BIOCHEMISTRY AND BIOPHYSICS

Effect of β-Hydroxy-β-Methylglutaryl Coenzyme A Reductase Inhibitors and Antioxidant Vitamins on Free Radical Lipid Oxidation in Rat Liver

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 4, pp. 390-393, April, 2007
Original article submitted September 27, 2006

We studied the effects of two inhibitors of β-hydroxy-β-methylglutaryl coenzyme A reductase, simvastatin and lovastatin, on the lag phase of ascorbate-dependent lipid oxidation in rat liver. Oxidizability of liver biological membranes significantly increased in intact animals and rats with induced hypercholesterolemia after peroral administration of these statins. The lag phase of ascorbate-dependent lipid oxidation in liver biomembranes decreased by 2.1 times in hypercholesterolemic rats. In animals of the lovastatin group this parameter decreased by 4.4 times compared to the control. In intact rats receiving simvastatin, the lag phase of oxidation in biomembranes from the liver decreased practically by 2 times. At the same time, in animals receiving simvastatin in combination with antioxidant vitamins (vitamins E and C, provitamin A) and selenium, the period of induction of oxidation increased by 3.3 times. Our results indicate that β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitors produce a prooxidant effect on the liver, which can be prevented by administration of antioxidant agents.

Key Words: *hypercholesterolemia; free radical lipid oxidation; thiobarbituric acid-reactive products; β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitors; antioxidant vitamins*

β-Hydroxy-β-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors, or statins, are most effective cholesterol-lowering drugs used in the therapy for coronary heart disease (CHD) and atherosclerosis [6]. Hypercholesterolemia (HCH) is a risk factor for atherosclerosis [6]. Our previous studies showed that HCH contributes to the development of oxidative stress in CHD patients [1] and experimental animals [8]. Some published data suggest that statins produce an antioxidant effect, including activation of antioxidant enzyme glutathione peroxidase [11]. However, the results of our studies and other authorities contradict this hypothesis [3,12]. Apart from inhibition of cholesterol (CH) biosynthesis, statins block the synthesis of the major antioxidant compound ubiquinone (ubiphenol) Q_{10} [10] protecting plasma low-density lipoproteins (LDL) from atherogenic oxidative modification. Statins also block the synthesis of selenoproteins (*e.g.*, Se-containing glutathione peroxidase

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utilizing lipid hydroperoxides) [12]. The results of our experiments provide support for prooxidant activity of statins. We demonstrated accumulation of hydroxy-LDL in blood plasma and inhibition of erythrocyte glutathione peroxidase in CHD patients receiving statin therapy [2,3]. Damage to liver cells leading to significant elevation of plasma aminotransferase activity is a side effects of statin therapy [5]. We hypothesized that sharply increased oxidizability of biomembranes in the liver can be a factor determining hepatocyte damage during statin therapy. This experimental study was designed to test this hypothesis. Experiments were performed with widely used pharmaceutics simvastatin and lovastatin.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200±20 and feeding a standard briquetted diet. The animals were divided into 2 groups. HCH in group 1 rats (n=36) was induced by peroral administration oil suspension of CH (5 ml/kg olive oil, 1 g/kg CH (Sigma), and 100 mg/kg sodium cholate (Sigma) [7]. Group 1 animals were divided into 3 equal subgroups and received via a tube daily for 10 days olive oil (subgroup 1, control), or oil suspension of CH (subgroup 2, cholesterol), or oil suspension of CH and 40 mg/kg lovastatin (Mevacor, Merch Sharp&Dohme, subgroup 3, cholesterol and lovastatin). Subgroup 2 included intact animals (n=21) that were also divided into 3 equal subgroups: controls (subgroup 1) received 0.5 ml water via a tube daily for 30 days; subgroup 2 rats (simvastatin) received 0.5 ml aqueous suspension of simvastatin (Vasilip, KRKA) in a dose of 10 mg/kg; subgroup 3 rats (simvastatin, antioxidant vitamins, and selenium) received 0.5 ml aqueous suspension of 10 mg/kg simvastatin and complex antioxidant preparation Triovit (1 drop per kg, KRKA) containing vitamins E and C, provitamin A, and antioxidant element selenium.

