Effects of Simvastatin on the Phospholipid Composition of High-Density Lipoproteins in Patients with Hypercholesterolemia

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We studied the phospholipid composition of high-density lipoproteins in patients with hypercholesterolemia before and after treatment with simvastatin. Individual phospholipids were separated by thin-layer chromatography on glass plates coated with silica gel. It was found that apart from hypolipidemic effect, simvastatin changed the concentration and phospholipid composition of high-density lipoproteins, which improved their cholesterol-accepting and cholesterol-transporting properties.

Key Words: *lipoproteins; phospholipids; hypercholesterolemia; simvastatin*

High-density lipoproteins (HDL) are involved in reverse transport of cholesterol (CH). Antiatherogenic activity of HDL is associated with their ability to accept CH from cell membranes in organs and tissues and transport it to the liver for further excretion [4,8]. CH-accepting properties of HDL depend on their concentration in the blood and composition of the main components, in particular, phospholipids (PL), which determine physical and chemical characteristics of the surface layer of HDL particles [8]. The concentrations of HDL CH and total PL and the relative content of phosphatidylcholine (major HDL PL) are low in patients with coronary heart disease [1,8,9]. Fluidity of cell membranes and surface layer of lipoprotein particles depends on the ratio between individual PL. This surface layer determines functional properties of lipoprotein particles [5]. Experiments on liposomes showed that viscosity of carbohydrate chains in sphingomyelin surpasses that in phosphatidylcholine, while elevation of phosphatidylcholine/sphingomyelin ratio increases membrane fluidity [5,11]. This is of crucial importance for realization of CH-accepting properties of HDL [4,8].

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The use of new hypolipidemic drugs reducing the concentrations of total CH, triglycerides (TG), and atherogenic CH in the blood and increasing the content of HDL CH substantiates the necessity of studying the effects of these drugs on the content and composition of HDL PL, which determine functional activity of these particles and are involved in the regulation of reverse CH transport.

Here we evaluated the effects of highly effective hypolipidemic preparation simvastatin on the content of individual HDL PL in patients with hypercholesterolemia. Simvastatin (Zocor, MSD) reduces plasma contents of total CH and LDL CH by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key enzyme of CH synthesis in the liver, and by inducing the synthesis of low-density lipoprotein (LDL) receptors [10].

MATERIALS AND METHODS

Seventeen men (20-65 years) were included in the study. The total CH in all patients after 8-week hypolipidemic diet was above 250 mg/dl. The patients received simvastatin in a daily single dose of 10 mg for 12 weeks.

The blood from the cubital vein was taken from fasting men in the morning. Plasma contents of total

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CH, TG, and HDL CH were measured on an Airone 200 automatic analyzer using Human enzyme kits after precipitation of HDL with sodium phosphotungstate in the presence of magnesium chloride [3]. Lipid tests were qualitatively controlled at the Department of Standardization of Biochemical Assays (Research Center of Preventive Medicine). LDL CH concentration was calculated by the formula: LDL CH=total CH-TG/5-HDL CH. Plasma apolipoprotein A-I and B contents (apo A-I and apo B) were measured by immunonephelometry on a Beringh automatic analyzer using antisera and standards (Beringh).

The composition of HDL PL was estimated in the supernatant after LDL precipitation. PL were extracted with chloroform-methanol 2:1 mixture (v/v, Folch method). The content of total HDL PL was measured after mineralization followed by the reaction with ammonium molybdate and ascorbic acid [13]. 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 (17:7:1:0.5 v/v:v/v) [2], the plates were developed in iodine vapors, the spots corresponding to individual phospholipids were scraped, mineralization was performed, and phosphorus was assayed in the reaction with hydrazine hydrochloride [14]. The results were analyzed by paired Wilcoxon test.

RESULTS

After 8-week hypolipidemic diet the mean contents of total CH and LDL CH were high, while the concentrations of TG, HDL CH, and apo A-I did not differ from normal (Table 1).

Simvastatin decreased the contents of total CH, LDL CH, and TG by 24.3, 31.5, and 21.8%, respectively, but did not change HDL CH concentration (Table 1). Apo B content decreased by 20.1%, while apo A-I concentration remained unchanged. This effect of simvastatin on blood lipoproteins is consistent with published data [10]. In our experiments HDL CH level remained unchanged, which is probably related to its normal initial content in the blood (except for 1 patient, in whom HDL CH concentrations before and after simvastatin therapy were 32 and 37 mg/dl, respectively).

Simvastatin increased the total HDL PL content in the plasma by 15.4% (Table 1). The phosphatidylcholine/phosphatidylethanolamine ratio increased, the content of lysophosphatidylcholine and sphingomyelin decreased, and the content of cardiolipin remained unchanged. It should be emphasized that simvastatin increased the phosphatidylcholine/lysophosphatidylcholine (16.5±2.3 *vs*. 9.3±0.9 in the control, $p<0.001$) and phosphatidylcholine/sphingomyelin ratios (8.2±0.4 *vs*. 7.6±0.4 in the control, $p<0.02$).

TABLE 1. Concentrations of Plasma Lipids and Apolipoproteins and Phospholipid Composition of HDL in Patients with Hypercholesterolemia before and after Simvastatin Therapy (*M*±*m*)

Parameter	Before therapy	After therapy
CH, mg/dl	277.4±8.0	209.9±5.4*
TG, mg/dl	146.1±17.6	114.2±10.8**
LDL CH, mg/dl	198.1±7.9	135.7±4.9*
HDL CH, mg/dl	50.0±2.1	51.2±1.5
Apo A-I, mg/dl	156.8±4.6	149.9±6.9
Apo B, mg/dl	157.0±4.9	114.5±4.5*
HDL PL, mg/dl	123.0±3.4	142.1±4.1*
HDL PL composition, %		
phosphatidylcholine	74.6±0.9	79.0±0.6*
lysophosphatidylcholine	9.4±0.9	4.8±0.6*
sphingomyelin	10.2±0.5	9.7±0.4*
phosphatidylethanolamine	4.4±0.4	5.4±0.3*
cardiolipin	1.2±0.2	1.2±0.2

Note. *$p<0.01$ and **$p<0.05$ compared to the corresponding parameters before therapy.

Since phosphatidic acid is the common precursor of PL and TG, it can be assumed that simvastatin potentiates PL synthesis in the liver. This is confirmed by our finding that simvastatin increases the concentration of total HDL PL by increasing the relative content of phosphatidylcholine and phosphatidylethanolamine.

High relative content of choline and ethanolamine phosphatides in HDL improves their stability in the blood. Experiments on model systems showed that these phosphatides and sphingomyelin form complexes with CH [7,12], and therefore are involved in CH acception from cell membranes. However, only CH bound to phosphatidylcholine undergoes esterification in the blood catalyzed by lecithin-cholesterol acyltransferase [4]. At the same time, lysophosphatidylcholine destabilizes cell membranes and affects vascular smooth muscle cells, thus promoting the development of atherosclerosis [6]. These changes in the phospholipid composition of HDL improve CH-accepting and CH-transporting properties of these lipoproteins and, therefore, enhance their antiatherogenic activity.

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