IN VITRO STUDY OF THE EFFECT OF PURE AND LONG-STORED COMMERCIAL CHOLESTEROL ON BINDING OF VERY LOW- AND LOW-DENSITY LIPOPROTEINS BY RABBIT HEPATOCYTES

S. A. Volgushchev, V. A. Kosykh, E. A. Podrez, D. K. Novikova, R. S. Karpov, and V. S. Repin

UDC 577.175.5

KEY WORDS: cholesterol, oxidized derivatives of cholesterol, binding, very low-density lipoproteins, low-density lipoproteins.

Keeping rabbits on a high cholesterol diet leads to elevation of the plasma cholesterol level and, in particular, to the level of cholesterol esters, bound mainly with very low-density β-lipoproteins (β-VLDL), which are rich in apolipoprotein E [10]. Accumulation of these complexes in the blood stream is explained by reduction of their uptake by the liver [4] and a simultaneous increase in their production by the liver and (or) intestine [9]. It was recently shown that oxidized derivatives of cholesterol are present in preparations of cholesterol used to feed experimental animals, as well as food products [7, 11]. When rabbits are fed with cholesterol purified to free it from autooxidation products, hypercholesterolemia developed 1-1.5 months later than in animals fed with the same quantity of cholesterol, but containing its oxidation products [1]. In work published previously the writers showed that the rapid development of hypercholesterolemia in rabbits receiving long-stored commercial cholesterol is connected with hyperproduction of VLDL with a high content of cholesterol esters by the liver cells [5]. The aim of the present investigation was to study the effect of pure and long-stored dietary cholesterol on receptor activity of rabbit hepatocytes.

EXPERIMENTAL METHOD

Hepatocytes were isolated from the liver of Chinchilla rabbits weighing 2-3 kg, kept on different diets for 6 weeks. Rabbits of the control group received a standard pellet diet consisting of a combined formula with vegetables, to which olive oil was added (0.5 ml/kg). Animals of the experimental groups received purified or long-stored commercial cholesterol, suspended in olive oil (0.2 g cholesterol/kg body weight and 0.5 ml of olive oil), perorally. Hepatocytes were obtained by perfusion of the liver, using collagenase [8]. The cells were seeded in culture dishes with a density of $2 \cdot 10^5$ /cm^2 in Eagle's minimal medium containing 10% fetal calf serum, 100 μg/ml of kanamycin, and essential amino acids (1 mM), and cultured at 37°C in an atmosphere of 95% air and 5°C CO_2. Low-density lipoproteins (LDL) and β-VLDL were obtained by gradient ultracentrifugation for 48 h at 45,000 rpm and 4°C (50.2 rotor, "Beckman"). The protein concentration in the lipoproteins was determined by Lowry's method [6], using bovine serum albumin as the standard. The lipoproteins were labeled with ^{125}I with the aid of IC1 [2]. Binding (superficial binding and ingestion) of ^{125}I-LDL and ^{125}I-β-VLDL with hepatocytes [3] was determined. Specific binding ^{125}I-labeled LDL and β-VLDL by hepatocytes was calculated by subtracting from values of total binding, those values which were obtained in the presence of an excess of unlabeled lipoproteins. To separate lipids into classes the method of high-resolution thin-layer chromatography [9] was used. Cholesterol and its esters were assayed quantitatively by means of an automatic densitometer (Camag HPTLC/TLC Scanner, Switzerland) and integrator (SP4 100, West Germany) [10]. Each determination was accompanied by calibration, using cholesterol and cholesterol oleate as the standard. Total plasma cholesterol was determined enzymatically, using a kit from the firm "Medix" and an FP-901 analyzer ("Labsystems Oy", Finland). The results were subjected to statistical analysis by Student's t-test.

Research Institute of Cardiology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. Research Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 250-251, March, 1991. Original article submitted June 28, 1990.

0007-4888/91/0003-0307$12.50

TABLE 1. Content of Cholesterol and Its Esters (in μg/mg cell protein) in Rabbit Hepatocytes (M ± m, n = 4)

Group	Cholesterol	Cholesterol esters
Control	25,6±3,1	39,2±5,6
Rabbits receiving purified cholesterol	36,1±6,1	91,4±12*
Rabbits receiving commercial cholesterol	117±18**	949±120**

Legend. *p < 0.01, significance of differences from control group; **p < 0.01, significance of differences between experimental groups.

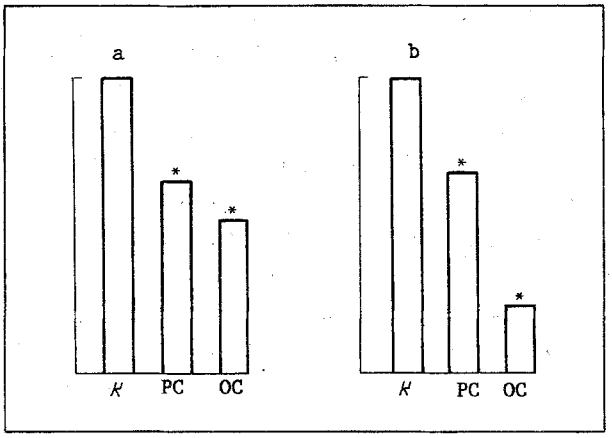

Fig. 1. Specific binding of ^{125}I-labeled β-VLDL (a) and LDL (b) by rabbit hepatocytes. PC) rabbits receiving purified cholesterol with the diet; OC) rabbits receiving old, oxidized cholesterol with the diet. *p < 0.05.

