Cerivastatin Modulates Antiatherogenic Properties of High-Density Lipoproteins in Patients with Coronary Heart Disease and Hyperlipidemia

I. N. Ozerova, D. M. Jeevan, A. P. Serdyuk, I. V. Paramonova, N. M. Akhmedzhanov, V. A. Metel'skaya, and N. V. Perova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 10, pp. 433-436, October, 2000
Original article submitted June 7, 2000

The effects of cerivastatin on antiatherogenic properties of high-density lipoproteins were studied in patients with coronary heart disease and hyperlipidemia. Apart from hypolipidemic effects, cerivastatin changed the phospholipid composition of high-density lipoproteins and improved their cholesterol-acceptor properties. This effect was most pronounced in the serum from patients with low content of high-density lipoprotein cholesterol. These data indicate that cerivastatin modulates antiatherogenic properties of high-density lipoproteins.

Key Words: *cerivastatin; high-density lipoproteins; phospholipids; cholesterol-binding capacity*

Dyslipoproteinemia increases the risk of atherosclerosis and cardiovascular diseases. High content of total cholesterol (CH) and low-density lipoprotein CH (LDL CH) and low concentration of high-density lipoprotein CH (HDL CL) play a role in the pathogenesis of atherosclerosis. It was shown that 1% decrease in the concentration of blood CH and 1% increase in the content of HDL CH reduce the risk of coronary heart disease (CHD) by 2.5 and 3-4%, respectively [9]. If diet is ineffective, hypolipidemic drugs should be used to normalize lipid content. Statins are the most potent hypolipidemic agents decreasing CHD mortality by 30-40% [12,15].

Unlike other statins, new synthetic preparation cerivastatin (Lipobay, Bayer) is used in very low doses (micrograms). Cerivastatin produces hypolipidemic effects by decreasing blood concentrations of total CH, LDL CH, and triglycerides (TG) [12]. This preparation slightly increases the content of HDL CH [12], which is inversely related to the incidence of CHD [2,9].

Department of Metabolic Disorders, Research Center of Preventive Medicine, Russian Ministry of Health, Moscow

Antiatherogenic properties of HDL are associated with their ability to perform reverse CH transport. HDL accept free CH from cell membranes in peripheral tissues and transport it in esterified form to the liver for further excretion [2]. CH-acceptor properties of HDL depend on their concentration in the blood and their major components (*e.g.*, phospholipids) [2,8]. Fluidity of membranes and surface layer of lipoprotein particles depends on the ratio between individual phospholipids and determines mobility and functional properties of its components. All individual phospholipids, phosphatidylcholine (PC), sphingomyelin, phosphatidylethanolamine, and cardiolipin form complexes with CH [6] and, therefore, are involved in CH uptake from cell membranes. However, only CH bound to PC is esterified by plasma lecithin-cholesterol acyltransferase (LCAT). The concentrations of HDL CH and phospholipids in the blood and the relative content of PC are low in CHD patients [4,10].

New approaches to the therapy of hyperlipidemia determine the necessity of studying the influence of various drugs on functional properties of HDL. Here we evaluated the effects of cerivastatin on antiatherogenic properties and chemical composition of HDL in CHD patients with hyperlipidemia.

0007-4888/00/0010-983$25.00

MATERIALS AND METHODS

We performed a randomized, double-blind, placebo-controlled trial in 34 men (30-70 years) with CHD and hyperlipidemia. After hypolipidemic diet, the level of HDL CH in all patients was above 130 mg/dl. The patients were randomly divided into 2 groups. Group 1 patients (n=17) were daily (in the evening) treated with 0.2 mg cerivastatin for 4 weeks; during the next 4 weeks, they received 0.4 mg/day cerivastatin. Group 2 patients (n=17) were given placebo for 8 weeks.

The blood from the cubital vein was taken from fasting men in the morning (12-14-h starvation). Serum content of total CH and TG was measured on an Airone 200 automatic analyzer using Human enzyme kits. HDL CH concentration in the supernatant was estimated after precipitation of LDL and very-low-density lipoproteins with sodium tungstate and magnesium chloride (similarly to the measurements of total CH) [1]. The content of LDL CH (mg/dl) was calculated by the formula: LDL CH=CH-TG:5-HDL CH.

