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QUANTITATIVE HISTOAUTORADIOGRAPHIC ANALYSIS OF CELL PROLIFERATION IN THE AORTIC WALL OF RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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UDC 616.12-004.6-092-07:
616.132-018.15-073.916

KEY WORDS: experimental hypercholesterolemia; cell division; autoradiographic investigation

Excessive deposition of apo-B-containing lipoproteins in the intima of arteries is regarded as a key phemomenon at the basis of atherogenesis [1, 2, 6]. Data obtained in recent years show that smooth muscle cells (SMC) and macrophages are quickly transformed into foam cells only after contact with aggregated or modified lipoproteins (LP), i.e., with modified particles [5]. Under these circumstances, an important place in the chain of events to be investigated is occupied by the problem of proliferation of the intimal cells that participate in assimilation of LP or in desmoplastic reactions. This process has not hitherto been studied, although a number of factors causing proliferation of cells of the vascular wall have been isolated [7]. Since the most convincing data, characterizing proliferation of cells of the vascular wall in atherogenesis can be obtained by the use of specific tritium labeling of the precursor of DNA synthesis, investigations of this kind are possible only by experiments using ^{3}H-thymidine.

The aim of this investigation was to study the character of proliferation of cells of the aortic wall in rabbits during the formation of atherosclerotic lesions against the background of hypercholesterolemia, and to determine the types of cells which participate in this process.

EXPERIMENTAL METHOD

Experiments were carried out on 49 male rabbits weighing 2.5-3 kg. Of this total number 32 animals were kept on an atherogenic diet (0.2 g cholesterol per kilogram body weight five times a week, in 5 ml of sunflower oil, given by gastric tube) for 1, 2, 3, 4, 6, 8, 12, and 16 weeks, five intact animals served as the control, and 12 rabbits were used in experiments with delayed labeling. For each of the times of the experiment studied, animals with approximately equal blood cholesterol levels were chosen. ^{3}H-thymidine (specific activity 19.8 Ci/mole) was injected intravenously in a dose of 0.3 μCi/g body weight into all the animals five times in the course of 24 h (at the end of the period on an atherogenic diet) at intervals of 6 h. The animals were killed 1 h after the last injection of the isotope. The aorta, cut longitudinally, was fixed in toto in Carnoy's fluid, and the material was prepared so that the whole aorta was accommodated on one section 3-4 μ thick. To determine whether the dividing cells could be transformed into foam cells experiments were carried out with delayed labeling. The animals were kept for 4 weeks on an atherogenic diet, after which they were given seven injections of ^{3}H-thymidine in the course of 36 h, at intervals of 6 h. Some of the rabbits were then killed, to serve as the control, whereas the remaining animals continued on the atherogenic diet for a further 4 weeks. Histoautoradiographs were prepared by the standard method, using Ilford K-5 emulsion, exposed for 18 days at 4°C, and then stained with hematoxylin and eosin. The number of labeled nuclei was counted separately for each type of lesion and for each zone of the atherosclerotic plaque, after which the index of labeled nuclei (ILN) was calculated. Low-density lipoproteins, labeled with ^{125}I (^{125}I-LDL), were in-

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 0007-4888/87/0103-0122$12.50

TABLE 1. Level of Cell Proliferation in Intima of Rabbit Aorta in Initial and Progressive Stages of Experimental Atherosclerosis (in %)

Duration of experiment, weeks	Unchanged intima	Prelipid stage (foci of proliferation)	Lipid stains	Atherosclerotic plaque						
				lipid	fibro-lipid			fibrous		
					surface part	central part	deep part	surface part	central part	deep part
0	0,5±0,1									
1	0,95±0,4	—	—							
2	2,3±0,8	16,5±1,7	—							
3	2,7±0,8	21,6±4,2	15,4±1,9	—						
4	7,6±1,9	22,5±1,5	20,3±1,3	19,0±1,6	—	—	—	—	—	—
6	—	28,5±1,2	20,1±1,9	24,8±2,0	—	—	—	—	—	—
8	—	—	17,3±1,7	14,8±1,8	12,2±3,0	6,1±1,6	1,7±0,4	24,8±0,4	9,9±0,2	13,2±1,9
12		—	19,8±3,8	19,8±3,1	18,6±2,4	7,2±1,1	3,0±0,8	18,3±2,8	9,9±1,5	20,2±2,2
16			21,9±3,3	19,3±4,4	24,1±2,2	10,0±1,1	7,0±1,0	25,8±3,0	7,0±1,8	24,5±1,3

From 6th week of experiment no unchanged regions of the aorta were observed.

