EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Micronized Fenofibrate (Lipanthyl-200M) on Cholesterol Synthesis in Peripheral Blood Lymphocytes in Hyperlipidemic Patients with Coronary Heart Disease

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The rate of cholesterol synthesis in peripheral blood lymphocytes and plasma lipid and lipoprotein spectra are studied in patients with isolated hypercholesterolemia and combined hyperlipidemias (IIa and IIb hyperlipidemias according to Frederickson classification). ^{14}C-acetate incorporation into cholesterol in peripheral blood lymphocytes is considerably higher in patients with type IIb hyperlipidemia. Lipanthyl-200M reduces the rate of cholesterol synthesis in lymphocytes of both groups. A direct correlation is established between serum triglyceride level and the rate of cholesterol synthesis.

Key Words: *lymphocytes; cholesterol synthesis; hyperlipidemia; micronized fenofibrate (Lypanthyl-200M)*

Fenofibrate and other fibric acid derivatives are effective hypolipidemic drugs. First-generation fibrates, in particular clofibrate (miscleron) now gave way to more effective drugs such as bezafibrate, gemfibrozil, cyprofibrate, and fenofibrate [4,6,14]. Micronized fenofibrate (Lypanthyl-200M) belongs to third-generation fibrates. This preparation is characterized by accelerated absorption and prolonged effect and therefore can be administered only one time per day and in a lower dose [11,14]. Lipanthyl-200M induces multiple metabolic effects: it inhibits cholesterol synthesis and stimulates β-oxidation of fatty acids, reduces synthesis of triglycerides (TG) and activates peripheral lipoprotein lipase [4,6,11,12].

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Mechanism of this hypocholesterolemic effect of fibrates is poorly understood. *In vivo* and *in vitro* experiments on rats showed that fenofibrate reduces cholesterol (CH) synthesis from 2-^{14}C-acetate in the liver and inhibits 3-hydroxy-3-methylglutaryl-CoA reductase, a rate-limiting enzyme of CH synthesis [12]. It was found that regulation of CH metabolism in peripheral blood lymphocytes is similar to that of hepatocytes [2,7,8,15]. Therefore, the rate of 2-^{14}C-acetate incorporation into cholesterol in lymphocytes is used as the indirect index of the intensity of CH synthesis in human liver. Previous studies showed that fenofibrate inhibits 3-hydroxy-3-methylglutaryl-CoA reductase in patients with familial hypercholesterolemia [13]. Mechanism of this effect remains unclear, since fenofibrate despite its dose-dependent hypocholesterolemic effect is not a competitive inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase [4,12].

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In the present study we measured the rate of CH synthesis in peripheral blood lymphocytes in different dyslipidemias and evaluated the effect of Lipanthyl-200M on CH synthesis in lymphocytes and serum lipid and lipoprotein spectrum in patients with coronary heart disease and isolated hypercholesterolemia or combined hyperlipidemias.

MATERIALS AND METHODS

Ten 31-60-years-old male patients with hypercholesterolemia (total CH exceeded 6.5 mmol/liter, or 250 mg/dl) were examined. All patients followed cardiologist and dietitian recommendations for nondrug correction of hyperlipidemia for 1.5 months before treatment [1]. If plasma concentration of CH remained above 6.5 mmol/liter, treatment with 1 capsule/day Lipanthyl-200M (200 mg, Laboratories Fournier) was started. Lipid parameters were determined before and 1, 2, and 3 months after the start of treatment. To this end blood samples were drawn from the ulnary vein after an overnight fast. The contents of total CH and TG were measured on a Centrifichem-600 analyzer using Randox kits. The CH content in high density lipoproteins (HDL) was determined after precipitation of low density (LDL) and very low density lipoproteins (VLDL) [1].

Immunonephelometric assay of apolipoproteins A-I and B was performed on a Behring analyzer using rabbit antibodies [1].

