Dabigatran and rivaroxaban inhibit thrombin generation much stronger than the growth of a fibrin clot

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BACKGROUND

Low amount of tissue factor (<5pM) produces in-vitro ~100 nM of thrombin, which is much greater than is required to form a clot. However, tissue factor is localized to a vascular wall, and thrombin concentration can decrease with distance. It is unknown how distribution of thrombin correlates with the rate of the fibrin cloth propagation from the damage site, particularly in the presence of anticoagulants.

CONCLUSIONS

Thrombodynamics-4D assay allows simultaneous measurement of clot growth and thrombin propagation in space from the local activator and reveals the differences between heparin, dabigatran and rivaroxaban on their effect on clot formation and thrombin distribution.

We showed that the amount of thrombin does not fully determine the fibrin clot formation process.

RESULTS

Spiking of normal plasma (*In-vitro*)

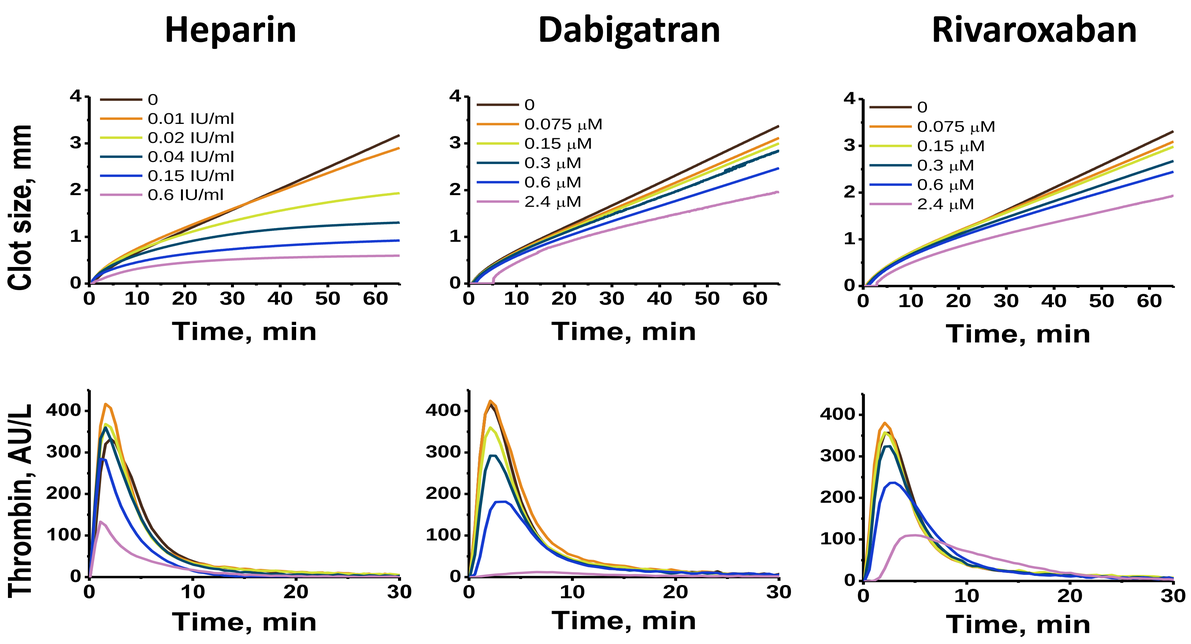


Fig.1 Effect of different anticoagulants on clot growth and thrombin generation on the TF-coated surface. Heparin does not induce the delay of thrombin generation, but has the strongest effect on the clot growth.

