PHARMACOLOGY

Effect of Long-Term Lovastatin Treatment on the Number of Glucocorticoid Receptors in Peripheral Blood Lymphocytes

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A reduced number of ^{3}H-dexamethasone-specific binding sites (glucocorticoid receptors) in lymphocytes was observed after 2 months of lovastatin treatment against the background of a lowered serum level of cholesterol and triglycerides and a reduced rate of cholesterol synthesis in lymphocytes. This phenomenon is considered as an adaptive reaction of the cells to relative cholesterol depletion due to inhibition of its intracellular synthesis.

Key Words: *glucocorticoid receptors; lymphocytes; dexamethasone; cholesterol; hypolipidemic drugs; hypercholesterolemia*

Glucocorticoid hormones (Gc) are known to inhibit cholesterol (Ch) synthesis in cells from peripheral tissues [4,5]. The sensitivity of the cell to Gc is determined by the number of specific receptors and their affinity to the hormone [14]. Changes in the number of receptors may be considered as an adaptive reaction to changes in environmental conditions. Peripheral blood lymphocytes of hypercholesterolemic (HCh) subjects have been shown to possess a lower number of glucocorticoid receptors (GcR) and a reduced sensitivity to Gc [10]. A decreased number of GcR was also observed in *in vitro* experiments after incubation of cultured human skin fibroblasts with HCh serum or isolated low- and very-low-density lipoproteins [10]. In line with the above investigations it seemed to be important to elucidate whether normalization of the Ch level in HCh subjects (for instance, due to hypolipidemic therapy) leads to changes in the number of GcR in blood cells.

The objective of the present study was to investigate the effect of long-term treatment with lovastatin, a hypolipidemic drug which is an inhibitor of HMG-reductase, on the number of GcR in peripheral blood lymphocytes.

MATERIALS AND METHODS

The study was performed on a group of male patients (36-59 years old) who had undergone medical examination at the Russian Research Institute of Preventive Medicine. Coronary heart disease was diagnosed in 15 patients: angina pectoris of functional class I in 5 patients, class II in 6 , and class III in 4 patients. Five patients had mild arterial hypertension and two had no cardiovascular

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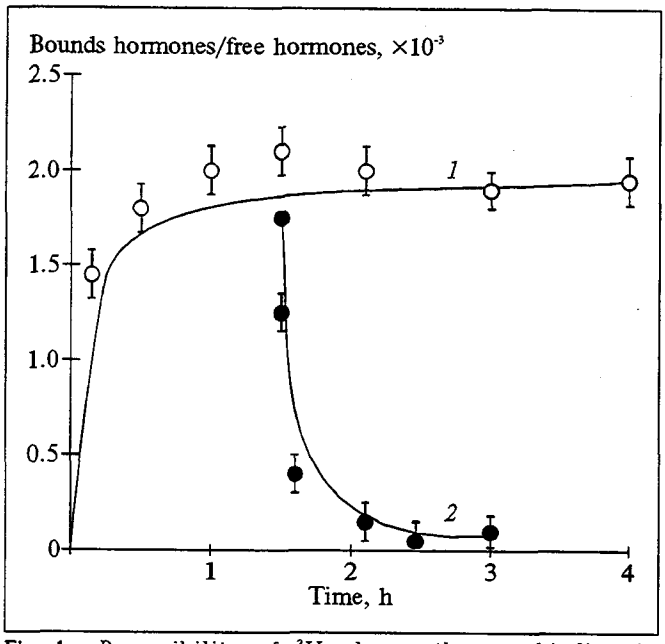

Fig. 1. Reversibility of ^{3}H–dexamethasone binding in peripheral blood lymphocytes. *1*) time course of ^{3}H–dexamethasone binding in lymphocytes; 2) ^{3}H–dexamethasone binding after addition of a 1000–fold excess of unlabeled dexamethasone; arrow indicates the time of addition of excess dexamethasone.