Lipid stains were first found in the 2rd week, but atherosclerotic plaques in the 4th week of experimental hypercholesterolemia.

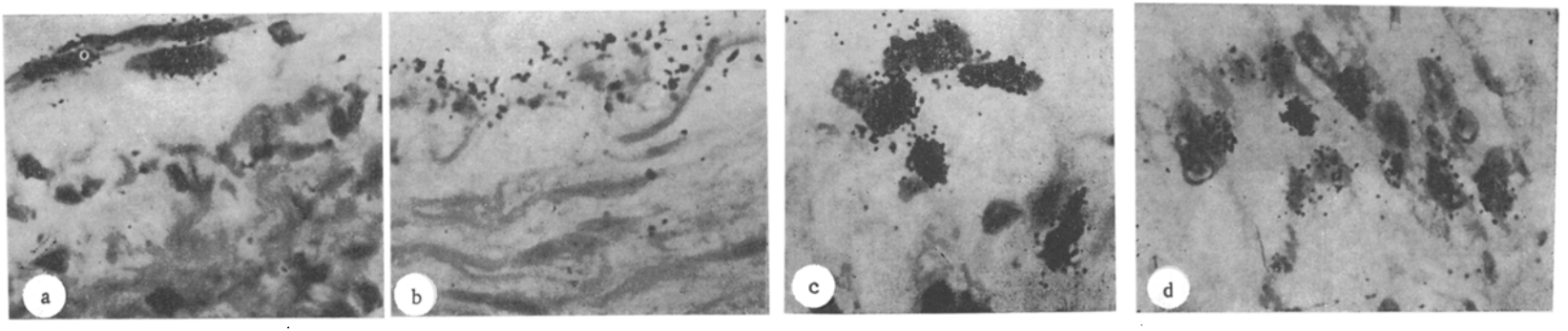

Fig. 1. Histoautoradiographic analysis of cell proliferation and infiltration of LP in initial stages of atherogenesis of the rabbit aorta. a) Sites (loci) of cell proliferation in subendothelial layer of intima. 1200 ×; b) accumulation of apo-B-containing LP in intima. 1400 ×; c) swelling and edema of intima in zone of cells intensively incorporating ^{3}H-thymidine, after 2 weeks of experiment. 1200 ×; d) incorporation of $_{3}$H-thymidine into nuclei of SMC in peripheral part of atherosclerotic plaque. 1000 ×.

jected intravenously into another 12 rabbits by the method described previously [2] in the early stages of the experiment. To discover to which cell type the DNA-synthesized belonged, and to determine their relative numbers in the atherosclerotic plaques, the method of electron-microscopic autoradiography [2] was used. In these cases the ^{3}H-thymidine was injected five times in the course of 24 h in a dose of 20 μCi/g body weight. Ilford L-4 emulsion was applied to the ultrathin sections, which were then exposed for 50 days. The material was examined in the JEM-100B microscope with a voltage of 75 kV.

EXPERIMENTAL RESULTS

The index of cell proliferation in the intima of the intact animals was very low (Table 1), and was mainly accounted for by division of undifferentiated SMC. The first discrete zones of proliferation, consisting of three to five dividing cells, appeared in the 2nd week of the experiment in the proximal part of the aorta (Fig. 1). Lipid deposits in the intima of the aorta were not found under these circumstances, but by the use of ^{125}I-LDL focal infiltration of the deposits could be observed (Fig. 1b). Thus the first sites (loci) of proliferation were found in the so-called "prelipid" stage of experimental atherosclerosis, characterized by focal edema of the intima and deposition of apo-B-containing LP. Histoautoradiography showed that the focal zones of proliferation are mainly connected with sites of infiltration of LP.

Cells taking part in division were not homogeneous and had different sources of origin. In the loci of proliferation intensive incorporation of ^{3}H-thymidine was observed into the nuclei of the endothelial cells and SMC and, what was particularly important, into nuclei of the monocytes which passed through the endothelial barrier into the intima (Fig. 1c). After 3 weeks of the experiment the number of foci of dividing cells in the proximal part of the aorta increased; so also did the index of proliferation (Table 1); the formation of single lipid stains could also be observed. Many of the cells in these newly formed lipid stains after 4 weeks of the experiment were in the S period. A high index of proliferation was observed in all parts of the intima: in the unchanged regions the index of proliferation was 14 times greater than initially, whereas in the lipid stains and the newly formed plaques, every fifth cell was taking part in division.