Lymphocytes were isolated on a Ficoll-Paque density gradient [3], suspended in Earle's medium containing 10,000 U/ml benzylpenicillin and 10 mg/ml streptomycin. Cell viability assessed by Trypan Blue exclusion was no less than 95%. One milliliter of lymphocyte suspension contained approximately 7×10^6 cells. The rate of CH synthesis was measured using the radiolabeled precursor 2-^{14}C-acetate [15]. Radioactivity was measured in a Rack-beta scintillation counter and expressed in pmol/10^6 cells/h; the rate of 2-^{14}C-acetate incorporation into CH remained linear during a 3-h incubation.

The data were processed statistically using the Student t test.

RESULTS

Of 10 subjects included in the study 4 patients had isolated hyperlipidemia (IIa) and 6 patients had combined hyperlipidemia (IIb, total CH>6.5 mmol/liter and TG>2.3 mmol/liter or 200 mg/dl).

Lipanthyl-200M treatment for 1 month lowered the content of total CH, TG, LDL-CH, apolipoprotein B and reduced the apolipoprotein B/apolipoprotein A-I ratio (Tables 1 and 2). As seen from Table 1, Lipanthyl-200M significantly reduced the rate of CH synthesis in lymphocytes. This was paralleled by a decrease in the total CH, TG, LDL-CH, and apolipoprotein B contents in the serum. A positive correlation between endogenous CH synthesis, plasma TG content, and VLDL formation was previously reported by other investigators [3,10].

Since Lipanthyl-200M is especially effective in parients with hypertriglyceridemia, we compared the rate of CH synthesis in lymphocytes from patients with types IIa and IIb hyperlipidemia. The combined type IIb hyperlipidemia is considered as a high-risk dyslipidemia. It is characterized by high concentrations of total CH, and LDL-CH, together with increased level of TG transported primarily by VLDL, and a reduced CH content in nonatherogenic HDL particles. In our experiments the rate of CH synthesis in lymphocytes was higher in type IIb than in type IIa hyperlipidemia (Fig. 1). In patients with type IIb hyperlipidemia the rate of CH synthesis in lymphocytes after 1 and 3 months of Lipanthyl-200M treatment decreased by 40 and 49%, respectively. In patients with type IIa hyperlipidemia, a significant

TABLE 1. Effect of Lipanthyl-200M on CH Synthesis in Lymphocytes and on Contents of Serum Lipids ($M\pm m$, n=10)

Duration of treatment, months	Synthesis of CH in lymphocytes, pmol 2-^{14}C-acetate/10^6 cells/h	Total CH	TG	HDL CH	LDL CH
		mmol/liter			
0	2.26±0.10	7.28±0.19	2.13±0.30	1.22±0.13	5.09±0.18
1	1.49±0.26**	6.01±0.32*	1.05±0.17*	1.29±0.05	4.26±0.28*
	(-34)	(-17)	(-51)	(6)	(-16)
2	1.38±0.29**	6.43±0.39*	1.27±0.20**	1.31±0.04	4.55±0.32*
	(-39)	(-12)	(-40)	(7)	(-11)
3	0.99±0.26**	6.23±0.41*	1.31±0.02*	1.31±0.05	4.30±0.35*
	(-56)	(-14)	(-39)	(7)	(-16)

Note. Here and in Table 2: percent of initial level is shown in parentheses. *p<0.05, **p<0.02 or 0.01 compared with the initial level.

inhibition of CH synthesis was noted after 2 and 3 months of Lipanthyl-200M therapy (by 40 and 41%, respectively). In CH patients with combined hyperlipidemia the rate of 2-^{14}C-acetate incorporation was higher than in patients with isolated hypercholesterolemia throughout the observation period (Fig. 1). It can be assumed that enhanced CH synthesis in lymphocytes in type IIb hyperlipidemia results from impaired receptor-mediated clearance of LDL particles [14]. It has been shown that small and dense LDL particles in hypertriglyceridemia are characterized by lower affinity for apolipoprotein B,E receptors [4,5,14]. This reduces the influx of exogenous CH to cells, while the decrease in cell CH content is a signal for activation of CH synthesis [5,14].

