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Cellular cardiomyoplasty in refractory angina: experimental substantiation of the optimal method and clinical experience

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Abstracts

Objective. Experimental substantiation of the optimal method of cellular cardiomyoplasty and familiarization with clinical experience.

Materials and methods. The study included experimental and clinical parts. The experimental part was performed on 122 rats of the Wistar–Cayote inbred line weighing 200 – 220 g, which were induced with myocardial infarction by stitching and ligation of the anterior interventricular artery. The animals were divided into five groups, and each group (except for the control) was treated with different routes of stem cell injection. Markers of neoangiogenesis in the blood serum, concentration of vascular endothelial growth factor, endothelin–1 level were studied in the dynamics, echocardiography was performed to calculate cardiac function. The clinical part of the study was based on the analysis of the results of treatment of 30 patients who were studied for the effectiveness of the use of autologous mesenchymal stem cells on myocardial remodeling. The diagnosis was made based on the clinical picture, anamnesis, and the results of invasive and non–invasive examination methods**.**

Results. Experimental studies have shown a positive effect of intramyocardial injection of autologous mesenchymal stem cells on the functional reserve and metabolism of cardiomyocytes due to the paracrine effect and stimulation of angiogenesis. This was confirmed by a statistically significant increase in the level of vascular endothelial growth factor, an increase in the concentration of nitric oxide derivatives with a simultaneous decrease in the concentration of endothelin–1 to normal values. Transplantation of autologous bone marrow stem cells increased left ventricular ejection fraction ($t = 2.5$, $p < 0.05$) and stroke volume ($t = 1.9$, $p < 0.05$).

Conclusions. Optimal transmyocardial injection of stem cells. The clinical study showed an improvement in total unipolar voltage from (7.3± 1.1) to (8.9± 1.8) mV after 6 months and (8.1± 1.3) mV after 12 months ($p= 0.03$), positive changes in intracardiac hemodynamics and reduction in the depth and area of myocardial perfusion defects, promotion of neoangiogenesis, and improvement of patients' quality of life.

Key words: refractory angina pectoris; stem cells; cellular cardiomyoplasty; experiment.

Coronary artery disease (CAD) is the leading cardiovascular disease in terms of complications and deaths, with one in five deaths in the United States [1]. In Ukraine, approximately 400 thousand patients are diagnosed with CAD annually [2, 3]. The standard methods of treatment for this category of patients are currently the following: drug therapy, direct myocardial revascularization, i.e. coronary artery bypass grafting (CABG) or angioplasty with stenting, and heart transplantation. However, current drug therapy is usually not effective enough in preventing myocardial remodeling [4, 5].

Heart failure (HF) caused by CAD or cardiomyopathy is one of the most serious diseases with a poor prognosis. Despite the large arsenal of medications and types of surgical interventions, there is still a significant number of patients with angina in whom surgery is not possible for various reasons, and drug therapy is not effective enough [6, 7].

The definition of refractory angina pectoris (PAR) was first proposed in 2002 by the European Society of Cardiology Joint Group on PAR Treatment: A chronic condition lasting more than 3 months and characterized by the presence of angina caused by coronary artery disease (with coronary artery disease), accompanied by severe clinical symptoms that cannot be controlled by combined drug therapy at the maximum allowable doses if myocardial revascularization, such as percutaneous coronary angioplasty or CABG, is not possible [8, 9].

Modern research in the field of stem cell (SC) biology has radically changed all ideas about the regenerative capacity of the myocardium and launched a new therapeutic area – cellular cardiomyoplasty, aimed at replacing damaged cardiomyocytes by implanting SCs [10], which many experts consider to be a potentially promising therapy for patients with chronic CAD. However, large–scale studies to evaluate the effectiveness of SC implantation in patients with HF have not yet been conducted [11].

Many fundamental questions of cell therapy remain unanswered: the mechanisms of homing, differentiation and engraftment of transplanted SCs, the role of cell fusion and

how transplanted cells affect the function and metabolism of the heart muscle. The most effective method of cell delivery to the myocardium, the timing of cardiomyoplasty, the number of cells in the graft, and the methods of graft preparation are also still under discussion, although many studies have been devoted to these issues [12].

The aim of the study is to experimentally substantiate the optimal method of cellular cardiomyoplasty and to familiarize with clinical experience.

Materials and methods

The experimental part of the work was performed on 122 rats of the Wistar–Cayote inbred line weighing 200 – 220 g, which were kept in the vivarium of the Gusak Institute of Emergency and Reconstructive Surgery of the National Academy of Medical Sciences of Ukraine and the Association of Biobanks of Ukraine. The animals were used in the experiment in accordance with the rules regulated by the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), the Council Directive of the European Community of 24.11.1986 and the order of the Ministry of Health of Ukraine No. 32 of 22.02.1988. The surgical interventions were performed in an experimental operating room under ketamine anesthesia (12.5 mg/100 g of body weight intramuscularly).

Induction of myocardial infarction (MI) was performed by stitching and ligation of the anterior interventricular artery. Only animals with transmural MI, the presence of which was confirmed by electrocardiography (ECG) and ultrasound (US), were included in the further experiment.

The effect of cellular cardiomyoplasty on the course of MI was studied in 100 surviving animals; 22 animals died in the first hours after modeling the pathological condition due to the development of life–threatening arrhythmia. Animals were withdrawn from the experiment by decapitation (under general anesthesia).

All 100 animals included in the experiment were divided into five groups (20 animals in each group). Animals in group 1 did not receive any treatment. Animals of group 2 received "empty" injections into the myocardium in the area of the ischemia zone, which was determined macroscopically. In the 3rd group, autologous mesenchymal SCs (auto–MSCs) were injected in the amount of 10 million. In the 4th group, auto–MSCs were injected intravenously in the same amount by tail vein puncture. In group 5, auto–MSCs were injected into the left ventricular (LV) cavity by puncture and catheter passage through the right femoral artery (in this way, we tried to create the maximum concentration of auto–MSCs in the mouth of the coronary vessel and simulate intracoronary injection.

