ATHEROGENIC EFFECT OF THE BETA-BLOCKER PROPRANOLOL EXHIBITED ON THE DE-ENDOTHELIZED RABBIT AORTA

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Beta-blockers are widely used to treat cardiovascular diseases. However, they may have an unfavorable action on the blood lipid composition [6]. Moreover, these preparations, and also the blood serum obtained from patients after taking a beta-blocker, stimulate proliferation and induce accumulation of cholesterol in cultured human aortic cells, i.e., they exhibit atherogenic properties [12]. Data on the effect of beta-blockers on the course of experimental atherosclerosis are few in number and contradictory in nature. The antiatherogenic action of parenterally administered propranolol on experimental atherosclerosis induced in rabbits and monkeys by hypercholesterolemia has been described [1, 5, 14]. In other published investigations no antiatherogenic effect of beta-blockers could be found [7] or the atherosclerotic changes were actually intensified [2]. The principal manifestations of atherosclerosic are lipidoidosis, namely the accumulation of lipids, and fibrosis, or thickening of the intima on account of local connective tissue proliferation. Mechanical damage to the vascular wall can be used as a method of producing a model of fibrosis.

The aim of this investigation was to study the effect of peroral administration of propranolol on the atherogenic properties of the blood serum and the formation of thickenings in the intima of the rabbit aorta induced by de-endothelization with a balloon catheter.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male chinchilla rabbits weighing 3.0-3.5 kg, aged 12-15 weeks, and kept under ordinary animal house conditions on a standard diet. In the experiments of series I blood was analyzed from four rabbits receiving propranolol (Khar'kov Zdorov'e Pharmaceutical Chemical Combine) in a single dose of 6 mg/kg body weight. Samples of blood serum were obtained before the propranolol was given and 1, 2, 3, 4, and 5 h thereafter. Blood serum was added to a culture of mouse peritoneal macrophages, obtained from ascites fluid in BALB/c mice [4]. All the experiments were carried out on the 2nd day of culture. The cells were washed with medium 199, then cultured for 4 h in medium 199 containing 10% of the test serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml of fungiosin (all reagents from Gibco Europe, Great Britain). At the end of culture with the test serum the cells were washed thoroughly with isotonic phosphate buffer. The total intracellular cholesterol level was determined by the method in [10]. In the experiments of series II, the abdominal aorta was de-endothelized by means of a balloon catheter on 16 rabbits anesthetized with pentobarbital. After the operation eight rabbits (the experimental group) received propranolol in a dose of 6 mg/kg. The propranolol was dissolved in 1 ml of distilled water and administered perorally by means of a syringe twice a day (at 10.30 a.m. and 4 p.m) for a period of 3 weeks Rabbits of the control group (n = 8) received 1 ml of distilled water at the same times. Before the operation and at the end of the 1st, 2nd, and 3rd weeks thereafter samples of blood serum were obtained to monitor the total cholesterol level and to determine the atherogenic properties of the serum on a cell culture. The aorta was excised 21 days after de-endothelization

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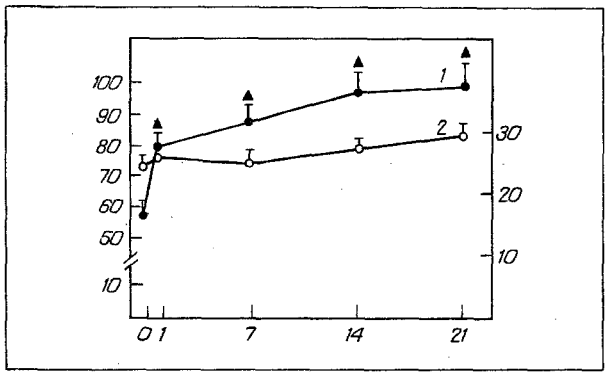

Fig. 1. Atherogenicity of serum and serum cholesterol concentration in rabbits receiving propranolol (n = 8). Abscissa, time of experiment (in days), ordinate: on right — cholesterol concentration in mouse macrophages in culture (in $\mu g/\mu g$ protein); on left — serum cholesterol concentration (in mg %). 1) Atherogenicity of serum; 2) serum cholesterol concentration; filled triangles — significant difference between values of atherogenicity ($p < 0.05$).

