Transplantation of Allogenic Cells in the Therapy of Patients with Dilated Cardiomyopathy

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We carried out a pilot study on intracoronary transplantation of prenatal allogenic skeletal myoblasts and multipotent bone marrow stromal cells to patients with dilated cardiomyopathy. Intracoronary transplantation of allogenic cells is a feasible and safe procedure: there were no life-threatening rhythm and conduction disturbances, symptoms of hemodynamic instability, and thromboembolic complications. Positive clinical effect of cell transplantation persisted for 6 months and consisted in decreased content of brain natriuretic peptide and improved tolerance to physical exercises. We observed no reliable dynamics of the major echocardiographic parameters and their correlations with patient's status.

Key words: *skeletal myoblasts; multipotent stromal cells; allogenic transplantation; dilated cardiomyopathy*

Chronic heart failure (CHF) is the leading cause of mortality in developed countries. CHF most often develops as a result of dilated cardiomyopathy, which is characterized by rapid progressing and unfavorable outcome. Heart transplantation is a golden standard in the therapy of dilated cardiomyopathy [4,8], but this procedure is not available for many patients due to severe shortage of donor organs. In light of this, principally new methods of CHF therapy are required.

Recent progress in biotechnology suggests that cell transplantation is a promising method for the treatment of CHF. In recent years, some experimental studies on transplantation of phenotypically different cells, such as myoblasts (MB), hemopoietic stem cells, multipotent stromal cells (MSC) and embryonic stem cells into the myocardium were performed. Impressive results in this field were obtained [3,10,13]. However, the possibility of using allogenic MSC and MB in dilated cardiomyopathy was not discussed despite the known fact that this pathology is associated with a number of genetic mutations [11] and the use of autogenic cells carrying these mutations can lead to degenerative changes in cardiomyocytes and produce no desirable clinical effect. The target of cell transplantation in dilated cardiomyopathy is not stimulation of angiogenesis, but recovery and stimulation of contractile elements of the myocardium, therefore transplantation of MB and MSC replacing damaged cardiomyocytes and restoring contractile function of the myocardium can be useful [7,14].

The absence of clear-cut concept on regeneration capacities of the myocardium and the risk of complications in allogenic cell transplantation hamper active use of cell technologies in the therapy of patients with dilated cardiomyopathy.

The aim of the present study was to evaluate the safety, dynamics of clinical and morphological state, size, and contractile functions of the heart in patients with dilated cardiomyopathy after intracoronary transplantations of allogenic MSC and MB.

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MATERIALS AND METHODS

This pilot uncontrolled study was approved by Academic Council and Ethical Committee of B. V. Petrovskii Russian Research Surgical Center, Russian Academy of Medical Sciences, and meets the principles of the Declaration of Helsinki. All patients signed informed consent for participation in the study.

Cell transplantation was performed in 16 men with idiopathic dilated cardiomyopathy: 9 patients (group 1) received prenatal MB, 7 patients (group 2) received prenatal MSC. The results were evaluated after 1, 3, and 6 months.

Clinical study included patients with clinically documented (according to anamnestic data and instrumental tests) idiopathic dilated cardiomyopathy; with dilated heart cavities (end-diastolic diameter and volume of the left ventricle 6.5 cm and 160 ml, respectively, according to transthoracic echocardiography data), decrease in heart pumping functions (ejection fraction, EF, not exceeded 35%, according to transthoracic echocardiography data); pronounced heart failure against the background of optimum drug therapy (NYHA functional class II-IV by the results of 6-min testing).

Exclusion criteria were: cardiac surgery less than 1 year before this study, history of autoimmune diseases, polyvalent allergy, decompensation of concomitant diseases, radical changes in drug treatment or surgery during the observation period, and neoplasms.

Clinical and functional state of all enrolled patients was severe (Table 1); 47% patients had NYHA class IV. Dilation of heart cavities was confirmed by instrumental studies. Initial level of brain natriuretic peptide (BNP) in the blood considerably surpassed the normal in all cases. All patients received optimal drug therapy approved for the treatment of CHF [2]. The therapy remained unchanged throughout the observation period.

Cell transplants, a suspension of 10^9 MB or MSC in 10% hydroxyethyl starch (Infukol GEK), were prepared under conditions of stem cell GMP laboratory (ReMeTeks Company).

