COAGULATORY AND FIBRINOLYTIC PROPERTIES OF PLATELETS IN RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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The thromboplastic activity of the platelets is lowered and their antifibrinolytic activity increased in rabbits with experimental cholesterol atherosclerosis. The changes in antifibrinolytic activity are due mainly to an increase in the content of substances with antiactivatory properties.

It is stated that the coagulatory properties of human platelets are modified in atherosclerosis [3, 4, 7, 17]. The object of the present investigation was to study the coagulatory and fibrinolytic properties of the platelets in rabbits with experimental atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 117 chinchilla rabbits, 61 of which received cholesterol with their food in a dose of 0.4 g/kg body weight daily for 4-6 months; 56 rabbits constituted the control group. By the end of the experiment, the serum cholesterol concentration in the rabbits receiving the atherogenic diet had risen to 500-1200 mg%. The tests were performed on platelets washed to remove plasma and on two fractions of platelets: fraction I, a thrombolysate; and fraction II, a suspension of platelet stroma.

Blood for analysis was taken from the marginal vein of the ear into cooled test tubes containing 1.34% sodium oxalate solution in the ratio of 4:1, and centrifuged at 4° for 15 min at 1000 rpm. Platelets were sedimented from the resulting platelet-rich plasma by repeated centrifugation for 30 min at 2000 rpm. The platelets were resuspended in 5 ml cold 1.34% sodium oxalate solution. The platelet suspension was centrifuged briefly at 1000 rpm for 3-5 min to remove any possible contamination by leukocytes and erythrocytes. The platelets were then washed twice with cold sodium oxalate solution and once with cold physiological saline. The final concentration of washed platelets in physiological saline was between 28,000 and 300,000/mm^3. The platelets were disintegrated by freezing and thawing three times. The supernatant obtained after centrifugation for 30 min at 8000 rpm was conventionally described as fraction I. The residue, containing platelet stromas, was washed six times with cold physiological saline (centrifugation each time at 8000 rpm for 30 min). The suspension of stroma was described as fraction II. All the apparatus used for the work with platelets was silicone-treated.

The coagulatory (thromboplastic) activity of the washed platelets and their fractions was determined from shortening of the recalcification time of fresh, platelet-free rabbit plasma after addition of the test material to it. The result was expressed as a percentage of the control time. Thrombin accelerator (platelet factor II) was detected from the shortening of the thrombin time of a bovine plasma substrate (a constant concentration of thrombin solution was always used, 1 unit/ml). Antiheparin activity was determined by a modified Jurgens' method [3]. The fibrinolytic properties of the platelets and their fractions were studied by the method of heated and unheated fibrin films [8]. To detect plasminogen proactivator,

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TABLE 1. Indices of Coagulatory and Fibrinolytic Activity of Platelets of Rabbits with Experimental Atherosclerosis

Index	Control rabbits						Experimental rabbits								
	susp. of platelets		fraction I		fraction II		suspension of platelets			fraction I			fraction II		
	n	$M \pm m$	n	$M \pm m$	n	$M \pm m$	n	$M \pm m$	P	n	$M \pm m$	P	n	$M \pm m$	P
Coagulatory (thromboplastic) activity (in %)	21	55,6±2,8	28	44,8±2,6	17	55,9±2,8	21	44,6±2,8	<0,02	33	32,2±2,5	<0,001	22	47,0±2,1	<0,02
Antithrombin activity	21	0	22	0	22	0	21	0	—	22	0	—	22	0	—
Platelet factor II (in sec)	21	2,8±0,5	22	2,7±0,5	22	2,9±0,5	21	2,0±0,6	>0,5	22	2,5±0,4	>0,5	22	2,57±0,54	>0,5
Antiheparin activity (in %)	22	57,0±3,6	25	63,3±3,2	16	57,2±4,2	24	50,4±3,3	<0,2	29	62,1±3,1	>0,5	20	58,3±2,2	>0,5
Plasmin	28	0	31	0	31	0	31	0	—	34	0	—	34	0	—
Plasminogen activator	28	0	31	0	31	0	31	0	—	34	0	—	34	0	—
Plasminogen proactivator	28	0	31	0	31	0	31	0	—	34	0	—	34	0	—
Total anti-fibrinolytic activity (index)	21	1,57±0,05	32	1,50±0,05	20	0	22	2,01±0,08	<0,001	37	2,05±0,04	<0,001	25	0	—
Antiplasmins (in %)	35	0	28	0	24	0	26	0	—	32	0	—	28	0	—
Antiactivators (in %)*	20	54,2±4,8	27	56,9±3,6	15	0	21	80,4±4,4	<0,001	30	76,2±4,0	<0,01	18	0	—
Antiactivators (in %)	14	36,4±3,4	14	41,2±3,1	14	0	14	60,8±3,6	<0,001	14	62,1±4,4	<0,01	14	0	—
Fibrin-stabilizing factor (index)	23	1,06±0,03	28	1,06±0,03	28	0	25	1,06±0,03	0	33	1,07±0,02	>0,5	33	0	—

Note. P calculated for difference between arithmetic mean values for control and experimental animals.