The animals were deprived of food and water over 14 h before decapitation. After decapitation, the blood and liver were sampled. The concentrations of total CH and high-density lipoprotein (HDL) CH in the serum were measured enzymatically using Lachema kits on an Airone 200 analyzer. The contents of LDL CH and very low-density lipoprotein (VLDL) CH were calculated as the difference between total CH and HDL CH. Total CH content in the liver was measured with these kits after lipid extraction by the method of Folch. For evaluation of ascorbate-dependent lipid oxidation, the liver was perfused with cold isotonic KCl and homogenized in an Ultra-Turrax SDT-1810 (Tekmar) at 4-8°C (15 mg wet tissue per 1 ml 0.154 M NaCl in 50 mM K,Na-phosphate buffer, pH 5.9). Homogenates were incubated with 0.5 mM ascorbate in the absence of Fe^{2+} ions with constant agitation and under aerobic conditions [4]. Aliquots of the incubation mixture were sampled at 2-5-min intervals. The amount of secondary LPO products in aliquots was estimated by the reaction with thiobarbituric acid (TBA). Optical density of samples was measured on a Hitachi 557 spectrophotometer at 532 nm [4]. Absorption of TBA-reactive products before incubation was subtracted from optical density of subsequent samples and ΔD_{532} as a function of incubation time was plotted. The lag phase of oxidation (induction period, τ) was evaluated from these curves [4]. Oxidizability of liver biological membranes was inversely related to the induction period ($1/\tau$). The results were analyzed by 2-sample Student's t test with various dispersions. The data are presented as means and standard errors.

RESULTS

Ten-day administration of a CH suspension to group 1 rats was followed by a significant increase in total CH concentration in the serum (practically by 2 times). The contents of LDL CH and VLDL CH increased by 3.8 and 1.2 times, respectively. Total CH concentration in the liver increased by 1.6 times (Table 1). Lovastatin in a dose of 40 mg/kg decreased serum content of total CH by 16%, LDL CH and VLDL CH by 24% (p<0.001), and total CH content in the liver by 33% (p<0.02). HDL CH content remained practically unchanged. These changes reflect the development of HCH in treated rats. This state can be accompanied by activation of free radical lipid oxidation [8]. Administration of CH suspension was followed by a 2.1-fold decrease in the lag phase of ascorbate-dependent oxidation in liver biomembranes. The induction period decreased by 4.4 times after administration of oil suspension of CH in combination with lovastatin (Table 1, Fig. 1). Oxidizability of liver biomembranes increased in animals of these groups (Table 1). Hence, as we expected, the use of lovastatin as a CH-lowering drug decreased antioxidant activity of the liver and sharply increased oxidizability of biomembranes in the liver.

Administration of simvastatin in a dose of 10 mg/kg for 30 days decreased the lag phase of oxidation in liver biomembranes from intact rats of group 2 (by 1.9 times). Hence, oxidizability of membranes increased under these conditions. At the same time, in rats receiving this statin in combina-

TABLE 1. Parameters of Lipid Metabolism and Free Radical Oxidation in Liver Biological Membranes from Group 1 Rats with Experimental HCH (*M*±*m*)

Parameters	Subgroups of group 1		
	1, control (olive oil)	2 (cholesterol)	3 (cholesterol and lovastatin)
Total CH, mg/100 m	61.6±5.3	120.0±8.4***	100.5±6.7+
HDL CH, mg/100 ml	44.4±1.4	54.3±1.7*	50.0±1.3
LDL CH and VLDL CH, mg/100 ml	17.2±0.8	65.7±1.2***	49.9±0.9++++
Liver CH, mg/g tissue	2.2±0.1	3.6±0.4**	2.4±0.2++
Induction period for oxidation in liver biomembranes, τ, min	12.2±1.6	5.9±1.1***	2.8±0.2***,+++
Oxidizability of liver biomembranes, 1/τ	0.08	0.17	0.35

Note. **p*<0.1, ***p*<0.02, and ****p*<0.001 compared to subgroup 1; +*p*<0.1, ++*p*<0.02, +++*p*<0.05, and ++++*p*<0.001 compared to subgroup 2.

tion with the preparation of antioxidant vitamins and selenium, the induction period increased by 3.3 times, *i.e.* membrane oxidizability in the liver sharply decreased (Table 2, Fig. 2). No differences were revealed between parameters of lipid metabolism in intact animals and rats receiving olive oil.

