Safety and Efficiency of Transplantation of Allogenic Multipotent Stromal Cells in Surgical Treatment of Dilatated Cardiomyopathy

T. Kh. Fatkhutdinov[2], A. V. D'yachkov[1], A. V. Koroteyev[1], D. V. Goldstein[2,3], and N. P. Bochkov[1,3]

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 1, pp. 10-16, January, 2010
Original article submitted July 1, 2009

Clinical study of intracoronary transplantation of allogenic multipotent bone marrow stromal cells was carried out in patients with severe chronic cardiac failure against the background of dilatated cardiomyopathy. The results indicate that intracoronary injection of allogenic multipotent stromal precursors is a safe procedure. No complications and side effects directly or indirectly related to cell transplantation were recorded during the immediate and delayed postoperative periods. The positive effect of cell transplantation developed from week 1 after transplantation and persisted for 6 months. It manifested in reduction of the level of brain natriuretic peptide and improvement of patient's functional status and quality of life. No appreciable changes in the main echocardiographic values were noted. Transplantation of allogenic multipotent stromal cells is effective as a component of combined therapy for chronic cardiac failure at the stage of preparation to surgery as a "bridge to surgical treatment".

Key Words: *multipotent stromal cells; allogenic transplantation; dilatated cardiomyopathy; chronic cardiac failure*

Chronic cardiac failure (CCF) is one of the main causes of death of the population of the Russian Federation. According to the data of EPOQUE-O-CCF epidemiological study, more than 8,100,000 residents of Russia have clinical symptoms of CCF. In 3,400,000 patients with CCF the disease runs the most severe course (NYHA functional classes, FC, III and IV), when drug therapy alone becomes ineffective. The mean life span of this numerous group of patients does not exceed 2-4 years [1,2].

Up to recent time, the only method for surgical treatment of severe CCF was heart transplantation, but the problem of CCF treatment cannot be solved by this method because of shortage of donor organs [3]. An alternative to transplantation, used at B. V. Petrovsky Center of Surgery, and a means due to which a grave patient can survive until this operation, is implantation of the extracardiac retinal carcass in combination with prostheses of the mitral valve and annuloplasty of the tricuspid valve. However, the application of these methods has some limitations, and the grave status of the patients often precludes these operations [4].

Cell therapy of the myocardium cannot become an alternative to traditional drug therapy and surgical methods for the treatment of heart diseases [8,15]. However, cell transplantation as a component of combined therapy can appreciably improve the results of therapy and prepare the patient to surgical intervention or improve its results. Numerous clinical and experimental studies showed that cell transplantation stimulates angiogenesis and reparation of the myocardium. Directed differentiation of stem/progenitor cells into blood vessel cells and cardiomyocytes [12,13] was

[1]B. V. Petrovsky Russian Center of Surgery, Russian Academy of Medical Sciences; [2]ReMeTex Company; [3]Medical Genetic Center, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** fatkhutdinov@gmail.com. T. Kh. Fatkhutdinov

0007-4888/10/1491-0119

demonstrated *in vitro* and *in vivo*. It is particularly important in CCF, when recovery of blood supply to damaged tissues does not normalize the contractile function of the myocardium. Hence, it can be hypothesized that stem cell transplantation can improve the efficiency of preoperative treatment and the results of surgical treatment of CCF in dilatated cardiomyopathy (DCMP).

MATERIALS AND METHODS

The study was carried out in 27 patients with CCF caused by DCMP. Two groups were formed: 14 patients with CCF treated without surgery and 13 patients operated for CCF. The patients treated conservatively were divided into 2 subgroups: control (group 1; *n*=8) receiving drug therapy alone and study group (group 2; *n*=6) receiving cell transplantation. The group of patients operated for CCF was also divided into 2 subgroups: control (group 3; *n*=8) receiving drug therapy and study group (group 4; *n*=5) receiving cell transplantation during the delayed periods after surgical treatment.

The study was approved by the Academic Council and Ethic Committee of B. V. Petrovsky Center of Surgery and conformed to Helsinki Declaration (2000). All patients gave voluntary informed consent to participation in the study.

The following criteria were used for selection of patients: 1) idiopathic DCMP, clinically confirmed by case history and results of instrumental examinations; 2) dilatation of heart cavities: end-diastolic size of the left ventricle ≥6.5 cm, end-diastolic volume of the left ventricle ≥160 ml (according to transthoracal echocardiography, EchoCG); 3) reduced pumping function of the heart: left-ventricular ejection fraction ≤35% (according to transthoracal EchoCG); 4) manifest cardiac failure against the background of optimal drug therapy corresponding to FC II-IV.

