Cardiac Contractility after Transplantation of Autologous Mononuclear Bone Marrow Cells in Patients with Myocardial Infarction

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Autologous bone marrow mononuclear cells were transplanted by intracoronary infusion to patients with myocardial infarction after recovery of coronary perfusion. Controls received traditional therapy alone. Echocardiography was carried out before and 3 and 6 months after cell therapy. Cell transplantation did not appreciably improved left-ventricular contractility in comparison with the control group. In none patient cell therapy provoked malignant ventricular arrhythmias. Intracoronary infusion of bone marrow mononuclear cells in patients with myocardial infarction did not improve cardiac contractility and did not aggravate the course of the disease.

Key Words: *myocardial infarction; transplantation; bone marrow mononuclear cells; cardiac contractility*

Until recently cardiomyocytes were considered terminally differentiated cells incapable of proliferation. However, findings of J. Kajstura *et al.* [7,13] led to revision of this opinion. It is now considered that heart cells are capable of normal division involving not only the nucleus (karyokinesis), but also the cytoplasm (cytokinesis) [3,4,7,13]. Proliferative activity of human cardiomyocytes increases 80-fold during myocardial infarction [7,13]. However, such mitotic activity is observed only in the periinfarction area; no dividing cardiomyocytes were detected in necrotic focus [7,13]. Similarly as myocardial cells, endotheliocytes actively proliferate only in the periinfarction area [7,13]. On the other hand, fibroblasts, less sensitive to hypoxia in comparison with endotheliocytes and cardiomyocytes, actively divide in the necrotic focus as well [7,13], and that is why infarction never eventuates in full-value regeneration of the heart, but in a cicatrix formation at the site of necrotic focus.

Experimental studies showed that myocardial regeneration can be appreciably stimulated by transplantation into the myocardium of regional stem cells (SC) isolated from the bone marrow or skeletal muscles (satellite cells) [3-5]. Clinical observations of patients with acute and subacute myocardial infarction showed that transplantation of autologous (isolated from the same patient) bone marrow mononuclear cells (BMMC) stimulated the pumping function of the heart and improved coronary perfusion of the myocardium [1,6,8,10,14]. Bone marrow mononuclear fraction contains up to 2% hemopoietic SC and up to 0.5% mesenchymal SC [14], and hence, it is hypothesized that improvement of contractility and coronary perfusion is due to transdifferentiation of transplanted SC into cardiomyocytes and endotheliocytes and hence, partial regeneration of infarcted heart [6,8,10,14]. Improvement of the pumping function of infarcted myocardium was observed also after intracardiac transplantation of autologous myoblasts [12]. However, in these stu-

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dies the positive effect of cell therapy was evaluated by comparing cardiac contractility and coronary perfusion parameters in the same patient before and after cell transplantation [6,8,10,12]. However, all these patients in addition to cell therapy, were subjected to coronary artery stenting [6,8,10, 14] or aortocoronary shunting [12], which improved coronary perfusion and pumping function of the myocardium. It remains unclear whether the positive clinical effect of treatment was due to cell transplantation or resulted from revascularization of infarcted myocardium. In addition, the number of patients with transplanted cells was no more than 9-15 in the majority of clinical studies [6,10,12, 14]. Only the study carried out by M. B. Britten *et al.* [8] covered 26 patients, but this group included patients receiving intracoronary infusion of mononuclear cells isolated from the bone marrow and of blood mononuclears, and it is not clear which of these cells brought about the therapeutic effect. These studies were carried out in two hospitals in Germany [6,8,10,14] and a hospital in France [12]. The study carried out in Russia included only 5 patients with acute myocardial infarction (AMI) [1]. Therefore, it is early to make a conclusion about high efficiency of cell therapy of myocardial infarction.

We tried to escape the flaws of previous studies and evaluated the effect of autologous BMMC transplantation on cardiac contractility in patients with myocardial infarction in a large group of patients (16 volunteers); initial (before transplantation) and final (after BMMC transplantation) parameters were compared in the same patients; comparative analysis of parameters in the control and main groups was carried out.

MATERIALS AND METHODS

Clinical characteristics of the patients are presented in Table 1. The diagnosis of myocardial infarction was verified by the presence of the following signs: 1) emergence of an attack of pain characteristic of myocardial ischemia of at least 30 min long; 2) emergence of pathological *Q* waves on the ECG; 3) creatine phosphokinase activity 2-fold higher than the upper threshold normal value. The size of myocardial infarction zone was evaluated by estimating *QRS* index in 12 standard ECG leads [15]. The main group was formed on the basis of the following criteria: 1) age under 75 years; 2) transmural infarction of the left ventricle; 3) no serious disorders in heart rhythm (ventricular tachicardia and ventricular fibrillation). The protocol of the study was approved by Ethical Committee of Institute of Cardiology. Cell transplantation was carried out after informed consent of the patients. The main group (BMMC transplantation) consisted of 16 patients, control group of 10 patients.

