Peculiarities of Changes in the Rate of Cholesterol Synthesis in Lymphocytes and Lipid and Lipoprotein Peripheral Blood Levels in Patients with Ischemic Heart Disease and Hypercholesterolemia in the Course of Combined Treatment with Lovastatin and Obsidan

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It is shown that the level of total serum cholesterol dropped and the rate of cholesterol synthesis in lymphocytes remained unchanged after 12 months of lovastatin treatment (20 mg/day) in patients with coronary heart disease. The administration of lovastatin abolished the deleterious effect of obsidan on the blood lipid spectrum.

Key Words: *lymphocytes; cholesterol synthesis; lovastatin; hypercholesterolemia*

Peripheral blood lymphocytes represent a convenient model for the investigation of the intensity of cholesterol (Ch) synthesis in patients with various dyslipidemias [10,14]. Competitive inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA-reductase), a rate-limiting enzyme of cholesterol synthesis, are used for the correction of hypercholesterolemia [10,11]. Among them lovastatin is the most widely used and its hypocholesterolemic effect has been studied at length [6,10,11]. It is attributed to an activation of B,E-receptors and accelerated catabolism of low-density lipoproteins (LDL) [13]. β-Adrenoblockers are commonly used for the management of coronary heart disease (CHD). However, they have been shown to affect the level of serum lipids and other parameters determining the development of the atherosclerotic process and CHD [1,15]. The objective of the present study was to evaluate the effect of different doses of lovastatin on the rate of cholesterol synthesis in lymphocytes and to investigate the dynamics of levels of serum lipids, lipoproteins, and A1 and B apolipoproteins in the serum of hypercholesterolemic patients treated with obsidan.

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MATERIALS AND METHODS

The material for the study consisted of serum and freshly isolated peripheral blood lymphocytes from CHD patients with angiographically verified atherosclerosis of the coronary arteries. In all, 37 male patients 40-60 years old with a level of total cholesterol higher than 250 mg/dl were examined. The therapeutic scheme is presented in Fig. 1. All patients were following the guidelines for hypolipi-

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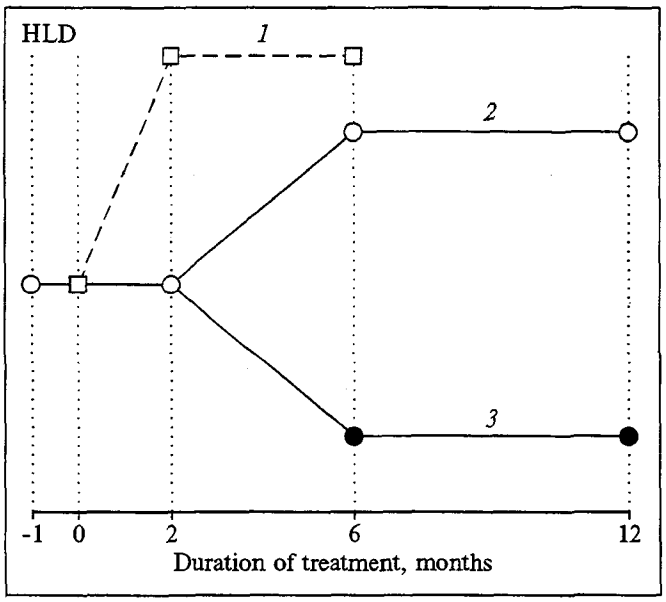

Fig. 1. Scheme of examination of CHD patients with hypercholesterolemia. *1*) patients receiving 40–80 mg/day obsidan ($n=9$); *2*) patients receiving 40–80 mg/day obsidan plus 20 mg/day lovastatin during 12 months ($n=9$); *3*) patients receiving 40–80 mg/day obsidan and lovastatin in a dose of 20 mg/kg during the first 2 months and 40 mg/day to the end of treatment ($n=11$).

