Inhibitor of β-Hydroxy-β-Methylglutaryl Coenzyme A Reductase Decreases Energy Supply to the Myocardium in Rats

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Hypocholesterolemic preparations, inhibitors of the key enzyme of cholesterol biosynthesis β-hydroxy-β-methylglutaryl coenzyme A reductase (statins), block the synthesis of ubiquinone Q_{10}, intermediate electron carrier in the mitochondrial respiratory chain. This should decrease energy supply to tissues. Daily peroral administration of β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitor simvastatin (24 mg/kg perorally) for 30 days had no effect on the contents of macroergic phosphates (ATP and creatine phosphate) in the liver, but decreased these parameters in the myocardium.

Key Words: *adenosine triphosphate; creatine phosphate; β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitors; cholesterol; ubiquinone Q_{10}; free radical oxidation*

Stating, inhibitors of β-hydroxy-β-methylglutaryl coenzyme A (HMG—CoA) reductase, the key enzyme of cholesterol biosynthesis, are widely used as hypocholesterolemic agents in the therapy of atherosclerosis [1,2]. These preparations decreasing blood cholesterol level in patients with coronary heart disease (CHD) should block biosynthesis of the lateral isoprenoid chain in ubiquinone Q_{10} (Fig. 1) [9]. Ubiquinone Q_{10} present in all human and animals tissues is involved in ATP synthesis-associated electron transfer in the mitochondrial respiratory chain, which is necessary for energy-dependent contractile activity of myocytes [5]. Thus, the inhibition of ubiquinone Q_{10} biosynthesis in tissues during simvastatin therapy can decrease energy supply to skeletal muscles and myocardium and provoke myopathies [6,8] or myocardial dysfunction [4]. Here we measured the content of macroergic phosphates in the liver and myocardium in rats perorally treated with the HMG—CoA reductase inhibitor simvastatin.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 240±20 g. Experimental animals (n=10) received 0.5 ml water suspension of simvastatin (24 mg/kg through a tube, Zokor, Merch Sharp & Dohme) daily for 30 days. Control animals (n=10) daily received 0.5 ml distilled water through a tube. The animals were deprived of food, but had free access to water 12 h before euthanasia. The rats were anesthetized with 2 g/kg urethane. The heart and liver were rapidly removed. Tissue samples were taken by a Wollenberg forceps cooled in liquid nitrogen. These samples were homogenized in cold 6% $HClO_4$ (10 ml/g tissue) on an ice bath using an Ultra-Turrax T-25 homogenizer (IKA-Labortechnik). Proteins were precipitated by centrifugation at 3000g for 10 min in a Beckman J-6B refrigerated centrifuge. Supernatants were neutralized

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with 5 M K_2CO_3 to pH 7.4. The dry weight of samples was estimated by weighing of precipitates after extraction with $HClO_4$ and drying to a constant weight at 110°C for 12 h. The contents of ATP and creatine phosphate (CrP) in tissue extracts were measured spectrophotometrically using glucose-6-phosphate dehydrogenase, hexokinase, and creatine kinase [10]. ADP content was estimated enzymatically using pyruvate kinase and lactate dehydrogenase [7]. Creatine (Cr) concentration in tissues was measured by the reaction with α-naphthol and diacetyl (Sigma) [11]. The measurements were performed on a Yanaco-2000 spectrophotometer. The total creatine content (ΣCr) was calculated by the formula: ΣCr=CrP+Cr. The concentrations of adenine nucleotides, CrP, and Cr in tissues were expressed in μmol/g dry weight. All used reagents were from Sigma.

RESULTS

In rats treated with simvastatin the contents of ATP, ADP, CrP, and Cr in the liver did not differ from the control (Table 1). ADP concentration and ATP/AP ratio did not differ between experimental and control animals (Table 1). At the same time, the contents of ATP, CrP, and Cr in the myocardium decreased by 13, 18, and 19%, respectively, after 1-month simvastatin treatment compared to the control (Table 1). The total content of myocardial Cr reflecting the integrity of cardiomyocyte sarcolemma decreased in simvastatin-treated animals, which attests to the development of myocardial dysfunction [11]. Thus, the HMG—CoA reductase inhibitor decreased the content of macroergic phosphates in the myocardium, but not in the liver. This was probably related to a relatively short period of observations. In clinical practice statins are used for 3-6 months. Our results are consistent with the data obtained by R. A. Wills *et al*. [12] on rats receiving a standard diet containing lovastatin (400 mg/kg food) *ad libitum* for 4 weeks. Taking into account the weight of animals and daily food consumption, we conclude that the daily dose of lovastatin in this experiment was similar to the therapeutic dose of simvastatin used in our experiments. Clinical studies showed that lovastatin and simvastatin in therapeutic

