Intensification of Free Radical Oxidation of Low-Density Lipoproteins in the Plasma of Patients with Ischemic Heart Disease Receiving β-Hydroxy-β-Methylglutaryl-Coenzyme A Reductase Inhibitor Cerivastatin and Inhibition of Low-Density Lipoprotein Peroxidation with Antioxidant Probucol

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Inhibitors of the key enzyme of cholesterol biosynthesis β-hydroxy-β-methylglutaryl-coenzyme A reductase (statins) decrease cholesterol content in atherogenic low-density lipoproteins in patients with coronary heart disease and hypercholesterolemia, but inhibited biosynthesis of ubiquinone Q_{10} protecting low-density lipoproteins from free radical oxidation. Cerivastatin in a daily dose of 0.4 mg markedly increased the content of lipid peroxides in low-density lipoproteins. However, complex therapy with cerivastatin and antioxidant probucol (250 mg/day) was accompanied by a sharp decrease in the content of lipid peroxides in low-density lipoproteins in patients with coronary heart disease *in vivo*. These data indicate that antioxidant agents should be used in combination with inhibitors of β-hydroxy-β-methylglutaryl-coenzyme A reductase (hypolipidemic preparations) for the therapy of patients with coronary heart disease.

Key Words: *low-density lipoproteins; β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitors; hypercholesterolemia; free radical lipid oxidation; antioxidants; ubiquinone Q_{10}; cerivastatin; probucol*

The major approach to the therapy and prevention of coronary heart disease (CHD) and atherosclerosis suggests the use of hypolipidemic preparations that decrease cholesterol level in atherogenic low-density lipoproteins (LDL) [6,7]. In the last years statins, inhibitors of the key enzyme of cholesterol biosynthesis β-hydroxy-β-methylglutaryl-coenzyme A (HMG—CoA) reductase, were widely used as hypolipidemic drugs [6,7]. However, our previous studies showed that statins intensify free radical oxidation in LDL *in vivo* [2]. These changes are accompanied by an increase in the degree of atherogenic oxidative modification of LDL [1]. Inhibition of HMG—CoA reductase blocks biosynthesis of mevalonate and farnesyl pyrophosphate, the common precursor of cholesterol and ubiquinone Q_{10} [3,12]. Reduced ubiquinone Q_{10}, ubiphenol Q_{10}, is a potent antioxidant. It protects LDL from oxidation in the circulation [13]. Our previous experiments demonstrated that HMG—CoA reductase inhibitor pravastatin *in vivo* increases the content of lipid peroxides in plasma LDL in patients with CHD, while ubiquinone Q_{10} abolishes this effect [2]. Probucol in low daily dose (250 mg) does not affect lipid

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metabolism [4], while in the standard therapeutic dose (1000 mg/day) this preparation produces a moderate hypocholesterolemic effect [5]. It should be emphasized that probucol in daily doses of 250 and 1000 mg is equally potent in protecting LDL from oxidation [4]. Here we studied whether the last-generation inhibitor of HMG—CoA reductase cerivastatin intensifies free radical lipid oxidation in LDL in patients with CHD and whether probucol in low daily doses (250 mg) protects LDL from atherogenic oxidative modification.

MATERIALS AND METHODS

A double-blind, randomized, placebo-controlled trial was performed on 32 men (53±5 years) with chronic CHD and primary type IIa hyperlipoproteinemia, patients of the A. L. Myasnikov Institute of Cardiology. The patients received no lipotropic drugs for 3 months before examination and followed a hypolipidemic diet for 2 months before the therapy. The diagnosis of hypercholesterolemia was made after two measurements of plasma lipid concentration. The measurements were performed before and 2 months after the start of the hypolipidemic diet. The patients with total plasma cholesterol level of 7.4±1.1 mmol/liter were randomly divided into 2 groups of 16 individuals each. Group 1 patients received cerivastatin in a daily dose of 0.4 mg (Lipobay, Bayer) and probucol placebo for 6 months. Group 2 patients received cerivastatin and probucol (Alcolex, ICN Pharmaceuticals) in daily doses of 0.4 and 250 mg, respectively, for 6 months. Venous blood was taken monthly from fasting individuals before and during the therapy (into tubes with 1 mg/ml EDTA). For isolation of LDL the plasma was centrifuged in a NaBr density gradient at 42,000 rpm and 4°C (2×2 h) using a Beckman L-8 refrigerated centrifuge equipped with a 50Ti angle rotor [15] and dialyzed at 4°C for 16 h. This rapid method of LDL isolation prevents oxidation of native LDL [14] compared to their isolation by standard methods [9]. Electrophoretic assay revealed no admixtures of other lipoprotein fractions and plasma proteins in LDL samples [15]. It should be emphasized that LDL isolated using this procedure did not differ from those obtained by standard methods in the size and lipid composition [9].