Patients with mitral insufficiency grade II and higher, ventricular arrhythmias of IVB class or higher after Lawn, autoimmune diseases, polyvalent allergies, malignant tumors, decompensated concomitant diseases, viral hepatitis, syphilis, HIV carriership or AIDS were not included in the study.

The patients were excluded from the study, if drug therapy was radically modified and if surgical interventions were carried out during the period of observation.

During selection for the study and during the study, all patients received drug therapy for CCF, recommended by the All-Russian Scientific Society of Cardiologists and Society of Specialists in Cardiac Failure [5]. The therapy was not much changed during the entire period of observation; if the treatment protocols had to be radically changed, the patients were excluded from the study.

All CCF patients who were not operated (groups 1 and 2) were comparable by the clinical, functional, EchoCG, and laboratory characteristics (Table 1). All patients presented with pronounced dilatation of the cardiac cavities seen from the mean end-diastolic volume of the left ventricle. This was paralleled by deterioration of the contractile function of the heart seen from the mean values of left ventricular ejection fraction. The level of brain natriuretic peptide (BNP; an objective parameter reflecting the severity of cardiac failure) surpassed the normal by 13.3 times in group 2 and by 15.7 times in group 1. Cell transplantation was carried out, if other methods of treatment proved to be ineffective or were associated with extremely high risk. The prognosis for these patients receiving drug therapy alone remained extremely unfavorable [9].

TABLE 1. Initial Characteristics of Patients

Parameter	Patients with CCF without operation		Patients operated for CCF	
	group 1 (*n*=8)	group 2 (*n*=6)	group 3 (*n*=8)	group 4 (*n*=5)
Gender, m/f	8/0	6/0	8/0	5/0
Age, years	49.1±18.1 (21-69)	56.0±7.4 (45-68)	49.1±11.6 (39-64)	48.0±8.6 (34-55)
NYHA FC	3.9	3.5	2.9	2.8
6-min test	170.5±21.3	155.0±23.9	310.5±18.9	258.0±27.7
Left-ventricular ejection fraction, %	26.1±6.0	20.5±1.6	25.6±7.1	22.8±8.0
End-diastolic size of left ventricle, cm	7.2±1.3	7.2±0.9	7.9±0.7	8.5±0.8
End-diastolic volume of left ventricle, ml	250.9±101.1	239.3±55.6	267.6±91.8	282.1±78.5
BNP, pg/ml	924.2±201.3	895.6±126.7	754.7±96.4	1132.6±153.9

All patients operated for CCF, included in the study (groups 3 and 4), were comparable by the main characteristics (Table 1). Heart cavities were dilated, the mean end-diastolic volume of the left ventricle in groups 3 and 4 was higher than in groups 1 and 2, respectively, the pumping function of the heart was reduced. The BNP level surpassed the normal value by 18.9 times in group 4 and by 12.6 times in group 3. In general, cell transplantation was carried out in patients after surgical treatment in extremely grave cases, when other therapeutic and surgical potentialities were exhausted.

According to modern concepts, DCMP is associated with genetic mutations [11], and hence, transplantation of autogenic cells carrying these mutations can fail to bring about good results. Allogenic donor mesenchymal multipotent stromal cells (MSC) were used. These cells are immunopriviledged, carry no type II main histocompatibility complex on their surface, and are characterized by immunosuppressive and antiinflammatory activities [14].

MSC were isolated and cultured in the Stem Cell Laboratory of ReMeTex Company according to the approved protocol [6]. Cell phenotype of the resultant cultures was evaluated by specific positive (CD44, CD90, CD105) and negative (CD34, CD45) markers. The percentage of these cells in the cultures was ≥80-90% according to the cell culture passport. Functional activity of multipotent MSC culture was evaluated by the capacity to directed differentiation into mesodermal cells (myogenesis, chondrogenesis, osteogenesis, adipogenesis) in standard media.

Several hours before transplantation, the cells were removed from Petri dishes, thoroughly washed (repeated centrifugation) from culture medium components, resuspended in 10 ml 10% hydroxyethylstarch solution (infucol), and transported at 4°C into angiographic laboratory.

