Changes in the Ratio between Apolipoprotein (a) and Lipids in Human Plasma during Interaction with Polyclonal Sheep Antibodies against Lipoprotein (a)

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Plasma contents of apolipoprotein (a), apolipoprotein B 100, cholesterol, triglycerides, and vitamin E were measured in 2 patients with lipoprotein (a) concentration >100 mg/dl during the interaction with the anti-lipoprotein (a) immunosorbent. Intraindividual heterogeneity of apolipoprotein (a)-containing particles in the plasma was demonstrated. Polyclonal antibodies against lipoprotein (a) immobilized on Sepharose CL-4B more effectively removed free apolipoprotein (a) than complexes containing apolipoproteins B 100, apolipoprotein (a), lipids, and vitamin E.

Key Words: *lipoprotein (a); apolipoprotein (a); apolipoprotein B 100; anti-lipoprotein (a) immunosorbent; vitamin E*

High plasma level of lipoproteins containing apolipoprotein (a), apo(a), is an independent risk factor for cardiovascular and cerebrovascular diseases. Lipoprotein (a), LP(a), is a heterogeneous group of lipoprotein particles in which apolipoprotein B 100 (apoB 100) is bound to apo(a) via a covalent disulfide bond. Heterogeneity of LP(a) particles is determined by different composition and content of their lipid and protein components. Genetically determined differences in the structure of apo(a) contribute to interindividual variability in the properties and structure of apo(a)-containing particles.

Human plasma includes apoB 100-apo(a)-containing particles that differ in lipid composition and content, ratio between protein and lipid components, and floating density [4]. These data suggests the existence of intraindividual heterogeneity in LP(a) particles. Low-density lipoprotein (LDL)-like LP(a) particles enriched with cholesterol esters, apoB 100-apo(a) lipoprotein complexes enriched with triglycerides, and apoB 100-apo(a) complexes with low and variable content of lipids can simultaneously circulate in human plasma [3]. The data on plasma levels of free (unbound) apo(a) not containing lipids are contradictory because of difficulties in the isolation of free apo(a) from complexes containing apo(a) antigen.

Immunosorbent based on polyclonal sheep antibodies against human plasma LP(a) effectively eliminates apo(a) antigen-containing compounds from the plasma in patients with genetically determined high level of LP(a) [6]. Expression of apo(a) epitopes mediating its interaction with immunosorbent depends on the content and composition of lipids in apo(a)-containing complexes. We hypothesized that apo(a)-containing complexes with different content of lipids display various binding affinity for the immunosorbent. Taking into account hydrophilicity of apo(a), we assumed that lipid-free apo(a) can be revealed in complexes containing apo(a) antigen and not containing hydrophobic compounds (*e. g.*, vitamin E).

Here we studied intraindividual heterogeneity of apo(a)-containing particles. To this end we analyzed

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changes in the ratio between the content of apo(a) antigen and concentrations of cholesterol, triglycerides, and vitamin E in two patients with high plasma content of LP(a) during anti-LP(a) immunosorption.

MATERIALS AND METHODS

Venous blood was taken from 2 patients with angiographically documented coronary heart disease and very high blood level of apo(a) antigen after 12-h starvation. LP(a) apheresis was performed at 2-week intervals using columns packed with anti-LP(A) immunosorbent (Pokard, Moscow) at the Laboratory of Hemodialysis and Plasmapheresis [1]. Blood cells were separated from the plasma after addition of heparin using a Cobe Spectra centrifuge plasma separator.

Plasma samples were taken at the inlet and outlet of the column to study the interaction of the plasma with polyclonal sheep antibodies against LP(a) immobilized on Sepharose CL-4B [6]. The placebo column without antibodies served as the control. The efficiency of immunosorption was estimated by changes in the concentration of test compounds in the venous blood before and after this procedure.

Plasma concentrations of cholesterol (total and in lipoprotein fractions) and triglycerides were measured enzymatically on a Kone biochemical analyzer.

Plasma concentration of apo(a) antigen was measured by sandwich immunoassay [2]. The content of apoB 100 was measured by immunoturbidimetry using Kone kits. The concentration of vitamin E was determined after extraction of hydrophobic compounds from the plasma containing 1 mg/ml EDTA as described previously [8] with modifications. DL-α-tocopherol (Serva) was used as the standard.

RESULTS

In patient 1, plasma levels of total cholesterol, high-density lipoprotein cholesterol, triglycerides, and vitamin E were 6.3, 0.9, and 1.0 mmol/liter and 27.4 μmol/liter, respectively. In patient 2 these parameters were 5.4, 1.3, and 1.5 mmol/liter and 23.8 μmol/liter, respectively.

Polyclonal sheep antibodies against LP(a) immobilized on Sepharose CL-4B used as the anti-LP(a) immunosorbent displayed high selectivity and effectively decreased LP(a) concentration in the venous blood (Fig. 1). The dynamics of the decrease in vitamin E content (hydrophobic compound circulating in lipoprotein particles) was similar to that of lipids. Thus, the efficiency of elimination of apo(a) antigen from the plasma 2.5-3-fold surpassed the efficiency of elimination of lipids and vitamin E. This suggests the presence of apo(a)-containing complexes with different lipid content.