Auto–MSCs were obtained from the peripheral blood of animals by the following method. A syringe containing 0.5 ml of phosphate–buffered saline, 50 units/mL of heparin and 0.25 mg/L of gentamicin sulfate was used to collect 0.5 ml of blood from the tail vein of the animal. The volume of blood taken from the animals is acceptable and safe, as it does not exceed 10% of the circulating blood volume (13.75 – 17.50 ml for rats, based on the recommendations for safe blood collection in mice and rats of the National Institutes of Health). The cell suspension was centrifuged at 1500 rpm for 5 min, the cell pellet was resuspended in erythrocyte lysis solution (114 mM ammonium chloride, 7.5 mM bicarbonate, 100 μM EDTA) for 3 min and centrifuged again. The hemolyzed supernatant was removed, and the cell pellet was resuspended in DMEM containing 10% fetal calf serum (HyClonegold, USA), 0.4 μM insulin, and 0.25 mg/L gentamicin sulfate. The resulting cells were sown in culture vials and transferred to an incubator with a 5% carbon dioxide concentration and 95% atmospheric air content and high humidity. 2 days after the primary culture was isolated, the non–attached cell suspension was removed, and the remaining cells with fibroblast–like morphology were continued to be cultured. The culture medium was replaced with fresh medium every 3 to 4 days. After 75 – 80% monolayer formation, the cells were washed once with Versailles solution, and then removed with Versailles solution with 0.25% trypsin solution, resuspended in growth medium and poured into new culture dishes. The cellular material, which was flattened fibroblast–like cells (mesenchymal stromal cells) fixed on plastic, retained population activity and did not contain dead cells, was considered suitable for use. The stromal origin of the SCs was confirmed by immunohistochemical method in culture by detecting type I collagen using rabbit monoclonal antibodies. MSCs of the first and second passages were used for the experiment. The viability of MSCs was determined before their introduction by trypan blue staining. To do this, a 0.1% trypan blue solution was added to the cell suspension and after 2–3 min, live cells were counted in a phase–contrast microscope without staining their membranes.

Markers of neoangiogenesis in the blood serum were studied in laboratory animals over time. The content of nitric oxide (NO) in the blood plasma was judged by the amount of its stable end metabolites, namely nitrite and nitrate (UNOX). The concentration of vascular endothelial growth factor (VEGF) was measured using a Luminex dual–laser flow cytometer (Luminex Corporation, USA) based on Simplex ProcartaPlexTM reagent kits (Affymetrix, USA). The level of endothelin–1 in blood plasma was determined by enzyme–linked immunosorbent assay using the Endotelin (1–21) kit from Biomedica (Austria) on a Stat Fax 2100 enzyme–linked immunosorbent assay analyzer.

Study periods: 1, 6, 24 hours after induction of MI and treatment, as well as on days 7 and 30.

Echocardiography (Echocardiography) was performed using a GE Vivid neonatal ultrasound machine (USA) with a 12 MHz transducer. Study period: 1 and 3 months of the experiment. The following rat heart parameters were studied in the experiment in different experimental groups: LV end–diastolic internal diameter (LVEDD); LV end–systolic internal diameter (LVESD); shortening fraction (SF); EF, stroke volume (SV). The ECG was performed on the ECO 1T apparatus on day 30 of the experiment.

The clinical trial was conducted on the basis of retro– and prospective analysis of the results of treatment of 30 patients with CAD, in whom the effectiveness of the use of auto–MSCs on myocardial remodeling was studied at the Department of Cardiac Surgery of the Gusak Institute of Emergency and Reconstructive Surgery of the National Academy of Medical Sciences of Ukraine. According to the objectives of the study, patients were divided into two groups. The control group consisted of 15 patients who were treated with standard conservative HF treatment regimens; the main group consisted of 15 patients who were prospectively studied for the effectiveness of the proposed treatment approach. The age of the examined patients was on average (53.2 ± 12.7) years (from 37 to 88 years). The male/female ratio was 2.47:1. Criteria for inclusion of patients in the study: presence of PAR with severe clinical manifestations and ineffective medical therapy; impossibility of revascularization of the infarct zone; patient's age not older than 70 years; high risk of CABG due to low LV contractile function; patient's informed consent to participate in the clinical trial. Criteria for not including patients in the trial: acute MI; "fresh" (less than 6 weeks before the start of the study) stroke; severe renal dysfunction (blood urea level of more than 50 mg/dL, creatinine 2.5 mg/dL or more); severe liver dysfunction (aspartate and alanine aminotransferase levels 5 times higher than the upper limit of normal); immunocompromised status; presence of an active infection of any type; severe pulmonary disease; alcoholism or drug addiction; patient's refusal to accept the proposed examination and treatment program.

The diagnosis of PAR was made based on the analysis of the clinical picture, anamnesis, and the results of invasive and non–invasive examination methods. According to the New York Heart Associatin (NYHA) classification, all patients were classified as having CHF functional class II to IV. The majority of patients (61.5%) had FC III of stable angina pectoris according to the Canadian Cardiovascular Society (CCS) classification. The study included mainly patients with multiple lesions of the distal cardiac arteries, and more than 50% of patients had shunt and stent closure, which explains the severe course of PAR in them. Patients were examined before and after the introduction of auto–MSCs according to the following protocol: standard ECG; treadmill test (according to the Bruce protocol); 6–minute walk test; Holter ECG monitoring; transthoracic echocardiography; electromechanical mapping with the NOGA XP navigation system; coronary angiography; ventriculography.

