CHANGES IN SURFACE CHARGE OF PLASMA LIPOPROTEINS DURING AUTO-OXIDATION

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One possible cause of atherosclerotic lesions of blood vessels is a disturbance of the mechanism of interaction between lipoproteins (LP), as a result of modification of their surface, and cell membranes of vessel walls [4]. In the first place this is associated with a change in the character of distribution of charges on the surface of LP. For instance, in patients with ischemic heart disease (IHD) the surface potential (i.e., the negative surface charge) of low-density LP (LDL) is reduced compared with that of normal controls, whereas that of high-density LP (HDL) is increased [8]. According to modern views on the atherogenic role of LDL and the antiatherogenic role of HDL [4], these two circumstances ought to lead to accumulation of cholesterol (Ch) in the smooth-muscle cells and to their atherosclerotic degeneration. The question arises, what is the cause of the disturbance of the charge topography on the surface of LP. According to one view, modification of the surface of LP and, in particular, disturbance of the distribution of the charges on their surface takes place as a result of activation of lipid peroxidation (LPO) processes in the blood [10-12]. An essential step in the elucidation of this problem is an investigation of the character of the change in surface potential (surface charge) of LP during their oxidation.

In the present investigation changes in the surface potential of the main classes of human plasma LP in the course of their auto-oxidation were studied. The EPR method, with a positively charged spin probe, was used for this purpose.

EXPERIMENTAL METHOD

LDL and HDL were isolated from plasma from healthy blood donors by the method described previously [13]. The preparations were dialyzed for 24 h at 4°C against a 5 mM solution of Tris-HCl, pH 7.3. The LP concentration was estimated on the basis of their phospholipid content [15]. Isolated LP were subjected to auto-oxidation by incubating them for several hours at 37°C with access of air. The LPO level was studied by determining the malonic dialdehyde (MDA) content in LP by the reaction with 2-thiobarbituric acid (TBA). For this purpose, 1 ml of 20% tricarboxylic acid (TCA) was added to 2 ml of a suspension of LP. After mixing, the sample was centrifuged for 10 min at 1500g. To 2 ml of the supernantant 1 ml of 0.5% TBA containing 0.3% sodium dodecylsulfate was added, and the contents were mixed and incubated for 15 min at 100°C. Optical density was then measured on a DU-7 spectrophotometer (from Beckman, USA) at 532 nm. The molar extinction coefficient of MDA was taken to be 1.56×10^5 M^{-1} cm^{-1} [1].

EPR spectra were recorded on an E-4 radiospectrometer (from Varian, USA) at 23 ± 1°C under the following conditions: microwave power 10 mW, amplitude of high-frequency modulation 0.1 mT, scanning of magnetic field 2.5 mT/min with time constant of 0.3-1.0 sec. The spin probe used was 7-spiro-2'-(N-oxy-4,5^1,5^1-trimethyl-Δ^3-imidazoline)-3-methylammonium octadenate.

The probe was added to the LP suspension in the form of a solution in ethanol. The concentration of the probe in the sample was 8.7×10^{-5} M, and that of ethanol 1 vol. %.

The surface potential of LP was estimated as described previously [8]. The results were subjected to statistical analysis on a programed EMG-666/B microcomputer (Hungary), using Student's test.

An increase in the degree of oxidation of lipids of both and LDL (Fig. 1) was accompanied

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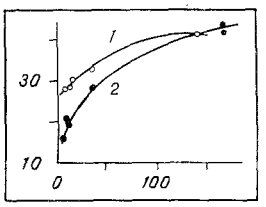

Fig. 1. Dependence of surface potential of HDL (1) and LDL (2) on the MDA content. Abscissa, MDA content (in nmoles/mg phospholipid); ordinate, absolute value of potential (in mV).

by an increase (in absolute value) of their negative potential. This was probably connected with an increase in the negative charge on their surface. In turn, the increase in the negative charge on their surface of LP could be the result of accumulation of polar lipid oxidation products and (or) a change in the character of distribution of charges on the surface of LP as a result of their modification during auto-oxidation.

