Simvastatin and Preparation of Polyunsaturated Phospholipids Produce Similar Changes in the Phospholipid Composition of High-Density Lipoproteins during Hypercholesterolemia

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We studied the phospholipid composition of high-density lipoproteins in patients with coronary heart disease and hypercholesterolemia treated with simvastatin (Zocor, inhibitor of the key enzyme of cholesterol synthesis) and preparation of polyunsaturated phospholipids (lipostabil forte). Simvastatin produced a hypolipidemic effect and modulates the phospholipid composition of high-density lipoproteins (similarly to lipostabil forte). These changes contribute to functional activity of high-density lipoproteins in the reverse cholesterol transport.

Key Words: *high-density lipoproteins; phospholipids; hypercholesterolemia; simvastatin (Zocor); polyunsaturated phospholipids (lipostabil forte)*

Antiatherogenic activity of high-density lipoproteins (HDL) is determined by their involvement in the reverse transport of cholesterol (CH): acceptance of CH from cell membranes, its esterification, transport to the liver, and catabolic transformation to bile acids [3,10]. Phospholipids (PL) determine fluidity of the HDL surface monolayer and CH-binding and transporting properties [4]. Phosphatidylcholine (lecithin) is a major HDL PL. PL can form complexes with CH, which contributes to the ability of HDL to accept CH from cell membranes, but only CH bound to phosphatidylcholine undergoes esterification catalyzed by lecithin-cholesterol transferase. The concentrations of HDL CH and HDL PL and relative content of phosphatidylcholine in HDL are low in patients with coronary heart disease (CHD) [6,7].

Preparations of polyunsaturated phospholipids (PUPL) produce a metabolic effect due to incorporation into cell membranes and surface monolayer of lipoproteins (particularly HDL) [9]. Some authors reported a moderate increase in blood HDL CH concentration under the influence of PUPL, while others revealed no such changes [1].

Lipostabil is the most commonly used PUPL preparation in Russia. Lipostabil is manufactured from soybeans with high content of phosphatidylcholine (76%) with polyunsaturated fatty acid (linoleic acid) in positions 1 and 2 (endogenous phosphatidylcholine contains polyunsaturated fatty acid only in position 2).

Various statins, including 3-hydroxymethyl-3-glutaryl coenzyme A reductase inhibitors, are used for the correction of dyslipoproteinemia. These preparations modulate the composition of lipoproteins and produce pleiotropic effects. They decrease the risk of acute cardiovascular episodes, mortality of patients with these disorders, and total mortality [1,5,8]. One of these preparations is simvastatin [8]. This drud produces a hypolipidemic effect and moderately increases blood HDL CH concentration. The increase in HDL CH concentration after treatment with simvastatin can be associated with changes in the phospholipid composition of HDL. PL are involved in the HDL-me-

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diated reverse transport of CH and promote its efflux from cell membranes. Our previous studies showed that simvastatin therapy produces a hypolipidemic effect, increases the concentration of HDL PL, and modulates their phospholipid composition in patients with hypercholesterolemia [2].

Here we compared the effects of simvastatin and PUPL preparation on blood lipids and phospholipid composition of HDL in patients with CHD and hypercholesterolemia.

MATERIALS AND METHODS

The study included 20 patients with CHD (40-65 years) with blood LDL CH >130 mg/dl (after hypolipidemic diet). The patients were randomly divided into 2 groups. Ten patients received simvastatin (Zocor, Merck Sharp & Dohme) in a single dose of 20 mg in the evening for 8 weeks. Ten patients received 2 capsules of PUPL (0.3 g, lipostabil forte, Rhone-Poulens Rorer) 3 times a day.

The blood was taken from the cubital vein after overnight fast. Serum contents of total CH, triglycerides, and HDL CH were measured on an Airone 200 automatic analyzer using Human enzyme kits. The measurements were performed after precipitation of LDL with sodium phosphotungstate in the presence of magnesium chloride. Lipid tests were qualitatively controlled at the Department for Standardization of Biochemical Assays (State Research Center of Preventive Medicine, Russian Ministry of Health). LDL CH concentration was calculated by the formula of Friedewald: LDL CH=total CH-$^1/_5$ triglycerides-HDL CH (mg/dl).

HDL PL were assayed after LDL precipitation. HDL PL were extracted with a chloroform-methanol (2:1, v/v) mixture (Folch method). The content of total HDL PL was measured after mineralization followed by the reaction with ammonium molybdate and ascorbic acid. Individual PL were separated by thin-layer chromatography on glass plates coated with silica gel in a system containing chloroform, methanol, aqua ammonia, and water (17.0:7.0:1.0:0.5 v/v) and developed in iodine vapors. The corresponding spots were scraped. PL standards were from Sigma. The relative content of individual PL was estimated by phosphorus content after mineralization in the reaction with hydrazine hydrochloride. The relative content of PL was calculated in percents of HDL PL concentration [2].

