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SPIN PROBE STUDY OF CHANGES IN SURFACE POTENTIAL OF PLASMA LIPOPROTEINS FROM PATIENTS WITH ISCHEMIC HEART DISEASE

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The plasma lipoproteins (LP) are the principal transport system of exogenous and endogenous lipids in the blood. Much clinical evidence has now been obtained to show that an increase in the plasma concentration of low-density (LDL) and very-low-density (VLDL) lipoproteins and a fall in the concentration of high-density lipoproteins (HDL) promote deposition of lipids in the arterial wall and the development of atherosclerotic changes in the vessels. Experimental results suggest that this is linked with the ability of LDL to transport lipids, including cholesterol (ChS) into cells of the vessel wall and the ability of HDL to accept ChS from cell membranes [2, 3]. Disturbance of the operation of this lipid transport mechanism leads to anomalies in lipid metabolism and ultimately to the development of atherosclerosis. It is not yet clear what is the mechanism triggering disturbances of interaction between LP and the cell membrane. One possible cause of this phenomenon is modification of the surface of LP, leading to a change in the surface charge density and the surface potential of LP. It has been shown, for instance [7], that feeding rabbits with ChS for 4 months leads to lowering of the negative surface charge of VLDL and LDL, without any change in the surface charge of HDL. Changes also have been found in the surface charge of LP in hyper-α-lipoproteinemia [6]. However, the problem of whether the surface potential of LP is changed in patients with ischemic heart disease (IHD) remains unsolved.

In the present investigation by the spin probe method the surface potential of the main classes of human plasma LP and its changed in IHD, a clinical manifestation of atherosclerosis, were studied.

METHODS

LDL and HDL (subfractions HDL_2 and HDL_3) were isolated from the blood plasma of patients with IHD and of healthy blood donors on the Zh-62 ultracentrifuge (USSR) in the RU-50 rotor as described previously [12]. The diagnosis of IHD was based on clinical manifestations of angina, ECG changes, and the results of graded physical exertion tests. The isolated LP were dialyzed for 24 h at 4°C against a 5 mM solution of Tris-HCl, pH 7.3. The LP concentration was determined on the basis of their phospholipid content [15]. EPR spectra were recorded on an E-4 radiospectrometer (Varian, USA) at 37°C [5]. The following imonoxyl radicals:

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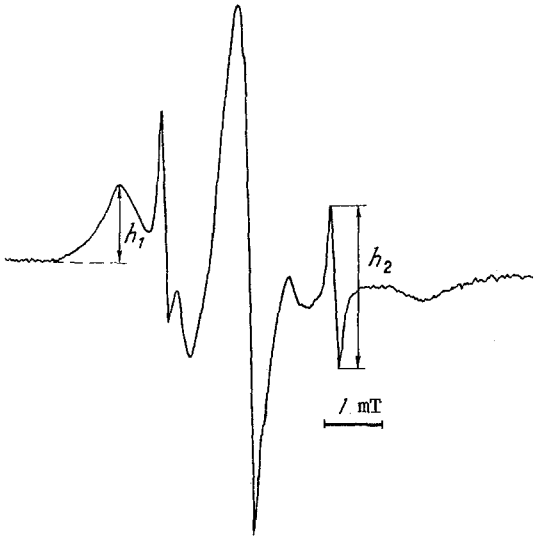
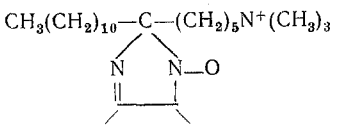
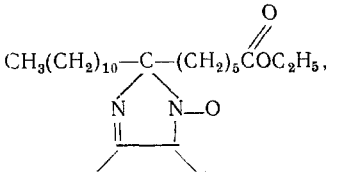


Fig. 1. EPR spectrum of spin probe I in suspension of LDL. Measuring medium: 5 mM Tris-HCl, pH 7.3 (37°C). Concentration of probe $8.7 \cdot 10^{-5}$ M; of LP 0.8 mg PL/ml.



(Probe I)

and



(Probe II)

synthesized by the method in [1], were used as spin probes. The probes were added to the LP suspension in the form of a solution in ethanol. The concentration of probe I in the sample was $8.7 \cdot 10^{-5}$ M, of probe II 10^{-4} M, and of ethanol 1 vol. %. In control experiments this ethanol concentration had no appreciable effect on the spectral parameters of probes I and II in LP. The distribution of the spin probes between LP and the aqueous environment was determined by the ratio h_1/h_2, where h_1 denotes half the amplitude of the low-polarity component of the EPR signal from probes bound with LP, and h_2 denotes the amplitude of high-polarity component of the EPR signal from probes dissolved in the aqueous phase (Fig. 1).

Surface potential can be determined by the equation [10, 11, 14]:

$$\varphi = \frac{RT}{zF} \ln \frac{\lambda}{\lambda_0},$$

where R is the universal gas constant; T the absolute temperature; z the charge of the probe; F the Faraday constant; λ the partition coefficient of the probe between the aqueous phase and LP; λ_0 the partition coefficient of the probe at $\varphi = 0$.

