LIPID PEROXIDATION PROMOTES INTRACELLULAR CHOLESTEROL ACCUMULATION IN ATHEROSCLEROSIS

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Much recent information shows that the development of atherosclerosis is accompanied by high cholesterol (Ch) levels in the blood cells [1, 2]. An important role in regulation of the intracellular Ch level is ascribed to plasma lipoproteins (LP) which are known to be the principal transport form of lipids in the blood. Low-density LP (LDL) transport lipids (including Ch) into blood cells and vessel walls, interacting with their surface by receptor-mediated endocytosis, or nonspecifically as a result of contact with the plasma membrane [3]. It is possible that Ch can be transported from LP into cell membranes by simple diffusion through the aqueous phase also [4]. This last process may play an important role in elevation of the Ch concentration not only in cells which have receptors for LP (monocytes, smooth-mucle and endothelial cells, etc.), but also in cells not containing specific receptors, such as erythrocytes. The opinion is held that a disturbance of the mechanism of interaction of LP with the cell surface lies at the basis of atherosclerotic degeneration of the cell [3]. If this is true, the question arises: what causes the change in character of LP–cell interaction. Atherosclerosis is accompanied by activation of lipid peroxidation (LPO) processes in the blood [4]. Experiments in vitro have shown that LPO can disturb protein–lipid interactions in LP, change their surface charge [5], and modify the structure of the lipids and apolipoproteins on the surface of LP particles [7]. It may be expected that such disturbances of organization of the proteolipid surface layer of LP also take place in vivo and may be a cause of the change in the mechanism of interaction between LP and the cell membrane and intensification of Ch transfer from LP into the cell.

It was accordingly decided to study Ch transfer between erythrocytes and the main classes of plasma LP (LDL, high-density LP — HDL) of healthy blood donors and patients with ischemic heart disease (IHD), the principal clinical manifestation of atherosclerosis.

In order to discover the possible effect of LPO on Ch transfer into cells in IHD, erythrocytes were incubated with LP previously oxidized to different degrees, after which changes in the intracellular Ch concentration were investigated. The principal classes of LP: LDL (which are believed to transport lipids into cell membranes, thereby exhibiting

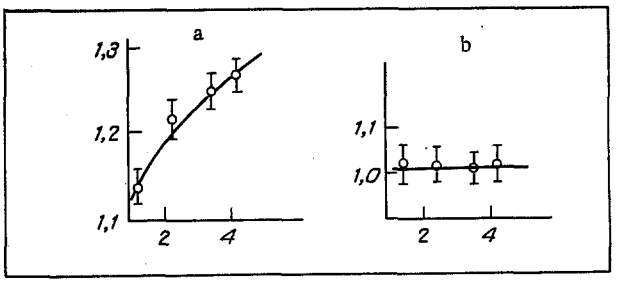

Fig. 1. Dependence of Ch(a) and PL (b) concentrations in erythrocytes after incubation with LDL on MDA content in LDL before incubation. Here and in Fig. 2: abscissa, MDA concentration (in μmoles/g PL); ordinate, Ch and PL levels, relative to initial level.

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TABLE 1. Changes in Ch Concentration in Erythrocytes after Incubation with Native and Previously Oxidized LDL, HDL_2, or HDL_3 from Healthy Blood Donors and Patients with IHD (M ± m)

Experimental conditions	$\frac{Ch - Ch_0}{Ch_0} \times 100\%$
LDL	
control (8)	11,4±3,4
IHD (6)	33,8±10,5**
LPO (6)	27,2±4,2**
HDL_2	
control (8)	—19,2±8,1
IHD (6)	— 3,1±2,1*
LPO (6)	— 1,2±2,1*
HDL_3	
control (8)	—26,2±7,4
IHD (6)	— 6,2±3,3*
LPO (6)	— 4,5±1,8*

Legend. Ch_0 and Ch represent Ch concentration in erythrocytes incubated in the absence and presence of LP respectively. Number of donors tested given in parentheses. —) Reduction of Ch concentration in erythrocytes. MDA concentration in control samples did not exceed 0.5 μmole/g PL; in samples subjected to LPO, 4.2 ± 0.4 μmole/g PL. *$p < 0.05$, **$p < 0.01$ Compared with control.

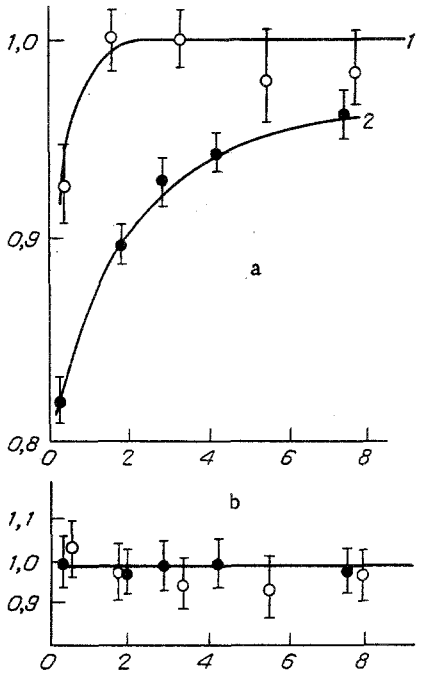

Fig. 2. Dependence of Ch (a) and PL (b) concentrations in erythrocytes after their incubation with HDL_2 (1) or HDL_3 (2) on MDA level in HDL_2 or HDL_3 respectively before incubation.

atherogenic properties) or HDL (subfractions HDL_2 and HDL_3), accepting Ch from cell membranes (antiatherogenic LP) [3], were used.

