EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Endur-Acine on Plasma Activity of Cholesterol Ester Transfer Protein in Subjects with Hypercholesterolemia

I. N. Gorshkova, N. G. Kiseleva, N. M. Akhmedzhanov, and N. V. Perova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 2, pp. 185-187, February, 1996
Original article submitted February 20, 1995

The activity of cholesterol ester transfer protein was measured in the plasma of hypercholesterolemic patients before and after treatment with endur-acine, a new prolonged-action preparation of nicotinic acid. The activity was measured by a substrate-independent radioisotope method. It was found that improvement of the lipid-apoprotein parameters of the plasma after the treatment was accompanied by a reduced activity of cholesterol ester transfer protein.

Key Words: *lipoproteins; cholesterol ester transfer protein; endur-acine; hypercholesterolemia*

Cholesterol ester transfer protein (CETP) performs a heteroexchange of cholesterol esters and triglycerides between plasma lipoproteins [9]. Transfer of an excess of cholesterol esters, which is formed in high density lipoproteins (HDL) as a result of the acceptance and subsequent esterification of free cholesterol, to low (LDL) and very low (VLDL) density lipoproteins represents an important step in the so-called reverse cholesterol transport. CETP plays a dual role in atherogenesis. On the one hand, this protein serves as an atherogenic bypass, since it transfers cholesterol esters from antiatherogenic HDL, which are able to transport them to the liver for subsequent catabolism, to atherogenic VLDL and LDL, which transport cholesterol and cholesterol esters to peripheral tissues. On the other hand, the removal of excess cholesterol esters from HDL particles may stimulate their cholesterol-acceptor function. The atherogenic role of CETP is confirmed by previous data demonstrating that the plasma activity and content of CETP directly correlate with the level of total cholesterol and VLDL cholesterol and inversely correlate with HDL cholesterol [7]. Marked atherogenic changes in the plasma lipoprotein profile are observed in patients with familial CETP insufficiency [6]. At the same time, the fact that CETP stimulates the efflux of cholesterol esters and triglycerides (TG) from lipid-loaded macrophages in culture suggests an antiatherogenic role of CETP [8].

For a better understanding of the role of CETP in atherogenesis it seems important to study the changes in CETP activity produced by hypolipidemic agents correcting atherogenic shifts in the plasma. Endur-acine, a new prolonged-action form of nicotinic acid, has been previously shown to be an effective drug for the correction of atherogenic dyslipidemias. However, there are no reports on the effect of endur-acine on the activity of CETP in patients with hypercholesterolemia.

MATERIALS AND METHODS

The trial comprised 25 patients (21 men and 4 women, mean age 49±3.5 years) with stable angina or essen-

State Research Center for Preventive Medicine, Russian Ministry of Health and the Medical Industry, Moscow (Presented by Yu. P. Nikitin, Member of the Russian Academy of Medical Sciences)

0007-4888/96/0002-0171$15.00

TABLE 1. Concentrations of Lipids and Apoproteins and CETP Activity in Blood of Hypercholesterolemic Patients before and after Treatment with Endur-Acine (*M±m*)

Parameter	Before treatment	After treatment
Total cholesterol, mg/dl	257.2±4.8	221.1±6.2**
Triglycerides, mg/dl	123.3±7.4	102.0±6.1*
HDL cholesterol, mg/dl	47.2±2.4	52.5±1.8**
LDL cholesterol, mg/dl	184.4±4.2	149.0±6.2**
Apoprotein A-1, mg/dl	131.6±3.0	150.4±3.7**
Apoprotein B, mg/dl	152.7±3.3	116.6±4.4**
CETP activity, nmol/h/ml	62.4±4.2	52.1±3.7**

Note. *$p<0.05$, **$p<0.01$ indicate the reliability of changes in the above parameters after the treatment according to the paired Wilcoxon test.

tial hypertension accompanied by hypercholesterolemia persisting against the background of an earlier-described hypolipidemic diet [1]. Total cholesterol exceeded 225 mg/dl in the men and 230 mg/dl in the women. Contraindications for participation in the trial were described in our previous study [1]. All patients followed the hypolipidemic diet throughout the study. The study was carried out using the double-blind method, the groups being randomized using the Random Number Table. The randomized groups were matched for sex, age, concomitant therapy (nitrates, calcium antagonists), and initial level of plasma lipids. Treatment schedules in these groups were as follows. The patients of group 1 received endur-acine (500 mg, 3 times a day) for 2 months, then a placebo (3 times a day) for the next 2 months, and then endur-acine (500 mg, 4 times a day) again for the last 2 months. The patients of group 2 received a placebo during the first two months and endur-acine (500 mg) 3 and 4 times a day during the second and third 2-month periods, respectively.

Plasma was separated from fasting blood taken from the ulnar vien and collected into tubes containing EDTA (1 mg/ml blood). The concentrations of total cholesterol, HDL cholesterol, TG, and apoproteins A-1 and B were measured and the plasma content of LDL cholesterol was calculated as described earlier [1].