Recanalization of coronary artery involved in infarction (in 4 and 5 patients of the main and control groups, respectively) was carried out by balloon angioplasty and stenting (Penta™ and Guidant stents) on a Coroskop+angiographic complex (Siemens). Other patients received systemic thrombolytic therapy (750,000 U streptokinase; Belmedpreparaty Firm) on admission. In this case the time of the antegrade bloodflow recovery in involved coronary artery was evaluated by indirect criteria of myocardial reperfusion: decrease of *ST* segment elevation in informative ECG leads by at least 26% of initial level within 60-90 min from the beginning of thrombolysis; relief or arrest of pain attack; presence of reperfusion arrhythmias; plasma CPK activity peak attained within the first 16 h from the disease onset. Reperfusion was considered realized in the presence of at least two signs, one of which (reduction of *ST* segment) was obligatory.

Cell transplantation was carried out during repeated angiography on days 7-21 of myocardial infarction. We chose this period on the basis of published data on the clinical efficiency of autologous BMMC transplantation to patients with myocardial infarction on days 5-10 after coronary arteries thrombosis [6,8,10,14]. Cell material was derived by puncture of the upper flaring portion of the ilium under local anesthesia by 10% lidocaine. Bone marrow aspirate (100 ml) was collected into two 60-ml syringes with 25,000 U heparin in 10 ml 0.1 M phosphate buffer (pH 7.4). Mononuclear cells were isolated by gradient centrifugation as described previously [14]. Sterile polysucrose and diatrizoate (HISTOPAQUE-1077, Sigma-Aldrich) with density gradient of 1.077 g/ml was used. The morphology of isolated cells was evaluated in smears stained with Azur-eosin. The cells were counted in a Goryaev cell. BMMC were then suspended in sterile heparin-treated phosphate buffer (0.1 M, pH 7.4) to final concentration of $2\text{-}4\times10^6$ cells/ml. The resultant suspension was injected into the coronary artery involved in infarction after recovery of the bloodflow in this artery. Intracoronary infusion of BMMC was carried out at a rate of 4-8 ml/min over 5 min under conditions of normal blood flow.

Clinical status and disease course were evaluated during repeated examination of patients 3 and 6 months after discharge from hospital. Functional class of cardiac insufficiency was evaluated by common criteria of New York Heart Association. Exercise tolerance was evaluated by 6-min walking test [2]. Quality of life was evaluated by common Min-

nesota Questionnaire. Echocardiography was carried out on a VIVID 7, GE ultrasonic system (GE Medical System) by standard methods. Ultrasonic study was carried out before cell transplantation and 3 and 6 months after AMI. ECG was recorded before and 6 months after cell transplantation.

The main and control groups were similar by clinical characteristics (Table 1). All patients received drug therapy, including aspirin, plavix, statins, angiotensin-converting enzyme inhibitors, and β-adrenoblockers. All procedures of the protocol of the study were well tolerated by the patients; no complications were caused by bone marrow aspiration, during and after BMMC infusion.

The data were analyzed using Statistics 6.0 software (Stat Soft, Inc.). The differences were considered significant at $p<0.05$.

RESULTS

One patient of the main group and one control developed repeated myocardial infarction within the first 3 months after AMI. These patients were excluded from subsequent analysis. Angina attacks persisted in 2 patients of the main group. Repeated coronary angiography detected no restenosis of coronary arteries in them. Persistent anginal attacks seemed to be caused by coronary microangiopathy, which could not be verified angiographically.

We failed to detect significant changes in the end-diastolic volume, end systolic volume, and left-ventricular ejection fraction in the main and control groups during a 6-month follow-up period (Fig. 1). No significant differences between these parameters in the two groups were detected. Hence, intracoronary transplantation of BMMC during the subacute period of myocardial infarction had no appreciable effect on cardiac contractility.

No significant differences between the groups in cardiac insufficiency functional class, exercise tolerance, or quality of life were detected during 6-month clinical observation (Table 2). Moreover, we detected no significant differences between the initial and final parameters (functional class of cardiac insufficiency, exercise tolerance, and quality of life). No ventricular tachycardia and ventricular fibrillation was detected in any of the patients after cell therapy.