In the later stages of the experiment, as the atherosclerotic lesions in the arteries progressed, a number of particular features of cell division could be distinguished. The first point to note was that the level of cell proliferation did not change in lipid stains formed at different times of atherogenesis (Table 1). Meanwhile, starting from the 8th week of the experiment, no new foci of proliferation were formed in the intima of the aorta, despite the high hypercholesterolemia. Delayed labeling experiments showed that it is the proliferating monocytes (macrophages and SMC) that come into contact with apo-B-containing LP and are transformed into foam cells. A redistribution of labeled cells was observed in the newly formed atherosclerotic plaques: fewer of them were found in the central part, more in the peripheral zones (Fig. 1d). This stage of affairs is connected with the structural features of the atherosclerotic plaques. In the central parts, because of disintegration of the foam cells, an atheroma was formed and only single dividing cells were preserved. In the peripheral zones, especially in the covering of the plaques, were collagen fibers predominate, the index of proliferation was high, and on average every fourth cell incorporated ^{3}H-thymidine. After 12 weeks of the experiment, of all the labeled and identified cells in the covering of the atherosclerotic plaques 68% were fibroblasts, 20% were SMC, and 12% were macrophages.

Proliferation of intimal cells of the aorta, an important link in the chain of events leading to the formation of atherosclerotic plaques, thus begins in the prelipid stage of atherogenesis. In the lipid stains 20% of cells take part in division. The conditions affecting active cell proliferation are evidently multifactorial and change depending on the stage of development of the pathological process.

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EFFECT OF INTRA-AORTIC BALLOON COUNTERPULSATION ON PLATELET ULTRASTRUCTURE AND FUNCTION

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UDC 616.2.-008.1-021.6-07:
617.155-25-008.1

KEY WORDS: counterpulsation; balloon pump; platelets; ultrastructure; functional properties

The intra-aortic counterpulsation method of assisted circulation is widely used in the treatment of acute heart failure. It has been found to be particularly effective in the treatment of cardiogenic shock complicating the course of acute myocardial infarction, and also during the intra- and postoperative periods in patients undergoing aortocoronary bypass surgery [1, 4].

The long-term use of counterpulsation may lead to trauma to the blood cells on account of mechanical action, and also as a result of contact between the blood and the material used in the construction of the intra-aortic balloon pump. Damage to platelets, which is connected with disturbances of clotting, rheology, vascular tone, and other important parameters of homeostasis, assumes particular importance under these conditions. Contact between blood and a foreign surface induces adsorption of plasma proteins and rapid (in the course of a few minutes) adhesion of circulating platelets to it. The resulting activation, under these circumstances, in the main factor in thrombus formation. When new models of balloon pumps are developed, attention must be paid not only to the hemodynamic efficiency of the design, but also the contact properties of polymer materials. Nowadays electron microscopy plays a leading role in the study of the mechanisms of thrombus formation and of surface-induced thrombogenesis, in order to assess the quality of polymers highly resistant to the development of thrombosis and of materials with a modified surface, that in the pure form do not possess this property.

We studied the effect of various materials (polyurethane, "Biomer") used in the intra-aortic balloon pumps developed in collaboration with the "Sever" LPTGO*, on the ultrastructure and function of platelets during experimental counterpulsation.

EXPERIMENTAL METHOD

Experiments were carried out on nine male mongrel dogs weighing 15-20 kg. The animals were anesthetized by intravenous injection of hexobarbital in a dose of 10-15 mg/kg body weight every 60 min. Adequacy of anesthesia was estimated by the usual clinical signs. Artificial ventilation of the lungs with moderate hyperventilation was used during the experiments, so that muscle relaxants were unnecessary. A balloon pump with a volume of 10 cm^3, made of polyurethane or "Biomer" was introduced through the common femoral artery and positioned in the descending part of the arch of the aorta and connected to an assisted circulation apparatus (AVK-5M) working on a 1:1 program. The duration of intra-aortic balloon counterpulsation was 5 h. Blood from the common femoral vein of the anesthetized dogs was collected in siliconized tubes before the experiment began and 5 h later, at its end. Aggregation of the platelets was determined [13] and their number counted. Platelets fixed with glutaraldehyde were separated from blood for electron-microscopic investigation by double centrifugation, post-fixed with OsO_4, dehydrated in increasing concentrations of alcohols, and embedded in Araldite

*Unidentified Russian abbreviation — Publisher.

No. 2 Department of Surgical Diseases, Electron-Microscopy Laboratory, and No. 1 Department of Internal Medicine, Academician I. P. Pavlov First Leningrad Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bekhtereva.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 107-108, January, 1987. Original article submitted February 12, 1985.

