Oxidation of Plasma Low-Density Lipoproteins from Coronary Patients with Various Forms of Hypercholesterolemia

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The duration of lag-phase of copper-induced free-radical oxidation of atherogenic LDL isolated from the plasma of coronary patients without hypercholesterolemia virtually does not differ from that of normal human LDL. On the other hand, lag-phase of plasma LDL oxidation was minimal in coronary patients with primary hypercholesterolemia without familial history and especially in patients with familial hypercholesterolemia. This can be attributed to sharply decreased content of natural lipid antioxidants in LDL of patients with familial hypercholesterolemia. However, therapy with natural antioxidant vitamin E did not modulate oxidizability of these LDL. By contrast, therapy with β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitor suppressing biosynthesis of ubiphenol Q induced sharp accumulation of lipoperoxides in LDL *in vivo*. These data suggest that reduced form of ubiquinone Q is the main antioxidant protecting LDL from free-radical oxidation.

Key Words: *low density lipoproteins; free-radical oxidation; lipoperoxides; antioxidants; vitamin E; ubiquinone Q; β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitors*

High plasma cholesterol level is a risk factor of atherosclerosis development and progress; cholesterol content in circulating atherogenic LDL is a still more informative parameter [2]. Free-radical oxidation of LDL leads to modification of their structure, modified LDL are recognized by scavenger receptors on macrophages penetrating into the interendothelial space of the vascular wall and are intensely captured by these cells [10]. Macrophages thus turn into the so-called "foam cells" and form lipoidosis zone in the aorta and coronary arteries, which can be regarded as the primary atherosclerotic damage to blood vessels.

Cholesterol and phospholipids are the main lipid components of natural membranes. Cholesterol concentration in the lipid bilayer is essential for free-radical oxidation of phospholipids, the most easily oxidized membrane lipids. We previously showed that changes in the content of lipoperoxides and cholesterol in bilayer lipid membranes (liposomes) produced opposite effects on their structural and dynamic parameters [3]. We hypothesized that induction of free-radical oxidation in biomembranes enriched with cholesterol can be regarded as a compensatory process aimed at the maintenance of certain structural and dynamic characteristics of the membrane essential for its normal functioning [3]. In contrast to the bilayer lipid membranes, the surface layer of LDL particles represent a phospholipid monolayer, where hydrophobic fatty acid chains are oriented inside and contact with the hydrophobic core consisting of nonpolar lipids (triglycerides and cholesterol esters) [2]. Surface phospholipids are first oxidized during free-radical oxidation of LDL, and only after lecithin peroxidation unsaturated cholesterol ester acyls from the hydrophobic core can be involved in oxidation. Hydroperoxides of cholesterol esters internalized by monocyte-macrophages with oxidized LDL or formed during enzymatic oxidation in the aorta *in situ* intensely accumulate

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in zones of atherosclerotic involvement of the vascular walls [9]. In light of this, we investigated the relationship between cholesterol content in plasma LDL from patients with atherosclerosis with different forms of hypercholesterolemia (HCS) and oxidizability of these lipoproteins *in vitro*.

MATERIALS AND METHODS

Thirty-eight coronary patients (30 males and 8 females) were included in the study: 15 patients (47±2 years) had familiar HCS, 14 patients (46±2 years) had primary HCS without clearly detected family history, and in 9 coronary patients (48±2 years) cholesterol levels were nearly normal (cholesterol level of 5.2 mmol/liter was taken for normal). Control group consisted of 7 men aged 45±5 years without signs of coronary disease and HCS. During observation the patients received standard antianginal therapy including β-adrenoblockers, calcium antagonists, and antiaggregants; cholesterol-reducing drugs, including β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA-reductases; statins) were excluded from the treatment protocol.

A special group of 29 men (47-59 years) with coronary disease and type IIa HCS received vitamin E (α-tocopherol acetate, L'vov Drug Factory) in a daily dose of 400 mg for 3 months. Another group of coronary patients with type IIa HCS (12 men, 3 women, aged 41-65 years) received a daily dose (20 mg) of HMG-CoA reductase inhibitor simvastatin (Vasilip, KRKA).