These facts suggest that intracoronary infusion of autologous BMMC did not provoke the development of malignant arrhythmias and did not aggravate the course of coronary disease after myocardial infarction. Hence, the use of BMMC is preferable to intracardiac transplantation of satellite cells, provoking the development of ventricular tachycardia resistant to antiarrhythmic drugs in many patients [12]. On the other hand, we failed to detect any positive effect of cell therapy. Intracoronary infusion of autologous BMMC during the subacute period of myocardial infarction had virtually no effect on cardiac contractility, cardiac insufficiency functional class, exercise tolerance, or quality of life. On the other hand, according to foreign authors, transplantation of BMMC improves cardiac contractility and has a positive impact on the course of cardiac insufficiency [6,8,10,14].

Virtually all foreign authors compared the initial values (before cell therapy) with resultant values (after transplantation) [6,8,10]. In addition to BMMC transplantation, recanalization of coronary

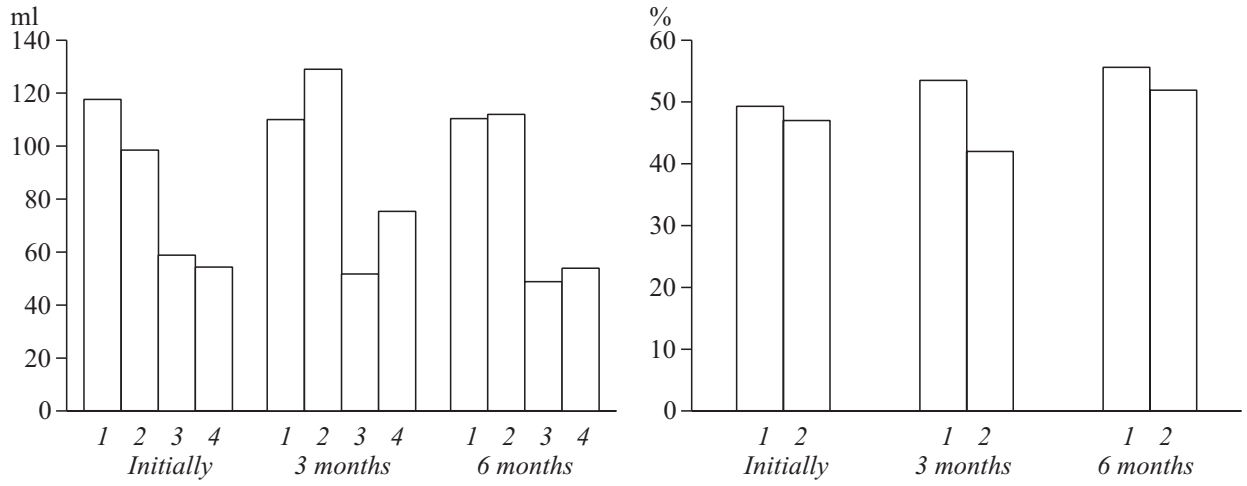

Fig. 1. Time course of end-diastolic volume (EDV) and end-systolic volume (ESV), (*a*); left-ventricular ejection fractions (EF) before and after therapy (*b*). Number of observations in the main group: 14; in control group: 9. For *a*: *1*) main group EDV; *2*) control group EDV; *3*) main group ESV; *4*) control group ESV. For *b*: *1*) main group; *2*) control group.

TABLE 1. Clinical Characteristics of Patients (*M*±*SD*)

Parameter	Control group, *n* (%)	Main group, *n* (%)	*p*
Number of patients	10	16	
Mean age	55.7±10.0	55.8±7.5	0.9
Male patients	7 (70)	15 (94)	0.2
Mean time of CAII recanalization, h	6.2±4.0	6.6±4.9	0.8
Anterior myocardial infarction	7 (70)	12 (75)	0.8
Distribution of patients depending on CAII: ADA/ACA/CA/LCA trunk	7(70)/3(30)/-/-	11(69)/3(19)/1(6)/1(6)	0.8
Number of patients depending on degree of coronary bed involvement 1/2/3 vessels involved	2(20)/4(40)/4(40)	2(12)/11(69)/3(19)	0.3
QRS index, % of LV involvement	7.3±3.9	9.1±4.2	0.3
Number of isolated BMMC, 10^6		88.5±49.2	
Signs of acute heart failure after T. Killip, FC I/II/III/IV	8(80)/1(10)/1(10)/-	9(56)/4(25)/2(13)/1(6)	0.3
Postinfarction angina	1(10)	1(6)	0.7

Note. FC: functional class; CAII: coronary artery involved in infarction; LV: left ventricle; ADA: anterior descending artery; ACA: anterior coronary artery; CA: circumflex artery; LCA: left coronary artery.