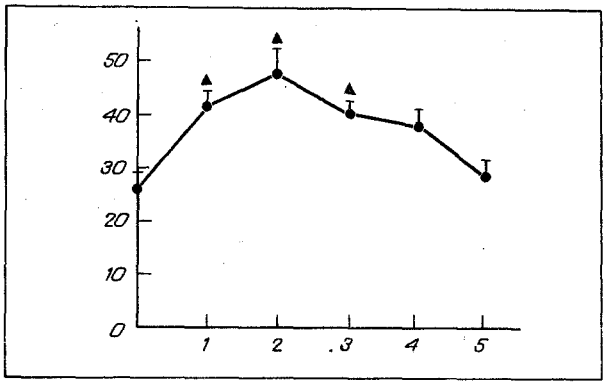

Fig. 2. Atherogenicity of serum from rabbits receiving a single dose of propranolol (n = 4). Abscissa, time of experiment (in h); ordinate, cholesterol concentration in mouse macrophages in culture (in $\mu g/\mu g$ protein). Filled triangles indicate significant difference between values of atherogenicity ($p < 0.05$).

under pentobarbital anesthesia. Part of the aorta corresponding to the zone of injury was used for biochemical and morphological tests. A piece of aorta 5 mm wide, taken from the center of the zone of injury, was fixed in 2.5% glutaraldehyde solution for morphological investigation. The thickness of the media and neointima was measured in semithin sections by means of an ocular micrometer and the intima/media index calculated. The rest of the zone of injury was used for biochemical tests. After mechanical removal of the intima from the aorta lipids were extracted from it by the method in [11]. Separate classes of lipids, namely phospholipids, triglycerides, and free and esterified cholesterol, were fractionated by thin-layer chromatography and determined quantitatively by densitometry [9]. The collagen level was determined as in [13]. Cells were separated from the fixed neointima by alcohol-alkaline dissociation [8]. The total cholesterol concentration in the sera was measured on a Technicon-MAAII automatic analyzer (USA), using a kit from Boehringer Mannheim (West Germany). The significance of differences was estimated by methods of dispersion analysis, using the BMDp package of statistical programs [3].

EXPERIMENTAL RESULTS

Parallel administration of propranolol to the rabbits caused no change in the total serum cholesterol level (Fig. 1). Meanwhile blood serum taken after a single dose of propranolol significantly raised the total cholesterol level in cell cultures

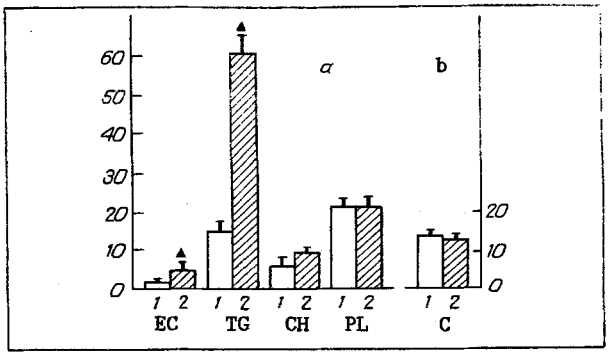

Fig. 3. Concentrations of lipids (a) and collagen (b) in neointima of rabbit aorta. EC) Esterified cholesterol; TG) triglycerides; CH) cholesterol; PL) phospholipids; C) collagen. ordinate: on right — lipid concentration (in μg/mg constant weight), on left — collagen content (in mg/mg constant weight). 1) Control (n = 8); 2) propranolol (n = 8); filled triangles indicate significant difference between values ($p < 0.05$).

(Fig. 2). The atherogenic potential of the serum was manifested 1 h after administration of the drug, in the form of a 100% increase in the intracellular cholesterol concentration, and this continued for 4 h. The maximal effect was found when the serum was tested 2 h after administration of the drug. Blood serum from rabbits receiving propranolol for 3 weeks likewise exhibited atherogenic properties (Fig. 1). In the course of propranolol treatment the atherogenicity of the serum did not change significantly.

Macroscopically, an elevated area could be identified in the zone of injury in all the animals 21 days after the operation. The neointima in rabbits receiving propranolol was twice as thick as that of the control group of rabbits. The intima/media index was 0.8 ± 0.1 and 0.4 ± 0.1 respectively ($p < 0.01$). The content of esterified cholesterol and triglycerides in the aorta of the experimental group of animals was higher than in the control group, whereas the concentration of free cholesterol, phospholipids, and collagen did not differ significantly (Fig. 3). The total number of cells in the neointima of the rabbits receiving propranolol was 1.5 times greater than in rabbits not receiving the drug, namely 2071 ± 2 compared with 1647 ± 3 ($p < 0.05$).

When propranolol was given perorally to the rabbits their blood serum was found to possess atherogenic potential, i.e., it could induce lipid accumulation in cells in culture. In rabbits in which experimental atherosclerosis was induced by deendothelilization of the aorta, a greater increase in the thickness of the intima of the aorta was observed in response to long-term propranolol administration, accompanied by accumulation of lipids in it and an increase in the number of cells.