EXPERIMENTAL RESULTS

The plasma cholesterol level of rabbits fed with purified and long-stored commercial cholesterol was 3 and 15 times higher respectively than in the control animals. Similar results were obtained previously [1]. The investigation to determine the lipid composition of the hepatocytes showed (Table 1) that the concentration of free cholesterol and its esters in the hepatocytes of rabbits receiving purified cholesterol was increased (by 1.4 and 2.3 times respectively; $p < 0.05$) compared with the control group, and sharply increased in rabbits receiving old commercial cholesterol (by 4.5 and 24 times respectively; $p < 0.05$).

Specific binding of ^{125}I-β-VLDL by hepatocytes of rabbits receiving purified and long-stored commercial cholesterol was reduced by 20 and 40% respectively compared with the control, whereas binding of ^{125}I-LDL was reduced by 32 and 77% (Fig. 1).

Early investigations showed that in rabbits receiving cholesterol with their diet, accumulation of free cholesterol in the liver leads to inhibition of synthesis of B- and E-receptors of the hepatocytes, which are responsible for the uptake of β-VLDL and LDL by the liver [4]. Expression of receptor activity depends on the cholesterol content in the hepatocytes. In rabbits receiving long-stored commercial cholesterol, containing oxidized derivatives, with their food, the more rapid development of hypercholesterolemia is observed than in rabbits receiving purified cholesterol with their diet. This is connected with a significant increase in the content of free and esterified cholesterol in the hepatocytes [12], leading to a more marked decrease in receptor-mediated uptake of β-VLDL and LDL in rabbits taking long-stored cholesterol with their diet.

LITERATURE CITED

1. S. M. Sogoyan, V. Z. Lankin, L. G. Rodina, et al., Byull. Éksp. Biol. Med., No. 6, 663 (1984).
2. D. W. Bilheimer, S. Eisenberg, and R. I. Levy, Biochim. Biophys. Acta, **260**, 212 (1972).
3. J. L. Goldstein, S. K. Basu, and M. S. Brown, Meth. Enzymol., **98**, 241 (1983).
4. P. T. Kovanen, M. S. Brown, S. K. Basu, et al., Proc. Nat. Acad. Sci, USA, **78**, 1396 (1981).
5. V. A. Kosykh, V. Z. Lankin, E. A. Podrez, et al., Lipids, **2** (1989).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, 265 (1951).
7. S.-K. Peng and C. B. Taylor, Wld. Rev. Nutr. Diet. (Basel), **44**, 117 (1984).
8. J. A. Reese and J. L. Byard, In Vitro, **17**, 935 (1981).
9. R. I. Roth, J. W. Ganbatz, A. M. Gotto, and J. R. Patsch, J. Lipid Res., **24**, 1 (1983).
10. B. Shore and V. Shore, Atherosclerosis Drug Discovery, ed. by C. E. Day, New York (1976), pp. 123-141.
11. G. Schmitz, G. Assman, and D. E. Bowyer, J. Chromatogr., **307**, 65 (1984).
12. G. Schmitz, M. Lenczyk, D. Ord, et al., J. Chromatogr., **307**, 81 (1984).
13. L. L. Smith, Cholesterol Autoxidation, New York (1981), pp. 369-458.
14. B. G. Stone, S. K. Erickson, W. Y. Craig, and A. D. Cooper, J. Clin. Invest., **76**, 1773 (1985).

NEUTRAL α-D-MANNOSIDASE ACTIVITY IN HUMAN GRANULOCYTES

A. L. Vlasova, M. E. Preobrazhenskaya, and N. D. Khoroshko

UDC 616.155.392.8-036.12-07:616.155.3-008.931-074

KEY WORDS: α-D-mannosidase, granulocytes, chronic myeloid leukemia.

Several forms of α-D-mannosidases, playing different functional roles in the processing and degradation of mannose-containing glycoproteins, exist in human and animal cells [6, 15]. In human blood cells two forms of α-mannosidases have been found: acid and neutral [11, 12]. Since the structure of the glycoproteins is modified in various forms of myelo- and lymphoproliferative diseases [9], the study of α-mannosidases, as the chief enzymes involved in degradation of mannose-containing N-bound glycoproteins, is of considerable interest. It was shown previously that in chronic myeloid leukemia (CML) activity of acid α-mannosidase is increased in the myeloid cells, whereas in chronic lymphatic leukemia activity of this enzyme in the lymphoid cells is sharply depressed [2, 4, 5, 10, 13]. Changes in activity of neutral α-mannosidase in these forms of diseases have not previously been studied. It was difficult to determine activity of neutral α-mannosidase because of, first, the great lability of this form of the enzyme [14] and, second, the presence of an acid form, interfering with determination of the neutral form. The study of the distribution of activities of acid and neutral α-mannosidases in healthy human blood cells showed that only the neutral enzyme is present in platelets, whereas both acid and neutral forms are present in erythrocytes and lymphocytes; only the acid form of α-mannosidase was found in granulocytes [11].

The aim of this investigation was to develop a method of determination of neutral α-mannosidase in blood cells and to study the presence of neutral α-mannosidase in mature and immature granulocytes in normal individuals and patients with CML.

Laboratory of Biochemistry and Pathochemistry of Carbohydrate Metabolism, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR. Hematologic Clinic, All-Union Hematologic Scientific Center, Ministry of Health of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 251-254, March, 1991. Original article submitted July 6, 1990.