Improvement of Cardiac Contractile Function in Rats with Postinfarction Cardiosclerosis after Transplantation of Mononuclear and Multipotent Stroma Bone Marrow Cells

O. N. Khokhlova[1], I. A. Ilyushkina[1], T. Kh. Fatkhudinov[2,3,5], G. A. Slashcheva[1], Yu. P. Baikova[3], G. B. Bol'shakova[3], T. B. Bukharova[2,4], V. I. Turobov[1], V. V. Glinkina[5], A. N. Murashev[1], and D. V. Gol'dshtein[2,4]

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 2, pp. 73-78, April, 2012
Original article submitted November 14, 2011

We compared the efficiency of autologous mononuclear cells and multipotent stromal cells of the bone marrow after their non-selective intracoronary transplantation on day 30 after acute coronary infarction in rats. Improvement of hemodynamic parameters of myocardial contractility (rates of left ventricular pressure rise and drop) in comparison with the initial values and deceleration of postinfarction prolongation of *QRS* and *QT* intervals were observed in rats of the experimental group in contrast to controls in 4 weeks after transplantation. These functional changes were more intensive after transplantation of multipotent stromal cells and were accompanied by more pronounced morphological signs of reverse myocardial remodeling: thickening of the scarred left ventricular wall, shrinkage of the scar, and decrease in left ventricular dilatation index.

Key Words: *mononuclear cells; multipotent stromal cells; myocardial infarction; myocardial contractility; reparation of the myocardium*

Despite evident progress in management of acute myocardial infarction (MI), the risk of postinfarction heart failure remains high. Pathological myocardial remodeling due to necrotic damage of heart tissues is characterized by dilatation and thinning of the scarred wall of the left ventricle, impairment of diastolic and systolic functions of the heart, and progressing ejection fraction decrease [3,6]. Cardiomyoplasty with stem/progenitor cells is now considered as an effective method of anti-remodeling therapy. Red bone marrow (BM) mononuclear cells (MNC) were first used as the transplant and were effective in acute MI in animal experiments and in clinical studies [10,13]. Multipotent stromal cells (MSC) capable of differentiating into cardiomyocytes and endothelial cells are promising material for cardiovascular regeneration and therapy of coronary heart disease [4,14,15]. However, MSC transplantation is less studied and the results are controversial [3,5]. Despite the choice of effective cell transplant remains an pressing problem of modern studies, direct comparison of the efficiency of different stem/progenitor cell populations receives insufficient attention. In most studies the cells are transplanted at the stage of acute MI [13]. The efficiency of different SC populations in chronic ischemia and congestive heart failure is less studied [3,8,12].

[1]Laboratory of Biological Testing, Branch of M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Pushchino; [2]ReMeTeks Company; [3]Laboratory of Growth and Development, Research Institute of Human Morphology, Russian Academy of Medical Sciences; [4]Laboratory of Stem Cell Genetics, Medical Genetic Research Center, Russian Academy of Medical Sciences; [5]Department of Histology and Embryology, N. I. Pirogov National Research Medical University, Ministry of Health and Social Development of the Russian Federation, Russia. ***Address for correspondence:*** khohlova@fibkh.serpukhov.su. O. N. Khokhlova

0007-4888/12/1534-0545

Here we compared the capacity of autologous MNC and MSC to improve contractile function of the heart in rats with postinfarction cardiosclerosis and pathological remodeling of the myocardium.

MATERIALS AND METHODS

Experiments were carried out on outbreed male rats (CD stock, Laboratory Animal Nursery, Pushchino Branch of Institute of Bioorganic Chemistry). All manipulations were approved by Bioethical Committee of Pushchino Branch of Institute of Bioorganic Chemistry).

Modeling of myocardial infarction. Transmural MI was modeled in 8-9-week-old rats by 20-min ligation of the left descending coronary artery followed by reperfusion. The animals were narcotized (100 mg/kg ketamine+10 mg/kg xylazin, intramuscularly) and artificially ventilated (Rodent Ventilator 7025, UGO BASILE). The development of infarction was controlled visually (by LV cyanosis) and by ECG.