pathology. Patients were considered as hypercholesterolemic if the level of total plasma Ch exceeded 200 mg/dl [13]. The experimental group comprised patients who had an initial Ch level of more then 240 mg/dl and whose Ch remained high even after 1 month of the hypolipidemic diet (stage I) recommended by the American Heart Association [2]. Thereafter all patients received lovastatin (Mevacor, MSD, USA) in a dose of 20 mg after the evening meal (the diet was continued). All patients continued other drugs as well: 7 patients - nifedipine in a dose of 30-100 mg/day, 17 patients - propranolol in a dose of 40-160 mg/day, 10 patients - prolonged nitrates, and 2 patients - verapamil. The number of GcR in peripheral blood lymphocytes and the concentration of lipids in the serum were determined at the initial point of the trial (after the diet and before lovastatin administration) and after 2 and 6 months of lovastatin treatment. The material for the study consisted of peripheral blood lymphocytes. Fasting blood for investigation was drawn from the ulnar vein into tubes with 1 mg/ml EDTA. Lymphocytes were isolated on a Ficoll-Paque (Pharmacia, Sweden) gradient [3] and suspended in medium 199 to a concentration of 2-4 mln. cells/ml. Cell viability, determined by trypan blue exclusion, was no less than 95%. The number of Gc specific binding sites was assayed using ^{3}H-dexamethasone (Amersham, UK, 70 Ci/mmol) after Scatchard [8] with some modifications [9]. Lymphocytes obtained from essentially healthy donors with normolipidemia (n=7) were used to choose the experimental conditions for the ^{3}H-dexamethasone specific binding assay. Five hundred milliliters of lymphocyte suspension were placed in glass tubes containing 50 μl ^{3}H-dexamethasone and 50 μl unlabeled dexamethasone. The final concentration of ^{3}H-dexamethasone was 1 nM, while the concentration of unlabeled dexamethasone varied from 0.5 to 50 nM. Nonspecific binding was determined in the presence of a 1000-fold excess of unlabeled hormone (10^{-6} M). In some experiments specific binding of ^{3}H-dexamethasone was assessed using a saturating concentration of the hormone (final concentrations of labeled and unlabeled hormone were 50 nM and 5×10^{-5} M, respectively. The samples were incubated at 37°C in 5% CO_2 and 80% humidity for 1.5 hours, and then washed twice by centrifugation with 2 ml phosphate buffer saline at 600 g, 4°C for 10 min. Thereafter the cell pellet was resuspended in buffer, 1 ml 96% ethanol was added, and the mixture was transferred to counting vials with 10 ml ZhS-8 scintillation liquid. The rate of intracellular Ch synthesis in lymphocytes was assayed by ^{14}C-acetate incorporation into digitonin-precipitated sterols as described earlier [11]. The content of total Ch, triglycerides (TG), and high-density lipoprotein Ch (HDL Ch) in blood serum was measured with a Centrifichem analyzer (UK) using Wako kits (Japan). Statistical processing of the results was performed using the Student t test and the Wilcoxon paired test (P_T).

TABLE 1. Number of GcR in Lymphocytes and Concentration of Plasma Lipids in Patients Treated with Lovastatin

Time of blood sampling	Number of ^{3}H-dexamethasone binding sites, ×10³ per well	Ch, mg/dl	TG, mg/dl	HDL Ch, mg/dl
Start point (n=22)	4.3±0.3	245.5±7.2	172.2±14.6	35.9±2.0
2 months (n=22)	3.4±0.3*	189.2±6.2*	117.9±11.2*	40.5±2.2
6 months (n=10)	5.0±0.9	185.9±8.4*	111.4±12.1*	41.8±5.8

Note. * – p<0.05 in comparison with the start point.

RESULTS

The trial revealed that 2-month lovastatin therapy led to a considerable drop of both total Ch and TG in the serum (Table 1). During the next 4 months of lovastatin therapy Ch and TG remained at this low level. The level of HDL Ch remained practically unchanged over the total period of treatment (Table 1).

The rate of Ch synthesis (from ^{14}C-acetate incorporation into digitonin-precipitated sterols) determined in 6 randomly selected patients after 1 month of lovastatin therapy was lower than before treatment and accounted for 30% of the initial level (378.5±±152.1 vs. 1172.8±500.7, respectively, $P_T<0.01$).

Prior to determination of GcR it was important to verify the suitability of the standard conditions for ^{3}H-dexamethasone specific binding in patients with different levels of blood lipids. In specially designed experiments we showed that ^{3}H-dexamethasone specific binding in peripheral blood lymphocytes is saturable and reversible. The addition of a 1000-fold excess of unlabeled dexamethasone almost completely eliminates ^{3}H-dexamethasone from the hormone-receptor complex (Fig. 1). In lymphocytes from both normolipidemic and HCh patients saturation was achieved during a 1.5-hour incubation with ^{3}H-dexamethasone (Fig. 2), and in both cases Scatchard analysis revealed one class of high-affinity binding sites (Fig. 3).

The initial mean number of GcR in lymphocytes of HCh patients was $4.3\pm0.5\times10^3$ ^{3}H-dexamethasone binding sites per cell and differed little from that of healthy normolipidemic donors determined in preliminary experiments ($4.9\pm0.4\times10^3$ bind. sites/cell) [1]. This may be attributed to the relatively low concentrations of total Ch in the blood of HCh patients after the diet.

Two months after the start of lovastatin therapy the number of GcR in lymphocytes was shown to be considerably lowered (Table 1), but after 6 months of treatment the number of GcR was restored and even exceeded the initial level.

