LIMITATION OF THE ZONE OF MYOCARDIAL INFARCTION IN RATS WITH CORONARY OCCLUSION BY ANTIOXIDANT THERAPY

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Ischemic damage to the tissues is accompanied by marked activation of free-radical lipid peroxidation (LPO) [3]. Activation of LPO also is observed during the formation of a myocardial infarct (MI) [5]. One cause of the intensification of LPO in infarction may be the decrease in activity of protective "antioxidant" enzyme systems [9], which detoxicate active forms of oxygen and lipid peroxides — superoxide dismutase (DOS), glutathione peroxidases (GP), and glutathione-S-transferases (GT) — in the ischemic and infarcted zone of the myocardium, discovered previously by the present writers [6] and others [2, 10]. A marked antinecrotic affect of natural and synthetic antioxidants has been demonstrated [5, 11].

Considering that antioxidants can exhibit their action *in vivo* indirectly through a change in the activity of "antioxidant" enzyme systems [7], it was decided to study the effect of the synthetic antioxidant dibunol (ionol, 2,6-di-tert-butyl-4-methylphenol), approved for pharmacologic use, on limitation of the zone of MI produced by coronary occlusion in rats and on DOS, GP, and GT activity in the heart muscle.

EXPERIMENTAL METHOD

Experiments were carried out on 100 male Wistar rats weighing 180 ± 20 g. MI was produced by ligation of the left coronary artery 2 mm below the level where it crosses the left border of the infundibulum at its base [4]. Operations were performed under endotracheal ether anesthesia. Ionol was administrered perorally through a tube in the form of a solution in sunflower oil in doses of 40, 80, and 120 mg/kg 24 h before the operation, and again 2 h before application of the ligature and daily for the next 7 days. Rats with MI receiving the solvent alone (sunflower oil, 1 ml/kg) served as the control. The animals were killed on the 7th day after coronary occlusion at the stage of scar formation [6]. The infarct was verified macroscopically by the presence of a zone of necrosis and a postinfarct scar. The presence of ischemia and an infarct was determined by characteristic changes in the ECG (elevation of ST and deepening of the Q wave) in three standard leads. The dimensions of MI were measured by the method [10] in the writers' modification. The heart was cut through the zone of the

TABLE 1. Changes in Size of MI in Rats with Coronary Occlusion Treated with Ionol

Parameter tested	Dose of ionol, mg / kg			
	0	40	80	120
Zone of infarct, % of weight of heart	27,83± ±1,99 (19)	27.38± ±2.6 (12)	23,44± ±1,46 (18)	21,1± ±1,35 (19)
P		>0,1	=0,05	<0,001

Legend. Here and in Table 2, number of animals shown in parentheses; P gives significance of differences from control.

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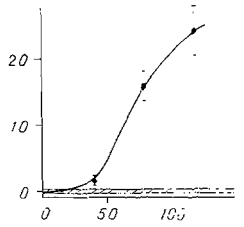


Fig. 1

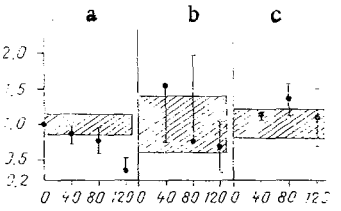


Fig. 2

Fig. 1. Effectiveness of protection of myocardium against infarction after coronary occlusion on dose of ionol. Abscissa, dose of ionol (in mg/kg); ordinate, zone of infarct (in %).

