular events were identified in 40 reports on 42 hemophilia A patients belonging to different families, varying from 7 to 79 years of age. A literature search going back 30 years on hemophilia B yielded 13 patients with myocardial infarction and 1 patient with a cerebral vascular event. Again, the majority of cases occurred after administration of factor VIII or IX concentrate, rFVIIa or PCC, but unprovoked events have been described. Furthermore, there is growing evidence that known risk factors for atherosclerosis and subsequent atherothrombosis, like hypertension, high cholesterol and smoking, also apply for hemophilia patients. Their extent of atherosclerosis, the presence of coronary artery disease and the lifetime prevalence of cardiovascular events in hemophiliacs are similar to the general population (35). These data suggest that the development of atherosclerosis is not influenced by factor VIII or factor IX deficiency. This means that these risk factors have to be treated in a similar fashion as for the non-hemophilia population (36). Epidemiological studies have revealed elevated factor VIII levels as a risk factor for thrombosis, suggesting a role for factor VIII in the thrombotic process (37). Since the bleeding tendency in hemophilia B patients is strongly correlated with factor IX levels (38), this raised the hypothesis that partial inhibition of factor IX could be a sensible way of reducing the risk of bleeding. This hypothesis was strengthened by the reduced incidence of ischemic heart disease in female carriers of hemophilia B, who have a factor IX activity of 50% of normal, without a severe bleeding tendency (39;40).
Advantages and disadvantages of inhibiting factor VIII or factor IX

Since spontaneous thrombosis is extremely rare in hemophilia A and B patients, factor VIII and factor IX appear to be attractive targets for anticoagulation. However, the obvious bleeding tendency in these patients is a reason of concern. As acknowledged by the developers of the factor VIII inhibiting antibody (TB-402), partial inhibition of the particular coagulation protein will be essential(20). Complete inhibition of either factor VIII or factor IX will induce medical hemophilia, with all its consequences and the exact target of inhibition has to be established in future research. The degree of inhibition needs to be established, before the efficacy and safety can be investigated in VTE treatment trials. Furthermore, it is important to emphasize that animal data cannot be translated to the human situation. For instance, the small molecule IX inhibitor TTP889 revealed a thrombo-protective effect in various animal models, but failed to reduce the risk of thrombosis in humans.

Inhibitors of factor XI

Factor XI knockout mice, developed in 1997 by Gailani et al, have a comparable phenotype as their wild type counterpart and a normal reproductive capacity(41). Despite a severely prolonged aPTT in the knockout strain, no increased bleeding time is present in these mice(41). Again, as with the factor VIII deficiency, this is in contrast with the human situation, in which severe factor XI deficiency is associated with a (mild) bleeding tendency, especially upon injury. Factor XI inhibition has been extensively studied in both arterial and venous thrombosis, in different animal models, with different kinds of factor XI inhibitors(42). Small molecule inhibitors, antibodies against factor XI and factor XI antisense oligonucleotides all show a protective effect in both arterial and venous thrombosis, without an increased risk of bleeding(42-45). As mentioned before, data derived from rodent studies cannot automatically be translated to humans, but the role of factor XI inhibition has also been studied in higher species. Tucker et al. used an anti-human factor XI monoclonal antibody to prevent vascular graft occlusion in a primate thrombosis model(46). A novel therapeutic agent, factor XI antisense oligonucleotides, successfully inhibited liver-derived synthesis of factor XI, without increasing the risk of bleeding in mice and cynomolgus monkeys(47;48). Similar studies are currently executed to ensure the safety of factor XI antisense oligonucleotides in humans.

