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ANTINECROTIC ACTION OF NATURAL AND SYNTHETIC ANTIOXIDANTS IN MYOCARDIAL INFARCTION DUE TO CORONARY OCCLUSION

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KEY WORDS: myocardial infarct; antioxidants; superoxide dismutase; glutathione peroxidase

Lipid peroxidation (LPO) in mammalian tissue takes place with the participation of antioxidant enzymes, which utilize active forms of oxygen and lipid peroxides (superoxide dismutase, glutathione-dependent lipoperoxidases) and bioantioxidants [4]. Disturbance of the normal regulation of LPO processes in extremal states may lead to the accumulation of toxic lipid peroxidation products in the tissues, which may give rise to oxidation of thiols, inactivation of various enzymes, destruction of biomembranes, and, ultimately, cell death [4]. The writers previously observed a sharp increase in the content of LPO products during ischemic tissue damage [1, 5], the cause of which could be an irreversible decrease in activity of the antioxidative enzymes superoxide dismutase (SOD), glutathione peroxidase (GP), and glutathione-S-transferase [1, 2, 6]. Accordingly, addition of SOD to the cardioplegic solution gives a marked protective effect in myocardial ischemia [13]. Other mechanisms of ischemic tissue damage connected with the accumulation of active forms of oxygen, capable of inducing LPO in biomembranes, also have been discussed in the literature. In particular, a decrease in the oxygen concentration in ischemic cells may lead to an increase in the degree of reduction of pyridine nucleotides, resulting in an increase of the rate of single-electron reduction of oxygen, with the formation of its semireduced form – the superoxide anion-radical [12]. During ischemia proteolytic conversion of xanthine dehydrogenase into xanthine oxidase [12] also takes place, together with stimulation of ATP catabolism, with the accumulation of the substrate of this enzyme (hypoxanthine) [11, 12], and this also promotes an increase in the concentration of active forms of oxygen

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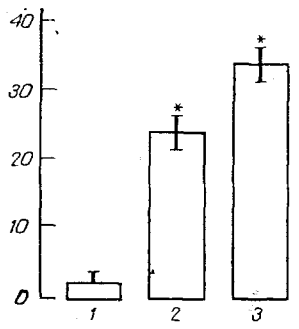

Fig. 1

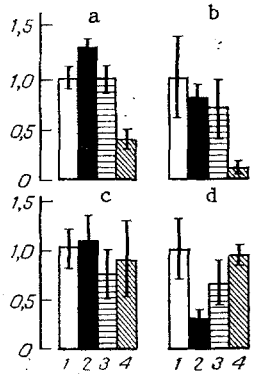

Fig. 2

Fig. 1. Effect of prophylactic administration of antioxidants on dimensions of postinfarct scar in rat myocardium. Ordinate, decrease in size of infarct (in percent of control). 1) SFN-6; 2) dibunol; 3) β-carotene.

Fig. 2. Effect of antioxidants on activity of SOD (a, c) and GP (b, d) in intact (a, b) and damaged (c, d) rat myocardium. Ordinate, enzyme activity (in relative units). 1) Control; 2) dibunol (120 mg/kg); 3) β-carotene (20 mg/kg); 4) SFN-6 (100 mg/kg).

in the ischemic organ. Taking these data into consideration, there can be no doubt about the fact that in ischemia and myocardial infarction, the conditions for intensification of LPO processes are created in the biomembranes of the cardiomyocytes. Attempts to use LPO inhibitors (antioxidants) as cardioprotective and antinecrotic agents must therefore be regarded as fully justified [3, 8]. The favorable effect of administration of antioxidants during ischemia may be connected not only with their direct action, limiting the rate of lipid oxidation in the tissues, but also with their effect on activity of antioxidative enzymes, as our previous experiments showed [7]. When the oxygen concentration of the cells is limited, the efficacy of antioxidants belonging to the carotenoid class ought to be increased because of the increased rate of rupture of the self-oxidation reaction chain on interaction between molecules of the inhibitor and the peroxy radicals of the substrate [10]. This fact suggests definite advantages in the use of carotenoids to protect cells against ischemia damage over phenolic antioxidants, whose effectiveness is reduced in the presence of a low partial pressure of oxygen in the tissues [10]. These considerations necessitate an investigation of the effect of these preparations on activity of antioxidative enzymes in ischemic cardiomyocytes and a comparison of the antinecrotic action of antioxidants belonging to the carotenoid class and sterically occupied phenols in experimental myocardial infarction.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 170 ± 10 g. A model of myocardial infarction was produced by ligation of the left coronary artery under endotracheal ether anesthesia [6]. A mock operation was performed at the same time on animals of the control group: thoracotomy and pericardiotomy, but without application of a ligature. The presence of a myocardial infarct was determined from the characteristic ECG changes in three standard leads and macroscopically by the presence of a zone of necrosis and the appearance of a postinfarct scar. The synthetic antioxidant dibunol (ionol, 2,6-di-tert-butyl-4-methylphenol), permitted for pharmacologic use, and produced by the Institute of Chemical Physics, Academy of Sciences of the USSR, its water-soluble analog — the sodium salt of 4-hydroxy-3,5-di-tert-butylphenyl-phosphonous acid (SFN-6), synthesized in the Department of Organic Chemistry, V. I. Lenin Moscow Pedagogic Institute [9], and the natural antioxidant β-carotene, synthesized at the "Vitaminy" Research and Production Combine, were used. Fat-soluble antioxidants (dibunol and β-carotene) were given to the animals perorally in the form of a freshly prepared solution (dibunol) or a fine suspension (β-carotene) in sunflower oil in doses of 120 and 20 mg/kg, respectively. SFN-6 was injected intraperitoneally in physiological saline in a dose of 100 mg/kg. All antioxidants used in the work were administered in accordance with a common schedule: 24 h before

