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## MORPHOLOGICAL AND MORPHOMETRIC CHANGES ON THE BACKGROUND OF CELL CARDIOMYOPLASTY IN EXPERIMENTAL MYOCARDIAL INFARCTION

S. Estrin, T. Kravchenko, A. Pechenko

**The aim:** to study the morphological and morphometric changes in the myocardium against the background of cellular cardiomyoplasty in experimental myocardial infarction.

**Materials and methods:** the experiment was carried out on 142 Wistar-Kyoto rats weighing 200–220 g, which were kept in the vivarium of the Department of Experimental Surgery of the State Institution “Institute of Emergency and Reconstructive Surgery named after V. K. Gusak of the National Academy of Medical Sciences of Ukraine” in the period from 2012 to 2013. The Wistar-Kyoto breed was used because it is inbred, which minimizes the rejection reaction, due to its genetic homogeneity. The animals were kept in a vivarium under conditions of 12-hour daylight hours, room temperature and access to water and food at libitum at an air temperature of +20 – + 22 °C, humidity no more than 50 %, in a light mode - day-night. The use of animals in the experiment was carried out in accordance with the rules regulated by the “European Convention for the Supervision and Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986), Directives of the Council of the European Union of November 24, 1986 and the order of the Ministry of Health of Ukraine No. 32 dated 02.22.88. The induction of myocardial infarction (MI) was carried out according to the technique developed by us under general anesthesia. A separate group consisted of 20 males, whom we used as donors of mesenchymal stem cells (MSC) for further research on the Y chromosome of cell homing in the body. Cultivation of MSCs was carried out in a mixture of nutrient media DMEM / F12, 1:1, (Sigma, USA). The material for morphological studies was the sections of the myocardium of laboratory animals. To assess the morphometric parameters, histochemical methods were performed according to the recipes, which are given in the instructions for histochemistry. Immunohistochemical study was performed on paraffin sections with a thickness of 5-6 µm by the indirect Koons method according to the Brosman method (1979).

**Results:** it was found that cellular cardiomyoplasty significantly improves the structure of the postinfarction heart, manifests itself in a decrease in the scar area and connective tissue, respectively, in an increase in the number of vessels and the percentage of preserved muscle fibers. The best results were achieved with intramyocardial injection, which requires confirmation of this fact in a clinical study.

**Conclusions:** cellular cardiomyoplasty with any method of introducing a cell graft has a positive effect both on the morphological substrate of the heart in the form of a decrease in the size of the scar during postinfarction remodeling, an increase in the number of newly formed vessels and an increase in the percentage of preserved cardiomyocytes. This occurs due to the homing of MSCs into the ischemic zone and the commonality of two mechanisms – direct differentiation into endothelial cells of the heart vessels, as well as due to the paracrine effect

**Keywords:** cellular cardiomyoplasty, experimental myocardial infarction, heart failure, stem cells

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### 1. Introduction

Coronary heart disease (CHD) ranks first among cardiovascular diseases in the frequency of complications and the number of fatalities. In the United States, it is the cause of one in five deaths. In Ukraine, CHD is diagnosed in approximately 400,000 patients annually [1–3]. Traditional treatments for this category of patients that exist are drug therapy, direct myocardial revascularization, and heart transplantation. Existing drug therapy is usually insufficiently effective in preventing myocardial remodeling processes [4].

There is a large group of patients who for one reason or another (distal occlusion, high risk of surgery, technical problems) can not perform direct myocardial revascularization. In addition, there is a group of patients with reversible, progressive angina in whom coronary

artery bypass graft surgery has already been performed and reoperation is not possible. There are also a number of unresolved issues regarding heart transplant donors, ethical aspects that significantly reduce the possibility of timely heart transplantation [5].

Research in the field of stem cell biology (SC) has radically changed all ideas about the regenerative abilities of the myocardium and marked the beginning of a new therapeutic direction – cellular cardiomyoplasty, which aims to replace damaged cardiomyocytes by implanting autologous bone marrow SC [4]. In recent years, this procedure has been introduced into clinical practice to improve the treatment outcomes of patients with ischemic myocardial dysfunction.

Bone cell transplantation is considered by many experts as a potentially promising therapy for patients with

chronic CHD. The results of recent studies have shown a reduction in angina symptoms, increased myocardial perfusion and improved myocardial contractile function.

However, many fundamental questions of cell therapy remain open: the mechanisms of homing, differentiation and engraftment of transplanted SC, the role of cell fusion and the mechanisms of influence of transplanted cells on the function and metabolism of the heart muscle. The most effective way to deliver cells to the myocardium also remains the subject of discussion [6].

**The aim** – to study morphological and morphometric changes of the myocardium on the background of cellular cardiomyoplasty in experimental myocardial infarction.

## 2. Materials and methods

The experiment was performed on 142 Wistar-Kyoto rats, weighing 200–220 g, which were kept in the vivarium of the Department of Experimental Surgery of the State Institution “Institute of Emergency and Reconstructive Surgery named after V. K. Gusak of the National Academy of Medical Sciences of Ukraine” in the period from 2012 to 2013. The breed Wistar-Kyoto was used because it is inbred, which minimizes the reaction of rejection, due to its genetic homogeneity.

Animals were kept in the vivarium under conditions of 12 hours of daylight, room temperature and access to water and food at libitum at air temperature +20 – +22 °C, humidity not more than 50 %, in light mode – day and night. The use of animals in the experiment was carried out in accordance with the rules regulated by the “European Convention for the Surveillance and Protection of Vertebrates Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986), Directive of the Council of the European Community of 24.11.86 and 32 of 22.02.88, the protocol of the experimental study was approved by the local ethics committee for medical and biological ethics of the State Institution “Institute of Emergency and Reconstructive Surgery named after V. C. Gusaka of the National Academy of Medical Sciences of Ukraine”, Donetsk, Ukraine (protocol No. 7, 2012) and corresponds to the Helsinki Declaration of the World Medical Association. Surgery was performed in an experimental operating room under ketamine anesthesia (12.5 mg/100 g body weight intramuscularly).

