Factor XI level is a determinant of plasma clot growth

C. Kluft [1], J. Begieneman[1], N. Podoplelova [2], N. Dashkevich [3], F. Ataullakhanov [3]

[1] Good Biomarker Sciences, Leiden, Netherlands, www.GBSLeiden.nl .
[2] Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia.
[3] Center for Theoretical Problems of Physicochemical Pharmacology, Moscow, Russia.

OBJECTIVES

- Clotting time in plasma in test tubes, started with contact activators homogeneously mixed in the plasma, is only markedly different for low levels of Factor XI.
- Clot formation from a surface coated with tissue factor, is another phenotype, with clot growth different from 0-100% Factor XI.
- We evaluated the characteristics of this growth process and its inhibition.

METHODS

Citrated plasma was depleted of microvesicles, and corn trypsin inhibitor and excess (4µM) lipids were added before recalcification was used to start the process together with exposure of the tissue factor bound to the surface.
Special plasma's, inhibitors and alkaline phosphatase were from commercial sources.

The layer formation can be analyzed for several variables which include:
(a) Thickness of the layer and rate of growth at each time point (notably at 2500 sec, fig 5,6)
(b) Thrombin formation in various layers of the clot (figure 4 A-C) at 0-4 mm distance from the TF-layer.

RESULTS

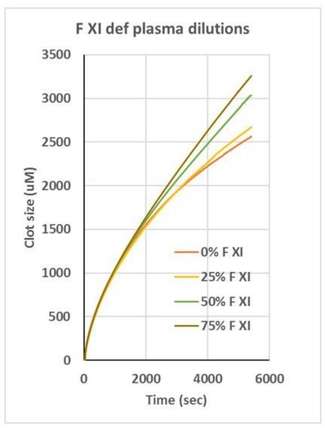

Figure 1: Clot growth in mixtures of pooled plasma with 100% FXI and FXI deficient plasma (mixing expressed in FXI %).

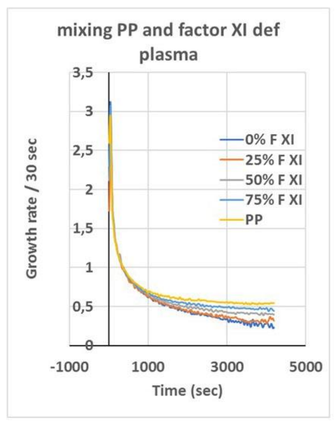

Figure 2: Clot growth rate (first derivatives) in mixture of pooled plasma and FXI def plasma (see figure 1).

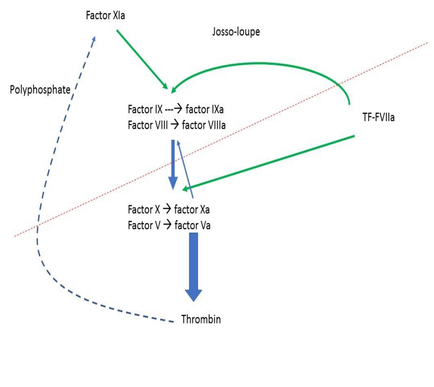

Figure 3: coagulation cascade
Below the dotted line = situation in Factor IX def plasma.

All factors in the scheme participate in normal plasma. Factor XII is excluded by addition of CTI.

Activation of Factor VIII is by Factor Xa

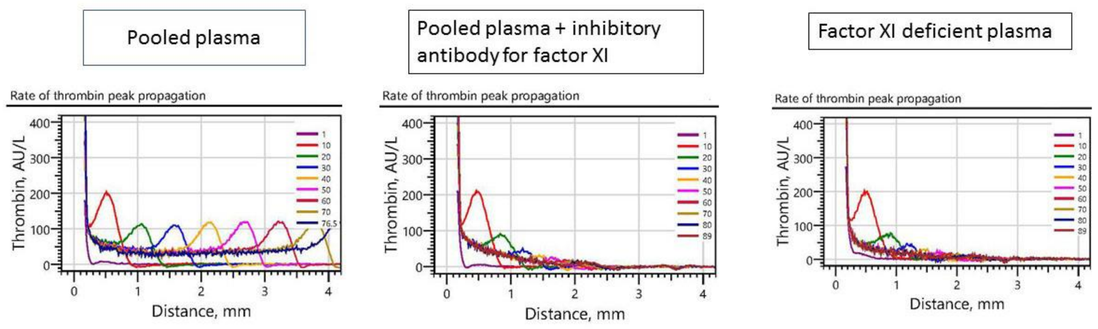

Figure 4 A-C: Thrombin peaks at various distances (X-axis) from the TF surface.
Thrombin substrate is present in the cuvette.
Legends at the right indicate timing of peaks