demic dietary therapy (HLD) of the European Atherosclerosis Society before they started treatment with obsidan (ISIS-Chemie GMBH) either alone or in combination with lovastatin (Mevacor, MSD). Fasting blood samples for the investigation were obtained from the ulnar vein in the morning. Total serum cholesterol and triglycerides were enzymatically measured using a Centrifichem-600 analyzer [12]. The concentration of high-density lipoproteins (HDL) was determined with a technicon AAII analyzer after Mg-heparin precipitation of LDL [3]. The content of A1 and B apolipoproteins was measured by the method of quantitative (rocket) immunoelectrophoresis [4]. Lymphocytes were isolated on a Ficoll-Paque gradient as described earlier [2] and suspended in Earl medium supplemented with antibiotics (10,000 IU/ml benzenepenicillin and 10 mg/kg streptomycin); 1 ml of the cell suspension contained about 7×10^6 cells. Cell viability, determined by trypan blue exclusion, was no less than 95%. The rate of Ch synthesis from sodium 2^{14}C-acetate (Radioizotop, St. Petersburg) in freshly isolated lymphocytes was measured as described elsewhere [13] and was expressed in pmol ^{14}C-acetate incorporated into digitonin-precipitated Ch per 10^6 cells during a 1-hour incubation. The rate of ^{14}C-acetate incorporation over a 3-hour incubation was linear. The results are presented as means±SEM. Statistical processing of the results was performed using the Student *t* test.

RESULTS

Figure 2 shows the dynamics of levels of serum lipids and the rate of Ch synthesis in lymphocytes of CHD patients who had followed the HLD for one month and then were administered either obsidan (40-80 mg/day, n=9) or obsidan+lovastatin (20 mg/day, n=28). In the group of patients

TABLE 1. Dynamics of Ch Synthesis and Lipoprotein Profile in CHD Patients after Combined Treatment with Obsidan and Different Doses of Lovastatin and after a Hypolipidemic Diet

Group	Time of treatment, months	^{14}C-Acetate incorporation, pmol/10^6 cells/hour	Content in serum, mg/dl					
			Ch	TG	LDL Ch	HDL Ch	ApoAI	ApoB
I	before HLD	1.58±0.07**	287±9.4*	179±34.8	217±6.8	38±3.9	128±3.1	156±4.3
	after 1 month HLD	2.66±0.08	251±10.7	140±14.0	180±8.7	34±2.4	127±3.6	145±5.2
Lovastatin, 20 mg/day	2	2.07±0.38	191±11.0**	124±33.2	154±22.9	38±3.8	124±4.1	122±4.3**
	6	2.23±0.48	204±10.9	134±24.9	143±10.3	39±3.0	126±6.0	119±5.5**
	12	2.95±0.47	226±17.7	119±21.0	150±11.7*	39±7.1	125±5.2	116±14.4*
II	before HLD	1.75±0.18**	300±12.3	182±29.3	220±11.4	43±3.2	135±10.1	148±12.9
	after 1 month HLD	2.48±0.09	273±15.9	175±33.8	194±20.8	44±2.6	137±16.7	139±8.4
Lovastatin, 20 mg/day	2	2.25±0.45	226±10.0*	163±21.3	161±7.1	42±3.3	118±4.7*	124±7.2
Lovastatin, 40 mg/day	6	2.16±0.49	205±26.0	174±21.4	133±12.6	37±1.1	114±3.4**	110±4.6*
	12	4.09±0.94**	197±12.5**	170±21.4	143±11.4*	38±2.0*	118±3.3**	114±7.6**

Note. * – $p<0.05$, ** – $p<0.01$ in comparison with the results obtained after 1 month of HLD.

