to some degree reflect the morphological and physiological state of the mast cells confirms our views that the mast cells are heparin producers, reorganizing cellular metabolism and exerting an antistressor and antihypoxic action, and actively influencing coagulation hemostasis [3, 8].

LITERATURE CITED

1. V. P. Baluda, S. I. Chekalina, G. N. Sushkevich, and O. Yu. Tokarev, Lab. Delo, No. 11, 653 (1976).
2. S. A. Georgieva, Heparin [in Russian], Moscow (1973), p. 70.
3. G. A. Zakharov and T. P. Pal'chun, The Microcirculatory and Blood Clotting Systems under Extremal Conditions [in Russian], Frunze (1987), p. 127.
4. B. A. Kudryashov, F. B. Shapiro, and A. M. Ul'yanov, Fiziol. Zh. SSSR, 68, No. 11, 1531 (1982).
5. K. M. Lakin, Current Problems in Hemostasis [in Russian], Moscow (1979), p. 288.
6. F. Z. Meerson, Pathogenesis and Prevention of Stress- Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
7. S. I. Pavlishchuk and N. I. Oleinik, Nauch. Trudy Kuban. Med. Inst., 48, 23 (1975).
8. V. V. Serov and A. B. Shekhter, Connective Tissue [in Russian], Moscow (1981).
9. T. M. Frolova, G. V. Leont'eva, L. A. Appolonova, and Yu. I. Bobkov, Patol. Fiziol., No. 3, 54 (1979).
10. V. I. Khavratovich and V. B. Gavrilov, Kardiologiya, No. 8, 89 (1985).
11. J. Y. Haft, P. D. Krans, and F. J. Albert, Circulation, 46, No. 4, 698 (1972).
12. H. Kammermeier and M. Ober, J. Mol. Cell. Cardiol., 17, No. 4, 371 (1985).
13. H. F. Scully and V. Fevis, Lancet, 2, 718 (1982).

EFFECT OF TEMPERATURE AND Ca^{++} ON INTERACTION BETWEEN HIGH DENSITY LIPROPROTEINS AND EPITHELIAL CELLS OF THE HUMAN SMALL INTESTINE

I. G. Safonova, D. D. Sviridov,
G. B. Men'shikov, and V. S. Repin

UDC 612.33.014.1:577.112.856].06:612.59].085.2

KEY WORDS: high-density lipoproteins; human enterocytes.

High-density lipoproteins (HDL) are responsible for reverse transport of cholesterol from the majority of organs and tissues and supplying it to organs synthesizing steroid hormones [4]. These two functions may perhaps be mediated through a specific receptor for HDL_3. Much evidence in support of the existence of specific binding sites (receptors) for HDL on the surface of many types of cells has been obtained in recent years [1, 5, 12].

The writers showed previously that epithelial cells of the human small intestine (enterocytes) have specific binding sites on their surface for HDL_3, possessing certain properties of a classical biological receptor. Binding of HDL_2 with human enterocytes is characterized by specificity, saturation, high affinity, and reversibility, it is controlled in response to saturation of the cells with cholesterol, and it is accompanied by biological effects: internalization, intensive degradation, and dissociation of particles with the release of cholesterol from the cells and stimulation of cholesterol synthesis [13, 14].

In the investigation described below, the effect of temperature and Ca^{++} on interaction of HDL_3 with enterocytes was studied in order to obtain a fuller idea of the characteristics of the receptors mediating this interaction and to elucidate their differences from receptors for low-density lipoproteins.

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimenteal'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 666-668, December, 1989. Original article submitted March 20, 1989.