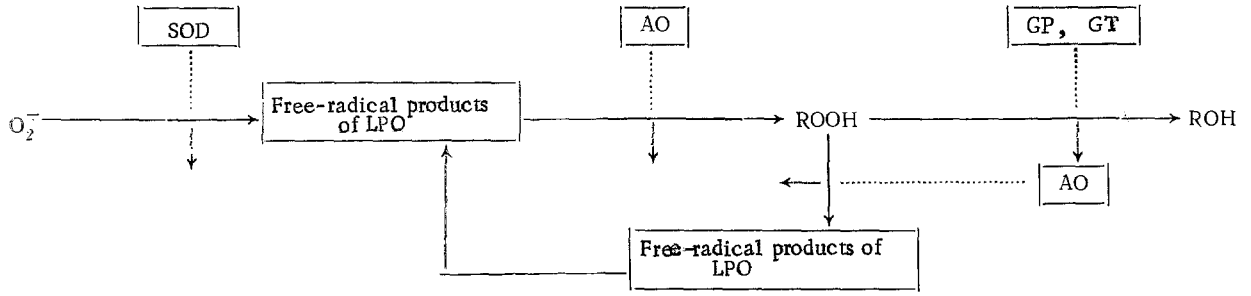
Fig. 2. Effect of dose of ionol on activity of "antioxidant" enzymes in myocardium. Abscissa, dose of enzyme (in mg/kg); ordinate, activity of enzymes (in relative units). a) GP; b) SOD; c) GT. Five animals were used to measure enzyme activity at each experimental point.

infarct into three transfer blocks, each of which was then quickly weighed on analytical scales, frozen on dry ice, and cut into sections 10-20 μ thick in the MK 25 cryostat at −20°C. Succinate dehydrogenase activity was demonstrated histochemically in sections obtained from each block by reduction of nitro-BT [1, 10]; the damaged zone of the myocardium was clearly distinguishable as unstained areas. The preparations thus obtained were projected on paper through a DM 4T epidiascope, and outlines of the section were traced to distinguish the region of the infarct, and then cut out. The percengage of tissue involved at this level of the myocardium was determined by weighing the corresponding projection zones of the section on analytical scales. Using the weight of the heart and the percengage of it involved in the lesion in each of the three blocks, the total weight of scar tissue in the heart was calculated [1, 2]. In parallel experiments the effect of daily peroral administration of ionol in a dose of 100 mg/kg for 7 days in intact rats was studied. The control for this gorup of animals consisted of intact rats and rats receiving the solvent (sunflower oil) only. DOS, GP, and GT activity also was investigated in frozen sections from the myocardium of rats with experimental infarction, receiving ionol in doses of 40, 80, and 120 mg/kg after the size of the zone of infarction had been determined as described above. Samples of heart muscle was homogenized in a glass homogenizer with Teflon pestle in 50 mM Tris-HCl, pH 7.4, centrifuged at 800g for 10 min in a refrigeration centrifuge at 4°C, and the supernatant was kept for 2-3 weeks in liquid nitrogen until the beginning of the investigation. SOD activity was determined as inhibition of reduction of nitro-BT in a xanthine–xanthine oxidase system [12], GP activity as oxidation of HADPH in a coupled glutathione reductase system, using tert-butyl hydroperoxide as the substrate [8], and GT activity was determined as conjugate formation from glutathione with 1-chloro-2,4-dinitrobenzene [13]. SOD activity was measured at 25°C on an Ultraspec 4050 spectrophotometer (LKB, Sweden), taking the quantity of enzyme necessary for 50% inhibition and reduction of nitro-BT under the conditions of determination as the unit of activity. GP and GT activity was measured at 30°C on an FP-901 chemical analyzer (Labsystems, Finland), taking the quantity of enzyme required to oxidize or conjugate 1 μmole of reduced glutathione respectively in 1 min as the unit of activity. The protein content in the enzyme preparations was determined by the microbiuret method, using test kits from Medix (Finland).

EXPERIMENTAL RESULTS

Data showing changes in the size of the experimental MI after injection of different doses of ionol are given in Table 1 and Fig. 1.

