Brief report

Factor XI–dependence of surface- and tissue factor–initiated thrombus propagation in primates

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Thrombin, generated through activation of factor XI (FXI) and/or tissue factor (TF)–factor VIIa, is essential for thrombosis and hemostasis. We investigated the role of FXI-dependent thrombus propagation under arterial flow conditions producing rapid thrombus growth that, after the initiation phase, could limit the availability of TF at the blood/thrombus interface. Thrombosis was initiated by knitted dacron or TF-presenting teflon grafts deployed into arteriovenous shunts in baboons treated with antihuman FXI polyclonal antibody (aFXI). Although aFXI did not prevent thrombus initiation, it markedly reduced intraluminal thrombus growth on both surfaces. The anti-thrombotic effect of aFXI was comparable with that of heparin at doses that significantly prolonged the partial thromboplastin time (APTT), prothrombin time (PT), and bleeding time (BT). aFXI also prolonged the APTT, but the PT and BT were unaffected. Thus, antithrombotic targeting of FXI might inhibit thrombosis with relatively modest hemostatic impairment versus strategies targeting other coagulation factors. (Blood. 2003;102:953-955)

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Introduction

Thrombin generation is initiated by exposure of blood to activated coagulation enzymes, tissue factor (TF), and/or foreign surfaces, and can be amplified through feedback activation of factor XI (FXI) and factor VII in the presence of platelets and TF, respectively. Thrombosis after knee replacement depends, in part, on extrinsic coagulation mechanisms that bypass FXI. However, the pathogenesis of thrombosis might be different in other cases. For example, large thrombi, which extend into the blood flow field, grow at the blood/thrombus interface that is removed from the initiating procoagulant factors present in the vessel wall. Hypothetically, the rapid radial and distal extension of such thrombi could depend importantly upon the activation of FXI, delivered continuously to the thrombus under blood flow. We therefore studied the effects of inhibition of FXI on the initiation and propagation of thrombi in primate arterial flow models, which produce robust thrombus growth.

Study design

In 35 nonterminal experiments, 8 nonanesthetized baboons (9-12 kg) were used. For in vivo anticoagulation, intravenous porcine heparin (1000 U/mL; Wyeth-Ayerst, Pearl River, NY) or affinity-depleted monospecific antihuman FXI polyclonal goat antibody (aFXI; GAFXI-AP, 5 mg/mL; Affinity Biologicals, Hamilton, ON, Canada) were used at 50 U/kg bolus plus infusion of 50 U/kg/h for 70 minutes and at 16 to 50 mg/kg bolus, respectively. Partial thromboplastin time (APTT) and prothrombin time (PT) were monitored using FDA-approved point-of-care diagnostic tests (Rapidpoint Coag, APTT and PT-One test cards; Bayer Diagnostics, Tarrytown, NY) as recommended, except 3.8% citrate was used as an anticoagulant. Template bleeding time (BT) (Surgicutt; ITC, Edison, NJ) was measured before and repeatedly during anticoagulation. The anti-thrombotic effects of heparin or aFXI were studied using 2 different thrombogenic devices deployed for 60 minutes into surgically placed high flow chronic arteriovenous (AV) shunts. In one setting, the device was composed of a 20-mm long dacron graft segment (0.25 mL) and a silicone extension chamber (1.3 mL) (DG), essentially as described. In another setting, the dacron/chamber was replaced with 20-mm long (0.25 mL) ringed expanded polytetrafluoroethylene (ePTFE, teflon) graft segments (WL Gore, Newark, DE) in the shunt. The hypothrombogenic ePTFE graft was converted into an acutely thrombogenic TF-dependent device (TG) for testing heparin and aFXI as follows. The ePTFE was wetted with ethanol, which was displaced by saline, followed by filling of the pores with thromboplatin (1:10 Innovin in saline; Dade Behring, Deerfield, IL) via transgraft perfusion until the reagent entered the graft lumen through the wall. The graft material retained approximately 0.2 μL/mm² thromboplastin. Prior to deployment, the TG was rinsed by passing 50 mL saline through the lumen. In order to limit the time of blood exposure to TF retained in the porous graft wall, the reagent content of the pores was flushed into the blood stream starting at 10 minutes after the start of flow by transgraft perfusion of saline at a rate of 2.4 μL/mm² graft surface/min using a pump. Blood flow was kept at 100 mL/min by proximal clamping. Thrombogenesis was
assessed by measuring radiolabeled fibrin and platelet contents of the thrombogenic devices, as described.\textsuperscript{3,4} In brief, the terminal fibrin content of graft/chamber thrombi (fibrin deposition) was determined by direct \textsuperscript{125}Iodine-labeled fibrin counting. The number of deposited platelets in 35-cm long 4-mm internal diameter shunt segments that incorporated the devices and associated thrombi was quantified by \textsuperscript{111}Indium-labeled platelet imaging with 5-minute data acquisition periods. Net platelet accumulation rate (NPAR) was calculated as the change in the platelet content of the device within one period. Experiments included at least 1 control and 1 heparin study, and 3 aFXI studies performed in each of the 6 animals that were used for comparative analyses. The thrombogenicity of native ePTFE grafts was tested in 5 animals. Results are given as the mean ± SD, and as the percentage of untreated controls where appropriate. Results were compared using the t test; \textit{P} < .05 was considered statistically significant.