We know that atherosclerosis and its clinical manifestation ischemic heart disease (IHD) are accompanied as a rule by accumulation of primary and secondary LPO products in the blood [6], whereas hypercholesterolemia leads to intensification of LPO [7] and to a decrease in activity of the antioxidant system of the cell [2, 5]. Meanwhile the surface potential of HDL (i.e., the negative surface charge) is increased in patients with IHD. Since the negative charge on HDL also is increased during LPO (Fig. 1), the cause of the increase (in absolute value) of surface potential of HDL discovered by the writers previously in patients with IHD compared with normal control subjects may perhaps be activation of LPO in the plasma.

It can be tentatively suggested that an increase in the negative charge on HDL, induced by LPO, disturbs the character of their interaction with cells of the blood vessel wall, the surface of which is negatively charged. In turn, this should lead to a change (increase) in the plasma Ch concentration, for it is now generally considered, on the basis of much clinical and experimental evidence, that HDL, by interacting with plasma membranes of cells in the vessel wall, accept Ch, and thus behave as an anti-risk factor in the development of atherosclerosis and IHD [4]. This hypothesis is confirmed by the fact that antioxidants have been used successfully in several cases to lower the plasma Ch level both experimentally [2] and clinically [2, 3]. Keeping animals on an "antioxidant-free" diet facilitates the development of experimental atherosclerosis [2]. Thus there is a definite link between hypercholesterolemia and LPO in the blood. Our data suggest that this link at the molecular level is realized as a disturbance of the character of interaction between LP (as a result of modification of their surface during lipid peroxidation) and cell membranes in the vessel wall. The train of events which we postulate can be represented as follows: activation of LPO in the blood, modification of HDL and an increase in their negative surface charge, weakening of interaction between HDL and vascular cells, inhibition of the Ch-acceptor properties of HDL, accumulation of Ch in the vessel wall, intensification of proliferation of smooth-muscle cells, the developement of an atheroma.

Unlike HDL, LDL are considered to be atherogenic, for by interacting with membranes of smooth-muscle cells, they transport lipids into them, and thereby promote accumulation of Ch in the vessel wall and, ultimately, the development of atherosclerotic lesions in the blood vessels [4]. The writers showed previously that the surface potencial (negative surface charge) of LDL is lower in patients with IHD than in normal healthy blood donors. This must contribute to strengthening of contact of LP belonging to this class with negatively charged glycosaminoglycans of the vessel wall. Meanwhile the results shown in Fig. 1 are evidence of an increase in the negative surface charge on LDL during their oxidation. With these two facts in mind it can be postulated that peroxidation of LDL is not the only cause of the change in topography of the surface charges of LDL. However, it must also be borne in mind that LPO significantly affects the surface organization of the lipoprotein layer of LDL. This is expressed, in particular, as modification of their chief protein apo-B [11] which, as we know [12], is responsible for interaction with receptors of biomembranes. Removal of apo-B from the surface of LDL during lipid peroxidation [11] exposes the lipid sites on their surface, and probably improves nonreceptor interaction with the cell membranes.

It can thus be concluded from these results that LPO at least is one of the causes of the increased negative surface charge on HDL in IHD. This, in turn, weakens interaction of HDL with negatively charged glycosaminoglycans of the vessel wall, i.e., it weakens the acceptor properties of HDL relative to Ch. Meanwhile peroxidation of LDL, if it exists, is not the only cause of the enhanced atherogenic role of this class of LP in IHD.

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END PRODUCTS OF LIPID PEROXIDATION IN ALCOHOL-INDUCED LIVER DISORDERS

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Lipid peroxidation (LPO) is a key factor in the development of various pathophysiological processes such as stress, ischemic heart disease, epilepsy, hypoxia, [3, 6, 10]. In particular, intensification of LPO has been demonstrated in patients with liver disease [5, 8]. This is quite understandable if it is recalled that the xenobiotic inactivation sytem of the hepatocytes is closely linked with the LPO enzyme system [1] and that activation of LPO is the initial stage of cytolysis [2].

One of the most widespread and serious forms of liver pathology is that due to alcohol, which is nowadays subdivided into several forms depending on the character of liver damage [4, 7, 9, 11]. However, there are no experimental data on the role of LPO processes in the various forms of alcohol-induced liver damage.

The aim of this investigation was to study the intensity of LPO in patients with different forms of alcohol-induced liver damage.

EXPERIMENTAL METHOD

Altogether 124 patients addicted to alcohol for a long perid of time (from 3 to 20 years) were investigated. All patients had a negative epidemiologic history of acute virus or drug-induced hepatitis, and no HBsAg was present in the blood serum. Clinical and morphological

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