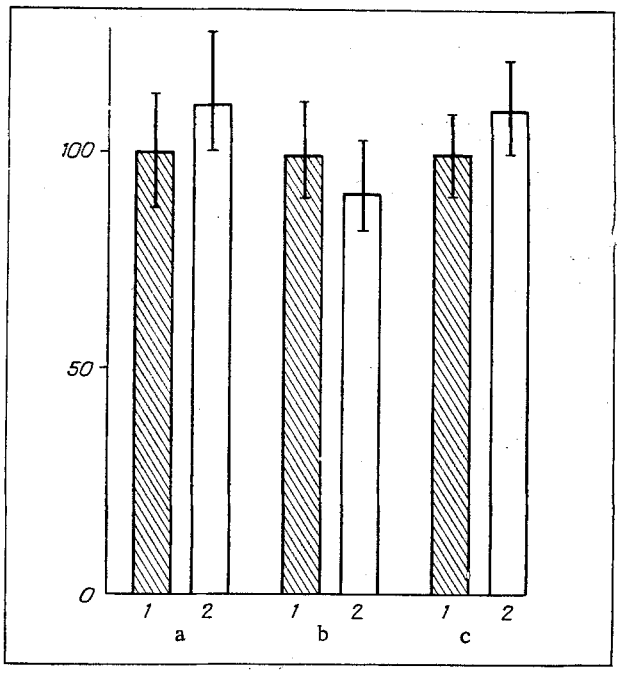

Fig. 3. MDA concentration in erythrocytes before (1) and after (2) incubation in absence (a) and in presence of LDL (b) or HDL (c) for 5 h at 37°C. Ordinate, MDA level (in % of control).

EXPERIMENTAL METHOD

LDL, HDL_2, and HDL_3 were isolated from blood serum from healthy donors or patients with IHD by ultracentrifugation [10]. The resulting preparation was dialyzed for 20 h against a 145 mM NaCl solution containing 10 mM Tris-HCl, pH 7.4, at 4°C. The purity of the LP fractions was verified by PAG electrophoresis and by comparison of the chemical composition with known data [3]. Erythrocytes were obtained from freshly donated blood by triple centrifugation in a tenfold excess of NaCl solution (145 mM), containing 10 mM Tris-HCl (pH 7.4), at 400g for 10 min. Erythrocytes were incubated with LP for 5 h at 37°C. The incubation sample (1.5 ml) contained: 145 mM NaCl, 10 mM Tris-HCl (pH 7.4), 20 mg/ml of human serum albumin, 4-6 million erythrocytes in 1 µl, and LP in the form of 0.2-0.8 mg phospholipids (PL) in 1 ml. Samples not containing LP were used as the control. After the end of incubation all the samples were centrifuged for 7 min at 400g, and the residue was washed 3 times by centrifugation in a 20-fold excess of 145 mM NaCl solution containing 10 mM Tris-HCl (pH 7.4) for 7 min at 400g. Preliminary experiments showed that washing the erythrocytes in this way does not lead to any change in their Ch and PL concentrations. Lipids were then extracted [9] from the washed erythrocytes and concentrations of Ch [8] and PL [13] in the extract were determined. In the course of incubation for 5 h 0.2-0.4% of the cells underwent hemolysis. However, this process caused no change in the concentration of either Ch or PL in the cells. The LP were oxidized by incubation at 37°C with access to air for several hours. The malonic dialdehyde (MDA) concentration was estimated by the reaction with 2-thiobarbituric acid [12]. In all cases the experiments were repeated 3-5 times with each sample within the series repeated 5 times. The results were subjected to statistical analysis on the EMG-666/B microcomputer (Hungary).

EXPERIMENTAL RESULTS

Table 1 gives the results of analysis of changes in the Ch concentration in erythrocytes after incubation with LDL, HDL_2, or HDL_3 from normal blood donors and patients with IHD. As a result of incubation of the cells with LDL from healthy donors the Ch concentration in the erythrocytes increased on average by 11%. In other words, LDL exhibit Ch-donor propertes relative to the membranes of cells not containing specific receptors to this class of LP. When erythrocytes were incubated with LDL from IHD patients a similar effect was found, although to a much more marked degree (34%). This means that LDL from IHD patients donate Ch more effectively to cell membranes.

Incubation of erythrocytes with HDL_2 or HDL_3, on the other hand, led to a fall in the intracellular Ch level (Table 1), i.e., both HDL_2 and HDL_3 accept Ch from cell membranes. If the erythrocytes were incubated with HDL_2 or HDL_3 from IHD patients, the intracellular Ch level also fell, but much less effectively. This indicates that Ch-acceptor ability of HDL from IHD patients is considerably depressed [6].

