In Vivo Intensification of Free-Radical Oxidation of Low-Density Lipoproteins in the Plasma of Patients with Coronary Heart Disease Treated with β-Hydroxy-β-Methylglutaryl Coenzyme A Reductase Inhibitor Pravastatin and Inhibition of Lipid Peroxidation with Ubiquinone Q_{10}

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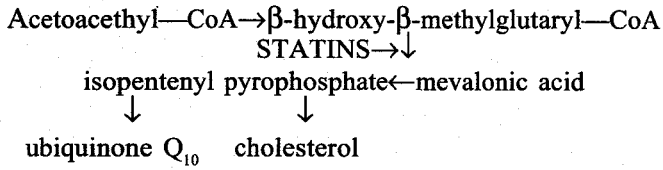
Pravastatin, an inhibitor of β-hydroxy-β-methylglutaryl coenzyme A reductase, the key enzyme of cholesterol biosynthesis, *in vivo* elevated the content of primary and secondary products of free-radical oxidation in low-density lipoproteins in patients with coronary heart disease, while combined treatment with pravastatin and ubiquinone Q_{10} sharply decreased this parameter. Ubiquinone Q_{10} prevented pravastatin-induced inhibition of antioxidant enzymes superoxide dismutase and lipid peroxidase utilizing reactive oxygen species in the blood. These data indicate that ubiquinone Q_{10} would be appropriate for use in combination with statins, inhibitors of β-hydroxy-β-methylglutaryl coenzyme A reductase, 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 peroxidation; ubiquinone Q_{10}*

Statins, the inhibitors of the key enzyme of cholesterol (CH) biosynthesis β-hydroxy-β-methylglutaryl-coenzyme A reductase (HMG—CoA reductase), are widely used as hypolipidemic drugs in the therapy of atherosclerosis [3,4,11]. These preparations reduce plasma content of CH in patients with coronary heart disease (CHD), but cause some delayed adverse effects. Inhibitors of HMG—CoA reductase suppress CH synthesis not only in the liver, but also in other organs, in particular in the brain, which is an undesirable effect because brain membranes are enriched with CH and it is intensively synthesized in the brain [6]. Furthermore, statins inhibit the synthesis not only of CH, but also of the isoprenoid lateral chain of ubiquinone Q_{10} (Q_{10}) [10]:



Q_{10} is present in all human and animal tissues and is involved in various metabolic processes, including electron transfer associated with ATP synthesis in the respiratory chain of mitochondria [1]. Thus, statin-induced inhibition of Q_{10} biosynthesis in tissues has adverse consequences, in particular, impaired energy supply to skeletal muscles and myocardium leading to myopathies [5,7]. Negative effects of statins extend further. Recent studies showed that oxidized low-den-

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sity lipoproteins (LDL) are involved in atherogenesis [14,15]. Monocytes and macrophages of the vascular wall internalizing oxidized LDL are transformed into foam cells forming the zone of lipidosis [15]. Until present, the major natural antioxidant α-tocopherol (vitamin E) was believed to play the major role in the antioxidant protection of circulating LDL involved in its transport in the body [12]. Recent studies demonstrated that Q_{10}, a component of LDL, is a more potent antioxidant, which is readily transformed into the corresponding phenol with high antioxidant activity [8,12]. This mechanism of LDL protection has a considerable biological significance, because the inhibition of LDL oxidation involves Q_{10} synthesized in the body, but not essential vitamin E. At the same time, the content of Q_{10} in LDL considerably decreases in patients with atherosclerosis treated with statins due to suppression of isoprenoid biosynthesis [5]. These data suggest that statins promote LDL oxidation by reducing the content of Q_{10}, a natural protector against free-radical oxidation. Here we tested this hypothesis and substantiated combined use of Q_{10} and HMG—CoA reductase inhibitors for the correction of hypercholesterolemia.

