STATE OF THE MICROCIRCULATION DURING LONG-TERM REGRESSION OF THE EARLY STAGES OF ATHEROSCLEROSIS

E. D. Klimenko, L. P. Kobozeva,
A. B. Michunskaya, and I. Yu. Khabarina

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Dyslipoproteinemia (DLP) is nowadays regarded as a leading risk factor in the pathogenesis of atherosclerosis. Disturbances in the microcirculatory system (MCS) in DLP arise before atherosclerotic plaques are formed in the main arteries and they lie at the basis of structural and functional changes in the target organs [3-5]. When the disturbances of lipid metabolism are overcome, atherosclerotic changes in the large arteries undergo regression [10, 12]. The possibility of regression of disturbances in the MCS has received insufficient attention in the literature. In the early stages of spontaneous regression of experimental atherosclerosis [6, 9] and during remission of ischemic heart disease, the microcirculatory disturbances (MCD) are not abolished [8].

The aim of this investigation was to analyze some parameters characterizing the state of MCS during long-term spontaneous regression of the early stages of atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 52 male Chinchilla rabbits weighing 2.5-3 kg. The animals were give cholesterol (Ch) in a dose of 0.3 g/kg body weight with vegetables. The rabbits of group 1 were kept for 2 months on an atherogenic diet (AD). Animals of group 2 were kept on AD for 2 months and then switched to the ordinary animal house diet for periods of 6, 9, and 12 months. The experimental animals were killed simultaneously with controls

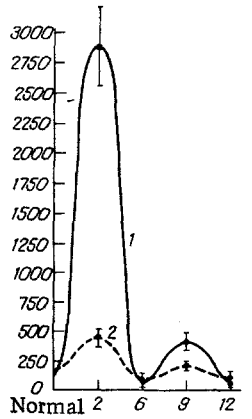

Fig. 1. Trend of content of various lipoprotein fractions in blood serum after 2 months on AD and at various stages of spontaneous regression. Ordinate, LP concentration (in mg%); abscissa, times on AD and of spontaneous regression (in months). 1) ALP fraction, 2) HDLP fraction.

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TABLE 1. Parameters Characterizing the State of MCS in the Early State of Atherogenesis and during its Regression (M ± m)

Group of animals	Area of cross section of microvessels, μ^2					Morphological parameters of erythrocytes, %							
	arterioles	venules	precapillaries	capillaries	postcapillaries	normocytes		flat disks		echinocytes		ETI	
						a	b	a	b	a	b	a	b
Intact ($n=12$)	205,6±7,2	310,30±13,62	40,36±2,32	35,95±1,2	85,63±4,95	42,0±6,38	44,8±3,2	25,7±3,2	22,7±3,2	54,7±3,1	2,5±1,0	1,38±0,1	1,26±0,1
1: 2 months on AD ($n=10$)	108,21±5,24	459,24±18,50	28,50±2,43	29,95±1,60	114,74±5,23	24,8±9,67	26,2±5,9	6,5±1,4	13,5±4,4	47,1±3,3	41,3±10,1	7,93±4,6	4,5±1,3
2 (regression): 6 months ($n=10$)	128,46±10,25	477,86±10,84	34,20±2,97	--	117,01±8,95	—	14,5±3,6	---	49,2±3,6	---	9,25±5,9	—	6,7±1,3
9 months ($n=10$)	142,36±6,31	428,08±16,89	37,07±1,71	31,75±1,91	88,18±3,86	25,1±2,9	30,2±4,0	29,7±7,5	25,7±3,3	17,87±4,0	20,4±5,6	2,9±0,6	2,67±0,2
12 months ($n=10$)	211,64±8,44	384,05±15,38	39,03±2,51	37,26±2,52	84,85±4,77	31,9±3,6	56,3±2,5	22,5±5,1	28,3±3,1	16,2±7,5	1,3±0,9	2,24±0,07	0,8±0,2

Legend. Asterisk indicates significant differences from corresponding value in animals of intact group at $p \leq 0.05$; n) number of animals in group.

