No statistically significant differences were found between the biochemical parameters in the different groups of animals receiving acetylcysteine. The results indicate that structural changes in the erythrocyte membranes during sensitization are closely linked with depression of thiol-disulfide equilibrium and with modification of the enzymes. Under the conditions used, thiol antioxidants were able to depress LPO and to normalize the thiol-disulfide balance in the sensitized animals. However, enzyme activity and erythrocyte resistance were partially restored only by the action of acetylcysteine, evidently due to the higher degree of oxidizability of unithiol compared with acetylcysteine and the greatest hydrophobicity of the latter. The stable tendency of G6PDH activity to decline in the animals under the experimental conditions will be noted, and a further study of the dose-dependent effects of the thiol antioxidants is called for during sensitization.

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ANTIOXIDANT EFFECT OF HIGH-DENSITY LIPOPROTEINS IN PEROXIDATION OF LOW-DENSITY LIPOPROTEINS

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KEY WORDS: lipoproteins; peroxidation; antioxidants

Increased sensitivity of low-density lipoproteins (LDL) to peroxidation *in vitro* under the influence of oxygen and Fe^{++} [6, 14], and of long-term dialysis in medium not containing antioxidants [15], are well-known facts. There is also evidence of peroxide modification of LDL *in vivo* in atherosclerotic plaques [1]. LDL isolated from blood plasma in the presence of antioxidants nevertheless contain large quantities of diene conjugates and fluorescent Schiff bases [15].

One result of peroxidation of polyene acyl groups of phospholipids, composing LDL, is that they acquire new properties: a change in the conformation of apoprotein B, the basic protein of LDL, which may be the cause of the appearance of antigenicity among them [5];

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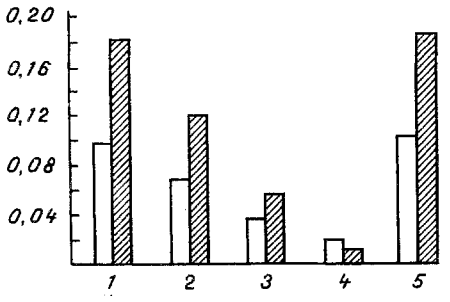

Fig. 1

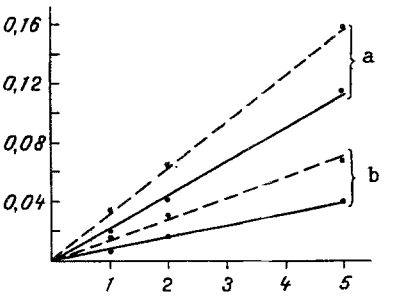

Fig. 2

Fig. 1. Effect of HDL on auto-oxidation of LDL in solution during incubation at 37°C for 72 h. 1) LDL; 2) LDL + HDL (2:1); 3) LDL + HDL (1:1); 4) LDL + HDL (1:2); 5) LDL + delipidized HDL (1:2). Unshaded columns — degree of turbidity of solution (D_{600}), shaded columns — MDA content (D_{532}). Here and in Figs. 2 and 3, mean results of three experiments are shown.

Fig. 2. Effect of HDL on LPO in solution of LDL during incubation at 37°C. a) LDL; b) LDL + HDL (1:1). Continuous line denotes MDA formation (D_{532}); broken line shows degree of turbidity of solution (D_{600}). Abscissa, incubation time (in min).

the discovery of lipid peroxidation (LPO) products, and, in particular, of 4-hydroxynonenal, which can easily pass through cell membranes, when it has a strong cytotoxic action in experiments on cell cultures [12], in the composition of LDL. However, in the intact organism, events do not develop so dramatically because of the presence of a complex of substances of protein and nonprotein nature in the blood plasma, organs, and tissues, which form the antioxidant protection system.

In view of modern ideas on the role of the superfluidity of LPO processes in mechanisms of injury to the vascular wall [2, 12], and the hypothetical atherogenic modification of LDL [6], and also arising from data on the antiatherogenic role of high-density lipoproteins (HDL) in the body [10], it was logical to suggest a possible antioxidant function of HDL.