Serum apolipoprotein AI content was measured by immunonephelometry on a Behring Nephelometer automatic analyzer.

The concentration and composition of HDL phospholipids were estimated after LDL precipitation. Phospholipids were extracted with chloroform-methanol (2:1) mixture [7]. The content of total HDL phospholipids was measured after mineralization followed by the reaction with ammonium molybdate and ascorbic acid [13]. Individual phospholipids were separated by thin-layer chromatography on glass plates coated with silica gel in a system containing chloroform, methanol, ammonia, and water (17:7:1:0.5 v/v), the plates were developed in iodine vapors, the spots corresponding to individual phospholipids were scraped and after mineralization, and phosphorus was assayed in the reaction with hydrazine dihydrochloride [14].

CH-binding capacities of serum HDL were estimated by the efflux of 1,2-^{3}H-CH from rat hepatoma Fu5AH cells (5% serum, 4 h incubation) [5]. The efficiency of reverse CH transport was evaluated by the ratio of the content of radioactive CH released in the incubation medium to the total amount of introduced radiolabel.

RESULTS

Before the start of therapy, the mean serum contents of total CH, LDL CH, TG, and HDL CH were similar in the placebo and cerivastatin groups. After 8-week treatment with cerivastatin, the content of total CH, LDL CH, and TG decreased by 27 (from 259.9±9.4 to 188.6±6.7 mg/dl, p<0.001), 34 (from 185.2±8.8 to 121.4±6.0 mg/dl, p<0.001), and 24% (from 154.7±17.2 to 115.1±11.6 mg/dl, p<0.01), respectively, which agrees with published data [12,15]. No changes were found in the placebo group.

Cerivastatin had no effect on the contents of HDL CH, HDL phospholipids, apolipoprotein AI, and the ratios of HDL CH/apolipoprotein AI and HDL CH/HDL phospholipids (Table 1), which indicated that serum HDL concentration remained unchanged. In HDL, the relative content of PC and phosphatidylethanolamine increased, while the content of sphingomyelin, lysophosphatidylcholine (LPC), and cardiolipin decreased. These changes were accompanied by a rise of PC/sphingomyelin and PC/LPC ratios. After

TABLE 1. Effects of Cerivastatin on HDL Components and CH-Acceptor Capacity in CHD Patients ($M\pm m$)

Parameter	Cerivastatin		Placebo	
	initial	8 weeks	initial	8 weeks
HDL CH, mg/dl	43.7±2.4	44.3±2.0	47.4±2.2	46.1±2.6
Apolipoprotein AI, mg/dl	145.0±5.1	141.7±5.0	145.2±4.4	146.0±4.5
Phospholipids, mg/dl	112.3±4.0	114.4±3.5	110.6±3.7	108.8±2.7
In %				
LPC	14.7±0.5	10.5±0.6*	14.4±0.8	12.7±0.7***
sphingomyelin	12.7±0.8	9.1±0.8*	9.1±0.6	8.8±0.7
PC	66.3±1.4	73.5±1.3*	72.2±1.0	73.3±1.2
phosphatidylethanolamine	2.5±0.4	3.9±0.5*	1.9±0.2	2.3±0.2**
cardiolipin	3.7±0.3	2.9±0.2*	2.9±0.2	2.7±0.2
PC/LPC	4.6±0.2	7.4±0.6*	5.3±0.3	6.2±0.5***
PC/sphingomyelin	5.5±0.4	9.3±1.1*	8.7±0.8	10.1±1.5
CH-acceptor capacity	25.7±2.1	29.7±2.5**	28.5±2.6	30.1±2.8

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to the initial level (Student's pairwise t test).