Bone marrow stromal MSC isolated from autopsy material of human fetuses (18-20 gestation weeks) obtained during medical abortions were cultured routinely. The phenotype of obtained cultures was evaluated by flow cytofluorometry by specific positive $CD44^+$, $CD90^+$, $CD105^+$ and negative $CD34^-$, $CD45^-$ markers. Not less than 80-90% MSC were detected in cultures. Functional activity of MSC culture was evaluated by the capacity to directed mesodermal differentiation (mitogenesis, chondrogenesis, osteogenesis, adipogenesis) in standard media.

Cultures of skeletal MB were also obtained from autopsy material of human fetuses. The cells were isolated and growth according to standard protocol [16]. The obtained cells were identified as skeletal MB by the formation of typical myotubes during culturing.

Several hours before transplantation the cells were harvested from plastic Petri dishes, thoroughly washed from culture medium components, resuspended in 10 ml 10% hydroxyethyl starch (Infukol GEK), and transported at 4°C to the angiography department.

The cells were transplanted intracoronary under conditions of the angiography department. Standard approach through the femoral artery was used. Coronarography was performed for evaluation of the state of the coronary bed. The cell suspension was adjusted to a volume of 50 ml with physiological saline and infused into the left and right coronary arteries over 30 min with repeated shaking (infusion rate 100 ml/h). The patients remained in the intensive care unit during the subsequent 24 h. For evaluation of possible disturbances in heart rhythm, conduction, and myocardial perfusion we used ECG monitoring both before the procedure and during the subsequent 24 h in the intensive care unit. Blood pressure (BP) monitoring was additionally performed.

The safety of the procedure was evaluated by the development of possible side effects (rhythm and conduction disturbances by the data of ECG monitoring and ECG recording in delayed terms, hemodynamics instability during the transplantation, pyrogenic and other reactions, negative dynamics in the clinical state of the patient).

Clinical efficiency was evaluated using the 6-min test, NYHA class, and echocardiographic data (stroke volume, EF, size and volume of heart cavities). The severity of heart failure was qualitatively evaluated by plasma level of BNP.

The data were processed statistically using MS Excel 2003 and Statistica 6.0 software. Significance of differences was evaluated using nonparametric Wilcoxon test.

RESULTS

ECG monitoring showed that cell transplantation was not associated with ventricular tachycardia or other life threatening rhythm and conduction disturbances. BP monitoring revealed no signs of hemodynamic instability. Angiography during intracoronary infusion of cells detected no signs of thrombotic complications.

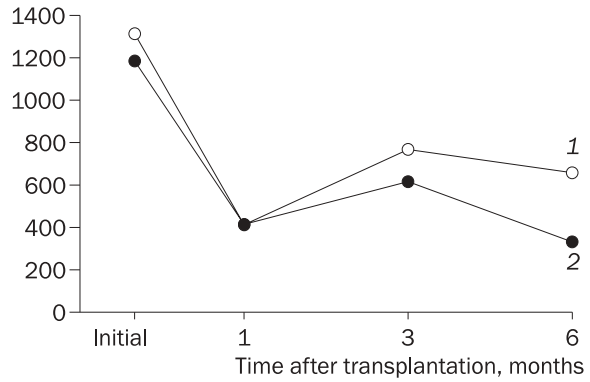

Fig. 1. Dynamics of BNP level in patients with dilated cardiomyopathy receiving transplantation of allogenic cells.

In group 1, heart failure progressed in 1 patient 5 months after the procedure and caused his death. In group 2, all patients survived.

Pronounced positive dynamics by the level of BNP was noted. In group 1 this parameter decreased from 1314.12±978.00 to 412.85±169.50 pg/ml after 1 month and to 658.28±189.80 after 6 months; in group 2 this parameter decreased from 1089.36± 602.97 to 541.34±335.34 and 294.36±240.30 pg/ml, respectively (Fig. 1).

Functional state of patients improved during observation (Fig. 2, 3). In group 2 this positive dynamics was more pronounced. In group 1, 6-min walking distance increased from 222.2± 87.0 to 345.71±91.99 m after 3 months and to 314.28± 121.50 m after 6 months, *i.e.* by 55.6 and 41.4%, respectively. NYHA class also significantly decreased from 3.00±0.87 to 2.1±0.7 after 3 months and to 2.4±0.8 after 6 months. In group 2, the distance significantly increased from 171.4±146.8 to 324.49±185.99 and 255.7±189.1 m after 3 and 6 months, respectively, *i.e.* by 89.2 and 49.2%. In this group, NYHA class after 3 and 6 months decreased from 3.30±0.76 to 2.14±1.34 ($p<0.05$) and 2.57±1.39, respectively.

