Autologous Mononuclear Bone Marrow Cells during Reparative Regeneratrion after Acute Myocardial Infarction

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A randomized controlled study included 44 patients with acute myocardial infarction. It was found that intracoronary injection of bone marrow mononuclear cells is safe, ensures fixation of the injected cells in the myocardium, reduces blood levels of IL-1β and TNF-α, increases the content insulin-like growth factor, and does not provoke malignant arrhythmias.

Key Words: *myocardial infarction; mononuclear bone marrow cells; cell cardiomyoplasty*

Processes of postinfarction remodeling of the left ventricle are a morphological substrate of chronic heart failure. These processes are regulated by many factors, but usually are determined by the size of necrotic zone in the myocardium and low proliferative capacity of cardiomyocytes [8]. The potentialities of cell cardiomyoplasty in modulating heart remodeling processes are extensively studied, but the data are controversial [1,4-7].

Here we studied the safety and efficiency of transplantation of bone marrow mononuclear cells (BMMC) in patients with acute myocardial infarction (AMI) and evaluated the effect of BMMC transplantation on reparative regeneration in AMI.

MATERIALS AND METHODS

Open randomized parallel controlled study included 44 patients (22 patients in the main and control groups, groups 1 and 2, respectively, Fig, 1). All patients gave informed consent for participation in the study. Inclusion criteria were: age below 75 years, primary transmural AMI, reperfusion time not less than 4 h after appearance of symptoms. The study protocol was approved by Ethical Committee of Institute of Cardiology of Tomsk Research Center. The groups were matched by parameters determining short- and long-term prognosis (Table 1). In 12 patients (5 and 7 patients from groups 1 and 2, respectively) primary balloon angioplasty and stenting of the coronary occlusion site were performed. Other patients received systemic thrombolytic therapy (750,000 U streptokinase). Cell cardiomyoplasty was performed during coronaroangiography on 7-21 day of AMI. In patients receiving thrombolytics delayed balloon angioplasty and stenting of the coronary artery were performed at the same terms. Apart from endovascular intervention, the patients of both groups received heparin, aspirin, plavix, statins, angiotensin-converting enzyme inhibitors, and β-adrenoceptor blockers in individual doses.

Iliac crest marrow aspirates (100 ml, 2×60-ml syringes) were obtained 4-6 h before cell cardiomyoplasty. BMMC were isolated by gradient centrifugation (HISTOPAQUE-1077 density gradient) [9]. Cell viability determined by trypan blue staining was

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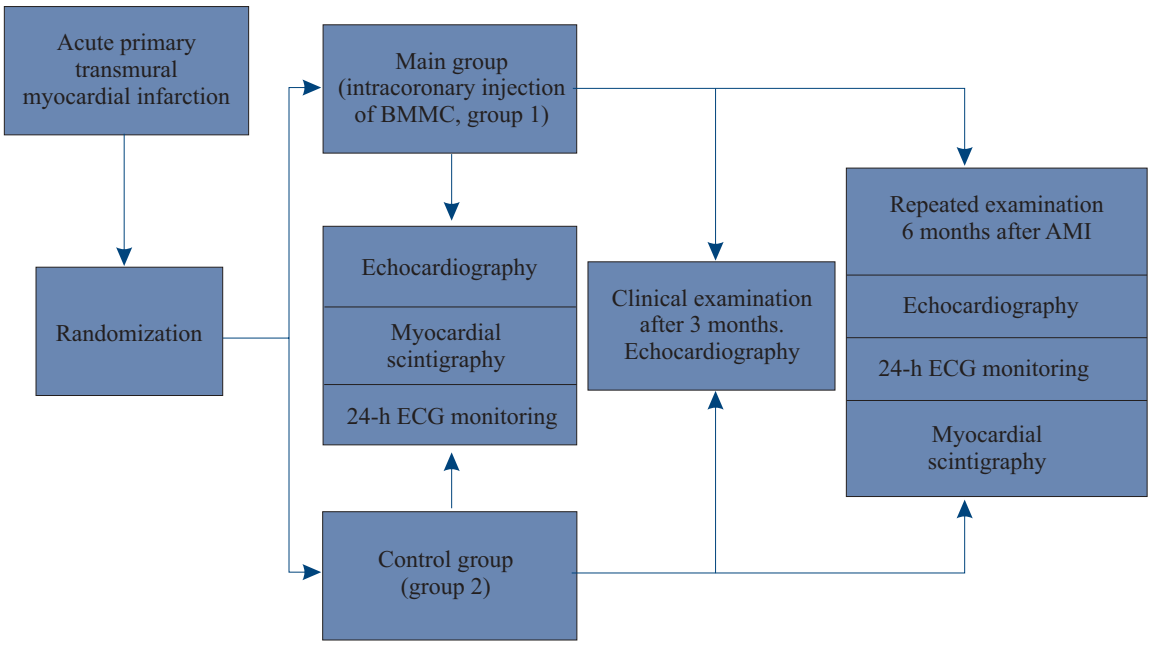

