Factor XI as target for antithrombotic therapy
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Chapter 8

Summary and future perspectives

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Summary

The potential of coagulation factor XI as antithrombotic target is reasonably well established. However, many research questions still remain: what is the best manner to inhibit factor XI? Is factor XI as effective as the current anticoagulant drugs in the prevention of thrombosis? And is there a difference in effectiveness between factor XI inhibition in venous and arterial vascular beds? What is the role of factor XI during atherosclerosis? These questions were addressed in this thesis.

In chapter 1, we provide an overview of the current literature on the intrinsic pathway (factors VIII, IX, XI, XII, prekallikrein and high-molecular weight kininogen) and thrombosis. We analysed 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 as well as pharmaceutical inhibitors of the intrinsic pathway. The combined results suggest that intrinsic coagulation factors could be suitable targets for anticoagulant drugs and especially factor XI seems promising.

Two novel inhibitory anti-human factor XI antibodies were characterized and described in chapter 2. These antibodies (αFXI-175 and αFXI-203) were generated in mice after repeated injections of human factor XI. Using calibrated automated thrombography, we found that both antibodies inhibited thrombin generation initiated via the intrinsic pathway. However, upon tissue factor (TF)-initiated thrombin generation, αFXI-203 did not inhibit thrombin generation, while αFXI-175 only inhibited thrombin generation at low concentrations of TF. Next we decided to investigate the antibodies in a murine ferric chloride induced thrombosis model. The vena cava inferior remained patent for 25 minutes in mice treated with αFXI-175 and for 12.5 min in αFXI-203 treated animals, which was significantly longer than in placebo-treated animals (5 min, p<0.05). Neither antibody caused severe blood loss in a tail bleeding assay. Therefore we concluded that the two inhibitory antibodies against factor XI prevented cessation of blood flow in a murine ferric chloride induced thrombosis model without inducing a bleeding tendency. However, both antibodies were also compared with enoxaparin (a low-molecular weight heparin) in this particular model and enoxaparin proved to be more effective in preventing thrombosis. Therefore, we continued our search for inhibiting factor XI antibodies. The results of this search are presented in chapter 3, where we describe again two novel inhibitory factor XI antibodies (Ab 34.2 and Ab 15F8.3). In comparison with the antibodies described in chapter 2, Ab 34.2 and Ab 15F8.3 have a 10-fold higher affinity for factor XI (200-400 pM), which probably makes them more suitable. Furthermore, these antibodies proved to be as effective as enoxaparin in the prevention of thrombosis in the ferric chloride induced thrombosis model. Interestingly, Ab 34.2 interacted with the catalytic domain of factor XI
which could indicate that Ab 34.2 inhibits the active site of factor XI, since the active site of factor XI is located in the catalytic domain. However, additional studies are necessary to confirm this observation. Because Ab 34.2 appeared to be a high affinity inhibitory factor XI antibody, we decided to adapt this antibody in such a way that is was suitable for administration to humans. As mentioned before, all our antibodies are murine derived and administration to humans would trigger the immune system of the recipient. Using composite antibody technology we produced an antibody (Ab pro-01) based on the murine sequence without T-cell epitopes. The characteristics of this antibody were the same as for the murine counterpart and the antibody was just as effective in preventing thrombosis as Ab 34.2, Ab 15F8.3 and enoxaparin. Surprisingly, we found an enhanced bleeding tendency in mice treated with Ab pro-01, which is currently not understood. Additional experiments are required to elucidate this unexpected finding. Ab pro-01 is in theory suitable for human administration, but future research is necessary to confirm this.

