EFFECT OF OXIDATION PRODUCTS OF CHOLESTEROL, POLYUNSATURATED FATTY ACIDS, AND THE SYNTHETIC ANTIOXIDANT IONOL ON EXPERIMENTAL HYPERCHOLESTEROLEMIA IN RABBITS

S. M. Sogoyan, V. Z. Lankin, L. G. Rodina, V. N. Ivanov, I. K. Filippov, I. S. Azhgikhin, and A. M. Vikhert

UDC 616.153.922-008.61-092.9-02:615.31:547.922

KEY WORDS: cholesterol and its oxidation products; polyunsaturated fatty acids; ionol; hyper- and hypocholesterolemia.

The development of atherosclerosis is frequently accompanied by hypercholesterolemia [3]. Anichkov's alimentary model [2, 3] is widely used as a model of experimental atherosclerosis, and by means of this model the hypocholesterolemic action of ethyl esters of polyunsaturated fatty acids (PUFA) has been demonstrated [9] and shown to increase with an increase in the degree of their unsaturation [7].

Encouraging results have been obtained in the treatment of atherosclerosis by various combinations of PUFA and antioxidants [4]. The attention of research workers has recently been drawn to elucidation of the role of free-radical peroxidation of PUFA in atherogenesis [5, 6]. During free-radical oxidation of unsaturated acyl residues of phospholipids, cholesterol may be subjected to auto-oxidation, with the formation of several molecular products including hydroperoxides of varied structure [6, 10, 12]. Oxidation products of unsaturated lipids have been shown to contribute to atherogenesis [8, 14]. Under aerobic conditions cholesterol is oxidized spontaneously fairly rapidly; commercial preparations of cholesterol, moreover, usually contain large quantities of its oxidation products [11, 13].

In view of the facts described above, the development of hypercholesterolemia in rabbits under the influence of commercial cholesterol preparations and also of cholesterol preparations subjected to special purification, combined with PUFA and a synthetic antioxidant, was studied.

EXPERIMENTAL METHOD

Male Chinchilla rabbits weighing 3.4 ± 0.2 kg were kept on standard general animal house diet consisting of pelleted kombikorm (a formulation used in animal feeding) and fresh vegetables. Every day the animals were given the test preparations perorally via a tube in the form of solutions in sunflower oil; control animals received an equal volume of sunflower oil (500 mg/kg). The dose of cholesterol was 200 mg/kg and the preparation of ethyl esters of PUFA, obtained by the method of the All-Union Research Institute of Sea Fisheries and Oceanography from lipids of hydrobionts [1], was administered in a dose of 200 mg/kg, and the synthetic antioxidant ionol (2, 6-di-tert-butyl-4-methylphenol; dibunol) was given in a dose of 100 mg/kg. Cholesterol was used in the form of the commercial preparation or was recrystallized from hot ethanol.

At definite time intervals blood samples were taken from the auricular vein of the rabbits and total cholesterol determined in the serum by an enzymic method on an "Olli-3000" analytical system (Finland), using standard kits obtained from "Medix" (Finland). The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

When the commercial preparation of cholesterol was used, a significant increase in the blood cholesterol level (by more than threefold) was observed in the rabbits 1 week after the beginning of feeding, but after 2 months the hypercholesterolemia reached its maximum, which was more than 18 times higher than the control

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 6, pp. 663-664, June, 1984. Original article submitted April 20, 1983.

 0007-4888/84/9706-0724$08.50

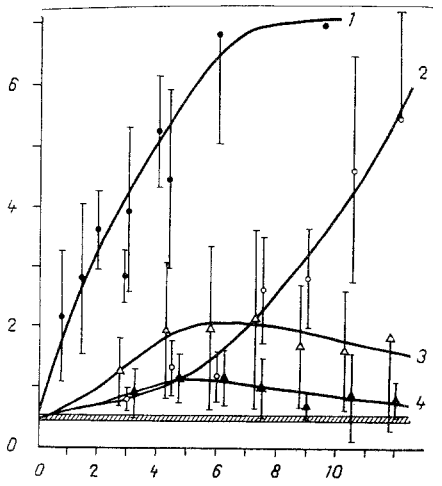

Fig. 1. Effect of various cholesterol preparations, PUFA, and ionol on experimental hypercholesterolemia in rabbits. 1) Commercial cholesterol, 2) purified cholesterol, 3) purified cholesterol + preparation of ethyl esters of PUFA + ionol. Shaded band denotes cholesterol level in intact animals and also in animals receiving solvent (sunflower oil), preparation of ethyl esters of PUFA, or ionol. Abscissa, time (in weeks); ordinate, serum cholesterol concentration (in mg/ml).

(Fig. 1). Feeding rabbits with the recrystallized cholesterol preparation, with a reduced content of its oxidation products, caused lasting hypercholesterolemia much later – 6-8 weeks after the beginning of the experiment; the serum cholesterol level of the rabbits of this group, moreover, remained lower than in animals receiving commercial cholesterol, even 3 months after the beginning of the experiment. The dynamics of the rise of the blood cholesterol level in rabbits receiving purified cholesterol was characterized by a well-marked lag period, of about 1.5 months (Fig. 1). Administration of ethyl esters of PUFA appreciably reduced (by 2.5 times) the hypercholesterolemia evoked by feeding with recrystallized cholesterol, 10 weeks after the beginning of the experiment.

