PAPAVERINE ABOLISHES THE ATHEROGENIC EFFECT OF THE BETA-BLOCKER PROPRANOLOL

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Beta-blockers are highly effective in the treatment of arterial hypertension and of some types of cardiac arrhythmias. However, although they improve the clinical picture of myocardial ischemia, they act unfavorably on the blood lipid composition [6]. Moreover, these preparations, as well as blood serum obtained from patients after taking a beta-blocker, stimulate proliferation of human aortic cells in culture and induce cholesterol accumulation in them, i.e., they exhibit atherogenic properties [14]. Data on the effect of β-blockers on the course of experimental atherosclerosis are limited and contradictory. An antiatherogenic action of parenterally administered propranolol (PR) on experimental atherosclerosis induced in rabbits and monkeys by hypercholesterolemia has been described [1, 5]. In other studies no antiatherogenic effect of beta-blockers could be found [7], or intensification of atherosclerotic changes was even found [2]. We have shown recently that peroral administration of the beta-blocker PR stimulates growth of the neointima in rabbits with mechanical damage to the aorta [14]. The atherogenicity of beta-blockers, demonstrated at the level of arterial cells in culture, can be abolished if the beta-blocker is used in combination with a calcium antagonist [12].

The aim of this investigation was to study the effect of peroral PR on the atherogenic properties of the blood serum and on the formation of thickening of the intima of the rabbit aorta, and also the possibility of abolishing this effect by a combination of beta-blocker and calcium antagonist (papaverine – PA).

EXPERIMENTAL METHOD

Experiments were carried out on 34 male chinchilla rabbits weighing 3.0-3.5 kg, and aged 12-15 weeks. The animals were kept under ordinary animal house conditions and on a standard diet. In one series of experiments blood was analyzed from nine rabbits receiving a single peroral dose of 20 mg PR (n = 5) or a combination of PR and PA (n = 4) in the same dose (all preparations were from the Zdorov'e Pharmaceutical Chemical Preparations Combine, Khar'kov). PA was chosen for this investigation as a calcium antagonist with moderate antiatherosclerotic action on arterial cells in culture [12]. Samples of blood serum were taken before and 1, 2, 3, 4, and 5 h after its administration. The blood serum was added to a culture of mouse peritoneal macrophages, obtained from the ascites fluid of BALB/s 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 Fungizone (amphotericin) (all the reagents were obtained from Gibco Lurope, Great Britain). At the end of culture with the test serum the cells were vigorously washed with isotonic phosphate buffer. The total intracellular cholesterol level was determined by an enzymic method [13]. In another series of experiments, de-endothelization of the abdominal aorta was carried out by means of a balloon catheter on 25 rabbits under pentobarbital anesthesia. After the operation seven rabbits (group 1) received PR in a sessional dose of 20 mg. Rabbits of group 2 (n = 6) received PA in a sessional dose of 20 mg. Rabbits

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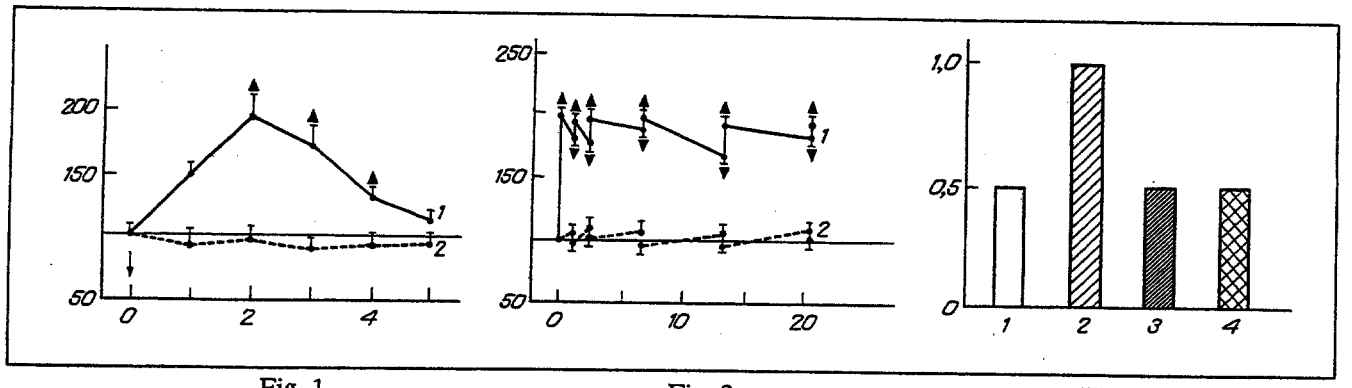

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Effect of papaverine on atherogenic properties of rabbit blood serum evoked by a single dose of propranolol (n = 9). Abscissa, time of experiment (in h); ordinate, cholesterol content in cultured mouse macrophages (in % of control): 1) propranolol (n = 5), 2) propranolol + papaverine (n = 4); triangles indicate significant difference between values of parameters ($p < 0.05$).

Fig. 2. Effect of papaverine on atherogenic properties of rabbit blood serum evoked by repeated administration of propranolol (n = 25). Abscissa, time of experiment (in days); ordinate, cholesterol content in mouse macrophage cultures (in % of control); 1) propranolol (n = 7), 2) propranolol + papaverine (n = 7), triangles) significant difference between values of parameters ($p < 0.05$).

