Factor XI as target for antithrombotic therapy
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Chapter 3

Two novel inhibitory anti-human factor XI antibodies prevent cessation of blood flow in a murine venous thrombosis model

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Abstract

**Background:** Coagulation factor XI is a promising target for anticoagulation, because of its major role in thrombosis and relatively minor role in hemostasis. This implies that inhibition of factor XI can prevent thrombosis without causing bleeding.

**Aim:** To investigate the antithrombotic properties of two novel inhibitory anti-human factor XI antibodies (αFXI-175 and αFXI-203).

**Methods:** The *in vitro* properties of both antibodies were analyzed using standard clotting assays and calibrated automated thrombography. For the *in vivo* model we used factor XI knock out mice, in which factor XI plasma levels were restored with purified human factor XI. Thrombosis was induced by applying ferric chloride to the vena cava inferior, after which time to occlusion was analyzed. A tail bleeding assay was used to investigate the safety of both antibodies.

**Results:** Using calibrated automated thrombography, both antibodies inhibited thrombin generation initiated via the intrinsic pathway. In contrast, upon tissue factor (TF)-initiated thrombin generation, αFXI-203 did not inhibit thrombin generation, while αFXI-175 inhibited thrombin generation only at low concentrations of TF. In the murine thrombosis model, the vena cava inferior remained patent for 25 minutes in mice treated with αFXI-175 and for 12.5 minutes in αFXI-203 treated animals, which was significantly longer than in placebo treated animals (5 minutes, p<0.05). Neither antibody caused severe blood loss in a tail bleeding assay.

**Conclusions:** The two inhibitory antibodies against factor XI prevented cessation of blood flow in a murine thrombosis model without inducing a bleeding tendency.
Introduction

Currently available anticoagulant medication, such as vitamin K antagonists (VKA), low-molecular-weight heparin (LMWH) and small molecule factor Xa or thrombin inhibitors all target the extrinsic or common pathways of coagulation(1,2). These drugs are all associated with an increased risk for severe bleeding even in spite of careful dose monitoring(3). Several lines of evidence suggest that inhibition of the intrinsic pathway, and in particular factor XI, might be an attractive target of anticoagulation to overcome these difficulties(4,5). 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(6,7). Furthermore, increased plasma factor XI levels have been associated with DVT, ischemic stroke and myocardial infarction, indicating that factor XI contributes to pathological thrombus formation(8-10). In addition, factor XI deficient mice are protected against several forms of artificially induced arterial and venous thrombosis(11-14). On the other hand factor XI deficiency in humans is associated with a mild bleeding tendency and in some individuals even may be unnoticed(15). One may therefore postulate that inhibition of factor XI may be an effective therapeutic approach for anticoagulation without a risk for severe bleeding.

Factor XI plays a minor role during normal hemostasis; normal clot formation occurs via the tissue factor (TF)-factor VIIa complex, which can activate factor X and factor IX leading to prothrombin activation and ultimately producing a fibrin clot at the site of injury(1). Factor XI can be activated by thrombin creating a positive feedback loop which forms additional thrombin and thereby stabilizes the clot via the activation of thrombin-activatable fibrinolysis inhibitor, which protects the clot against fibrinolysis(16,17). Furthermore, factor XI can also be activated by factor XII during the intrinsic or contact pathway of coagulation(5,18).

The most common indication for anticoagulation is atrial fibrillation (AF), since VKA and factor Xa or thrombin inhibitors can significantly reduce the incidence of ischemic stroke in these patients(2,19). A second major indication for anticoagulation is the treatment and prevention of DVT and pulmonary embolism (PE)(20,21). The pathophysiology of these two entities however, is significantly different: AF and subsequent stroke arises in the arterial vasculature and is primarily the consequence of atherosclerotic plaque rupture, whereas thrombus formation in the venous vasculature (DVT and PE) is caused by alterations in blood flow, composition of the blood and damage to the endothelial alignment of the vessel wall. Furthermore, risk factors for arterial thrombosis, like smoking, hypercholesterolemia and hypertension do not apply for venous thrombosis, indicating that the underlying mechanism is different. Most of the previously mentioned murine models investigated the influence of factor XI inhibition on ferric chloride (FeCl₃).
induced carotid arterial thrombosis(22), while in this study we focused on the venous vasculature and blood flow, which is substantially different from the arterial vasculature. Here, we evaluated the potential of two novel inhibitory anti-human factor XI antibodies (αFXI-175 and αFXI-203) in vitro and in vivo.