EXPERIMENTAL METHOD

LDL (d = 1.019-1.063 g/ml) and HDL (d = 1.067-1.210 g/ml) were obtained from the blood plasma of donors and isolated by ultracentrifugation [9] on a Beckman L8-55 (USA) ultracentrifuge. The lipoproteins were then dialyzed against physiological saline with Na_2EDTA (1 mg/ml) for 24 h, and then against Hanks' solution for 12 h at 4°C. Solutions of lipoproteins were sterized by filtration on an Amicon (USA) apparatus through filters with a pore diameter of 0.45 μ. Both spontaneous LPO, during incubation of solutions of LDL and HDL at 37°C, and LPO induced in a xanthine–xanthine oxidase system, were studied. The LDL concentration in all experiments was 2 mg/ml (as protein). HDL (initial concentration 28 mg/ml as protein) were added to the solution of HDL in the ratio (as protein, by weight) of 2:1, 1:1, and 1:2. In the last case the incubation mixture contained an extra 150 μg/ml of xanthine and 0.06 IU/ml of xanthine oxidase (from Serva, West Germany). The total volume of the mixture was made up with 0.05 M Tris-HCl buffer (pH 8.0) to 2.5 ml. The incubation time of the mixture was 1 h at 37°C. Delipidation of the HDL was carried out with a mixture of alcohol and ether. The formation of LPO products was judged by the increase in concentration of malonic dialdehyde (MDA), which was determined with the aid of 2-thiobarbituric acid (TBA) by the method in [4], with minor modifications: to 1.4 ml of the sample were added 0.6 ml of a 17% solution of TCA and 1.0 ml of a 0.8% solution of TBA. After boiling for 10 min the samples were cooled to 4°C and centrifuged for 10 min at 4000g. The optical density of the transparent supernatant was measured at 532 nm on an automatic spectrophotometer (Leitz, Switzerland). The turbidity formation was measured on the same spectrophotometer at 600 nm, using water as the control.

EXPERIMENTAL RESULTS

During incubation of LDL solutions at 37°C an increase in turbidity was found with a parallel increase in MDA concentration, compared with solutions of LDL kept at 4°C. HDL inhibited both processes, and intensity of the effect, moreover, depended on their concentra-

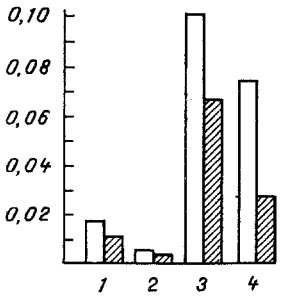

Fig. 3. Effect of HDL on LPO in LDL, induced by xanthine oxidase reaction. 1) Initial LDL; 2) initial HDL; 3) LDL after incubation in system containing xanthine–xanthine oxidase; 4) LDL + HDL (1:1) after incubation under the same conditions. Incubation time 1 h. Remainder of legend as to Fig. 1.

tion (Fig. 1) and on the incubation time (Fig. 2). Meanwhile, the delipidized HDL had no antioxidant action, which is evidence that HDL have a certain inhibitory effect of LPO when acting on LDL in the form of a whole particle, but not its protein moiety (Fig. 1). A similar picture also was found in experiments with induction of LPO in LDL in the xanthine–xanthine oxidase system (Fig. 3), in which the antioxidant action of HDL also was clearly manifested.