Preparation of cell transplant. MNC were isolated by density gradient centrifugation of red BM specimens drawn from the femoral and tibial bones through the knee joint cavity puncture [8]. The isolated nucleated cells were resuspended in 0.9% NaCl to a concentration of 5×10^6 cells/ml. MSC were isolated from BM aspiration biopsy specimens obtained routinely 4-5 weeks before MI modeling [3] and cultured in DMEM/F12 (1:1) supplemented with 10% embryonic calf serum, 2 mM L-glutamine, and 0.5 mg/ml amikacin. Immunophenotyping of MSC was performed on a FACS Caliber cytofluorometer (BD Biosciences) and their functional activity was evaluated by the capacity to directed mesodermal differentiation (myogenesis, chondrogenesis, osteogenesis, adipogenesis) in induction media. The cells were frozen in liquid nitrogen and resuspended in 0.9% NaCl to a concentration of 5×10^6 cells/ml before transplantation.

Cell transplantation. In 4 weeks after MI modeling, the animals received transplantation of autologous MNC or MSC (25×10^6 cells/kg body weight) through a catheter introduced into the left ventricle (the aorta was clumped at the moment of during infusion). This procedure ensures settling of most cells in coronary arteries [2]. The procedure was performed under general anesthesia. Controls received 1 ml 0.9% NaCl.

ECG recording and calculation of myocardial contractility indexes. Narcotized animals were fixed in the supine position and electrodes for ECG recording in standard lead II were applied on the right fore paw and left hind paw. LV blood pressure was recorded using a DTX™ Plus TNF-R transducer (Becton Dickinson) through a catheter introduced into LV. ECG and LV pressure signals were processed using a HemoDynamics 1.1 computer-assisted system (Institute of Cell Biophysics, Russian Academy of Science). ECG records were used for measuring of *PQ*, *QRS*, *QT* intervals (Fig. 1, *a*) and calculation of myocardial contractility indices: first derivatives of LV pressure rise and drop (+dP/dt; -dP/dt) and ratio of maximum contraction change rate to instant blood pressure (dP/dt/Pi).

Scar morphometry. After euthanasia, the heart was removed, weighed, and fixed in 10% formalin. Serial paraffin cross-sections of the left and right ventricles at 10 levels with an interval of 500 μ were prepared and stained with picrosirius red. For evaluation of reverse LV remodeling, scar size (ratio of scar area to LV wall area×100), index of LV dilatation (ratio of LV cavity area to total LV area×100), and index of

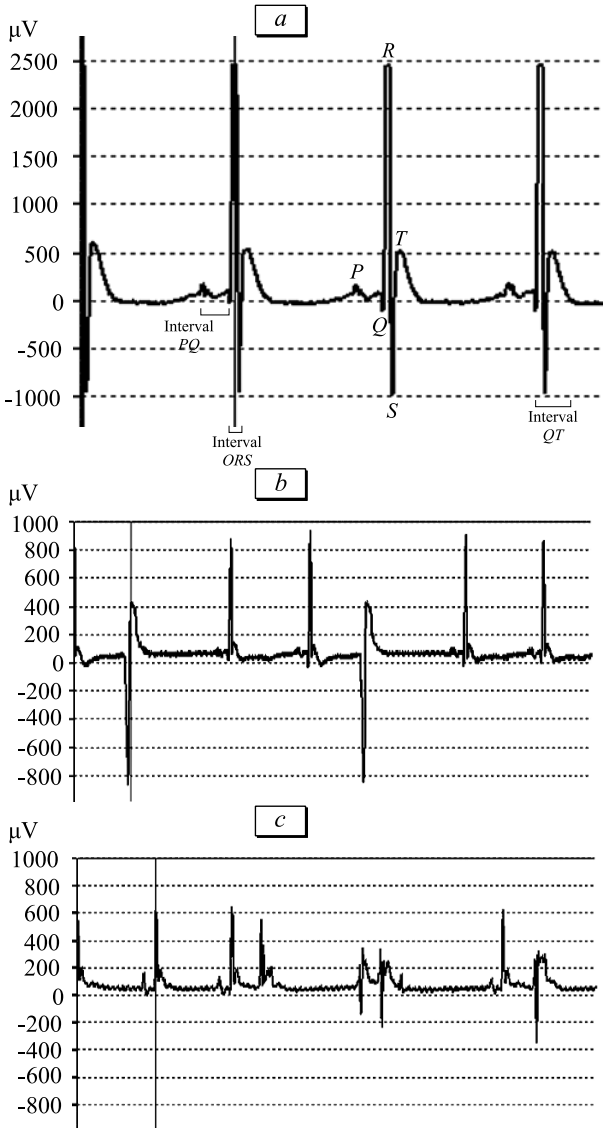

Fig. 1. ECG before (*a*) and 4 weeks after MI modeling (*b*, *c*). *b*) pathological *QRS*; *c*) injury currents in T wave.

scarred wall thickness (ratio of thicknesses of scarred LV wall to that of perifocal area×100) were calculated.