0007-4888/89/0012-0000$12.50

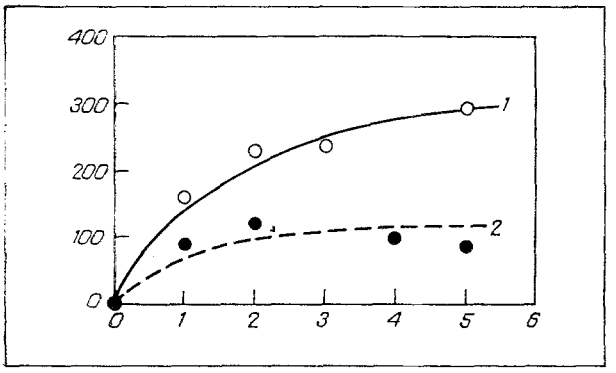

Fig. 1. Kinetics of binding of ^{125}I-HDL$_3$ with human enterocytes at 37°C (1) and 4°C (2). Abscissa, time (in h); ordinate, quantity of ^{125}I-HDL$_3$ bound with cells (in ng/mg cell protein). Here and in Fig. 2 specific binding is shown; each point represents mean value of two measurements.

EXPERIMENTAL METHOD

The small intestine (middle part of the jejunum) of children aged from a few days to 14 years was removed during autopsy, performed not later than 1-2 h after death. To isolate the enterocytes, a hypotonic solution containing 1% polyvinylpyrrolidone was used, by the method described previously [13]. HDL$_3$ (d = 1.125-1.216 g/ml) were obtained from healthy blood plasma by Lindgren's method by successive preparative ultracentrifugation [9]. Their characteristics were published previously [13, 14].

Delipidized serum was produced by ultracentrifugation of fresh pooled blood serum of healthy donors with a density above 1.25 g/ml for 48 h at 105,000g. For the iodination of lipoproteins we used the iodide-monochloride method in D. W. Bilheimer's modification [2].

To study binding of ^{125}I-HDL$_3$ with enterocytes, 0.5 × 10^6 cells were added to incubation medium containing 50 µl of delipidized serum, 3-50 µl of ^{125}I-HDL$_3$, and Eagle's medium (Flow Laboratories, Great Britain) up to a total volume of 0.5 ml. To determine nonspecific binding, the samples were treated with a 20-fold excess of unlabeled HDL$_3$. The mixture was poured into the wells of a "Multiwell" panel ("Nunc") and incubated at 37°C in a CO_2 incubator ("Forma Scientific") with CO_2 concentration of 5% or at 4°C in a cold box, with mixing on an orbital shaker ("Tek-Tator") at the rate of 60 rpm for 3 h. At the end of incubation the mixture was transformed into plastic test tubes. The cells were washed 3 times with 10 ml of Eagle's medium containing 1 mg/ml of bovine serum albumin (Sigma," USA) each time, by centrifugation at 1100g for 5 min at 4°C. The cells were then washed once with 10 ml of phosphate-salt solution ("Flow") without Ca^{++} and Mg^{++}, by centrifugation at 500g for 10 min at 4°C, resuspended in 0.9 ml of 0.05% trypsin–0.002% EDTA ("Flow"), and incubated for 5 min at 37°C. The reaction was stopped by the addition of 100 µl of embryonic calf serum ("Flow"). The cells were sedimented by centrifugation at 1100g for 5 min at 4°C. The supernatant (binding) and residue (internalization) were transferred into gamma-vials and their radioactivity was determined on a gamma-counter ("Compugamma," LKB). Absorption was determined as binding + internalization. Specific binding, internalization, and absorption were determined as the difference between total and nonspecific binding, internalization, and absorption.

EXPERIMENTAL RESULTS

The effect of temperature on the kinetics of binding of ^{125}I-HDL$_3$ with enterocytes is illustrated in Fig. 1. At both 4°C and 37°C binding flattened out on a plateau after 30 min. At 37°C, however, the number of particles bound with the cells was approximately 3 times greater than with binding at 4°C. The results of analysis of dose-dependence of binding of ^{125}I-HDL$_3$ with enterocytes between Scatchard coordinates at 4 and 37°C are given in Fig. 2. The dissociation constant at 4°C was 12.5 ± 2 µg/ml, or (7.4 ± 1.2) × 10^{-8} M, i.e., it was virtually identical with the dissociation constant at 37°C [20 ± 8 µg/ml, or (11.8 ± 4.7) × 10^{-8} M], and the maximal number of binding (B_{max}) was 77 ± 5 mg/mg protein, or (5.4 ± 0.3) × 10^4 binding sites per cell, and it was 15 times less than B_{max} at 37°C [1087 ± 244 mg/mg protein, or (3.3 ± 0.8) × 10^5 binding sites per cell].

