Anniversary Issue Contribution

Factor XI in haemostasis and thrombosis: Past, present and future

Uri Seligsohn

The Amalia Biron Research Institute of Thrombosis and Hemostasis, Chaim Sheba Medical Center, Tel-Hashomer and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

The discipline of haemostasis and thrombosis has set an example, perhaps more than other disciplines of Medicine, in discoveries based on observations of the so called "experiments of nature". During a relatively short time of 13 years (1947 - 1960), most coagulation factors were identified in patients who presented with a bleeding tendency and were worked up by rather simple laboratory assays. This era of discoveries was followed by the characterization of the biochemical reactions involved in blood coagulation, delineation of the sequence of reactions in the coagulation pathways and more recently by unraveling the molecular genetic aspects of the various coagulation components. The objective of this review is to describe some of the major developments in understanding the role of factor (F)XI in haemostasis and thrombosis that have occurred over more than 50 years of research, and to highlight recent evidence which suggests that FXI deficiency can have an antithrombotic effect.

The early years

Soon after the distinction of haemophilia B (FIX deficiency) from haemophilia A (FVIII deficiency), Rosenthal et al. described in 1953 and 1955 a third haemophilia (FXI deficiency) which was associated with a mild to moderate bleeding tendency(1,2). The presence of the disorder in two sisters and their maternal uncle was regarded as an indication that it is inherited as an autosomal dominant trait (2). However, a seminal study by Rapaport et al. in 1961 clearly established that transmission of the disorder was autosomal recessive and distinguished between subjects with a major deficiency exhibiting FXI levels of less than 20 U/dl from subjects with a minor deficiency (FXI level 30-60 U/dl) (3). This study as well as others indicated that FXI deficiency is particularly prevalent in Jews. This was indeed proven in 1978 by me in two large surveys of Ashkenazi (European) Jews indicating that the estimated frequency (95% confidence interval) of major FXI deficiency was 0.1 - 0.3% and of minor FXI deficiency 5.5 - 11% (4).

In the classical sequence of the coagulation reactions designed in 1964, FXI was assigned with a role in the initial contact activation phase. It was shown that negatively charged surfaces

trigger activation of FXII, later found to occur in the presence of prekallikrein (PK) and high-molecular-weight kininogen (HK). FXIIa then activates FXI, and FXIa activates FIX leading through additional reactions to thrombin generation. However, this sequence of the so-called intrinsic coagulation pathway was difficult to reconcile with the observations that patients affected by severe deficiencies of FXII, PK or HK did not have a bleeding tendency, whereas FXI-deficient patients did present with an injury-related haemostatic defect. This apparent paradox was resolved in 1991 by the demonstration that thrombin is the physiological activator of FXI (5, 6) bypassing the initial contact induced reactions. These seminal studies lead to the design of a revised scheme of the coagulation pathway in which FXII, PK and HK play no role in physiologic haemostasis, while FXI is important for amplifying thrombin generation following its initial formation by the tissue factor (TF)-FVII pathway (5).

Biochemistry of FXI

FXI is a 160 kDa homodimer in which each 80 kDa monomer comprises 607 residues organized in a heavy chain with four tandem repeats of 90 - 91 residues (apple domains), and a light chain harboring a serine protease (7).

Apple 1 domain contains binding sites for HK forming a FXI:HK complex in the circulation and a thrombin binding site, apple 3 domain bears binding sites for FIX and platelet glycoprotein Ib α , and apple 4 harbors the binding site for FXII (8, 9) and contains the interface between the monomers as recently defined in the FXI crystal structure (10). FXI is activated by thrombin that cleaves an Arg369-Ile370 bond yielding a 47 kDa heavy chain bound by a disulfide bond to a 33 kDa light chain. FXIa activates FIX in the presence of calcium ions by sequential cleavages of Arg146 - Ala147 and Arg180 - Val181 bonds yielding activated FIXa β and an activation peptide.

The FXI gene consists of 15 exons and 14 introns (GenBank M 18295) and is located on chromosome 4q34-35 (11, 12). FXI is produced in the liver and circulates in blood at a concentration of 3-7 µg/ml.