Incidence of venous and arterial thrombosis in patients deficient in factor XI

Patients with a severe factor XI deficiency (factor XI <15%) have a significant lower incidence of ischemic stroke(49). Since stroke is a potential deadly disease and data were collected retrospectively, the factor XI deficient cohort might have missed some individuals who had a fatal stroke. In contrast, the incidence of myocardial infarction
(MI), matched for sex and age, is the same as in the general population (50). This was a retrospective study in which the expected incidence (Poisson distribution) was compared with the observed incidence. However, no information on (long term) outcome was provided, which could theoretically be different. For instance all 16 factor XI deficient patients with verified MI survived, while one might expect a higher mortality among acute MI patients. However, this study may have some bias, since factor XI levels are not routinely determined in patients suffering from myocardial infarction. It should be noted that known risk factors for atherosclerosis were present in most of these patients, confirming the need for treatment of atherosclerotic risk factors in patients with a congenital bleeding disorder. The reason for these discrepant results between ischemic stroke and myocardial infarction is not clear. In 2011, Salomon et al. studied the incidence of deep-vein thrombosis (DVT) in 219 unrelated patients with severe factor XI deficiency, aged 20 to 94 (51). No cases of DVT were observed in the factor XI-deficient cohort, which proved to be significantly less than could be expected from the normal population. This is a single observation, so no definite conclusions can be drawn yet, but it provides a good indication that the prevalence of venous thrombosis may be lower in factor XI deficient patients. The bleeding tendency of factor XI deficient patients is usually mild and injury-induced. Spontaneous bleedings are rare, with the exception of menorrhagia and post-partum haemorrhage in factor XI deficient women. Bleeding typically occurs after trauma or surgery, especially when surgery involves tissues with high fibrinolytic activity, such as oral and nasal cavities, tonsils and the urinary tract (52-54). However, the management of factor XI deficient patients is troublesome, because the bleeding tendency is unpredictable and not clearly associated with factor XI plasma levels (55). Patients with a severe factor XI deficiency (activity < 15 U/dL) should always be treated in case of surgery or trauma (56). Management of partial factor XI deficiency varies among centers and depends on the bleeding history and the presence of other hemostatic disorders. Treatment options are antifibrinolytic drugs like tranexamic acid, desmopressin, fresh frozen plasma, recombinant factor VIIa and factor XI concentrates (57). In general, a target factor XI level of 45 U/dl for 5 days after surgery is advisable in high risk patients (56). Furthermore, the infusion of factor XI concentrate (Hemoleven (Laboratoire français du Fractionnement et des Biotechnologies [LFB], Les Ulis, France) and factor XI concentrate (Bio Products Laboratory [BPL], Elstree, Hertfordshire, United Kingdom) are available in Europe) is related with various side effects, especially thrombosis. Nevertheless, levels of factor XI within the range of 10-50% (which may occur in heterozygous patients) hardly cause an enhanced bleeding tendency (58).
Advantages and disadvantages of inhibiting factor XI
Factor XI deficient patients have a less severe bleeding tendency when compared to hemophilia A and B patients. This indicates that factor XI might be a safer target for anticoagulation than factor VIII or factor IX. Epidemiological data from Israel showed that factor XI deficient patients have a lower incidence of venous thrombosis and stroke, which suggests that targeting factor XI will have thrombo-protective effects. However, the incidence of myocardial infarction in these patients was not significantly different from the normal population. This might indicate that factor XI inhibition is not effective in all vascular beds and suggests that other mechanisms than fibrin clot formation are involved in the process of MI. Again, one must be cautious when translating animal data to humans, as factor XI knockout mice have no bleeding tendency at all. In contrast, humans with less than 15% factor XI plasma levels have a mild bleeding disorder, especially upon injury. Thus, complete drug induced factor XI deficiency might cause bleeding problems in patients.

Inhibitors of factor XII
Since the factor XII deficient index patient John Hageman died of pulmonary embolism, there has not been much interest for factor XII as an antithrombotic target. Therefore, the available data on factor XII is much more limited when compared to, for instance, factor XI. In recent years, however, there is renewed interest for factor XII and its role in thrombosis. In part, this is due to unexpected observations in factor XII knockout mice. These mice were protected against artificially induced thrombosis, both arterial and venous, in combination with normal bleeding times. Like humans, these mice have significantly prolonged aPTT clotting times without an obvious bleeding tendency. This led to the development of several inhibitors of factor XII. Inhibition of factor XII proved thromboprotective in mouse models for chemically and mechanically induced arterial and venous thrombosis. Again, no bleeding was observed in any of these studies. So apparently, inhibition of factor XII is a safe and efficient way of thrombosis prevention without side effects, at least in mice.

Inhibitors of prekallikrein
Because prekallikrein is essential for the activation of factor XII, this protein is also taken in account in the study by Revenko et al. Inhibition of prekallikrein proved thromboprotective in mouse models for chemically and mechanically induced arterial and venous thrombosis, without bleeding. More recently, Bird et al. presented a study in which they obtained similar results with prekallikrein knockout mice. However, there are some contradictory results in literature since inhibition of plasma kallikrein with different inhibitors produced a prothrombotic state, which is a relevant observation for the further development of kallikrein inhibitors.
Inhibitors of high-molecular weight kininogen

In 2008, Merkulov et al. knocked out the murine kininogen gene 1, creating a mouse without high and low molecular weight knininogen(67). These mice displayed a delayed time to carotid artery occlusion and normal bleeding times. Furthermore, targeting HK reduced thrombus formation in ischemic vessels and improved cerebral blood in mice(68) and this was accompanied by a less severe inflammatory response.