MATERIALS AND METHODS

A double-blind, randomized, placebo-controlled trial was performed on 20 men (49±2.5 years, total plasma CH 7.2±0.4 mmol/l, type IIa hyperlipidemia) with chronic CHD subjected to out-patient treatment at A. L. Myasnikov Institute of Cardiology. The patients received no lipotropic drugs for 3 months before examination and followed a low-cholesterol diet for 1 month before the therapy. All patients were treated with pravastatin (Lipostat, Bristol-Myers Squibb) and Q_{10} (Bioquinone, Phrama Nord) in daily doses of 40 and 60 mg, respectively, or placebo. Fasting venous blood was taken (in tubes with 1 mg/ml EDTA) monthly before the start and during the therapy. LDL were isolated as described elsewhere [13]. Protein content in LDL was measured by the method of Lowry [13]. The concentration of lipid peroxides in LDL was estimated by the reaction with xylenol orange before and after hydroperoxide reduction with triphenylphosphine on a Hitachi 557 spectrophotometer at 560 nm [9]. The content of secondary free-radical oxidation products (malonic dialdehyde, MDA) in the plasma was evaluated by the reaction with thiobarbituric acid [2]. To estimate activity of antioxidant enzymes in erythrocytes (containing about 95% enzyme activity), whole blood (0.1 ml) was diluted 1:9 with 5 mM hypotonic K,Na-phosphate buffer (pH 7.4). After hemolysis, the mixture was frozen and stored at -20°C for no more than 1 month [2]. The cold chloroform-ethanol mixture (3:5) in a volume of 0.5 ml was added to 1 ml sample on ice bath, shaken, and precipitated by gem centrifugation. Superoxide dismutase activity (SOD) was estimated by the inhibition of HCT reduction with superoxide anion radical generated in the xanthine-xanthine oxidase system in a Hitachi 557 spectrophotometer at 560 nm [2]. The amount of SOD causing 50% inhibition was taken as a unit of activity [2]. Glutathione peroxidase activity was determined by the kinetics of oxidized glutathione formation estimated from NADPH oxidation in coupled glutathione reductase reaction using tert-butyl hydroperoxide as the substrate on a FP-900 chemical analyzer (Labsystems OV) at 340 nm [2]. The amount of glutathione peroxidase oxidizing 1 μmol reduced glutathione per 1 min was taken as a unit of activity [2]. The contents of total CH, LDL CH, and high-density lipoprotein (HDL) CH in the plasma were estimated on a Kone Progress chemical analyzer using Boehringer kits (Laboratory of Clinical Biochemistry, A. L. Myasnikov Institute of Cardiology).

TABLE 1. Effects of Pravastatin Alone and in Combination with Q_{10} on Lipid Metabolism and Free-Radical Oxidation in the Blood of Patients with CHD (*M±m*)

Parameter	Pravastatin+placebo		Pravastatin+Q_{10}	
	before therapy	6-month therapy	before therapy	6-month therapy
Total CH, mmol/l	7.1±0.39	5.3±0.21* (-26)	7.2±0.40	5.1±0.20* (-29)
LDL CH, mmol/l	5.1±0.59	3.6±0.40* (-29)	5.3±0.59	3.4±0.26* (-36)
HDL CH, mmol/l	1.2±0.14	1.3±0.13* (+13)	1.2±0.12	1.3±0.15* (+13)
Glutathione peroxidase, U/g hemoglobin	6.36±0.59	1.3±0.21* (-79)	6.2±0.68	3.6±0.30* (-42)
SOD, U/g hemoglobin	1081±239	790±166* (-27)	1073±103	1191±194
Lipid peroxides in LDL, μmol/ml	5.78±0.31	7.4±0.40* (+29)	6.27±0.55	1.5±0.07* (-77)
MDA, nmol/l	0.295±0.041	0.35±0.034* (+18)	0.30±0.040	0.16±0.016* (-48)

Note. *$p<0.05$ compared with the corresponding parameter before therapy. Changes in parameters (%) are shown in brackets.