Fig. 1. Scheme of the study. AMI: acute myocardial infarction; BMMC: bone marrow mononuclear cells.

98-99%. A suspension of 2-4×10^6 BMMC per 1 ml heparinized solution was prepared (20 U heparin per 1 ml) and injected into the stented artery.

BMMC distribution in the organism of patients with AMI was determined 30 min, 2.5 h, and 24 h after intracoronary injection of labeled (40-60 mCi ^{99m}Tc-HMPAO, Ceretec) cell suspension.

The size of infarction zone was assessed by calculation of *QRS* index by standard ECG; clinical state, exercise tolerance [2], and quality of life were evaluated (Fig. 1). Echocardiography and 24-h ECG monitoring were performed. Myocardial perfusion was evaluated by single-photon emission computed tomography with tallium-199 chloride.

Serum contents of fatty acid-binding protein (myocardial damage marker), TNF-α, IL-1β and insulin-like growth factor were measured by immunoenzyme assay before and on day 2, 5, and 12 after cell transplantation.

The data were analyzed using Statistica 6.0 software. The differences were significant at $p<0.05$ [3].

RESULTS

All procedures were well tolerated by patients. No complications were observed during bone marrow aspiration and infusion of BMMC into the coronary artery. The content of fatty acid-binding protein after interventions was similar in both groups. All patients were observed for 6 months. The data of 7 patients (4 and 3 patients in groups 1 and 2, respectively) were excluded from the analysis because of repeated AMI, restenosis of the infarction-related artery, and microcoronary angiopathy.

Viability of radiolabeled BMMC was 96±4%. Intracoronary infusion of the cell suspension ensured their fixation in the myocardium (Table 2). Table 3 presents the data on extracardiac distribution of BMMC not fixed in the myocardium. The majority of cells after intracoronary injection migrated into the

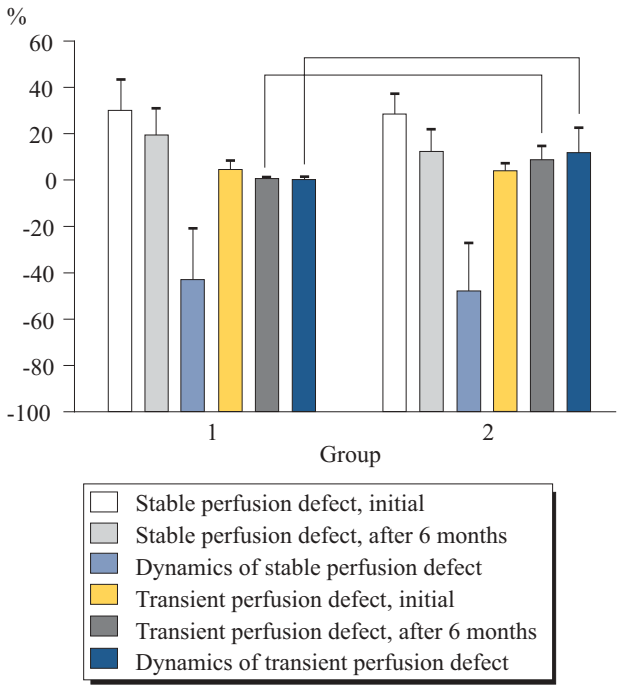

Fig. 2. Dynamics of myocardial perfusion defects. The data are presented as mean±standard deviation.

