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STATE OF THE MAST CELL POPULATION IN RATS WITH EXPERIMENTAL ATHEROSCLEROSIS

B. A. Umarova, S. M. Strukova,
G. G. Bazaz'yan,* T. G. Khlebnikova,
and E. G. Kolokol'chikova

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Heparin is the principal humoral agent of the anticlotting system and it enters the blood stream during activation of that system. Heparin is synthesized, stored, and secreted in the mast cells (MC) which, in response to various stimuli, secrete it from granules along with histamine and other biologically active substances [7]. It has been shown [9] that excitation of the function of the anticlotting system by α-thrombin and its analog, DIP-α-thrombin, is characterized both by elevation of the anticoagulant potential of the blood and by a marked fall in the saturation index of MC with heparin. These results are evidence that the MC population is involved in the effector response of the anticlotting system. However, the problem of the status of the MC population in various functional states of the anticlotting system has received little study. We know that if animals are kept for a long time on a diet rich in cholesterol and fat, this leads to the development of atherosclerosis and depression of the function of the anticlotting system [1]. The mast cell population was previously stated to play a role in the development of atherosclerosis [3].

In connection with the facts described above it was decided to investigate changes in the state of the MC population of animals with experimental atherosclerosis and the effect of DIP-α-thrombin, a specific activator of the function of the anticlotting system, on this state. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Male albino rats were kept for 8 months on a diet rich in cholesterol and fat, which contained animal fat with added cholesterol, methylthiouracil, vitamin D_2, and cholic acid

*Deceased.

Department of Human and Animal Physiology, M. V. Lomonosov Moscow University. Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 3, pp. 366-369, March, 1987. Original article submitted September 15, 1986.

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TABLE 1. Changes in Parameters of State of Anticlotting System of Rats Kept for 8 Months on an Atherogenic Diet

Parameters	Experimental conditions	
	ordinary diet	atherogenic diet
Cholesterol, mg %	94,5±9,1 (6)	905,2±39,8 (9)
Plasma heparin tolerance, min	24±1,0 (9)	5,5±0,6 (9)
Fibrinogen, mg %	378±27,3 (7)	520,6±34,2 (8)
Activity of factor XIII, units/ml	28,6±2,7 (9)	106,6±11,9 (9)
Total fibrinolytic activity, mm^2	55±3,4 (9)	15±1,9 (8)
Nonenzymic fibrinolysis, mm^2	27,4±1,8 (9)	3,2±1,0 (9)

Legend. Number of animals given in parentheses; $p < 0.001$.

TABLE 2. Changes in Parameters of Clotting and Anticlotting Systems of Rats with Experimental Atherosclerosis 5 Min after Intravenous Injection of DIP-α-Thrombin

Parameters	Substance injected	
	0.85% NaCl solution	DIP-α-thrombin
Thrombin time, sec	29,8±0,8 (9)	30,2±0,8 (11)
Fibrinogen, mg %	542,4±70,5 (7)	626,6±69,0 (9)
Soluble fibrin, μg/ml	133,3±16,3 (9)	136,4±22,4 (11)
Total fibrinolytic activity, mm^2	25,0±3,7 (7)	29,6±4,9 (10)
Nonenzymic fibrinolysis, mm^2	14,1±4,3 (7)	14,5±2,1 (10)

Legend. Number of animals given in parentheses; $p > 0.05$.