Echocardiography was performed in M– and B–modes, as well as in pulsed–wave and color Doppler modes according to the standard methodology on Toshiba SSA–380A (Japan), Toshiba–Applio (Japan) using sectoral sensors with a radiation frequency of 2.5–5.0 MHz. Additionally, strain mapping was performed. The following indicators of myocardial systolic function were evaluated: LV end–diastolic dimension – maximum LV size (in cm) in diastole according to the R wave on the ECG; LV end–systolic dimension – LV cavity size (in cm) in systole according to the T wave on the ECG; LV posterior wall thickness (in cm) in systole and diastole; interventricular septal thickness (in cm) in systole and diastole; size of the aortic root and left atrium (LA) (in cm).

For further work, the following indicators of the functional state of the myocardium, cardiac and systemic hemodynamics, structural state of the myocardium and heart chambers were calculated: end–diastolic and end–systolic volumes, LV volume and EF by the modified Simpson method. Patients' quality of life (QOL) was assessed by the Minnesota Living with Heart Failure Questionnaire (MLHFQ).

Patients of both groups underwent catheter–based electromechanical mapping of the LV using the Noga XP system (Cordis, USA) after obtaining written consent for the study. All procedures were performed using the Prucka Engineering electrophysiological laboratory (CardioLab 6.5, GE, USA) and the Noga XP navigation system, which allows for volumetric reconstruction of the LV and at the same time determines myocardial viability. The LV chamber was reconstructed on the basis of taking virtual points that formed small triangles to perform a homogeneous reconstruction; color interpolation; and the threshold for filling the map with color. The method of map construction and its color filling was based on the principle of triangulation, when the so–called lattice of triangles was initially built. The reliability of the reconstructed surface and its color decreased if the distance between the nodal points increased. The electrophysiologist chose the scale or threshold for filling the map with color – the lower the threshold, the more "voids", i.e. the map is the most accurate and detailed. The points were taken at sinus rhythm under the conditions of stable catheter placement, stable local activation time, stable cycle length (no more than 3 mm), and in the absence of ST–segment elevation on the unipolar reference channel. Thus, an electroanatomical map of the LV was constructed, reflecting its geometry and the sequence of electrical activation. Then it was transferred to the mode of unipolar voltage and mechanical map. On the unipolar volt map, myocardial segments with a spike amplitude below 7 mV were considered to be scarred. On the

mechanical map, areas with a wall motion amplitude of less than 12% of the maximum were considered scar or insufficiently vascularized myocardium. By comparing the unipolar voltages and mechanical maps, viable myocardial segments that were in a state of ischemia and whose contraction amplitude was significantly reduced were determined – these are the zones of the so–called hibernating myocardium. The unipolar volt map allowed to identify red zones with low–amplitude potentials (scar). Purple zones are zones of viable myocardium, in which high–amplitude electrical activity is recorded. The mechanical map shows the amplitude of wall motion. Red zones are segments that contract poorly or unevenly. Purple zones are myocardium that contracts well. The red zones on the maps of the two types may not coincide, because high–amplitude electrical activity is recorded in areas of hibernating myocardium, but they contract poorly.

After completion of the LV mapping process, patients in the main group were implanted with a Myostar catheter (Biosense Webster, USA) designed for intramyocardial injection of active agents. The length of the needle advancement was pre–adjusted using an aortic arch simulator (the length of the needle advancement was the myocardial thickness). Auto–MSCs were injected into the areas of hibernating myocardium, avoiding injections into the area of the mitral valve apex to prevent a high risk of perforation, and in the area of recording of His bundle potentials because of the risk of blockage. The injection rate did not exceed 0.1 ml per 15 seconds. Typically, 8–10 injections of 0.2 ml were performed. Cells were injected in a total amount of 50 million. MSCs were obtained from the patient's peripheral blood by magnetic separation. Quality control and infection control were mandatory [11].

All patients included in the study received the necessary drug therapy: statins, cardiomagnesium, diuretics and angiotensin–converting enzyme inhibitors (100% of patients), beta–blockers were used in 99.1% of patients, nitrates in 96.7%, warfarin in 55.8%, cardiac glycosides in 54.9%, amiodarone in 50.6%, Plavix in 42.9%, inotropic drugs in 6.9%, and sotalex in 7.8%. All patients included in the study were taking atorvastatin for the correction of hypercholesterolemia.

The standard SPSS software package (version 20.0 for Windows) was used for statistical processing of the data.

Results

Experimental part of the research

When studying the levels of vasoconstrictor endothelin–1, vasodilator OA and EFRP in the dynamics during 1 month after modeling MI, a general pattern was determined for all three indicators: they reached their maximum value on the 1st day after modeling MI (*Table 1*).

The experimental data obtained showed a decrease in the degree of myocardial ischemia after transplantation of auto–MSCs, which reduced the level of such a powerful vasoconstrictor as endothelin–1.

When studying the dynamics of the concentration of EPPF, which reflects the intensity of angiogenesis, it was found that by 6 hours after induction of MI, this index was almost no different from the control values. After 6 hours, in the 1st group of animals, the content of EPFR increased and reached a peak by the end of the 1st day. By the end of the experiment, the level of EPPF decreased to (89.74 ± 21.38) pg/ml, which was not statistically significantly different from normal values ($t = 0.91$; $p > 0.05$). In the 2nd group of animals, a significant increase in the level of EFRS was also observed by the 6th hour of the study, reaching a maximum by the end of the 1st day. Then this indicator decreased slightly and by the end of the experiment amounted to (132.74 ± 19.87) pg/ml, which was 2 times higher than normal values ($t = 2.8$; $p < 0.05$).

The analysis of the effect of auto–MSC transplantation on vascular tone after MI showed that cellular cardiomyoplasty from the 1st day showed a pronounced vasodilator effect, which was reflected in an increase in the level of OA with a simultaneous decrease in the content of the vasoconstrictor endothelin–1. It is characteristic that the increased level of OA persisted until the end of the experiment.