Methods

Materials
Enoxaparin (Clexane) was from Sanofi-Aventis (Paris, France). Human coagulation factor XI (Hemoleven) was purchased from LFB Biomedicaments (Les Ulis, France). Factor XI and factor XII deficient plasmas were obtained from Siemens Healthcare Diagnostics (Marburg, Germany). Purified factor XII and high molecular weight kininogen (HK) were from Haematologic Technologies Inc. (Essex Junction, VT, USA). Chromogenic substrate S-2366 was from Chromogenix (Milano, Italy). Thrombin was provided by Dr. W. Kisiel, University of Albuquerque, NM, USA. Factor XI was purified from human plasma as described before(17). Corn trypsin inhibitor (CTI) was from Sigma-Aldrich (St. Louis, MO, USA).

Antibodies
Monoclonal antibodies were generated by conventional methods after injection of Balb/c mice with a mixture of purified human factor XI and factor XIa. Antibody-producing clones were tested for coagulation inhibitory properties in an aPTT coagulation test. Two antibodies were identified, designated αFXI-175 and αFXI-203.

Coagulation assays
Normal pooled plasma, derived from more than 200 healthy volunteers, was supplemented with antibodies and incubated for 30 minutes at room temperature before analysis. Prothrombin time (PT), activated partial thromboplastin time (aPTT) and factor XI levels were measured on an automated coagulation analyzer (Behring Coagulation System, BCS) with reagents and protocols from the manufacturer (Siemens Healthcare Diagnostics).

The Calibrated Automated Thrombogram assays the generation of thrombin in clotting plasma. The assay was carried out as described(23) and the Thrombinscope manual. Coagulation was triggered by recalcification either in the presence of 1, 5 or 20 pM recombinant human tissue factor (Innovin, Siemens Healthcare Diagnostics), or aPTT reagent (8 times diluted; Pathromtin SL, Siemens Healthcare Diagnostics), 4 μM phospholipids, and 417 μM fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem,
Two novel inhibitory anti-human factor XI antibodies prevent cessation of blood flow in a murine venous thrombosis model

Bubendorf, Switzerland). Fluorescence was monitored using a Fluoroskan Ascent fluorometer (ThermoLabsystems, Helsinki, Finland), and the ETP, peak, time-to-peak, lag time and velocity index were calculated using Thrombinscope® software (Thrombinscope, Maastricht, The Netherlands).

**Factor XI activation by thrombin or factor XIIa**

50 nM factor XI was incubated with either αFXI-175 or αFXI-203 (500 nM) in Tris-buffered saline (TBS; pH 7.4) and incubated for 30 minutes at room temperature. Then, 10 nM of thrombin or 5 nM of factor XIIa was added, thrombin was incubated overnight at room temperature and factor XIIa was incubated for 2 hours at 37°C. Subsequently, 1 unit of hirudin was added to inhibit thrombin or 0.2 units of CTI to inhibit factor XIIa and incubated for 20 minutes at room temperature. Chromogenic substrate S-2366 (f.c. 0.5 mM) was added and changes in OD 405 nm were monitored for 5 minutes. Activation of factor XI by factor XIIa was performed in absence or presence of 50 nM HK.

**Solid-phase binding assay**

The interaction of the monoclonal antibodies with factor XI was determined with a solid-phase binding assay. The individual apple domains of factor XI as fusion protein with tPA(24) were coated on microtiter wells (100 μl, 5 μg/ml in coating buffer; 50 mM Na₂CO₃, pH 9.6) and incubated overnight at 4°C. After washing the plate 3 times with TBS, the plate was blocked with 200 μl blocking buffer (TBS/1.5% BSA) for 30 minutes at room temperature, followed by incubation with the different antibodies (αFXI-175 and 203, 2 μg/ml in TBS/1.5% BSA) for 1 hour at room temperature. Antibodies were diluted to 2 μg/ml in blocking buffer. Then, the plate was washed 3 times with TBS/Tween-20 (0.1%), followed by detection with peroxidase-labelled goat anti-mouse immunoglobulins (Dako, Glostrup, Denmark). O-phenylenediamine dihydrochloride (OPD) tablets (Sigma-Aldrich, St. Louis, MO, USA) dissolved in phosphate-citrate buffer (50 mM citric acid, 114 mM NaH₂PO₄, pH 4.5) was used as substrate after which the reaction was stopped with 50 μl 1.0 M H₂SO₄. Absorption was read at 490 nm.