Protein content in LDL was measured by the method of Lowry. The content of lipoperoxides in LDL was determined. To this end, lipoperoxides were oxidized with Fe^{2+} and the content of Fe^{3+} was determined before and after reduction of organic hydroperoxides with triphenylphosphine in the reaction with xylenol orange [10]. Plasma levels of total cholesterol and high-density lipoprotein (HDL) cholesterol were measured on a Kone Progress chemical analyzer using Boehringer kits. The concentration of LDL cholesterol was calculated from these values. Biochemical reagents were obtained from Sigma.

The results were analyzed by Student's *t* test.

RESULTS

In clinical practice probucol is used as a moderate hypolipidemic drug. For reduction of the cholesterol level this preparation should be used in a daily dose of 1000 mg. Our previous studies showed that probucol in a daily dose of 250 mg had no effect on lipid metabolism in the plasma from patients with CHD, but the antioxidant effect of this dose did not differ from that of 1000 mg probucol [4]. Taking these data into account, we used probucol in low daily doses (250 mg) as the antioxidant agent. The concentrations of total cholesterol, LDL cholesterol, and HDL cholesterol were similar in groups 1 (7.4±0.86, 5.2±0.86, and 1.3±0.48 mmol/liter, respectively) and 2 (8.1± 0.92, 5.7± 0.88, and 1.3±0.43 mmol/liter, respectively). LDL cholesterol concentration decreased by 38% after 6-month therapy with cerivastatin. In patients receiving cerivastatin and probucol the concentration of LDL cholesterol decreased by 49% (Table 1). The concentration of total cholesterol decreased by 25% after treatment with cerivastatin; in patients receiving cerivastatin and probucol this parameter decreased by 39%. It should be emphasized that the more potent hypolipidemic effect of combination therapy with cerivastatin and probucol did not reach the level of significance (Table 1) probably due to low number of examined patients. Our results and previous observations [2] indicate that cerivastatin is much more potent than other HMG—CoA reductase inhibitor pravastatin in decreasing cholesterol concentration. This is consistent with published data [7]. Probably, cerivastatin more drastically inhibits biosynthesis of cholesterol and ubiquinone Q_{10} than pravastatin. Theoretically,

TABLE 1. Changes in the Content of Plasma LDL Cholesterol in Patients with CHD after 6-Month Therapy with Cerivastatin Alone or in Combination with Probucol (*M*±*m*)

Therapy, months	Cerivastatin and probucol	Cerivastatin
0	5.67±0.89	5.27±0.86
1	4.63±1.73*	4.68±1.25
2	4.21±1.14*	4.27±0.98*
3	3.25±0.9*	4.39±1.18*
4	3.21±0.64*	4.15±0.57*
5	3.19±0.75*	3.95±1.37*
6	2.88±0.63*	3.26±1.09*

Note. *$p<0.05$ compared to the parameter before therapy.

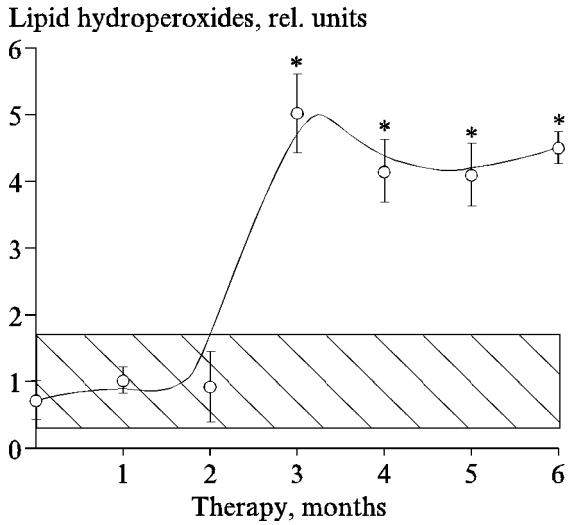

Fig. 1. Changes in the content of LDL lipid hydroperoxides in patients with CHD after 6-month therapy with cerivastatin alone (curve) or in combination with probucol (shaded area; results of 6 measurements during 6-month combination therapy).

