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MORPHOLOGY AND PATHOMORPHOLOGY

Proliferative Activity of Cardiomyocytes and Specific Features in the Course of Acute Experimental Myocardial Infarction in Rats with Chronic Alcohol Poisoning

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Vascular mesenchymal changes are less expressed in rats preexposed to alcoholization, and the fibrillogenesis associated with these changes occurs later and is less severe. In rats suffering myocardial infarction in the presence of chronic alcohol poisoning the proliferative activity of cardiomyocytes is reduced.

Key Words: *experimental myocardial infarction; chronic alcohol poisoning; cardiomyocytes; proliferation*

Previously we revealed a depression of manifestations of a regeneratory nature on the part of muscular elements of the heart in both humans and rats with chronic alcoholism [3]. We thought it interesting to study the course of myocardial infarction in rats with chronic alcohol poisoning.

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MATERIALS AND METHODS

Forty-five outbred adult male rats weighing 255-320 g were subjected to semiforced alcoholization with a 15% ethanol solution with a 0.08% tasty saccharine additive [4]. After 20 weeks of alcoholization the thorax was opened under ether mask anesthesia and after removal of the pericardial leaflet the descending branch of the left coronary artery was ligated at the level of the middle third.

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For control 27 male rats weighing 275-310 g were taken in which the descending branch of the left coronary artery was similarly ligated without preliminary alcoholization. The animals were decapitated 1 to 45 days after the operation. The whole isolated heart was fixed in 10% neutral formalin and embedded in paraffin. The resultant slices 6 to 8 μ thick were stained with hematoxylin-eosin, after Van Gieson, with ferric hematoxylin after Heidenhain, Schick's reaction was performed (with amylase control). The activities of some redox enzymes and of acid and alkaline phosphatases were assessed by the azo coupling method.

For assessment of proliferative processes in the heart the counts of binuclear cardiomyocytes per 3000 cells were estimated near the necrosis zone and at a distance from it. Using a screw ocular micrometer the areas of 200 cardiomyocytes, 100 cells per zone, were estimated in the same zones on transverse sections. Quantitative analysis of enzymatic activities was performed with a LYuMAM-R-2 device with an FML-1 photometric adapter and FEU-79A photoamplifier at 1200 V power in arbitrary optical density units at wavelength 680 nm. The reliability of differences in the results was assessed using Student's *t* test.

RESULTS

In the control the morphological pattern of heart injury in the rats conformed in general to the course of experimental myocardial infarction described in literature [1,2]. On the first day extensive areas of necrotic cardiac muscle were observed at the focus of infarction. Hemorrhages and a few neutrophilic leukocytes and lymphoid cells were detected along the margin of the necrotic zone adjacent to the intact myocardial tissue. On days 3-5 the infarction zone was represented by a variety of cellular elements among which neutrophilic leukocytes, red cells, lymphoid cells, and macrophages predominated. Islets of unresorbed necrotic muscular tissue were seen as well as small groups of cardiomyocytes with intact normal structure. On days 7-11 fibroblasts predominated in the zone of infarction and the number of fibrous structures increased. From day 15 on, at first developing and then mature fibrous connective tissue was detected at the site of infarction. As a rule, the infarctions were localized subepicardially and intramurally, and only in isolated animals were they transmural. They involved 8-11% of the left ventricle surface.

The morphological characteristics of myocardial infarction in animals preexposed to chronic alcohol poisoning had some distinguishing features. Measurement of the size of the necrotic area suggested a somewhat smaller zone of infarction in alcoholized animals (6-9% of the left ventricle surface) than in the controls. Foci of necrosis in all the animals were localized under the epicardium and intramurally; transmural infarction was not detected in any of the cases. Vascular mesenchymal changes in the infarction zone were far less pronounced in alcoholized animals. The number of cells per arbitrary square unit was smaller in them, and fibrous structures were detected in the involved focus 3 to 5 days later than in the controls. The

