Morphometric Analysis of the Lymph System in Rat Heart during Myocardial Infarction

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Simulation of myocardial infarction in random-bred rats showed that lymph vessels play the leading role in the drainage of the myocardium during the acute period of ischemic damage and compensate for the developing insufficiency of interstitial drainage at the expense of venous component of the coronary bed. A pronounced enlargement of the lymph collectors and I, II, and order III vessels in the left and right ventricles of the heart was detected.

Key Words: *experimental myocardial infarction; lymph system; morphometry*

Cardiac lymph system plays an important role in the maintenance of exchange between parenchyma cells and tissue fluid in health and disease [4,8,10]. Subepicardial lymph vessels collect the lymph from all layers of the heart and carry it off from the heart [6,11], and therefore their structure and function can serve as the integral indicator of lymph formation and lymph passage from all heart layers.

Since the lymph removes products of cell metabolism and foreign particles from tissues, the lymphatic bed is directly involved in the elimination of necrotic products during myocardial infarction [12] and some other conditions. Elucidation of the role of the lymph system in the detoxification and regeneration processes will help to develop new methods for active modulation (including drug treatment) of this system and is important for the diagnosis and treatment of myocardial infarction [15].

We investigated the type and severity of changes in the cardiac lymph system in experimental myocardial infarction (EMI).

MATERIALS AND METHODS

EMI was induced in 60 random-bred male rats (150-250 g). The skin was cut along the median sternal line under ether narcosis and the skin flap was prepared for 1 cm. Musculus pectoris major was dissected parallel to the sternum between the median clavicular and sternal lines from rib II to rib VI. The dissected edges of the muscle were fixed with provisory sutures and ribs IV and V together with intercostal muscles were dissected simultaneously along the parasternal line. The heart was exposed and the anteriolateral descending branch of the left coronary artery was ligated after G. Selie under visual control. The heart was placed back into the thorax. The skin and muscles were sutured with continuous suture. The animals were sacrificed on day 7 after surgery by decapitation under light ether narcosis. Sham-operated and intact rats served as controls.

The subepicardial lymph bed was visualized by injection of Gerot blue mass (1-ml Luer syringe, 0.5/16 mm stainless steel needle). Morphotopographic characteristics of subepicardial lymphangions (SEL) were studied under an MBS-10 binocular microscope. The length and width of SEL were measured simultaneously using an ocular micrometer and their volume was estimated using a simplified formula for ellipsoid:

$$V = \frac{1 \times d^2}{2},$$

where l and d are the length and diameter, respectively. We regarded a lymphangion as a portion of the

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lymph vessel between two valves, the peripheral valve belongs to this lymphangion and the central to the next one. Muscle cuff, valvular sinus wall, and valvular shaft were distinguished in the lymphangion.

The lymphangion myoarchitectonics was studied on total preparations of lymph vessels stained with hematoxylin and eosin, azane after Heidenhein, and Weigert resorcin-fuchsin. Total preparations of the lymph collectors and I, II, and order III vessels of both heart ventricles were examined. Smooth muscle cells (SMC) were counted under an MBI-10 microscope using a Stefanov grid.

The data were processed using Biostat software.

RESULTS

Segments of the lymph vessels between valves (lymphangions) are structural and functional units responsible for active lymph outflow from the cardiac walls [1]. In intact albino rats SMC were seen in the muscle cuff starting from order I lymphangions. Depending on the angle of SMC orientation with lymphangion axis, flat, intermediate, and steep spirals orientation was distinguished, which was confirmed by the analysis of azane-stained sections (after Heidenhein). The number of SMC in order I lymphangions varied from 18.0±2.5 to 20±3 depending on their location in the right or left ventricle. The muscular cuff of the lymphangion in order II and III vessels contained 35.0±5.5 and 67±7 SMC, respectively. The muscular cuff of the right-ventricular lymphangions contained 34±4 and 60±6 SMC, respectively.

SEL of the right and left ventricles and left and right collectors contained 20 and 18 SMC in I order lymphangions, 35 and 33 SMC in order II lymphangions, 67 and 60 SMC in order III lymphangions in the left and right ventricles, respectively; 13.0 SMC in the left collector and 120 SMC in the right one. The number of SMC in the muscular cuff tended to increase with increasing the order of lymphatic vessel. Comparison of the right and left ventricles showed an increase in the number of SMC in vessels of the left ventricle, but this difference was statistically insignificant.

