Factor XI and traveling waves: the key to understanding coagulation in hemophilia?


Fazoil I Ataullakhanov
Author for correspondence:
Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, 4 Kosygina St., Moscow, Russia and HemaCore LLC, 3, 4th B Marta St., Moscow, Russia and National Research Center for Hematology, Ministry of Healthcare and Social Development, 4a Novyi Zykovskyi Pr., Moscow, Russia
Tel.: +7 495 258 2538 ataullakhanov.faily@gmail.com

Natalia M Dashkevich
Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, 4 Kosygina St., Moscow, Russia and HemaCore LLC, 3, 4th B Marta St., Moscow, Russia and EA 4174 Unité de Recherche sur l’Hémosthèse, Université Lyon 1, Lyon, France

Claude Negrier
EA 4174 Unité de Recherche sur l’Hémosthèse, Université Lyon 1, Lyon, France and Unité d’Hémostase Clinique, Hôpital Edouard Herriot, Lyon, France

Mikhail A Panteleev
Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, 4 Kosygina St., Moscow, Russia and HemaCore LLC, 3, 4th B Marta St., Moscow, Russia and National Research Center for Hematology, Ministry of Healthcare and Social Development, 4a Novyi Zykovskyi Pr., Moscow, Russia

Hemophilia A, B and C are hereditary bleeding disorders that result from congenital deficiencies or defects of blood coagulation factors VIII (FVIII), IX (FIX) and XI (FXI), respectively. These proteins are part of the so-called intrinsic pathway of the coagulation cascade. FIX is activated by FXIa, and FXI is in turn activated by factor XIIa (FXIIa) that, in its turn, is spontaneously autoactivated when it is in contact with a negatively charged surface. The FXII pathway, however, is not presently considered to be physiologically relevant for normal hemostasis [1]. Currently, extravascular membrane protein tissue factor (TF) is well established as a major factor initiating clotting via the extrinsic pathway. This pathway starts from TF–FVIIa complex, extrinsic tenase, which is formed upon TF exposure at the site of injury. Extrinsic tenase activates FV and factor X, and factor Xa (FXa) activates prothrombin into thrombin, the main enzyme that fulfills the actual coagulation – that is, formation of a fibrin clot. Thrombin also performs other functions, in particular by rapidly activating factor V and FVIII, elements of positive feedbacks that accelerate FXa and thrombin formation by three to five orders of magnitude. So FVIII and FIX are more or less explicitly involved in both pathways.

By contrast, there was no natural activator for FXI other than FXIIa reported for many years. However, deficiency of FXII is not associated with bleeding. It seemed strange, as a deficiency of FXI (hemophilia C) leads to bleeding, although clinical manifestation is milder compared with deficiency of FIX and FVIII. It meant that FXI had a function in hemostasis independent of FXII.

A critical progress was achieved two decades ago with the finding that FXI can be activated, albeit slowly, by thrombin [1]. This activation can occur in vivo and enhance FIX generation. However, there is a problem: thrombin cleaves fibrinogen to form fibrin much faster than it activates FXI [8], so FXIa seems to have no considerable effect on clot formation. Thrombin formation is affected more significantly [2,3]. Still, both effects can be observed only in a limited range of experimental conditions: absence of FXI plays a role when there is low or zero TF concentration [2–5]. In these cases, FXI deficiency can lead to delayed clot formation and decreased thrombin generation.

The question of the meaning of thrombin generation actually goes beyond the problem of hemophilia. While various forms of thrombin generation assays have become an established clinical tool, it is also established that fibrin clot is already formed when only 5–10% of thrombin is activated [6]. This raises a major question: why thrombin, produced after the fibrin clot formation, is important?

With respect specifically to hemophilia C, it was proposed that the need for additional thrombin can be explained by the observation that bleeding usually occurs in the tissues with increased fibrinolysis rate [7]. Decreased thrombin generation can reduce protection of the clot to fibrinolysis by the following mechanisms:

“...the ‘spatial’ concept can provide a new point-of-view for understanding the bleeding mechanism and hemophilia treatment strategies.”
Structure of fibrin clot depends on thrombin concentration, as lower thrombin concentrations (i.e., in case of hemophilia) form a clot with thicker fibers that are easier to lyze [8];

Thrombin protects the clot from fibrinolysis by activating TAFI [9];

Thrombin activates FXIII, which is necessary for stable clot formation.

However, this does not solve all of the problems. As mentioned earlier, thrombin generation depends on FXI in cases of very low TF concentrations (less than 1 pM). The density of TF present at the site of injury, especially during surgery or in severe trauma, however, is very high (e.g., amount of TF on the surface of fibroblasts is ~100,000 molecules/cell) [10]. If we try to relate concentrations and densities, however, another possible solution arises. TF in vitro is not dissolved, in contrast to the in vitro prothrombin time and thrombin generation assays. It is localized on the damaged tissues so that the clot grows from the surface of the TF-bearing cells to the volume of blood where there is no TF. Therefore, FXI can possibly perform its function in the propagation of coagulation from the activator, because far from the activator there is no another source of FIXa.

