Influence of Fibrinolysis System on ADP- and Serotonin-Induced Aggregation of Human Platelets

M. Yu. Men'shikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 2, pp. 158-161, February, 1998
Original article submitted December 26, 1996

Urokinase, a component of the fibrinolytic system, induces a time-dependent decrease in platelet aggregation activated by ADP and serotonin. Significant inhibition of ADP-induced aggregation was observed on the 30th-60th min and serotonin-induced on the 3th-10th min of preincubation with urokinase and depended on urokinase concentration. The plasmin inhibitor aprotinin partially abolished urokinase-induced reduction of the amplitude and rate of ADP-induced aggregation and had no effect on serotonin-induced aggregation. Our results favor multiple mechanisms of urokinase influence on platelet activity.

Key Words: *urokinase; platelets; aggregation*

Platelets, together with the coagulant and anticoagulant blood systems and the fibrinolytic system, are one of basic factors of homeostasis. Platelet-mediated thrombus formation is initiated by the inducers of aggregation causing change in shape, aggregation, and release reaction [15].

Platelets, in addition to their role in thrombus formation as structural component, also regulate thrombus formation and lysis: on the one hand, their granules contain a number of coagulants and fibrinolysis-inhibiting factors [4], on the other hand, their membranes have sites for the binding and activation of the fibrinolytic system components: plasminogen, plasmin, and urokinase-type and tissue-type plasminogen activators [5,7,14].

The main biological function of fibrinolytic system is the lysis of fibrin clots. This function is performed by plasmin-serine protease that is formed under the influence of urokinase- or tissue-type plasminogen activators. Urokinase-mediated activation of plasminogen is linked to cell migration, embryogenesis, malignization, and other biological processes [13].

Plasmin modifies platelet functions, affecting the rate of intracellular processes, aggregation, and release reaction [2,3,8,10]. These effects may be associated with structural components (glycoproteins Ib and IIb/IIIa) and plasma factors participating in aggregation [9,12].

It is difficult to define the unique one mechanism by which the fibrinolytic system affects platelet activation. The possibility cannot be exculed that fibrinolytic agents have several different sites for action, wich is associated with specific stimulation of platelets by individual inducer of aggregation.

We examined the effect of urokinase-type plasminogen activator on platelet aggregation induced by ADP and serotonin, which differ by the mechanism of action on platelets.

MATERIALS AND METHODS

Experiments were performed on platelet-rich plasma (PRP). For preparation of PRP blood from the untecubital vein of healthy donors was collected into 130 mM trisodium citrats (pH 7.4) 9:1 and then centrifuged at 190*g* for 15 min. After PRP separation, the residual blood was centrifuged again (2000*g*, 15 min) to prepare platelet-poor plasma (PPP).

Platelet aggregation was measured in PRP at 37°C with constant stirring in 300 μl aliquots in 5-mm glass quvettes. A Biola 230 LA two-channel laser

Institute of Cardiology, Cardiology Research Center, Ministry of Health, Moscow

0007-4888/98/0002-0135$20.00

analyzer of aggregation (Cardiology Research Center) equipped by a thermostat, cell counter, and computer was used for measurements. Special software allowed us to record simultaneously lightabsorbance of cell suspension and the average radius of platelet aggregates by analysing lightabsorbance fluctuations [1]. Lightabsorbance of PPP was taken as 100%. In the beginning of each experiment platelet concentration was measured and adjusted to a final concentration of 2×10^5/μl by dilution with PPP.

The amplitude and the rate of ADP-induced platelet aggregation were estimated by light absorbance of PRP. Platelet response to serotonin was assessed by changes in average radius of platelet aggregates.

To examine the effect of urokinase on ADP- and serotonin-induced aggregation, PRP without urokinase preincubated under identical condition (37°C) for the same time was used as control. In some experiments aprotinin was added in the incubation mixture immediately before urokinase.

High-molecular-weight recombinant urokinase-type plasminogen activator was produced at the Laboratory of Genetic Engineering (Cardiology Research Center). ADP, serotonin, dihydrous Na_3 citrate, and aprotinin (10,000 U/ml) were obtained from Sigma.

RESULTS

Urokinase-type plasminogen activator inhibits ADP- and serotonin induced platelet aggregation. A decrease in the amplitude and the rate of platelet response depends on the urokinase preincubation time (Fig.1).

Significant inhibition of ADP-induced aggregation was observed on the 30th-60th min and of serotonin-induced aggregation on the 3th-10th min of preincubation. The inhibition depended on urokinase concentration. Persistent and reproducible decrease in ADP-induced aggregation required prolonged preincubation (30-60 min) with urokinase (Fig. 1, *a*). At the same time, a weaker and reversible serotonin-induced aggregation required a 3-10-min preincubation with urokinase for sufficient inhibition which can be observed up to 50 min of preincubation time (Fig. 1, *b*).