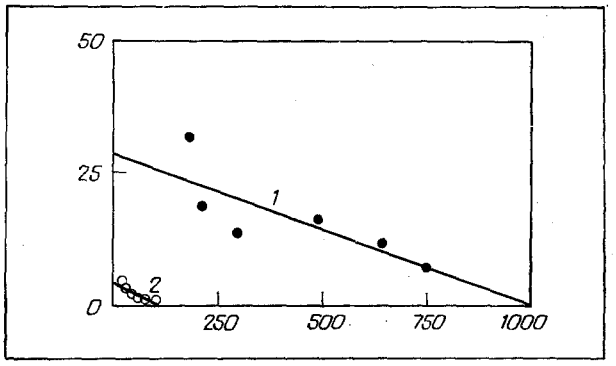

Fig. 2. Dose-dependence of binding of ^{125}I-HDL_3 with human enterocytes at 37°C (1) and 4°C (2), in Scatchard coordinates. Abscissa, quantity of HDL_3 bound with cells (in ng/mg cell protein); ordinate, ratio of bound HDL_3 to free HDL_3 × 10^3.

TABLE 1. Effect of Ions on Interaction of HDL_3 with Human Enterocytes

Experimental conditions	Binding of ^{125}I-HDL_3, % of control	Absorption of ^{125}I-HDL_3, % of control	p
Control	100	100	
EDTA (10 mM)	108	116	>0,05
$CaCl_2$:			
0,5 mM	100	117	>0,05
2 mM	120	125	>0,05
5 mM	122	126	>0,05

Legend. Value of p calculated by Student's t test.

The results are evidence that binding of HDL_3 with human enterocytes depends on temperature. Similar results were obtained on bovine hepatocyte membranes [10]. However, several investigations have shown that the dissociation constant is increased at 4°C, whereas the number of binding sites is unchanged [6] or both parameters of binding are changed [3].

Table 1 shows the effect of Ca^{++} ions and EDTA on binding and absorption of ^{125}I-HDL_3 by human enterocytes. Addition of EDTA and $CaCl_2$ in the concentrations specified to the medium did not lead to any significant change in binding or absorption of ^{125}I-HDL_3 by human enterocytes.

Other investigations have shown that binding of HDL with different cells is independent of concentrations of ions in the medium [3, 7, 11, 12] or that it increases only slightly [6]. In this respect binding of HDL with cells differs from binding of low-density lipoproteins, for which the presence of bivalent cations is essential [8].

LITERATURE CITED

1. R. Bieshroeck, J. F. Oram, J. J. Alpers, et al., J. Clin. Invest., 71, 525 (1983).
2. D. W. Bilheimer, S. Eisenberg, and R. Levi, Biochim. Biophys. Acta, 260, 212 (1972).
3. Y. J. Chen, F. B. Kraemer, and G. M. Reaven, J. Biol. Chem., 255, 9162 (1980).
4. S. Eisenberg, J. Lipid Res., 25, 1017 (1984).
5. N. H. Fidge, P. G. Nestel, and N. Suzuki, Biochim. Biophys. Acta, 753, 14 (1983).
6. J. M. Hoeg, S. J. Demosky, S. B. Edge, et al., Arteriosclerosis, 5, 228 (1985).
7. A. Kagami, N. Fidge, N. Suzuki, and P. Nestel, Biochim. Biophys. Acta, 795, 179 (1984).
8. P. T. Kovanen, M. S. Brown, and J. K. Goldstein, J. Biol. Chem., 254, 11367 (1979).
9. F. F. Lindgren, Analysis of Lipids and Lipoproteins, New York (1975), p. 204.
10. C. M. Mendel, S. T. Kunitake, and J. P. Kane, Biochim. Biophys. Acta, 875, 59 (1986).
11. J. B. Mitchell, V. A. Rifici, and H. B. Eder, Biochim. Biophys. Acta, 917, 324 (1987).
12. L. Ose, I. Rocken, K. R. Norum, et al., Scand. J. Clin. Lab. Invest., 41, 63 (1981).