The idea of the spatial separation of the reactions of initiation and propagation of coagulation was proposed by Hoffman and Monroe [11,12] as a cell-based model of coagulation and by the authors’ group [13,14] as a spatial propagation model. Despite the different experimental systems, it was shown that initiation and propagation of coagulation are regulated by different reactions of the coagulation cascade [12,15].

The basic idea of this approach, as it appears today, is the following: FXa, which is necessary to produce thrombin, is generated by extrinsic tenase on the surface of TF-expressing cells. However, this factor is inhibited very rapidly in plasma, and hence cannot get far from the activator by diffusion (it is protected from the inhibition on the platelet surface, but it needs to get there to get this protection). Without another source of FXa, its generation would be confined to a thin layer of the TF-expressing cells, and no solid 3D fibrin clot would be formed. So, there should be some source of FXa that can diffuse and produce FXa in a new location. This function is performed by FIXa, which has a much lower rate of inactivation by the plasma inhibitors [15] and can diffuse much further from the point of activation. In addition, when FIXa diffusion is not enough, FXIa comes into play. FXIa can be activated by thrombin and thus form a source of FIXa that would be independent of TF-initiated coagulation. The clotting process thus could propagate even when TF is no longer available.

Typical coagulation assays simulate the coagulation process close to TF-bearing cells where TF is always present. In these models, the role of FXI is diminished. To emphasize the propagation phase of coagulation, the experimental system should be considered where it is possible to observe clot growth from the TF-coated surface in plasma. In this system, hemophilia A and B were shown to be associated with abnormal clot growth, while initiation by TF was not affected [10].

Recently, we were able to directly measure thrombin distribution in the growing clot in vitro [16]. High concentration of thrombin is produced near the activating TF-coated surface. The concentration of thrombin produced is dependent on TF concentration and is similar to the thrombin peak obtained in regular thrombin generation assay. However, there is the second phase of thrombin generation in the spatial system. Clot propagation in space is driven by a moving peak of high thrombin concentration. This peak is fully independent from the TF and is propagating with a constant speed far from the activator. In addition, in these conditions, the only way to sustain thrombin generation independent from TF is activation of FIX by FXIa.

This self-sustaining wave of thrombin is not formed in the absence of factor XI (as well as FVIII), showing that this TF-independent mechanism is required for clot growth. This mechanism of coagulation propagation can spread the coagulation process far enough from the damaged tissue. It can be especially important in cases of large-scale injury such as trauma or surgery, in situations when bleeding is observed in hemophilia C.

This concept is in accordance with the previous results [3,4,9] and can reinforce the ‘fibrinolysis’ hypothesis. In the absence of FXI, the moving thrombin peak is not formed and the local concentration of thrombin far from the activator at the time of clot formation is much lower [16]. This can lead to an increased fibrinolysis rate for the reasons mentioned above.

Considering coagulation as a spatially heterogeneous process shows that thrombin production near the TF-covered surface and far from it are completely different processes controlled by different reactions of the coagulation cascade. FXI is important in the TF-independent propagation phase of clot formation, not in the initiation process.

This new concept of bleeding in hemophilia can lead to another view on diagnostics and therapy. Specifically, experiments with existing bypassing agents [17] and potential new ones [18] indicate that this should be kept in mind: thrombin produced near activation site is not equivalent to that produced far from it. The ‘spatial’ concept can provide a new point-of-view for understanding the bleeding mechanism and hemophilia treatment strategies. Additional experiments, in particular in animal models, should be performed to elucidate this in more detail.

Financial & competing interests disclosure
The authors are financially supported by ‘Bourse de Thèse en Cotutelle’ from the French Embassy in Russia (NM Dashkevich), the Russian Foundation for Basic Research Grants 12-04-00438 (FI Ataullakhanov), 11-04-00303 and 12-04-33055 (MA Panteleev) and the Russian Academy of Sciences Presidium Basic Research Programs ‘Molecular and Cellular Biology’ (MA Panteleev), ‘Basic Science for Medicine’, ‘Integrative Physiology’ and ‘Molecular Mechanisms of Physiologic Functions’ (FI Ataullakhanov). NM Dashkevich, MA Panteleev and FI Ataullakhanov are affiliated with HemaCore LLC, which holds several patents and patent applications on the diagnostic use of coagulation assays in spatially distributed systems currently developed under the trade name of Thrombodynamics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.
Factor XI & traveling waves

References
9 von dem Borne PA, Meijers JC, Bouna BN. Feedback activation of factor XI by thrombin in plasma results in additional formation of thrombin that protects fibrin clots from fibrinolysis. Blood 86(8), 3035–3042 (1995).