Plasma activity of CETP was evaluated using a radioisotope substrate-independent method by measuring the transfer of ^{3}H-cholesterol esters from HDL_3 to LDL in the presence of an aliquot of the test plasma. Lipoproteins were separated from pooled plasma of healthy donors with normal lipid indexes. HDL_3 containing radiolabeled cholesterol esters were prepared as follows [2]: 10 μCi 1α,2α-^{3}H cholesterol dissolved in benzene were dried under nitrogen and redissolved in 20 ml 95% ethanol. The resultant solution was slowly added to 120 ml freshly isolated and dialyzed plasma free of VLDL, LDL, and HDL_2 under constant stirring. The mixture was incubated at 37°C for 24 hours for esterification of cholesterol by lecithin-cholesterol acetyltransferase and then HDL were separated from the labeled plasma by ultracentrifugation at d=1.21 g/ml. The isolated HDL were incubated with an excess of LDL (≈240 mg protein content) for 5 h at 37°C in order to remove unesterified labeled cholesterol from HDL_3 and then again isolated from LDL by ultracentrifugation. Thin-layer chromatography of the obtained HDL_3 demonstrated that esterified ^{3}H-cholesterol accounts for 96% of the total ^{3}H-cholesterol. The specific activity of the obtained radiolabeled HDL_3 was 32 cpm/nmol total cholesterol.

For determination of CETP activity the test plasma (50 μl) was incubated with labeled HDL_3 (0.2 μmol total cholesterol) and LDL (0.5 μmol total cholesterol) in the presence of 2 mM dithionitrobenzoic acid (Sigma), an inhibitor of lecithin-cholesterol acetyltransferase. Incubation was carried out in phosphate buffer containing 0.01% NaN_3, pH 7.4, in a total volume of 600 μl, in tubes placed in a water bath with a shaker, at 37°C for 16 hours. Then LDL were precipitated with dextran-sulfate and $MgCl_2$ as described elsewhere [5]. The precipitant was separated by centrifugation at 3500 g for 10 min, 1 ml supernatant was added to 10 ml scintillation liquid, and radioactivity was counted on a Mark III (Delta Medicum). The samples were incubated in duplicates. Each series of the test samples included samples of a control serum. Two incubation mixtures in which test plasma was replaced with buffer served as a blank. In these samples less than 1% of labeled cholesterol esters was transferred from HDL to LDL.

The amount of cholesterol esters transferred from HDL to LDL was calculated by the formula:

$$\text{Cholesterol esters (nmol)} = \frac{(R_{blank} - R_{plasma})}{R_{blank}} \times \text{HDL cholesterol esters},$$

where R_{blank} and R_{plasma} are the radioactivity of the supernatant of samples containing buffer and plasma, respectively, and HDL cholesterol esters is the content of cholesterol esters (nmol) in labeled HDL_3 present in the incubation mixture.

CETP activity was expressed as the amount of cholesterol esters (nmol) transferred from HDL_3 to LDL in the presence of 1 ml plasma in 1 hour of incubation.

RESULTS

There were no differences between the two groups in terms of the concentrations of plasma lipids and apoproteins or in CETP activity both before and af-

ter endur-acine treatment, and therefore the experimental data are presented for the entire group of patients included in the trial (Table 1). As is seen from the table, endur-acine lowered total cholesterol by 14% on average, triglycerides by 21%, LDL by 19%, and apoprotein B by 24%, whereas the content of HDL cholesterol and apoprotein A-1 was increased by 11% on average. This positive dynamics of lipid and apoprotein indexes was accompanied by a 16.5% reduction of CETP activity.

It is interesting to note that endur-acine treatment produced a maximal drop in CETP activity (by 30% on average) and a more marked improvement of lipid indexes (18% drop of total cholesterol and 35% increase of HDL cholesterol) in the three hypercholesterolemic patients with an initially high level of TG (>175 mg/dl) and a low content of HDL cholesterol (<35 mg/dl). These data are in conformity with previous reports on a more pronounced correcting effect of hypolipidemic preparations in cases where atherogenic shifts in the plasma lipid profile are maximal [3] and, in addition, they demonstrate a maximal drop of CETP activity in these cases.

The above data corroborate the atherogenic role of CETP and suggest that the hypolipidemic effect of endur-acine is partially mediated through a reduction of CETP activity. Recent data indicate that preparations of nicotinic acid are able to inhibit the direct synthesis of LDL, activate lipoprotein lipase, and lower HDL catabolism [1]. The antiatherogenic impact of the reduced CETP activity may be mediated through at least two pathways: first, a reduced redistribution of cholesterol esters from antiatherogenic HDL to atherogenic LDL, and second, a reduced exchange of cholesterol esters to TG between LDL and VLDL [9]. This prevents the formation of triglyceride-enriched LDL which may be converted to small "atherogenic" LDL due to the action of liver lipase [4]. Thus, the reduced activity of CETP results in a lowered content of small atherogenic LDL.

REFERENCES

1. R. G. Oganov, N. G. Kiseleva, D. M. Aronov, et al., *Kardiologiya*, № 10, 54-59 (1993).
2. J. J. Albers, J. H. Tollefson, C.-H. Chen, and A. Steinmetz, *Arteriosclerosis*, **4**, 49-56 (1984).
3. M. C. Cheung, M. A. Austin, P. Moulin, *et al., Atherosclerosis*, **102**, 107-119 (1993).
4. B. A. Griffin, D. J. Freeman, G. W. Tait, *et al., Ibid.*, **106**, 241-253 (1994).
5. J. E. M. Groener, R. W. Pelton, and G. M. Kostner, *Clin. Chem.*, **32**, 283-286 (1986).
6. A. Inazn, M. L. Brown, C. B. Hesler, *et al., N. Engl. J. Med.*, **323**, 1234-1239 (1990).
7. R. McPherson, C. J. Mann, A. R. Tall, *et al. Arterioscler. Thromb.*, **11**, 797-804 (1991).
8. R. E. Morton, *J. Lipid Res.*, **29**, 1367-1377 (1988).
9. A. R. Tall, *Ibid.*, **34**, 1255-1274 (1993).