13. D. D. Sviridov, I. G. Safonova, V. A. Gusev, et al., Metabolism, 35, 588 (1986).
14. D. D. Sviridov, I. G. Safonova, V. P. Tsybulsky, et al., Biochim. Biophys. Acta, 919, 266 (1987).

ANTIOXIDATIVE ACTION OF ANTICATARACT REMEDIES

L. V. Chasovnikova, V. E. Formazyuk,
V. I. Sergienko, and Yu. A. Vladimirov

UDC 615.2.[3.03:617.741-004.1].017:
615.615.272.014.425].076.9

KEY WORDS: anticataract remedies; antioxidative action.

One possible trigger mechanism leading to the development of senile cataract, in the modern view [4], is destruction of components of the lens by active forms of oxygen. Consequently, their antioxidative action may be an important aspect of the pharmacologic activity of anticataract agents.

The aim of this investigation was to determine whether the anticataract remedies widely used at the present time in ophthalmology, and certain other drugs which have not yet achieved popularity, are in fact antioxidants. Oxidative processes can develop directly in the lens, and they may also involve components of the aqueous humor which bathes the lens. Quantity of aqueous humor in the eyes of animals is insufficient for the study of antioxidative properties of anticataract agents. For that reason rat serum, which contained high-density lipoproteins and fatty acids in the form of complexes with serum albumin [6], which are lipid-containing components common to both fluids, was used as the test object.

EXPERIMENTAL METHOD

The reaction system in which control oxidation of the rat serum was carried out consisted of 10 ml of buffer (0.105 M KCl, 0.02 M KH_2PO_4, pH 7.45), 0.4 ml of serum, and 1 ml of 0.05 M $FeSO_4$. The reaction was carried out at room temperature with constant mixing. To determine the antioxidative activity of the therapeutic preparation it was added to the reaction mixture in an amount sufficient to reveal the range of concentrations in which a change in the antioxidative activity of the preparation takes place from maximal effect of complete suppression of the reaction to absence of effect. After fixed time intervals samples of 0.2 ml were taken and mixed with 0.8 ml of 30% TCA. After centrifugation (20 min, 10,000g) 0.8 ml of supernatant was mixed with a 0.5% solution of 2-thiobarbituric acid (TBA) and kept for 1 h at 90-95°C. The level of TBA-active products was determined on a "Hitachi-323" spectrophotometer (Japan) at the absorption maximum of 534 nm [1]. The quantity of total lipids was measured as in [11].

EXPERIMENTAL RESULTS

In the first stage of the work oxidation of rat serum, induced by ferrous ions, was studied. For this purpose, the rate of accumulation of TBA-active products in the reaction system was measured for 2 h. It was found that the kinetic curves characterizing sera of different animals differ very substantially (Fig. 1), as a result of differences in the state of activity of the antioxidative system in the blood of these animals. Characteristically, the wide scatter of the experimental data did not correlate with the very small fluctuations in the content of total lipids, which we determined in parallel tests in the sera of these animals. The total lipid content was 3.5 $\pm$ 0.4 mg/ml. Curves characterizing accumulation of TBA-active products for the same serum were found to remain very close together for 5-7

Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, Moscow.
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 668-670, December, 1989. Original article submitted December 25, 1988.