Statistical analysis. The data are presented as mean±standard deviation. Significance of differences from the initial values were evaluated using Mann–Whitney (for ECG parameters) and Wilcoxon tests (for contractility indexes), the differences between the groups were analyzed using one-way Kruskal–Wallis test. Statistical processing of the results was performed using GraphPad Prizm 5.0 software. The differences were significant at $p<0.05$.

RESULTS

MI-related mortality over the first 3 days after left coronary artery ligation was 34%. Autologous MNC and MSC did not significantly improve animal survival after MI.

In 4 weeks after ligation of the left coronary artery, ECG signs typical of subacuite stage of MI were revealed: pathological *Q* wave, *ST* segment shift (Fig. 1, *b*, *c*), and lengthening of *QT* interval (Table 1). The observed *R* wave depression agrees with the data on progressive decrease in *QRS* amplitude after MI due to replacement of the myocardial tissue with fibrous tissue [9,11]. The mean duration of *QRS* complex tended to increase in comparison with the corresponding parameter in healthy animals, which together with considerable lengthening of *QT* interval attests to decelerated conduction in ventricles. Prolonged T wave in animals with MI can indicate injury currents (Table 1, Fig. 1, *c*).

In the experimental group, no significant changes in ECG parameters in comparison with both the control group and parameters before transplantation were observed 4 weeks after intracoronary cell transplantation. Insignificant P wave elevation observed after MNC transplantation is typical of MI scar stage [11]. No significant increase in *R* wave amplitude attesting to improvement of ventricular conduction was recorded. The duration of *QRS* and *QT* complexes did not change significantly after MNC and MSC transplantation. However, comparison of these parameters in groups with the corresponding values before MI revealed less rapid increase in these intervals after cell transplantation, especially after MSC transplantation (Fig. 2).

The absolute values of contractility indexes after transplantation of MNC and MSC did not significantly differ from those in animals receiving 0.9% NaCl (Table 2). However, +dP/dt and -dP/dt indexes after cell transplantation were higher than before it (in contrast to the control group). It should be noted that after MSC transplantation, the percentage of systolic function index +dP/dt was higher that in the control group.

The process of postinfarction myocardial remodeling is characterized by progressive LV dilatation, thinning of its wall in the scarred area, and myocardial hypertrophy [6]. In our experiments, the heart weight (% of body weight) 4 weeks after injection of 0.9% NaCl, MNC, and MSC was 0.387±0.085, 0.358±0.046, and 0.357±0.013, respectively. No significant differences between the groups were found, but the mean values after transplantation were lower than in the control. MNC transplantation did not affect the scar size and index of LV dilatation, but thickening of scarred LV wall was noted (Table 3). After MSC transplantation, more pronounced signs of myocardial remodeling in the scarred area (apart from LV wall thickening) were seen: scar size and index of LV dilatation de-

TABLE 1. ECG Parameters in CD Rats with Cardiosclerosis before and 4 Weeks after Intracoronary Transplantation of MNC, MSC, or 0.9% NaCl (Control)

Parameter	Before MI (*n*=25)	4 weeks after MI (before transplantation) (*n*=20)	4 days after transplantation		
			control (*n*=6)	MNC (*n*=6)	MSC (*n*=6)
P, μV	91±44	60±30*	60±26	103±33+	67±32
R, μV	1348±617	502±181*	465±148	826±579	330±67
T, μV	370±158	227±122*	215±156	221±248	235±96
PQ, msec	37±17	41±13	50±18	36±16	44±8
QRS, msec	31±20	42±28	63±37	54±37	36±14
T, msec	45±23	63±20*	60±28	43±22	43±29
QT, msec	76±19	105±26*	123±30	98±35	79±26

Note. Summary ECG parameters before MI modeling are presented for comparison. $p<0.05$ in comparison with: *before MI; +before transplantation.

creased in comparison with both the control and MNC transplantation.