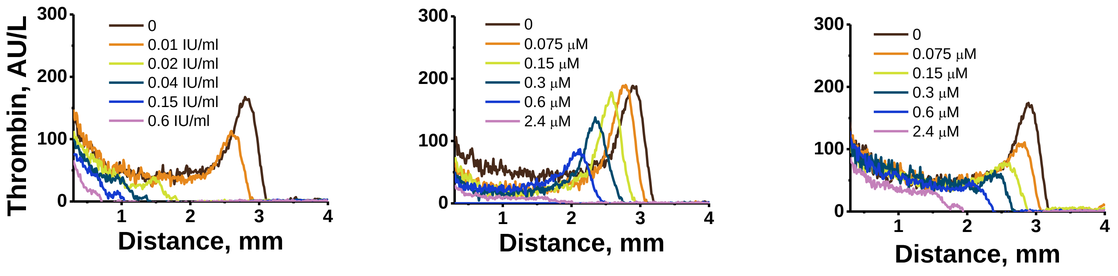


Fig.2 Effect of different anticoagulants on thrombin spatial distribution in 60 min after coagulation initiation. Anticoagulants demonstrate pronounced difference between the patterns of thrombin distribution.

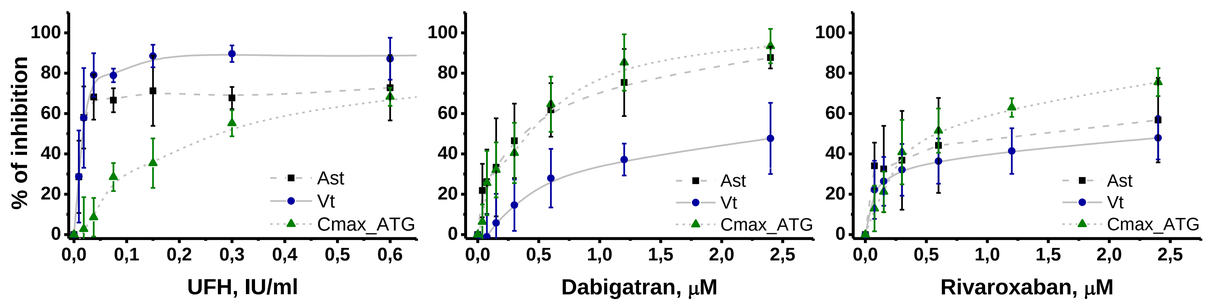
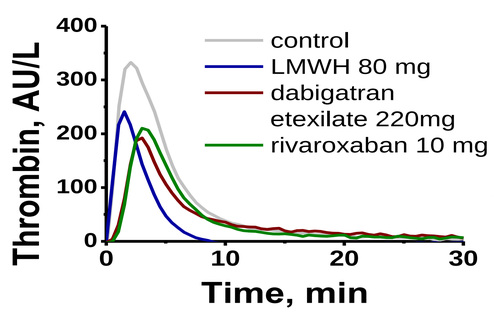


Fig.3 Effect of different anticoagulants on the maximal thrombin peak height on the activator and the moving peak height and its propagation rate. Mean and SD values for N=6-8 healthy individuals are shown.

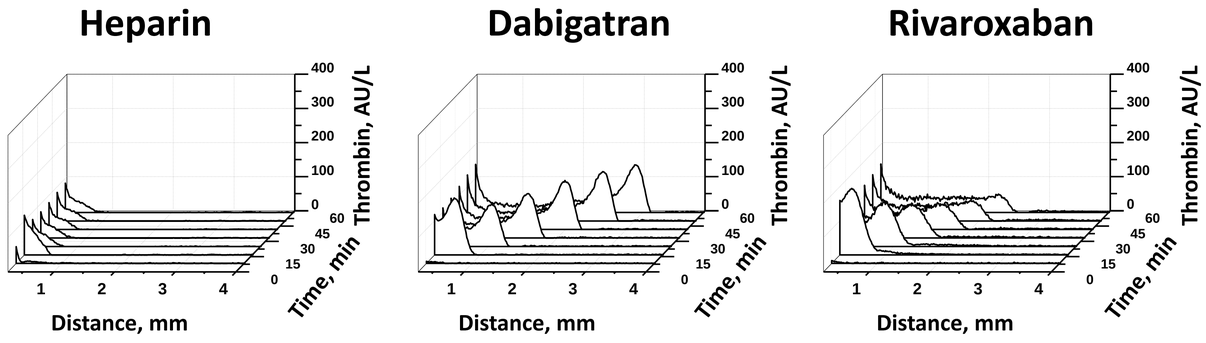
Patients after the orthopedic surgery (*Ex-vivo*)

Anticoagulant prophylaxis after total hip or knee replacement.