Beta-blockers are highly effective in the treatment of arterial hypertension and some types of cardiac arrhythmias However, besides their positive action on the clinical manifestations of myocardial ischemia, they act unfavorably on the blood lipid composition [6]. The results of this investigation, relating to the atherogenic properties of the blood serum of rabbits receiving propranolol, are in agreement with the results of experiments with patients' blood serum [12]. Data on the atherogenic effect of propranolol obtained in vivo in rabbits and monkeys kept on a high cholesterol diet are contradictory [1, 5, 14]. However, in the investigations cited the action of the beta-blockers was studied in animals with an intact endothelium and with hyperlipidemia, whereas in the present investigation a different model of atherosclerosis was used, namely de-endothelization of the aorta. The mechanisms of the atherogenicity of propranolol are not clear. It can be tentatively suggested that propranolol acts, on the one hand, on the lipid-transporting system and, on the other hand, directly on cells of the intima. The atherogenic effect of the beta-blocker propranolol both in vitro and in vivo is evidence that long-term treatment with beta-blockers may stimulate the development of existing and induce the development of new atherosclerotic lesions.

LITERATURE CITED

1. V. Chobanian, P. Brecher, and C. Chan, Circulat. Res., **56**, 755 (1985).
2. H. R. Davis, I. F. Mullan, and T. Lichtor, Arteriosclerosis, **5**, 526a (1985)
3. W. J. Dixon and M. B. Brown, Biomedical Computer Programs, P Series, Berkeley (1977), p. 185.
4. J. L. Goldstein, G. K. Ho, S. K. Basu, et al., Proc. Nat. Acad. Sci. USA, **78**, 333 (1979).

5. J. R. Kaplan, Eur. Heart J., **8**, 928 (1987).
6. P. Leren, Am. J. Med., **82**, 31 (1987).
7. P. Lindqvist, C. Olsson, C. Nordborg, et al., Atherosclerosis, **72**, 163 (1988).
8. A. N. Orekhov, E. R. Andreeva, V. V. Tertov, et al., Acta Anat. (Basel), **119**, 99 (1984).
9. A. N. Orekhov, V. V. Tertov, and I. D. Vovikov, Exp. Molec. Path., **42**, 117 (1985).
10. A. N. Orekhov, E. R. Andreeva, A. V. Krushinsky, et al., Am. J. Path., **125**, 902 (1986).
11. A. N. Orekhov, V. V. Tertov, S. A. Kudryashov, et al., Atherosclerosis, **60**, 101 (1986).
12. A. N. Orekhov, M. Ya. Ruda, G. N. Baldenkov, et al., Am. J. Cardiol., **61**, 1116 (1988).
13. H. Stegemann and K. Stalder, Clin. Chim. Acta, **18**, 267 (1967).
14. P. J. Whittington-Coleman, O. Carrier, jr., and B. H. Douglas, Atherosclerosis, **18**, 337 (1973).

Laminaria saccharina EXTRACT LENGTHENS SURVIVAL OF MICE DURING ACUTE EXPOSURE TO COLD

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Hypothermia in man is a widespread pathological process which in some occupations occurs on a massive scale [1-5]. Under the conditions of the Arctic, Antarctica, and the Far North, even the warmest clothing can maintain a positive heat balance in an ambient temperature of below −12°C only for a strictly limited time [1, 3, 9]. Attempts at pharmacologic correction of cooling have been limited in number, and have included administration of sodium bicarbonate in order to counter cold-induce acidosis [8], vitamin C and protein–vitamin concentrates [3], and sydnocarb with glutamic acid [2]. However, we know that animals living in the polar region, the tundra, and the Arctic Ocean have developed in the course of evolution a combination of protective reactions and adaptive substances, regulating resistance to exposure to cold [1, 5]. These substances include natural antifreezes, which are glycoproteins and glycolipids, that enable Arctic fishes to exist with a blood temperature of −2.2°C [9].

The aim of this investigation was to look for agents against hypothermia among preparations isolated from *Laminaria saccharina,* caught in the Barents Sea.

EXPERIMENTAL METHOD

Experiments were carried out on 100 (CBA × C57BL/6)F_1 mice, divided into five groups with 20 in each group. The pharmacologic agents were injected intraperitoneally 30 min before the experiment began, dissolved in 0.1 ml. The model of acute hypothermia consisted of placing the animals in plastic cages measuring 8 × 8 × 8 cm, which were placed in the Minsk-18 cold chamber at −18°C. Every 15 min the number of animals dying in each group was noted. The preparation isolated from *Laminaria saccharina* (LSP) was the batch end product obtained on crystallization of mannitol from the aqueous solution remaining after distillation of ethyl alcohol from an extract of *Laminaria saccharina.* The LSP was a brown opaque liquid with density 1.28-1.40, pH 3.5-5.0, and containing 40-60% of dry substance. Its composition was as follows: amino acids – asparagine

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