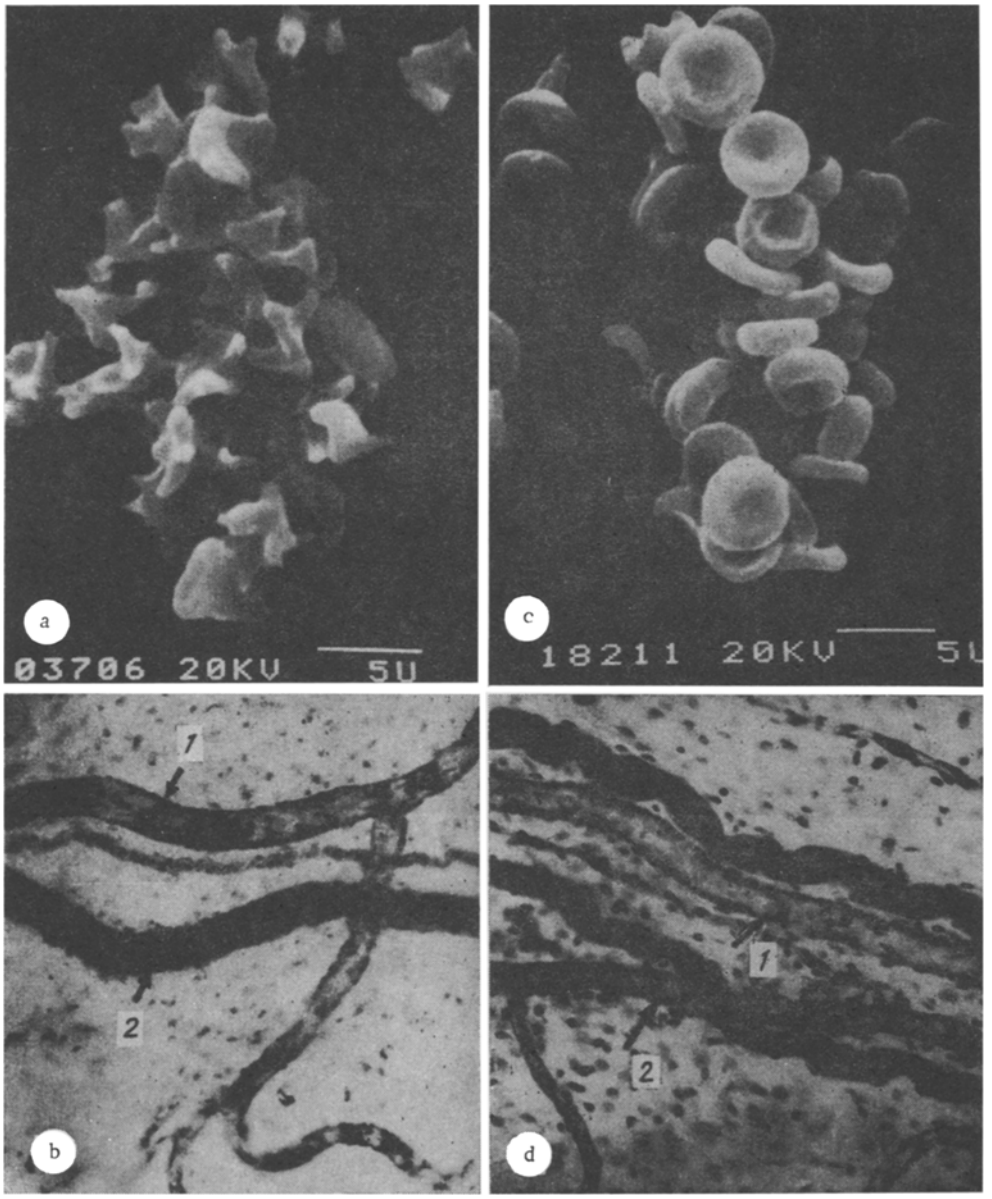

Fig. 2. Erythrocytes and microvessels of mesentery of rabbit small intestine after 2 months on AD and 12 months of spontaneous regression: on left — after 2 months on AD; on right — after 12 months of spontaneous regression; a) aggregate of echinocytic forms of erythrocytes. Scanning electron microscopy. 3000×; b) aggregates of blood cells in lumen of microvessels (1 — arteriole, 2 — venule). Impregnation by Kupriyanov's method. 200×; c) aggregate of stomatocytes, flat disks, and normocytes. Scanning electron microscopy. 3000×; d) microvessels from mesentery of small intestine. Impregnation by Kupriyanov's method. 200×.

by air embolism. The state of the microcirculatory bed (MCB) was studied in total film preparations of the mesentery of the small intestine (V. V. Kupriyanov's method). The area of cross section of the microvessels was determined on a Leitz ASM system. The index of atherosclerotic involvement of the aorta (IAA) was calculated by Avtandilov's method. The ratio between the various lipoprotein fractions was investigated in the blood serum [7]. Erythrocytes, treated by the standard method [1], were studied in a Hitachi scanning electron microscope. The dynamics of the morphological changes in the blood cells were assessed with the aid of the erythrocyte transformation index (ETI) [2].