*Aqueous extract of adventitia of human aorta used as source of plasminogen activator.

†Extracts from pig's heart, prepared in potassium thiocyanide solution, used as source of plasminogen activator.

an equal volume of streptokinase solution with an activity of 250-1000 units/ml was added to the samples. Total antifibrinolytic activity was determined from the degree of lengthening of the time required for lysis of prepared clots in the presence of the euglobulin fraction of platelet-free dog's plasma [6]. The result was expressed by an index calculated by dividing the time of lysis of the clot in the experimental sample by the time of its lysis in the control. Antiplasmin activity was estimated from changes in the zones of lysis of heated bovine fibrin films by a solution of fibrinolysin (800-1500 units/ml) preliminarily mixed with an equal volume of physiological saline in the control and with the test material in the experimental sample [6]. The results were expressed as percentages. The content of antiactivators was determined in a similar manner, but unheated bovine fibrin films were used, and the source of plasminogen activator was aqueous extracts of the adventitia of the human aorta and extracts from a pig's heart prepared in potassium thiocyanate solution [9]. Fibrinase activity of the platelet material was determined by the method of Baluda et al. [2]. The calculation was made just as for the index of antifibrinolytic activity.

EXPERIMENTAL RESULTS

Platelets washed free from plasma, and their two fractions, considerably accelerated coagulation of recalcified platelet-free plasma, due chiefly to the fact that the platelets contain platelet factor III (thromboplastic). The stroma of the platelets evidently contains more of this factor (Table 1). The procoagulant activity of the platelets was decreased in rabbits receiving cholesterol. This was observed in experiments both with the suspension of washed platelets and with the two fractions ($P < 0.02$).

No antithrombin substances were detected in rabbit's platelets. On the addition of platelet material to substrate plasma, not only was the thrombin time not prolonged, as it would have been had the platelets contained antithrombins, but on the contrary, it was shortened. This result indicates that platelets contain thrombin accelerator (platelet factor II). No significant abnormality in the activity of this factor was found in the rabbits with hypercholesteremia. Similar results were obtained for platelet factor IV (antiheparin) (Table 1).

Particular attention was paid to the investigation of the fibrinolytic properties of the platelets, for they have received least study. Fibrinolytically active substances (plasmin, plasminogen proactivator and activator) were not found in the rabbit's platelets. Antifibrinolytic substances which, in the mechanism of their action, were of the antiactivator type, were detected. These were found only in fraction I; fraction II containing mostly the stroma of the platelets, did not possess any inhibitory action on fibrinolysis. No antiplasmins were found in the rabbit's platelets, and only traces of fibrinase could be detected.

Antifibrinolytic activity of the platelets in animals receiving a cholesterol-rich diet was considerably increased. Special methods of investigation showed that this was due to an increased content of antiactivators (Table 1).

The thromboplastic activity of the platelets is thus lowered and their antifibrinolytic activity increased in rabbits with experimental atherosclerosis. With respect to the antifibrinolytic activity of the platelets, similar results were obtained in man by Gushchina [3] and Korotkova [4]. However, these workers did not establish which components were responsible for the increase in antifibrinolytic activity of the platelets. So far as the decrease in thromboplastic activity of the platelets in atherosclerosis is concerned, no relevant data could be found in the literature. However, reports have been published which describe a decrease in the clotting activity of the blood plasma of rabbits with alimentary cholesterol atherosclerosis [15, 16]. Similar results have also been obtained by studies of the walls of arteries affected by atherosclerosis [1, 5, 10, 14]. The mechanism of weakening of thromboplastic activity of the platelets in the plasma and arterial walls is probably the same. The writers suggest that this is due mainly to changes in the composition of the phospholipids: an increase in the content of sphyngomyelin, which possesses a marked antithromboplastic action, and a decrease in the components of the cephalin fraction, responsible for the thromboplastic effect [11, 12].

The mechanism of increase of the antifibrinolytic activity of the platelets in atherosclerosis remains unexplained. It may perhaps be due to the accumulation of β-lipoprotein complexes, which have the property of inhibiting fibrinolysis [16, 17].

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