DEPOLYMERIZATION OF FIBRIN AND NONENZYMIC FIBRINOLYSIS IN RABBITS ON AN ATHEROGENIC DIET WITH ADDED ANTIOXIDANTS

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Experimental and clinical studies have demonstrated involvement of the cells of the vascular wall, blood lipoproteins, and thrombogenic factors in the genesis of atherosclerosis [3]. Thrombotic processes are among the complications of atherosclerosis and, in turn, they contribute to its progression [12].

Fibrin formation can be activatd in hypercholesterolemia [13]. Reduction of the fibrinolytic and anticoagulant properties of the blood [1], increased platelet aggregation [2], and limitation of complex formation by heparin and blood proteins and amines [3] have been found in animals on an atherogenic diet. Initiation and progression of atherosclerosis are brought about not only by changes in plasma-platelet reactions, but also by deposition of fibrin on the vascular wall. Morphologic studies have demonstrated massive accumulations of fibrin at sites of maximal lipid deposition (cited in [8]).

An important step in atherogenesis and, in particular, of membrane pathology associated with dyslipoproteinemia, is activation of lipid peroxidation. Products of lipid oxidation are known to accelerate aging of erythrocytes and to slow the blood flow at the level of small and medium-sized vessels [7]. It was decided to investigate processes of fibrin polymerization during the development of experimental atheroscloersis and also to study the state of plasma hemostasis during treatment of atheroscloersis with antioxidants.

The aim of this investigation was to study the degree of depolymerization of unstabilized fibrin and the level of nonenzymic fibrinolysis of blood plasma in rabbits with experimental atheroscloersis and during treatment with the antioxidants 3-hydroxypyridine (3-HP) and α-tocopherol.

EXPERIMENTAL METHOD

Experiments were carried out on 15 adult male chinchilla rabbits weighing 2.5-3 kg. The animals of group 1 received a diet with added cholesterol in a dose of 0.3 g/kg for 2 months. Animals of group 2, after 1 month on an atherogenic diet, received 3-HP together with α-tocopherol in a dose of 10 mg/kg during the subsequent month in addition to their diet. Rabbits of group 3 were kept on a standard diet. Blood was taken from the auricular vein 2 months after the beginning of the experiment for analysis and added to 3.8% sodium citrate in the ratio of 9:1.

Total fibrinolytic activity (TFA) and nonenzymic fibrinolysis (NEF) were determined in the blood plasma by the method in [5], the heparin level as in [9], and nonenzymic activity of the antithrombin III–heparin–thrombin complex as in [6]. Depolymerization activity of the plasma was determined by the use of purified fibrin monomer, obtained by the method in [11] and dissolved in acetate buffer, pH 5.2, containing 10% urea, as the substrate. The fibrinogen concentration was determined by the method in [10].

The 3-HP used in the investigation was of USSR origin: it is a water-soluble antioxidant of the 3-hydroxypyridine class.

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TABLE 1. Parameters of Hemostasis System in Rabbits Receiving Atherogenic Diet with the Addition of Antioxidants (M ± m, n = 5)

Parameters	Group of animals		
	1	2	3
TFA, mm²	33,5 ±5,2*	51,7±6,5	54±2,3
NEF, mm²	19,2±3,6*	35±5,3	31±7,8
Depolymerization activity, %	23±1,0*	49,1±4,7	39±5,1
Fibrinogen, mg %	348±24*	—	250±18
NEF activity of antithrombin III–heparin-thrombin complex, mm²	14,5±0,22*	31,0±5,3	23,1±0,9
Heparin, μg/ml	4,3±0,3*	9,8±0,3	10,0±0,3

Legend. *p < 0.05 Compared with control.

EXPERIMENTAL RESULTS

It will be clear from the data given in Table 1 that in the animals of group 1, not receiving antioxidants, the basic signs of development of depression of function of the anticlotting system and of a prethrombotic state were discovered, in confirmation of previous findings [1, 2, 4]. There was a marked lowering of the TFA and NEF levels in the blood plasma by 1.3 and 2 times respectively, the heparin level was lowered by half, and nonenzymic fibrinolytic activity of the antithrombin III – heparin–thrombin complex, isolated from blood plasma, also was reduced by half compared with blood plasma from healthy animals of the control group (group 3). The depolymerization activity of the blood plasma relative to unstabilized fibrin in rabbits on an atherogenic diet was only half of that in the blood of animals kept on a standard diet. The fibrinogen concentration in the blood plasma showed a tendency to rise. Thus lowering of th eparameters of fibrin depolymerization and of activity of the antithrombin III–heparin–thrombin complex, isolated from blood plasma, can be regarded as additional criteria for evaluation of the degree of depression of function of the anticlotting system during the development of atherosclerosis.