Combined administration of PUFA and ionol suppressed the hypercholesterolemia induced by feeding with purified cholesterol even more strongly; throughout the whole duration of the experiment, moreover, the serum cholesterol level of rabbits of this group did not differ significantly from the control (Fig. 1).

Administration of the solvent (sunflower oil), whether separately or in combination with ionol and PUFA, throughout the course of the experiment had no significant effect on the serum cholesterol level of the rabbits; the blood cholesterol level in animals receiving these preparations, moreover, did not differ significantly from its level in intact rabbits.

The results thus indicate the effectiveness of the hypocholesterolemic action of PUFA, especially in conjunction with the antioxidant ionol. It must also be pointed out that feeding with a commercial preparation of cholesterol, known to contain a certain quantity of its oxidation products, caused a sharp rise in the serum cholesterol concentration of the animals, whereas similar doses of purified cholesterol, freed from oxidation products, caused a much slower rise in the plasma cholesterol level. The impression is created that minor components (probably cholesterol oxidation products) contained in commercial cholesterol have a very significant hypercholesterolemic action.

LITERATURE CITED

1. I. S. Azhgikhin, V. G. Gandel', S. D. Mekhtikhanov, et al., Farmatsiya, No. 5, 18 (1979).
2. N. N. Gorev (ed.), Experimental Atherosclerosis and Age [in Russian], Moscow (1972).
3. A. N. Klimov, in: Preventive Cardiology [in Russian], Moscow (1977), pp. 260-321.
4. V. I. Kalmykova, "Lipid peroxides and antioxidants in the pathogenesis and treatment of atherosclerosis," Author's Abstract of Doctoral Dissertation, Moscow (1978).
5. V. Z. Lankin, Kardiologiya, No. 2, 23 (1976).
6. V. Z. Lankin, Kardiologiya, No. 8, 42 (1980).

7. F. M. Rzhavskaya, Fats of Fish and Marine Mammals [in Russian], Moscow (1976).
8. M. G. Culter and R. Schneider, Atherosclerosis, 20, 383 (1974).
9. S. G. Kahn, J. Nutr., 83, 262 (1964).
10. J. R. Mitton, N. A. Scholan, and G. S. Boyd, Eur. J. Biochem., 20, 569 (1971).
11. S.-K. Peng, C. B. Taylor, P. Tham, et al., Arch. Pathol. Lab. Med., 102, 57 (1978).
12. L. L. Smith and J. I. Teng, Fed. Proc., 33, 1428 (1974).
13. L. L. Smith, W. S. Mathews, J. C. Prise, et al., J. Chromatogr., 27, 187 (1967).
14. K. Yagi, H. Ohkawa, N. Ohishi, et al., J. Appl. Biochem., 3, 58 (1981).

CORRELATION BETWEEN ACTIVITY OF ANTIOXIDANT SYSTEMS AND ENDOGENOUS LIPID PEROXIDATION IN THE LEFT AND RIGHT VENTRICULAR MYOCARDIUM

V. E. Kagan, V. M. Savov,
V. V. Didenko, Yu. V. Arkhipenko,
and F. Z. Meerson

UDC 612.173.1:612.397.2/.014.464

KEY WORDS: lipid peroxidation; superoxide dismutase; glutathione peroxidase; catalase; antioxidant enzymes.

It has been conclusively proved in recent years that activation of lipid peroxidation (LPO) caused by insufficiency of the antioxidant regulatory systems or excessiveness of the systems generating active forms of oxygen and lipid peroxides, plays an important role in injury to the heart muscle during stress [3] and infarction [4]. The harmful action of LPO in the muscle cell is based on inhibition of the work of the ion pumps responsible for creating and maintaining transmembrane gradients of Na^+ and Ca^{++}, and, consequently, for regulating muscle contraction [1]. This suggests that, other conditions being the same, those parts of the heart which contract more rapidly and strongly may also be characterized by greater power of their antioxidant systems, the more efficient utilization of oxygen in oxidative phosphorylation and, as a result, comparatively low intensity of release of active oxygen and LPO products. The left ventricular myocardium is known to contract faster and more strongly than the right ventricular myocardium [5].

In the investigation described below a comparative study was made of activity of antioxidant enzyme systems and the concentration of LPO products in the myocardium of the left and right ventricles in rats.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Lipids were isolated by Folch's method [10]. Accumulation of lipid hydroperoxides (primary LPO products) was assessed by absorption of a solution of the lipids in a methanol: hexane (5:1 v/v) mixture at 232 nm [7], using a Perkin-Elmer (USA) spectrophotometer. The level of LPO end products (Schiff bases) was determined from the intensity of fluorescence of solutions of the lipids in chloroform at 440 nm and excitation at 360 nm [8] on the MPF-1 spectrofluorometer (Hitachi, Japan).

Superoxide dismutase (SOD) activity was estimated by the method in [11] and glutathione peroxidase (GP) activity by Emerson's method in the modification of Lankin et al. [2]. Catalase activity was determined by Luck's method [2].

Laboratory of Physical Chemistry of Biomembranes, Biological Faculty, M. V. Lomonosov Moscow University. Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 6, pp. 664-666, June, 1984. Original article submitted April 1, 1983.

 0007-4888/84/9706-0726$08.50