TABLE 2. Parameters Reflecting the Severity of Chronic Cardiac Insufficiency (*M*±*SD*)

Parameter	Control group	Main group	*p*
CCI FC before transplantation	1.3±0.5 *n*=10	1.5±0.8 *n*=16	0.2
CCI FC 3 months after AMI	1.5±0.9 *n*=9	1.2±0.4 *n*=15	0.1
CCI FC 6 months after AMI	1.5±0.8 *n*=9	1.2±0.4 *n*=15	0.1
6-min test before transplantation, m	432.9±124.5 *n*=10	429.6±116.0 *n*=16	0.5
6-min test 3 months after AMI, m	475.0±148.5 *n*=9	498.5±90.0 *n*=15	0.3
6-min test 6 months after AMI, m	493.0±118 *n*=9	563.0±143.0 *n*=15	0.09
Quality of life before transplantation, score	19.0±8.3 *n*=10	18.5±20.0 *n*=16	0.5
Quality of life 3 months after AMI, score	29.3±16.9 *n*=9	26.5±16.2 *n*=15	0.4
Quality of life 6 months after AMI, score	26.0±14.1 *n*=9	33.1±21.9 *n*=15	0.2

Note. CCI FC: chronic cardiac insufficiency functional class.

arteries by stenting or ballooning was carried out in all patients [6,8,10]. Hence, it is impossible to differentiate positive effect of cell therapy from positive effect of coronary angioplasty. Only B. E. Strauer *et al.* [14] compared the values in controls (stenting) and in the main group (stenting+BMMC transplantation). This clinical study detected a positive effect of cell therapy on the cardiac pumping function and course of cardiac insufficiency [14].

One of the causes of positive effect of BMMC transplantation in our study can be low content of mesenchymal SC in the isolated bone marrow mononuclear fraction. According to B. E. Staruer *et al.* [14], BMMC population contains up to 2% hemopoietic stem cells and up to 0.5% mesenchymal SC [14]. On the other hand, only mesenchymal, but not hemopoietic SC can participate in heart regeneration after transplantation into the myocardium

[9,11]. Presumably, the content of mesenchymal SC in BMMC fraction isolated in our study was significantly lower than in the study carried out by B. E. Strauer *et al.*, and that is why we detected no positive effect of cell therapy.

Inefficiency of cell therapy in our observations can be also explained by the fact that survival of transplanted SC largely depends on such growth factors as granulocytic CSF, erythropoietin, and SC factor. Analysis of relevant published data is presented in our review [4]. Insulin-like growth factor can be important for cell therapy efficiency. This factor does not modulate SC, but stimulates cardiomyocyte proliferation, emerging in the myocardium as a result of transdifferentiation of transplanted SC [4]. Protein synthesis is an obligatory condition of cell division and growth. The intensity of this process largely depends on blood levels of insulin and testosterone. Full-value regeneration of the heart is impossible without neoangiogenesis, which depends on the intensity of NO formation in ischemic organs and tissues [3,4]. It is known that glucocorticoids induce apoptosis of lymphocytes, monocytes, and their mononuclear precursors. Hence, high blood level of hydrocortisone in the patients can be responsible for apoptosis of transplanted mononuclear cells. Presumably, the final effect of BMMC transplantation in patients with myocardial infarction depends not only on the number of transplanted mesenchymal SC, but also on the blood and tissue levels of insulin, hydrocortisone, testosterone, growth factors, and NO. The levels of these bioactive substances in patients with myocardial infarction can vary within a wide range. Presumably, the effect of BMMC transplantation is strictly individual. The results of clinical observations confirm this hypothesis. For example, 6 months after BMMC transplantation the end-systolic volume of the left ventricle decreased by 17% and did not change in the control (Fig. 1). Exercise tolerance in the main group increased by 31% after 6-month observation, *vs.* 14% in the control group (Table 2). During the same period the quality of life in the main group increased 1.8 times *vs.* 1.3 times in controls (Table 2). However, this effect of BMMC transplantation was observed not in all patients, and we failed to detect significant differences between the main group and controls. The findings of foreign scientists are to some extent in line with our results [6,8,10,14]. Positive effect of autologous BMMC transplantation was not observed in all patients receiving this treatment modality [6,8,10,14]. Hence, the effect of BMMC transplantation is most likely determined by individual characteristics of the patient. Further clinical observations will show the causes of individual reactions to BMMC transplantation.

Hence, we draw the following preliminary conclusions from our findings: intracoronary infusion of autologous BMMC during the subacute period of myocardial infarction had virtually no effect on cardiac contractility, functional class of cardiac insufficiency, exercise tolerance, or quality of life; intracoronary transplantation of BMMC did not provoke ventricular tachycardia and ventricular fibrillation; transplantation of BMMC into infarcted myocardium did not augment the course of coronary disease in patients with myocardial infarction.

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