The Amalia Biron Research Institute of Thrombosis and Hemostasis Chaim Sheba Medical Center, Tel Hashomer 52621, Israel Tel.:+972 3 530 2104. Fax: +972 3 535 1568 E-mail: Seligson@sheba.health.gov.il

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Correspondence to:

Uri Seligsohn, MD

Mutations, founder effects and ethnic distribution

In 1989 the first three mutations causing FXI deficiency were identified in six Jewish patients (13). Two of the three mutations, Glu117X (type II) and Phe283Leu (type III) predominate in Jews; they were found to be harbored by 98% of 295 unrelated patients with severe FXI deficiency (14). The third mutation, type I, is located at the last splice site of the FXI gene and has so far been identified in seven unrelated patients (14). Polymerase chain reaction and restriction analysis for detection of the two common mutations in Jews (15) as well as a series of polymorphisms (16) enabled us to obtain the following information: i) Homozygotes for the Glu117X mutation have a mean FXI level of 0.012 U/ml, homozygotes for the Phe283Leu have a mean FXI level of 0.097 U/ml and compound heterozygotes for Glu117X and Phe283Leu have a mean FXI level of 0.033 U/ml; ii) the allele frequency of the Glu117X mutation in Ashkenazi and Iraqi Jews was 0.0217 and 0.0167, respectively (16), while among other Jewish ethnic groups and Palestinian Arabs it was significantly lower, i.e. 0.0026 and 0.0065, respectively (17); iii) the Phe283Leu mutation was only present in Ashkenazi Jews in whom the allele frequency was 0.0254 (16, 17); iv) distinct founder effects for the Glu117X and Phe283Leu mutation were defined by haplotype analysis (17); v) mutation age estimates revealed that the Glu117X mutation occurred approximately 2,500 years ago when all Jews resided in the Middle East before they were exiled by the Romans in 70 A.D., whereas the Phe283Leu occurred more recently (18). These age estimates fit with the current distribution of Glu117X in various Jewish ethnic groups, and with the confinement of Phe283Leu to Ashkenazi Jews who separated from other Jewish ethnic groups approximately 1,900 years ago.

Two additional clusters of FXI-deficient patients were recently ascertained by us. One was identified in French Basques, a Cys38Arg mutation, and the second, a Cys128X mutation, was identified in Britons, with allele frequencies of 0.005 and 0.009, respectively (19, 20). For each mutation, haplotype analysis was consistent with a founder effect, but an age estimate was not possible to perform because of insufficient data.

As of February 2007, 120 mutations causing FXI deficiency have been described in peer-reviewed journals. All are listed in a database of rare clotting factor deficiencies (www.med.unc.edu/ isth/welcome). Characterization of these mutations has shed light on understanding the structure-function relationships of FXI and has defined several dysfunctional mutations such as Gly555Glu and Pro520Leu (21, 22). Interestingly, four mutations were shown to exert a dominant negative effect which stems from impaired secretion of heterodimers consisting of a normal and a mutant monomer (23, 24). Such heterozygotes may present with bleeding episodes caused by FXI levels that are considerably lower than observed in usual heterozygotes in whom dimerization of a normal monomer and a mutated monomer does not impair the secretion of FXI dimer from producing cells.

Discovery of a new function of FXI

The procoagulant activity of FXI was further refined in 1995 by an elegant in-vitro study performed by the group of B. Bouma

from the Netherlands (25). At high TF concentrations, the rate of fibrin formation in plasma was independent of FXI, but at decreasing amounts of TF, there was a gradual increase in the contribution of FXI to the rate of fibrin formation. This observation and a more recent study (26) are consistent with a model in which initial clot formation occurs at the site of TF exposure indepentently of FXI, while at the propagation phase, when the clot increases in size, the process becomes dependent on FXI. Another novel finding was that thrombin-induced activation of FXI leads to inhibition of tissue plasminogen activator (tPA)-induced fibrinolysis of the clot (25). This effect was mediated by FXI-dependent generation of substantial amounts of thrombin after clot formation, which in turn activates the thrombin-activatable fibrinolysis inhibitor (TAFI) that removes carboxy-terminal lysine residues from fibrin, the sites of plasminogen binding to fibrin (27). In the presence of a monoclonal antibody to TAFI, this effect was abolished as in the presence of a monoclonal antibody to FXI implicating TAFI as a mediator of the antifibrinolytic activity of FXI. Further support for the role of FXI in inhibiting fibrinolysis comes from observations in patients with severe FXI deficiency who are particularly prone to bleed following injury at sites where there is augmented local fibrinolysis like the oral cavity, nose, tonsils and urinary tract (15, 28, 29). Furthermore, tooth extractions in such patients can be carried out uneventfully by using tranexamic acid instead of replacement therapy (30).