Fig. 3. Thickness of intima. Ordinate, intima/media ratio. 1) Control, 2) propranolol, 3) papaverine, 4) propranolol + papaverine; triangles indicate significant difference between values of parameters ($p < 0.05$).

of group 3 (n = 7) received PR together with PA, in a sessional dose of 20 mg. All preparations were dissolved in 1 ml distilled water and given by the peroral route 3 times a day (at 10 a.m. and 2 and 6 p.m.) for 3 weeks. Rabbits of the control group (n = 5) received 1 ml distilled water at the same times. Before and 1, 2, 7, 14, and 21 days after the operation, samples of blood serum were taken to monitor the total cholesterol level and to determine its atherogenic properties on the cell culture. The aorta was excised under pentobarbital anesthesia 21 days after de-endothelization. The aorta was excised 21 days after de-endothelization, under pentobarbital anesthesia. A region of aorta 5 mm wide, taken from the middle of the zone of injury, was fixed in 2.5% glutaraldehyde solution for morphologic investigation. The thickness of the media and neointima was measured in semithin sections by means of an ocular-micrometer and the intima/media ratio was determined. The rest of the zone of injury was used for biochemical investigations. After mechanical separation lipids were extracted from the intima of the aorta by the method described previously [13]. Individual classes of lipids, namely triglycerides, and free and esterified cholesterol, were fractionated by thin-layer chromatography and determined quantitatively by densitometry [13]. The collagen content was determined by the method in [15]. Cells from the fixed neointima were isolated by alcohol-alkaline dissociation [10]. The total cholesterol content in the sera was measured on a Titertek Multiskan MC apparatus (LKB, Sweden), using a kit from Boehringer Mannheim (Germany). The significance of differences was estimated by dispersion analysis, using the BMDP statistical package [3].

EXPERIMENTAL RESULTS

Peroral administration of PR and of the combination of PR with PA caused no change in the total serum cholesterol concentration. The total serum cholesterol was 34 ± 12 mg %. Meanwhile blood serum taken after a single administration of PR significantly raised the total cholesterol level in the cultured cells (Fig. 1). The atherogenic potential of the serum was exhibited 1 h after injection of the preparation and reached a maximum after 2 h, in the form of a twofold increase in the intracellular cholesterol, which continued for 4 h. The blood serum of rabbits receiving PR for 3 weeks also exhibited atherogenic properties (Fig. 2). This atherogenicity remained at a high level throughout the period of investigation. Fresh doses of the preparation did not significantly increase the atherogenicity acquired after the first dose.

TABLE 1. Effect of Papaverine on Atherogenic Effects of Propranolol in Rabbits (n = 34, % of control)

Parameter	Control	Propranolol	Papaverine	Propranolol + papaverine
Number of cells	100	305±9*	116±4	127±8
Cholesterol esters		573±135*	107±11	94±16
Free cholesterol		244±32*	124±13	95±10
Triglycerides		355±31*	161±38	158±26
Collagen		172±20*	104±8	99±10

Legend. *$p < 0.05$: Significant increase in values of parameters.

The blood serum of a rabbit receiving PR and PA simultaneously did not induce intracellular cholesterol accumulation (Fig. 1). For 3 weeks of regular administration of PR in combination with PA, the rabbits' blood serum was not found to have atherogenic properties (Fig. 2).

Macroscopically, elevation was observed in the zone of injury in all animals 21 days after the operation. The thickness of the neointima in rabbits receiving PR was almost twice that in the control group. When PR was used against the background of PA, thickening of the neointima was virtually the same as the control. In rabbits receiving PA alone the thickness of the neointima likewise was the same as in the control rabbits (Fig. 3).

Compared with the control PR increased the number of cells in the neointima, stimulated accumulation of cholesterol esters and free cholesterol and triglycerides, and also increased the collagen content in the affected region of the aorta (Table 1). PA had virtually no effect on these parameters. On the other hand, PR in combination with PA completely lost its atherogenic properties (Table 1).

The investigation thus showed that when administered perorally, PR causes the appearance of atherogenic properties in rabbits' blood serum. Long-term administration of PR induces stable atherogenicity in rabbits' blood (just as with long-term treatment of atherosclerotic patients [8]), a marked increase in thickness of the intima of the de-endothelized aorta, accumulation of lipids and collagen in it, and an increase in the number of cells. A combination of PR with PA completely abolished the atherogenic effect of beta-blocker.

Atherogenic properties of the blood serum of rabbits taking PR, revealed by the present investigation, agree with data obtained on human patients' blood serum [14]. Data on the effect of PR on the course of experimental atherosclerosis in animals are contradictory [1, 2, 5, 7]. The cause of these contradictions may lie in differences between the models used.

The mechanisms of atherogenicity of PR are not clear. It can be tentatively suggested that, on the one hand, PR acts on the lipid-transporting system, on the other hand, it acts directly on intimal cells. Since we found an atherogenic effect of the beta-blocker PR both in vivo and in vitro, it can be postulated that prolonged treatment with beta-blockers may stimulate the development of existing atherosclerotic lesions and may facilitate the appearance of new ones.

The ability of PA, discovered in this investigation, to completely abolish the atherogenic effect of PR confirms data obtained previously on models in vitro [12]. This action of the combination of PR with PA is not simple addition of the atherogenic effect of PR to the antiatherogenic effect of PA, for PA by itself did not prevent the development of atherosclerotic manifestations in the injured rabbit's aorta.

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