It is now accepted that paracrine mechanisms play the key role in therapeutic activity of progenitor BM cells [12,16]. Studies on dogs with chronic MI demonstrated more active angiogenesis after transplantation of heterogeneous mononuclear population, which probably explains higher efficiency of these cells in comparison with MSC [8]. In our experiments, intracoronary administration of MSC was more efficient in comparison with MNC, which contradict previous findings [8]. Other authors showed that expression of angiogenic factor bFGF positively correlating with cardiac function improvement in the MI demarcation zone after MSC transplantation was higher than after MNC administration [7]. Moreover, the results obtained by us on the model of postinfarction cardiosclerosis in rats agree with published data on pronounced stimulation of cardiac contractility in dogs [14] and swine [12] with experimental chronic MI. We have previously reported that BM mononuclear cells after intracoronary transplantation were primarily located in the scar, where they differentiated into fibroblasts, proliferated, and actively synthesized extracellular matrix components of the scar tissue, thus promoting thickening and strengthening of the scar [1,2]. It was also hypothesized that stromal MNC fraction primarily migrated into the scar [1]. Higher efficiency of MNC detected into our experiments can be explained by more pronounced stimulation of fibroplastic processes in MI zone due to more active migration of MSC into the scar, its higher cellularity and maturity, and more intensive angiogenesis processes. Moreover, we cannot exclude the possibility of MSC differentiation into specialized heart cells as a mechanism of their therapeutic activity. Although *in vivo* differentiation capacity of donor MSC is questionable [3], it was demonstrated in some experiments [4,15]. It should be noted that MSC after intracoronary administration had fibroblast phenotype in the scar and cardiomyocyte phenotype in the non-infarction zone [17].

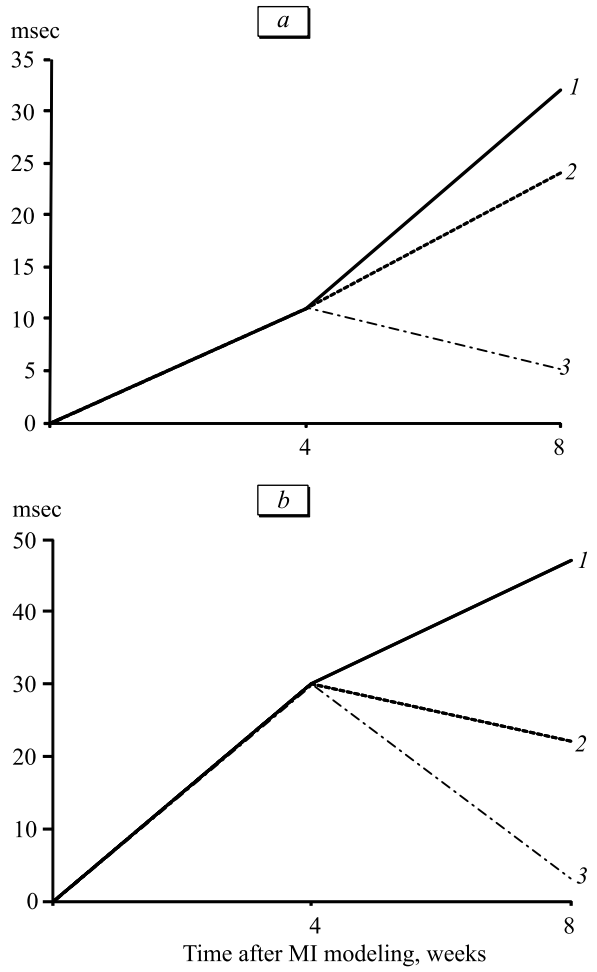

Fig. 2. Changes in *QRS* (*a*) and *QT* (*b*) intervals after administration of 0.9% NaCl (*1*), MNC (*2*), or MSC (*3*) in 4 weeks after MI modeling. 0: before MI modeling.

