blood advances

Factor XI contributes to myocardial ischemia-reperfusion injury in mice

Christina U. Lorentz,[1,2,*] Norah G. Verbout,[1,2,*] Zhiping Cao,[3] Lijuan Liu,[3] Monica T. Hinds,[2] Owen J. T. McCarty,[2,4] Ivan Ivanov,[5] Erik I. Tucker,[1,2] David Gailani,[5] and András Gruber[1,2,4,6]

[1]Aronora, Inc., Portland, OR; [2]Department of Biomedical Engineering, [3]Department of Anesthesiology, and [4]Department of Medicine, Oregon Health & Science University, Portland, OR; [5]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN; and [6]Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR

Key Points

- Inhibiting contact activation of factor XI during reperfusion of acute myocardial ischemia reduces infarct size in mice.
- Factor XII/XI contact axis inhibition may improve the outcome of coronary artery recanalization in acute myocardial infarction.

Introduction

Factor XI (FXI) is the zymogen of a plasma protease (FXIa) that serves a limited role in formation of normal blood clots at sites of injury (hemostasis).[1,2] In vitro, FXI is converted to FXIa by the enzymes thrombin or factor XIIa (FXIIa).[1] Given the absence of abnormal bleeding in FXIIa-deficient individuals,[2] FXI activation by thrombin is likely more relevant for hemostasis. FXIIa-dependent FXI activation, while not required for hemostasis, may contribute to coagulation during the host-response to various inflammatory stimuli.[3-5]

In humans, plasma FXI levels are associated with risk for venous thromboembolism and ischemic stroke.[6-12] Some data also support a link with myocardial infarction.[13-15] Work with rodent and primate models support an important role for FXI in thrombosis,[16-18] and strongly suggest that FXIIa-mediated FXI activation is a contributor.[17,18] In addition to its effects on thrombus growth, FXI contributes to inflammation. In mice, FXI deficiency or inhibition blunts the cytokine response to certain types of infections,[3-5] improving survival, and reduces cerebral ischemia-reperfusion injury after transient middle-cerebral artery occlusion.[19,20]

Preclinical and epidemiologic data suggest that interfering with the FXIIa-FXI interaction could inhibit thromboinflammatory processes without compromising hemostasis.[3-7] In support of this hypothesis, an anti-FXI antibody (14E11) that preferentially interferes with FXI activation by FXIIa is antithrombotic in mice and baboons[17-20] and reduces the cytokine response during polymicrobial sepsis in mice.[4,5] The dual roles of FXI in thrombosis and inflammation suggest that inhibiting the FXIIa-FXI interaction may be beneficial in acute myocardial infarction (MI). Using mice, we investigated whether inhibiting FXI activation with 14E11 reduces myocardial ischemia-reperfusion injury.

Methods

Proteins

Human FXI and FXIa were from (Haematologic Technologies, Essex Junction, VT). Human FXII was from Enzyme Research Laboratory (South Bend, IN). Genomic DNA was isolated from human leukocytes. Generation and purification of the monoclonal anti-FXI antibody 14E11 has been reported.[17]

Chromogenic assays

Purified human FXII (200 nM) and FXIa (10 nM) or FXI (30 nM) in the absence or presence of 5 μg/mL DNA were incubated with 14E11 (5-200 nM) in 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.4, 100 mM NaCl, 0.1% polyethylene glycol 8000, and $ZnCl_2$ (10 μM) at 37°C for 60 min. FXII activation was stopped by aprotinin (10 μM), and ΔOD_{405nm} in the presence of S-2302 (500 μM) was monitored. FXI activation was terminated by mixing aliquots with polybrene (0.2 mg/mL) and S-2366 (1 mM), and ΔOD_{405nm} was monitored. Results were compared with a standard curve prepared with purified FXIa.

Myocardial ischemia-reperfusion injury model

We used an established murine model of nonthrombotic acute MI,[21,22] with minor modifications. Male C57BL/6 mice (17-20 weeks) were intubated and ventilated under isoflurane anesthesia, the jugular vein was catheterized, and body temperature was maintained at 37°C. Lead II electrocardiogram (ECG)

Submitted 18 January 2017; accepted 6 December 2017. DOI 10.1182/bloodadvances.2017004879.