Collagen and elastic fibers were detected in all layers of lymphangions. Collagen fibers in the muscle layer tightly adhered to SMC dividing them into bundles and layers. Elastic fibers were arranged in parallel with SMC.

Seven days after EMI modeling necrotic segments of the myocardium in the infarction zone underwent resorption and granulation tissue appeared. Fibroblasts predominated among proliferating cells, new fine argirophilic fibers and small bundles of collagen fibers appeared. In the periinfraction zone degenerative changes in cardiomyocytes were accompanied by hemodynamic disorders.

EMI was associated with a decrease in the number of SMC in the left lymph collector (from 130 to 90 cells per visual field), which was due to lymphangion dilatation. Simultaneously all morphometric parameters characterizing the subepicardial lymphangions increased (Table 1). The diameter and length of order I lymph vessels increased significantly (by 64 and

TABLE 1. Morphometric Parameters of Rat Heart Lymphatic Structures in EMI ($M\pm m$)

Parameter	Intact	Sham-operated	EMI
Length of lymph vessels, mm:			
order I	0.290±0.012	0.35±0.03	0.48±0.01
order II	0.330±0.023	0.46±0.03	0.59±0.02
order III	0.400±0.017	0.54±0.03	0.67±0.02
lymph collector	0.500±0.047	0.57±0.05	0.70±0.05
Diameter of lymph vessels, mm:			
order I	0.140±0.014	0.15±0.01	0.230±0.007
order II	0.18±0.02	0.20±0.02	0.350±0.009
order III	0.20±0.02	0.20±0.02	0.39±0.01
lymph collector	0.24±0.03	0.30±0.02	0.420±0.024
Volume of lymph vessels, mm^3:			
order I	0.006±0.015	0.008±0.002	0.025±0.002
order II	0.010±0.003	0.020±0.004	0.070±0.006
order III	0.020±0.003	0.020±0.005	0.110±0.009
lymph collector	0.04±0.01	0.04±0.01	0.15±0.03

Note. All differences from intact Wistar rats are significant ($p<0.05$).

66%, respectively) in comparison with intact rats. Differences from sham-operated rats were less pronounced (by 53 and 37%, respectively). For order II vessels the same parameters increased in myocardial infarction by 94 and 79% compared to intact animals and by 75 and 28%, respectively, compared to sham-operated animals. The diameters and length of order III vessels increased in myocardial infarction by 95 and 68% in comparison with intact animals and by 95 and 24%, respectively, in comparison with sham-operated ones. The diameter and length of the lymph collector increased in EMI by 75 and 40% compared to intact and by 40 and 23% compared to sham-operated animals, respectively.

The volume of SEL increased significantly by 317% for order I vessels, by 600% for order II vessels, and by 450% for order III vessels in comparison with the corresponding parameters in intact rats and by 212, 250, and 450%, respectively, in comparison with sham-operated rats. The volume of lymph collector increased by 275% in comparison with intact and sham-operated rats.

These data indicate that lymph vessels play the key role in the drainage of the myocardium during the acute period of myocardial infarction and compensate for impaired drainage of interstitial liquid via coronary veins.

Disturbances in coronary circulation after ligation of the coronary artery affect cardiomyocytes and capillary bed, are associated with massive necrosis of cardiomyocytes, capillary endotheliocytes, and remodeling of the interstitium in the infarction zone, which determine the general processes of postinfarction myocardium remodeling [14]. Edema is a key phenomenon of the structural and functional rearrangement of the myocardium under conditions of EMI. Pronounced and persistent interstitial, intra- and perivascular edema promotes, together with other factors, the development of myocardial and vascular fibrosis. It was shown, among other things, that the development of myocardial edema in the left and right ventricles after pulmonary artery ligation in rats led to hyperproduction of types I and III collagen by fibroblasts [7]. All these events promote the development of alterative heart failure [3,9].

Long-term lymphostasis, in turn, is a pathogenetic factors leading to the development of coronary arteriopathy [13]. Blockade of lymphatic drainage causes subendothelial and interstitial edema, plasma impregnation of arterial walls, SMC degradation, dilatation of lymph vessels, and eventually fibrosis of interstitial connective tissue and vascular walls. Accumulation of cell degradation products and toxic metabolites in the lymph during EMI impairs endotheliocytes of lymph capillaries [5], which lead to their destruction and eventually to decompensation of the lymphatic drainage system [2].

Hence, the lymph vessels play the key role in draining of the myocardium during the acute period of infarction. Disorders in lymphatic draining promote damage to coronary vessels and decelerate regeneration of the parenchymal and stromal cells in the periinfarction zone.

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