Induction of myocardial infarction (MI) was carried out according to the method developed by the authors under general anesthesia. In the position of the animal on its back, a sternotomy and pericardiotomy were performed, after which the anterior interventricular artery was sutured and ligated. The sternotomy wound was sutured in layers. During the operation, the body temperature of the animals was maintained at 37.0±0.5 °C due to an external heat source. 22 animals died in the first hours after modelling the pathological condition as a result of the development of life-threatening arrhythmias. Thus, the experimental study was performed on 120 animals, which were divided into 6 groups (20 in each series). The five study groups included females (20 in each series).

A separate group consisted of 20 males, which were used as donors of mesenchymal stem cells

(MSC) for further study on the Y-chromosome of homing cells in the body. Of the five groups: in the 1st group did not carry out any treatment, in the 2nd performed “empty” injections into the myocardium in the area of the ischemia zone, which was determined macroscopically, in the 3rd group performed injections of MSC in a dose of 10 million cells also in the myocardium in the area of the ischemia zone, in the 4th group MSC was administered intravenously at the same dose by puncture of the caudal vein, in the 5th group MSC was injected into the left ventricular cavity (LV) by puncture and catheterization right femoral artery (thus trying to create the maximum concentration of SC in the mouth of the coronary vessel).

MSC cells were obtained from the bone marrow of animals with the addition of 625 IU / ml heparin (Darnytsia, Ukraine). Cultivation of MSC was performed in a mixture of nutrient media DMEM/F12, 1:1, (Sigma, USA) with the addition of 10 % fetal calf serum (Biolog, Russia), 0.75 mg/ml glutamine (Institute of Polio and Viral Encephalitis, Russia), 2 ng/ml of the main fibroblast growth factor (Sigma, USA) and 100 IU / ml of penicillin and streptomycin (Darnytsia, Ukraine) in a CO<sub>2</sub> incubator (Jouan, France) at 37 °C and 5 % CO<sub>2</sub> atmosphere. The medium was changed every 3–4 days of cultivation. Cultures reached the primary monolayer at 8–11 days of cultivation, depending on the seeding density of the initially isolated cell suspension, individual characteristics of donors and the level of proliferative activity of cells. Passage was performed using a mixture of solutions of trypsin/EDTA (Biolog, Russia) in a ratio of 0.05 %: 0.02 % in FSB, pH 7.4 (Sigma, USA). The passage rate was 1:2 or 1:3. The cells were then cultured in a CO<sub>2</sub> incubator under the same conditions. As a result of these manipulations received uncommitted cell culture MSC.

The material for morphological studies were areas of the myocardium of laboratory animals. Histological examination was performed by microscopy with magnification ×100–×400 with preliminary staining of histological specimens with hematoxylin and eosin in order to characterize the changes in the myocardium. The material was fixed in 10 % neutral formalin, after which it was subjected to alcohol wiring and paraffin pouring, sections with a thickness of 5–6 μm were made. Review drugs stained with hematoxylin and eosin were used to assess the overall condition of the tissues studied. Staining of preparations with fuxelin on elastic fibers according to Weigert's addition with picrofuxin staining by the method of Van Gizon, as well as Mallory staining were used to identify and differentiate connective tissue structures.

To assess the morphometric parameters performed histochemical techniques according to the regulations, which are given in the instructions on histochemistry. For quantitative analysis of the condition of the scar, we performed a morphometric study. As at scarring of a myocardial infarction there is a decrease in its size, first of all determined degree of contraction of the MI center. To do this, determine the outer and inner diameters of normal myocardial tissue bordering the site of infarction.

Thus, the theoretically possible area of the myocardium in the total section was calculated by the formula:

$$S = \pi (R_2^{\text{external}} - r_2^{\text{internal}}).$$

Immunohistochemical study was performed on paraffin sections, 5–6  $\mu\text{m}$  thick by the indirect method of Koons according to the method of Brosman (1979).

Statistical processing of data from the results of the experimental study was performed using a licensed software package Microsoft Excel 2010, Statistica 6.0. The Shapiro-Wilk test (W) was used to check the distribution of data for normality, which allowed to use it even with a small sample ( $n < 30$ ). To identify significant differences between the average values of different sets of comparable groups used methods of variation statistics using Student's *t*-test with Bonferroni correction for multiple comparisons with a probability of error of the first kind  $p = 0.05$ . Data were considered reliable at  $p < 0.05$ . Correlation analysis was used to detect the presence of a statistical relationship between a pair of traits.

### 3. Research results

Experimental modelling of acute MI in combination with modern and classical research methods is indispensable in solving the problem for understanding the pathomorphophysiological mechanisms of this disease in humans and developing new ways to treat it. Ligation of the anterior left interventricular artery in laboratory animals led to the formation of successive changes that resemble the picture of acute MI in humans. By the end of 1 day of modelling of acute MI in the area of ischemia, all the signs characteristic of the stage of alteration in the inflammatory reaction, accompanied by swelling and edema of cardiomyocytes, with the first signs of dystrophy and initial degenerative signs. Cardiomyocytes began to lose their transverse delineation, in the interstitial tissue and between the muscle fibers appeared cellular infiltrates containing neutrophils, monocytes and lymphocytes. Blood cell stasis, hemorrhage and leukocyte infiltration were observed in the vessels of the microcirculatory tract. Clear boundaries of necrosis in this period were not observed.