Under these circumstances

$$\lambda = N_W/N_{LP}$$

where N_W and N_{LP} denote the quantity of probe in the aqueous phase and LP respectively. N_W and N_{LP} are connected with the corresponding amplitudes of the EPR spectra (Fig. 1) by the equations:

$$N_W = K_W \cdot h_2, \quad N_{LP} = K_{LP} \cdot h_1,$$

where K_W and K_{LP} denote coefficients of proportionality, i.e.:

$$\lambda = \frac{K_W}{K_{LP}} \cdot \frac{h_2}{h_1} = K \cdot \frac{h_2}{h_1}, \quad \lambda_0 = K_0 \cdot \frac{(h_2)_0}{(h_1)_0},$$

TABLE 1. Values of Parameter h_1/h_2 for Probes I and II in Suspension of LP from Patients with IHD and Healthy Donors

Experimental conditions	LDL		HDL_2		HDL_3	
	probe I	probe II	probe I	probe II	probe I	probe II
Control (10)	0,57±0,13	1,81±0,05	0,13±0,03	0,74±0,05	1,47±0,10	1,30±0,11
IHD (12)	0,32±0,03*	2,03±0,11	0,14±0,02	0,82±0,04	1,89±0,06†	1,36±0,10

Legend. Concentrations (in mg PL/ml): LDL 0.8, HDL_2 0.4, HDL_3 1.0. *$P < 0.02$, †$P < 0.01$. Number of subjects tested shown in parentheses.

where the index 0 related to the case with $\varphi = 0$. If the change in h_2/h_1 was due only to a change in the surface charge of LP,

$$\varphi = \frac{RT}{zF} \ln \left[\frac{(h_2/h_1)}{(h_2/h_1)_0} \cdot \frac{K}{K_0} \right],$$

but the change in the surface potential of LP in IHD compared with LP of healthy blood donors is given by:

$$\Delta\varphi = \varphi_{\text{H}} - \varphi_{\text{IHD}} \frac{RT}{zF} \ln \frac{h_2/h_1}{(h_2/h_1)_{\text{IHD}}} = \frac{RT}{zF} \ln \frac{(h_1/h_2)_{\text{IHD}}}{h_1/h_2}. \quad (1)$$

This equation was used to determine the change in surface potential of LP of patients with IHD compared with that of the healthy control group. The results were subjected to statistical analysis by Student's test on an EMG-666B programed microcomputer (Hungary).

RESULTS

Spin probe I is an amphiphilic compound with a hydrophobic carbon chain and a positively charged group at its end, capable of interacting with negatively charged groups of lipids and proteins located on the surface of LP. The affinity of the carbon chain of the probe with the fatty acid residues of the phospholipids facilitates insertion of the hydrophobic part of probe I into the phospholipid region on the surface of LP. Consequently the distribution of probe I in the suspension of LP should depend at least on the surface charge of LP and on the character of molecular packing of the lipids in the surface monolayer of LP. If the probe is uncharged (for example, probe II), its distribution should not depend on the surface charge of LP, but should be determined only by the structural features of the lipoprotein layer. Investigation of the distribution of probes I and II in a suspension of LP of different classes, isolated from the plasma of IHD patients and healthy blood donors of the control group, showed that values of the parameter h_1/h_2 for probe II in a suspension of LDL, HDL_2, and HDL_3 from patients with IHD and healthy blood donors were identical (Table 1). Meanwhile, probe I differed in its distribution in LDL and HDL_3 from patients with IHD and the blood donors (in the case of HDL_2 this difference was not statistically significant, see Table 1). Incidentally, in a medium of high ionic strength (140 mM NaCl) no difference was found in the distribution both of the charged probe I and of the uncharged probe II in a suspension of LP from patients with IHD compared with the control. This fact, and also the absence of difference in the distribution of probe II and the difference in the distribution of probe I in the LP suspension from healthy blood donors and patients with IHD, lead to the conclusion that these differences are caused by changes in the surface charge, i.e., the surface potential, of LP with the development of IHD. Accordingly, equation (1) can be used to estimate changes in the surface potential of LP from IHD patients compared with that of healthy donors. As these data show, IHD is accompanied by a decrease (in absolute terms) of the negative surface potential (i.e., a decrease in the negative surface charge) of LDL, on the one hand, and an increase (i.e., an increase in the surface negative charge) in the case of HDL_3, on the other hand. The increase found in the surface potential of HDL_2 was not statistically significant.

It can be tentatively suggested that a change in the surface potential of LP will have a significant influence on the character of their interaction with cell membranes of the vessel wall. Since we know that positively charged apo-B sites are responsible for interaction of LP with cell membrane receptors [8, 9, 13], reduction of the surface potential of LDL in

IHD ought to facilitate contact of this class of LP with glycosaminoglycans on the surface of the vascular cells and blood cells, carrying a negative charge. Meanwhile an increase of the surface potential of HDL (i.e., an increase in their surface negative charge) probably impedes their interaction with arterial cell membranes. As we know, modern views on the atherogenic role of LDL and the antiatherogenic role of HDL [2, 3] are based on the assertion that these two facts may lead to accumulation of ChS in the vascular wall, i.e., to atherosclerotic changes in the vessels.

It must be emphasized that the development of IHD leads to an increase in the surface potential of HDL_3 only, and not of HDL_2. On the assumption that changes in surface potential are the main cause of disturbance of the properties of HDL in IHD, the antiatherogenic role of HDL is probably due mainly to subfraction HDL_3, for an increase in the surface potential of LP of this class leads to reduction of the antiatherogenic role of HDL and accompanies the development of IHD. This is in good agreement with the hypothesis that HDL_3 themselves, by interacting with cells of the vessel wall, accept lipids (including ChS), and are converted under these circumstances into HDL_2 [4].

Thus the surface potential (negative surface charge) of LDL is lower (in absolute value) in patients with IHD than in healthy subjects, whereas that of HDL_3 is higher. This may probably be one of the causes of the atherogenic role of LDL and of the antiatherogenic role of HDL.

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