cerivastatin therapy, the changes in individual phospholipids were more pronounced in patients with low blood content of HDL CH (equal to or below 40 mg/dl). The relative content of PC increased from 64.2±1.4 to 72.5±1.3% (by 8.3%, $p<0.001$) in patients with low HDL CH content; in patients with normal HDL CH content, this parameter increased from 70.1±0.5 to 75.3±1.0% (by 5.2%, $p<0.001$). In patients with low and normal levels of HDL CH, the relative content of sphingomyelin decreased from 13.5±0.9 to 9.5±0.8% (by 4%, $p<0.001$) and from 11.2±0.3 to 8.2±0.6% (by 3%, $p<0.001$), respectively. In the placebo group, the relative contents of PC and sphingomyelin remained unchanged, the amount of LPC slightly decreased, and the level of phosphatidylethanolamine increased (to a lesser degree than in cerivastatin-treated patients). Cerivastatin-induced changes in phospholipid composition probably affect HDL functions. High PC/sphingomyelin and PC/LPC ratios determine higher fluidity and less pronounced destruction of the surface layer of HDL particles, which improved the functions of these lipoproteins. The efficiency of the first stage of reverse CH transport (*in vitro* efflux of ^{3}H-CH from hepatoma Fu5AH cells) after cerivastatin therapy increased by 16% (Table 1). In patients with low serum HDL CH concentration, the increase in CH-acceptor capacity (from 22.4±1.2 to 32.7±4.4%, by 25%, $p<0.01$) was more pronounced than in patients with normal HDL CH levels (from 31.7±5.2 to 32.7±6.4%, differences are insignificant). In the placebo group, there were no changes in CH-acceptor properties. In cerivastatin-treated patients with low HDL CH concentration, increased CH-acceptor capacity of HDL was associated with a pronounced increase in the relative content of PC. Thus, the relative content of PC (substrate for LCAT [2]) increased, while the content of sphingomyelin inhibiting LCAT activity [3] decreased. These changes promote CH esterification in the blood, the next stage of the HDL-mediated reverse CH transport. The increase in the relative contents of PC and phosphatidylethanolamine (substrates for hepatic lipase [11]) indicates that cerivastatin inhibits this enzyme. Relatively low content of LPC also indicates low activity of hepatic lipase catalyzing the hydrolysis of PC to LPC. In addition, low relative content of LPC improves structural stability of the surface layer of HDL particles and modulates their functional properties.

Hence, cerivastatin not only produces hypolipidemic effects, but also improves CH-acceptor and CH-transporting properties of HDL even at a constant level of CH. Our findings indicate that cerivastatin is a very potent hypolipidemic statin modulating antiatherogenic properties of HDL, which can be used in the therapy of patients with CHD and hyperlipidemia.

REFERENCES

1. G. Assman, H. Schkiewer, G. Schmitz, *et al.*, *Clin. Chem.*, **29**, 2026-2030 (1983).
2. Ph. J. Barter and K. A. Rye, *Curr. Opin. Lipidol.*, **7**, 82-87 (1996).
3. D. Bolin and A. Jones, *J. Biol. Chem.*, **271**, 19,152-19,158 (1996).
4. P. Bovet, R. Darioli, A. Essinger, *et al.*, *Atherosclerosis*, **80**, 41-47 (1989).
5. M. de la Llera Moya, V. Atger, J. L. Paul, *et al.*, *Arterioscler. Thromb.*, **14**, 1056-1065 (1994).
6. R. A. Demel, J. W. Jansen, P. W. Dijek, *et al.*, *Biochem. Biophys. Acta*, **465**, 1-10 (1977).
7. I. Folch and M. J. Lees, *J. Biol. Chem.*, **226**, 497-509 (1957).
8. N. Fournier, M. de la Llera Moya, *et al.*, *J. Lipid. Res.*, **37**, 1704-1711 (1996).
9. I. J. Holme, *Circulation*, **82**, 1916-1924 (1990).
10. F. Kunz, C. Pechlaner, R. Erhart, *et al.*, *Arterioscler. Thromb.*, **14**, 1146-1150 (1994).
11. H. M. Laboda, J. M. Glick, and M. C. Phillips, *Biochem. Biophys. Acta*, **876**, 233-242 (1986).
12. E. A. Stein, *Drugs*, **56**, Suppl. 1, 25-31 (1998).
13. A. Svannborg and L. Svennercholm, *Acta Med. Scand.*, **169**, 43-45 (1961).
14. V. E. Vaskovsky, E. Y. Kostetsky, and J. M. Vasendin, *J. Chromatogr.*, **114**, 129-141 (1975).
15. C. J. Vaughan, M. B. Murphy, and B. M. Buckley, *Lancet*, **348**, 1079-1082 (1996).