

Autologous bone marrow-derived progenitor cell transplantation for myocardial regeneration after acute infarction

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Background. Experimental and first clinical studies suggest that the transplantation of bone marrow derived, or circulating blood progenitor cells, may beneficially affect postinfarction remodelling processes after acute myocardial infarction. **Aim.** This pilot trial reports investigation of safety and feasibility of autologous bone marrow-derived progenitor cell therapy for faster regeneration of the myocardium after infarction. **Methods and results.** Four male patients (age range 47–68 years) with the first extensive anterior, ST elevation, acute myocardial infarction (AMI), were treated by primary angioplasty. Bone marrow mononuclear cells were administered by intracoronary infusion 3–5 days after the infarction. Bone marrow was harvested by multiple aspirations from posterior crista iliacae under general anesthesia, and under aseptic conditions. After that, cells were filtered through stainless steel mesh, centrifuged and resuspended in serum-free culture medium, and 3 hours later infused through the catheter into the infarct-related artery in 8 equal boluses of 20 ml. Myocardial viability in the infarcted area was confirmed by dobutamin stress echocardiography testing and single-photon emission computed tomography (SPECT) 10–14 days after infarction. One patient had early stent thrombosis immediately before cell transplantation, and was treated successfully with second angioplasty. Single average ECG revealed one positive finding at discharge, and 24-hour Holter ECG showed only isolated ventricular ectopic beats during the follow-up period. Early findings in two patients showed significant improvement of left ventricular systolic function 3 months after the infarction. There were no major cardiac events after the transplantation during further follow-up period (30–120 days after infarction). Control SPECT for the detection of ischemia showed significant improvement in myocardial perfusion in two patients 4 months after the infarction. Echocardiographic assessment in these two patients also showed significant improvement of systolic function three months after the infarction. **Conclusion.** Preliminary results of the study showed that the transplantation of bone marrow-derived progenitor cells into the infarcted area was safe, and feasible, and might improve myocardial function. Further follow-up will show if this treatment is effective in preventing negative remodeling of the left ventricle and reveal potential late adverse events (arrhythmogenicity and propensity for restenosis).

Key words: myocardial infarction; stem cell transplantation; transplantation, autologous; ventricular remodeling; regeneration.

Introduction

Several dogmas about myocardial regeneration after infarction have been changed in the past few years. Bel-

trami et al. (1) showed that heart is not a postmitotic organ and that myocytes proliferated after infarction, especially in the zone adjacent to the infarct, raising the possibility that the regeneration of myocytes could contribute to the in-

crease in viable and functional mass of myocardium. The origin of cells which could regenerate the cardiomyocytes is not yet well defined. For the first time, Hierlihy et al. (2) provided evidence that adult heart retained an endogenous stem cell-like population, which was activated during growth challenge. The possibility that circulating stem cells could contribute to the formation of solid-organ tissue, derived from the studies of solid organ transplantation. Quaini et al., and Müller et al. reported male chimerism in heart allografts from female donors (3, 4). The sources of progenitor cells, which can differentiate to cardiomyocytes, or endothelial cells, are probably mobilized from bone marrow and skeletal muscles (5, 6). Skeletal muscle contains myoblasts, which have the capacity for self-renewal, differentiation into mature cells, and regeneration of muscle upon injury (7). Bone marrow has a variety of progenitor cells, including endothelial and mesenchymal precursors, which can participate in cardiac myocyte regeneration and angiogenesis in the ischemic heart (8). In the suitable environment both skeletal myoblasts and bone marrow adult stem cells might differentiate to cells with characteristics very similar to cardiomyocytes (9–12). However, several very serious experimental studies have questioned the possibility of bone marrow-derived cells to transdifferentiate into cardiac myocytes (13, 14). The direct incorporation of endothelial progenitors during new vessel walls formation was also examined (15). However, the benefit from bone-marrow progenitor cell transplantation may be a result of paracrine signalling of these cells to local tissue damage. Bone marrow-derived progenitor cells promoted neovascularization of ischemic myocardium by secreting various growth factors (16–18) and enhanced cytoprotection of residual cells upregulated by the expression of several heat shock proteins (19).