All data characterizing the cell culture, including the results of cytogenetic analysis, bacteriological and virological data, were listed in the cell transplant passport attached to the case history.

The cell transplant was injected intracoronarily under conditions of angiographic laboratory. The immediate results were evaluated 1 week and 1 and 3 months after cell transplantation, late results were evaluated after 6 and 12 months.

The data were statistically analyzed using Excel 2003 and Statistica 6.0 software. The data were mainly presented as the means and standard deviations. The significance of differences between the groups was evaluated using nonparametric Mann–Whitney test, the significance of differences between the characteristics before and after the intervention in the same group was evaluated using nonparametric Wilcoxon test.

RESULTS

Safety. Not a single anginal attack, elevation or depression of *ST* segment, or changes in the final part of the ventricular complex were recorded during the procedure and in the immediate postoperative period. Laboratory markers of acute myocardial infarction were within the normal range of values. Hypokinesia and akinesia zones were evaluated 1 week after cell transplantation. In none patients differences between the initial values and the parameters during the immediate postoperative period were recorded. Coronarography carried out directly after injection of the cell transplant showed no signs of embolism, all arteries were patent. Cell transplantation did not lead to appreciable changes in hemodynamics: the initial mean systolic blood pressure (BP) was 97.5±15.8 mm Hg and did not change much during the procedure and the subsequent 24 h; after 24 h, BP was 100.1±21.8 mm Hg.

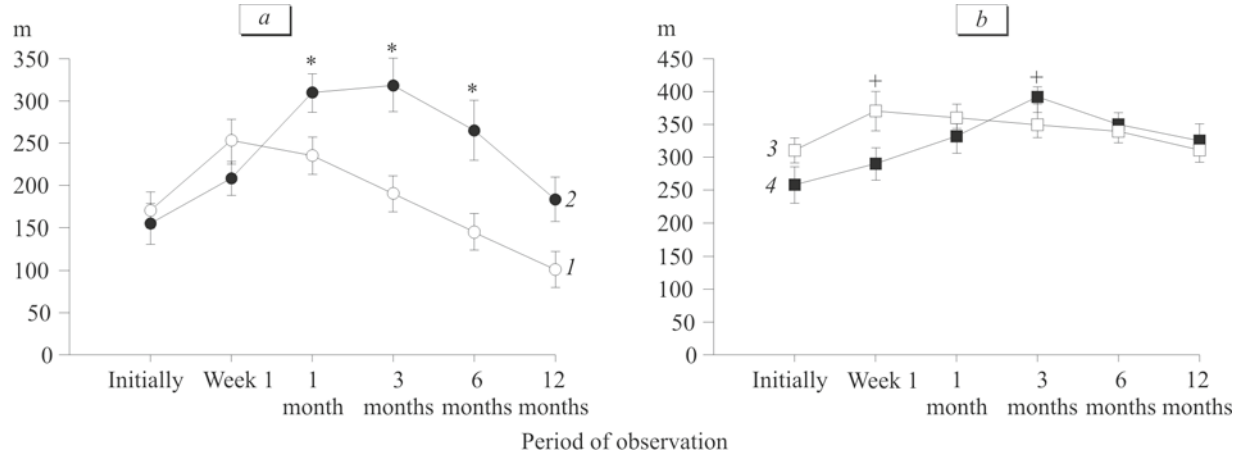

Fig. 1. Dynamics of the mean distance in the 6-min test in patients without operation (*a*) and in patients operated for CCF (*b*). Here and in Figs. 2-6: *1*) group 1 (no operation; no transplantation); *2*) group 2 (no operation; transplantation); *3*) group 3 (operation; no transplantation); *4*) group 4 (operation; transplantation). $p<0.05$ compared to: *group 1, +group 3.

Inflammation markers did not appreciably change after cell transplantation. No allergic or pyrogenic reactions were observed. No increase of ventricular arrhythmia class after Lawn or other complications of cell therapy were recorded during the delayed period.

Survival. No lethal outcomes were recorded during transplantation and the immediate postoperative period. All patients survived for 1 year after cell transplantation. In group 3, four patients died by month 12 of observation from progressive cardiac failure.

Cell transplantation efficiency. The functional status improved starting from week 1 after transplantation in all patients of group 2. The improvement reached the maximum values by month 3 and persisted until month 6 posttransplantation (Fig. 1, *a*). The distance in the 6-min test increased significantly (more than 2-fold: from 155.0±23.9 to 318.3±31.3 m), the functional status also improved (from FC 3.5 to 2.2; Fig. 1, *a*; Fig. 2, *a*).