From our previous studies, which had demonstrated the ability of low-density lipoproteins (LDL) to reduce the number of GcR in peripheral blood lymphocytes [10], it seemed logical to expect that normalization of the lipid composition of the plasma in the course of lovastatin therapy would increase the number of GcR. A tendency toward a rise was revealed only after 6 months of lovastatin treatment (Table 1), whereas after 2 months, when the hypolipidemic effect of the drug was completely manifested, we observed a statistically reliable drop of the number of GcR. This

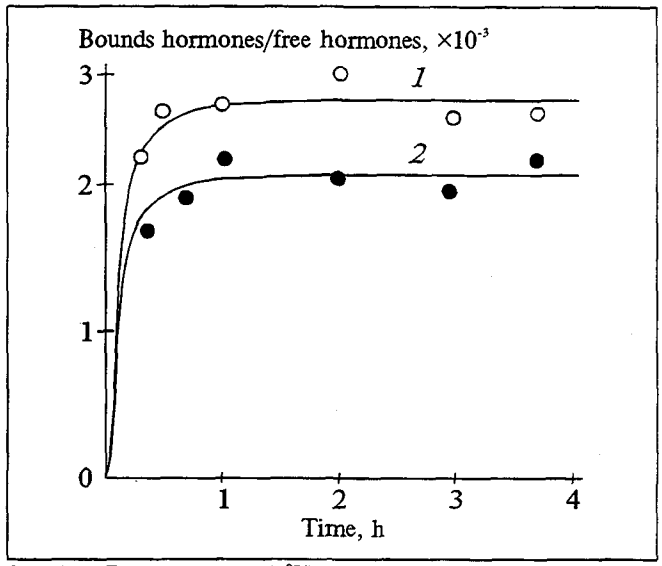

Fig. 2. Time course of ^{3}H–dexamethasone binding at a saturation concentration (50 nM). *1*) lymphocytes of healthy donor with normolipidemia; *2*) lymphocytes of hypercholesterolemic patient.

result (the decreased number of GcR after 2 months of lovastatin therapy) suggests a possible independent effect of lovastatin on the number of GcR, which is apparently not related to the changes in the level of plasma Ch. This assumption is based on the following facts.

Previously we found a low level of GcR in cultured human skin fibroblasts obtained from patients with familial HCh [1]. These cells lack normal LDL receptors and, correspondingly, receptor-mediated endocytosis of LDL, the Ch requirements of the cell being met through accelerated endogenous synthesis [7], which is partially effected through a reduced sensitivity of the cell to Gc [10].

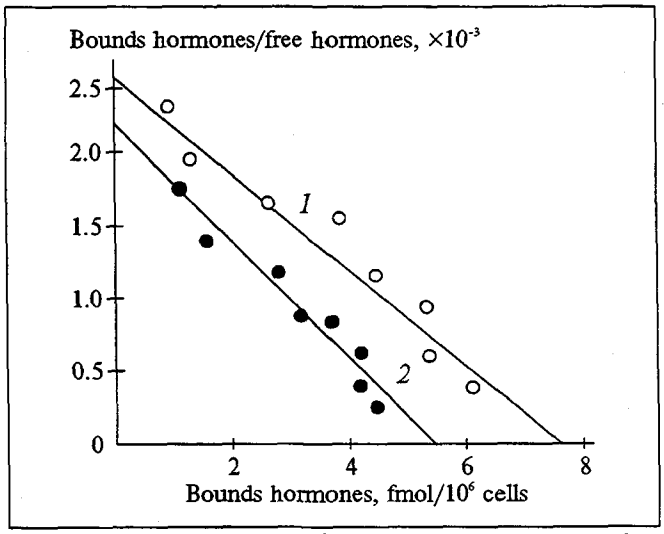

Fig. 3. Specific binding of ^{3}H–dexamethasone in lymphocytes in Scatchard coordinates. *1*) lymphocytes of healthy donor with normolipidemia; *2*) lymphocytes of hypercholesterolemic patient.

On the cellular level lovastatin acts via inhibition of intracellular Ch synthesis due to competitive inhibition of HMG-CoA reductase [6,12]. Under our experimental conditions this was confirmed by the considerably reduced incorporation of ^{14}C-acetate into digitonin-precipitated sterols. It may be thought that in our study, similarly to the case with familial HCh, the reduced number of GcR and, correspondingly, the reduced sensitivity of the cell to GC reflect an adaptive reaction of the cell directed toward compensation for Ch depletion, except that in familial HCh this deficit is due to the absence of LDL uptake, while in the case of hypolipidemic therapy it is due to inhibition of intracellular Ch synthesis.

Thus, our results suggest that the effect of lovastatin on the number of GcR receptors in blood lymphocytes derives from at least two factors: a reduced content of Ch (or LDL) in blood plasma and inhibition of endogenous Ch synthesis in lymphocytes. The detailed mechanisms of these phenomena are a subject for further investigation.

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