Factor XI as target for antithrombotic therapy
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Chapter 1

Introduction and outline of the thesis

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General introduction

Thrombosis is one of the most prevalent disorders in Western society with a high morbidity and mortality(1). Depending on the type of blood vessel in which the clot is formed, thrombosis is divided into arterial or venous thrombosis. Arterial thrombosis can lead to disorders like myocardial infarction or stroke and is usually associated with atherosclerosis(2). Risk factors for atherosclerosis are for instance smoking, high cholesterol levels and hypertension. Venous thrombosis is most often found in the lower legs and/or in the lung circulation and is associated with different risk factors, for example immobilisation, cancer or oral contraceptives(1). Despite differences between arterial and venous thrombosis, treatment with anticoagulant drugs is the cornerstone of thrombosis management(1;2). At present, several anticoagulant drugs are on the market, which target one or more coagulation factors that are involved in thrombus formation. These drugs are all very effective in the treatment and prevention of both arterial and venous thrombosis(3). However, they also share a common disadvantage as they profoundly increase the tendency to bleed(4). In fact, every year thousands of patients treated with anticoagulant drugs are admitted to the hospital because of spontaneous bleeding and some of them even die(4). This indicates that new safer anticoagulant drugs, which do not increase bleeding, are urgently needed for the treatment of thrombosis. In this thesis we explore the effectiveness and safety of several novel anticoagulant drugs, which inhibit coagulation factor XI. To explain why we have chosen coagulation factor XI as target for anticoagulation, some basic knowledge on coagulation is necessary.

Coagulation occurs when the plasma protease activated factor VII comes into contact, and subsequently forms a complex, with tissue factor (TF). For this, TF must come into contact with blood, for instance upon injury or inflammation. 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. Fibrin molecules then combine to form long fibrin threads that entangle platelets building up the blood clot. 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. A second amplification loop is formed by thrombin-mediated factor XI activation. Factor XI can be activated by thrombin which leads to enhanced thrombin formation via the activation of factor IX (figure 1).
Figure 1. Simplified schematic presentation of coagulation activation in vivo. Initiation of coagulation occurs when the plasma protease activated factor VIIa (FVIIa) comes into contact and forms a complex, with tissue factor (TF). The principal route of thrombin generation proceeds by the direct activation of factor (F)X by the TF–FVIIa complex. A first positive feedback loop consists of the activation of FIX by the TF–FVIIa complex followed by subsequent activation of FX by this activated FIX (in the presence of cofactor FVIIIa). A second amplification pathway is formed by thrombin-mediated activation of FXI. Activated FXI activates FIX. In the presence of cofactor FVa, activated FX converts prothrombin into thrombin, which in turn catalyzes the conversion of fibrinogen into fibrin.

Additionally, a TF-independent coagulation pathway has evolved in vertebrates. Coagulation factor XII can be activated on charged surfaces (for instance polyphosphate, RNA(5)) by a process called contact activation. Following auto-activation, 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 coagulation may result in bleeding disorders. For example, Hemophilia A (factor VIII deficiency) or Hemophilia B (factor IX deficiency) results in severe and often spontaneous bleeding(6). 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(7), where factor XII-deficient patients do not have a bleeding tendency at all(8). These observations suggest that contact activation is not essential for normal hemostasis in vivo. Two other proteins are involved in 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(9). HK forms a non-covalent complex with factor XI, which is necessary for the binding of factor XI to negatively charged surfaces and for its activation to factor XIa by factor XIIa;
HK serves as a non-enzymatic cofactor in this reaction(10). 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(8)(figure 2).

Figure 2. Activation of the contact system. Factor XII (FXII) can be activated by negatively charged surfaces such as polyphosphates (polyP) derived from activated platelets or neutrophil extracellular traps (NETs). Prekallikrein can be activated to kallikrein (Kal) by prolylcarboxylase (PRCP) on endothelial cells. Factor XIIa will also activate prekallikrein and thereby allows reciprocal activation resulting in the generation of additional factor XIIa. Factor XIIa can initiate coagulation via the activation of factor XI. Both prekallikrein and factor XI are in complex with their cofactor high molecular weight kininogen (HK).

So why choose factor XI as target for anticoagulation? Several lines of evidence suggest that inhibition of the intrinsic pathway, and in particular factor XI, might be an attractive target for anticoagulation to overcome the previously mentioned bleeding problems(9;11). Arguments in favor of this strategy are the lower incidence of ischemic stroke and deep vein thrombosis (DVT) in factor XI deficient patients when compared to the normal population(12;13). Furthermore, increased plasma factor XI levels have been associated with DVT(14), ischemic stroke(15) and myocardial infarction(16), indicating that factor XI contributes to pathological thrombus formation. In addition, factor XI deficient mice are protected against several forms of artificially-induced arterial and venous thrombosis(17). On the other hand, factor XI deficiency in humans is associated with a mild bleeding tendency and in some individuals even may be unnoticed. We therefore postulated that inhibition of factor XI may be an effective therapeutic approach for anticoagulation without a risk for severe bleeding.
**Thesis outline**

The focus of this thesis is on the various ways to inhibit factor XI and the influence of factor XI inhibition on both arterial and venous thrombosis. Chapter 2 reviews the literature on the association between the intrinsic coagulation factors and thrombosis. Available studies in both animals and humans are reviewed. Chapter 3 presents the results of an animal study evaluating the antithrombotic properties of two novel inhibiting factor XI antibodies. These antibodies were first extensively characterized in the laboratory, before they were tested in a murine thrombosis model. Chapter 4 is a continuation of this study using two other antibodies that inhibit factor XI. This study presents the first inhibiting factor XI antibody that is suitable to be administered to humans. In chapter 5, we investigated the antithrombotic potential of factor XI antisense oligonucleotides in an atherosclerotic mice model. Atherosclerotic plaques were acutely ruptured using ultrasound and ensuing thrombus formation was registered. The results of this study were the prelude to chapter 6, where the effect of factor XI inhibition on atherogenesis was studied. Finally, in chapter 7 we investigated the role of activated neutrophils and circulating nucleosomes on thrombus formation.
References