[1]. Animals of the control group received an ordinary laboratory diet. Before use in the experiments the blood cholesterol level [1], plasma heparin tolerance [12], fibrinogen concentration [10], activity of factor XIII [11], total fibrinolytic activity, and nonenzymatic fibrinolysis [4] were determined. DIP-α-thrombin (1 μM) with residual clotting activity not exceeding 0.04 NIH unit/ml, was obtained by the method in [5]. The preparation for testing was injected into the jugular vein. After 5 min blood samples were taken from the same vein and the fibrinogen concentration, soluble fibrin level [14], thrombin time, total fibrinolytic acitivty, and nonenzymatic fibrinolysis were determined. The total number of animals used in the investigation was 38. The animals were decapitated 6 min after injection of the preparation. MC (about 800 from each animal) in film prepartions of the serous membranes from the mesentery, omentum, renal capsule, and pericardium were investigated. The films were fixed in buffered formalin solution and stained with 0.1% toluidine blue solution, pH 4.9. The MC population was characterized by a combined morphometric approach [8]. The number of dark and pale cells was counted and the saturation index of the cells with heparin and the degranulation index also were calculated, distinguishing between weak, moderate, and strong degrees. For electron-microscopic analysis MC were isolated from the peritoneal cavity of some animals by the method in [15]. The cells were fixed in a 2.5% solution of glutaraldehyde and treated by the method in [13]. For the electron-microscopic investigation of the sections the IEM-100B electron microscope was used. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results of keeping the animals on a high cholesterol-fat diet were elevation of the blood levels of pre-β- and β-lipoproteins by 2-4 times and of cholesterol by 10 times, and

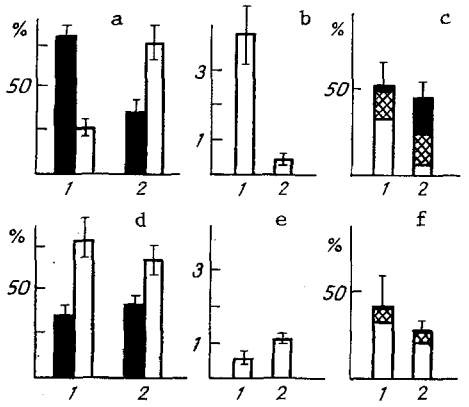

Fig. 1. Morphometric criteria of state of MC population in rats 5 min after intravenous injection of physiological saline (1) or of DIP-α-thrombin (2). a, b, c) Rats kept on ordinary laboratory diet; d, e, f) rats with experimental atherosclerosis. a, d) Relative frequency (in %) of dark (black columns) and pale (white columns) cells; b, e) saturation index of cells with heparin; c, f) degranulation index and relative frequency (in %) of its forms: unshaded part of columns — weak form, cross-hatched — moderate, part shaded black — strong form.

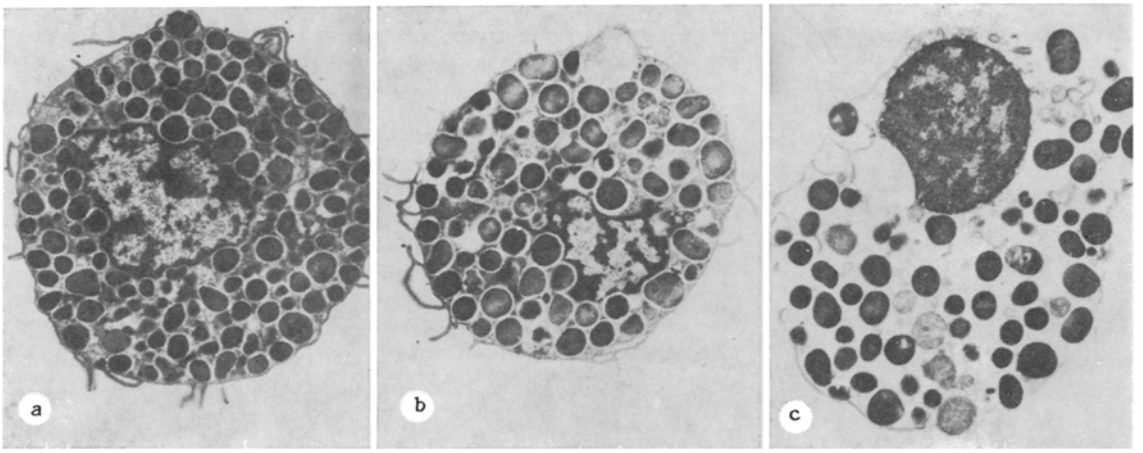

Fig. 2. MC isolated from peritoneal cavity of intact animals (a) and animals with experimental atherosclerosis (b, c). b) Cell with weak degree of degranulation. Formation of electron-transparent zones can be seen; c) pale cell with partially destroyed membrane; completely emptied granules predominate. 13,000 ×.

