Fibrinolysis in thrombodynamics

S. Talens[1], S. Naji[1], J. Begieneman[1], S. Karamzin[2], F. Ataullakhanov[3,4], C. Kluft[1]

[1]Good Biomarker Sciences, Leiden, the Netherlands, [2]Hemacore, Moscow, Russia, [3]Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia, [4]Center for Theoretical Problems of Physicochemical Pharmacology, Moscow, Russia.

Introduction

Traditional coagulation tests determine the clotting time, but do not completely reflect the complex cascade of coagulation and fibrinolysis. In addition, the spatial diffusion of plasma components is not considered, which can be analyzed using thrombodynamics, a novel global hemostasis test.

Aim

The aim of this study was to investigate the analysis of fibrinolysis using a thrombodynamics analyzer.

Materials and methods

Fibrinolysis was analyzed together with coagulation propagation using the thrombodynamics analyzer (Hemacore, Russia) by addition of tissue-type plasminogen activator (tPA, 200 ng/ml) to normal pooled plasma. The lysis parameters lysis initiation time (LOT, min) and the lysis progression (LP, %/min) were calculated by the thrombodynamics software. The clot lysis time (CLT) was the time between 50% clotting and 50% lysis, in minutes. The effect of thrombomodulin (TM) was analyzed in several deficient plasma's (PC-, PS-, TAFI-depleted) with added tPA (200ng/ml).

Results

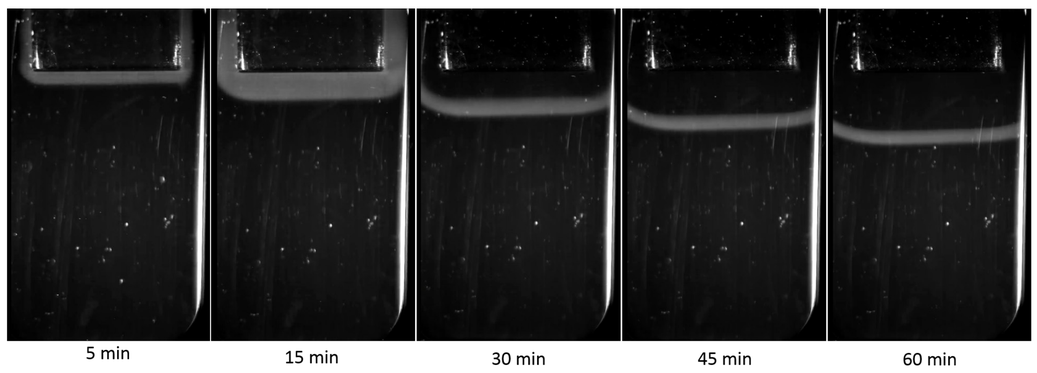

Fig 1: Representative images of thrombodynamics analysis in time of pooled normal plasma with added tPA (200 ng/ml), with a LOT of 24 (±3) min, a LP of 27 (±3) %/min and a CLT of 22 (±3) min (n=5). Fibrinolysis starts at point of activation and follows coagulation propagation.

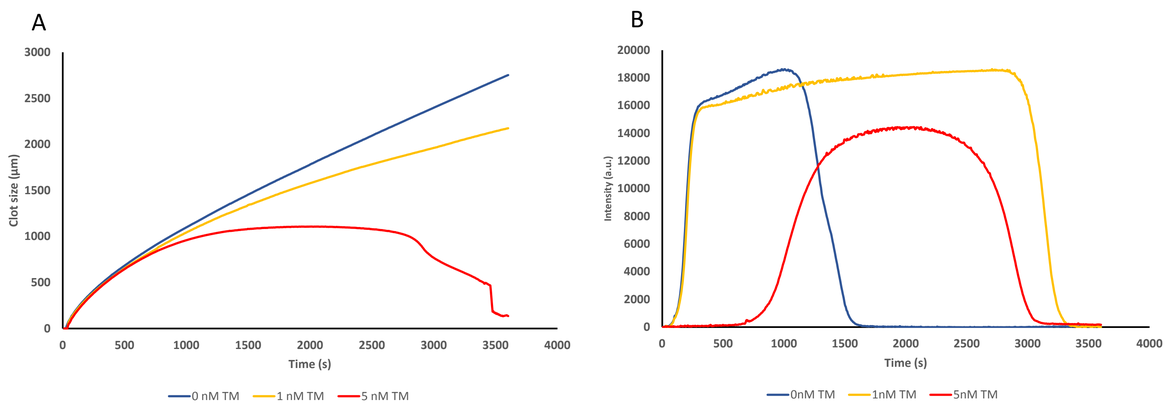

Fig 2: Clot growth (A) and fibrinolysis (B) curves of pooled normal plasma with added tPA (200 ng/ml) and TM (1 nM or 5 nM). With a LOT of 23 min, 51 min, 46 min, a LP of 27 min, 24 min, 14 min, a CLT of 19 min, 49 min and 29 min for 0 nM, 1 nM and 5 nM TM, respectively. TM decreases the clot growth, resulting in smaller clot and inhibits fibrinolysis, particularly at low concentrations. In the presence of TM the fibrinolysis occurs from the outside of the clot inwards.