Incidence of venous and arterial thrombosis in patients deficient in factor XII, high molecular weight kininogen and prekallikrein

Deficiencies of factor XII, HK or prekallikrein (the contact phase proteins) are not associated with a bleeding tendency, not even during extensive surgery(59;69). Apparently, these proteins are not necessary for clot formation during normal haemostasis. Paradoxically, these patients have an immense in vitro clotting defect, with prolonged to immeasurable aPTT clotting times(69). Factor XII is an important factor for the aPTT clotting time and this test requires factor XII autoactivation. However, the molecular basis for factor XII autoactivation in vivo is not known. Since factor XII, HK and prekallikrein deficiencies are rare disorders, large epidemiological studies concerning the incidence and prevalence of venous thrombosis are lacking. Publications on venous thrombosis in factor XII deficient patients are limited and are in almost every case related with a thrombotic risk factor like pregnancy, surgery or factor V Leiden, suggesting a negligible role for factor XII in thrombosis and haemostasis(70). Some argue that deficiencies of the contact phase proteins are a risk factor for venous thrombosis itself, but the available data is insufficient and non-conclusive (70-72). Girolami et al studied all the reported cases of thrombosis in patients with a contact system deficiency in 2011(59). They concluded that deficiencies of the contact phase do not protect against thrombosis. More epidemiological evidence was provided by Cushman et al. who studied coagulation factors IX through XIII and the risk for thrombosis(73). Also, there are data on the most common factor XII polymorphism (F12 –4C>T) and the risk for thrombosis. The results of these analyses provided only weak evidence for factor XII deficiency as risk factor for thrombosis. At best an odds ratio of 1.13 with 95% confidence intervals between 1.00 and 1.27 was found for the risk of myocardial infarction in factor XII deficient patients(74). Based on these observations, factor XII does not seem the most suitable candidate for targeting anticoagulation. Interestingly, high levels of factor XII even decreased the risk of myocardial infarction in a cohort from the Netherlands (75), contrary to elevated levels of factor VIII, IX and XI which increased this risk. In addition, low levels of factor XII-inhibitor or kallikrein-inhibitor complexes were associated with increased risk of both MI and stroke in middle-aged men(76). This would suggest that the effect of factor XII on risk is not by activation of factor XI, but by an alternative mechanism, which has not yet been elucidated. Furthermore, the role of
factor XII may be sex and age-specific, as young women (18 to 50 years) with high levels of factor XII do have an increased risk of stroke(77). Additional confusion concerning factor XII function was raised after the publication of a large Austrian study, investigating the relevance of factor XII plasma activity as predictor for all-cause mortality (78). In this study, hazard ratios for death continuously increased to almost 5-fold in patients with levels between 10% and 20% factor XII, when compared to individuals with factor XII levels above 100%. Intriguingly, in patients with severe factor XII deficiency (plasma levels <10%) mortality was comparable to the Austrian population. Thus, although factor XII has been implicated in mechanisms beyond coagulation, such as fibrinolysis, angiogenesis and inflammation(79), the exact biological function of factor XII is not clear. The question is whether the previously mentioned mice data can be translated to the human situation. As stated above, large epidemiological studies concerning deficiency of the proteins of the contact system are lacking, but based on what is available, the contact system plays a negligible role during normal hemostasis. The influence of the contact system in human thromboembolic disease has yet to be elucidated, but it appears that factor XII deficient patients do not have a thrombotic risk when compared to healthy individuals with normal factor XII levels(59;73;75;78). There are several indications that the human coagulation system is functionally different from the coagulation system of mice, and the contact system may well be one of these differences.

Advantages and disadvantages of inhibiting proteins from the contact system
Since a deficiency of factor XII, prekallikrein or HK is not associated with bleeding, targeting these proteins might be a safe alternative to the currently available antithrombotic drugs. However, there is no evidence in humans that deficiency of one of these proteins reduces the risk of either venous or arterial thrombosis. Factor XII null mice are protected from artificially induced thrombosis, but this cannot automatically be translated to humans. Although prekallikrein and HK knockout mice are protected against thrombosis, the underlying mechanism needs to be elucidated. It is very well possible that targeting factor XII, prekallikrein or HK will not be sufficiently effective to treat and prevent thrombosis, but more research is needed to confirm this. Furthermore, inhibition of the contact system will also influence the inflammatory system, which is a potentially dangerous side-effect.