Venous blood for isolation of LDL was collected after overnight fasting in tubes with 1 mg/ml EDTA. Plasma was centrifugated twice in a NaBr density gradient for 2 h at 42,000 rpm in a 50 Ti angular rotor at 4°C in a Beckman L-8 ultracentrifuge as described elsewhere [12] and then dialyzed at 4°C for 16 h. Protein content was measured by the method of Lowry, and LDL concentration was adjusted to 50 μg protein/ml with a solution containing 0.154 M NaCl and 50 mM phosphate buffer (pH 7.4). LDL oxidation

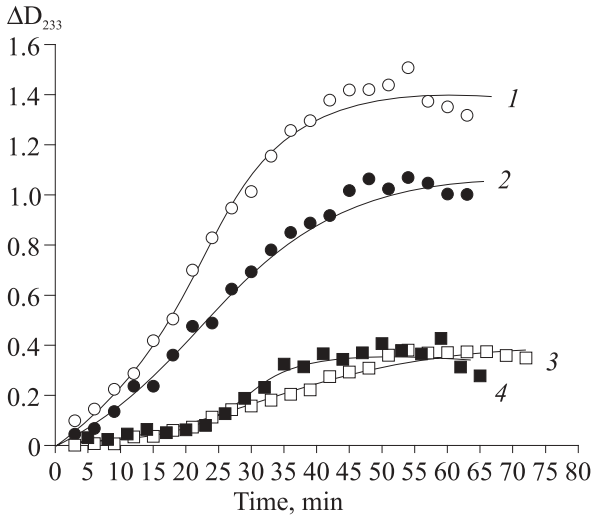

Fig. 1. Typical kinetic curves of Cu^{2+}-induced oxidation of plasma LDL isolated from coronary patients with familial hypercholesterolemia (*1*), coronary patients with primary hypercholesterolemia without proven familial history (*2*), coronary patients without hypercholesterolemia (*3*), and normal subjects without signs of coronary disease and hypercholesterolemia (*4*).

was induced by adding 3×10^{-5} M $CuSO_4$ at 37°C, accumulation of lipohydroperoxides was measured after fixed periods at λ=233 nm on a Hitachi 220A spectrophotometer [5,8]. Kinetic curves were constructed and the duration of the oxidation lag-phase was determined. Total cholesterol and HDL cholesterol were measured by the enzymatic method on an Airone-200 chemical analyzer using Biocon kits, and LDL cholesterol content was deduced from these data. The results were statistically processed using Mann—Whitney nonparametric test.

RESULTS

The highest LDL cholesterol level was detected in patients with familial HCS (2.6-fold surpassed the control, $p<0.001$). LDL cholesterol concentration in patients with primary HCS without proven familial history was notably lower (1.4-fold surpassed the control, $p<0.001$). LDL cholesterol level in coronary pa-

TABLE 1. Total and LDL Cholesterol Content and Oxidizability of Plasma LDL in Coronary Patients with Various Forms of HCS

Group of patients	Total cholesterol, mmol/liter	LDL cholesterol, mmol/liter	Atherogenicity index	Lag-phase, sec
Control (no coronary disease or HCS, *n*=7)	5.10±0.03	3.30±0.33	5.2±0.5	945±23
Coronary disease+fHCS (*n*=15)	10.50±0.63**	8.9±0.9**	13.60±2.87***	188.8±49.0*
Coronary disease+HCS_{prim} (*n*=14)	6.50±0.17^{+}	4.90±0.26^{+}	7.10±0.84^{+++}	715.7±151.0^{+++}
Coronary disease without HCS (*n*=9)	4.8±0.1^{+}	3.10±0.15^{+}	4.60±0.23^{+}	936.6±207.0^{++}

Note. fHCS: familial hypercholesterolemia; HCS_{prim}: primary hypercholesterolemia without proven family history; *n*: number of patients in the group. *p=0.007, **p=0.008, ***p=0.015 compared to the control; $^{+}p<0.001$, ^{++}p=0.005, ^{+++}p=0.006, ^{++++}p=0.012 compared to coronary disease+fHCS group.

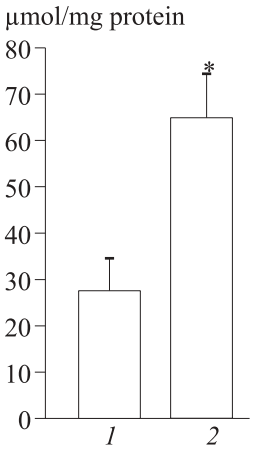

Fig. 2. Content of lipoperoxides in plasma LDL isolated from coronary patients treated with simvastatin (β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitor) in a daily dose of 20 mg. *1*) before therapy; *2*) after 2-month therapy. **p*=0.003 compared to this parameter before therapy.

tients without HCS virtually did not differ from that in the control group. Similar results were obtained for the total cholesterol and atherogenicity index (Table 1).