In both groups, the contractile function of the heart tended to improve over 3 months, but by the 6th months parameters of the pumping function returned to the initial level (Figs. 4 and 5). The maximum increase in heart contractility was observed 3 months after transplantation (echocardiography data). In group 1, EF increased from 23.4± 6.7 to 25.4±11.1% and stroke volume from 61.5± 30.9 to 77.6±59.8 ml; in group 2, EF increased from 24.4±10.3 to 26.0±7.6% and stroke volume from 56.8±41.2 to 72.3±57.7 ml (Table 1, 2).

The experience is now accumulating on application of cell technologies in the therapy of heart failure and the possibility of combined approach to the treatment of heart failure including surgery and stem cell transplantation [12]. However, there is no consensus on the safety and efficiency of cell therapy, especially in allogenic transplantations, *e.g.* for the therapy of CHF. Some authors report that stem cell transplantation considerably increases myocardial contractility and improved patients' status [13,15], while others observe no positive dynamics after stem cell transplantation and warn that this technology can led to serious complications [9], *e.g.* heart rhythm disturbances, immunological complications, thrombotic complications and embolism, allergic reactions. We observed no undesirable effects in our study.

We used prenatal allogenic skeletal MB and bone marrow MSC for the therapy of dilated cardiomyopathy. This choice was determined by experimentally proven capacity of MSC to differentiation into not only vascular cells (endothelial and smooth muscle cells), but also into cardiomyocytes. The possibility of restoration of contractile elements of the myocardium after transplantation of MB and MSC is of critical importance in the choice of cell transplant for the therapy of dilated cardiomyopathy, because this therapy is aimed at recovery of the contractile function, but not angiogenesis. The

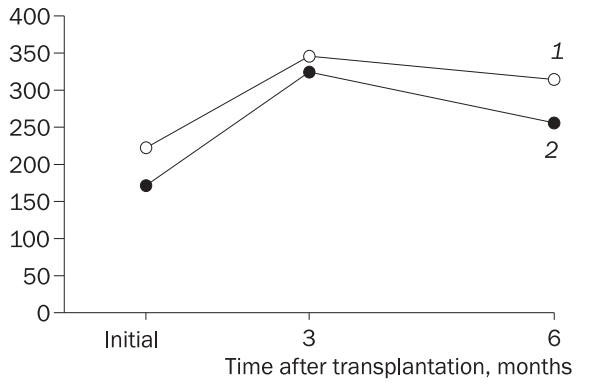

Fig. 2. Dynamics of 6-min test class in patients with dilated cardiomyopathy receiving transplantation of allogenic cells.

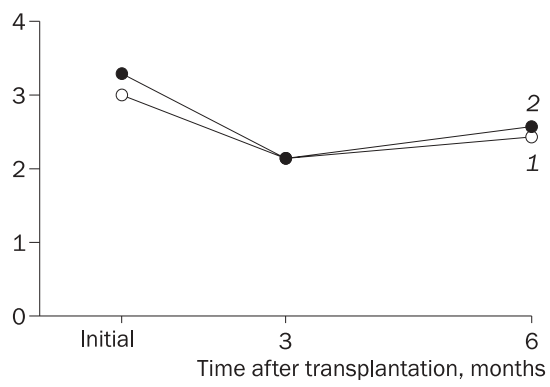

Fig. 3. Dynamics of NYHA class in patients with dilated cardiomyopathy receiving transplantation of allogenic cells.

TABLE 1. Initial Characteristics of Patients Receiving Transplantation of Allogenic Cells

Parameter	Group	
	1	2
Age, years	38.3 (22-65)	53.3 (45-68)
NYHA class	3.00±0.87	3.30±0.76
6-min test, m	222.2±87.0	171.4±146.8
EF, %	23.4±6.7	24.37±10.28
EDD, cm	7.54±1.10	7.9±1.2
EDV, ml	265.02±94.60	235.93±66.47
BNP, pg/ml	1314.12±978.00	1089.36±602.97

Note. Median values for the specified age are shown in parentheses. Here and on Fig. 2 and Table 2: EDD and EDV are end-diastolic diameter and volume of the left ventricle, respectively.

use of autogenic skeletal MB and MSC in dilated cardiomyopathy is inexpedient, because this variant of cardiomyopathy is associated with gene mutations [11] and transplantation of cells carrying pathological alleles can be safe, but ineffective. Myoblasts and MSC do not carry class II histocompatibility molecules, while MSC exhibit immunosuppressive and anti-inflammatory activity, which was demonstrated in *in vivo* and *in vitro* experiments [1,5].