The studies mentioned so far focused on the effect of factor XI inhibition on venous thrombosis. However, many thrombotic events occur in the arterial vascular bed after plaque rupture of diseased atherosclerotic vessels and the role of factor XI in this process has hardly been studied. To mimic acute atherosclerotic plaque rupture, we used a novel murine model in which plaque rupture was induced using ultrasound and the results of this study are described in chapter 4. Factor XI was inhibited by factor XI antisense oligonucleotides, a new therapeutic strategy which prevents protein transcription and protein translation on the mRNA level. Treatment with factor XI antisense oligonucleotides induces a 80 to 90% reduction in factor XI plasma levels. After plaque rupture, the subsequent thrombus formation was visualized and quantified by intravital microscopy and immunohistochemistry. Initial platelet adhesion and platelet plug formation were not impaired in animals treated with factor XI antisense oligonucleotides. However, the ensuing thrombus formation and fibrin deposition were significantly lower after 5 to 10 minutes (P<0.05) in factor XI antisense oligonucleotide-treated animals without inducing a bleeding tendency. Furthermore, thrombi from antisense-treated animals were less stable than thrombi from nonsense oligonucleotides-treated animals (control). Therefore we concluded that factor XI antisense oligonucleotides safely prevent thrombus formation on acutely ruptured atherosclerotic plaques in mice. However, the long term effects of factor XI inhibition on atherosclerosis are not known and this was studied in chapter 5. Apolipoprotein E (apoe) knock-out mice fed on a Western-type diet were treated either with factor XI antisense oligonucleotides, dabigatran (a thrombin inhibitor) or nonsense oligonucleotides (placebo control). After 12 weeks the animals were sacrificed and the level of atherosclerosis was determined. Atherosclerotic lesions in animals treated with either dabigatran or factor XI antisense oligonucleotides were
significantly reduced when compared to placebo treated animals. Furthermore, treatment with dabigatran was accompanied by an anti-inflammatory phenotype and more stable plaques. The mechanism behind factor XI inhibition and reduced atherosclerosis still needs to be elucidated. Furthermore, we performed arterial and venous thrombosis models in these mice, to investigate the importance of factor XI during thrombosis in mice with an atherosclerotic phenotype. Inhibition of factor XI with factor XI antisense oligonucleotides proved to be effective in preventing thrombosis both in ferric chloride induced arterial thrombosis as well as in venous thrombosis, which was induced using vena cava ligation. The combined data suggest that there is no reason to doubt the effectiveness of factor XI inhibition in patients with atherosclerosis and related diseases.

This thesis focuses on factor XI as target for anticoagulant therapy. One could also argue that other proteins of the intrinsic pathway are suitable targets for anticoagulation. Especially factor XII has regained new interest the last decade. Intriguingly, the physiological activator(s) of the intrinsic pathway and the relevance of the intrinsic pathway in vivo is largely unknown. To identify these activators of the intrinsic pathway, we performed a case control study in patients with a suspicion of deep-vein thrombosis. We found that these patients had higher levels of circulating nucleosomes and activated neutrophils in their plasma, which is presented in chapter 6. This is in line with the observation that nucleosomes released by activated neutrophils and apoptotic cells can trigger the contact system thereby inducing thrombosis. This study suggests an association among circulating nucleosomes, activated neutrophils, and presence of DVT in humans, which might have implications for treatment and prevention of thrombosis.
Perspectives

With the introduction of the direct oral anticoagulant drugs (DOAC’s) a new era in the treatment of thrombosis has started. The DOAC’s are as effective as the traditional anticoagulant drugs, vitamin K antagonists and heparin, but with a superior safety profile. In spite of a reduced number of (major) bleedings in patients treated with a DOAC, bleeding problems are still present as a side-effect. This is reason for concern and indicates that the ideal anticoagulant has not been developed yet. In my opinion, any pharmaceutical that blocks tissue factor-induced coagulation pathways will be accompanied by bleeding. Therefore, to prevent bleeding it is essential to preserve this tissue factor pathway, a goal which can be accomplished by inhibiting the intrinsic pathway of coagulation. The main question is whether inhibition of factor XI will be as effective as the vitamin K antagonists, heparins or DOAC’s. All the evidence for factor XI as antithrombotic target has been obtained in rodents and primates. We know that the coagulation system of mice and rats is substantially different from the human coagulation system, especially when it comes to the intrinsic pathway. Therefore, the results from rodent studies are difficult to translate to the human situation. For example, two inhibitors of factor IX worked perfectly in mice, but failed in humans.

At the time of writing, the first phase 1 trials with factor XI antisense oligonucleotides in humans have been performed with excellent results. The drug produced dose-dependent statistically significant reductions of greater than 80 percent in factor XI protein levels. Furthermore, subjects tolerated the antisense oligonucleotides well with no increase in bleeding. A phase 2 study was initiated in 2012, evaluating factor XI antisense oligonucleotides in patients undergoing knee replacement surgery. This study will compare the safety and activity of factor XI antisense oligonucleotides to enoxaparin, a low-molecular weight heparin, in preventing thrombosis in these patients. This trial will be a key study for the future of factor XI as antithrombotic target.

Irrespective of the results of this trial, inhibitors of factor XI are potentially useful as additional therapy in patients with myocardial infarction or stroke. Not so much as a primary treatment, but as a preventive measure. The same applies for the extended treatment of patients with deep-vein thrombosis and/or pulmonary embolism. Another indication may be catheter thrombosis, as catheter thrombosis is strongly driven by contact activation and inhibition of factor XI might prevent this complication. Future studies on the effectiveness and safety of factor XI inhibitors will furnish the landscape of thrombosis treatment in the next decade.