In group 1, the functional status also improved from week 1 until month 1. The improvement of the function in both groups of non-operated patients during these periods can be explained by the choice of the optimal therapy and its more stringent monitoring. In group 2, the function improved more significantly, and later this parameter further improved in these patients in comparison with group 1 patients demonstrating negative dynamics of the functional status.

Positive changes in the functional status were recorded after cell transplantation in patients operated for CCF (group 4; Figs. 1, *b*; 2, *b*). A statistically significant increase in the distance in the 6-min test was recorded in group 4 starting from week 1 until month 12 of observation. The maximum improvement was noted 3 months after transplantation: the distance increased more than 1.5 times (from 258.0±27.7 to 392.0±15.9 m) and the functional status improved from FC 2.8 to 1.8. In group 3, the functional status virtually did not change throughout the entire period of observation.

Persuasive positive changes in BNP level were observed. One week after transplantation it decreased by 23.3% (from 895.6±126.7 to 687.3±70.8 pg/ml; Fig. 3, *a*) in non-operated patients (group 2). Then, BNP concentration smoothly increased, but remained below the initial level. In group 1, negative changes with

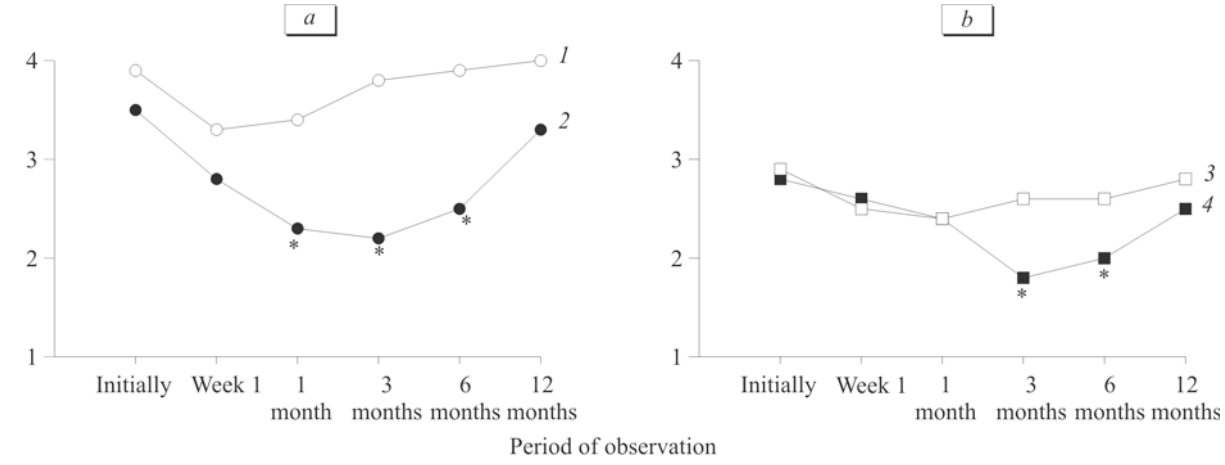

Fig. 2. Dynamics of mean NYHA functional class in non-operated (*a*) and operated patients (*b*).