As these results show, the infarct was significantly reduced in size after injection of ionol in doses of 80 and 120 mg/kg. Because of the sharp increase in the effect of ionol with an increase in dose, it may be expected that a low therapeutic dose of the compound is 50-60 mg/kg. Considering that LD_{50} for ionol is 1700-2450 mg/kg, and that the maximal tolerated dose is 500 mg/kg, the doses of the compound which we used (80 and 120 mg/kg) can be interpreted as average therapeutic doses, and the possibility cannot be ruled out that the effect of ionol will increase with a further increase in the dose. In experiments in [11] on a similar model of MI in rats, but with a different scheme and method of injection (intra-



Scheme 1

peritoneally) of ionol (120 mg/kg) a greater effect was obtained (almost 50% narrowing of the zone of infarction), and in our opinion this was attributable to the method used by these workers to determine the area of the infarct (dimensions of the zone of infarction on the outer surface of the wall of the left ventricle were estimated on the 2nd and 5th days after ligation by measuring the area of the lesion with a binocular loop and an ocular grid). The method which we used to determine the size of the infarct [10], incidentally gives highly reproducible results and has been extensively used in similar experimental investigations in our laboratory [1].

Our previous investigations showed that ischemia and myocardial infarction cause a decrease in SOD, GP, and GT activity in the affected zone of the myocardium [6]; administration of antioxidants can significantly change the activity of "antioxidant" enzymes in the tissues [7]. It might accordingly be suggested that the protective action of ionol is exerted indirectly, on account of its effect on enzyme systems detoxicating active forms of oxygen and lipid peroxides. Nevertheless, peroral administration of ionol in the doses studied to intact rats or to rats with experimental myocardial infarction either did not change or significantly reduced the activity of GP and GT — enzymes responsible for detoxication of lipid peroxides in the myocardium (Table 2). Meanwhile, SOD (the enzyme responsible for detoxication of the superoxide anion-radical in the myocardium) activity was increased after administration of ionol to intact rats, but was unchanged when ionol was given to animals with an experimental MI (Fig. 2). Incidentally, the level of SOD activity in the heart muscle of rats with an NI due to coronary occlusion was unchanged after administration of ionol in doses (80 and 120 mg/kg) which gave subbtantial protection to the myocardium in our experiments (Fig. 2).

After administration of ionol in doses limiting the area of myocardial infarction, activity of the "antioxidant" enzymes studied in the zone of infarction thus was either unchanged or reduced. The most likely points of application of the action of antioxidants (AO) during free-radical LPO are indicated in Scheme 1.

It will be clear from Scheme 1 that the sites of action of ionol and "antioxidant" enzymes of LPO differ; moreover, if ionol does not affect activity of the "antioxidant" enzymes in the myocardium in infarction, its protective action is due to its antiradical activity. If this hypothesis is correct, it can be submitted that an important contribution to myocardial damage after coronary occlusion is made by an increase in the content of free-radical products in the ischemic and infarcted zone, and for that reason the use of preparations from

TABLE 2. Activity of "Antioxidant" Enzymes (in units/mg protein) in Myocardium of Intact Rats on 7th Day after Daily Peroral Administration of Ionol in a Dose of 100 mg/kg

Dose of ionol, mg/kg	SOD	GP	GT
0	27,7±1,19 (3)	0,28±0,045 (5)	0,014±0,0022 (4)
100	35,2±0,81 (4)	0,22±0,007 (5)	0,004±0,0034 (4)
P	0,05	>0,1	<0,05

the bioantioxidant class to protect the myocardium, in the writers' opinion, is very promising.

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EFFECT OF HYPEROXIA ON THE OXYHEMOGLOBIN DISSOCIATION CURVE AT DIFFERENT AGES

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The widespread use of oxygen therapy in geriatric practice [8] on the one hand, and the frequent absence of a therapeutic effect [15] or even the occurrence of side effects during its use in elderly and old patients [3], on the other hand, necessitate a penetrating study of the effect of oxygen on the senile organism. Investigation of the effect of hyperoxia on the respiratory function of the blood, which plays a major role in the maintenance of gaseous homeostasis of the body, is particuly interesting in this connection. The aim of this investigation was to study parameters of the oxygen transport function of blood in elderly subjects during inhalation of oxygen.

EXPERIMENTAL METHOD

The investigation was conducted on nine clinically healthy elderly subjects (aged 60-74 years) and on nine young subjects (aged 19-32 years) who formed the control group. As the model of hyperoxia, the subjects inhaled a gas mixture containing 95% oxygen and 5% nitrogen for 20 min from the closed circuit of a SG-1M spirograph. The oxygen content in the working

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