When studying the concentrations of OA derivatives in groups of animals with different methods of auto–MSC

Table 1. **Dynamics of angiogenesis markers levels in the blood serum of rats of groups 1st and 2nd (M ± m)**

Indicator.	Animal group	Terms of the study					Control
		1 hour	6 h	24 h	7 days	30 days	
OA, μg/ml	1st	0,86±0,04***	0,92±0,03	1,12±0,05***	0,99±0,04*	0,88±0,03*	0,58±0,03
	2nd	0,89±0,05***	1,04±0,04*	1,26±0,03***	1,24±0,04	0,96±0,05***	
EFRS, pg/ml	1st	70,21±11,36	126,72±24,05*	220,45±22,13*	134,89±25,24	89,74±21,38	66,98±12,47
	2nd	71,42±13,45	134,86±28,11*	288,22±23,46***	189,57±28,47*	132,74±19,87	
Endothelin-1, mol/ml	1st	10,6±0,7***	12,8±0,5*	12,9±0,4	8,8±0,3***	5,3±0,4***	5,1±0,4
	2nd	10,4±0,5***	12,8±0,6**	9,1±0,3***	6,9±0,4***	5,1±0,2***	
Note.	Statistical significance of the difference compared to the previous indicator: * - p<0.05; ** - p<0.01; *** - p<0.001.						

Table 2. **Dynamics of angiogenesis markers levels in rat serum 30 days after modeling MI (M ± m)**

Indicator.	Control	Animal group				
		1st (n=20)	2nd (n=18)	3rd (n=20)	4th (n=20)	5th (n=20)
OA, μg/ml	0,58±0,03	0,88±0,03***	0,85±0,02***	0,99±0,04***	0,96±0,05***	0,92±0,03***
EFRS, pg/ml	66,98±12,47	89,74±21,38***	132,74±19,87***	144,22±14,59***	132,74±19,87***	111,43±12,22***
Endothelin-1, mol/mL	5,1±0,4	5,9±0,2*	5,8±0,2*	4,9±0,2	5,1±0,2	5,0±0,3
Note.	Statistical significance of the difference in comparison with the control: * - p<0.05; ** - p<0.01; *** - p<0.001.					

Table 3. **Ultrasound parameters of heart function in rats (M ± m)**

Indicator.	Control	Animal group				
		1st (n=20)	2nd (n=20)	3rd (n=20)	4th (n=20)	5th (n=20)
KDVDLL, mm	6,16±0,12	7,19±0,16	7,21±0,14	6,18±0,11	6,34±0,14	6,81±0,15
KVDLL, mm	2,82±0,18	3,82±0,11	3,76±0,13	2,85±0,14	2,93±0,15	3,62±0,13
FU, %.	45,3±1,7	26,8±0,8	27,3±0,7	45,0±0,9	42,6±0,12	31,2±0,9
FF, %	76,9±2,5	55,3±3,4	56,2±3,2	75,8±3,2	70,6±2,3	66,5±3,3
UO, ml	0,25±0,08	0,13±0,03	0,15±0,05	0,24±0,07	0,21±0,02	0,18±0,02
Note.	The statistical significance of the difference is given in the text.					

administration, it was found that after 1 month in rats of the 1st and 2nd groups they increased to (0.88 ± 0.03) and (0.85 ± 0.02) μg/ml, respectively (t = 6.4, p < 0.05). In the 3rd, 4th and 5th groups, a more significant increase in the studied parameters was found – up to (0.99 ± 0.04), (0.96 ± 0.05) and (0.92 ± 0.03) μg/ml, respectively, with the OA content in the 3rd group being higher than in the 5th group (t = 1.99, p < 0.05).

A similar trend was observed with regard to the concentration of the EFRD (*Table 2*). *2*): in group 1 it slightly exceeded the normal values, in groups 2, 3, 4 and 5 the studied indicator was significantly higher than normal and amounted to (132.74 ± 19.87), (144.22 ± 14.59), (132.74 ± 19,87) and (111.43 ± 12.22) pg/ml, while by analogy with the dynamics of OA level in animals of group 3, the maximum level of EFRS was determined in comparison with the corresponding indicator in animals of group 5 (t = 1.97, p < 0,05).

The analysis of the dynamics of endothelin–1 concentration showed that in groups 3, 4 and 5, 1 month after induction of MI, the values of this indicator did not differ from the norm, and in groups 1 and 2 they were slightly higher than normal and amounted to (5.9 ± 0.2) and (5.8 ± 0.2) mol/mL, respectively.

When studying the index of LVEF (*Table 3*), it was found that in the 1st group of animals it increased compared to the control value, which was (6.16 ± 0.12) mm, to (7.19 ± 0.16) mm (t = 5.15, p < 0.001). In group 2, a similar trend in the dynamics of this indicator was determined: it increased to (7.21 ± 0.14) mm (t = 5.7, p < 0.001). In animals of the 3rd and 4th groups, LVEDD remained close to the control (t = 0.12 and 0.98, respectively). In group 5, this indicator increased to (6.81 ± 0.15) mm (t = 3.4, p < 0.01). The study of the index of LVEF showed that in the 1st group of rats it increased from control (2.82 ± 0.18) mm to (3.82 ± 0.11) mm (t = 4.7, p < 0.05), and in the 2nd group of animals – to (3.76 ± 0.13) mm (t = 4.2, p < 0.01). It should be noted that in the 3rd and 4th groups, the values of LVEDD did not differ from the control ones, and in the 5th group this indicator increased to (3.62 ± 0.13) mm (t = 3.5, p < 0.05). At the same time, the LVADL in group 5 did not differ significantly from the corresponding values recorded in rats of groups 1 and 2, and was higher than in animals of group 3 (t = 4.0, p < 0.01). Thus, the best values of LVEF and LVEF–CS were obtained in animals of groups 3 and 4.