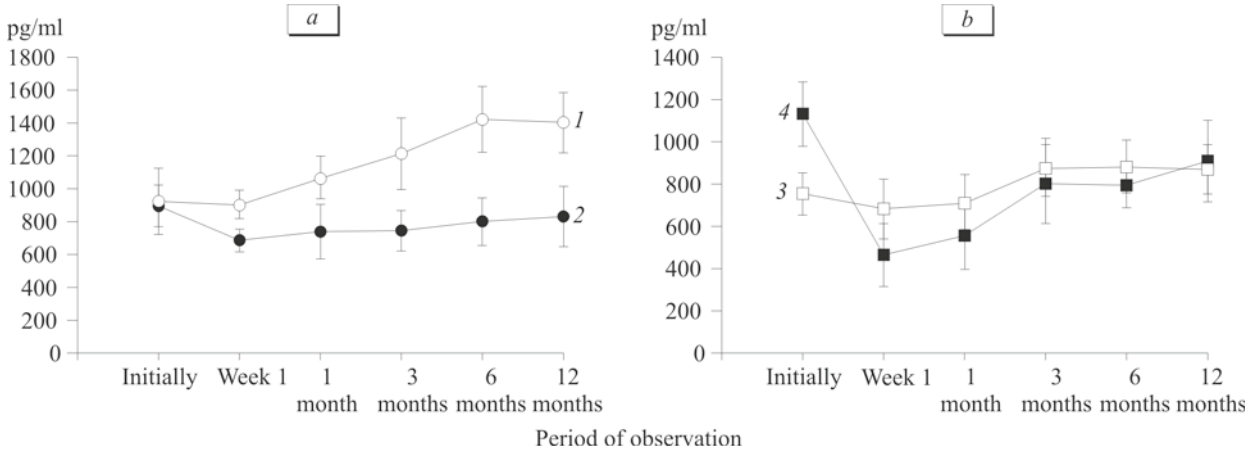

Fig. 3. Dynamics of the mean BNP concentration in non-operated (*a*) and operated patients (*b*).

increase in BNP level by 53.8% (from 924.2±201.3 to 1421.5±198.2 pg/ml) by month 6 were recorded.

A pronounced (58.9%) decrease in BNP concentration (from 1132.6±153.9 to 465.6±147.8 pg/ml; Fig. 3, *b*) was observed after cell transplantation in operated patients (group 4). Later, the peptide concentration increased, but remained far below the initial level, while in group 3 (no transplantation) this parameter virtually did not change.

Changes in the pumping function of the heart were noted in non-operated patients with CCF throughout 6 months after transplantation (group 2; Fig. 4, *a*). By month 3, the ejection fraction increased by more than 5%, but the differences were statistically insignificant. By month 12, the parameters returned to the initial level. An opposite trend was observed in group 1. The ejection fraction decreased by more than 5% by month 12 of observation. Presumably, cell transplantation decelerated deterioration of cardiac pumping function characteristic of the natural course of CCF in DCMP.

Parameters of the left-ventricular pumping function were stable throughout 12 months in patients previously operated for CCF (groups 3 and 4; Fig. 4, *b*). Changes during various periods of observation were negligible and did not surpass 5%.

The size and volume of the left ventricle of the heart virtually did not change in all the groups. A trend to a slight reduction of the left-ventricular size was observed after cell transplantation, but the difference did not surpass the error in measurements. In group 1 (no operation, no transplantation), a trend to dilatation of heart cavities was observed.

A statistically significant improvement of the quality of life was observed after cell transplantation. It was evaluated using the Minnesota questionnaire ("Life with Heart Failure") and DASI questionnaire (physical activity index) evaluating the peak oxygen consumption. According to MLHFQ questionnaire, the maximum and significant effect manifested in non-operated patients with CCF by month 3 (Fig. 5, *a*). According to DASI questionnaire, the initial peak oxygen consumption was very low in these patients and corresponded to NYHA FC III-IV. By month 3 after cell transplantation (group 2), this parameter increased

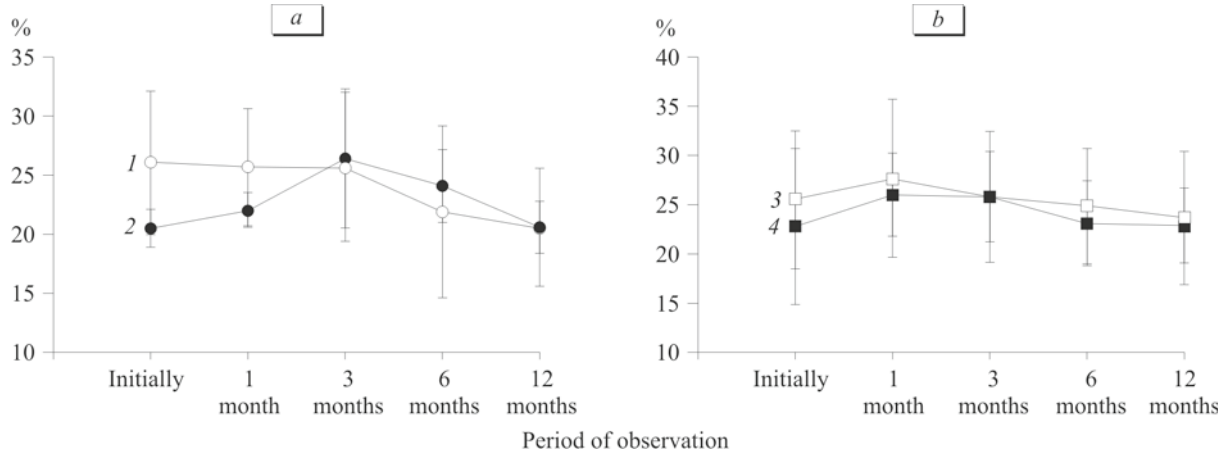

Fig. 4. Dynamics of left-ventricular ejection fraction in non-operated (*a*) and operated patients (*b*).