Up to 3–4 days there was a continuation of the classical inflammatory reaction in the form of infiltration of the damaged area by tissue macrophages, leukocytes and lymphocytes, also the formation of granulation tissue in the form of a barrier around the damaged area, where there were myocytolysis. In Fig. 1 shows fresh coagulation myocardial necrosis with severe perifocal inflammation on day 3 of the experiment.

Simultaneously, there was increased activity of the stromal component in the form of proliferation of stroma cells and activation of endothelial cells. There were phenomena of scar formation in the form of collagen synthesis without the formation of collagen fibers. Short thick collagen structures were oriented correctly. MI progressed as a result of peripheral myocytolysis, which significantly expanded the affected area. In the extrinfarction zone there was edema of cardiomyocytes and the formation of erythrocyte sludge in the capillaries.

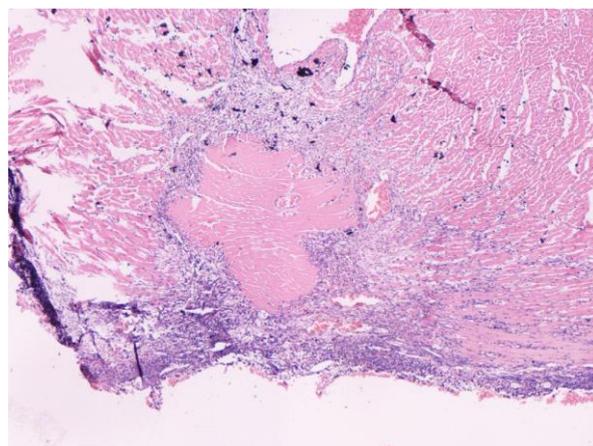


Fig. 1. Coagulation necrosis of the myocardium in rats without treatment for 3 days after ligation of the anterior left interventricular artery. Staining with hematoxylin and eosin,  $\times 10$

Up to 7 days after modelling of the pathological condition, the organization of the infarction was determined. In the area of damage, there were individual islands of cardiomyocytes that underwent myocytolysis. The process of infiltration decreased, in the area of damage there were much fewer macrophages, leukocytes, lymphocytes than for 3–4 days. Muscle cells began to be resorbed, apparently due to phagocytosis and secretion of lysosomal enzymes. The initial stages of connective tissue formation in the damaged area were observed. Connective tissue was formed in the stratum, multiple young thin-walled vessels and fibroblasts were found. It should be emphasized that the proliferative reaction of the connective tissue occurred at a time when instead of granulocytes in the myocardium began to dominate mononuclear cells (monocytes, macrophages and lymphocytes). Collagen fibers were constructed in the central areas of the necrotic region. Outside the lesion area, functional load and intracellular edema of cardiomyocytes were observed.

By day 21, the infiltration process ended and tissue macrophages, leukocytes and lymphocytes were detected only in the perivascular space. In the area of damage there was an active formation and structuring of scar connective tissue, collagen fibers and a small number of individual elastic fibers. A large number of sinusoidal vessels with a thin, easily stretched wall were noted in the connective tissue. Pathological processes involved not only the area of necrosis, but also cardiomyocytes in the border area, where there was intercellular edema. The edema was provoked by a “no reflow” situation, when the blood flow was disturbed in the area outside the ligation, as the vessels were compressed by cardiomyocytes. In this case, there is a high probability of recurrent heart attack in the intact area.

By 30–35 days in the area of necrosis scar tissue was formed. The connective tissue contained sinusoidal vessels. Signs of progressive peripheral myocardial damage were observed in the border zone: infiltration, activation of the stromal component, for-

mation of granulation tissue. In the extrainfarction zone there was a functional load and edema of cardiomyocytes. In some cases, perivascularly located islets of muscle fibers were observed.

Thus, in animals without treatment, a large scar was formed, which spread to all layers of the myocardium and its properties resembled the picture of transmural MI in humans (Fig. 2).

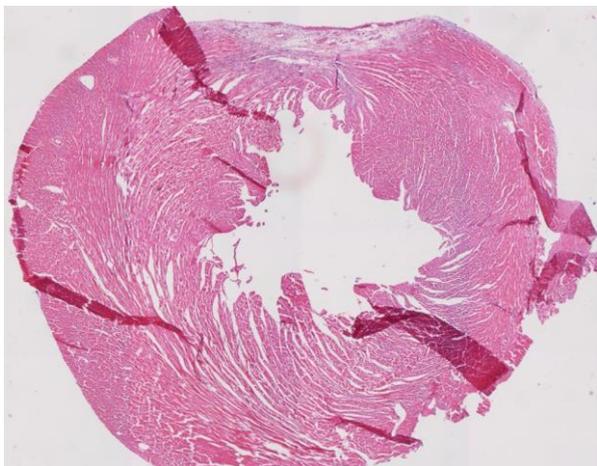


Fig. 2. The scarred area of the myocardium at the apex of the left ventricle on day 30 of the experiment in rats without treatment. The big scar with formation of an aneurysm is defined. Staining with hematoxylin and eosin,  $\times 5$

Due to the fact that experimental MI in rats was accompanied by surgery in the form of stitching and ligation of the coronary vessel, some animals had pericarditis as a result of pericardial incision and aseptic inflammation as a reaction to sterile suture material at the ligation site. Accordingly, if the epicardial layer of myocytes got into the area of stitching, coagulation necrosis was observed in the area of the ligature.