It can thus be concluded from the results that IHD, on the one hand, is accompanied by increased ability of LDL to donate Ch to cell membranes, and on the other hand, by inhibition of the Ch-acceptor capacity of HDL_2 and HDL_3. Both these effects should lead to Ch accumulation in the cell membranes and promote atherogenic degeneration of the cells.

To study the effect of LPO on Ch transfer between LP and the erythrocyte membrane, erythrocytes were incubated with LDL, HDL_2, or HDL_3 from healthy blood donors, oxidized beforehand to different levels, monitored on the basis of the content of MDA, a product of LPO, in LP. It was found that LPO leads to greater accumulation of Ch within the cell than native LP (Table 1). These results suggest that LPO can facilitate release of Ch from them and its insertion into the erythrocyte membrane. To confirm this hypothesis, we studied dependence of the observed effect on the MDA content in LDL. Dependence of the change in Ch concentration in the erythrocytes as a result of incubation for 5 h with LDL on their MDA content before the beginning of incubation is shown in Fig. 1a. An increase in the MDA content in LDL led to increased Ch accumulation in the erythrocytes. Meanwhile the PL concentration in the erythrocytes did not change with an increase in the degree of preliminary oxidation of LDL (Fig. 1b). These data are evidence of increased ability of LDL to donate Ch to biomembranes during intensification of LPO in the blood. The results (Table 1) show that preliminary oxidation both of HDL_2 and of HDL_3 leads to inhibition of their Ch-acceptor capacity. The more highly oxidized the HDL, the less the degree by which they can accept Ch from erythrocyte membranes (Fig. 2). The PL concentration in the erythrocytes did not depend on oxidation of HDL (Fig. 2b).

The results are evidence that preliminary oxidation of LP has a marked influence on Ch transfer between LP and biomembranes, similar to that observed in IHD.

The question arises: what causes modification of LP leading, on the one hand, to potentiation of the Ch-donor properties of LDL and, on the other hand, to inhibition of the Ch-acceptor ability of HDL_2 and HDL_3? Is it the result of a disturbance of the structure of LP in the course of their preliminary oxidation, or is it due to preradical processes of LPO taking place during incubation of the erythrocytes with LP? The results of a study of the MDA concentration in erythrocytes incubated in the absence of LDL or HDL are given in Fig. 3. During incubation of the erythrocytes with LDL or with HDL, accumulation of MDA in the system was not observed. This is evidence that incubation is not accompanied by activation of LPO and is probably linked with the presence of an effective antioxidative protective system in the erythrocytes [11]. The results suggest that activation of the Ch-donating properties of LDL (Fig. 1) and inhibition of the Ch-acceptor properties of HDL (Fig. 2), which we found, are the result of modification of the structure of LP during their preliminary oxidation.

The results thus explain the possible correlation in vivo between two important features of atherosclerosis: accumulation of Ch in cells of the blood and vessel walls, on the one hand, and activation of LPO processes in the blood, on the other hand. In all probability LPO, taking place during artherosclerosis, can substantially modify the structure of both LDL and HDL. This in turn may lead to disturbance of the mechanism of interaction with the cell surface and, as a result, to potentiation of the ability of LDL to transport Ch into cell membranes and to inhibition of the Ch-acceptor function of HDL. Ultimately this must inevitably lead to Ch accumulation in cells of the blood and vessel walls.

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CHANGES IN THE MICROCIRCULATORY SYSTEM IN THE PAIN SYNDROME OF SPINAL ORIGIN

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Pathological pain leads to severe autonomic disturbances affecting the sympathico-adrenal system [1, 3-5, 8, 9]. Clinical and experimental studies have shown that in trigeminal neuralgia, facial pains of cervical genesis [3], reticulitis [1], and migraine [12] disturbances of the hemostasis system are observed. However, the state of the microcirculatory system has not been studied in central pain syndromes caused by the appearance of a generator of pathologically enhanced excitation (GPEE) in certain parts of the nociceptive system [8]. This paper is devoted to an explanation of this phenomenon.

EXPERIMENTAL METHOD

Experiments were carried out on 30 male Wistar rats weighing 200-250 g, divided into three groups (10 animals in each group): 1) control animals, 2) animals with a pain syndrome of spinal origin (PSSO), and 3) animals undergoing a mock operation.

A PSSO was induced in rats by the formation of a GPEE in the posterior horns of the lumbar division of the spinal cord by application of an agar wafer (1.5 × 3 × 6 mm) containing penicillin in a dose of 7.5 U/mm^3 [7]. Various substances which disturb inhibitory mechanisms in a neuron population or induce neuron depolarization are used nowadays to produce models of central pain syndromes based on the formation of a GPEE. The use of penicillin for this purpose is based on the fact that, as an antagonist of the inhibitory mediator GABA, it disturbs inhibition. Penicillin also acts directly on neuronal membranes by blocking chloride channels or reducing the transmembrane Cl^- gradient [10, 11].

Six components of PSSO (vocalization, general motor response, frequency of attacks, duration of attacks, local response, response to a stimulus) were evaluated on a 3-point

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