When comparing the values of FU, it was found that in animals of the 1st and 2nd groups they decreased to (26.8 ± 0.8) and (27.3 ± 0.7)%, respectively, when compared with the control (t = 9.8, p < 0.001). In group 3, the value of EF did not differ from the control and amounted to (45.0 ± 0.9)% (t = 0.16, p > 0.05). In group 4, FU was slightly lower than normal and amounted to (42.6 ± 0.12)% (t = 1.98, p < 0.05). In the 5th group of animals, FU was (31.2 ± 0.9)% and was below normal values (t = 7.3, p < 0.001). At the same time, the stud-

Table 4. **Indicators of chronotropic function of the heart under stress-simulated load (M ± m)**

Heart rate, per 1 min	Norma	Animal group				
		1st (n=10)	2nd (n=10)	3rd (n=10)	4th (n=10)	5th (n=10)
Output.	485±43	503±23*	507±18*	489±11*	482±14	501±16 **
Maximum	517±35	525±15*	522±11**	526±8**	528±9*	527±7**
Increase	32	22*	24**	38**	39**	28**
Final	490±23	495±12	510±13**	494±10**	499±15**	503±11**
Note.	Statistical significance of differences: * - between the studied indicator and previous data ($p < 0.05$); ** - between the studied indicator and the norm ($p < 0.05$).					

ied index was higher in animals of group 5 than in animals of groups 1 and 2 ($t = 3.7$ and 3.4, respectively, $p < 0.05$) and lower than in animals of groups 3 and 4 ($t = 10.8$ and 12.6, respectively, $p < 0.001$). In addition, in animals of group 3, PF was higher than in animals of group 4 ($t = 2.6$, $p < 0.05$).

A similar trend was observed when analyzing changes in LVEF. Thus, in group 1, it decreased from control (76.9 ± 2.5) to (55.3 ± 3.4)% ($t = 5.1$, $p < 0.05$), in group 2, the same picture was observed, with no statistically significant difference between these indicators in group 1 and 2. In animals of group 3, EF did not differ from the norm, and in animals of group 4, this indicator was below the norm and amounted to (70.6 ± 2.3)% ($t = 1.9$, $p < 0.05$). In animals of group 5, LVEF also decreased to (66.5 ± 3.3)% ($t = 2.5$, $p < 0.05$) compared with the control value. It should be noted that the EF value in group 5 was higher than in groups 1 and 2 ($t = 2.4$ and 2.2, respectively, $p < 0.05$), but lower than in group 3 ($t = 2.02$, $p < 0.05$). PEF values in groups 4 and 5 did not differ statistically.

Changes in the UE index in the experimental groups of animals were as follows: in group 1, UE decreased from control (0.25 ± 0.08) ml to (0.13 ± 0.03) ml ($t = 1.9$, $p < 0.05$), and in other groups this index did not differ statistically from its control value. However, in group 3, the UO was closest to normal and higher than in groups 1, 2, and 5 of laboratory animals. Thus, the closest to normal values were obtained in animals of the 3rd, 4th and 5th groups, while in the 3rd group they practically did not differ from the norm.

Table 5. **Dynamics of QOL indicators (scores) of patients in the study groups according to the MLHFQ questionnaire (M±m)**

Patient group	Terms of the study		
	before treatment	after treatment, months	
		3	6
Main (n=15)	40,8±12,6	56,6±18,7*	73,2±6,9*
Control group (n=15)	68,3±9,5	77,1±5,4*	71,3±5,8
Note.	* - p≤0,005.		

During the modeling of stress load, a positive chronotropic effect was observed in experimental animals of all groups, the severity of which differed significantly in animals of different groups. Thus, in intact rats, at the 1st minute of isopropylnorepinephrine loading, the absolute increase in heart rate (HR) was about 32 per 1 min. Against the background of a high baseline heart rate in animals of group 2, the stress–simulated increase in this indicator was 24 per 1 min. In an-

Table 6. **Distribution of patients with coronary artery disease by NYHA class 3 and 6 months after cellular cardiomyoplasty**

FC	Patient group	Terms of the study						p <
		before treatment		after treatment, months				
				3		6		
		abs.	%	abs.	%	abs.	%	
II	Main (n=15)	3	20	5	33,3	10	66,7	0,05
	Control (n=15)	3	20	1	6,7	0	0	0,05
III	Main (n=15)	9	60	9	60	4	26,7	0,05
	Control (n=15)	9	60	12	80	11	73,3	0,05
IV	Main (n=15)	3	20	1	6,7	1	6,7	0,05

Table 7. **Dynamics of physical activity tolerance according to the treadmill test (M±m)**

Patient group	Indicator, MET		
	before administration	after 6 months	p<
Main (n=15)	2,8±0,46	3,8±0,47	0,05
Control (n=15)	3,2±0,47	2,3±0,48	0,05

Note. MET - metabolic unit; p - statistical significance of the difference between baseline and 6 months later.

Table 8. **Dynamics of LV EF parameters (M±m)**

Patient group	LVEF, %.					
	before entering	after the introduction, Ms.				
		3	p1	6	p2 >	p3 >
Main (n=15)	33,8±3,6	42,8±4,8	<0,05	40,3±5,1	0,05	0,05
Control (n=15)	42,4±4,2	40,3±3,8	>0,05	36,5±3,8	0,05	0,05

Note. Statistical significance of the difference: p1 - between baseline and 3 months later;
p2 - between the indicators after 3 and 6 months; p3 - between the initial indicators and indicators after 6 months.

imals of the 3rd group, the same indicator was 38 per 1 min. Already by the 3rd minute of the experiment, there was a decrease in heart rate in animals of all groups with stabilization at the level of 490 – 495 per 1 min (*Table 4)*.