the operation, 2 h before ligation of the artery, and thereafter daily for 7 days. The animals were killed 7 days after the experiment, the heart was removed and the dimensions of the postinfarct scar were determined, using the histochemical reaction or succinate hydrogenase activity [2]. Quantitative evaluation of the myocardial lesion was carried out by planimetry of sections using the IBAS-1 computerized system for biological image analysis (West Germany). SOD and GP activity in the myocardial homogenate was determined as described previously [2, 6]. SOD activity was measured on a "Hitachi 220A" spectrophotometer (Japan), and GP activity and protein were determined on an FP-901 chemical analyzer (Finland).

EXPERIMENTAL RESULTS

Data on the effect of the antioxidants studied on the dimensions of the postinfarct scar are given in Fig. 1. Injection of the water-soluble dibunol analog SFN-6 in a dose of 100 mg/kg did not lead to any decrease in size of the zone of the postinfarct scar, whereas dibunol in a dose of 120 mg/kg caused a reduction in size of the experimental myocardial infarct in the rats by 24% (Fig. 1). Meanwhile prophylactic administration of the fat-soluble antioxidant β-carotene in a much smaller dose (20 mg/kg) was followed by reduction of the size of the myocardial infarct by 34%. The fact must be noted that in these experiments the water-soluble antioxidant was ineffective, and of the two fat-soluble antioxidants, β-carotene had the strongest cardioprotective action, which might be expected on the grounds that it exhibits its antioxidative activity at low partial pressures of oxygen [10]. When the results are explained it must be remembered that antioxidants, on entering the body, can give rise to significant metabolic changes, as a result of which their protective effect may be attributed not to their direct antioxidative action, but indirectly, for example, to a change in activity of antioxidative enzymes [7]. It was therefore decided to investigate activity of antioxidative enzymes in the intact and infarcted myocardium following administration of antioxidants used to restrict ischemic damage to the myocardium.

The results given in Fig. 2 indicate that SFN-6, injected in a dose of 100 mg/kg, can give rise to a sharp decrease in antioxidative enzyme activity in intact animals, whereas its fat-soluble analog dibunol, in a dose of 120 mg/kg, lowered GP activity only in the infarcted myocardium, by more than two-thirds. β-carotene in a dose of 20 mg/kg, which exhibited the maximal cardioprotective action, at the same time did not change antioxidative enzyme activity in the intact myocardium and did not cause a further decrease in antioxidative enzyme activity in the ischemic myocardium (Fig. 2).

The antinecrotic efficacy of β-carotene revealed by this investigation may thus be linked to a certain degree with the absence of inhibition of the natural protective systems of the myocardium. Moreover, considering that dibunol and β-carotene, while exerting an antinecrotic action in the doses used, did not cause an increase in antioxidative enzyme activity in the infarcted myocardium, the cardioprotective action of these antioxidants is probably mainly attributable to their antiradical activity.

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SYNTHETIC ENKEPHALIN ANALOGS AS ANTIATHEROGENIC AGENTS

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Definite progress in the search for new pathogenetically based methods of treatment of atherosclerosis has been made by the study of endogenous protective systems and their metabolites, which prevent development of the atherosclerotic process. In particular, according to one hypothesis [13], endogenous opioid peptides can be regarded as antiatherogenic factors, and this view is confirmed by data on the hypolipidemic action of enkephalins and endorphins [11].

In the investigation described below, the possibility of using synthetic analogs of opioid peptides as antiatherogenic agents was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male Wistar rats weighing 200-250 g. Hypercholesterolemia was produced by administration of 5% cholesterol solution in a dose of 500 mg/kg body weight by the gastric route through a PVC tube [2]. The animals were decapitated under superficial ether anesthesia 6 h after a single dose and 24 h after the last of 20 daily doses of cholesterol indicated above. The substances for testing were injected intraperitoneally in a dose of 0.1 mg/kg. In acute experiments the preparations were given once, 3 h before the animals were taken from the experiment; in experiments with chronic cholesterol feeding the preparations were injected in the above dose on alternate days. Arginine-containing analogs of Leu-enkephalin (dalargin and DFEN) were obtained from the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Head of Laboratory Professor M. I. Titov). Altogether 52 patients (men aged 42-56 years) with obliterative atherosclerosis (OA) of the lower limbs in stage II-III, with concomitant arterial hypertension, were investigated. All the patients had received the traditional treatment [1, 3]; 24 patients had received dalargin in a dose of 2 mg intravenously daily for 5 days as a hypotensive agent, and the patients of the comparison group (28) had received papaverine and dibazol (2-benzylbenzimidazole hydrochloride). The control group consisted of 25 clinically healthy persons aged 37-50 years. Blood samples were taken in the morning before breakfast, before and 7 days after the beginning of treatment. Plasma parathormone (PTH) levels were determined by radioimmunoassay using kits from "Byk-Mallinckrodt" (West Germany). Radioactivity was counted on a "Tracor" gamma-spectrometer (USA). The blood cholesterol concentration was determined by kits from "Bio-La-Chema" (Czechoslovakia). Serum lipoprotein fractions were separated by electrophoresis in gel, using kits of reagents from "Miles" (USA) and relative percentages of high- (HDL), low- (LDL), and very low- (VLDL) density lipoproteins were calculated. The blood lactate concentration was determined by an enzymic method using kits from

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