Immunohistochemical staining for actin and troponin T most vividly visualized the total death of muscle fibers in the scar area. On day 30, the wall of the scarred area was completely represented by connective tissue. At the same time in all terms found proliferating cells of a connecting fabric and a vascular wall that testifies that for this term processes of incomplete regeneration and remodelling still proceed (Fig. 3–5). Also attracted the attention of many sinusoidal vessels in the early post-infarction period, which later acquired a wall, but nevertheless their lumen remained wide.

During histological examination after cell cardiomyoplasty, we studied the amount of connective tissue, blood vessels and their qualitative characteristics, as well as the percentage of preserved myocardial mass and MSC homing.

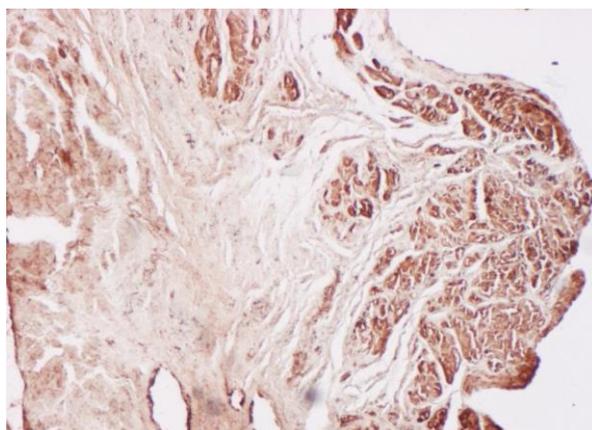


Fig. 3. The area of the scarred myocardium on day 30 of the experiment in rats without treatment. Large scar with preservation of single bundles of muscle fibers in the subepicardial departments. IHC-staining with antibodies to troponin T,  $\times 30$

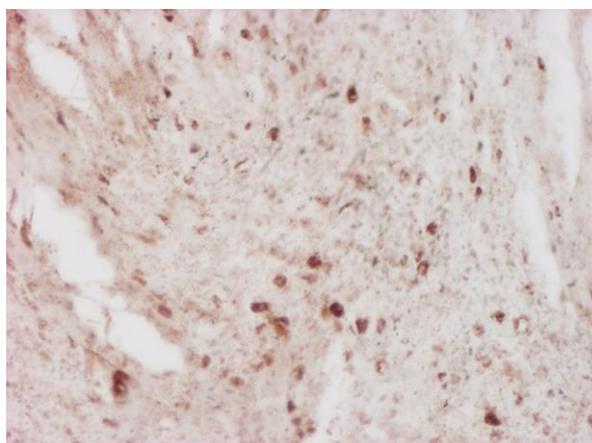


Fig. 4. The area of the scarred myocardium on day 30 of the experiment in rats without treatment. A large scar with a large number of connective tissue cells that proliferate. IHC-staining with antibodies to PCNA,  $\times 150$

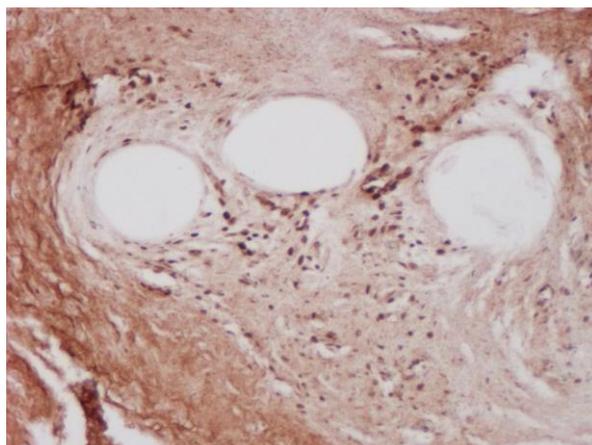


Fig. 5. The area of the scarred myocardium on the 30th day of the experiment in rats without treatment. Active proliferation of connective tissue cells around blood vessels. IHC-staining with antibodies to PCNA,  $\times 75$

It should be noted that already at qualitative morphological research after transplantation of autologous MSC significant difference of a morphological picture in the damaged site was defined. First of all, in the area of the infarction there was an alternation of preserved muscle areas and fields of scar tissue. These changes are confirmed by the fact that in no case after MSC transplantation we did not observe the formation of aneurysms. When immunohistochemical staining for actin and troponin T, we most clearly saw the alternation of preserved muscle areas and fields of scar tissue (Fig. 6, 7). In addition, starting from the term of 21 days, we visualized only a single proliferating cells in the scar, which indicates the completion of the scarring process for this period. In addition, the different condition of the vessels in animals that received and did not receive treatment attracted attention. In animals after cell transplantation, the vessels were larger per unit area, their lumen was smaller, they had a well-formed wall.

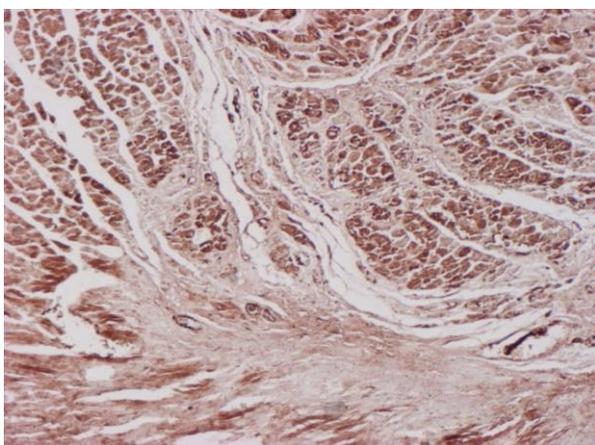


Fig. 6. The area of the scarred myocardium on day 30 of the experiment in rats after MSC transplantation. Scar at the site of intramural infarction. IHC-staining with primary antibodies to troponin T,  $\times 30$

