PROTECTIVE EFFECT OF SIMULTANEOUS ADMINISTRATION OF FIBRINOLYSIN WITH HEPARIN AGAINST THROMBUS FORMATION IN ANIMALS IN THE PRETHROMBOTIC STATE AND IN EXPERIMENTAL ATHEROSCLEROSIS

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It has been established [4, 5] that the intravascular coagulation of blood occurs in animals by the depression of a physiologic anticoagulation system. The condition of depression may be provoked by various stimuli [2, 6]. It has been shown that in the experimental animal the concentration of antihemophilic globulin in the blood rises sharply. The administration of a lethal dose of thrombin evokes intravascular clotting and subsequent death [6].

The state of depression of the anticoagulation system, evidently, is expressed in the inability of the organism to neutralize exogenous or endogenous thrombin and to lyse fibrin clots.

The question arises: is it impossible to administer the corresponding agents of the anticoagulation system in order to imitate the state of its physiological functioning in the sick organism?

In this work are presented the results of experiments dealing with the prophylactic administration of heparin and fibrinolysin to animals which were in the prethrombotic state and in experiiental atherosclerosis.

METHODS

The experiments were performed on white male rats weighing 280-360 g, maintained both on the natural rations and on an experimental fat diet according to Wilgram [11], and on rabbits with atherosclerosis experimentally produced by feeding them cholesterol for 20 weeks. In all experimental rats on the day prior to the experiment all signs of the prethrombotic state were established. The fibrinogen level and the fibrinolytic activity of the blood were determined by the method of Bidbell [1] and the blood plasma tolerance to heparin by the method of Gormsen [9]. The total cholesterol content in the blood was studied according to Grigant [10], the level of antihemophilic globulin in the blood by the method of Pool and Robinson as modified by our laboratory [7].

The experimental rats were divided into four groups: 23 rats in the first group received intravenous mixture of 2.2 units of heparin and 100 units of fibrinolysin per 100 gm of body weight or 2.2 units of heparin and 400 units of fibrinolysin subsequently with a five-minute interval;* 19 animals in the second group received intravenously only the heparin solution in a dose of 2.2 units; the third group—only the fibrinolysin solution in a dose of 100 units, the fourth group—physiological saline.† Within 5 min a thrombin solution calculated as a mean of 0.4 ml per 100 g of body weight was administered to all four groups.

Heparin was used as a solution from the commercial Gedeon—Richter preparation. Thrombin isolated from horse blood was used, with an activity of 3-4 sec and fibrinolysin as a preparation obtained from the III fraction of placental blood serum by the method of G. V. Andreenko and B. A. Kudryashova.

*Results of survival are equal, therefore the animals are combined into one group.
†In addition, there was still a controlled (fifth) group of animals.

TALBE 1. Content of Fibrinogen, Cholesterol and Antihemophilic Globulin (AHG), Fibrinolytic Activity and Blood Plasma Tolerance to Heparin in Rats Maintained on an Atherogenic Diet (Mean Data)

Group of animals	Mean weight of animals (in g)	Fibrinogen concentration (in mg %)	AHG (in %)	Fibrinolytic activity (in %)	Blood plasma tolerance to heparin	Blood cholesterol content (in mg %)
Experimental (70 rats) Rats in the pre-thrombotic state provoked by prolonged (5 month) use of an atherogenic fat diet according to Wilgram	260	671	429	14.7	2 min 39 sec	719
Control (40 rats) Normal rats of the same age maintained on the regular laboratory rations	270	519	109	33.5	14 min 10 sec	125

TABLE 2. Prophylactic Protective Effect of Fibrinolysin in Combination with Heparin during Intravenous Injection of Lethal Doses of Thrombin into Rats Maintained on an Atherogenic Diet

Group	Preparation used	Mean weight of animals (in g)	Number of animals			Lethality (in %)
			total	survivors	dead	
1st	Heparin + fibrinolysin + thrombin	300	23	18	5	21.6
2nd	Heparin + physiologic saline + thrombin	280	19	13	6	31.6
3rd	Physiologic saline + fibrinolysin + thrombin	280	18	12	6	33
4rd	Physiologic saline + thrombin	284	17	5	12	70.6

Rabbits, separated into similar groups as the rats were given a five ml mixture of fibrinolysin and heparin from a calculation of 150 units of fibrinolysin and 2.2 units of heparin per ml and heparin and fibrinolysin separately in the same doses. The dose of thrombin contained 1.25 ml per kg of rabbit body weight.

RESULTS

The prethrombotic state in the experimental rats was indicated by the fact that they showed, in comparison with the control, a higher concentration of fibrinogen, greater heparin tolerance of the blood plasma, higher concentration of antihemophilic globulin, and reduced fibrinolytic activity of the blood (Table 1).

The simultaneous administration of fibrinolysin with heparin to the experimental white rats appeared to have a good protective effect against a fatal amount of thrombin solution (Table 2). With a statistical treatment of the results by the "chi-square" test the proof of their validity was established.

Administration of heparin and fibrinolysin in the above indicated doses separately also appeared to have a protective effect but in lesser degree than with simultaneous injection, although the difference with the first group appeared statistically negligible.

The administration of thrombin to the control animal, which receive only physiologic saline, produced death in 70% of cases. In all dead rats thrombin was found in the veins of the liver, kidney, pulmonary arteris, aorta and pericardium.

The protective effect was also seen with simultaneous injection of fibrinolysin and heparin to experimental rabbits with experimental atherosclerosis during the administration of thrombin (Table 3). All rats which had received fibrinolysin and heparin lived. The majority of the control animals which received heparin, fibrinolysin and physiological saline separately, died.

TABLE 3. Survival of Rabbits with Experimental Atherosclerosis after Intravenous Injection of Thrombin Against a Background of Fibrinolysin and Heparin Injected Together and Separately

Group	Preparation used	Number of animals		
		total	survivors	dead
1st	Heparin + fibrinolysin + thrombin	7	7	0
2nd	Heparin + physiologic saline + thrombin	7	2	5
3rd	Physiologic saline + fibrinolysin + thrombin . .	5	3	2
4rd	Physiologic saline + thrombin	8	1	7
5th (control—normal animals)	Thrombin + physiological saline	17	12	5

The greater protective effect of simultaneous administration of fibrinolysin and heparin is explained by the fact that inclusion of the latter in the medium where the fibrinolytic process occurs, hastens clot lysis [5]. In this regard heparin, evidently, inhibits the effect of antifibrinolysin and opens a pathway to the interaction of fibrinolysis with fibrinogen, which leads to relative hypofibrinogenemia and decreases the possibility of thrombogenesis during the injection of thrombin [8].

The separate injection of animals with fibrinolysin and heparin is less effective, because the optimal conditions are not created for the imitation of the reaction of the physiologic anticoagulation system.

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