*C.U.L. and N.G.V. contributed equally to this study.

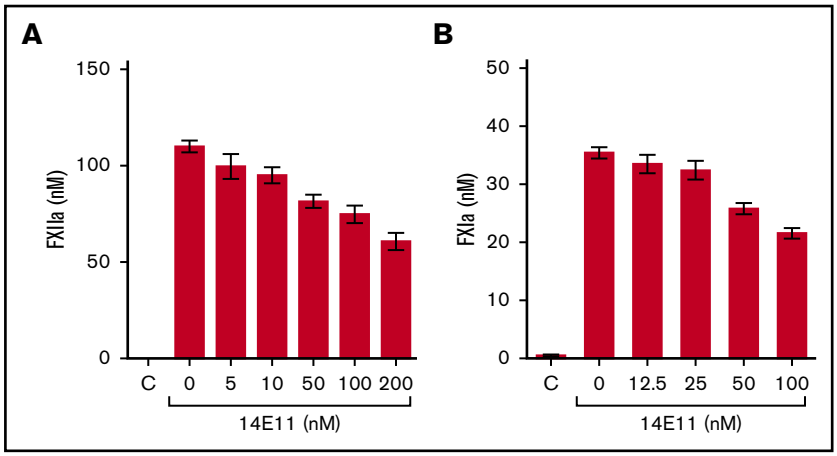

Figure 1. Effects of anti-FXI IgG 14E11 on FXII activation by FXIa and FXI autoactivation, in vitro. The 14E11 antibody binds the A2 domain of mammalian FXI and inhibits its activation by FXIIa. Experiments were performed to elucidate additional effects of 14E11 on the FXII-FXI axis. (A) FXIIa activity is shown as a function of 14E11 antibody concentration. A mixture of purified human FXII and FXIa was incubated with 14E11, and FXIIa amidolytic activity was measured and expressed as the concentration of FXIIa generated in the reaction mixture. The antibody inhibits activation of purified human FXII by purified human FXIa in a concentration-dependent manner, in vitro. (B) FXI autoactivation is shown as a function of 14E11 antibody concentration. Purified human FXI was mixed with purified leukocyte-derived DNA in the presence of various concentrations of 14E11. FXIa amidolytic activity was measured and expressed as the concentration of FXIa that was generated in the reaction mixture. The antibody inhibits the DNA-induced autoactivation of purified human FXI in a concentration-dependent manner, in vitro.

was monitored. After thoracotomy, the left anterior descending artery (LAD) was gently ligated near the origin with a suture loop. Ischemia was verified by pale left ventricular myocardium (LVM) surface and ST-segment elevation. After 35 minutes, surviving mice received phosphate-buffered saline (100 μL) with or without 1 mg/kg 14E11 intravenously (n = 10, each). At 40 minutes, the ligature was loosened to allow LVM reperfusion, verified by ECG and LVM color.

After 2 hours of reperfusion, the LAD was religated, and an 800-μL suspension of fluorescent microspheres (4% [weight/volume], 2-8 μm diameter, in water, 0.01% polysorbate-80; Duke Scientific, Palo Alto, CA) were infused over 4 minutes into the LVM apex to occlude vessels in perfused capillary beds surrounding the zone of ischemia, without entering the ischemic tissue itself, to identify the area at risk (AAR) for infarction during LAD ligation.[22]

The heart was immediately excised, cut into 1-mm slices, and photographed under UV light to identify the AAR. Tissue slices were then stained for 10 min with 1% 2,3,5-triphenyltetrazolium chloride solution (TTC; Sigma) and placed in 10% neutral-buffered formalin. Myocardium not taking up TTC was considered infarcted. Tissue-section images were analyzed (Photoshop) in a blinded fashion to measure the infarcted region as a percentage of LVM. Infarct volume was normalized to the AAR in the corresponding slides. Statistical significance was calculated ($P \leq .05$) by analysis of variance, and these are expressed as the means ± standard error of the mean (SEM) (Prism GraphPad). All animal experiments were approved by the institutional animal care and use committee of Oregon Health & Science University.