**Animal experiments**

Eight-week-old male and female factor XI knock out-mice on C57BL/6 background(11) were given plasma derived human factor XI concentrate (Hemoleven) prior to the surgical procedure, which completely restores factor XI levels(14). Dosage of Hemoleven was based on our previous experiments with this compound(14). Thereafter, the mice were intravenously injected with either αFXI-175, αFXI-203 or saline. Enoxaparin (LMWH) was injected subcutaneously 6 hours before the procedure. Dosages for the antibodies were based on the in vitro experiments presented here. Enoxaparin concentration was based on the dosage used in humans treated for DVT or PE (1 mg/kg).
Mice were housed in micro-isolator cages on a constant light-dark cycle and were given access to food and water ad libitum. All animal procedures were performed at the Academic Medical Center (Amsterdam, The Netherlands) and approved by the Animal Care and Use Committee of the institute. At the end of the study period the mice were killed by cervical dislocation.

**Collection of mouse plasma samples**

Blood samples were collected by cardiac puncture under anaesthesia. Blood was quickly withdrawn from the heart using a 1-mL plastic syringe with a 27-G needle and collected into a final ratio of 9 parts of whole blood to one part of 3.2% sodium citrate. Blood samples were immediately mixed by tapping and inverting the tube 5 times to ensure proper anticoagulation and then centrifuged for 15 minutes at 600g at room temperature. Plasma was stored at -80°C until assayed.

**FeCl₃-induced inferior vena cava thrombosis**

The antithrombotic properties of αFXI-175 and αFXI-203 were studied using a well-established ferric chloride induced inferior vena cava (IVC) thrombosis model(13). In short, mice anesthetized with 2.5% inhalant isoflurane and a mixture of ketamin/xylazin (2:1), received a midline incision after which the IVC was exposed by blunt dissection. A filter paper soaked in a 10% FeCl₃ solution was placed below the renal veins on the IVC for 3 minutes after which the paper was removed and venous flow was measured for 45 minutes using a tissue perfusion monitor (type BLF22; Transonic Systems Inc. Ithaca, NY, USA). The flow before administration was set at 100% after which the decline in flow was calculated accordingly.

**Tail vein bleeding assay**

A mouse tail bleeding assay was used as described(13). Bleeding was assessed by determining the time until bleeding stopped with a maximum recording time of 30 minutes. Furthermore, the total amount of blood loss was analyzed as described(25).

**Statistical analysis**

Statistical comparisons were made using student t tests or Mann-Whitney U tests. P values less than .05 were considered significant. Data were analyzed using SPSS software package for Windows, Version 19.0 (SPSS). Graphics were constructed using GraphPad Prism, Version 5 for Windows (GraphPad Software).
Results

Both antibodies were able to (dose-dependently) prolong coagulation in normal plasma with a maximum inhibition of ~85% (Fig. 1). Complete inhibition of factor XI (<1% factor XI activity) was achieved by adding the combination of αFXI-175 and αFXI-203 to normal human plasma (data not shown). The antibodies reacted with both factor XI and factor XIa with dissociation constants of 3-5 nM (data not shown).

Figure 1. Effect of αFXI-175 and αFXI-203 on factor XI activity and aPTT in human plasma. The antibodies were added to normal human plasma after which the aPTT (a) and factor XI activity (b) were determined. The experiment was performed twice, and a representative experiment is depicted.
Factor XI is a homodimer with a molecular size of 160-kDa, consisting of 4 tandem repeats called apple domains (apple1-4) and a catalytic domain. We tested the binding of the antibodies to the individual apple domains (Fig. 2) using solid-phase binding assays. αFXI-175 predominantly binds to apple domain 4 and αFXI-203 interacts with apple domain 2.

**Figure 2. Binding properties of αFXI-175 and αFXI-203 to factor XI.** The binding site of both antibodies was assessed with solid-phase binding assays using individual human factor XI apple domains linked to tPA. Individual apple domains were coated on microtiter wells and incubated with either αFXI-175 (2 μg/ml; a) or αFXI-203 (2 μg/ml; b) after which binding to the apple domains was determined. The experiment was performed three times, and a representative experiment is depicted.