The results were analyzed by Student's *t* test and paired Wilcoxon test. The differences were significant at $p<0.05$.

RESULTS

Before therapy group 1 and 2 patients did not differ by the levels of total CH, triglycerides, LDL CH, and HDL CH (Table 1). Simvastatin therapy decreased total CH and LDL CH (by 30.3 and 42.5%, respectively), had no effect on triglyceride concentration, and increased HDL CH content (by 6.8%). PUPL had no effect on blood lipid content. We revealed only a slight increase in the concentration of HDL CH (by 4.7%).

After therapy the concentration of HDL CH significantly increased, while HDL PL content remained unchanged in both groups. At the same time, we re-

TABLE 1. Concentration of Lipids in Serum Lipoproteins and Phospholipid Composition of HDL in Patients with Hypercholesterolemia before and after Therapy (*M*±*m*)

Parameter		Simvastatin		PUPL	
		before therapy	after therapy	before therapy	after therapy
Total CH, mg/dl		241.0±5.6	168.0±3.1*	241.0±6.2	245.0±4.8
Triglycerides, mg/dl		148±15	127.0±20.3	138.0±13.4	149.0±19.1
LDL CH, mg/dl		167.0±4.5	96.0±2.6*	174.0±5.4	174.0±6.4
HDL CH, mg/dl		44.0±2.5	47.0±2.7**	40.0±1.2	42.0±0.9**
HDL PL, mg/dl		156±11	156.0±9.9	108.0±10.4	120±11
HDL PL, %	phosphatidylcholine	69.10±2.32	76.40±1.47*	61.30±2.52	64.8±2.0**
	lysophosphatidylcholine	13.20±0.94	8.30±0.62*	14.5±1.3	13.4±1.0**
	sphingomyelin	13.00±1.68	9.30±1.15*	14.7±2.0	10.50±1.18**
	phosphatidylethanolamine	1.40±0.28	2.60±0.27*	4.30±1.22	5.70±1.44**
	cardiolipin	3.40±0.43	3.00±0.33**	5.30±0.69	4.00±0.59**

Note. *$p<0.01$ and **$p<0.05$ compared to the corresponding parameter before therapy (Wilcoxon test).

vealed changes in the composition of individual PL: increased relative content of phosphatidylcholine and phosphatidylethanolamine, and decreased relative content of lysophosphatidylcholine, sphingomyelin, and cardiolipin (Table 1). The increase in the relative content of HDL phosphatidylcholine was accompanied by a decrease in the content of lysophosphatidylcholine. It was associated with an increase in the phosphatidylcholine/lysophosphatidylcholine ratio after therapy with simvastatin (from 5.2 to 9.2) and PUPL (from 4.2 to 4.8). Therefore, the phosphatidylcholine/lysophosphatidylcholine ratio increased to a greater extent after simvastatin therapy. It was previously demonstrated that increased lysophosphatidylcholine content leads to destruction of cell membranes and surface monolayer of lipoproteins; hence, the decrease in the relative content of lysophosphatidylcholine stabilizes molecular packing of the surface monolayer in HDL. The decrease in the relative sphingomyelin content also contributed to the increase in the phosphatidylcholine/sphingomyelin ratio after therapy with simvastatin (from 5.3 to 8.2) and PUPL (from 4.2 to 6.2). The increase in the relative content of HDL phosphatidylcholine and decrease in the content of sphingomyelin determine higher fluidity of the surface monolayer in these lipoproteins and improve their antiatherogenic activity. The increase in the relative content of phosphatidylcholine (the major HDL PL) in patients treated with lipostabil can be explained by the fact that lipostabil consists of polyunsaturated phosphatidylcholine. This compound is easily incorporated into the surface monolayer of HDL, which can modulate the relative content of other HDL PL. Simvastatin was more potent in modulating the composition of HDL PL compared to PUPL. After treatment with simvastatin and PUPL the relative content of HDL phosphatidylcholine increased by 7.3 and 3.5%, respectively. However, the relative content of HDL lysophosphatidylcholine decreased by 4.9 and 1.1%, respectively. Probably, the hypolipidemic preparation simvastatin not only modulates the synthesis and catabolism of CH and triglycerides, but also affects the synthesis of individual PL, thus modulating phospholipid composition of HDL and improving their CH acceptor and transporting capacities. The influence of simvastatin on HDL PL can be attributed to pleiotropic antiatherogenic activity. Therapy with simvastatin (Zocor) produces a hypolipidemic effect and promotes phospholipid-dependent HDL-mediated reverse CH transport.

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