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Hydroperoxides of ApoB-Lipoproteins Isolated by Immunoaffinity Chromatography

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Studies using affinity chromatography and ultracentrifugation indicate that in human blood 40-50% of lipid hydroperoxides are transported by apoB-lipoproteins, 20% by high-density lipoproteins, and 30-40% by albumins and other proteins. Affinity-isolated apoB-lipoproteins from umbilical blood contain smaller amounts of lipid hydroperoxides than do those from the blood of healthy adults, while the content of lipid hydroperoxides in apoB-lipoproteins from the blood of patients with ischemic heart disease is often higher. Aortal apoB-lipoproteins are considerably enriched in these hydroperoxides.

Key Words: *peroxidation; hydroperoxides; lipoproteins; affinity chromatography; atherosclerosis*

Modification of plasma low-density lipoproteins (LDL) by lipid peroxidation (LPO) products is a major contributor to atherosclerotic lesions of coronary arteries in man [15]. One of the LPO products reflecting initial stages of LPO is lipid hydroperoxides (LHP) [9].

According to some authors, the bulk of blood plasma LHP occurs in albumins and in high-density lipoproteins (HDL) in healthy people and also in LDL in patients with coronary atherosclerosis. Other authors came to the conclusion that LPO products are present only in LDL [13].

The objectives of this study were to examine the distribution of LHP among LDL, HDL, and other plasma components and to measure their levels in apoB-lipoproteins (apoB-LP) isolated by immunoaffinity chromatography or ultracentrifugation.

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MATERIALS AND METHODS

Blood was taken from the cubital vein with EDTA used as anticoagulant (final concentration 1 mg/ml). Plasma was separated by low-velocity centrifugation (600 g), adding butylated hydroxytoluene (final concentration 1 μM) to plasma samples to inhibit peroxidation.

LDL were isolated using an affinity carrier containing as a ligand monospecific polyclonal sheep antibodies against human apoB-100 (4-5 mg IgG/ml gel) which is used as a sorbent for LDL in patients treated in the Cardiology Research Center of the Russian Academy of Medical Sciences [3,12]. Each column of 1 cm in diameter contained 4-6 ml of the sorbent and was equilibrated with 0.15 M NaCl solution containing 0.05 M phosphate buffer, pH 7. Unbound proteins were eluted with this buffer and then with 0.5 M NaCl, while bound proteins were eluted with a 0.2 M glycine-HCl buffer, pH 2.5. From 2 to 4 ml of plasma were applied per column. The completeness of elution was checked spectrophotometrically at 280 nm using a flow-through ultraviolet sensor.

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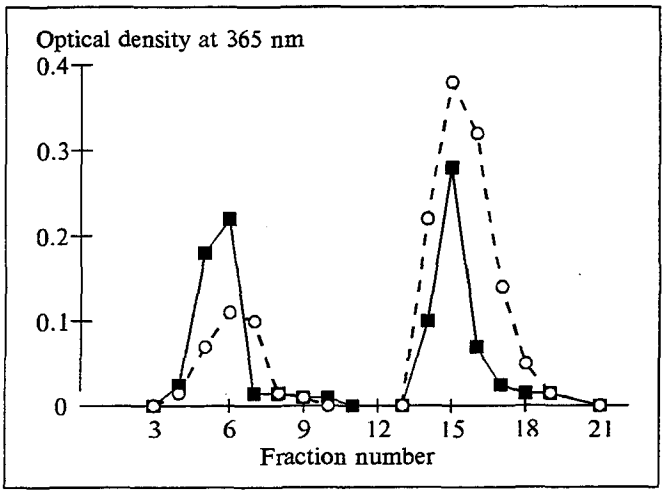

Fig. 1. Distribution of lipid hydroperoxides between unbound plasma components (first peak) and those bound to the affinity apoB-LP gel (second peak). Solid line: plasma; dashed line: total LP fraction (d<1.21 g/ml).

LHP were determined spectrophotometrically [6]. For this purpose 1.3 ml water and 1.5 ml iodine reagent were added to 0.2 ml plasma. After the plasma samples were kept in the darkness for 30 min, their optical density was measured on an SF-26 spectrophotometer, using titrated H_2O_2 solutions for calibration and a molar extinction coefficient of triiodide ion equal to 2.45×10^4 $M^{-1}cm^{-1}$ for calculations [6].

Protein was determined by the Lowry method in the presence of sodium dodecyl sulfate using bovine serum albumin as the standard. Total LP and their individual classes were obtained by sequential centrifugation in an NaBr density gradient [10] and then dialyzed against 0.0001 M EDTA.

Plasma from healthy people (without diseases of internal organs), umbilical serum, and plasma from patients with ischemic heart disease documented by standard methods were used [2].

Extracts of human atherosclerosis-affected aortal intima-media were prepared as previously described [1].