The use of skeletal myoblasts for cardiac regeneration was examined in more than 30 experimental studies, primarily involving animal models, and was reviewed in several general articles (20, 21). Overall, they consistently showed the differentiation of implanted myoblasts into multinucleated myotubes, the lack of coupling between engrafted myotubes and host cardiomyocytes, but also the improvement in local and global left ventricular function (10, 11, 22). Thus, several pilot clinical trials, using either surgical or transcatheter-based implantation method, have been performed. They all showed that autologous myoblast transplantation was technically feasible, could be implemented without procedural complications, and the results of differentiation into myotubes seemed to be sustained over time. However, engrafted islands probably represented arrhythmogenic substrates (22–24). The multicenter placebo-controlled efficacy trial, named MAGIC (the acronym for myoblast autologous grafting in ischemic cardiomyopathy), is currently under way, and should include 300 patients in whom myoblasts will be implanted in the myocardial scar during the by-pass surgery.

Orlic et al. showed in the mice model of myocardial infarction that bone marrow-derived stem cells (injected

into the peri-infarction areas of myocardium), or cytokine-induced mobilization of bone marrow stem cells (25, 26), decreased mortality, infarction size, cavitory dilatation, and diastolic stress.

So far, several clinical trials (27–35) examining the role of bone marrow-derived progenitor cells in the treatment of either acute or chronic ischemic heart disease have been published. Four studies have entailed the direct intracoronary injections of bone marrow mononuclear cells (27–30), or mobilized progenitors derived from circulating blood in patients with acute myocardial infarction (29). However, in these trials, the grafted areas were also subjected to angioplasty, which made the assessment of the respective benefits of revascularization versus cell therapy virtually impossible. Three other studies have used similar mononuclear cells injected directly through the left ventricle for the treatment of intractable ischemia (31, 32) or ischemic heart failure (33), without additional procedure. Finally, two surgical studies have entailed direct epicardial injections of mononuclear cells (34) or CD133+ progenitor cells during coronary artery bypass (35). All this studies showed that the procedures were safe, and probably improved myocardial perfusion and systolic function. Nevertheless, these studies didn't identify the most efficient phenotype of progenitor cells adapted to the clinical indication. Thus, acute myocardial infarction, refractory angina, and heart failure might need different type of progenitor cells. Cell dosing is another unsettled issue. Cytokine mobilization raises the safety issue of cytokine treatment in the acute phase of myocardial infarction, or in patients in end-stage heart failure. The application of G-CSF could provoke severe reperfusion injury mediated by higher number of mobilized, activated neutrophils, or for instance influence the high incidence of restenosis in one clinical study (29).

In this preliminary communication we presented the clinical project of the Clinic of Emergency Internal Medicine of the Institute of Radiology and the Institute of Transfusiology of the Military Medical Academy, Belgrade. In the project the application of bone marrow-derived progenitor cells for regeneration enhancement after acute, extensive myocardial infarction was investigated. We reported the preliminary results with the first 4 patients in whom bone marrow-derived progenitor cell transplantation was performed into the infarct-related artery a few days after the acute myocardial infarction.

Methods

On the admission, after explaining the procedure, and obtaining the written consent, 4 male patients (age-range 47–68 years), were submitted to autologous bone marrow-derived progenitor cell therapy 3–5 days after extensive acute myocardial anterior infarction, treated with primary angioplasty. The basic premorbid and periprocedural characteristics of patients are presented in Table 1. Initial electrocardiograms of all patients showed extensive myocardial

Table 1

Patients' characteristics				
Case	No. 1	No. 2	No. 3	No. 4
Age	61	68	57	47
Hypertension	Yes	No	Yes	No
Diabetes	Yes	No	No	No
Current smoker	No	Yes	Yes	Yes
Hypercholesterolemia	Yes	No	Yes	No
Hypertriglyceridemia	Yes	No	Yes	No
Family history of AMI	Yes	No	Yes	No
Previous infarction	No	No	No	No
Time from the pain onset to re-perfusion (h)	3	12	4	3
Infarction related artery	LAD	LAD	LAD	LAD
TIMI flow at presentation	0	0	0	0
TIMI flow after PCI	3	3	3	3
Early ST segment resolution	No	Yes	Yes	Yes
Stents –number	2	2	1	1
Stents diameter (mm)	3	3	3	4
Stents whole lenght (mm)	29	38	23	16
Residual stenosis	<20%	<20%	<20%	<20%
Multivessel disease	No	No	Yes	Yes

ischemia with ST elevation in almost all precordial leads, and in DI and aVL leads. Patients were hemodinamically stable on admission. All the patients had high occlusions of left anterior descendent artery, and in all of them normal TIMI-3 flow through the infarcted artery was achieved after stent implantation with residual stenosis less then 20%.