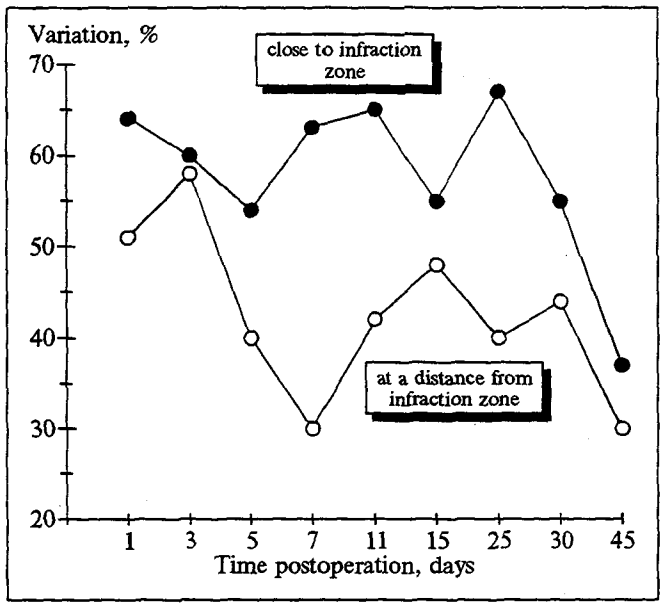

Fig. 1. Number of binuclear cardiomyocytes near to and at a distance from infarction zone in experimental rats.

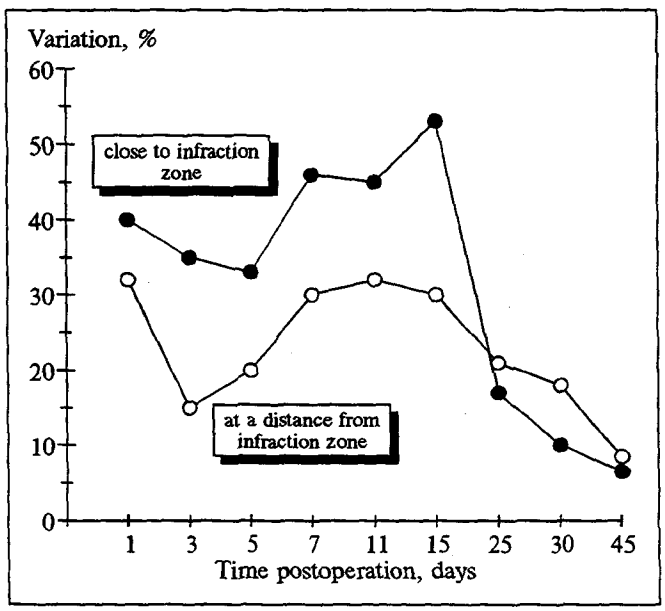

Fig. 2. Number of binuclear cardiomyocytes close to and at a distance from infarction zone in control rats.

infarction seemed to have completely healed in the alcoholized animals 1.5-2 weeks later.

The activity of redox enzymes (succinate, NADH and NADP dehydrogenases) was lower in alcoholized rats than in the controls throughout the experiment both near to and at a distance from the infarction focus. At late stages of infarction healing the same enzymes as in the cardiomyocytes were found in small quantities in the connective tissue developing at the site of dead myocardium in both groups of animals.

Quantitative analysis of cardiomyocyte proliferative activity showed no myocyte mitoses in experimental rats, whereas in control animals 0.25-0.4% of myocyte mitoses were observed mainly in the early periods after injury. Estimation of binuclear cardiomyocytes permitted us to assume that in alcoholized rats they were somewhat more numerous near the necrosis zone than at a distance from it (Fig. 1), but the total count of binuclear cardiomyocytes was higher in controls than in experimental animals (Fig. 2). Measurement of the cardiomyocyte area confirmed the data of visual examination concerning the absence of muscle cell hypertrophy near the focus of injury. In control animals marked cardiomyocyte hypertrophy was observed at the boundary of the focus of injury ($p<0.01$).

Study of the course of acute experimental myocardial infarction in rats preexposed to alcoholization showed less expressed vascular mesenchymal changes in the zone of involvement as against control animals, a fact which was probably reflected in the healing time. Moreover, our results indicate that an experimental myocardial infarction proceeding against the background of chronic alcohol poizoning is associated with a marked reduction of proliferative activity of parenchymatous elements of the heart.

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