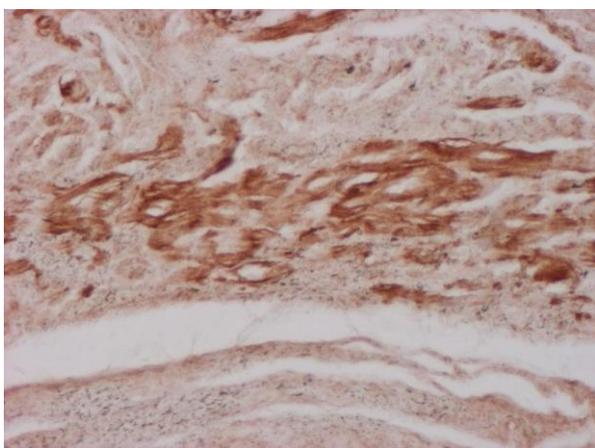


Fig. 7. The area of the scarred myocardium on day 30 of the experiment in rats after MSC transplantation. Alternation of preserved bundles of cardiomyocytes and scar tissue. IHC-staining with primary antibodies to actin,  $\times 150$

When using in situ hybridization in female rats, we found in the rumen staining of chromosome 12, which is formed, cells with the presence of the Y chromosome in the nucleus, i.e. the successor cells of the transplanted MSCs. When staining tissues of females that were not transplanted, we did not observe any staining when using samples to the Y chromosome (Fig. 8), but the control was positive in both females and males. We found Y-chromosome cells among endothelial cells, in the wall of vessels that are formed, and among scar fibroblasts (Fig. 9–11). We did not detect positive cells in the myocardial bundles adjacent to the scar.

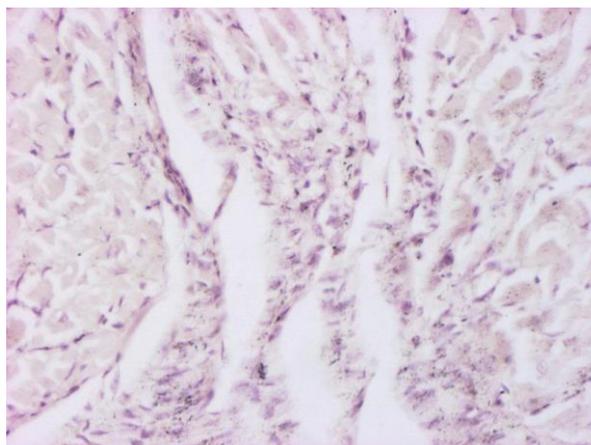


Fig. 8. Rat without transplantation. The cells in the scar do not contain a Y chromosome. In situ hybridization with a marker to the Y chromosome,  $\times 150$

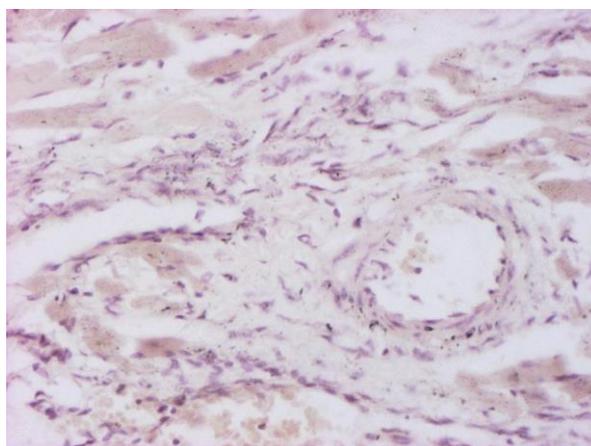


Fig. 9. Rat without transplantation. Cells in the scar and vessel walls do not contain the Y chromosome. In situ hybridization with a marker to the Y chromosome,  $\times 150$

For quantitative analysis of the condition of the scar, we performed a morphometric study. Since the infarct area covered a certain sector of this ring, the theoretical initial area of the infarct area was determined by dividing the area of the entire ring by the specific volume of the infarcted area relative to the total area of the ring. After that, the specific volume of the scar was calculated by dividing its area in the section by the theoretical initial area. Also morphometrically calculated the specific volume of connective tissue and vessels in the scar.

We calculated the area of the infarct (by dividing the area of the whole ring by the specific volume of the

infarcted area relative to the total area of the ring), the specific volume of the scar by dividing its area by the initial initial area, the specific volume of connective tissue and vessels in the scar (Fig. 12, 13).

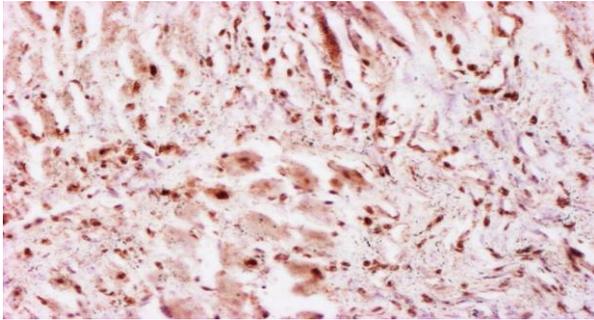


Fig. 10. Positive control (chromosome 12) in the rat myocardium after MSC transplantation for 30 days. In situ hybridization with a marker up to chromosome 12, ×150