TABLE 2. Hemodynamic Parameters of Heart Contractility in CD Rats with Cardiosclerosis before and 4 Weeks after Intracoronary Transplantation of MNC, MSC, or 0.9% NaCl (Control)

Parameter	Control (*n*=6)			MNC (*n*=7)			MSC (*n*=6)		
	before injection	after 4 weeks	change, %	before injection	after 4 weeks	change, %	before injection	after 4 weeks	change, %
+dP/dt, mm Hg/sec	7261±2066	7245±893	4±19	6842±1094	8861±1256^{+}	32±30	3984±1448	6723±698^{+}	91±82*
-dP/dt, mm Hg/sec	3744±1442	4316±988	22±30	3916±1004	5730±1023^{+}	52±36	2470±874	4451±443^{+}	98±64
dP/dt/Pi, 1/sec	113±23	112±18	0±9	111±12	120±11	9±18	103±14	115±10	14±22

Note. *p<0.05 in compariсon with values *before transplantation, $^{+}$control.

TABLE 3. Hemodynamic Parameters of Heart Contractility in CD Rats with Cardiosclerosis before and 4 Weeks after Intracoronary Transplantation of MNC, MSC, or 0.9% NaCl (Control)

Parameter, %	Control	MNC	MSC
Scar size, %	7.1±4.9	9.7±7.7	4.9±5.5*+
Index of dilatation, %	28.4±8.1	33.4±13.5	8.2±4.9*+
Wall thickness index in the scarred zone, %	26.5±15.7	30.6±16.4*	43.1±19.9*+

Note. *p<0.05 in comparison with: *control, +MNC transplantation.

Thus, improvement of hemodynamic parameters of myocardial contractility and slightly decelerated postinfarction prolongation of *QRS* and *QT* intervals were observed in rats with postinfarction cardiosclerosis after intracoronary transplantation of MNC and MSC. These functional changes were more intensive after MSC transplantation and were accompanied by more pronounced morphological signs of reverse myocardial remodeling.

REFERENCES

1. Yu. P. Baikova, T. Kh. Fatkhudinov, G. B. Bol'shakova, *et al., Kletoch. Tekhnol. Biol. Med*., No. 4, 203-210 (2010).
2. T. Kh. Fatkhudinov, G. A. Slashcheva, G. B. Bol'shakova, *et al., Kletoch. Tekhnol. Biol. Med*., No. 4, 222-229 (2009).
3. J. Bartunek, J. D. Croissant, W. Wijns, *et al., Am. J. Physiol. Heart Circ. Physiol.*, **292**, No. 2, H1095-H1104 (2007).
4. W. Dai, S. L. Hale, B. J. Martin, *et al., Circulation,* **112**, No. 2, 214-223 (2005).
5. L. Y. Eun, H. Song, E. Choi, *et al., Tissue Cell.,* **43**, No. 4, 238-245 (2011).
6. K. Kurrelmeyer, D. Kalra, B. Bozkurt, *et al., Clin. Cardiol.*, **21**, No. 12, Suppl. 1, I14-I19 (1998).
7. S.-R. Li, X.-Y. Qi, F.-L. Hu, *et al., Chin. Med. J.,* **121**, No. 23, 2403-2409 (2008).
8. M. Mathieu, J. Bartunek, B. El Oumeiri, *et al., J. Thorac. Cardiovasc. Surg*., **138**, No. 3, 646-653 (2009).
9. A. Miranda, R. H. Costa-e-Sousa, J. P. S. Werneck-de-Castro, *et al., Ann. Acad. Bras. Cienc.*, **79**, No. 4, 639-648 (2007).
10. D. Orlic, J. Kajstura, S. Chimenti, *et al., Nature*, No. 410, 701-705 (2001).
11. P. E. Santos and M. O. Masuda, *Braz. J. Med. Biol. Res*., **24**, No. 11, 1173-1177 (1991).
12. T. Sato, Y. Iso, T. Uyama, *et al., Lab. Invest*., **91**, No. 4, 553-564 (2011).
13. C. W. Siu, S. Y. Liao, Y. Liu, *et al., Thromb. Haemost.* **104**, No. 1, 7-12 (2010).
14. G. V. Silva, S. Litovsky, J. A. Assad, *et al., Circulation,* **111**, No. 2, 150-156 (2005).
15. C. Toma, M. F. Pittenger, K. S. Cahill, *et al., Circulation,* **105**, No. 1, 93-98 (2002).
16. H. F. Tse, C. W. Siu, S. G. Zhu, *et al., Eur. J. Heart Fail.,* **9**, No. 8, 747-753 (2007).
17. J. S. Wang, D. Shum-Tim, J. Galipeau, *et al., J. Thorac. Cardiovasc. Surg*., **120**, No. 5, 999-1005 (2000).