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Effects of Lipid Peroxidation on Structure of Human Plasma Lipoproteins (Magnetic Resonance Spectroscopy)

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Magnetic resonance spectroscopy revealed structural modifications of human plasma lipoproteins during peroxidation induced by copper sulfate *in vitro*. Decreased molecular mobility of fatty acid chains in lipoprotein lipids was demonstrated.

Key Words: *peroxidation; lipoproteins; lipid mobility*

Lipoproteins (LP) transporting dietary fats into the plasma are presented by globules consisting of a hydrophobic core (nonpolar triglyceride and cholesterol ester molecules) and surface ambipolar layer (apolipoproteins and phospholipids). Low polar cholesterol molecules are present in both the core and surface layer of LP [9]. Particular interest in LP is determined by their involvement in lipid metabolism. Each type of LP, including very-low-density LP, intermediate-density LP, low-density LP (LDL), and high-density LP, plays a specific role in this process [8]. Studies of LP are important for understanding of the pathogenetic mechanisms of cardiovascular diseases and diagnostics of these disorders and tumors.

New approaches to the study of LP were provided by nuclear magnetic resonance technique. This method allows measurement of the concentration of compounds in test samples and their mobility. The concentrations of LP lipids [3,4], LP with different density [7], and various subtypes of LP [11,13] were measured in the plasma by proton magnetic resonance (PMR) spectroscopy. Specific features of PMR spectra of LP with different density were revealed in patients with cardiovascular diseases, tumors, and other disorders and in healthy individuals [6].

Oxidized LP play a key role in the pathogenesis of atherosclerosis and participate in the formation of atherosclerotic plaques. Moreover, oxidized LP induce functional disturbances in blood cells and inhibit coagulation and fibrinolysis. Here we studied structural changes in LP during lipid peroxidation (LPO).

MATERIALS AND METHODS

Reagents were purchased from Sigma. The blood was taken from 10 fasting donors and the plasma was obtained by centrifugation. The plasma from 6 donors (1-ml samples) was incubated with $CuSO_4 \times 5H_2O$ (final concentration 10 μmol/ml) at 37°C for 24 and 48 h. Oxidation was stopped by adding sodium ethylenediaminetetraacetate (EDTA, final concentration 200 μmol/ml) at 4°C. In the control series, the plasma was incubated without $CuSO_4$ at 37°C for 24 and 48 h, cooled to 4°C, and then EDTA (200 μmol/ml) and $CuSO_4$ (10 μmol/ml) were consecutively added.

Methanol extracts were obtained from lyophilized plasma samples. After extraction, methanol was evaporated to dryness on a rotor evaporator. The sediment was dissolved in 0.3 ml deuterium-labeled methanol.

LP lipids in oxidized and nonoxidized plasma samples and extracts were assayed by PMR spectroscopy. Heavy water was added to the samples (10% v/v) to

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obtain PMR spectra for the plasma. PMR spectra were recorded on a Brucker AM-400 WB nuclear magnetic resonance spectrometer at 37°C. Magnetic field homogeneity was evaluated by deuterium signals. We summarized 128 signals of free induction decay at 32 K. Signals of water protons in plasma samples were suppressed under conditions of selective presaturation. Single-impulse spectra were accumulated with the relaxation time and pulse duration of 25 sec and 90°, respectively. Spin-echo spectra were recorded using Hahn sequence:

$$D(90^{o}x\text{-}t\text{-}180^{o}y\text{-}t),$$

where t=68 msec, and D=3 sec.

RESULTS

Various proton-containing compounds with chemically similar structural fragments are present in the plasma and therefore the PMR spectrum of normal plasma recorded at 400 MHz is presented by a complex curve resulting from superposition of wide signals from macromolecules (immunoglobulins, albumin, and LP) and narrow signals from low-molecular-weight compounds (lactate, alanine, valine, and glucose). Relatively narrow signals from NCH_3 protons of phospholipids have a chemical shift of δ=3.25 ppm [7,10]. Signals with chemical shifts of 0.9 and 1.3 ppm are superpositions of resonances (CH_3 and CH_2 protons, respectively), LP with different density, and protons of CH_3 groups in cholesterol, lactate, and valine [7,10]. The spin-echo technique selectively detects resonances of mobile protons, recognizes signals from lipids, lactate, alanine, and valine and, therefore, markedly simplifies the spectra.