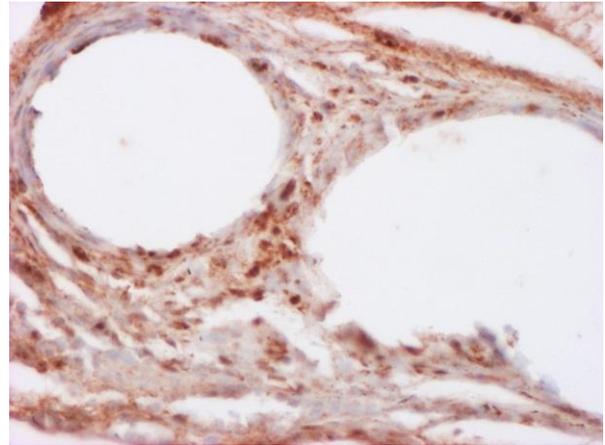


Fig. 11. Cells containing the Y chromosome in the walls of blood vessels and connective tissue around them in female rats on day 30 after MSC transplantation. In situ hybridization with a marker to the Y chromosome, ×180

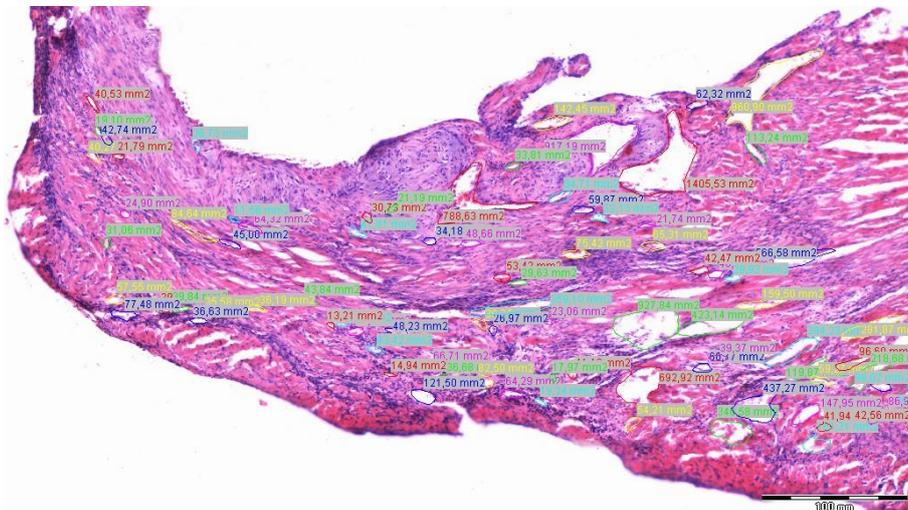


Fig. 12. Myocardial infarction with scar formation. Vascular morphometry

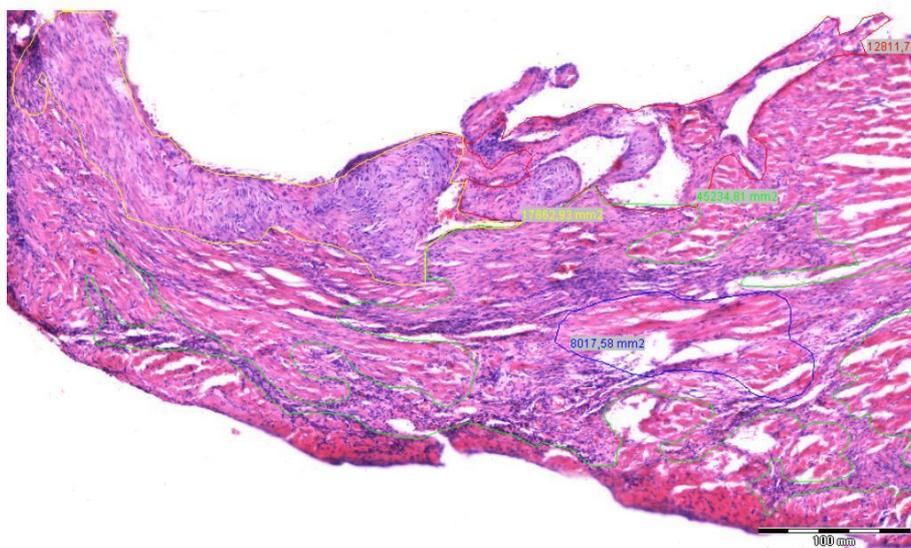


Fig. 13. Myocardial infarction. Connective tissue morphometry

As shown in tab. 1 data, in groups 3, 4 and 5 after MSC transplantation, almost all studied indicators achieved significantly better results compared with animals of group 1, while the level of significance of differences was usually less than 0.01.

Particularly impressive results were obtained in the study of the average number of vessels per 100,000  $\mu\text{m}^2$  (10.21 $\pm$ 1.26 without treatment compared with 68.2 $\pm$ 4.64 in rats with a model of acute MI after administration of MSC - group 4). In animals without treatment, wide vessels with a large lumen were detected, while in unvarnished animals they were vessels of small diameter.

Therefore, we also calculated the specific number of vessels per 100,000  $\mu\text{m}^2$  of scar tissue. Thus, the number of vessels per 100,000  $\mu\text{m}^2$  in the group with MSC transplantation became 6 times more. It should be noted that the specific volume of the MI in group 1 was (65.89 $\pm$ 8.21 %), and in group 3 – (24.02 $\pm$ 1.04 %), i. e. the area of myocardial infarction decreased by almost 3 times. Favourable vascular dynamics was accompanied by a significant improvement: a decrease in the volume of connective tissue in the MI area from 33.78 % to 17.73 % (1.9 times), an increase in the percentage of preserved muscle fibers from 15.9 to 45.04 % (2.8 times).