Thus, our experiments confirmed the possibility of prooxidant effect of HMG-CoA reductase inhibitors on the liver and showed that the adverse effect of statins in this tissue can be prevented by antioxidants. Our previous studies showed that ubiquinone Q_{10} is more potent than the complex of antioxidant vitamins and selenium in inhibiting free radical oxidation in liver biological membranes [4]. These data indicate that ubiquinone Q_{10} not only inhibits the accumulation of atherogenic hydroxy-LDL in statin therapy for CHD and HCH [3,9], but also holds promise for the prevention of liver aminotransferase release into the blood during hepatocyte damage under conditions of aggressive (high-dose) treatment with HMG-CoA reductase inhibitors.

This work was supported by the Russian Foundation for Basic Research (grants No. 03-04-48344 and 05-04-49854).

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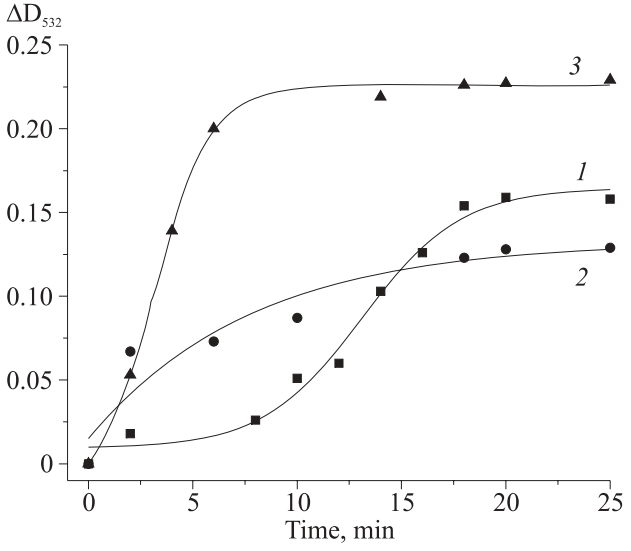

Fig. 1. Typical kinetic curves for ascorbate-dependent free radical oxidation in liver homogenates from rats with experimental HCH after administration of lovastatin (group 1). Control (*1*); CH (*2*); and CH and lovastatin (*3*).

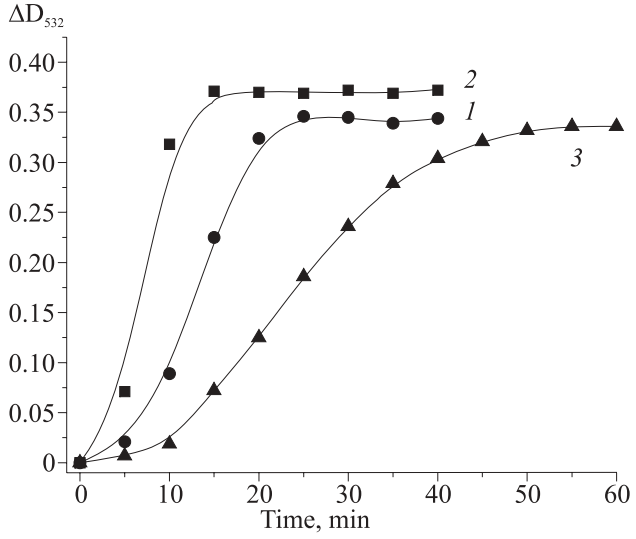

Fig. 2. Typical kinetic curves for ascorbate-dependent free radical oxidation in liver homogenates from intact rats after administration of simvastatin and preparation of antioxidant vitamins and selenium (group 2). Control (*1*); simvastatin (*2*); and simvastatin and Triovit (*3*).

TABLE 2. Parameters of Free Radical Oxidation in Liver Biomembranes from Intact Rats after Administration of Simvastatin and Preparation of Antioxidant Vitamins and Selenium (Group 2, *M*±*m*)

Parameters	Subgroups of group 1		
	1, control (olive oil)	2 (cholesterol)	3 (cholesterol and lovastatin)
Induction period for oxidation in liver biomembranes, τ, min	10.2±2.4	5.3±0.6*	17.6±2.7*+
Oxidizability of liver biomembranes, 1/τ	0.10	0.19	0.06

Note. *$p<0.001$ compared to subgroup 1; +$p<0.05$ compared to subgroup 2.

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