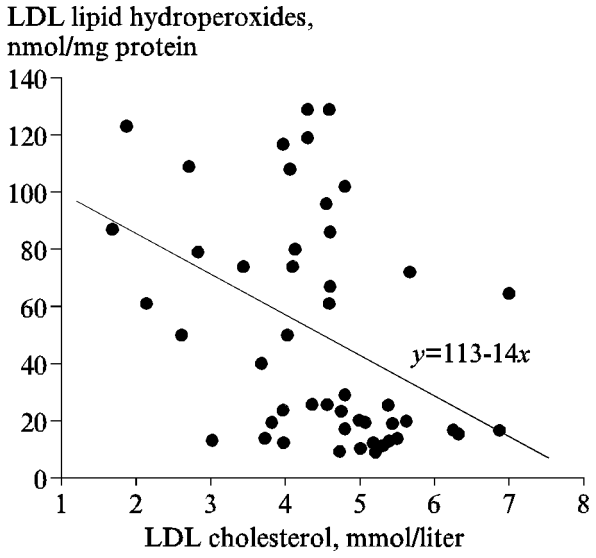

Fig. 2. Correlation between the contents of LDL cholesterol and lipid hydroperoxides in patients with CHD receiving cerivastatin (from the 3rd to 6th month of therapy).

cerivastatin used for the therapy of patients with CHD should markedly intensify free radical oxidation in LDL. We found that lipid peroxide content in LDL increased by 5 times after 3-month therapy with cerivastatin and remained high to the end of observations (more than a 4-fold increase, Fig. 1). However, our previous observations showed that after 6-month therapy with pravastatin the content of lipid peroxides in LDL increases only by 30% [2]. The content of lipid peroxides did not increase in patients with CHD receiving combination therapy with cerivastatin and probucol (Fig. 1). These results indicate that probucol in a daily dose of 250 mg completely blocks intensification of free radical oxidation in LDL *in vivo* (Fig. 1), induced by cerivastatin and enhances their atherogenic modification [1]. Recent studies showed that statins produce an antioxidant effect in model systems [6]. The therapy with these preparations *ex vivo* inhibits oxidation of LDL [6]. However, our observations indicate that HMG—CoA reductase inhibitors markedly increase the content of primary oxidation products in LDL *in vivo*. It should be emphasized that direct measurements of lipid peroxide content in LDL more adequately reflect the intensity of oxidative processes in LDL *in vivo* than Cu^{2+}-induced LDL oxidation *in vitro* [6]. Our results are consistent with published data that statins decrease plasma ubiquinone Q_{10} level in patients [8,11,12]. If this hypothesis is true, in patients with CHD receiving HMG—CoA reductase inhibitors a negative correlation should be observed between the content of plasma LDL cholesterol, which decreases due to the inhibition of its biosynthesis, and lipid hydroperoxide concentration in these particles, which increases due to reduction of the amount of the natural antioxidant ubiquinone Q_{10}. After the therapy with cerivastatin we revealed a negative correlation between the contents of cholesterol and lipid peroxides in LDL (r=-0.42, p=0.003, Fig. 2). The correlation coefficient was low probably due to an incomplete inhibition of the synthesis of cholesterol and ubiquinone Q_{10} with cerivastatin (Table 1) and uncontrolled supply of patients with cholesterol and ubiquinone Q_{10} from food products.

Our results indicate that the positive effect of HMG—CoA reductase inhibitors (reduction of cholesterol level) is associated with enhanced atherogenic oxidative modification of LDL due to the decrease in ubiquinone Q_{10} content. Therefore, the therapy with HMG—CoA reductase inhibitors increases the risk of atherogenic oxidative modification of LDL. The negative effect of statins should be corrected with antioxidants.

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