LPO sharply decreased the intensity of signals from CH_3 and CH_2 groups in LP lipids (Fig. 1, *a*). *In vivo* and *in vitro* γ-irradiation produced the same effect in human blood plasma [1]. The intensity of signals in spin-echo spectra depends on the concentration of fragments in molecules and their molecular mobility. To estimate the influence of fragment concentration we used single-impulse spectra of methanol plasma extracts. In these extracts the macrostructure of LP was broken, and the intensity of signals was proportional to the concentration of the corresponding groups. The spectra of extracts contained signals of CH_3 and CH_2 groups in lipids, cholesterol signals of CH_3 groups in C-18, C-21, C-26, and C-27, and 2 signals of C-19 from cholesterol and cholesterol ester of linoleic acid (Fig. 1, *b*) [10]. The intensity of multiplets at 0.9 and 1.3 ppm in control and oxidized samples was similar (Fig. 1, *b*). Therefore, the concentration of CH_3 and CH_2 groups of lipids in oxidized LP did not differ from that in nonoxidized LP. These results are consistent with published data that oxidation of LDL with $CuSO_4$ produces an insignificant decrease in the amount of CH_3 and CH_2 groups in LP lipids (no more than 15%) [4].

These results show that the disappearance of signals from echo-spectra at 0.9 and 1.3 ppm reflects the decrease in mobility of CH_3 and CH_2 groups in LP lipids, rather than changes in their concentration. Studies of echo spectra from extracts of oxidized and control plasma samples showed that the intensity of signals at 0.9 and 1.3 ppm remained unchanged during oxidation (Fig. 1, *c*). Therefore, the effect was associated with changes in the macrostructure of LP during LPO. By contrast, the contribution of structural changes in lipid molecules was insignificant. Therefore, LPO decreases mobility of alkyl chains in LP lipids. In echo spectra these changes are manifested in disappearance of resonances from CH_3 and CH_2 groups.

The effect was observed in samples oxidized for 48 h, but not 24 h. At the same time, the dynamics of accumulation of thiobarbituric acid-reactive products showed that $CuSO_4$-induced LPO reached the stationary phase 1 day after the start of the experiments [4]. In our experiments the reaction can be evaluated by the ratio of signal intensity from protons of isolated C=C bonds in polyunsaturated fatty acid fragments of lipids (δ=5.4 ppm) and conjugated double bonds in conjugated dienes formed during LPO (δ=5.72 ppm) [2]. The signal at 5.72 ppm was not detected in control samples, but appeared the plasma oxidized for 24 and 48 h. The ratio between the concentrations of lipid fragments with isolated and conjugated double bonds calculated as the ratio between the intensity of signals at 5.4 and 5.72 ppm in spectra of plasma extracts oxidized for 24 and 48 h was similar (8:1). Therefore, the stationary phase was reached after 24-h incubation. Mobility of CH_3 and CH_2 groups in LP decreased only after 48 h. Hence, this effect depended on the time of incubation, but was not directly related to LPO. When oxidation was stopped after 24 h by the addition of EDTA and the reaction mixture was kept at 37°C for 24 h, changes in spin-echo spectra were similar to those observed after 48-h oxidation. Signals from alkyl fragments of lipids disappeared. It should be emphasized that this effect was not observed in control samples incubated for 48 h.

Our results indicate that oxidation modifies the macrostructure of plasma LP, which is followed by a considerable decrease in mobility of CH_3- and CH_2-containing fragments in lipid molecules. The effect can result from aggregation of LP, which leads to the appearance of close contacts between surface monolayers of individual particles. These conglomerates are characterized by low mobility [5]. Therefore, mobility