In the rabbits of group 2, receiving an atherogenic diet with the addition of antioxidants, by contrast with the animals of group 1, whose diet was not supplemented by antioxidants, all the parameters tested including depolymerization activity of the plasma relative to unstabilized fibrin were at the same level as the corresponding parameters in the healthy control group (group 3, see Table 1).

The test of depolymerization activity of the plasma relative to unstabilized fibrin, incidentally, showed changes linked with those of NEF. This test also enabled the state of the process of polymerization of unstabilized fibrin in the blood stream to be quantified. Lowering the level of fibrin depolymerization is evidence of the beginning of possible formation of fibrin structures during atheroscloerosis.

These experiments demonstrated the absence of a prethrombotic state in rabits receiving antioxidants simultaneously with an atherogenic diet. The antioxidant 3-HP, together with α-tocopherol promote normalization of the level of fibrin depolymerization, of NEF, and of ther hemostatic parameters in the blood plasma of rabbits receiving an atherogenic diet.

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QUANTITATIVE DETERMINATION OF TRANSPLANTABLE HEMOATOPOIETIC STEM CELLS BY THE LIMITING DILUTIONS METHOD

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Totipotent hematopoietic stem cells (THSC), giving rise to all categories of cells of the hematopoietic and immune systems, have received less study than all other categories of colony-forming hematopoietic precursors, including polypotent myeloid precursors (CFU-s), which can easily be determined quantitatively, for direct methods of their quantitative analysis are not yet available. An attempt at a direct quantitative approach was undertaken by Boggs and co-workers [2], who used the limiting dilutions method [10] during transplantation of normal bone marrow into unirradiated genetically anemic W/W^V mice. We tested the limiting dilution method during transplantation of bone marrow into lethally irradiated recipients, assuming that restoration of hematopoiesis by donors' cells and, consequently, survival of the recipient, are possible if even only one THSC should find its way to hematopoietic territories.

EXPERIMENTAL METHOD

As recipients we used female (CBA × C57B1/6)F_1 (CBF_1) mice aged 5-10 months. The donors were CBF_1 or (C57B1/6 × CBA/T6/F_1 ($BCT6F_1$) mice, and their age and sex are given in Table 1. Total irradiation was carried out on the IPK (^{137}Cs) apparatus with a dose rate of 19 Gy/min, in two sessions separated by an interval of 3 h; the total dose was 15.2 Gy. Under these conditions not a single control mouse, not receiving bone marrow cells, survived more than 18 days. The mice were kept under ordinary conditions. After irradiation they were given acidified water (pH 3.5) and antibiotics with their food (polymyxin sulfate and monomycin, 500,000 U/500 g of each). Bone marrow cells were taken from the femur by flushing out with Hanks' solution. A suspension of single cells was obtained by passing the suspension repeatedly through a fine needle. After enumeration of the nucleated cells and dilution, the cell suspensions were injected intravenously into recipients 1-2 h after irradiation in 2 or 3 doses, at least 10 mice being used for each dose. Survival of the irradited mice was monitored for 28 days or more. Proliferation of the donors' cells in the bone marrow, spleen, and thymus was determined by the presence of the T6-chromosome in metaphase plats. To describe the THSC which, on transplantation, repopulated the irradiated recipient, and consequently ensured its survival after lethan irradiation, we used the functional term "hematopoiesis restoring unit" (HRU) [1]. The principle of the limiting dilutions method as applied to determination of hematopoietic precursors was described previously [1-3]. Briefly, the probability that a sample of homogeneous cell suspension does not contain HRU is determined by the equation $P_0 = e^{-KX}$, where K is the fraction of HRU in 10^6 cells and X the dose of cells injected (in millions). P_0 denotes the proportion of samples not containing HRU. This will be identical (in the case under discussion) with the proportion of mice which did not survive. The concentration of HRU in the sample is determined by the equation $K = -\ln P_0/X$.

EXPERIMENTAL RESULTS

Examples of determination of the HRU fraction in bone marrow samples from healthy mice are given in Table 1. In three bone marrow samples from female CBF_1 mice we obtained values

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