We observed considerable positive dynamics by all studied parameters in the group with MSC transplantation, most pronounced during the 3rd month of observation. Despite initial similarity of the experimental groups, patients' survival was higher in group 2 (MSC transplantation); 6 months after the procedure this parameter was 100%. Plasma level of BNP considerably decreased in all patients included in the study. At the same time, echocardiographic parameters of the pumping function of the heart improved insignificantly. Our results are consistent with the data obtained in other studies where no correlation between the dynamics of BNP level and EF were found [6,15]. EF is a calculated echocardiographic parameter affected by various intra- and extracardiac factors. The level of

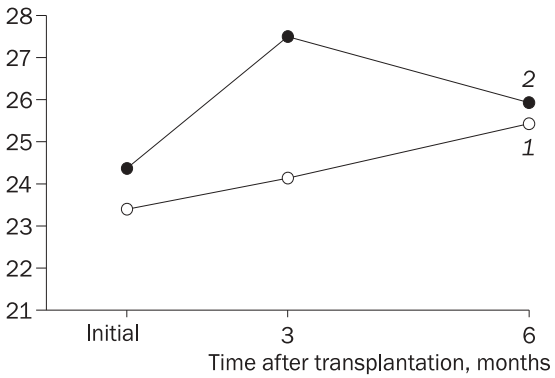

Fig. 4. Dynamics of EF of the left ventricle (echocardiography data) in patients with dilated cardiomyopathy receiving transplantation of allogenic cells.

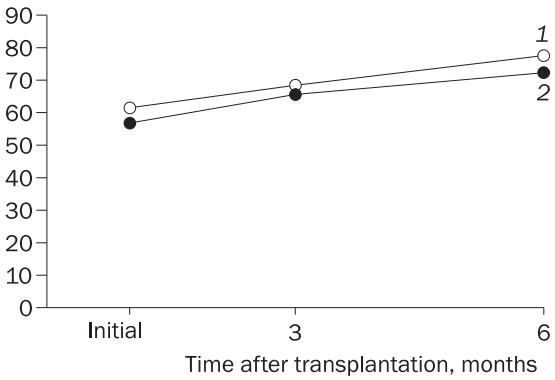

Fig. 5. Dynamics of stroke volume in patients with dilated cardiomyopathy receiving transplantation of allogenic cells.

BNP is determined automatically and does not depend on investigator's subjective evaluation. Taking these reasons into account we conclude that blood level of BNP is a more objective criterion of the state of myocardium and functional state of the cardiovascular system on the whole.

Thus, transplantation of allogenic MB and MSC can be safe and effective procedure and can be included into complex therapy of heart failure caused by dilated cardiomyopathy. There are some bioethical and legal limitations for the use of prenatal cells. Taking into account the positive experience of transplantation of prenatal allogenic cells

TABLE 2. Dynamics of Heart Size and Volume in Patients Receiving Transplantation of Allogenic Cells

Parameter	Group 1			Group 2		
	initial	after 3 months	after 6 months	initial	after 3 months	after 6 months
EDD, cm	7.54±1.10	7.8±1.2	7.8±1.3	7.9±1.2	7.5±1.3	7.1±1.3
ESD, cm	6.69±0.90	6.66±1.10	6.5±1.2	6.63±1.20	6.34±0.95	6.09±1.01
EDV, ml	265.02±94.60	280.86±81.80	298.86±95.60	235.93±66.47	229.71±69.84	235.86±92.32
ESV, ml	201.51±71.40	212.40±65.99	218.57±70.07	179.34±58.76	164.14±43.61	166.00±43.96

Note. ESD and ESV are end-systolic diameter and volume of the left ventricle, respectively.

in dilated cardiomyopathy we will continue our studies with allogenic MSC obtained from healthy adults.

The results of this pilot study drove us to the following conclusions. Intracoronary transplantation of prenatal allogenic skeletal MB and MSC is safe for clinical use and leads to improvement in clinical and functional state of patients with dilated cardiomyopathy. The positive clinical effect of cell therapy was observed within 6 months after transplantation.

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