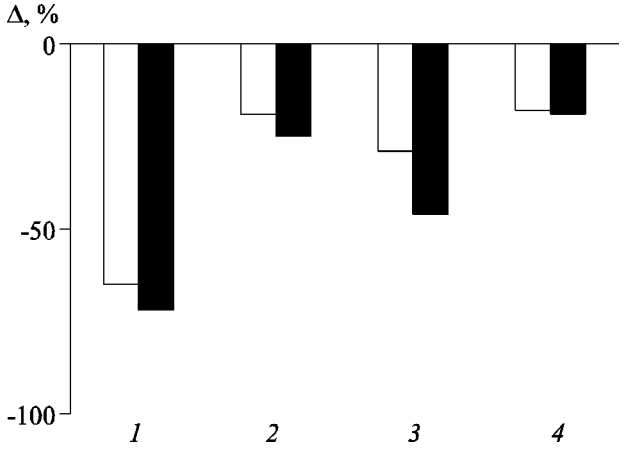

Fig. 1. Decrease in the content of lipoprotein (a), LP(a), and lipids in the venous blood after anti-LP(a) immunosorption: LP(a) (*1*), total cholesterol (*2*), triglycerides (*3*), and vitamin E (*4*). Here and in Fig. 2: patient 1 (light bars) and patient 2 (dark bars). The means for 15 procedures for each patient are presented.

Since the concentration of test compounds in the venous blood depends on blood dilution during the immunosorption procedure, the dynamics of apo(a), apoB 100, and lipid concentrations in the venous blood are clinically important, but does not reflect peculiarities of anti-LP(a) immunosorption. For evaluation of the efficiency of anti-LP(a) polyclonal antibodies in eliminating apo(a)-containing complexes, plasma samples were taken at the inlet and outlet of the anti-LP(a) immunosorbent-packed column. Taking into account the fact that vitamin E is present only in lipid-containing of apoB 100-apo(a) complexes, but not in amphiphilic molecule of free apo(a), changes in the ratio between vitamin E concentration and contents of the apo(a) antigen, apoB 100, cholesterol, and triglycerides in the plasma were estimated before and after immunosorption. The ratio between vitamin E content and levels of apoB 100-containing particles, cholesterol, and triglycerides changed insignificantly during immunosorption (Fig. 2). A sharp increase in the ratio between vitamin E and apo(a) antigen concentrations after immunosorption attests to higher affinity of polyclonal antibodies for free apo(a) compared to that for lipid-containing complexes of apoB 100 and apo(a). This is probably related to partial masking of apo(a) epitopes responsible for the interaction with the sorbent in lipid-enriched apoB 100-apo(a) lipoprotein particles. We found no changes in placebo columns. Our experiments demonstrated intraindividual heterogeneity of apo(a)-containing particles in plasma samples from both patients.

Recent studies showed that apo(a) molecule can induce synthesis of I-309 chemokine in endothelial cells. This chemokine produces a chemotactic effect on monocytes [7]. Monocyte chemoattractant activity induced by apo(a) differs from activity of monocyte

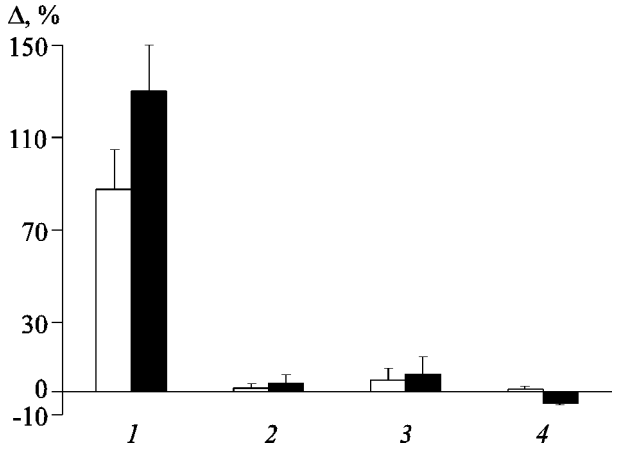

Fig. 2. Changes in the ratio between vitamin E content and concentrations of plasma lipids and apolipoproteins after interaction with anti-LP(a) immunosorbent: vitamin E/LP(a) (*1*), vitamin E/total cholesterol (*2*), vitamin E/triglycerides (*3*), and vitamin E/apoB 100 (*4*).

chemoattractant protein-1, whose synthesis in endothelial cells is initiated by lipid components of minimally oxidized LDL [5].

Among apo(a)-containing complexes, free apo(a) most actively interacts with matrix components of the vascular wall and is rapidly accumulated in inflammatory foci. The proinflammatory activity and adhesive properties of free apo(a) suggest that the increase in the ratio of lipid-free apo(a) enhances plasma atherogenicity (even at the same level of apo(a) antigens). The mechanisms underlying intraindividual heterogeneity of apo(a)-containing particles are poorly understood. Polyclonal antibodies against LP(a) can be used as a tool in further studies in this field.

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