TABLE 1. Basic Clinical and Demographic Parameters of Patients (*M*±*SD*; *n*, %)

Parameter	Main group (*n*=22)	Control group (*n*=22)	*p*
Mean age	55.2±8.6	52.1±9.2	0.3
Males	20 (90)	16 (73)	0.08
Time of IRCA recanalization, h	6.7±4.7	5.5±3.9	0.4
Patient distribution by IRCA, ADA/RCA/CA	14(63)/5(23)/3(14)	14(64)/7(32)/1(5)	0.8
Number of patients with 1/2/3 involved arteries	2(5)/14(74)/4(21)	8(47)/6(35)/3(18)	0.3
QRS index	9.5±4.1	7.9±4.0	0.2
Number of isolated BMMC, 10^6	88.5±49.2		
Acute heart failure, FC I/II/III/IV (after T.Killip)	10(45)/8(36)/2(9)/2(9)	11(47)/8(38)/3(14)/—	0.3
Postinfarction angina	5(23)	5(23)	0.7
CHF after 6 months, FC I/II/III	14(78)/3(17)/1(6)	14(76)/3(15.5)/2(14.5)	0.7
CHF FC on AMI day 21	1.5±0.8	1.3±0.5	0.2
CHF FC after 3 months	1.2±0.4	1.5±0.9	0.1
CHF FC after 6 months	1.2±0.4	1.5±0.8	0.1
6-min walking test on AMI day 21, m	429.6±116.0	432.9±124.5	0.5
6-min walking test after 3 months, m	498.5±90.0	475.0±148.5	0.3
6-min walking test after 6 months, m	563.0±143.0	493.0±118	0.09
Score (AMI day 21)	18.5±20.0	19.0±8.3	0.5
Score (3 months)	26.5±16.2	29.3±16.9	0.4
Score (6 months)	33.1±21.9	26.0±14.1	0.2

Note. IRCA: infarction-related coronary artery; ADA: anterior descending coronary artery; RCA: right coronary artery; CA: circumflex artery; CHF FC: chronic heart failure, functional class; *n*: number of patients.

TABLE 2. Fixation of Labeled BMMC in the Myocardium of AMI Patients

Time after injection	% of total radioactivity; total number of cells
30 min	7.8; 9.4×10^6
2.5 h	6.8; 8.2×10^6
24 h	3.2; 3.8×10^6

liver, and then some cells were transported into the spleen. In 90% cases intensive accumulation of BMMC at the site of aspiration was observed.

The size of stable perfusion defect decreased in both group by the 6th month (Fig. 2), but transient perfusion defect appeared in group 2 patients (but not in group 1). Analysis of volume parameters and ejection fraction of the left ventricle revealed no differences between the groups.

The incidence and severity of chronic heart failure, heart rhythm disturbances, exercise tolerance, and quality of life were similar in both groups (Table 1).

On day 2 after intervention plasma content of IL-1β decreased by 70% in group 1 patients, while in group 2 this parameter increased by 32% ($p<0.02$). On day 5 after intervention, the content of IL-1β in group 1 patients increased by 17% compared to the initial level, but did not attain the level observed in group 2. The content of TNF-α demonstrated a similar dynamics. On day 12 after intervention, the plasma content of insulin-like growth factor in group 1 patients surpassed that in group 2 individuals (237±96 *vs*. 138±64, $p=0.01$).

TABLE 3. Extracardiac Distribution of Labeled BMMC after Intracoronary Injection

Time after injection	Intensity of ^{99m}Tc-HMPAO-mononuclears in organs (% of total activity)			
	lungs	liver	spleen	bone marrow
30 min	14.4±3.7	29.3±4.2	7.0±1.4	3.6±0.8
2.5 h	9.6±1.5	25.5±3.8	14.1±2.1	4.6±1.1
24 h	5.3±1.1	22.4±3.3	7.4±1.9	7.2±1.2

Thus, intracoronary injection of BMMC in AMI ensures penetration and fixation of cells in the myocardium without causing additional damage and provoking malignant arrhythmias. Transplantation of BMMC reduces the plasma content of IL-1β, TNF-α, and increases the content of insulin-like growth factor. Intracoronary injection of BMMC in patients with AMI does not affect the contractile function of the left ventricle.

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