Oxidizability of plasma LDL was evaluated by lag-phase of copper-induced oxidation: this parameter was minimum in coronary patients with familial HCS and maximum in coronary patients without HCS (Fig. 1, Table 1). The lag-phase of plasma LDL oxidation in patients with familial HCS was 5-fold shorter and in patients with primary HCS without familial history 1.3-fold shorter than in control individuals. In coronary patients without HCS this parameter virtually did not differ from the control. Hence, our data suggest that the lag-phase (induction period) of LDL oxidation depends on the content of natural lipid antioxidants, rather than on cholesterol content in these structures. However, even after 3-month treatment with vitamin E in a daily dose of 400 mg (the dose by more than an order of magnitude higher than the daily need in this vitamin) we observed no significant changes in oxidizability of plasma LDL isolated from coronary patients. The lag phase of LDL oxidation before and after 3-month vitamin E therapy was 30.5±6.3 and 42.4±9.2 min, respectively ($p>0.05$). This can be explained by the fact that circulating LDL are protected from oxidation not by α-tocopherol (vitamin E) transported by these particles, but by another natural antioxidant, ubiphenol Q_{10} (reduced form of ubiquinone Q_{10} synthesized in the body) [11] present in LDL in minor amounts. Ubiphenol Q_{10} (QH_2) can directly react with lipid radicals ($LO_2^{\bullet}$, $LO^{\bullet}$) with the formation of ubisemiquinone radical ($^{\bullet}QH$):

$LQ_2+QH_2 \rightarrow LOOH+{}^{\bullet}QH$,
$LQ_2^{\bullet}+{}^{\bullet}QH \rightarrow LOOH+Q$
or $LO^{\bullet}+QH_2 \rightarrow LOH{}^{\bullet}QH$,
$LO^{\bullet}+{}^{\bullet}QH \rightarrow LOH+Q$.

The same mechanism underlies ubiphenol-dependent bioregeneration of α-tocopherol phenoxyl free radicals (α-$TO^{\bullet}$), forming during interactions of vitamin E (α-TOH) with lipid radicals:

$LO_2^{\bullet}+\alpha\text{-TOH} \rightarrow LOOH+\alpha\text{-}TO^{\bullet}$,
or $LO^{\bullet}+\alpha\text{-TOH} \rightarrow LOH+\alpha\text{-}TO^{\bullet}$,
$\alpha\text{-}TO^{\bullet}+QH_2\text{-}\alpha \rightarrow TOH+{}^{\bullet}QH$,
$\alpha\text{-}TO^{\bullet}+{}^{\bullet}QH\text{-}\alpha \rightarrow TOH+Q$.

As we see from these schemes, high efficiency of antioxidant and tocopherol-sparing effects of reduced form of ubiquinone Q_{10} is determined by the possibility of reducing two tocopheroxyl or lipid radicals by one molecule of ubiphenol Q_{10}. The biological essence of this process is that ubiquinone Q_{10} (but not an essential component vitamin E) is utilized in the reaction; the required amount of ubiquinone Q_{10} can be replenished via its biosynthesis [7] or via reduction of ubisemiquinone radicals in mitochondrial electron transport chain or in the reaction with ascorbic acid. Bioregeneration of semidehydroascorbate and dehydroascorbate is easily realized with participation of various enzyme systems [1,4]. It is noteworthy that ubiquinone Q_{10} is present in normal human plasma predominantly (by 95-100%) in reduced antioxidant form [10], while in atherosclerosis the plasma concentration of reduced ubiquinone Q_{10} notably decreased.

It seems that ubiphenol Q_{10}, but not α-tocopherol, is responsible for LDL protection from free-radical oxidation. If so, the differences in LDL oxidation in the studied groups of coronary patients (Fig. 1, Table 1) are probably determined by different levels of ubiphenol Q_{10} in these particles. On the other hand, hypolipidemic drugs belonging to HMG-CoA-reductase inhibitors (statins) suppress both cholesterol and ubiquinone Q_{10} biosynthesis [5,7], and some authors reported a significant decrease in the content of ubiquinone Q_{10} LDL [7]. Hence, we can expect that the decrease of cholesterol level in coronary patients treated with HMG-CoA-reductase inhibitors is paralleled by an increase in LDL oxidation. Our results indicate that 2-month therapy with an HMG-CoA-reductase inhibitor simvastatin was associated with intensive accumulation of lipoperoxides in LDL *in vivo* (Fig. 2), which confirms our previous data [5,6]. Hence, improvement of the antioxidant status of patients can improve the efficiency of hypolipidemic therapy.

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