EXPERIMENTAL RESULTS

After the animals had been kept on AD for 2 months (group 1) the level of atherogenic lipoproteins (ALP) in the blood was elevated by 27 times, that of antiatherogenic lipoproteins (HDLP) by four times compared with initially (Fig. 1). IAA was 22%. In all components of the MCB marked vascular, intravascular, and extravascular changes were found. The most significant vascular disturbances were inequality of caliber, tortuosity of the venules, venular sacculations, a net like structure of the microvessels, and micro-aneurysms; the ratio of the diameters of the arterioles and the venules parallel to them was disturbed. There was a sharp increase in the number of functioning capillaries, but their lumen was significantly reduced (Table 1). Intravascular aggregation of erythrocytes was observed in various parts of MCB. Extravascular changes consisted of foci of cellular infiltration and perivascular edema. Scanning electron microscopy showed a marked decrease in the number of normocytes and a marked increase in the number of echinocytes and in their powers of aggregation in arterial and venous blood, accompanied by a sharp increase in ETI (Table 1; Fig. 2a, b).

After 6 months of regression (group 2) the blood ALP level was down to its initial values and HDLP was 50% below normal. IAA was reduced by 10 times to 2.2%. In MCB, despite reduction of the constrictor response in the arterioles and precapillaries, inequality of their caliber and increased tortuosity remained, and in the capacitive component dilatation progressed (Table 1). However, the intravascular disturbances were less marked and were predominantly local in character compared with those in group 1. The number of normocytes in the peripheral blood continued to fall and the number of flat disks rose sharply; ETI was considerably increased. The echinocyte effect was less marked (Table 1).

After 9 months a new rise of the ALP fraction was observed, accompanied by a very small rise of the blood HDLP level (Fig. 1). IAA was 2.7%. In MCS, besides restriction of arterioles, dilatation of venules, and reduction of the capillary lumen, marked intravascular disturbances were present. Aggregates of blood cells, unlike at the previous stage of regression, were found not only in postcapillaries and venules, but also in arterioles and precapillaries.

The presence of a large number of echinocytic forms of erythrocytes in the blood, especially venous, was noted. Meanwhile the increase in the number of normocytes and the decrease in the number of flat disks were accompanied by lowering of ETI.

After 12 months of regression the ALP and HDLP levels were 40-50% below their initial values. IAA was almost unchanged compared with the previous times (2.3%). By this stage a tendency was observed for the structure of the different components of MCB to be restored to normal, but the changes in MCS were not completely abolished. Dilatation of the venules and disturbance of the state of aggregation of blood in individual microvessels still remained. A tendency toward an increase in the number of normocytes and a decrease in the number of flat disks was intensified in arterial and, in particular, in venous blood, and the number of echinocytic forms of erythrocytes was reduced; ETI came close to its initial level (Table 1; Fig. 2, c, d).

The increase in the ALP fraction in the blood was thus accompanied by progression of changes in MCB and by an increase in the number of pathological forms of erythrocytes in the arterial and venous blood (2 months on AD + 9 months regression). The increase in the total lipoprotein fraction after 9 months of regression was evidently due not only to resorption of lipids from plaques and increased endogenous Ch synthesis, but also to an increase in the membrane Ch pool. The echinocytic effect of DLP and of pathological transformation of the erythrocytes was undoubtedly connected with a change in their ability to undergo deformation and to form aggregates, and disturbance of the blood flow in MCS. It was shown previously that an important role in disturbances of MCD during the development of DLP is played by changes in the hematocrit index, activity of the clotting system of the blood, and osmotic resistance of the erythrocytes [11]. An inevitable consequence of the longterm constriction of arterioles, a decrease in the area of cross section of the precapillaries and capillaries, and changes in the rheologic properties of the blood arising during DLP is disturbance of the metabolic blood flow.

After 12 months of regression, with lowering of the ALP and HDLP levels below their initial values, the structure of the various components of MCB and the area of cross section of the microvessels were restored to normal; reversibility of the changes in the erythrocytic component of hemostasis was more complete. Since disturbances in MCS during DLP are generalized in character [5], it can be tentatively suggested that during long-term regression a

tendency toward normalization of the structural and functional disturbances in MCS developed in various organs and tissues. The facts described above are evidence of possible correction of disturbances in MCS in the late stages of regression after abolition of DLP, and this must be borne in mind in the prevention of atherosclerosis. If one of these components in the pathogenesis of the disease is underestimated, therapeutic measures may not be sufficiently effective.

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