TABLE 1. Distribution of Lipid Hydroperoxides (LHP) Between Lipoproteins (LP) and Proteins in the Plasma of Healthy Subjects (n=3)

Fraction	Density, g/cm³	LHP distribution, %
VLDL+LDL	1.006-1.063	42
HDL_2	1.063-1.125	15
HDL_3	1.125-1.210	10
VHDL and other proteins	>1.210	33

Note. VHDL = very high density lipoproteins.

RESULTS

It was found that approximately one half of LHP occurred in plasma outside apoB-LP and the other half within these lipoproteins (Fig. 1). Chromatography of total LP revealed that 20% of the LHP were transported unbound to the LP (HDL) sorbent, while 80% were transported in the bound form, i.e., as apoB-LP [very low-density lipoproteins (VLDL), LDL, and LP(a)]. These findings agree well with the results obtained in analyzing the LP isolated by ultracentrifugation: about 40% of the hydroperoxides occurred in the LDL+VLDL fraction, 25% in HDL, and 35% in other plasma proteins (Table 1).

The concentration of LHP was lowest in the affinity-isolated apoB-LP of umbilical blood (1 nmol/mg protein). In healthy subjects, the LHP concentration was 4 times higher and corresponded to 4 nmol/mg protein of the LDL obtained by centrifugation.

Among patients with ischemic heart disease (n= 22), there were individuals with high LHP levels in the apoB-LP (in 78% of the cases) and those with levels characteristic of healthy people (in 22% of the cases). In general, individuals with elevated LHP levels predominated among the patients, the mean LHP level being 9 nmol/mg protein (Table 2).

The content of LHP in the aortal wall apoB-LP from healthy subjects was 4.2 times higher than in their plasma apoB-LP (Table 2).

These results agree with the previously reported data that aortal LDL are more peroxidated than plasma LDL [1].

LHP levels in the LP rose sharply during storage even at 4°C, and 14 days after LDL were isolated their particles were found to have divided into two fractions, a gel-bound fraction and a gel-unbound one. Both fractions contained elevated LHP levels, exceeding 5-fold and 14-fold, respectively, those in the plasma LP from healthy subjects (Table 2). This indicates that isolated LP undergo spontaneous peroxidation during storage and that a proportion of the apoB-LP are so strongly modified as to be incapable of being recognized by polyclonal antibodies.

It is now known that apoB-LP can be damaged by free radicals [4], LHP [13,17], and LHP destruction products (aldehydes) [7]. Modification of the LDL by free radicals does not yet result in the acquisition of atherogenic properties by LDL particles, as was found when the endpoint used was interaction with macrophages [7,10]. In the case of aldehydes, such an effect was detected and its magnitude depended on their concentration and hydrophobicity; thus, 4-hydroxynonenal was more effective in

TABLE 2. Lipid Hydroperoxide (LHP) Levels in Affinity-Isolated Lipoproteins (LP)

Source of LP	*n*	LHP level, nmol/mg of altered LP protein	Change, %
Umbilical plasma	3	1±1	25
Plasma from healthy subjects	11	4±2	100*
Plasma from patients with ischemic heart disease:			
normal LHP level	5	4±2	100
high LHP level	17	9±2	225
Aortal extract	3	17±4	425
Apo-LP after 14 days**	2		
gel-bound		21±4	525
not gel-bound		58±7	1450

Note. *LHP and LP levels in adult plasma were taken to be 100%. **LP were affinity-separated from plasma of healthy subjects and stored at 4°C in 0.15 M NaCl (pH 7.4) in the presence of 5 μM methylphenylsulfonyl fluoride.

damaging LDL than were hexanal and malonic dialdehyde in the same concentration [10].

The accumulation of LHP in an LP particle appears to reflect the intensity of LPO processes as a whole, so that the more primary LPO products the particle contains, the higher are the levels of intermediate and end products in it. The degree to which protein is modified by LPO products seems to be proportional to their total content in the particle. These conclusions are based on the results of a study where the distribution of various aldehydes between erythrocyte membrane lipids and proteins was considered in relation to their total content in the erythrocyte membrane [5].

About 15 years ago a correlation was found to exist between LPO product levels in human plasma LDL and the area of coronary vessels affected by atherosclerosis [14].

The results of the present study also highlight the relationship between LHP levels in apoB-LP and atherogenesis. For isolation of LP to determine their content of LHP, two methods may be used — immunoaffinity chromatography and ultracentrifugation. However, the unquestionable advantages of the affinity method are rapidity of analysis (≈30-40 min) and its potentially complete automatization using standard blocks of high performance liquid chromatography.

Since LPO products accumulate not only in ischemic heart disease but also in many other conditions [16], it seems important to utilize the affinity method for isolating both apoB-LP and other plasma components, such as albumin, HDL, and LP(a), to assess how LHP are transported in various pathological states by LP of different classes and also by proteins.

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