Results and discussion

Since the point-of-care PT and APTT cards use dry chemistry, these tests were not affected by sample dilution. The use of 3.8\% citrate in this test resulted in somewhat longer than typical PT values. High-dose heparin produced hemostatic impairment consistent with expectations based on human experience.\textsuperscript{5,6} The APTT (seconds) was prolonged from 28 ± 8 to 105 ± 54 (\textit{P} < .001), the BT (minute) from 4.0 ± 1.0 to 5.6 ± 1.2 (\textit{P} < .01), and the PT (seconds) from 21 ± 5 to 51 ± 17 (\textit{P} < .001). Coagulation tests following aFXI administration suggested impairment of the intrinsic pathway, as expected. aFXI pretreatment prolonged the APTT to 89 ± 58 (\textit{P} < .001) for at least 25 hours but BT (4.1 ± 1.2) and PT (23.3 ± 7.5) were unaffected. There was no clinically apparent bleeding following administration of either anticoagulant.

All shunts remained patent during the experiments. All devices initiated thrombosis in both untreated and treated animals. Fibrin deposition (mg) averaged 0.36 ± 0.19, 5.08 ± 0.35, and 2.66 ± 0.09 in untreated ePTFE, DG, and TG controls, respectively. These differences were likely related to differences in geometry, volume, texture, surface, and/or chemistry. The antithrombotic effects of heparin and aFXI in the DG or TG were comparable. Heparin reduced fibrin deposition to 1.58 ± 0.58 (31\%; \textit{P} < .001) and 1.03 ± 0.38 (38\%; \textit{P} < .02) in the DG and TG, respectively. Pretreatment with aFXI reduced fibrin deposition to 1.45 ± 0.45 (29\%; \textit{P} < .001) and 1.02 ± 0.55 (38\%; \textit{P} < .01) in the DG and TG, respectively. Thrombogenesis was minimal in the native ePTFE grafts with peak NPAR (billions)/(5-minute period) at (0.21 ± 0.18)/(60-55), compared with (1.49 ± 0.4)/(50-45) and (2.87 ± 0.46)/(40-35) in untreated DG and TG controls, respectively (Figure 1A-B). Heparin reduced NPAR between 10 to 60 minutes in DG and 15 to 60 minutes in the TG, with peak values reaching (0.38 ± 0.14)/(35-30) (26\%; \textit{P} < .02) and (0.30 ± 0.08)/(20-15) (10\%; \textit{P} < .01) in the 2 devices, respectively. Pretreatment with aFXI also reduced NPAR between 10 to 60 minutes in the DG and 20 to 60 minutes in the TG, with peak values of (0.44 ± 0.23)/(35-30) (30\%; \textit{P} < .05) and (0.587 ± 0.117)/(15-10) (20\%; \textit{P} > .05), respectively. Incorporated into untreated ePTFE, DG, and TG thrombi by 60 minutes were 1.57 ± 1.70, 10.78 ± 1.86, and 17.9 ± 2.01 billion platelets, respectively (Figure 1C-D). Following heparin, the DG and TG thrombi contained only 2.93 ± 0.62 (27\%; \textit{P} < .001) and 1.56 ± 0.56 (9\%; \textit{P} < .001) billion platelets, respectively. aFXI reduced the platelet contents of the DG and TG to 3.79 ± 1.2 (35\%; \textit{P} < .001) and 1.19 ± 1.11 (7\%; \textit{P} < .01) billion platelets, respectively. Antithrombotic effects of anticoagulants, such as heparin and aFXI, could result from reduced fibrin formation and platelet activation and/or from enhanced thrombolysis and/or embolization. FXI, unlike coagulation factors of the extrinsic and common pathways, is not essential for hemostasis.\textsuperscript{7} However, plasma FXI activity is required to occlusion following transvascular FeCl3-induced arterial injury and thrombosis in mice as well as the early postnatal mortality observed in protein C–deficient mice.\textsuperscript{8,9} Moreover, FXI is an independent risk factor for thrombosis in humans.\textsuperscript{10,11} About 0.5\% of Ashkenazi Jews exhibit severe FXI deficiency.\textsuperscript{12} Interestingly, medical history of acute myocardial infarction (AMI) in such patients corresponds to the incidence of AMI in a matched general population.\textsuperscript{13} Since AMI has 30\% to 50\% mortality,\textsuperscript{14} it remains unclear whether FXI deficiency modifies the outcome of AMI. In our model, anticoagulation did not prevent initiation of thrombogenesis, but it markedly reduced the extent of thrombus propagation over time irrespective of the initiating trigger or the anticoagulant used. Thus, while FXI inhibition may not reduce the frequency of initial thrombotic events, thrombus growth rate, overall thrombus mass, and thrombo-occlusion may be reduced, thereby leading to more favorable outcomes. Accordingly, FXI appears to be a rational target for antithrombotic therapy.

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