GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Comparative Assessment of Heart Remodeling in Rats after Experimental Coronary Stenosis and Cryodestruction

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Comparative analysis of myocardial changes due to heart remodeling after experimental coronary stenosis and cryodestruction in rats was performed. Similar picture of heart remodeling was observed in all animals on day 45 irrespective of the type of destruction: hypertrophy of intact myocardium of the left ventricle, formation of extensive connective tissue cicatrix, and similar structural changes in the myocardium adjacent to the damage area. The type of the damaging influence possibly plays a role at the stage of lesion formation. We concluded that the proposed method of cryodestruction induces heart remodeling comparable to that observed after coronary occlusion.

Key Words: *cryodestruction; coronary occlusion; heart remodeling*

The use of models adequately representing real pathology is an essential part of investigations of pathophysiological processes and protective effects of bioactive compounds and physical factors on the damaged tissues. Coronary stenosis is often applied for modeling acute myocardial infarction and postinfarction heart remodeling in laboratory animals [3,6,11]. This model completely reproduces changes occurring in the heart muscle after coronary catastrophe.

However, coronary stenosis not always fits the aim of the experimental study. This approach does not allow localization of the peri-infarction area at the stage of damage, which is sometimes essential for testing new methods of cardioprotection. For instance, it is currently accepted that timely transplantation of stem cells into the peri-infarction area of the myocardium increases regenerative capacities of the myocardium [2,7]. For this reason, cryodestruction is now used for evaluation of the protective effects of cell transplants [4,5,8,10]. Despite its artificiality, this method provides clear visual control of the demarcation area even during damaging.

However, the possibility of obtaining comparable myocardial changes due to heart remodeling in animals after using these models was not studied.

Here we studied the possibility to obtaining comparable myocardial changes due to heart remodeling after cryodestruction and coronary occlusion.

MATERIALS AND METHODS

Experiments were carried out on 25 male Wistar rats weighting 230-250 g. The animals were kept in convenience with European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The animals were divided into two groups (10 rats per group). In group 1, coronary artery ligation was used. To this end, the animals narcotized

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with ether were fixed on dissecting table, surgical area was prepared, the thorax and pericardium were opened. The heart was taken out of thorax and a ligature was applied onto the upper third of the left descending coronary artery. The wound was treated with antibiotic and sutured layer-by-layer after air discharge from the thorax. The total duration of the whole procedure from thorax opening to last suture did not exceed 4 min.

In group 2, destruction was obtained by applying a massive aluminum rod cooled in liquid nitrogen to the myocardium of the left ventricle (LV). Flat face of the rod, 6 mm in diameter, served as a working surface and was gently applied to the heart surface. The contact lasted 10 sec in all cases; the same heart area was exposed in different animals. These parameters were determined in preliminary experiments and provided good survival of experimental animals. Similarly to group 1, the wound was treated with antibiotic and sutured. All manipulations on animals were performed under sterile conditions using sterile instruments. The operated animals were maintained under vivarium conditions.

After 45 days, the animals of the experimental group and 5 intact animals comprising group 3 (control) were killed by cervical disposition. Body weight and heart weight were determined, the LV was isolated and weighted [1]. In 5 animals from each group, the weight of the damaged myocardium was also determined. The myocardium was stained with nitroyellow tetrazolium [9] for clear visualization of the damaged area in the myocardium. Not stained hearts were histologically examined. LV fragments obtained from the destruction zone and intact area were fixed in 10% neutral formalin. Serial paraffin sections were stained with hematoxylin and eosin. The data was analyzed using non-parametric Mann—Whitney *U* test.

RESULTS

Forty-five days after destruction, heart weight in animals of groups 1 and 2 increased 1.6 fold compared to that in intact animals (Table 1). An increase in LV weight was observed. In should be noted, that the ratio of heart weight increment to LV weight increment was virtually the same in animals of both experimental groups. This suggests that heart hypertrophy was determined by hypertrophy of LV myocardium irrespective of the applied model.

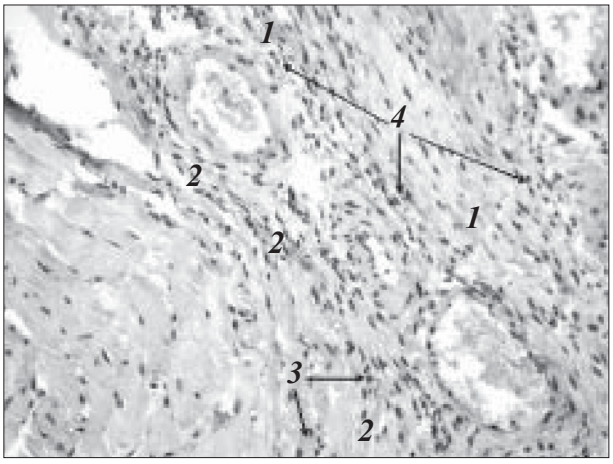

Fig. 1. Morphology of LV myocardium after coronary occlusion in rats. Hematoxylin and eosin staining, ×200. *1*) postinfarction cardiosclerosis area; *2*) peri-infarction zone of the myocardium; *3*) mild mononuclear infiltration in peri-infarction zone; *4*) small amount of mononuclears in post-infarction cardiosclerosis area.