Results and discussion

FXI activation by FXIIa triggers coagulation in the aPTT assay used in clinical practice.[2] Despite this, the reaction is not required for hemostasis, because total absence of FXIIa does not cause abnormal bleeding, even with trauma or surgery.[2] However, evidence from animal models support a role for FXIIa activation of FXI in thromboinflammatory conditions, including ischemia-reperfusion injury in the central nervous system.[19,20] Our goal was to test the ability of a monoclonal immunoglobulin G (IgG) (14E11) that specifically interferes with the FXIIa-FXI axis, to reduce myocardial ischemia-reperfusion injury.

The 14E11 antibody binds to an epitope on the FXI A2 domain and cross-reacts with FXI from most mammalian species.[17] It preferentially interferes with FXI activation by FXIIa, prolonging the aPTT of human and mouse plasmas ~2.5-fold. FXI activation by thrombin and FXIa-catalyzed activation of factor IX are not inhibited by 14E11. In most plasma coagulation models the interaction between FXIIa and FXI is unidirectional (FXIIa activates FXI).[2] However, evidence from sepsis models supports the premise that FXIa can activate FXII, the precursor of FXIIa.[5] The 14E11 antibody has a modest inhibitory effect on FXII activation by FXIa (Figure 1A) and slows FXI autoactivation in the presence of a polyanion (DNA) (Figure 1B). This may be relevant for FXI activation during inflammatory processes.[23]

The 14E11 antibody attenuates thrombus formation in mouse and primate models[17] and improves survival in a mouse sepsis model.[4] Interestingly, during sepsis, the beneficial effect of FXI inhibition/deficiency may be largely due to blunting of cytokine-mediated inflammation, rather than an effect on coagulation.[5] FXI may also play a role in leukocyte migration.[24] FXI deficiency reduced vessel wall infiltration by leukocytes in atherosclerotic (apolipoprotein E deficient) mice.[25] These preclinical data indicate 14E11 has anti-inflammatory properties in addition to antithrombotic properties, making it an attractive agent to test in myocardial ischemia-reperfusion injury.

In the present study, LAD occlusion was confirmed by sustained ST segment elevation and by observing regional pallor and ventricular wall motion abnormalities consistent with focal perfusion deficits. During reperfusion, natural color returned to the LVM distal to the ligation. Analysis of AAR (Figure 2A; defined as the area devoid of microspheres normalized to ventricular volume) revealed no significant

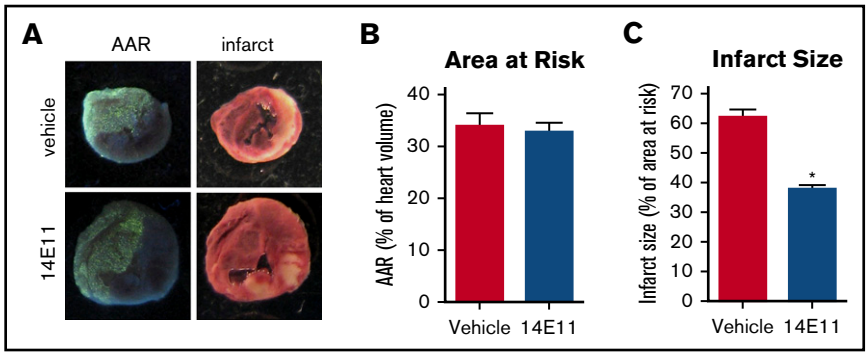

Figure 2. Effects of anti-FXI IgG 14E11 in the murine myocardial ischemia-reperfusion model. (A) Representative sections of mouse hearts from animals treated during ischemia with vehicle (top) or 14E11 (bottom), followed by 2-hour reperfusion. The images on the left show the AAR of ischemia as areas devoid of fluorescent microspheres, whereas the TTC-stained images at right show infarcted tissue (paler areas in which the TTC stain is not taken up). (B) AARs for each group were calculated and are presented as percentages of total ventricular volume. (C) Treatment with 14E11 reduced the infarct size (percentage of AAR undergoing infarction) after regional myocardial ischemia-reperfusion. Data are shown as the means ± SEM of infarct size normalized to the AAR (n = 10 mice/group; *$P \leq .0001$, 14E11 vs vehicle).