The qualitative response of the heart to the stress–simulated load in animals of different groups was the same, but the heart rate increase of 32 per 1 min was most closely approached in groups 3, 4, and 5, which was 38, 39, and 28 per 1 min, respectively. In the 1st and 2nd groups, these figures were only 22 and 24 per 1 min, respectively. Thus, in the groups of rats with untreated MI and animals that received "empty" injections into the myocardium, compensation of the necessary blood flow is impossible, despite the stress, which is manifested by the HF clinic.

Clinical part of the research

The study groups of patients were compared in terms of QOL assessed by the MLHFQ questionnaire (*Table 5)*.

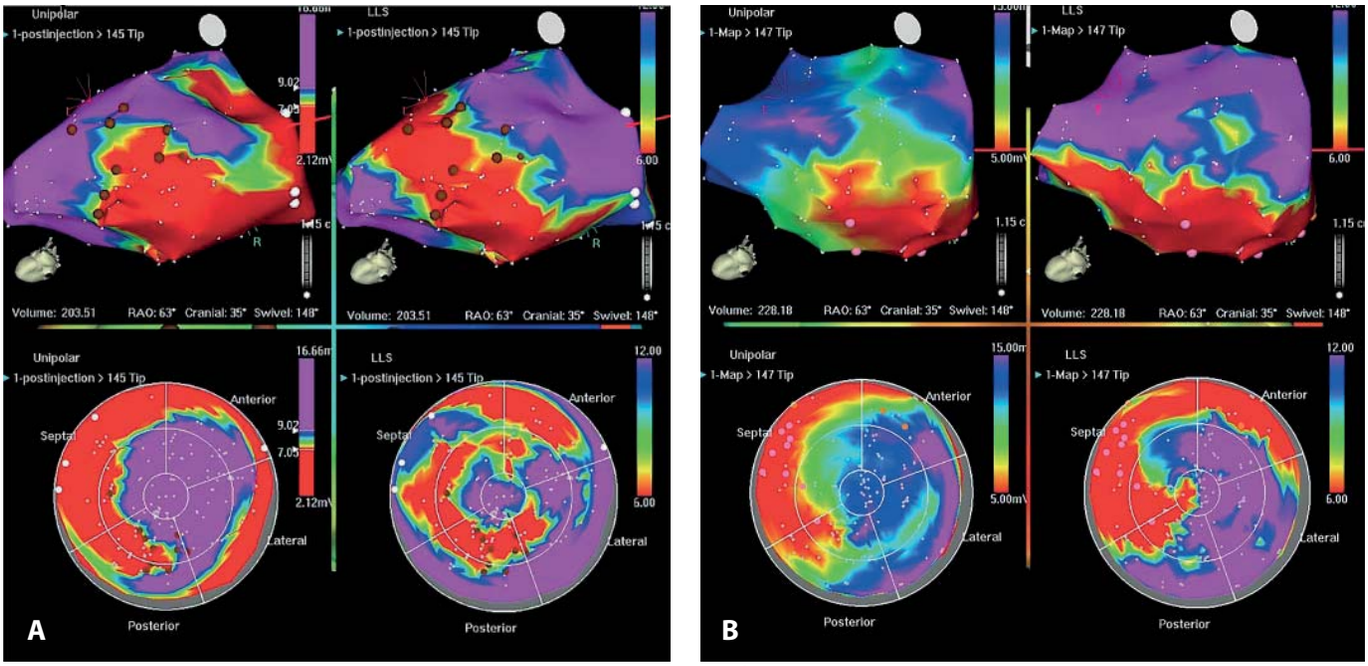
In the control group, 3 months after the course of conservative therapy, the QOL score increased from (68.3 ± 9.5) to (77.1 ± 5.4) points, but after 6 months, patients reported a decrease in QOL score to an average of (71.3 ± 5.8) points. At the same time, patients in the main group noted a statistically significant improvement in QOL – its score increased by 1.4 times. This indicator continued to improve and 6 months after treatment exceeded the baseline values by 1.8 times.

When studying the dynamics of angina FC (*Table 6*) in the control group, the following data were obtained: 3 months after the course of drug therapy, the number of patients with FC III increased from 60 to 80% due to a decrease in the number of patients with FC II from 20 to 6.7%, the number of patients with FC IV decreased from 20 to 13.3%. However, after 6 months, the number of patients with FC II decreased to 0, and the number of patients with FC IV increased to 26.7%. Thus, standard medical treatment of PAR led to a deterioration in the general condition of patients and an increase in its FC.

Supplementation of conservative therapy with transendocardial injection of auto–MSCs yielded the following results: after 3 months, the number of patients with FC III did not change significantly, while the number of patients with FC IV decreased from 20 to 6.7% and the number of patients with FC II increased from 20 to 33.3%. After 6 months, the number of patients with FC IV did not change significantly, and the number of patients with FC III decreased to 26.7% and the number of patients with FC II increased to 66.7%.

The effect of reducing the manifestations of HF and lowering the NYHA score in the main group was maintained for 2 to 4 months ($p < 0.05$).

Thus, in the main group, a positive trend was observed by 3 months in the form of a statistically significant increase in the number of patients with FC II and a decrease in the number of patients with FC IV. After 6 months, the number of patients with FC IV did not change significantly, the number of patients with FC III decreased significantly, and the number of patients with FC II increased by 50%. That is, a positive effect in the course of the disease was clearly determined. In the course of drug treatment, at first, a false–positive dynamics of PAR was noted starting from 3 months in the form of an increase in the number of patients with FC III and a decrease in the number of patients with FC IV. However, after 6 months, the number of patients with FC II was leveled and the number of patients with FC IV



Electromechanical map of patient K., 60 years old (main group).
Diagnosed with coronary artery disease: FC III angina pectoris, postinfarction and atherosclerotic cardiosclerosis.
Condition after CABG-2 and LIMA in 2008. Occlusion of venous shunts. Chronic HF grade I.
Panel A (baseline mapping) demonstrates a large area of hypokinesis in the basal-lateral and posterior-basal regions (hibernating myocardium).
Panel B (control mapping): the hypokinesis zone in the basal-lateral region has significantly decreased.