Clinical features

The common presentation of severe FXI deficiency is injury-related bleeding, e.g. tooth extractions, tonsillectomy, nasal surgery, or prostatectomy. Alternatively, a prolonged activated partial thromboplastin time found in a routine examination leads to the diagnosis. Bleeding occurs less frequently following surgery performed at sites where there is no local fibrinolytic activity, e.g. orthopedic surgery, appendicectomy, circumcision or herniorrhaphy (15, 29). In contrast, surgery at sites with local fibrinolysis (vide supra) is associated with excessive bleeding in 49–67% of cases (29). Regarding post-partum haemorrhage, a retrospective analysis of 62 women with severe FXI deficiency revealed that 43 (70%) had 93 uneventful deliveries (85 vaginal and 8 caesarian) without blood component therapy (31). Consequently, an on demand policy of blood component therapy was proposed for such patients.

Whether or not heterozygotes with partial FXI deficiency display a bleeding tendency is controversial. A bleeding risk assessment in a large cohort of patients with FXI deficiency yielded an odds ratio (OR) of 13 (95% CI 3.8–45) in patients with FXI levels less than 0.2 U/ml and an OR of 2.6 (95% CI 0.8–9) in patients with FXI levels of 0.2–0.69 U/ml (32). Hence, major FXI deficiency confers a markedly higher risk of bleeding than minor deficiency. Patients with a minor deficiency who have a bleeding history should be tested for an additional hemostatic disorder. Guidelines for therapy in FXI-deficient patients can be found elsewhere (14, 33).

Development of inhibitors to FXI in patients with severe FXI deficiency has been described. In our recent study of 118 unrelated patients with severe FXI deficiency, seven were found with an inhibitor (34). The seven patients were among 21 patients who 50th Anniversary (1957–2007)

had received replacement therapy and were homozygotes for the Glu117X mutation that is associated with extremely low FXI levels. Since none of the 43 patients with other genotypes associated with higher FXI levels ranging between 0.02–0.15 U/ml developed an inhibitor despite replacement therapy, it was concluded that development of an inhibitor to FXI can be expected in one third of patients with an extremely low FXI level following treatment with blood components. Interestingly, patients with an inhibitor usually do not bleed spontaneously but do present a challenge when surgery is needed. Recombinant FVIIa is helpful under such circumstances.

The prothrombotic effect of elevated levels of FXI

The procoagulant and antifibrinolytic effects of FXI could hypothetically imply that increased FXI levels confer an increased risk of venous and or arterial thrombosis. Indeed, in the Leiden thrombophilia study of 474 patients with venous thromboembolism and 473 controls, the adjusted OR for patients whose FXI was above the 90th percentile was 2.2 (95% CI 1.5–3.2) compared to patients with FXI levels below 90th percentile (35). In another study, recurrence of venous thrombosis was significantly more frequent in patients in whom both FXI activity and TAFI antigen level were increased (36).

Regarding arterial thrombosis, a recent study of 200 women with acute myocardial infarction before the age of 49 years and 626 controls indicated that elevated FXI levels were not associated with an increased risk (37). Another study of 174 men and 26 women with myocardial infarction at an age range of 32–72 years also did not reveal an increased relative risk for subjects in the upper quartile of FXI levels (adjusted OR 0.8; 95% CI 0.2 – 2.7) (38). However, another study of 560 men with an acute myocardial infarction before the age of 70 and 646 controls showed that the adjusted OR in those subjects whose FXI level was in the highest quintile was 1.8 (95% CI 1.2-2.7) compared to those with the lowest quintile (39). Interestingly, the highest risk was observed in a subgroup of patients with both high FXI level and low FXII level (adjusted OR 6.4, 95% CI 2.2-18.0). The discrepancy among these studies and the question whether an increased FXI level confers a risk of myocardial infarction in men but not in women will await further studies.