Table 1

Morphometric analysis of the results of the experimental part of the study

Indicators	Groups of animals				
	1	2	3	4	5
Specific volume of the infarct from the original tissue, %	65.8 $\pm$ 8.2	61.2 $\pm$ 3.45	19.1 $\pm$ 2.3***	24 $\pm$ 1***	32 $\pm$ 4.6**
Specific volume of connective tissue, including blood vessels, %	33.8 $\pm$ 1.7	30.1 $\pm$ 2.2*	15.2 $\pm$ 1.8***	17.7 $\pm$ 5.7**	23.4 $\pm$ 4.2*
Specific volume of vessels, %	6.3 $\pm$ 0.2	6.8 $\pm$ 1.1	10.1 $\pm$ 2.4**	9.4 $\pm$ 3.3*	8.1 $\pm$ 2.3*
Average number of vessels per 100,000 $\mu\text{m}^2$	10.2 $\pm$ 1.3	13.1 $\pm$ 1.5*	73.0 $\pm$ 3.1***	68.2 $\pm$ 4.6***	44.9 $\pm$ 5.3**
% of preserved muscle fibers (from the original)	15.9 $\pm$ 0.3	20.2 $\pm$ 1.2*	49.2 $\pm$ 3.2***	45.0 $\pm$ 9.8**	37.8 $\pm$ 6.1**

Note: \* –  $p < 0.05$ , \*\* –  $p < 0.01$ , \*\*\* –  $p < 0.001$  – between the study group and group 1

Therefore, MSC transplantation in experimental MI in rats leads to a decrease in the MI area by 3 times, while the connective tissue component of the infarct area decreased by 1.9 times, due to an increase in the number of vessels by 6 times and preservation of muscle fibers by 2.6 times more than in 1 group.

In group 2, in comparison with the indicators of group 1, the studied indicators actually remained unchanged, except for the specific volume of connective tissue, including vessels, which in group 1 was equal to (33.78 $\pm$ 1.72) %, and in group 2 less – (30.12 $\pm$ 2.21) %. The average number of vessels per 100,000  $\mu\text{m}^2$  also increased compared to group 1 from (10.21 $\pm$ 1.26) to (13.12 $\pm$ 1.51), in addition, the percentage of preserved muscle fibers was 4.3 above in group 2. Thus, in general, in all probability, damage to myocardial cells still enhances angiogenesis, which leads to improved cardiac perfusion and preservation of full muscle tissue.

In group 3, the maximum values were achieved, which was expressed as an increase or decrease in the studied parameters during morphometry. Thus, the specific volume of the infarct from the original tissue was equal to (19.05 $\pm$ 2.29) % against (65.83 $\pm$ 2.29) % in 1 group. Specific volume of connective tissue, including blood vessels, which in group 3 was equal to (15.21 $\pm$ 1.8) %, in group 1 much more – (33.78  $\pm$ 1.72) %. The average number of vessels per 100,000  $\mu\text{m}^2$  increased compared to group 1 from (10.21 $\pm$ 1.26) to (72.99  $\pm$ 3.1), in addition, the percentage of preserved muscle fibers was 33.3 % above in group 3.

In group 5, the specific volume of the infarct from the original tissue was equal to (32.03 $\pm$ 4.6) % against (65.83 $\pm$ 2.29) % in group 1. Specific volume of connective tissue, including blood vessels, which in group 5 was equal to (23.4 $\pm$ 4.2) %, in group 1 was much higher – (33.78 $\pm$ 1.72) %. The average number of vessels per 100,000  $\mu\text{m}^2$  increased compared to group 1 from (10.21 $\pm$ 1.26) to (44.9 $\pm$ 5.3), in addition, the percentage of preserved muscle fibers was 21.9 % higher than in group 3.

The main parameters of morphometric analysis of rat hearts in different groups were analysed (Fig. 14).

When studying these indicators in different groups, it was found that the specific volume of MI (SVMI) was the lowest in group 3, and the following data were achieved: SVMI 3 groups less than 2 groups ( $p < 0.05$ ). In the data of the specific volume of connective tissue in groups 3 and 4 there was no difference, as there was no difference between groups 4 and 5, but the indicators of group 5 were less than group 3 ( $p < 0.05$ ). Interestingly, the proportion of vessels was the same in groups 3, 4 and 5. The number of vessels in groups 3 and 4 was the same, and in 5 less than in group 4 at  $p < 0.01$  and, accordingly, in group 3 they were more in comparison with group 5 at  $p < 0.00$ . The number of vessels per 100,000  $\mu\text{m}^2$  in groups 3 and 4 was the same, in group 5 they were significantly less than in groups 3 and 4 at  $p < 0.001$ . The percentage of preserved muscle fibers also did not differ in groups 3 and 4, but in group 5 was less compared with group 3,  $p < 0.05$ .

