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EFFECT OF THYMALIN AND HETEROLOGOUS TRANSFUSION ON BLOOD CLOTTING AND FIBRINOLYSIS IN THYMECTOMIZED RATS

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UDC 616.438-089.87-092.9-07:[616.151.5+616.153.962.4]-02:[615.38-615.361.438

KEY WORDS: thymalin; thymectomy; hypercoagulation; fibrinolysis.

The writers showed previously that in thymectomized rats 10 min after transfusion with heterologous (human) blood hypocoagulation develops rather more intensively but fibrinolysis is activated to a lesser degree [13, 14]. These findings can be explained to some extent on the grounds that after removal of the thymus in rats destruction of heterologous erythrocytes takes place less intensively and, consequently, the process of intravascular blood clotting, which arises in heterologous transfusion shock, follows a milder course [7, 8, 13]. A low-molecular-weight factor, with a modulating action on the state of cellular immunity, has been isolated from the thymus [9, 10]. This compound, to which the name "thymalin" has been given, subsequently has achieved wide application in experimental and clinical medicine [2, 5, 10]. It has been shown, in particular, that thymalin restores adequate responses of the hemostasis system in thymectomized animals to adrenalin [5], thrombin [3], and histamine [4].

In the investigation described below the state of the blood clotting system and of fibrinolysis was studied in thymectomized animals with heterologous transfusion shock treated beforehand with thymalin.

EXPERIMENTAL METHOD

Experiments were carried out on 46 rats: 28 experimental (thymectomized at the age of 1.5 months) and 18 control. All the experiments were carried out 3 months after removal of the thymus, when a frank deficiency of cellular immunity had developed.

For a period of 1 week 10 thymectomized rats were given intramuscular injections of 1 mg thyamlin (Lenmyasoprom Medical Preparations Factory) in 0.5% procaine solution intramuscularly in a dose of 1 mg. The control animals, both intact and thymectomized, received a corresponding dose of procaine. The jugular vein was exposed in all the animals under superficial ether-halothane anesthesia and 2-3 ml blood was withdrawn into 0.2-0.3 ml of 3.8% sodium citrate solution. The intact animals (control) and thymectomized rats not receiving (experiment 1) and receiving (experiment 2) thymalin, were all given an injection

Department of Normal Physiology, Chita Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 2, pp. 26-28, February, 1983. Original article submitted April 12, 1982.

 0007-4888/83/9502-0192$07.50

TABLE 1. State of Hemostasis in Control and Thymectomized Rats before (A) and after (B) Injection of Heterologous Blood

Parameter studied	Control		Thymectomized rats (experiment 1)		Thermectomized rats receiving thymalin (experiment 2)	
	A	B	A	B	A	B
Blood clotting time, sec	247,4±9,48	289,8±17,16*	165,68±7,41†	260,0±19,2*	230,6±12,36	288,67±16,07*
Plasma recalcification time, sec	78,85±1,17	96,46±4,08*	70,17±2,59†	88,63±3,57*	79,7±3,37	99,3±3,4*
Prothrombin time, sec	18,95±1,05	23,27±0,67*	16,91±0,62	24,73±1,33*	18,18±2,67	23,73±0,76*
Cephalin time, sec	66,64±2,28	75,93±2,76*	59,44±2,91	64,44±3,4	73,0±4,33	77,89±4,0
Kaolin time, sec	58,95±2,5	62,47±3,13	54,42±1,52	58,64±1,65	55,33±3,71	59,33±4,27
Thrombin time, sec	29,33±0,82	27,8±0,67	29,92±1,04	28,82±0,71	29,0±0,84	26,71±1,12
Total antithrombin activity, sec	9,08±0,92	8,92±0,92	9,0±0,67	8,27±1,05	9,0±0,62	6,8±0,5
Factor V, sec	20,0±0,72	22,5±1,94	18,0±1,15	20,75±2,18	20,16±1,12	24,87±0,99
Factor VII, sec	57,4±4,8	57,6±4,5	58,1±5,1	64,3±4,3	55,63±1,35	58,14±1,86
Plasma fibrinogen, mg	12,78±0,8	9,8±0,6*	14,11±0,79	10,41±0,57*	13,0±0,77	12,14±0,84*
Hageman-dependent fibrinolysis, sec	24,29±1,4	14,17±1,45*	25,67±2,12	16,7±2,91*	24,3±2,27	13,52±1,57*
Total euglobulin fibrinolysis, min	184,28±16,54	110,0±10,81*	259,33±28,58	217,83±25,89	191,56±20,22	130,59±59,0*
Hematocrit, %	43,83±1,61	36,83±1,77*	41,0±0,97	30,0±1,13*	41,86±0,7	34,29±1,12*
Degree of hemolysis		0,27 100%		0,18 66%		0,24 88%