Effect of αFXI-175 and αFXI-203 on thrombin generation in human plasma

Both antibodies inhibited thrombin generation initiated via the intrinsic pathway in normal plasma (Fig. 3a/b), indicating that the antibodies inhibited factor XIIa-dependent activation of factor XI, factor Xla-mediated activation of factor IX or both. Upon tissue factor (TF)-initiated thrombin generation, αFXI-203 did not inhibit thrombin generation (Fig. 3d/e/f), while αFXI-175 was able to inhibit thrombin generation at TF concentrations up to 1 pM (Fig. 3c), but not at higher concentrations of TF (Fig. 3e/f).
Two novel inhibitory anti-human factor XI antibodies prevent cessation of blood flow in a murine venous thrombosis model.

Figure 3. Effect of αFXI-175 and αFXI-203 on thrombin generation. The antibodies were added to normal human plasma. Thrombin generation was assessed in recalcified plasma in the presence of antibodies (a, c, e and f; αFXI-175 and b, d, e and f; αFXI-203). Coagulation was initiated by aPTT reagent (a and b) or different concentrations of TF (1 pM in panels c and d; 5 pM in panel e and 20 pM in panel f). Concentrations of the antibodies in plasma are indicated, except for e and f, where 100 μg/ml antibody was used. The experiment was performed twice, and a representative experiment is depicted.
This suggests that αFXI-203 inhibits factor XIIa-mediated activation of factor XI, and that αFXI-175 inhibits factor Xla-mediated activation of factor IX or activation of factor XI by either factor XIIa or thrombin. To further elucidate the working mechanism, we tested the effects of the antibodies in a factor XIa-initiated thrombin generation assay in the absence or presence of endogenous factor XI. As expected, no effect of αFXI-203 could be observed on thrombin generation, whereas αFXI-175 inhibited factor XIa-initiated thrombin generation, both in normal plasma (with an intact thrombin-mediated factor XI feedback loop) and in factor XI deficient plasma (Fig. 4). αFXI-175 inhibits thrombin generation in both systems, most likely indicating that this antibody interferes with factor IX activation by factor Xla.

![Graph a.](image1)

**Figure 4.** Effect of αFXI-175 and αFXI-203 on thrombin generation in plasma initiated by FXIa. Thrombin generation either in normal plasma (a) or in factor XI deficient plasma (b) was initiated by addition of FXIa (50 ng/ml) in presence or absence of anti-FXI antibody (60 μg/ml). The experiment was performed three times, and a representative experiment is depicted.
Factor XI activation studies were performed in a purified system. αFXI-175, but not αFXI-203, inhibited factor XI activation by thrombin (Fig. 5a). On the other hand, both antibodies were able to inhibit factor XII mediated activation of factor XI (Fig. 5b, closed bars) in the presence of HK. Strikingly, in the absence of HK, αFXI-203 was not able to inhibit factor XI activation by factor XIIa (Fig. 5b), indicating that this antibody prevents the binding of factor XI(a) to HK. This could be confirmed in direct binding assays with HK (data not shown).

Figure 5. Effect of αFXI-175 and αFXI-203 on factor XI activation by thrombin and factor XIIa in a purified system. Factor XI (50 nM) was incubated with αFXI-175 or αFXI-203 and 10 nM thrombin and subsequently factor Xla activity was measured using a chromogenic substrate S-2366 (a). A similar experiment was performed using factor XIIa (5 nM) as activator of factor XI in the absence or presence of HK (50 nM) (b). The experiment was performed twice, and a representative experiment is depicted.
Factor XI suppression by inhibiting antibodies in mice

The antibodies were raised in mice against human factor XI(a), and in order to perform animal experiments in mice, it was necessary to check cross-reactivity of the antibodies for mouse factor XI. In functional assays, it was established that the antibodies did not inhibit mouse factor XI (data not shown). To allow testing of αFXI-175 and αFXI-203 in a mouse thrombosis model, we reconstituted factor XI-/- mice with human factor XI. Thus, all the circulating factor XI in these mice is human derived and receptive for both antibodies. This reversal of factor XI plasma levels in factor XI-/- mice with human derived factor XI concentrate was investigated prior to the thrombosis model. Intravenous administration of 5 U factor XI immediately restored factor XI plasma levels to more than 100% (Fig. 6b). The subsequent administration of inhibiting factor XI antibodies and enoxaparin decreased factor XI activity levels in mice, with a concomitant increase in aPTT (Fig 6a/b).