For equal preincubation times the effect of urokinase depends on its concentration. The minimal concentration of urokinase that significantly decreased amplitude and rate of ADP-induced aggregation after 50-min preincubation was 35 nM (Fig. 2, *a*). At the same time, the decrease in the maximum amplitude and the rate of serotonin-induced aggregation for the same urokinase concentration was observed after a 10-min preincubation (Fig. 2, *b*).

According to current views, influence of plasminogen activator on cell activity is mediated by plasmin synthesis [2]. We suppose that in our experiments the inhibitory effect of urokinase-type plasminogen activator is mediated by the plasminogen-plasmin transformation followed by proteolytic modification of plasma components participating in aggregation or platelets.

There are several options to explore this suggestions, the use of the plasmin inhibitor aprotinin [2,6,11] is one of them.

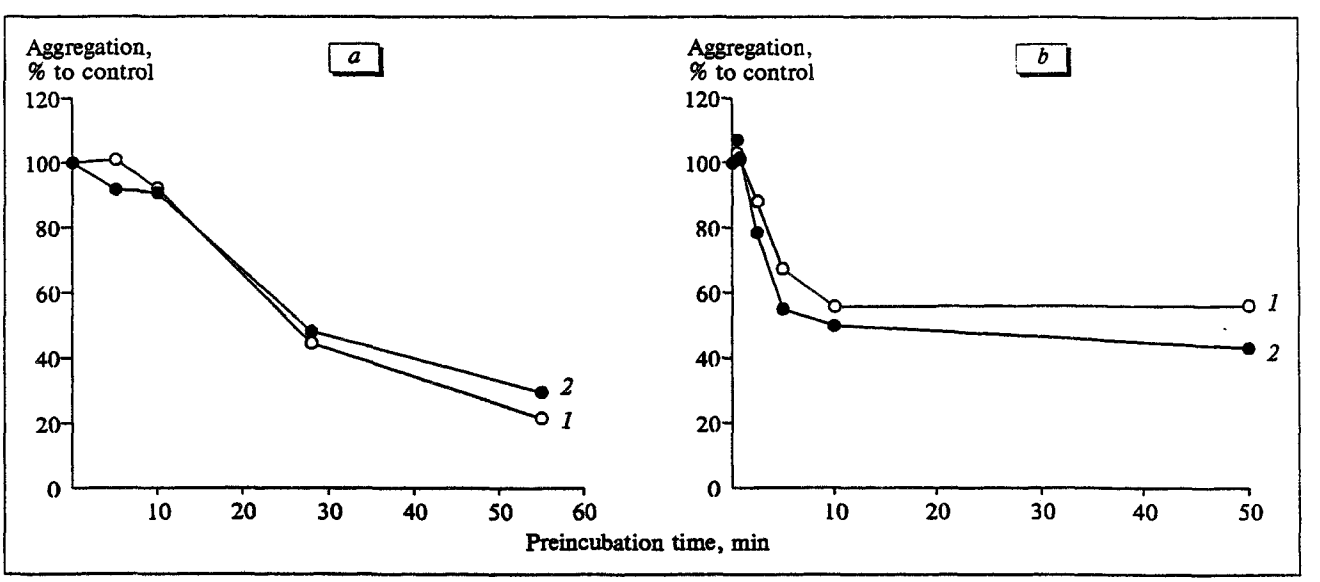

Fig. 1. Dependence of platelets aggregation on preincubation time with 200 nM urokinase. Aggregation induced by 3 μM ADP (*a*) and 5 μM serotonin (*b*). Amplitude (*1*) and rate (*2*) of platelets aggregation after addition of the inducer.