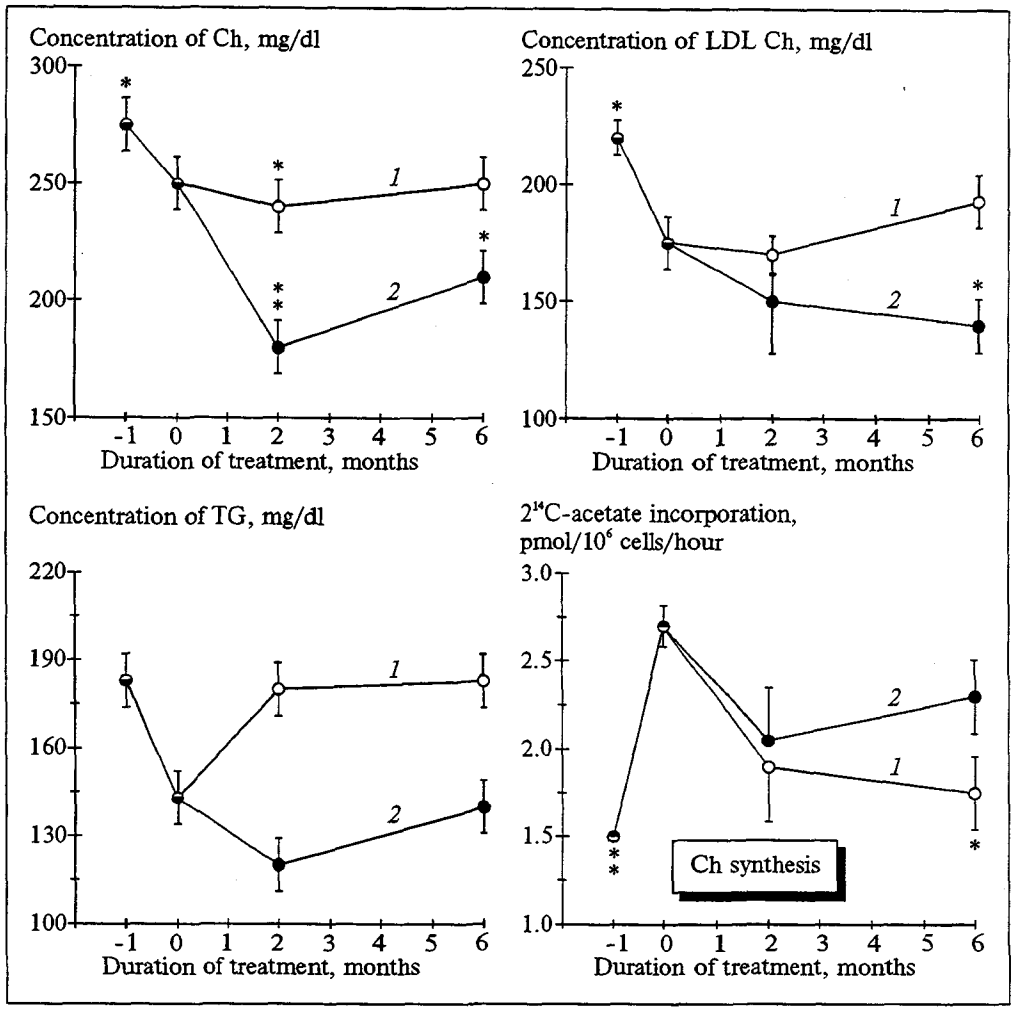

Fig. 2. Dynamics of serum lipids and rate of Ch synthesis in freshly isolated peripheral blood lymphocytes in CHD patients after administration of obsidan alone (*1*), and in combination with lovastatin in a dose of 20 mg/day (*2*). * – $p<0.05$,** – $p<0.01$ in comparison with the results obtained after 1 month of treatment.