Study protocol

When the diagnosis of the acute myocardial infarction was established, the patients were administered aspirin 300 mg orally, morphine 10 mg subcutaneously, and 80 mg of enoxaparin intravenously. After that, within 60 minutes, patients were submitted to coronarography and percutaneous

coronary angioplasty. GP inhibitors were not used. Subsequently, patients were treated with combined antiaggregatory therapy (aspirin 150 mg per day, and ticlopidin 250 mg twice a day). Anticoagulation therapy was continued for the next 5 days with enoxaparin 1 mg/kg of body mass, subcutaneously twice daily. Ramipril and carvedilol were titrated according to arterial blood presure and heart rate, and simvastatin according to low density lipoprotein serum levels. The basic scheme of study protocol is shown in Figure 1.

Bone marrow harvesting and cell transplantation

Between 3–5 days after the infarction, patients underwent general anesthesia, and bone marrow aspiration from

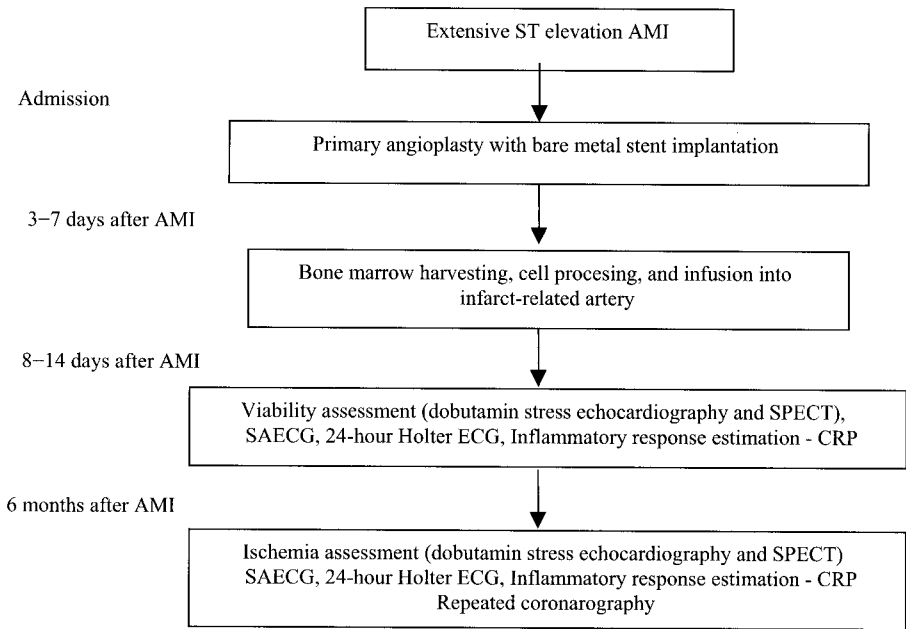


Fig. 1 – Study protocol

the posterior iliac crests was performed several times in aseptic conditions. The sample volume in the first patient was 104 ml, and in all the others was little above 200 ml. The harvested bone marrow cells were placed into a sterile container with an anticoagulant solution and tissue culture media. Anticoagulation was provided by using citrate solution and heparin diluted in normal saline (36). Immediately after the cell collection, the marrow aspirates were filtered consecutively through stainless steel mesh screens to remove blood clots, bone and other tissue fragments, lipid particles, cell aggregates and fibrin. They were then transferred into the plastic satellite bags for processing. The primary mononuclear cell suspension (MNC) was obtained from aspirate samples by purification techniques, such as red blood cell reduction, as well as plasma/additive solution, and platelet depletion. Purification was accomplished with centrifugation ($377 \times g$ for 10 min) by using Hettich-Roto Silenta RP (Hettich, Germany), combined with hydroxyethyl starch (HES) induced sedimentation, and using cell separation manual technique (37, 38). Processed MNCs were resuspended in serum-free culture medium up to optimized hematocrit, and immediately afterwards administered as an intermittent infusion (divided into 8 equal volumes) into the infarcted coronary artery across the coronary catheter, 3–4 hours after bone marrow harvesting. Quality control of the final cell suspension included the quantification of total mononuclear cells, and the number of CD34+/CD45+ cells using flow cytometry. The cell suspension consisted of heterogeneous cell populations including hematopoietic progenitor cells, which were determined by fluorescence-activated cell sorter (FACS) analysis, using directly conjugated antibodies against anti-human CD34 (FITC; Becton Dickinson), and anti-CD45 (Becton Dickinson). Cell viability was investigated by the use of trypan blue dye exclusion test, as previously described (36–38). Culture of bacterial and fungal contamination was also obtained from the samples of cell suspension.

