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EFFECT OF A PEPTIDE PREPARATION FROM THE HEART ON THE ISCHEMIC MYOCARDIUM

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The writers showed previously that cordialin, a polypeptide preparation from myocardial tissue, can limit the size of an ischemic focus [4] and have a beneficial effect on the state of cells in the peri-infarct zone [5] in animals with experimental myocardial infarction. However, to determine whether treatment of a myocardial infarct (MI) with cordialin is indicated, a more detailed study of its effects on the formation of the zone of necrosis and on the various stages of the healing process is essential.

The aim of this investigation was to study the effects of cordialin on mortality of animals with experimental MI, the degree of heart damage, and the time course of healing of the pathological focus.

EXPERIMENTAL METHOD

The possibility of a direct effect of cordialin on physiological and biochemical parameters characterizing the state of the myocardium was studied on isolated hearts. Experiments were carried out on 150 noninbred albino rats and 20 guinea pigs. MI was produced by ligation of the left coronary artery. The size of the zone of necrosis (ZN) in the myocardium was determined by the method in [1]. The size of the unperfused zone (UPZ) was determined by filling the coronary arteries [2]. Cordialin was injected intraperitoneally in a dose of 0.5 mg/kg, 1, 2, and 6 h after coronary occlusion. Fragments of myocardium were fixed in 10% neutral formalin, dehydrated in alcohols of increasing concentration, and embedded in paraffin wax. Paraffix sections 5-7 μm thick, were stained with hematoxylin and eosin and by Van Gieson's method. The guinea pigs' hearts were perfused by Langendorff's method at 30°C and with saturation of the perfusion fluid with carbogen. Perfusion was carried out without stimulation of the heart muscle, at a constant flow rate of 10 ml/min, which was assigned by a peristaltic pump. One hour after the beginning of perfusion, during which the work of the heart became adapted and stabilized, total ischemia was created for a period of 30 min by compressing the tube introducing the solution. The change in the parameters of contraction (frequency and amplitude) was assessed and the malonic dialdehyde (MDA) concentration determined in biopsy material from the myocardium.

EXPERIMENTAL RESULTS

Table 1 shows that by the end of the first day the ZN in animals receiving cordialin was smaller than in the control, but on the second day the dimensions of ZN in the groups compared were identical and did not differ from the control values obtained 24 h after coronary occlusion. One hour after creation of MI total death of the cells was observed in the control group

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TABLE 1. Mortality of Rats and Size of ZN and UPZ during Development of Experimental MI (in per cent of mass of left ventricle)

Substance injected	Time after coronary occlusion, h			
	6	24	48	72
Physiological saline (n = 90)				
ZN	43,5±2,2	55,6±1,7	55,4±2,0	30,0±1,8
UPZ	40,1±1,9	43,6±2,4	45,2±1,6	—
Mortality	29	2	1	1
Cordialin (n = 60)				
ZN	37,4±1,9*	50,0±1,3*	55,6±2,1	34,2±2,2
Mortality	10	1	0	2

Legend. *$p < 0.05$ compared with corresponding values in control; data on mortality of rats given disregarding numbers from previous column; *n*) number of observations.

TABLE 2. MDA Concentration in Biopsy Material from the Heart and Amplitude of Contractions (AC) (in per cent of initial level) of the Isolated Heart

Parameter	Before ischemia	After ischemia and reperfusion, min	
		15	30
MDA			
Control	226	218	—
Cordialin, μg/ml			
0,2	216	233	—
0,5	179*	213+	—
1	197*	256*,+	—
5	160*	274*,+	—
AC			
Control	100	70,4	72,8
Cordialin, μg/ml			
0,2	100	70,7	90,3
0,5	100	85,0	82,0
1	100	63,0	50,7
5	100	19,0	13,3

Legend. Mean values of MDA concentration in cardiac biopsy specimens given. Significance of differences in groups determined by nonparametric tests. Asterisk indicates significant difference compared with control group before ischemia + $p < 0.01$ denotes significance of differences within the group calculated by the "signs" test.

in the zone lying within the region deprived of its blood supply, whereas in animals receiving cordialin, viable cells were still found in UPZ. Thus cordialin leads to an increase in the survival time of cells in a state of hypoxia.

The results are evidence that the definitive formation of a focus of MI in the control group occurred toward the end of the first day, and that cordialin delayed these processes, but without reperfusion it did not limit the size of ZN. Moreover, 3 days after coronary occlusion the relative mass of necrotic myocardium was greater in the group of animals receiving cordialin, possibly indicating slowing of healing processes in the affected zone. Thus on the basis of this morphological analysis of the state of the myocardial region adjacent to ZN, the following conclusions were drawn: whereas during the first and second days an inflammatory process began to take place in the control animals around the periphery of ZN with infiltration of leukocytes, the number of which reached a maximum on the second day, after injection of cordialin the morphological signs of the inflammatory reaction were preserved but reached their maximum somewhat later, namely by the 3rd or 4th day. A fibroblastic reaction occurred in both groups. In the control, however, it began on the 3rd or 4th day, whereas in experiments with cordialin the first fibroblasts differentiated in the wound zone not before the 4th or 5th day. In this case, a rapid transition from young forms of fibroblasts to fibrocytes was observed in the control and the pool of the latter began to predominate rapidly against a back-

ground of intensive collagen formation. In the experiments with cordialin the number of fibroblasts rose progressively but the fibrocytic reaction was weak, and on the 6th day young granulation tissue with many fibroblasts and with only a few collagen fibres was still present.

In our previous publications we gave data showing a less severe degree of damage to cells of the peri-infarct zone in animals receiving cordialin and also higher energy reserves in these cells compared with animals not treated with cordialin on the first day of development of MI [4, 5]. Higher survival rates and longer survival under the influence of cordialin also have been recorded in animals with heart damage due to isoproterenol [3]. It will be clear from Table 1 that the survival rate of animals after coronary occlusion was higher in the group receiving cordialin, especially before 6 h. It can be tentatively suggested that delay of death of the cells in the ischemic zone in the early periods of MI is a positive effect of this preparation.

An important factor in cordialin therapy is its ability to reduce the intensity of the inflammatory reactions, and in principle this can be used in the treatment of MI in the later stages.

During perfusion of the isolated heart the presence of cordialin in the perfusion fluid in doses of 1 and, in particular, of 5 μg/ml causes a negative inotropic effect, which was more marked after ischemia with reperfusion (Table 2). In doses of 0.5 to 5.0 μg/ml cordialin reduced the MDA concentration in the myocardium during perfusion conditions. However, after ischemia with reperfusion the content of LPO products increased with an increase in the dose of cordialin, and was higher than the control level.

Data obtained on the isolated heart agree to a definite degree with the results of a study of development of experimental MI. A feature of both is that cordialin can depress cardiomyocyte function and lower the intensity of LPO processes in the intact and ischemic myocardium. During reperfusion, however, cordialin activates LPO, and this takes place parallel with even stronger inhibition of contractile activity than in the control.

Cordialin was thus shown to increase the survival rate of animals after coronary occlusion. The results of these investigations may serve as experimental proof of the justification of using cordialin on the first day of development of MI. Later, however, administration of the preparation must be stopped or its ability to delay the processes of healing of the necrotic zone must be corrected.

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