An increased relative risk of ischemic stroke in patients aged less than 55 years was also recently reported to be associated with increased FXI levels (40). FXI above the 95th percentile was observed in 17 (22%) of 78 patients with stroke (n = 65) or transient ischemic attack (n = 13) versus two of 40 controls (5%), OR 5.3 (95% CI 1.2–24.1).

Is severe FXI deficiency protective against thrombosis?

Severe inherited bleeding disorders have for many years served as paradigms for understating the pathogenesis of thrombosis, and for development of antithrombotic therapy. Reports of thrombosis in patients with severe hemostatic defects have introduced some skepticism about the value of mimicking these experiments of nature. However, because of the limited number of observations in such patients and because thrombosis is multicausal, interpretation of these anecdotal cases is difficult. One matter has been clarified; patients with profound defects in haemostasis, e.g. haemophilia A, haemophilia B, severe type 3 von Willebrand disease and Glanzmann thrombasthenia are not protected against atherosclerosis (41–43). Nevertheless, longitudinal studies in the Netherlands showed that haemophilia A and hemophilia B are associated with a decreased incidence of myocardial infarction (44, 45), which suggests that the low coagulability does prevent thrombotic occlusion. Whether or not severe FXI deficiency also provides protection against thrombosis has been recently addressed by observations in animal models and patients.

Animal studies

Several animal models have been employed for addressing the question whether FXI deficiency confers a fibrinolytic and or an antithrombotic effect. An initial study revealed that a jugular vein thrombus in rabbits treated locally or systemically by a FXI antibody underwent thrombolysis two-fold faster than a control thrombus (46). A similar effect was elicited by an anti-TAFI antibody, suggesting that thrombolysis induced by FXI deficiency was mediated by diminished TAFI activation. In a murine model of FXI deficiency, FeCl₃-induced injury of the carotid artery resulted in significantly better blood flow than observed in wild type mice (47, 48). Full protection was also observed in FIX-deficient mice, but while tail bleeding time was normal in FXI-deficient mice, FIX-deficient mice had a markedly prolonged tail bleeding time (48). This suggests a selective antithrombotic effect imparted by FXI deficiency without a demonstrable anti-haemostatic effect. In another thrombosis model in baboons, the growth of a thrombus initiated by knitted dacron or TF presenting teflon grafts deployed in arteriovenous shunts was markedly reduced by infusion of a goat anti-human FXI antibody (49). This antithrombotic effect was comparable to heparin infusion. Similar protective effects by FXI deficiency were obtained in an iliac artery balloon injury model in rabbits (50), and in FeCl₃-induced thrombosis of the inferior vena cava in mice (51). In another study in mice, the antithrombotic effects of FXI or FXII deficiency was examined by four thrombosis models, i.e. pulmonary embolism induced by infusion of collagen and epinephrine, FeCl3-induced injury of mesenteric arterioles, mechanical injury of the aorta and carotid artery injury accomplished by ligation with a surgical filament. Striking antithrombotic effects were discerned in FXII-deficient mice that were examined by all four models of thrombosis (52). A recent study by the same investigators demonstrated that both FXII-and FXI-deficient mice were similarly protected from transient middle cerebral artery occlusion (53). Collectively, these data suggest that, at least in animals, FXII and FXI play a role in inducing thrombosis, but are dispensable for haemostasis.