Figure 4 A-C:
4A = normal plasma
4B = normal plasma with excess Factor XI quenching antibody
4C = Factor XI deficient plasma

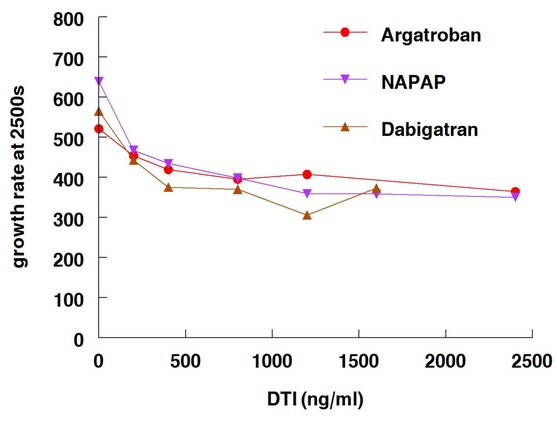

Figure 5: Dose effect of growth rate inhibition in pooled plasma by reversible thrombin inhibitors (DTI).
Final concentration in ng/ml on X-axis.

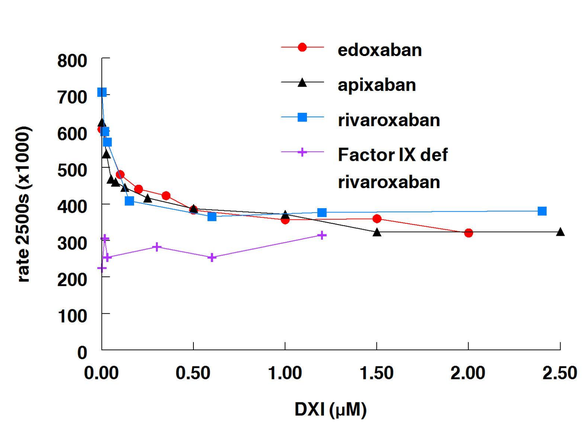

Figure 6: Dose effect of growth rate inhibition in pooled plasma by reversible Factor Xa inhibitors (DXI).
Example of effect of rivaroxaban in factor IX deficient plasma
Final concentration in µM on X-axis

- Factor XI contributes to prolonged clot growth causing a constant rate (figure 2). Effect is dependent on the level of Factor XI between 0-100%.
- Factor XI is activated by thrombin, requiring polyphosphate present in plasma. Addition of more has no effect, degrading polyphosphate by alkaline phosphatase (30 min 37°C) renders normal plasma similar to Factor XI deficient plasma (not shown).
- Absence of Factor XI, or inhibition by antibodies results in reduced or absence of thrombin formation in more distant layers (Figure 4 A-C).
- Inhibition by high dosage of CTI (23 µM) did not shown an effect; protease nexin II at high concentrations did inhibit Factor XIa contribution (erratum relative to abstract).
- Inhibition of thrombin is attributes to inhibition of activation of Factor XIa and similar for known reversible thrombin inhibitors (Figure 5)
- Inhibition of Factor Xa is attributed to prevention of Factor VIIIa formation, preventing Factor XI to exert effect (figure 6 and 3). For all reversible Factor Xa inhibitors effects in Factor IX deficient plasma is absent (example for rivaroxaban shown in Figure 6)

- Apparently Factor XI contribution to growth is lower with lower levels and can be inhibited by direct inactivation of Factor XI(a), inhibition of its activation by thrombin by thrombin inhibitors and degradation of polyphosphate, and by Factor Xa inhibitors preventing action of Factor XI further in the clotting cascade.

CONCLUSIONS

• The role of Factor XI in coagulation is expressed dose-dependently in clot growth and involves activation by thrombin supported by sufficient polyphosphate present in plasma. Alkaline phosphatase treatment removes this option.

• Factor XI contribution is inhibited directly by reversible thrombin and indirectly by Factor Xa inhibitors; the latter block the access to Factor X (see figure 3).

• It is suggested to employ clot growth as pharmacodynamic model for Factor XI contribution in haemostasis.

Contact details

Email

C. Kluft: kluft@kluft.in
J. Begieneman: j.begieneman@gbsleiden.nl
N. Podoplelova: podoplelovan@yandex.ru
N. Dashkevich: dashkevichnm@gmail.com
F. Ataullakhanov: ataullakhanov.fazly@gmail.com

You are invited to visit GBS-Leiden for a demonstration of the clot growth analysis and testing of interesting clinical samples.