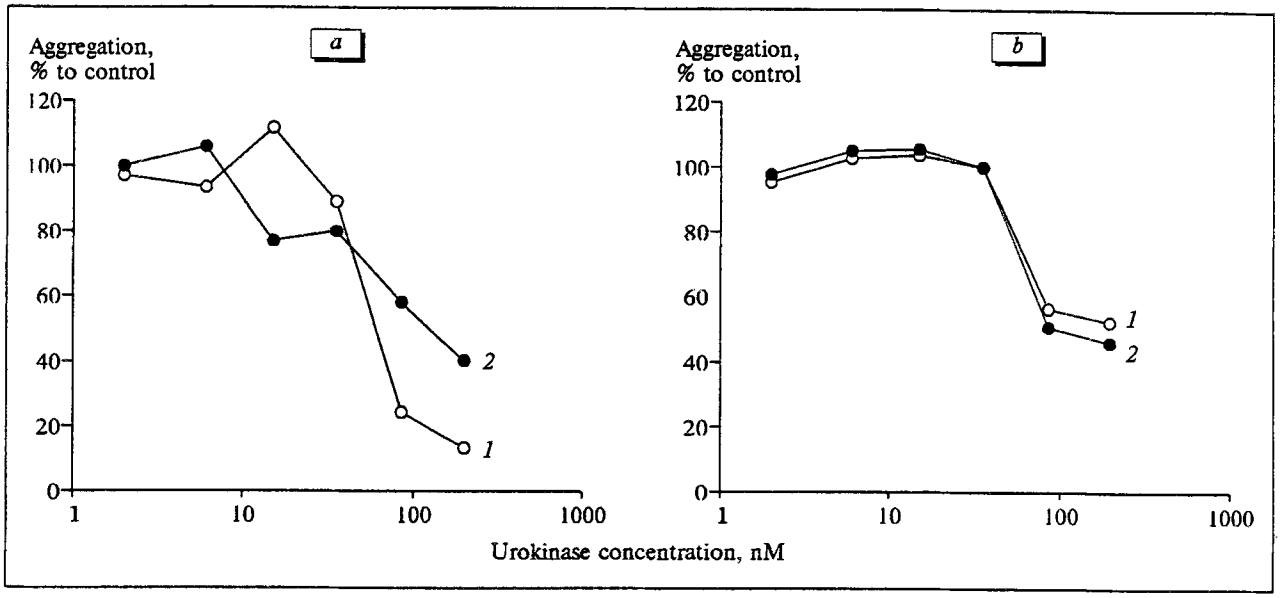

Fig. 2. Dependence of platelets aggregation on urokinase concentration. *a*) aggregation induced by 3 μM ADP after 50 min preincubation with urokinase; *b*) aggregation induced by 5 μM serotonin after 10 min preincubation with urokinase.

As shown in Table 1, aprotinin modifies the effects of urokinase on ADP-induced aggregation and has no effects on urokinase inhibition of serotonin-induced aggregation. It can be suggested that the influence of urokinase on platelet response to serotonin does not strongly depends on plasmin synthesis and is mediated by other urokinase-coupled mechanisms.

Thus, platelet aggregation induced by different activators varies considerably in the sensitivity to urokinase and mechanisms of its influence. One of possible reasons for such differences is the existence of several mechanisms mediating the influence of urokinase on cell activity.

The study was supported by the Russian Foundation for Basic Research (Project No. 96-04-49206).

REFERENCES

1. Z. A. Gabbasov, E. G. Popov, I. Yu. Gavrilov, *et al.*, *Laboratornoe Delo*, No. 10, 15-18 (1989).
2. L. Y. Chen, J. L. Mehta, *Thromb. Res.*, **74**, No. 6, 555-563 (1994).
3. M. Yamaguchi, L. A. Bunce, L. A. Sporn, and C. W. Francis, *Blood*, **84**, No. 4, 1143-1150 (1994).
4. P. Harrison and E. M. Cramer, *Blood Rev.*, 7, No. 1, 52-62 (1993).
5. Y. Jiang, R. Pannell, J.-N. Liu, and V. Gurevich, *Blood*, **87**, No. 7, 2775-2781 (1996).

Table 1. Influence of Urokinase and Aprotinin on Platelet Aggregation (*M±m*)

Experimental conditions		Aggregation index	
		amplitude	rate
ADP-induced aggregation	control	100±5.1	100±5.6
	urokinase	30.0±7.7	40.4±4.3
	aprotinin	90.6±3.6	93.8±5.5
	aprotinin+urokinase	66.4±3.6	68.2±3.7
Serotonin-induced aggregation	control	100±4.8	100±12.5
	urokinase	69.2±1.1	63.8±5.9
	aprotinin	86.7±7.9	64.6±3.3
	aprotinin+urokinase	60.6±2.6	35.1±8.9

Note: Parameters of platelet aggregation were measured after preincubation of PRP at 37°C without urokinase and aprotinin (control), in the presence of urokinase (200 nM) or aprotinin (400 U/ml), or both. Preincubation time before adding ADP (3 μM) was 50 min and before adding serotonin (5 μM) was 10 min.

6. M.A. Orchard, C. S. Goodchild, C. R. Prentice, *et al., Br. J. Haematol.*, **85**, No. 3, 533-541 (1993).
7. H. Ouimet and J. Loscalzo, *Thromb. Res.*, **70**, No. 5, 355-364 (1993).
8. R. Paolini, A. Casonato, G. Boeri, *et al., J. Med.*, **2**, No. 2-3, 113-130, (1993).
9. B. Pasche, H. Ouimet, and S. Francis, *J. Loscalzo, Blood*, **83**, No. 2, 404-414 (1994).
10. W. F. Penny and J. A. Ware, *Ibid.*, **79**, No. 1, 91-98 (1992).
11. B. D. Royston, D. Royston, and J. D. Pearson, *Blood. Cell. Fibrinolysis*, **3**, No. 6, 737-742 (1992).
12. R. B. Stricker, D. Wong, D. T. Shiu, *et al., Blood*, **68**, No. 2, 1280-1284 (1986).
13. J. D. Vassali, *Fibrinolysis*, **8**, Suppl. 1, 172-181 (1994).
14. C. Watala, T. Pietrucha, E. Dzieiatkowska, *et al., Chem. Biol. Interact.*, **89**, No. 2-3, 115-127 (1993).
15. M. B. Zucker and V. T. Nachmias, Arteriosclerosis, **5**, No. 1, 2-18 (1985).