*P < 0.05 between A and B.
†P < 0.05 between control (A) and experiment 1 (A).

of 0.3 ml of human blood (mainly group IV) and a further 2-3 ml blood was withdrawn 10 min later. Heterologous transfusion shock thus developed in the rats after preliminary blood loss. It must be pointed out that the experiments on intact and thymectomized rats receiving and not receiving thymalin were always performed on the same day. The following blood clotting and fibrinolysis parameters were determined: blood clotting time and plasma recalcification time, cephalin and kaolin time, thrombin time, total antithrombin activity, activity of factors V and VII, fibrinogen, and total and Hageman-dependent euglobulin fibrinolysis [1]. The degree of hemolysis caused by injection of heterologous blood also was determined spectrophotometrically (wavelength 540 nm) in all the rats. The extinction coefficient in plasma from intact (control) rats was taken conventionally as 100%.

The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The thymectomized rats developed hypercoagulation and their fibrinolysis was considerably inhibited (Table 1). Meanwhile in the thymectomized rats a tendency was observed for the concentrations of plasma factors and fibrinogen to increase. Similar results were obtained by the writers previously [4-6, 12, 14]. Under the influence of thymalin the blood clotting and fibrinolysis parameters were restored in the thymectomized rats to the characteristic levels for intact animals.

Animals of all three groups developed hypocoagulation after injection of heterologous blood (0.3 ml per rat). The blood clotting time and plasma recalcification time were lengthened, the kaolin and cephalin times were lengthened a little, and the fibrinogen level fell. Meanwhile total and Hageman-dependent fibrinolysis were stimulated in all the animals.

However, careful analysis of the data showed that the picture of the changes in the hemostasis system after injection of heterologous blood was by no means uniform in intensity. For instance, in thymectomized animals not receiving thymalin the blood clotting time was lengthened more, whereas fibrinolysis was stimulated much less strongly than in intact and thymectomized rats receiving thymus polypeptide factor. Hageman-dependent fibrinolysis was stimulated by a lesser degree in the thymectomized animals.

In our opinion injection of heterologous blood is accompanied by intravascular clotting. There is much evidence in the literature in support of this view [7, 8, 11-15]. If the intensity of intravascular hemolysis is used as the criterion, destruction of the heterolo-

gous erythrocytes takes place more slowly in thymectomized rats and, consequently, the conditions are created for a weaker degree of intravascular blood clotting. This can be explained on the grounds that the thymus controls the formation of natural antibodies — agglutinins and hemolysins. There is no doubt that the development of hypocoagulation in heterologous transfusion shock is partly attributable to consumption of plasma blood clotting factors and platelets, and also the appearance of degradation products of fibrinogen and fibrin.

However, such an explanation is biased. In heterologous transfusion shock in different animals degranulation of mast cells takes place and heparin is released into the tissue fluid and plasma. As a result, the disorders of blood clotting observed after injection of heterologous blood are considerably aggravated.

Meanwhile both in the control animals and in thymectomized rats not receiving (experiment 1) and receiving (experiment 2) thymalin, the total antithrombin concentration did not rise in the presence of heterologous transfusion shock. Similar results were observed by the writers previously [12, 14]. These findings can be explained as follows : 1) in rats, just as in rabbits and cats, in response to injection of heterologous blood very small quantities of anticoagulants are released ; 2) the absence of an increase in antithrombin activity may be due to consumption of antithrombin III during intravascular blood clotting [1, 3]; 3) natural anticoagulants secreted during heterologous transfusion shock bind with the antiheparin factor of the erythrocytes [7, 8, 11].

It is worth noting that after preliminary injection of thymalin into rats their adequate response to injection of heterologous blood is restored so far as the blood clotting and fibrinolysis parameters studied in this investigation are concerned. This action is undoubtedly largely due to the normalizing effect of thymalin on the production of natural antibodies — agglutinins and hemolysins. For instance, the intensity of hemolysis in thymectomized animals receiving thymalin rises considerably after injection of heterologous blood and approaches its initial level.

These results are of fundamental importance for they not only give some idea of the role of the thymus in regulation of the blood clotting and fibrinolysis system, but they also demonstrate its important role in the regulation of agglutinin and hemolysin production.

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