More pronounced differences between the experimental groups were revealed for the ratio of scar tissue formed in the damaged area. Scar zone was compact after cryodestruction, whereas coronary stenosis resulted in more diffuse scar zone. Generally, no significant differences between the groups were observed.

One could expect that differences in nature of damage applied and possible associated peculiarities of the heart remodeling process will manifest in LV myocardium morphology. Since coronary stenosis is more conventional method for modeling of myocardial infarction and postinfarction heart remodeling, myocardium morphology in group 1 was considered as the etalon. By day 45, a focus of sclerosis presented by loose connective tissue was formed in the myocardium; cell elements in the zone of postinfarction cardiosclerosis were presented by fibrocytes and fibroblasts

TABLE 1. Weight Indices in Animals of Control and Experimental Groups (*M*±*m*)

Parameter	Group		
	1 (*n*=5)	2 (*n*=5)	3 (*n*=5)
Body weight, g	242.45±11.17	236.60±3.07	287.00±23.07
Heart weight, mg	1519.27±55.48*	1501.20±20.96*	955.90±44.68
Weight of LV, mg	1035.73±33.21*	1020.20±13.09*	620.50±35.37
Weight damaged area, mg	113.64±1.62	111.10±1.44	0

Note. *$p<0.05$ compared to group 3.

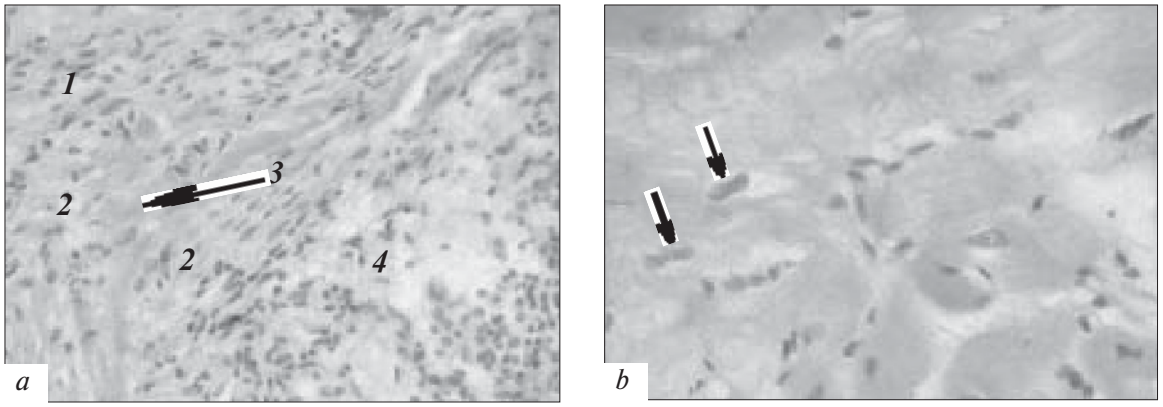

Fig. 2. Morphology of LV myocardium after cryodestruction in rats. Hematoxylin and eosin staining. *a: 1*) postinfarction cardiosclerosis area; *2*) marked dense irregular polymorphocellular infiltration; *3*) focus of destroyed cardiomyocytes (arrow); *4*) serous inflammation in the pericardium, ×200. *b*: marked interstitial edema, irregular cardiomyocyte hypertrophy, mild mononuclear infiltration, peri-nuclear vacuoles in the cytoplasm of individual cardiomyocytes (arrows).

(mean count of fibroblastic elements 45.7±4.6 per field of view), lymphocytes, monocytes, and histiocytes were less numerous (mean number of mononuclear 18.5±3.7 per field of view), newly formed vessels were not observed (Fig. 1). At the periphery of the damaged area, slight edema of the stroma, minor mononuclear infiltration (mean number of mononuclears 26.2±3.1 per field of view), and hyperemia were noted. Along with myocyte dystrophy, their focal atrophy (groups of 2-5 cardiomyocytes in field of view). Irregular hypertrophy of muscle fibers, mild interstitial edema (1.4±0.3 points by 4-point scale), and marked capillary and venous plethora were observed in adjacent intact myocardium.

Microscopy of the heart muscle after cryodestruction revealed damage zone (mean number of fibroblasts and fibrocysts 39.4±5.2, mononuclears 22.4±4.1), its border is clearly defined by cellular infiltration, which forms a demarcation zone between the damaged and intact myocardium (mean number of mononuclears 27.4±2.9 per field of view; Fig. 2, *a*). Microscopy at higher magnification showed that the demarcation zone is characterized by the presence of destroyed cardiomyocytes and cardiomyocytes with preserved structure, the latter formed isolated islets (3-4 per field of view; Fig. 2, *b*). Marked interstitial edema (3.5±0.5 points by 4-point scale) and irregular muscle hypertrophy were observed in the adjacent intact myocardium. It should be noted that newly formed blood vessels were absent in the damaged area after both coronary occlusion and cryodestruction during the studied period.

Thus, our study revealed similar pattern of heart remodeling characterized by hypertrophy of intact LV myocardium, formation of extensive connective tissue cicatrix, and similar structural changes in sites of the myocardium adjacent to the damaged area in animals of the experimental groups on day 45 of the study. The type of damaging agent possibly plays a role at the stage of lesion formation.

Thus, cryodestruction allows investigators to fit the conditions providing initiation of heart remodeling comparable to that after coronary occlusion.

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