**Figure 5.** Effect of αFXI-175 and αFXI-203 on factor XI activation by thrombin and factor XIIa in a purified system.

Factor XI (50 nM) was incubated with αFXI-175 or αFXI-203 and 10 nM thrombin and subsequently factor XIa activity was measured using a chromogenic substrate S-2366 (a). A similar experiment was performed using factor XIIa (5 nM) as activator of factor XI in the absence or presence of HK (50 nM) (b). The experiment was performed twice, and a representative experiment is depicted.

**Figure 6.** Reconstitution of factor XI in FXI-/- mice. FXI-/- mice were injected with human-derived factor XI concentrate, which restored aPTT (a) and factor XI levels (b). Subsequent administration of the inhibiting factor XI antibodies or enoxaparin increased aPTT and decreased factor XI activity. Each symbol represents one animal and the horizontal line indicates median.

**Ferric chloride induced IVC thrombosis**

Enoxaparin treatment of mice completely prevented ferric chloride induced IVC thrombosis, while animals treated with saline revealed a rapid decline in flow (Fig. 7a). Figures 7b and 7c show the influence of αFXI-175 and αFXI-203 on venous blood flow after applying FeCl₃. Both inhibitory antibodies significantly prolonged the time to obstruction, 12.5 minutes for αFXI-203 and 25 minutes for αFXI-175 (P<0.05).
Two novel inhibitory anti-human factor XI antibodies prevent cessation of blood flow in a murine venous thrombosis model

**Figure 6. Reconstitution of factor XI in FXI-/- mice.** FXI-/- mice were injected with human-derived factor XI concentrate, which restored aPTT (a) and factor XI levels (b). Subsequent administration of the inhibiting factor XI antibodies or enoxaparin increased aPTT and decreased factor XI activity. Each symbol represents one animal and the horizontal line indicates median.

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**Figure 7. Mouse inferior vena cava thrombosis model.** FXI-/- mice were given human derived factor XI concentrate followed by a single dose of either saline (control), enoxaparin (1 mg/kg), αFXI-175 (8 mg/kg) or αFXI-203 (8 mg/kg). Thrombosis was induced by applying a filter paper soaked in 10% ferric chloride for 3 minutes to the inferior v. cava, and venous blood flow was measured for 45 minutes. Each symbol represent the mean value of 6 animals, bars indicate SEM, *P<0.05, ns=non-significant.

**Tail bleeding assay**

Infusion of the antibodies did not result in prolonged bleeding times compared to the saline control (Fig. 8a). This is in contrast to the markedly prolonged bleeding times in the enoxaparin treated animals. Also, the total amount of blood loss (Fig. 8b) was increased with enoxaparin and if anything, decreased in the animals treated with the factor XI antibodies.
inhibiting factor XI antibodies and enoxaparin on bleeding in mice. FXI-/- mice were given human derived factor XI concentrate followed by a single dose of either saline (control), enoxaparin (1 mg/kg), αFXI-175 (8 mg/kg) or αFXI-203 (8 mg/kg). Subsequently, tail bleeding (a) and amount of blood loss (b), depicted as haemoglobin (Hb) content, were evaluated as described in the method section. Each symbol represents one animal, and the horizontal line indicates median. ***P<0.0001, ns=non-significant.

Discussion

The last two decades the medical need for effective antithrombotic drugs with a greater safety profile than VKA and LMWH led to the development of several small molecule factor Xa and thrombin inhibitors(2). However, these drugs have some major drawbacks, paving the way for new innovations(3). Coagulation factor XI has been a target of interest for many years and there was steady increase in publications dealing with this subject(4;5;18), most of which used mice as experimental animals to obtain preclinical data. Because mice do not develop spontaneous thrombosis, an artificial trigger is warranted(26). The most commonly used trigger in drug development studies is ferric chloride, because it works immediately while leaving the vasculature and circulation intact(22).