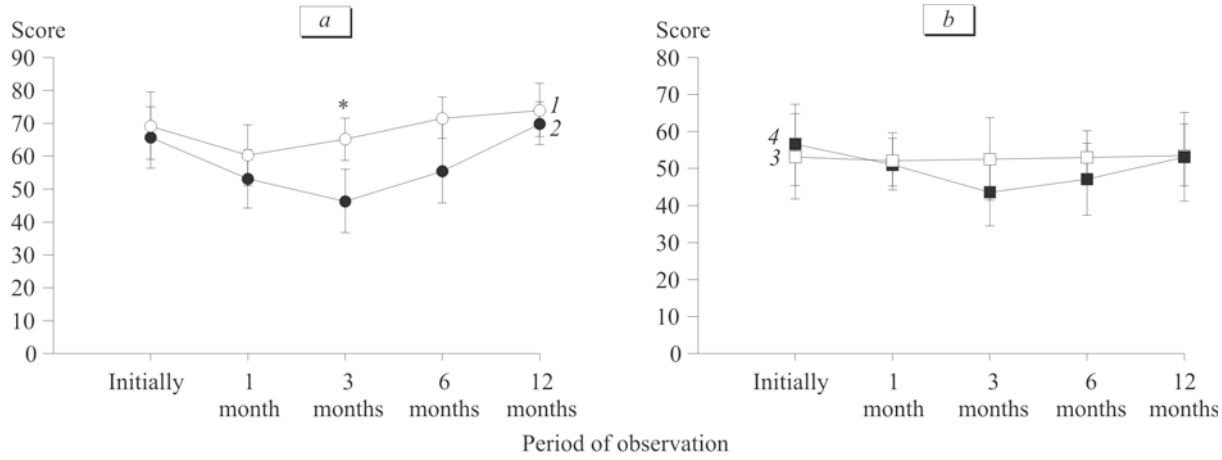

Fig. 5. Dynamics of quality of life according to MLHFQ in non-operated (*a*) and operated patients (*b*).

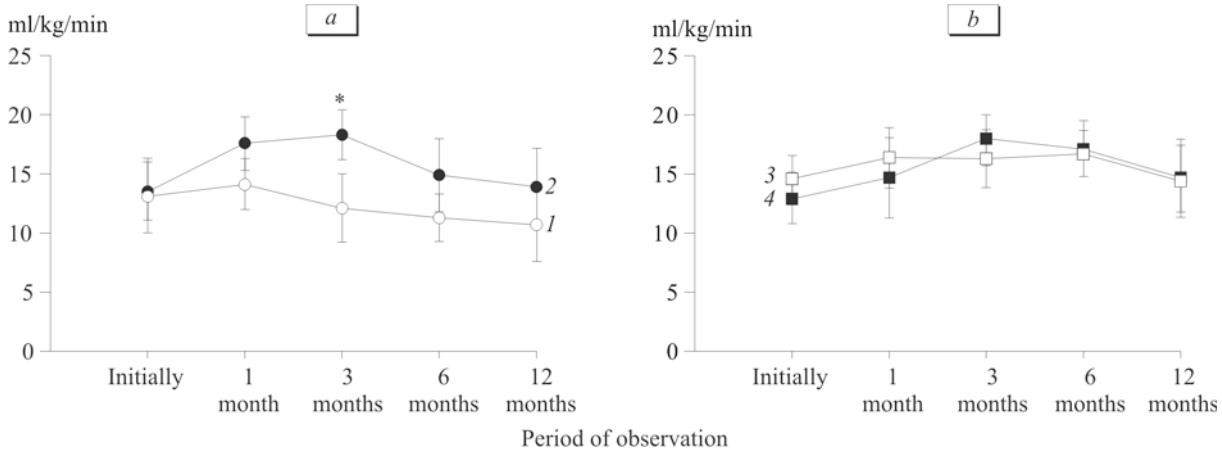

Fig. 6. Dynamics of peak oxygen consumption according to DASI questionnaire in non-operated (*a*) and operated (*b*) patients.

significantly, which reflected improvement of the functional status by at least 1 FC (Fig. 6, *a*).