difference between mice treated with 14E11 or vehicle (Figure 2B; 33.0% ± 5.5% vs 34.0% ± 7.6% of ventricular volume, respectively; $P = .86$). There was a 39% reduction in the volume of infarcted tissue within the ischemic zone (AAR) in 14E11-treated animals in comparison with vehicle-treated controls (Figure 2C; 37.8% ± 4.2% vs 62.2% ± 7.5% of the AAR; $P \leq .0001$). This protective effect is comparable to that observed previously for 14E11 in a model of cerebral ischemia-reperfusion injury.[20]

Our results suggest that contact activation contributes to myocardial ischemia-reperfusion injury and that injury can be partially mitigated by inhibiting FXIIa activation of FXI. Given the mechanism of action of 14E11, this beneficial effect could be mediated by reducing contact activation, thrombin generation, inflammation, or any combination of these. An advantage of therapeutic targeting of the FXIIa/FXI axis is that, unlike currently available anticoagulants, it should not compromise hemostasis. Given this, it may be possible to use this type of therapy in addition to standard of care to improve outcomes.

Acknowledgments

The authors gratefully acknowledge Donna Van Winkle for providing access to her laboratory and equipment.

This work was supported by National Institutes of Health, National Heart, Lung, and Blood Institute grants HL095315 (A.G. and E.I.T.), HL117589 (E.I.T. and N.G.V.), HL128016 (A.G. and E.I.T.), HL101972 (O.J.T.M. and A.G.), and HL081326 (D.G.).

Authorship

Contribution: C.U.L., N.G.V., and A.G. designed the study, interpreted the data, and wrote the manuscript; L.L., I.I., and Z.C. executed the experiments; A.G., D.G., E.I.T., M.T.H., and O.J.T.M. oversaw the project, critically reviewed the data, and wrote the manuscript.

Conflict-of-interest disclosure: A.G., C.U.L., E.I.T., and N.G.V. are employees of Aronora, Inc., a company that may have a commercial interest in the results of this research. A.G., E.I.T., and Oregon Health & Science University (OHSU) have a financial interest in Aronora, Inc. This potential conflict of interest has been reviewed and managed by the OHSU Conflict of Interest in Research Committee. The remaining authors declare no competing financial interests.

Correspondence: András Gruber, Departments of Biomedical Engineering and Medicine, CH13B, Oregon Health & Science University, School of Medicine, 3303 SW Bond Ave, Portland, OR 97239; e-mail: grubera@ohsu.edu.

References

1. Mohammed BM, Matafonov A, Ivanov I, et al. An update on factor XI structure and function. *Thromb. Res.* 2017;161:94-105.
2. Gailani D, Neff AT. Rare clotting factor deficiencies. In: Hoffman RBE, Shattil S, Weitz J, Heslop H, Silberstein L, McGlave P, eds. Hematology: Basic Principles and Practice. 6th ed. New York, NY: Churchill Livingstone; 2013:1971-1986.
3. Tucker EI, Gailani D, Hurst S, Cheng Q, Hanson SR, Gruber A. Survival advantage of coagulation factor XI-deficient mice during peritoneal sepsis. *J Infect Dis.* 2008;198(2):271-274.
4. Tucker EI, Verbout NG, Leung PY, et al. Inhibition of factor XI activation attenuates inflammation and coagulopathy while improving the survival of mouse polymicrobial sepsis. *Blood.* 2012;119(20):4762-4768.
5. Bane CE Jr, Ivanov I, Matafonov A, et al. Factor XI deficiency alters the cytokine response and activation of contact proteases during polymicrobial sepsis in mice. *PLoS One.* 2016;11(4):e0152968.
6. Key NS. Epidemiologic and clinical data linking factors XI and XII to thrombosis. *Hematology Am Soc Hematol Educ Program.* 2014;2014:66-70.
7. Weitz JI, Fredenburgh JC. Factors XI and XII as targets for new anticoagulants. *Front Med (Lausanne).* 2017;4:19.
8. Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med.* 2000;342(10):696-701.