Conclusions

- Fibrinolysis starts at the coagulation activation point and follows coagulation propagation in the absence of TM
- In the presence of TM the fibrinolysis is from the outside of the clot inwards
- The CLT of the clot decreases down from the activator site
- Regulators of fibrinolysis show expected effects as seen with other coagulation tests

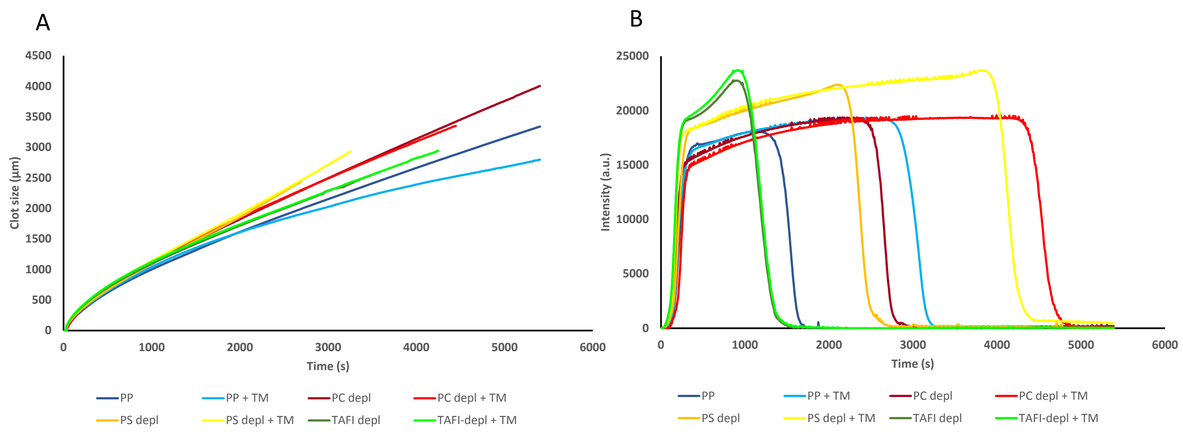

Fig 3: Clot growth (A) and fibrinolysis (B) curves of pooled normal plasma, PC-depleted plasma, PS-depleted plasma and TAFI-depleted plasma in the presence and absence of TM (1nM). The effects seen on fibrinolysis by the deficient plasma's are as expected.

	TM	LOT	LP	CLT
PP	-	24.6	27.7	22
	+	49.3	20.1	47.5
PC-depl	-	43.4	29.5	41.4
	+	74.6	22.2	71.9
PS-depl	-	38.6	26.8	35.6
	+	67.9	22.7	66.2
TAFI depl	-	18.7	20.3	17.3
	+	18.8	20.2	17.5

Table 1: LOT in min, LP in %/min and CLT in min from the different plasma's shown in figure 3.

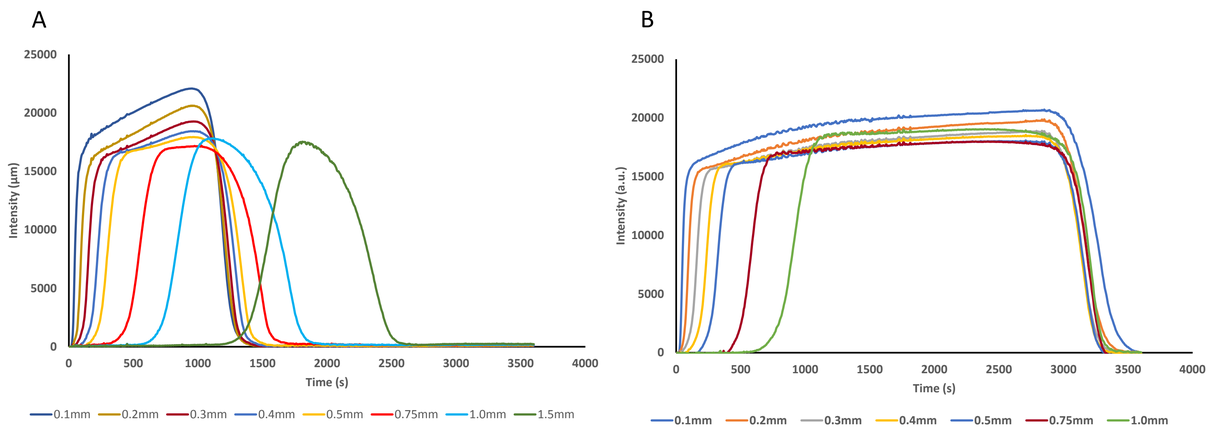

Fig 4: Intensity throughout the plasma clot, in mm from the activator insert. The intensity is shown for normal pooled plasma with added tPA (200ng/ml) (A) and with added tPA (200ng/ml) and TM (1nM) (B). In general, the further down in the clot the faster the fibrinolysis, both in absence and presence of TM.

	TM	LOT	LP	CLT
0.1mm	-	19.1	33.3	19
	+	52.9	18.3	53.6
0.2mm	-	19.3	31.8	18.6
	+	51.7	22.3	51.4
0.3mm	-	19.7	32.4	18
	+	51.2	23.4	49.7
0.4mm	-	20.3	29.5	17.5
	+	51.2	24.5	48.4
0.5mm	-	21	27.4	17.1
	+	51.4	25.6	47.2
0.75mm	-	22.7	20	15.1
	+	52	25.6	43.6
1.0mm	-	25.8	14.7	13.9
	+	52.2	25.9	38.2
1.5mm	-	36.6	14	13.3
	+	-	-	-

Table 2: LOT in min, LP in %/min and CLT in min from the different distances from the activator through the clot, shown in figure 4.

Contact:
s.talens@gbsleiden.nl
kluft@kluft.in