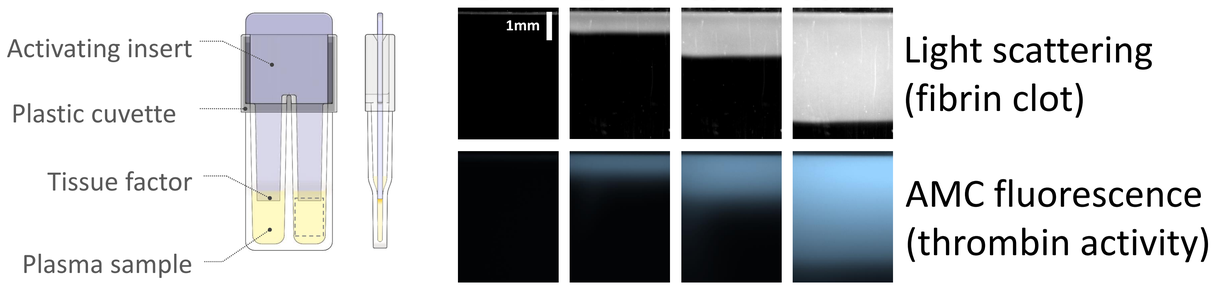
Ex-vivo effect of the anticoagulants on thrombin generation on the TF-coated surface and in space 3h after the drug administration.



Patterns of thrombin distribution differ between anticoagulants and are similar to those observed *in-vitro.*



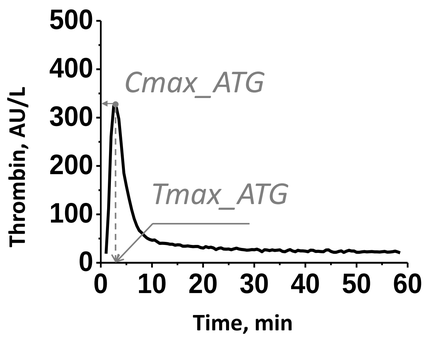
METHODS



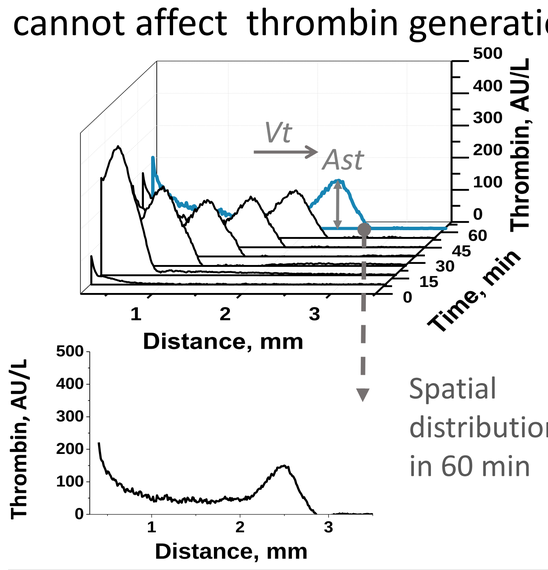
Thrombodynamics-4D assay. Plasma sample is supplemented with 4μM of phospholipids, 400 μM of fluorogenic AMC-based substrate and a contact phase inhibitor. Coagulation is activated in a thin layer of plasma by immobilized TF. AMC distribution in space and time is transformed into thrombin distribution [1, 2].

Initial thrombin and fibrin formation occurs on the TF-coated surface of the activator.

Thrombin generation curve is measured in the volume of plasma that has direct contact with TF (<0.2 mm from the surface).



In normal plasma thrombin further propagates in space from the activator as a moving peak followed by fibrin clot formation, while the TF-surface is covered with fibrin and cannot affect thrombin generation.



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Conflict of Interest Disclosure

N.D., R.O., Y. K., and F.A. are employees of HemaCore Labs LLC which is currently developing a diagnostic assay based on spatial dynamics of thrombin generation under the trade name of Thrombodynamics-4D

References

[1] Kondratovich et al BBA 2002
[2] Dashkevich et al, Biophys J 2012