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LIMITING THE AREA OF ISCHEMIC NECROSIS BY THE ANTIOXIDANT IONOL

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The fact that the antioxidant ionol limits the area of the zone of necrosis in experimental myocardial infarction was first established comparatively recently [3] and direct confirmation has been obtained more recently [1]. However, these investigations with injection of ionol before or after the creation of an experimental infarct leave a number of questions unanswered. For instance, data on the pharmacokinetics of ionol show that only traces of the antioxidant remain in the body 24 h after its administration [9]. Consequently, the protective effect of ionol in the investigations cited above can hardly be dependent on its preventive action, but is realized mainly after coronary occlusion has taken place and an ischemic focus has been formed, i.e., when virtually no blood flow is present in the ischemic zone, into which significant quantities of antioxidants are unable to penetrate. It is thus not clear how in fact the zone of necrosis is reduced in size. Another very important factor is that the area of the zone of necrosis is usually measured by one of the existing methods: planimetrically or by manual or computerized morphometry.

The aim of this investigation was, first, to compare the action of ionol as an inhibitor of lipid peroxidation in and outside the zone of ischemia, and second, to assess the effect of the method used to determine the area of the zone of necrosis on the results of the investigation by using methods of manual and computerized morphometry simultaneously but independently.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. Experimental myocardial infarction was created by Selye's method by ligation of the descending branch of the left coronary artery. Animals with experimental myocardial infarction were divided into two groups. Ionol was given perorally in olive oil in a dose of 100 mg/kg daily for 3 days before creation of the infarct and 2 and 24 h thereafter. The hearts of animals receiving ionol and, at the same time, hearts of control animals not receiving the antioxidant, were taken 48 h after creation of the experimental myocardial infarct. Biochemical tests were carried out for diene conjugates [5], Schiff bases [6], and activity of antioxidative enzymes, namely glutathione peroxidase, as in [7], and catalase, as in [8]. Serial histotopographic sections through the heart, stained with nitro-BT for succinate dehydrogenase, were used to measure the area of the necrotic tissue. Sections were cut every 1.5 mm, starting from the apex of the heart and in the direction of its base.

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TABLE 1. Effect of Ionol on Concentration of LPO Products and Antioxidative Enzyme Activity in Zone of Necrosis in Experimental Myocardial Infarction

Experimental conditions	Schiff's bases, relative units	Diene conjugates, relative units	Catalase, nmoles H_2O_2/mg·min	Glutathione peroxidase, nmoles NADPH/mg·min
Control (n = 18)	1,00±0,14	1,00±0,08	432±30	207±13
Infarct (n = 18)	4,30±0,19*	2,91±0,24*	198±16*	54±11*
Infarct + ionol (n = 18)	4,18±0,14*	2,79±0,24*	219±23*	65±16*

*Significant differences compared with control ($p \leq 0.05$); n) number of animals.

TABLE 2. Effect of Ionol on Concentration of LPO Products and Antioxidative Enzyme Activity Outside Zone of Necrosis in Experimental Myocardial Infarction

Experimental conditions	Schiff's bases, relative units	Diene conjugates, relative units	Catalase, nmoles H_2O_2/mg·min	Glutathione peroxidase, nmoles NADPH/mg·min
Control (n=18)	1,37 ± 0,11	1,36 ± 0,15	356 ± 23	173 ± 11
Infarct (n=18)	2,46 ± 0,22*	2,43 ± 0,29*	247 ± 17*	97 ± 11*
Infarct + ionol (n=17)	1,41 ± 0,13	1,70 ± 0,25	298 ± 18**	139 ± 10**

*Significant differences compared with control ($p \leq 0.05$).
**Significant differences compared with infarct series ($p \leq 0.05$).
Note. n) Number of animals.

Determination of the relative value of necrotic tissue was based on the Delaisse principle, according to which the fraction of necrotic tissue was calculated by the equation:

$$V_v = \frac{P_i}{P_t},$$

where V_v is the volume of the tissue structure, P_t the number of test points when using the point counting method, P_i is the total number of points corresponding to the necrotic zone.

In the parallel experiments morphometry of the sections obtained by the same method was carried out by automatic image analysis on the Leitz TAS system (West Germany) [4]. The results of measurements of individual sections were averaged in the computer's memory and, after processing by the appropriate program, they were printed out in the form of general parameters for the series of experiments. As a result of computer analysis, a corresponding parameter was obtained, namely the relative volume of necrotic tissue. Differences between the parameters were assessed by Student's test.