9. Salomon O, Steinberg DM, Koren-Morag N, Tanne D, Seligsohn U. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. *Blood*. 2008;111(8):4113-4117.

10. Suri MF, Yamagishi K, Aleksic N, Hannan PJ, Folsom AR. Novel hemostatic factor levels and risk of ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Cerebrovasc Dis*. 2010;29(5):497-502.

11. Salomon O, Steinberg DM, Zucker M, Varon D, Zivelin A, Seligsohn U. Patients with severe factor XI deficiency have a reduced incidence of deep-vein thrombosis. *Thromb Haemost*. 2011;105(2):269-273.

12. Preis M, Hirsch J, Kotler A, et al. Factor XI deficiency is associated with lower risk for cardiovascular and venous thromboembolism events. *Blood*. 2017; 129(9):1210-1215.

13. Berliner JI, Rybicki AC, Kaplan RC, Monrad ES, Freeman R, Billett HH. Elevated levels of factor XI are associated with cardiovascular disease in women. *Thromb Res*. 2002;107(1-2):55-60.

14. Doggen CJ, Rosendaal FR, Meijers JC. Levels of intrinsic coagulation factors and the risk of myocardial infarction among men: opposite and synergistic effects of factors XI and XII. *Blood*. 2006;108(13):4045-4051.

15. Salomon O, Steinberg DM, Dardik R, et al. Inherited factor XI deficiency confers no protection against acute myocardial infarction. *J Thromb Haemost*. 2003;1(4):658-661.

16. Gruber A, Hanson SR. Factor XI-dependence of surface- and tissue factor-initiated thrombus propagation in primates. *Blood*. 2003;102(3):953-955.

17. Cheng Q, Tucker EI, Pine MS, et al. A role for factor XIIa-mediated factor XI activation in thrombus formation in vivo. *Blood*. 2010;116(19):3981-3989.

18. van Montfoort ML, Meijers JC. Recent insights into the role of the contact pathway in thrombo-inflammatory disorders. *Hematology Am Soc Hematol Educ Program*. 2014;2014:60-65.

19. Kleinschnitz C, Stoll G, Bendszus M, et al. Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. *J Exp Med*. 2006;203(3):513-518.

20. Leung PY, Hurst S, Berny-Lang MA, et al. Inhibition of factor XII-mediated activation of factor XI provides protection against experimental acute ischemic stroke in mice. *Transl Stroke Res*. 2012;3(3):381-389.

21. Motoki A, Merkel MJ, Packwood WH, et al. Soluble epoxide hydrolase inhibition and gene deletion are protective against myocardial ischemia-reperfusion injury in vivo. *Am J Physiol Heart Circ Physiol*. 2008;295(5):H2128-H2134.

22. Redfors B, Shao Y, Omerovic E. Myocardial infarct size and area at risk assessment in mice. *Exp Clin Cardiol*. 2012;17(4):268-272.

23. Ivanov I, Shakhawat R, Sun MF, et al. Nucleic acids as cofactors for factor XI and prekallikrein activation: different roles for high-molecular-weight kininogen. *Thromb Haemost*. 2017;117(4):671-681.

24. Itakura A, Verbout NG, Phillips KG, et al. Activated factor XI inhibits chemotaxis of polymorphonuclear leukocytes. *J Leukoc Biol*. 2011;90(5):923-927.

25. Shnerb Ganor R, Harats D, Schiby G, et al. Factor XI deficiency protects against atherogenesis in apolipoprotein E/factor XI double knockout mice. *Arterioscler Thromb Vasc Biol*. 2016;36(3):475-481.