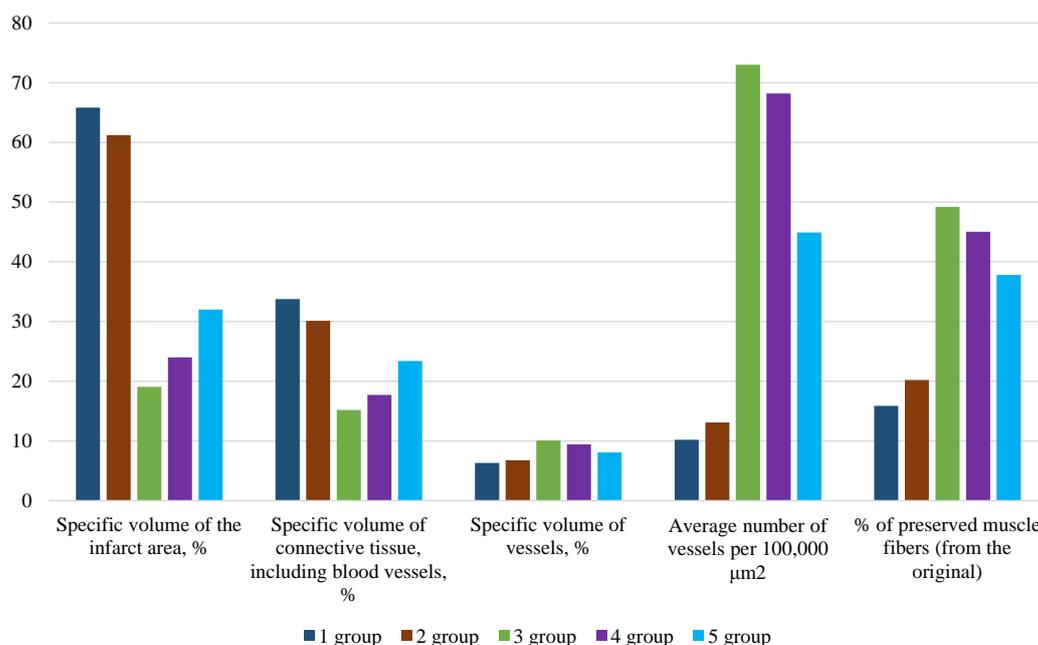


Fig. 14. Dynamics of the main morphometric parameters of the hearts of rats after various cardiomyoplasty

#### 4. Discussion of research results

MSC transplantation can significantly improve the contractile function of the myocardium of mice after induced heart attack [7]. In the work of Wang J.S. et al. [8] for the first time hypothesized that myocardial infarction in the heart creates certain conditions and signals under the interaction of which MSCs differentiate into cardiomyocytes (CMC). In the works of Rangappa S. Et al. [9] it was shown that human MSCs transplanted into the myocardium of rats after 8 weeks turned into CMC. Among other things, it was found that transplantation of MSCs not cultured with 5 – azacytidine can isolate the growth factor of SCF stem cells [10].

The experiment proposed a new approach to enhance myocardial regeneration, based on stem/progenitor cell transplantation, which in simulated MI has shown its effectiveness and viability for the treatment of the effects of myocardial ischemia. In the modern literature, the main question remains open – what is the mechanism of protective action of stem/progenitor cells. It is important to note that there are quite a number of types of stem/progenitor cells that can be characterized by potency, sources of origin, etc. Multipotent mesenchymal stromal cells are one of the most attractive cell types for cell therapy due to their proven cardioprotective properties and low immunogenicity. To date, stem/progenitor cell researchers have been divided into two camps: those who believe that after administration, stem cells and progenitor cells differentiate and replace dead or damaged cells; and those who consider the main paracrine activity of SC and progenitor cells, i.e. the synthesis and secretion of certain signalling molecules, key in the implementation of the therapeutic effect of these cells. Both groups of scientists recognize the positive therapeutic effect of the introduction of stem or progenitor cells in the treatment of pathological conditions of various organs. The study of protective effects in several studies conducted in rats, showed that the introduction of MSC after experimental

infarction leads to a decrease in its volume and improve the functional recovery of the myocardium. In our study, transplantation of MSCs isolated from bone marrow 24 hours after simulation of the pathological condition also led to a decrease in the amount of damage to the heart muscle.

MSC transplantation significantly improves vascularization in the infarct area, which may reduce ischemia in the border areas with infarction, reduce ischemic damage to cardiomyocytes in these areas, resulting in reduced scar area and prevention of heart aneurysm formation. It is proved that transplanted cells are actively involved in the formation of blood vessels and connective tissue in the area of scarring, which ends on the 21st day after MI modelling. In a routine morphological study, we did not identify significant differences between groups of animals after MSC transplantation. In the study of the myocardium of animals after intravenous administration of MSC culture, we visualized changes similar to those in animals without transplantation. Therefore, to objectify the differences between groups of animals, we conducted morphometric studies.

It was found that cellular cardiomyoplasty significantly improves the structure of the postinfarction heart, which is manifested in a decrease in the area of the scar and connective tissue, respectively, an increase in the number of vessels and the percentage of preserved muscle fibers. The best results were achieved with intramyocardial administration, which requires confirmation of this fact in a clinical study.

According to the authors, this effect is due to the fact that the intracoronary administration of MSCs, which have high adhesive properties, there is a partial thrombosis of the vessels of the microcirculatory tract, which leads to the expansion of the area of ischemia. Intramyocardial administration is accompanied by the maximum concentration of the cell graft locally in the area of ischemia and hibernating myocardium, which enhances the therapeutic effect. Intravenous graft admin-

istration showed averages between the two above-mentioned methods of cell graft administration.

**Study limitations.** The study included only rats with the presence of actually proven myocardial infarction in order to investigate the morphological and morphometric changes of the myocardium on the background of cellular cardiomyoplasty in experimental myocardial infarction.

**Prospects for further research.** This work is the first stage of an extended study. Based on the conclusions of the first stage, the second stage will be performed, which will study the effectiveness of MSC in patients with refractory angina.

## 5. Conclusions

Cellular cardiomyoplasty with any method of cell graft administration has a positive effect on the morphological substrate of the heart in the form of reducing the size of the scar in postinfarction remodelling (from 33.78

% to 17.73 % (1.9 times)), increasing the number of newly formed vessels ( $10.21 \pm 1.26$  without treatment compared with  $68.2 \pm 4.64$  in rats with a model of acute MI after administration of MSC – group 4) and an increase in the percentage of preserved cardiomyocytes (from 15.9 to 45.04 % (in 2.8 times)). This is due to MSC homing in the area of ischemia and direct differentiation into endothelial cells of the heart vessels.

Therefore, MSC transplantation in experimental MI in rats leads to a decrease in the MI area by 3 times, while the connective tissue component of the infarct area decreased by 1.9 times, due to an increase in the number of vessels by 6 times and preservation of muscle fibers by 2.6 times more than in 1 group.

## Conflict of interests

The authors declare that they have no conflicts of interest.

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