Lipanthyl-200 markedly reduced serum concentration of TG in both groups of patients (Fig. 2). This can be attributed to stimulation of TG-rich lipoproteins due to activation of lipoprotein lipase in peripheral tissues [6,14]. Lipanthyl increases the subfraction of large LDL particles, which are better ligands for apolipoprotein B,E receptors. This enhances LDL CH influx into cells and induces intracellular feed-back inhibition of CH synthesis.

Apart from reduction of LDL atherogenicity and inhibition of CH synthesis, activation of apolipoprotein B,E receptors decreases nonspecific clearance of LDL. This leads to a rise of plasma LDL content in patients with hypertriglyceridemia treated with Lipanthyl-200M [5,9,15]. For instance, during the first month of Lipanthyl-200M therapy the content of TG and total CH in both groups decreased by 50 and 20%, respectively, whereas the initially lower level of LDL CH in patients with combined hyperlipidemia decreased more slowly that in patients with isolated hypercholesterolemia (Fig. 2). After 2-3-months Lipanthyl-200M treatment, plasma concentration of TG and the content of apolipoprotein B in patients with hypertriglyceridemia decreased by 44% and 23%, respectively, while the concentrations of total CH and LDL CH did not differ significantly from the initial values. In contrast, serum contents of total CH and LDL CH in patients with isolated hypercholesterolemia considerably dereased after 2-3 months of Lipanthyl-200M treatment. This suggests that inhibition of CH synthesis in isolated hypercholesterolemia is a result of enhanced LDL clearance and increased CH influx to the cell.

Since accelerated synthesis and metabolism of TG and VLDL in the plasma of hypertriglyceridemic patients is accompanied by enhanced CH synthesis [3,10], the inhibition of CH synthesis can be due to inhibition of TG and VLDL production. This effect is apparently more pronounced in type IIb hyperlipidemia characterized by VLDL hyperproduction.

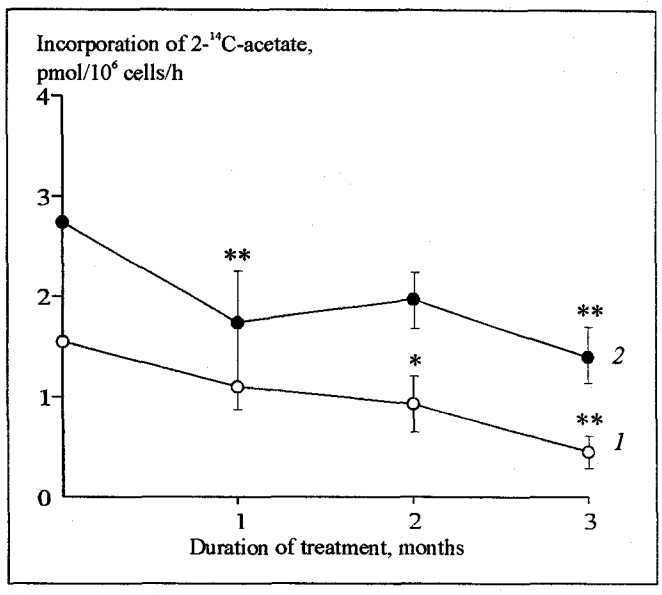

Fig. 1. Rate of cholesterol synthesis in freshly isolated peripheral blood lymphocytes of patients with hypercholesterolemia (*1*) and combined hyperlipidemia (*2*) before and during Lipanthyl-200M therapy. Here and in Fig. 2: *p<0.05, **p<0.01 compared with the initial level.

Regulation of CH synthesis is similar in the liver and peripheral blood lymphocytes. Therefore, it can be assumed that Lipanthyl-200M-induced inhibition of VLDL formation is primarily responsible for reduced rate of CH synthesis in type IIb hyperlipidemia. The decreased serum concentration of apolipoprotein B together with lowered TG level confirms the assumption on a reduced production of VLDL particles in the liver against the background of Lipanthyl-200M treatment (Fig. 2).