The obtained cell viability was 98% in all the patients, and the count of the infused MNCs and CD34+/CD45+ cells was $1.12 \times 10^9 \pm 0.5 \times 10^9$ and $10.1 \pm 4.8 \times 10^6$, respectively.

Estimation of infarction size

Creatine kinase-MB (CK-MB) isoenzyme was determined before PCI, and every 6 hours during the period of two days, and daily thereafter. CK-MB maximum value and the area-under-the-curve value (CK-MB for 72 hrs) were used as a surrogate measure for infarction size, as previously described (39, 40).

Selvester QRS scoring system was evaluated by the standard 12-lead ECG 5–10 days from AMI. Infarction size was presented as the percentage of left ventricle involved (41).

Estimation of infarction size was performed because we wanted to compare the results of the group of patients who were administered stem cells to historical control group, which consisted of patients treated only with primary angioplasty.

Echocardiographic viability and systolic function assessment

Low dose dobutamine echocardiography was performed in patients at a mean of 10 days (range 8–13 days) after the onset of myocardial infarction (42). Beta-blockers were suspended 24–48 hours before the test. After baseline cross-sectional echocardiography, dobutamine was administered intravenously at the doses of 5 and 10 mg/kg/min, each dose within five minutes. Standard parasternal and apical views were recorded on VHS videotape at baseline and at the end of dobutamine infusion for subsequent off-line analysis.

Echocardiograms were reviewed by two experienced observers. Wall motion was evaluated using a 16-segment model of the left ventricle and a four-point scoring system: 1) normokinesia; 2) hypokinesia; 3) akinesia; 4) dyskinesia. Myocardial viability was defined as an improvement in thickening during dobutamine infusion in two or more contiguous infarction zone segments. Improved thickening was defined as a change from akinesia or dyskinesia to hypokinesia or normal wall thickening, and from hypokinesia to normal wall thickening. The wall motion score index was calculated by summing the scores for each segment and dividing them by the number of segments analyzed.

Left ventricular end-diastolic and end-systolic volumes were determined from apical two- and four-chamber views using the acoustic quantification method every three months for at least one year during the follow-up. The ejection fraction was calculated as $[(\text{end-diastolic volume} - \text{end-systolic volume}) / \text{end-diastolic volume}] \times 100\%$. Baseline echocardiography parameters will be compared to the follow-up measurements.

Radionuclide ventriculography and single photon emission computed tomography (SPECT)

Nuclear imaging was performed at a mean of 12 days (range 10–15 days) from the onset of myocardial infarction. The control radionuclide ventriculography was planned 6 months after the infarction. The standard *in vivo* equilibrium radionuclide angiocardiology technique was used for ejection fraction assessment (43). The injection of 2–3 mg stannous pyrophosphate was administered intravenously, 30 minutes later followed by 20 mCi Tc-99m pertechnetate. Another 30 minutes later, gated planar imaging was performed in the left anterior oblique position (best septal separation), by using LEAP (Low Energy All Purpose) collimators and 16 frames per cardiac cycle. LVEF was calculated by using left ventricular ROIs to determine the end-systolic, end-diastolic, and background counts, with the standard formula: $\text{LVEF} = (\text{End-diastolic counts} - \text{End-systolic counts}) / \text{End-diastolic counts}$. Radionuclide ventriculography will be repeated after 6 and 12 months.