Human studies

The rather low prevalence of uprovoked venous thromboembolism at different age groups (1:300-1:1,000) makes it hard to assess whether patients with severe FXI deficiency are protected against venous thrombosis. Five anecdotal cases of venous thromboembolism were included in a recent review (54) of whom two occurred following infusion of a FXI concentrate (known to sometimes induce thrombosis) and one had systemic lupus erythematosis.

ciency (55). Of 96 unrelated patients (55 women and 41 men) who were more than 35 years old, 16 had a history of acute myocardial infarction, of whom six underwent coronary artery bypass grafting and four had recurrent events. One or more athero-

The incidence of acute myocardial infarction was recently assessed systematically in Israeli patients with severe FXI defi-



Figure 1: Revisited schemes of the coagulation cascade highlighting the potential differences between haemostasis and thrombosis. The schemes (A and B) are based on recent experiments in animal models of thrombosis which underscore the importance of the contact phase of coagulation for propagation of thrombi, and on the clinical observations that patients with a severe deficiency of FXII, PK or HK do not have a bleeding tendency which renders the contact phase of coagulation unimportant for haemostasis. Alternative sites for triggering FXII activation are activated platelets and clot surface (see text). Coagulation factors are depicted in Roman numbers, activated factors are shown in red, and cofactors are in green. PL indicates phospholipids mainly provided by activated platelets, HK – high-molecular-weight kininogen, PK – prekallikrein, KAL – kallikrein, TAFI – thrombin activatable serine protease inhibitor, u-PA - urokinase plasminogen activator, t-PA tissue plasminogen activator.

sclerotic risk factors were observed in 13/16 (81%) of patients who had a myocardial infarction compared to 44/47 (56%) in patients who had no myocardial infarction (p<0.001). The calculated annual rate of acute myocardial infarction in male patients was similar to the expected rate in the general Israeli population, whereas in female patients it was almost two-fold higher but did not reach statistical significance. These data indicate that severe FXI deficiency confers no protection against acute myocardial infarction. Whether or not severe FXI deficiency protects against ischemic stroke is at the time of this writing under investigation.

Quo vadis

More than 50 years of research on FXI and FXI deficiency have witnessed oscillations in understanding the role of FXI in haemostasis and thrombosis. The first conceptual change emanated from the discovery that FXI can be activated by thrombin rather than by FXIIa. This finding and the observations that FXI-deficient patients have a bleeding tendency, while patients deficient in FXII, HK or PK do not, led to a revision of the clotting cascade assigning no role for the initial contact reactions in haemostasis (Fig. 1A), and making them relevant only for in-vitro coagulation until proven otherwise. The second conceptual change was that FXI is not only a procoagulant but also an antifibrinolytic factor. The third conceptual change emanated from the observations in animal models of arterial and venous thrombosis in which both FXII or FXI deficiency exhibited an antithrombotic effect. These findings are consistent with a "thrombosis scheme" of the coagulation pathways (Fig. 1B) that swings back the pendulum to the originally described cascade. Recent evidence indicates that apart from negatively charged surfaces at injury sites of the vessel wall, activated platelets or clot surface can serve as templates for triggering FXII activation and propagation of the growing thrombus (52). Moreover, polyphosphates released from dense granules of activated platelets (56), and RNA re-

leased from damaged cells at injury sites (57) were recently shown to augment contact activation. Whether or not this third conceptual change is relevant for thrombosis in humans remains to be established. Supportive evidence is that elevated FXI levels confer a risk of venous thromboembolism (35), myocardial infarction in men (39) and stroke (40). Evidence which does not support the concept includes: i) Lack of protection conferred by severe FXI deficiency against myocardial infarction (55); ii) Elevated FXI levels in women are not associated with an increased risk of myocardial infarction (37); iii) Decreased rather than increased levels of FXII are associated with a risk of myocardial infarction in men (39). iv) Patients with severe FXII deficiency are not protected against thrombosis (58, 59). Thus, more observations on arterial and venous thrombosis, particularly in patients with severe FXI or FXII deficiency would be helpful before accepting the revised scheme. If indeed FXI deficiency will be found to be protective against one or more types of thrombosis, development of humanized monoclonal antibodies against FXI or other agents that interfere with FXI function could serve as excellent antithrombotic drugs that would have no or minimal haemorrhagic potential.

Also foreseen are improvements in the treatment of patients with severe FXI deficiency who undergo surgery. A more parsimonious use or no use of plasma or FXI concentrate for certain types of surgery and labor will decrease the frequency of transmission of infectious agents and development of a FXI inhibitor. Also, use of tranexamic acid without replacement therapy during surgery at sites with increased fibrinolytic activity seems justified to evaluate given the experience gained with tooth extractions. Finally, development of rFXI is anticipated.

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