Thus, our findings suggest that hypocholesterolemic effect of Lipanthyl-200M is mediated though inhibition of CH synthesis, in particular, though inhibition of 3-hydroxy-3-methylglutaryl CoA reductase. This mechanism can be realized indirectly

TABLE 2. Effect of Lipanthyl-200M on Serum Content of Apolipoproteins (Apo) ($M\pm m$, n=10)

Duration of treatment, months	apo B	apo A-I	apo B/apo A-I
	g/liter		
0	1.56±0.18	1.25±0.12	1.22
1	1.21±0.07*	1.46±0.06	0.83
	(-21)	(17)	
2	1.29±0.09*	1.52±0.07	0.85
	(-18)	(22)	
3	1.23±0.08*	1.56±0.04*	0.79
	(-22)	(25)	

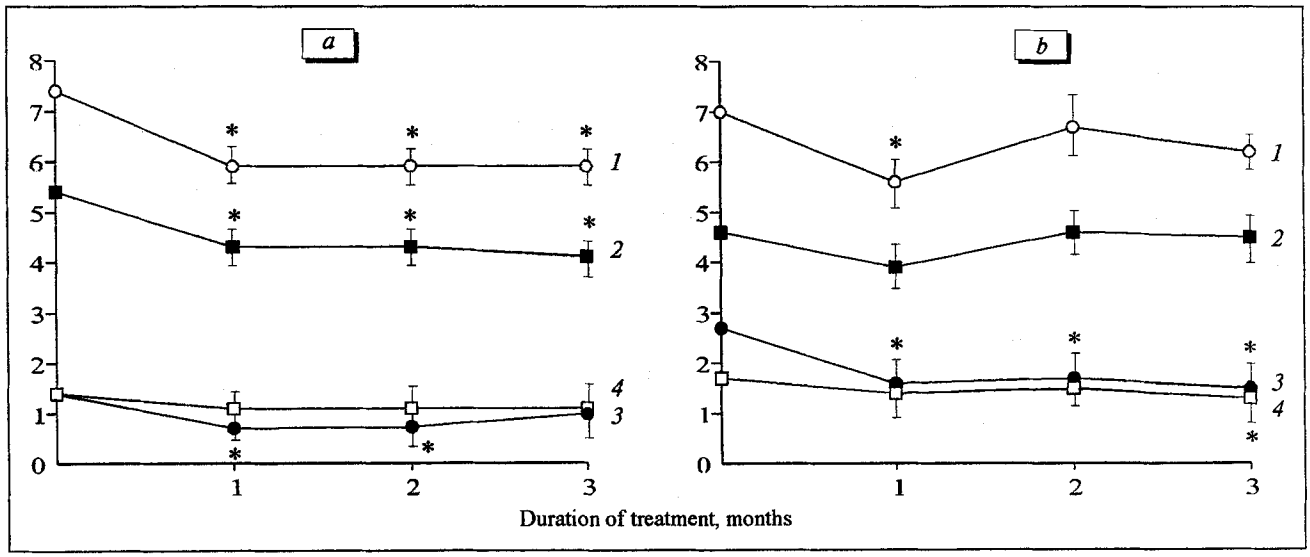

Fig. 2. Content of serum lipids (mmol/liter) and apolipoprotein B (g/liter) in patients with ischemic heart disease and different types of hyperlipidemia before and during Lipanthyl-200M therapy. a) isolated hypercholesterolemia (type IIa). b) combined hyperlipidemia (type IIb). *1*) total cholesterol; *2*) low density lipoprotein cholesterol; *3*) triglycerides; *4*) apolipoprotein B.

through enhanced influx of CH or CH esters to cells due to stimulation of apolipoprotein B,E receptor-mediated uptake of LDL particles and feed-back inhibition of this enzyme. Another possibility is a direct effect of fibric acid formed intracellularly from Lipanthyl-200M on transcription and translation of 3-hydroxy-3-methylglutaryl CoA reductase or their noncompetitive interaction.

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