In this study we focused on the venous vasculature and the influence of factor XI inhibition on thrombus formation. We investigated two novel antibodies, one of which inhibited the factor XIIa-mediated factor XI activation (αFXI-203) and the other interfered with the activation of factor IX by factor Xa and thrombin-mediated factor XI activation (αFXI-175). In a mouse model for venous thrombosis, we have shown a rapid decline in venous blood flow after inducing thrombosis with a 10% ferric chloride solution placed for 3 minutes on the inferior vena cava, which was prevented completely by LMWH and prevented partially with the two inhibiting factor XI antibodies. Previous experiments
with factor XI−/− mice and FeCl₃ applied to the carotid arteries revealed 30 minutes as mean time to occlusion (27). Our αFXI-175 data are consistent with these observations, αFXI-175 prolonged the time to 50% of flow significantly for 25 minutes. At 30 minutes, venous blood flow was still higher in the αFXI-175 group than in the saline group, but this difference was not significant (P=0.25). Also, αFXI-203 significantly delayed flow reduction, but the delay induced by the antibody was considerably shorter than that by αFXI-175. In contrast to αFXI-175, αFXI-203 did not inhibit TF driven thrombin generation \textit{in vitro} (Fig 3.). This suggests a relatively minor role for factor XIIa-mediated factor XI activation in FeCl₃ induced thrombosis models, which is in accordance with the publication by Cheng \textit{et al} (27). In this study, the antibody 14E11 (with a similar working mechanism as αFXI-203) was unable to protect mice from carotid artery occlusion induced by 10% FeCl₃.

Since factor XI−/− mice do not have a bleeding tendency (28), we did not expect an effect of our inhibiting factor XI antibodies in a tail bleeding assay. Indeed, bleeding in mice was unaffected by administration of αFXI-175 or αFXI-203 as compared to control mice treated with saline. In contrast, animals who received enoxaparin revealed a significantly longer bleeding time (Fig. 8a), indicating that factor XI inhibition may have a better safety profile over LMWH drugs like enoxaparin. The tail bleeding assay consists of cutting the tip of the mouse tail, followed by measuring the time until bleeding stops. In our hands, making a big wound induces major blood loss with a relatively short bleeding time, while a small wound causes less blood loss with a longer bleeding time. To correct for this, we also quantified the blood loss by analyzing the haemoglobin (Hb) content as described (25). A similar, albeit not identical, pattern as the bleeding time was observed when the hemoglobin content was analyzed (Fig. 8b).

Inhibiting factor XI antibody αFXI-203 prevented the activation of factor XI by factor XIIa in the presence of HK. Since factor XII displays a negligible role during normal human hemostasis (29), it is difficult to directly translate this observation to the human situation; further research is needed to establish whether inhibition of factor XIIa mediated factor XI activation is an effective strategy to treat thrombosis. Antibody αFXI-175 appears to be a more suitable candidate for anticoagulation, since this antibody inhibits factor IX activation by factor Xa, which is linked to pathological thrombus formation. Furthermore, αFXI-175 inhibits factor XI activation by thrombin, thereby blocking the feedback amplification loop in coagulation. Initiation of thrombin formation is still preserved, allowing the fibrin network to be formed. Interestingly, αFXI-175 showed a high affinity for the apple 4 domain of factor XI. Apple 4 is not involved in the binding of either factor IX or thrombin; therefore the exact working mechanism of αFXI-175 is not entirely clear. Puy and colleagues described an antibody (Ab 1A6) with a similar working mechanism as αFXI-175, but with a different binding site on factor XI (apple 3).
(30). Both antibodies block factor IX activation by factor Xla, as well as factor XI activation by factor XIIa. However, αFXI-175 also inhibits thrombin-mediated factor XI activation, suggesting that the apple 4 domain is involved in the activation of factor XI by thrombin. This is not the first study which proposes factor XI as alternative target for anticoagulation. We previously reported the use of antisense factor XI oligonucleotides (14), other research groups also used an antibody approach (27;31-33). The advantage of antibodies is the fast mechanism of action, which is particularly convenient in the treatment of DVT and/or PE. A possible disadvantage is the immunological reaction towards the antibodies. The novel factor Xa and thrombin inhibitors are small molecule inhibitors; the major drawback of these types of drugs is the lack of an antidote. We did not perform reversal studies in this research, but antibodies could have similar problems concerning an antidote as the small molecule inhibitors although factor XI concentrate is available as potential antidote. Furthermore, antibodies require parenteral administration which is less desirable in thrombosis patients who often need treatment for a long period of time. However, due to the long half-life of the antibodies, administration once weekly would suffice, which also ensures patient compliance.

In conclusion, we have generated two inhibitory antibodies against factor XI with different specificities. Both antibodies prevent flow restriction in a mouse IVC thrombosis model.
## References