The results confirm that LDL can be modified by peroxidation products and, at the same time, they afford evidence of the antioxidant ability of HDL, manifested both during incubation of a mixture of lipoproteins at 37°C and during induction of a peroxidation reaction in the xanthine–xanthine oxidase system. The most powerful physiological antioxidant, α-tocopherol (TP), is known to be bound mainly with lipoproteins in the blood [6]. Under these circumstances LDL contain 20% of the total TP of blood plasma, whereas HDL contain approximately 50% [11]. Accordingly, the results of the present investigation can be interpreted from the standpoint of the effect of TP, contained in high concentrations in HDL, on the velocity of LPO in LDL, more especially because, according to available data [7], experiments *in vitro* have demonstrated the possibility of TP exchange between HDL and LDL. Nevertheless, there may be other, more complex mechanisms of antioxidant protection of LDL by HDL. However, the available data unfortunately are insufficient to provide arguments in favor of other views regarding the antioxidant role of HDL during LPO in LDL.

Hence, besides the known ability of HDL to carry out cholesterol transport into the liver for its subsequent oxidation into bile acids [8], there are also grounds for considering another, hitherto unknown, mechanism of the antiatherogenic action of HDL as an antioxidant relative to LDL.

The increase in turbidity of the LDL solution on account of aggregation of lipoprotein particles was evidently due to peroxidation of LDL which, in our opinion, can take place not only in model systems. This conclusion is confirmed by other workers, who found aggregated LDL and quite high concentrations of products of peroxide nature in the vascular wall [1, 3]. In turn, the presence of LDL, undergoing peroxidation and aggregation, in the vascular wall facilitates their uptake by cells of macrophagal type, with subsequent transformation into foam cells, a dominant morphological element of the resulting atherosclerotic lesions [10].

It was not by accident that xanthine oxidase was chosen as inducer of LPO in the lipoprotein solution. First, the characteristics of xanthine oxidase as a leading pro-oxidative enzyme in ischemic damage to the myocardium, liver, and gastrointestinal trace, were confirmed. Second, a considerable (by 20 times or more) increase in xanthine oxidase activity has been found in the human aorta in the presence of atherosclerotic lesions [13]. In the most recent investigations (preliminary data) the present writers have shown increased activity of this enzyme in the blood plasma of persons with hypertrigylceridemia. In this connection the role of the xanthine oxidase system in the intensification of peroxidation in hyperlipoproteinemias of types IIb and IV, for which an increase in the quantity of products of peroxide nature has been demonstrated, and for which progression of atherosclerosis is a characteristic feature, cannot be ruled out.

On the whole, the results given in this paper and their analysis are evidence of the pathogenetic importance of an uncontrolled intensity of peroxidation reactions of LDL in the formation of the atherosclerotic vascular lesion, for excessive concentrations of peroxidation products in this case behave as a factor directly injuring the vascular cells, and as a factor in peroxide modification of LDL, which makes them an object for active ingestion by cells of macrophagal type.

Hence, in addition to the function of reverse transport of cholesterol into the liver, and also the inhibitory effect of HDL on permeability of the rabbit aorta for LDL, the anti-

oxidant effect of HDL will add a new dimension to our understanding of their antiatherogenic action.

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LEUKOCYTIC THERMOSTABLE α-GLYCOPROTEIN: IMMUNOCHEMICAL STUDY AND INVESTIGATION OF ITS ENZYME ACTIVITY

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KEY WORDS: leukocytic α-glycoprotein; granulocytic elastase; amidolytic activity; granulocytic antigens

Leukocytic thermostable α-glucoproetin (LTαG), with mol. wt. of 90 ± 7 kilodaltons, identified in 1982 [4], is a specific protein of human granulocytes [7]. A particularly important fact is that in certain diseases [1-4, 7], and also after operations involving the use of an assisted circulation [6], LTαG is found in raised concentrations in the patients' blood serum. It is impossible to assess the importance of this factor and the possibility of using it for diagnostic purposes and for studying the pathogenesis of diseases and their complications, without a study of the biological function of LTαG, but this has not hitherto been undertaken.

The investigation described below was devoted to the systematic immunochemical identification and characterization of the protein components of peripheral blood leukocytes, namely soluble leukocytic antigens (SLA) [5], LTαG is one of the proteins included in this system (SLA-2). The approach chosen to assess the functional state of the leukocytes was by determining concentrations of individual SLA in hemolysates from patients, for it has been shown

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