SPECT was performed using a pharmacological stress reinjection protocol with lower dipyridamol dose (0.28 mg/kg) for the viability assessment 7–12 days after the in-

fraction, and with higher dipyridamol dose 0.56 mg/kg for the ischemia assessment in the fourth month after the infarction (42). A 370 MBq dose of ^{99m}Tc -tetrafosmin was injected 2 minutes after intravenous infusion of 0.28 mg/kg body mass of dipyridamol, and a 740 MBq dose 3 hours later. Images were acquired 15–30 minutes after ^{99m}Tc -tetrafosmin injection by Siemens Orbiter gamma camera. ^{99m}Tc -tetrafosmin uptake was analyzed in 17 segments, as previously reported. A four grade scale (1 = normal, 2 = mild, 3 = moderate, 4 = severely decreased uptake) was used for the qualitative analysis of viability. Segments with a reinjection score ≥ 3 were considered to be necrotic, and those with a score < 3 to be viable. The images were analyzed by one observer blinded to all other data. Segments were secondarily assigned to the infarct-related artery based on the coronary angiogram. Infarct risk area was defined as the number of segments assigned to the infarct-related artery. Myocardial viability was assessed semiquantitatively, by a presence of at least 4 viable segments for left anterior descending artery distribution area, and 3 viable segments for other two arteries distribution areas within the infarct risk area.

Non-invasive electrophysiology assessment

Before being discharged at days 10–14 (mean day 12), and after three months, patients underwent 'time-domain analysis' signal-averaged electrocardiography (SAECG) recording on X, Y, Z orthogonal derivations. Taking a noise limit $< 1 \mu\text{V}$, at least 500 complexes were filtered with frequency range 40–250 Hz. Ventricular late potentials were considered to be present if filtered QRS duration (QRSD) was longer than 120 ms, or if any of two of the three criteria were met: 1) $\text{QRSD} > 114 \text{ ms}$, root-mean-square voltage of the last 40 ms (RMS-40) of the QRS complex $\text{RMS-40} < 20 \mu\text{V}$, and 2) the duration of low amplitude $> 40 \mu\text{V}$ signal of the terminal portion of the QRS (LAS) $> 38 \text{ ms}$ (44).

24-hour Holter ECG monitoring was performed after 1, 3, and 6 months after the infarction, as a standard two-channel outpatient examination.

Electrophysiologic assessment will be performed every 2 months during the first year after the infarction to reveal arrhythmias or the propensity for them.

Assessment of inflammatory response

C-reactive protein (CRP) was determined 48 hours after the infarction, 24 hours after the transplantation, and 7, 14, and 30 days after the infarction. Standard immunoassay (Behringer) was used for CRP serum level measurement. Increment of inflammatory response after cell transplantation only a few days after primary angioplasty could contribute to the development of restenosis, or could enhance the reperfusion injury and influence myocardial damage.

Study end-points

Primary end-points of systolic function of left ventricle and myocardial viability improvement assessed at the second week and three months after the infarction were evaluated.

Secondary end-points were a composite of coronary death, reinfarction, revascularization of the target vessel as a result of ischemia, or disabling stroke during the first six months after the index procedure. We also evaluated secondary end-points as the infarction size, incidence of positive late potentials and malignant arrhythmias during 3 months, as well as the presence of all kind of complications during this period.

Reinfarction was defined by the presence of recurrent ischemic symptoms or electrocardiographic changes accompanied by a creatine kinase level that was of more than twice the upper limit of the normal range (with an elevated MB isoform level), or more than 50 percent higher than the previous value obtained during hospitalization. Revascularization of the target vessel was considered to have been prompted by ischemia, if there was an evidence of ischemia during functional testing, or angina recurrence. Disabling stroke was defined as an acute neurologic deficit that lasted more than 24 hours and affected the ability to perform daily activities or resulted in death. Episodes of bleeding were defined as major or minor, according to the TIMI classification (45).

Results

Preprocedural angiogram showed proximal occlusion of left anterior descending artery in all 4 patients. Predilatation of 2 mm diameter balloon was performed, and after reopening the arteries, bare metal stents were implanted in the infarct-related lesion. According to guidelines, only infarction-related artery was treated. In the first patient, two stents were implanted because of the long lesion. The second patient had early stent thrombosis on the day of cell transplantation, but half an hour before the infusion of bone marrow MNCs, with the small raise of CK-MB afterwards. This patient was treated with the second angioplasty and stent implantation. After the restoration of TIMI-3 flow he was administered intracoronary infusion of MNCs. In other 3 patients there was no increase of CK-MB after intracoronary cell infusion. Normal TIMI-3 flow with less than 20% residual stenosis was achieved in all the cases. No other periprocedural and in-hospital complications were detected.