Table 9. **Efficiency of auto-MSCs application according to mapping data**

Group of patients	Effectiveness of treatment			Together
	unchanged	improvement	significant improvement	
Control (n=15)	9	6	-	15
Main (n=15)	-	14	1	15
In total ...	9	20	1	30

increased significantly, while the number of patients with FC III remained almost unchanged. Thus, the negative course of PAR with drug treatment in the dynamics was once again confirmed.

The dynamics of physical activity tolerance was also studied (*Table 7*).

In the main group, a statistically significant ($p < 0.05$) increase in treadmill test scores from (2.8 ± 0.46) to (3.8 ± 0.417) MET was noted, while in the control group, exercise tolerance decreased from (3.2 ± 0.47) to (2.3 ± 0.48) MET ($p < 0.05$).

In the analysis of global LV contractility by echocardiography, a statistically significant decrease in LV volume was observed in the main group of patients compared with the corresponding index of patients receiving standard conservative treatment, with a moderate increase in LV EF compared with its baseline values.

The study of the dynamics of LV EF according to ultrasound (*Table 8*) in the main group showed an increase in the period of 3 months from (33.8 ± 3.6) to (42.8 ± 4.8)% with a subsequent slight decrease. Thus, cell transplantation increased the index of LV EF. Patients in the control group did not show any dynamics in LVEF.

According to the results of catheter electromechanical mapping (*see Figure*) *of* the LV using the Noga XP system, the effectiveness of auto–MSC transplantation was judged (*Table 9*).

Thus, transplantation of auto–MSCs has a positive effect on the electromechanical properties of the myocardium of patients.

The number of daily angina episodes in patients of the main group during the 6–month period decreased from 2.8 ± 4.1 to 1.0 ± 1.5 and amounted to 0.6 ± 1.2 after 12 months ($p = 0.022$). Accordingly, the need for sublingual nitrates decreased from (1.8 ± 2.8) tablets per day before the auto–MSC implantation procedure to (0.5 ± 1.2) tablets per day after 6 months and to (0.5 ± 1.3) tablets per day after a year ($p = 0.043$). In contrast, patients in the control group showed

a false–positive trend after 3 months, which was leveled after 6 months.

There were no complications of transendocardial transplantation of auto–MSCs. There was no mortality in the main group within 6 months. In the control group, 3 patients died during the six–month period.

Against the background of stabilization of LV contractile function, 3 patients in the main group underwent myocardial revascularization within 4–6 months after auto–MSC transplantation. The course of the postoperative period in all patients was smooth, no mortality was recorded.

Discussion

Multipotent MSCs are among the most attractive cell types for cell therapy due to their proven cardioprotective properties and low immunogenicity.

To date, SC researchers have been divided into two groups: some believe that after introduction into the body, SCs and progenitor cells differentiate and replace dead or damaged cells; others believe that the paracrine activity of SCs and progenitor cells, i.e. the synthesis and secretion of certain signaling molecules, is key to 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 after the introduction of MSCs in experimental MI, its volume decreases and functional recovery of the myocardium improves [12].

It has been recognized that MSCs can promote tissue regeneration by secreting growth factors such as brain–derived neurotrophic factor, nerve growth factor, VEGF, and other cytokines [13]. In addition, they can regulate the inflammatory response, which plays an important role in postischemic myocardial injury, as SCs secrete a number of immunomodulatory factors, such as prostaglandin E2, tumor necrosis factor alpha, transforming growth factor beta, interleukin–6, and others. However, the mechanisms underlying the regenerative potential of MSCs are not fully understood and require further study.

One way to study the mechanisms of SCs' influence on organ function is to directly study the interaction between the body's cells and transplanted cells, to look for chemical compounds that SCs can secrete into the intercellular space after they are co–cultured with differentiated cells. The same applies to recipient cells, as they can respond to the presence of transplanted cells by synthesizing and secreting various biological compounds. Such "communication" between neighboring cells or even distant organs within the same organism is very likely to occur. Numerous data have been obtained confirming the existence of this phenomenon. However, intercellular communication as a result of the secretion of biologically active compounds and their "capture" by neighboring cells is limited by diffusion. This limitation can be minimized in the case of directed transfer of cellular components from cell to cell as a result of the formation of intercellular contacts. Examples of such contacts include gap junctions formed with the participation of the protein connexin–43, which provides intercellular communication, electrical coupling of neighboring cells, and transmission of depolarization signals. Among the intercellular interactions, the migration of cytoplasmic components between contacting cells along cell processes, in particular, tunneling nanotubes, deserves special attention. For a number of differentiated cells in direct contact with progenitor cells, the exchange of cytosolic components has been shown [14], as well as intercellular transport of mitochondria. Direct intercellular contact probably provides the least expensive way of exchanging chemical/biological information between cells, but the efficiency of such exchange is limited by the very low ratio of the number of SCs to the number of differentiated cells and, as a result, the low number of intercellular contacts. A more costly method of information exchange for cells is the secretion of biologically active molecules [8] into the intercellular space (into the culture medium in vitro and into the extracellular space/interstitial fluid in vivo). At a higher level, communication between cells in different organs is possible. This involves the secretion of certain factors into the bloodstream, which then travel to distant organs with the bloodstream.