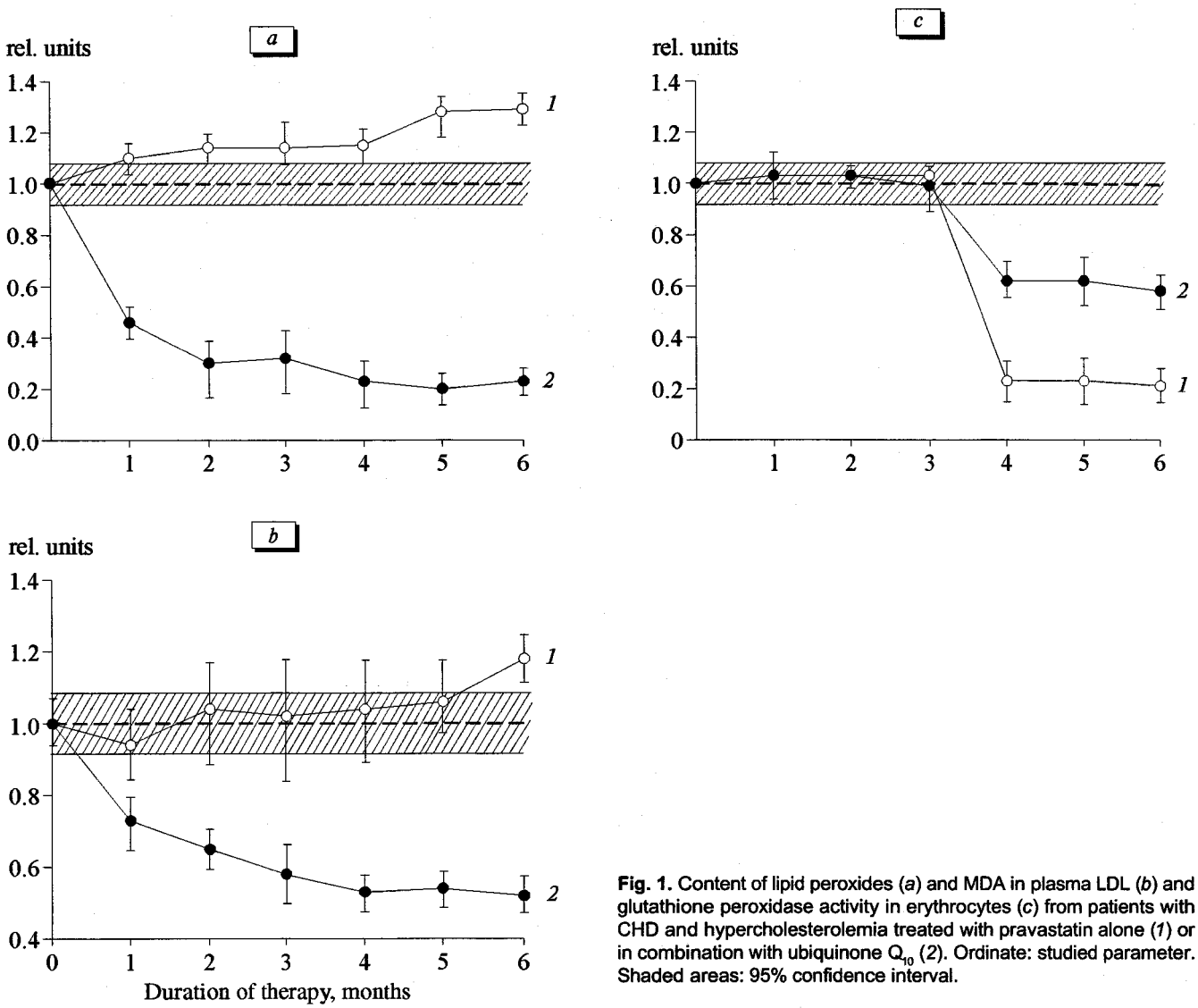

Fig. 1. Content of lipid peroxides (*a*) and MDA in plasma LDL (*b*) and glutathione peroxidase activity in erythrocytes (*c*) from patients with CHD and hypercholesterolemia treated with pravastatin alone (*1*) or in combination with ubiquinone Q_{10} (*2*). Ordinate: studied parameter. Shaded areas: 95% confidence interval.

RESULTS

Six-month therapy with pravastatin alone or in combination with Q_{10} decreased the contents of total CH and LDL CH, and increased the concentration of HDL CH (Table 1). The content of lipid peroxides in LDL increased during monotherapy with pravastatin, but decreased after combined treatment with pravastatin and Q_{10} (Table 1, Fig. 1, *a*). Pravastatin alone and in combination with Q_{10} reduced the content of MDA in LDL, but these changes were less pronounced (Table 1, Fig. 1, *b*). It should be emphasized that glutathione peroxidase activity in the blood remained unchanged during the first 3 month of monotherapy with pravastatin, but then decreased by 80% (from the third to fourth month) and remained unchanged to the end of observations (Table 1, Fig. 1, *c*). During combined therapy, enzyme activity changed similarly, but by month 4 it decreased to a lesser extent (Table 1, Fig. 1, *c*). SOD activity in the blood decreased after 6 month monotherapy with pravastatin, but remained unchanged after combined therapy with pravastatin and Q_{10}. These data suggest that HMG—CoA reductase inhibitors *in vivo* intensify LDL oxidation, while Q_{10} inhibits this process via activation of antioxidant enzymes. Q_{10} would be appropriate for use in combined therapy of patients with CHD and hypercholesterolemia for preventing adverse effects of statins, which promote atherogenic oxidation of LDL.

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