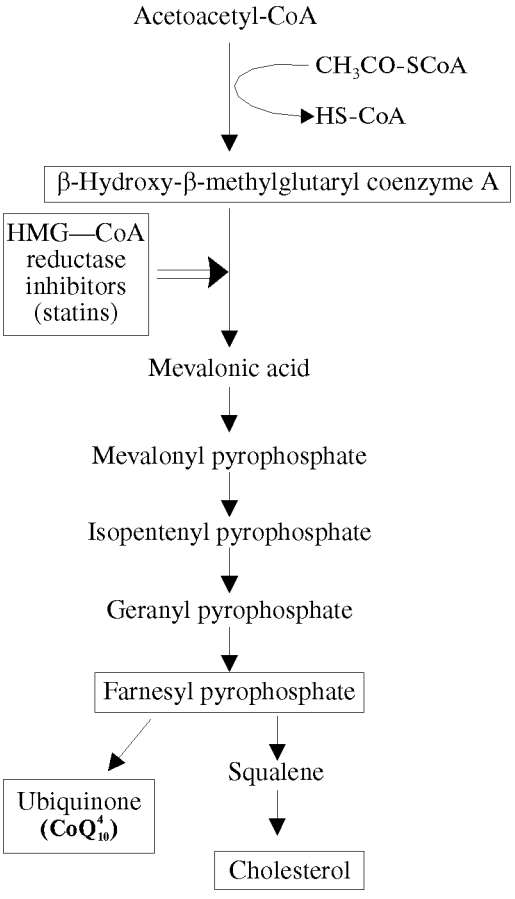

Fig. 1. Biosynthesis of cholesterol and ubiquinone Q_{10} in mammalian tissues.

TABLE 1. Effects of Simvastatin on Adenine Nucleotides, CrP, and Cr Content in Rat Liver and Heart (μmol/g Dry Weight, *M*±*m*)

Parameter	Liver		Heart	
	control	treatment	control	treatment
ATP	7.37±0.42	8.86±0.56	19.39±0.23	16.79±0.99*
ADP	6.38±0.16	6.56±0.26	4.10±0.22	4.31±0.15
ATP/ADP	1.16±0.07	1.38±0.12	4.79±0.26	4.25±0.40
CrP	0.21±0.04	0.25±0.03	24.49±0.65	20.05±0.75*
Cr	0.59±0.02	0.66±0.03	33.97±2.26	27.39±1.00*
ΣCr	0.80±0.05	0.91±0.02	59.22±2.20	47.45±1.63*
CrP/Cr	0.35±0.40	0.38±1.00	0.75±0.05	0.73±0.03

Note. *p<0.05 compared to the control.

doses produce similar effects on lipid metabolism and decrease the content of low-density lipoprotein cholesterol in patients [3]. Hence, lovastatin and simvastatin in similar doses should be equally potent in inactivating HMG—CoA reductase and inhibiting ubiquinone Q_{10} biosynthesis in animal tissues. Myocardial ubiquinone Q_{10} content in rats receiving lovastatin for 1 month decreased by 14% [12], which is consistent with the reduction of myocardial ATP concentration in rats treated with simvastatin (Table 1). Our findings indicate that HMG—CoA reductase inhibitor suppresses biosynthesis of ubiquinone Q_{10}, decreases its content in the myocardium and, therefore, impairs energy supply to the heart. Our results are confirmed by published data that 1-month lovastatin therapy not only decreases blood ubiquinone Q_{10} content in patients, but also impairs energy-dependent myocardial functions: stroke volume, cardiac output, and contractile index decreased [4]. These data indicate that long-term administration of statins to patients with CHD and hypercholesterolemia suppresses ubiquinone Q_{10} biosynthesis in myocytes [12], causes myopathies [6,8], and impairs myocardial energy supply [4], which decreases the efficiency of therapy.

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