EXPERIMENTAL RESULTS

The data given in Table 1 show marked activation of lipid peroxidation (LPO) in the zone of ischemia 24 h after the appearance of the myocardial infarct, manifested as a three-fourfold increase in the content of diene conjugates and Schiff bases and accompanied by a marked decrease in activity of the antioxidative enzymes catalase and glutathione peroxidase. These changes are characteristic of experimental myocardial infarction. It is important to note that administration of a large dose of the antioxidant ionol did not affect activation of LPO or depression of antioxidative enzyme activity in the zone of ischemia.

Table 2 gives the results of determination of the same parameters for parts of the heart located outside the ischemic zone. It shows that weaker, although quite significant activation of LPO was present there: the concentration of LPO products there was increased by 70-80% and activity of the antioxidative enzymes studied was reduced. The

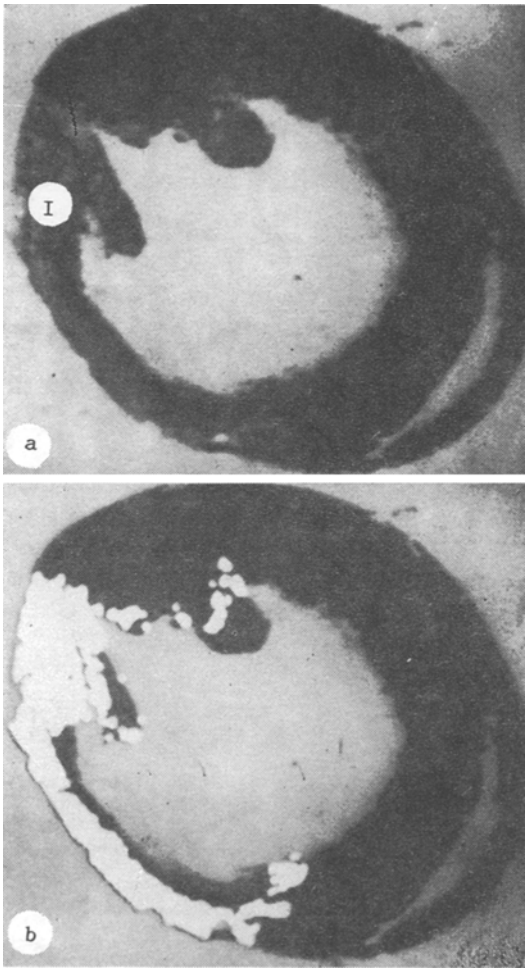

Fig. 1. Comparison of results obtained by point counting method and by computerized image analysis.

TABLE 3. Results of Manual and Computerized Morphometry during Determination of Effect of Ionol on Volume of Necrotic Tissue

Experimental conditions	Volume of necrosis, in % of volume of heart muscle	
	point counting method	method of computerized image analysis
Myocardial infarct	38,5±2,9 ($n = 9$)	48,1±2,8 ($n = 8$)
Myocardial infarct + ionol	28,8±1,4* ($n = 12$)	36,6±1,7* ($n = 13$)

*Significant differences ($p < 0.05$) compared with control; n) number of animals.

antioxidant ionol completely prevented activation of LPO and significantly reduced the degre of depression of antioxidative enzyme activity.

Comparison of the results of these experiments shows that ionol did not exhibit its antioxidative action in the zone of ischemia and necrosis of the myocardium. Outside the ischemic zone, where activation of LPO regularly occurred as a result of the accompanying stressor adrenergic effect and of reoxygenation, which always takes place immediately after a transient disturbance of the blood flow [2, 3], the action of ionol as an LPO inhibitor, on the other hand, was realized to a considerable degree. Bearing this fact in mind, the data in Table 3 on the effect of ionol on the

volume of necrotic myocardium in experimental infarction can be evaluated. The results of morphometry show that the volume of necrotic tissue in the hearts of the animals receiving ionol was 25% less than in the control animals.

Comparison of the results obtained by the point counting method and by computerized image analysis (Fig. 1) shows that they agree qualitatively (Table 3). The volume of the zone of necrosis, determined by computer, was 23-25% greater than during manual counting, the result of the greater sensitivity and objectivity of the automatic method of analysis (its greater efficiency due to the speed of operation of the computer must also be noted).

The initial data showing that ionol limits the area of the zone of ischemic myocardial necrosis [3] are thus confirmed. Meanwhile it can be tentatively suggested that this cardioprotective effect is largely realized in the peri-infarction zone, where ionol inhibits activation of LPO after the initial ischemia has been replaced by reoxygenation, accompanied by the action of high concentrations of catecholamines, which is characteristic of an infarct. This view is in agreement with the known fact that ionol protects the heart against reoxygenation damage [3], and also against damage induced by high catecholamine concentrations. These protective effects, taking place on the boundary of the ischemic zone, lead ultimately to a decrease in size of the area of established necrosis, actually observed in the experiments described above.

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