treated with obsidan alone 2 months after the start of therapy the level of total Ch continued to decline below the value observed after HLD, but after 6 months of obsidan it returned to the initial value ($n=5$). When patients were administered obsidan in combination with lovastatin, the level of total serum Ch reliably dropped ($n=19$) and remained at this level over 6 months of treatment, which was connected with a simultaneous drop of the level of LDL Ch. After 2 months of obsidan therapy, triglycerides (TG) stopped dropping as they had done during the HLD period, and after 6 months their content returned to the initial value. In the group administered obsidan+lovastatin the drop of the TG level was statistically unreliable, probably due to wide deviations in individual values. After 1 month of HLD the drop of the level of total serum Ch (from 285±9.4 to 251±10.7 mg/dl) and LDL Ch (from 217±6.8 to 180±8.7 mg/dl) was accompanied by accelerated incorporation of sodium 2¹⁴C-acetate into Ch in lymphocytes (from 1.58±0.07 to 2.66±0.08 pmol/10⁶ cells/hour, Fig. 2). After 6 months of treatment with obsidan+lovastatin the rate of Ch synthesis in lymphocytes did not differ reliably from that after HLD, whereas obsidan alone reduced the incorporation of ¹⁴C-acetate into Ch in lymphocytes. It should be noted that the level of total Ch and LDL Ch in obsidan-treated patients was reliably higher than that in the group treated with obsidan in combination with lovastatin (259±15.9 vs. 204±10.5 mg/dl, $p<0.05$, for total Ch and 183±14.5 vs. 143±10.3 mg/dl, $p<0.05$, for LDL Ch, respectively). If the level of total Ch in the group treated with lovastatin remained above 250 mg/dl after 2 months of treatment, the dose was increased to 40 mg/day. In Table 1 we compare the changes in the rate of Ch synthesis in lymphocytes and in the serum lipid spectrum in two groups of CHD patients who received either 20 or 40 mg lovastatin per day together with obsidan. When lovastatin was administered in a dose of 20 mg/day, incorporation of ¹⁴C-acetate into Ch in lymphocytes did not differ reliably from that observed soon after the beginning of treatment. On the other hand, when lovastatin was administered in a dose of 40 mg/

day, the rate of acetate incorporation into Ch in lymphocytes was reliably elevated after 12 months of treatment. Lovastatin in a dose of 40 mg/day lowered the level of LDL Ch, whereas the dose of 20 mg/day did not affect this parameter. The combination of obsidan with 20 mg/day lovastatin over 2-6 months reliably lowered the level of apoB ($p<0.01$) but did not affect the content of apoA1, the apoB/apoA1 ratio being reliably reduced from 1.14 ± 0.05 to 0.93 ± 0.05 ($p<0.05$). Lovastatin in a dose of 40 mg/day reduced the content of apoA1 and apoB without affecting their ratio.

These results indicate the existence of an inverse relationship between the rate of Ch synthesis in lymphocytes and the level of serum Ch. The synthesis of Ch in lymphocytes depends on the concentration of Ch in the cell, i.e., it is regulated by feedback [5,8]. The elevation of the total Ch level observed after 6 months of lovastatin treatment was accompanied by a decreased rate of Ch synthesis in the lymphocytes. The treatment with lovastatin abolished the adverse effects of obsidan on the blood lipid spectrum. The hypocholesterolemic effect of lovastatin administered against the background of obsidan depends on its diurnal dose. There are diverse published data on the effect of lovastatin on the rate of Ch synthesis in peripheral blood lymphocytes [7,8,13]. We showed that this effect depends on the diurnal dose of the preparation and on the degree of the drop of total serum Ch. Increasing the diurnal dose of lovastatin to 40 mg/day stimulates the 2^{14}C-acetate incorporation into Ch and produces a more pronounced reduction of both total and LDL Ch in comparison with the effect of 20 mg/day. Just as in other cells, in lymphocytes transport of exogenous Ch is mediated through apoB,E receptors on the cell membrane, which bind and internalize LDL particles [5]. The stimulation of Ch synthesis in lymphocytes may be attributed to a Ch depletion related to an induction of receptor-mediated binding of LDL and their elevated internalization, degradation, and removal from the circulation [6]. This suggestion has been confirmed by data on the induction of HMG-CoA-reductase in lymphocytes and the synthesis of specific LDL receptors against the background of long-term lovastatin treatment [5,8,9]. Thus, measurement of the rate of Ch synthesis in freshly isolated lymphocytes of hypercholesterolemic patients with CHD reveals new additional characteristics of the effect of hypolipidemic drugs and makes it possible to optimize their dosage.

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