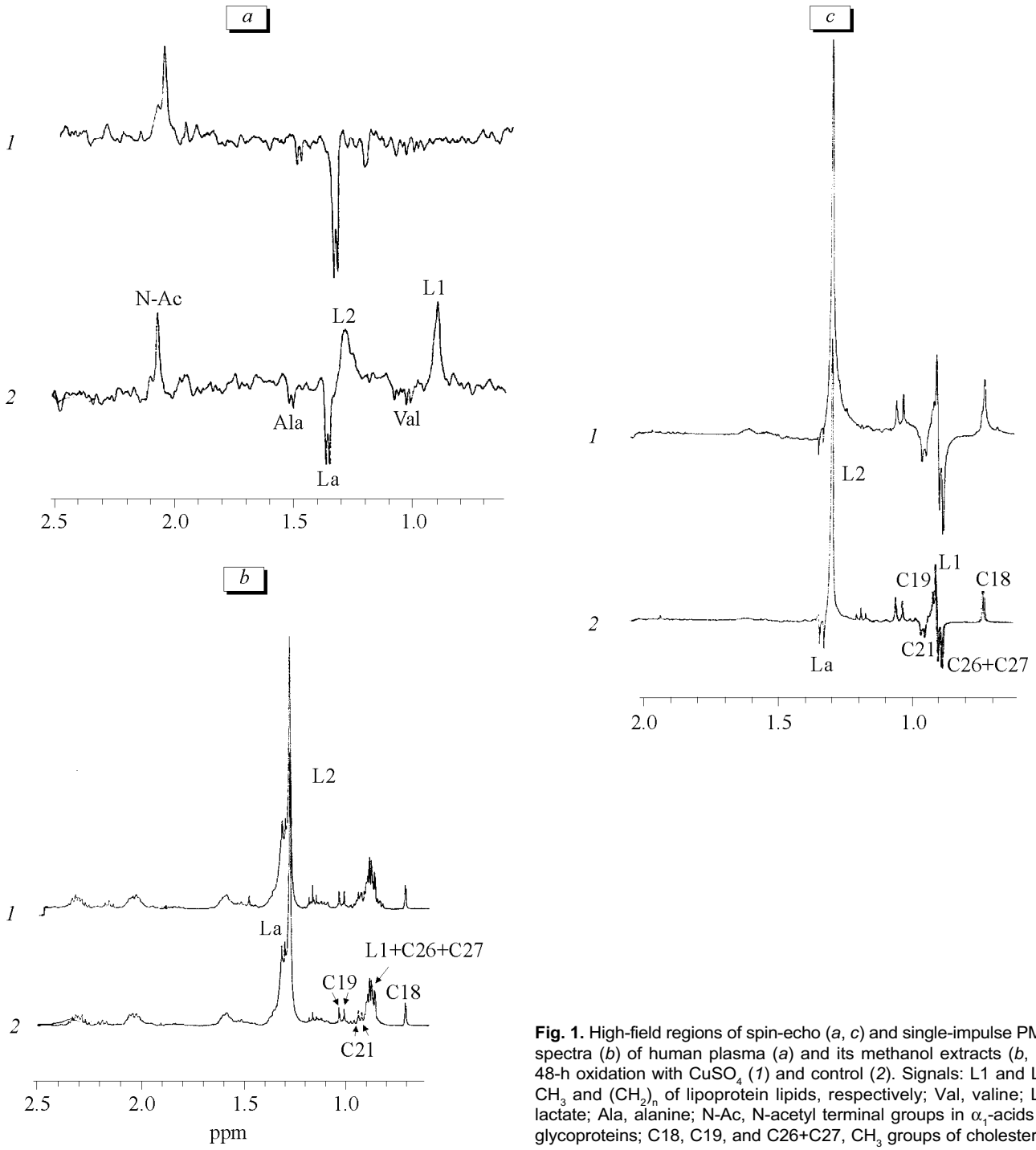

Fig. 1. High-field regions of spin-echo (*a*, *c*) and single-impulse PMR spectra (*b*) of human plasma (*a*) and its methanol extracts (*b*, *c*): 48-h oxidation with $CuSO_4$ (*1*) and control (*2*). Signals: L1 and L2, CH_3 and $(CH_2)_n$ of lipoprotein lipids, respectively; Val, valine; La, lactate; Ala, alanine; N-Ac, N-acetyl terminal groups in α_1-acids of glycoproteins; C18, C19, and C26+C27, CH_3 groups of cholesterol.

of their lipid components decreases. It should be emphasized that in echo spectra of oxidized plasma samples the intensity of signals at 3.25 ppm (amino groups of phospholipid) remained unchanged. Therefore, this signal was not associated with LP globules. Phosphatidylcholine hydroperoxide is one of the major primary products formed after peroxidation of LP [12]. Structural modification of surface layers in LP during oxidation is probably accompanied by the release of choline-containing fragments from LP. This process compensates the loss of signals at 3.25 ppm produced by a decrease in mobility of LP during aggregation.

Our results indicate that peroxidation causes structural modification of LP. These data hold much promise for the early diagnostics of atherosclerosis.

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