Interaction between inflammation and coagulation during thrombosis
Recently, the relation between inflammation and thrombosis has received a lot of consideration. The role of inflammation in atherosclerotic plaque formation and subsequent arterial thrombosis is reasonably well established, but this does not apply for venous thrombosis. Attention has been drawn to the role of white blood cells in initiating
as well as propagating the coagulation cascade by a study by von Brühl et al. (80). Following exogenous stimuli, the vein wall has a large capacity to attract white blood cells(81). These cells contribute to a local pro-inflammatory environment, which results in platelet-monocyte complexes providing a template for activation of the coagulation cascade. Concomitantly, inflammatory cytokines also directly stimulate activation of the coagulation cascade(82). Thus, multiple, pro-inflammatory pathways are incriminated in enhancing coagulation. Neutrophils are the most abundant inflammatory cells and the first leukocytes mobilized towards invading bacteria, to phagocytose and eventually kill these bacteria in the extracellular space. To do so, neutrophils can form so-called NETs (neutrophil extracellular traps), webs of chromatin fibers in which bacteria are exposed to high concentrations of proteases (83). These NETs link inflammation with thrombosis through several distinct mechanisms, as recently discussed by Fuchs et al (84). NETs are proposed to be involved in initial thrombus formation by recruiting and assembling platelets, red blood cells and coagulation factors, subsequently they might also enable thrombus stability through interaction with fibrin and von Willebrand factor (vWF). Since NETs are negatively charged, they might serve as an initiator of coagulation via factor XII. Indeed von Brühl and colleagues found evidence that NETs can bind and activate factor XII (80). This interaction between the contact system and leukocyte function is not new. In the 1980s several articles were published in which factor XIIa and prekallikrein were identified as activators of neutrophils(85;86). At this time, NET formation by neutrophils was not yet discovered, but these observations might suggest that proteins of the contact system are involved in the inflammatory response via neutrophil activation. Furthermore, the surface of the human neutrophil membrane has been identified as circulating platform for components of the contact system(87). Taken together, there is substantial evidence that leukocytes and in particular neutrophils can initiate coagulation and this is most likely facilitated via the contact system. This new concept creates several possibilities for the development of novel therapeutic agents, for instance by an extracellular deoxyribonuclease (DNase), which dismantles NETs and thus can prevent the initiating coagulation steps by these NETs. Another option could be to inhibit monocyte recruiting chemokines (e.g. CCL2 or 5), to prevent the previously described monocyte induced coagulation activation. Coagulation and inflammation are also linked via the contact system. Once factor XII is activated it converts prekallikrein into its active form kallikrein and this releases the vasoactive proinflammatory peptide bradykinin. This peptide causes vasodilatation, thereby increasing the vascular permeability and induces contraction of smooth muscles. The major regulator of this series of events is plasma C1-esterase inhibitor, a member of the serpin family, which can inhibit serine proteases (88;89). Remarkably, the physiological activator of factor XII (and thus of the contact system) is not identified yet. Negatively charged surfaces
like kaolin or silica are used for in vitro assays, but these substances are not present in human plasma. A possible candidate might be polyphosphates, an inorganic substance consisting of anionic polymers of phosphates. Polyphosphates can be secreted from activated human platelets and this might explain the coagulation stimulating properties of platelets(90). Furthermore, polyphosphates can accelerate both factor V and factor XI activation, and can directly initiate the contact system. Long-chain polyphosphates, which can be secreted by several microorganisms, triggers the contact pathway in a factor XII-dependent manner. However, platelet size polyphosphate is not capable of directly activating factor XII and the contact system. The subsequent kallikrein activation has been shown to activate complement components C3 and C5, major participants of the immune system (90,91). These prohaemostatic and prothrombotic effects designate polyphosphates as an attractive new target for antithrombotic therapy.

Conclusion
The social and economic burden of thrombotic disorders is immense and demands appropriate treatment strategies. The current drugs target the backbone of coagulation, namely factors II, VII, IX and X, which are essential for clot formation. These drugs are undeniable good in terms of effectiveness, but the side effects are such that identification and characterization of new drugs is warranted. Based on epidemiology, pathophysiology and animal work, inhibition of the intrinsic pathway may fulfill the goal of anticoagulation without bleeding side effects. Future studies will demonstrate which coagulation protein will be the best target for anticoagulation, but it appears that the intrinsic pathway provides an attractive option to effective anticoagulation with significantly reduced or even without side effects.
References


Anticoagulation beyond direct thrombin and factor Xa inhibitors: indications for targeting the intrinsic pathway?


Renne T. The procoagulant and proinflammatory plasma contact system. Semin Immunopathol 2012;34:31-41.