Cell transplantation to patients previously operated for CCF (group 4) promoted improvement of the quality of life throughout the entire period of observation (12 months). The maximum and statistically significant effect manifested by month 3 of observation. The quality of life in patients without cell transplantation (group 3) virtually did not change.

Presumably, induction or the paracrine effect played the major role in reduction of the severity of heart failure after cell transplantation. Transplanted cells release numerous bioactive substances modulating microenvironment and stimulating cardiomyocytes of the recipient and the whole body by affecting the humoral mechanisms of CCF development [7]. We observed a reduction of cardiac failure severity, which was seen from the level of BNP and the functional status.

However, we observed no relationship between the dynamics of BNP and left-ventricular ejection fraction. Our results are in line with published data, indicating that left-ventricular ejection fraction increases after stem cell transplantation by no more than 5% [8,15]. On the other hand, other diagnostic methods (measurement of plasma BNP level, 6-min test, peak oxygen consumption) better correlate with clinical improvement and prognosis in CCF [10].

It is noteworthy that the parameters of the cardiac pumping function and the size and volume of the left ventricle did not deteriorate, but even somewhat improved throughout the entire period of observation after cell transplantation. Hence, it seems that stabilization of patients' status and prevention of further progress of heart failure were due to cell transplantation.

Intracoronary transplantation of allogenic MSC to patients with severe CCF (NYHA FC III-IV) in the presence of DCMP is not associated with complications and side effects and hence, is a safe procedure. Cell transplantation reduced the severity of cardiac failure. The positive effect develop starting from week 1 after transplantation and persisted up to 6 months, and hence, repeated cell transplantations are advisable. Cell therapy with allogenic MSC improves the efficiency of preoperative treatment of patients and the results of surgical treatment of CCF in the presence of DCMP.

REFERENCES

1. F. T. Ageyev, M. O. Danielyan, V. Yu. Mareyev, *et al.*, *Serdechn. Nedostat.*, **5**, No. 1, 4-7 (2004).
2. Yu. N. Belenkov, V. Yu. Mareyev, and F. T. Ageyev, *Ibid.*, **4**, No. 3, 116-121 (2003).
3. Yu. N. Belenkov and V. Yu. Mareyev, *Philosophy of Rational Therapy for Heart Failure* [in Russian], Moscow (2000).
4. A. V. Koroteyev and I. E. Belyanko, *Surgical Treatment of Heart Failure* [in Russian], Moscow (2007), pp. 204-223.
5. *National Recommendations of VNOK and OSSN for Diagnosis and Therapy of CCF (Second Revision)*, *Serdechn. Nedostat.*, **8**, No. 2, 1-35 (2006).
6. A. A. Rzhaninova, S. N. Gornostayeva, and D. V. Goldstein, *Byull. Eksp. Biol. Med.*, Suppl., 39-43 (2003).
7. J. Burchfield and S. Dimmeler, *Fibrogenesis Tissue Repair*, **1**, 4 (2008).
8. J. Hescheler, A. Welz, and B. K. Fleischmann, *Lancet*, **364**, 121-122 (2004).
9. H. Krum, *Eur. Heart J.*, **26**, No. 22, 2472 (2005).
10. N. Li and J. A. Wang, *J. Zhejiang. Univ. Sci.* B, **7**, No. 9, 766-768 (2006).
11. T. M. Olson, *Acta Paediatr. Suppl.*, **95**, No. 452, 17-21 (2006).
12. D. Orlic, J. Kajstura, S. Chimenti, *et al.*, *Nature*, **410**, No. 5, 701-705 (2001).
13. E. C. Perin, G. V. Silva, J. A. Assad, *et al.*, *J. Mol. Cell. Cardiol.*, **44**, No. 3, 486-495 (2008).
14. J. M. Ryan, F. P. Barry, J. M. Murphy, and B. P. Mahon, *J. Inflamm.* (London), **2**, 8 (2005).
15. V. Schächinger, B. Assmus, M. B. Britten, *et al.*, *J. Am. Coll. Cardiol.*, **44**, No. 8, 1690-1699 (2004).