lowering of lipolytic enzyme activity and of the blood heparin level [2]. It will clear from Table 1 that in rats kept for 8 months on the atherogenic diet, besides elevation of the blood cholesterol (by 9.6 times) and fibrinogen (by 1.3 times) levels and of activity of factor XIII (by 3.6 times), and high plasma heparin tolerance, the total fibrinolytic activity (by 3.6 times) and nonenzymic fibrinolysis (by 8.5 times) were sharply reduced. The low level of nonenzymic fibrinolysis is evidence of the virtually total absence of heparin and its complexes from the blood stream. These data confirm the development of atherosclerosis by the animals, accompanied by depression of function of the anticlotting system.

Injection of thrombin in animals with atherosclerosis caused intravascular blood clotting and did not lead to activation of the anticlotting system because of a disturbance either of thrombin reception by the altered vascular wall or of the humoral component of the anticlotting system, permitting heparin to enter the blood stream [1]. To estimate the state of the anticlotting system in experimental atheroscloersis, DIP-α-thrombin, which is a highly effective stimulator of the anticlotting system and is completely without proteolytic or other enzyme activity, was chosen for injection in this investigation. It was

shown that after injection of 1 μM DIP-α-thrombin no changes characteristic of activation of the anticlotting system were recorded in the blood (Table 2). Meanwhile injection of DIP-α-thrombin in the same dose into animals receiving an ordinary laboratory diet, caused a marked response of the anticlotting system [6]. One of the causes of the absence of activation of the anticlotting system in response to injection of DIP-α-thrombin may have been insufficiency of the release of heparin into the blood stream. This situation thus called for analysis and a comparative study of the state of the MC population in intact animals and in animals with experimental atherosclerosis.

Morphometric analysis of the state of the MC population of rats kept on an ordinary laboratory diet showed that dark cells, filled with densely packed metachromatic granules (Fig. 1a: 1) with a high index of saturation with heparin (Fig. 1b: 1) predominated in the control animals receiving physiological saline. After intraveous injection of DIP-α-thrombin into the rats, pale cells free from heparin predominated in the MC population (Fig. 1a: 2). The saturation index of the cells with heparin fell to 0.35 (Fig. 1b: 2). Changes in the character of degranulation were observed: a decrease in the weak and a considerable increase in the moderate and strong degrees of degranulation compared with the control (Fig. 1c).

In animals with atherosclerosis sharp changes were observed in the state of the MC population. Analysis of the morphometric criteria in the MC population of the control rats receiving physiological saline revealed predominance of pale cells (Fig. 1d: 1). The saturation index of the cells with heparin was under 1 (Fig. 1e: 1). In addition, the appearance of cells of defective shape was observed. In the group of experimental animals receiving DIP-α-thrombin a high content of pale cells also was found in the MC population (Fig. 1d: 2). The saturation index of the cells with heparin in the experimental group did not differ significantly from that in the control (Fig. 1e: 2). The character of degranulation in this group likewise was unchanged compared with the control (Fig. 1f). Cells with a weak type of degranulation mainly predominated. These findings confirm the view that the MC pool of animals with atherosclerosis is defective.

The results of electron-microscopic analysis of MC isolated from the peritoneal cavity of animals kept on an atherogenic diet are given in Fig. 2. Whereas in intact animals the MC population was dominated by cells saturated with electron-dense granules (Fig. 2a), in animals with atherosclerosis mainly cells with a varied degree of emptying of the granules were observed (Fig. 2b, c). In cells with a weak degree of degranulation (Fig. 2b) the formation of electron-transparent zones and partial lysis of the contents of the granules were observed. In some cells completely emptied granules were found, with disturbance of the integrity of the cell membranes and granules (Fig. 2c).

Biochemical analysis of the blood and morphometric analysis of the MC population after injection of DIP-α-thrombin into animals with experimental atheroscloersis thus revealed profound depression of their anticlotting system. It can be tentatively suggested that heparin deficiency in the blood of animals kept for a long time on an atherogenic diet can be largely explained by a decrease in the heparin pool in MC.

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