In the experiment, we proposed a new approach to enhance myocardial regeneration based on SC transplantation, which in modeled MI showed its effectiveness and promise for the treatment of myocardial ischemia. It should be noted that quite often the positive effect of auto–MSCs is observed quite quickly after their transplantation. It is unlikely that within such a short period of time, MSCs can differentiate into functional cardiomyocytes and integrate into muscle tissue. Therefore, we hypothesized that neovascularization occurs at the site of myocardial injections, which may contribute to some improvement in the general condition of patients and a decrease in the FC of angina. The experiment proved that transplantation of auto–MSCs isolated from peripheral blood 24 hours after modeling the pathological condition led to a decrease in the amount of damage to the heart muscle. Based on the analysis of the experimental data obtained, we can assume two main directions of metabolic therapy of auto–MSCs in MI: optimization of energy production and consumption; normalization of the balance between the intensity of free radical oxidation and antioxidant protection.

In our studies, MI modeling was associated with a marked decrease in cardiac contractile function. However, a tendency to recovery was observed in the first hours after modeling the

pathological condition, suggesting the presence of powerful compensatory mechanisms both in the myocardium itself and in the cardiovascular system as a whole. This position is confirmed, in our opinion, by the heart's response to stress.

Animals examined a week after modeling MI showed a high baseline heart rate, which was significantly higher than that of intact animals. Stress loading did not reveal qualitative differences in the dynamics of heart rate, but quantitative differences in reactions exist. It can be assumed that these quantitative differences in myocardial reactions to stress are the manifestation of those destructive processes that contribute to the development of secondary disorders, accompanied by an increase in mortality under intense stress load.

According to our assumptions, this effect is due to the fact that intracoronary injection of auto–MSCs, which have high adhesive properties, partially thrombose the vessels of the microcirculatory bed, which leads to an expansion of the ischemic zone. Intramyocardial injection of auto–MSCs is accompanied by the maximum concentration of the cell graft locally in the zone of ischemia and hibernating myocardium, which enhances the therapeutic effect. Intravenous administration of the cell graft demonstrated its average performance between the other two methods mentioned above.

Clinical trials have shown that significantly fewer patients who received cell therapy require re–hospitalization for the treatment of decompensated HF than patients treated with standard regimens. In addition, auto–MSC transplantation improves myocardial perfusion in ischemic segments and does not increase the scar zone. Mapping with the Noga PX system in our study revealed an improvement in myocardial electrical function and contractility.

However, there were patients in whom angina EF decreased, HRQoL improved, but the increase in myocardial perfusion was statistically insignificant. Moreover, despite a decrease in NYHA angina class, not all patients had an increase in LVEF. In some patients, the improvement in QOL was the main manifestation of the treatment effect. In addition, the positive effect of cell therapy can be maintained for no more than 6 months.

Taken together, the results indicate the need for a more in–depth study of the mechanism of cell transplantation on the myocardium. A possible hypothesis is related to the activation of MSC angiogenesis through the production of angiogenic cytokines and upregulation of endogenous cytokine expression, which contributes to increased myocardial perfusion and function. Similar results have been obtained by other researchers [5, 7, 9]. However, these studies have a number of disadvantages, namely, a small number of patients, short study duration, and lack of randomization. The effectiveness of treatment was confirmed by our studies of electroanatomic mapping, LV function, and myocardial perfusion. Our results need to be confirmed in large multicenter randomized, double–blind, placebo–controlled trials involving a large cohort of patients with chronic postinfarction cardiosclerosis and significantly reduced LV function, but they demonstrate the value of cell therapy as an intermediate treatment.

Thus, transplantation of auto–MSCs can optimize oxygen metabolism by cardiomyocytes and stabilize aerobic and anaerobic glycolysis. It is mitochondrial transport rather than engraftment of auto–MSCs into tissues that can partially explain the positive therapeutic effect of their administration in experimental MI. It is known that the cytoprotective effect of SCs adjacent to damaged cells is due to their para– and endocrine activity [13]. For example, all paracrine factors secreted by SCs into the damaged myocardium have been described in detail. Similar examples also exist for lymphoid SCs (T– and B–cells), which secrete paracrine factors that promote the repair of damaged skeletal muscle, as well as for other types of SCs that secrete neurotrophic factors in response to brain cell damage or factors that stimulate the repair of damaged kidneys [14]. Summarizing all of the above, we can assume the following chain of events: during intercellular contacts, the cytoplasm of a neutral cell enters the auto–MSCs, inducing an increase in the production of myotrophic factors; then, more effective repair of the damaged tissue occurs due to the enhanced paracrine activity of auto–MSCs, and mitochondria are transferred from auto–MSCs to neutral cells damaged by ischemia.

Conclusions

1. Experimental studies have shown that cellular cardiomyoplasty, regardless of the method of SC administration, improves the structure of the post–infarction heart, which is manifested in a reduction of the scar zone, an increase in the number of vessels and the percentage of preserved cardiomyocytes.

2. Myocardial injections and intramyocardial injection of auto–MSCs have an advantage due to the functional reserve and metabolism of cardiomyocytes due to the paracrine effect and stimulation of angiogenesis.

3. Accurate electrical and mechanical mapping allows to choose the best place for SC implantation, which allows to achieve the maximum positive effect of cell therapy. The use of the NOGA XP electrophysiology unit and transendocardial injectors for the diagnosis and treatment of hibernating myocardium is effective and safe and avoids myocardial perforation and the development of malignant arrhythmias.

4. The technique of transendomyocardial injections of auto–MSCs in PAR according to the results of electroanatomical mapping of the LV showed an improvement in total unipolar voltage at 6 and 12 months ($p= 0.03$) with a slight increase in these indicators at 24 and 36 months.

5. The most effective method of endomyocardial implantation of auto–MSCs, which, according to electroanatomical mapping, contributes to a statistically significant reduction in the depth and area of myocardial perfusion defects with an improvement in the total perfusion index in the early and late posttransplantation period. The procedure is safe and effective, not accompanied by postoperative complications, and helps to increase survival time and improve patient QOL.

6. To achieve the maximum effect of treatment after 1 year, it is advisable to repeat the procedure of auto–MSC transplantation.

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