During the follow-up period, 30–120 days from the infarction, no deaths, reinfarctions, clinically manifested heart failure, or malignant arrhythmia was detected. The first patient had neutropenia caused by ticlopidin without any infection complications, with the normalization of leukocyte formula soon after drug cessation.

All the patients had viable myocardium 10 days after AMI in the infarction area detected either by SPECT or dobutamin stress echocardiography testing (Figure 2a). The increase of wall motion score index during dobutamine stress testing as a measure of myocardial viability is shown in Table 2. There was no significant reversible ischemia on ischemia-SPECT in 2 patients 3 months after the infarction. Control SPECT imaging showed significant improvement of viability in 2 patients who were followed three months

after the infarction. The control SPECT finding of the second patient is shown in Figure 2b.

Echocardiographic examination performed approximately 10 days after the infarction showed severe impairment of systolic function in all the patients (Table 2). Mean ejection fraction was $33.7\% \pm 1.3\%$ after 10 days of AMI. Follow-up echocardiographic examination was performed in two patients after three months after the infarction, at the time of writing this preliminary report. Both patients showed improvement in ejection fraction after 3 months (Table 2).

area was 10876 ± 3550 , and the mean Selvester score of the left ventricle was $14.25\% \pm 2.9\%$.

Late potentials were determined in all four patients at discharge, and one of them (the oldest patient with the longest period from the pain onset to reperfusion) had true-positive finding (QRSD 164 ms, RMS40 $14.93 \mu\text{V}$ and LAS 106 ms) showed in Figure 3, which was normalized 2 months later. 24-hour Holter monitoring was performed in all four patients before discharge, and the first patient had a number of isolated ventricular extrasystolas (195 ectopic

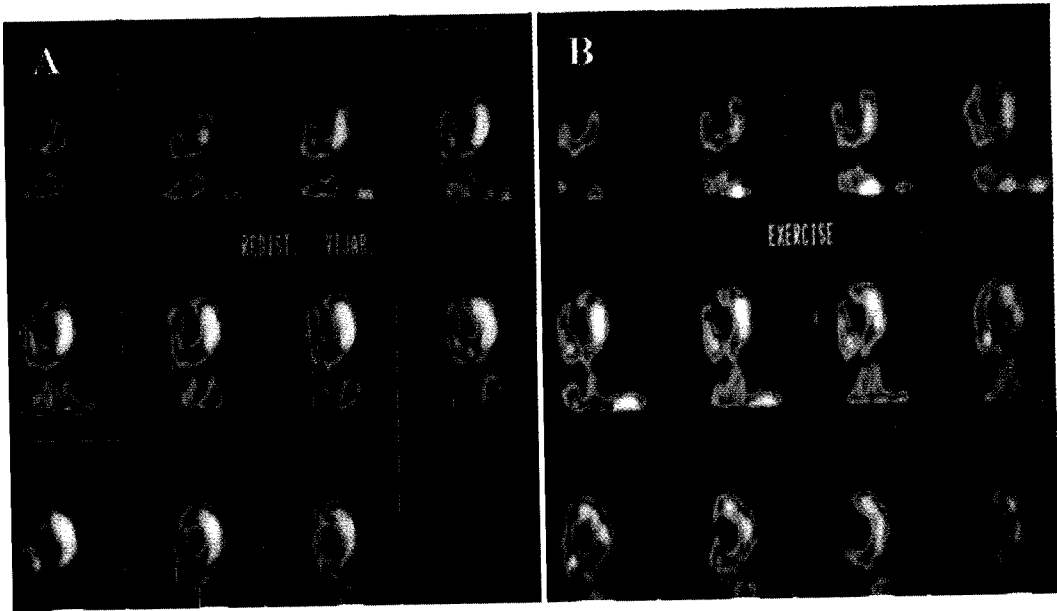


Fig. 2a and b.— Myocardial SPECT for viability assessment, 10 days after AMI, and for ischemia assessment 3 month after AMI

Table 2

Systolic function of left ventricle in patients with extensive anterior myocardial infarction treated with primary percutaneous coronary stent implantation and bone marrow derived autologous mononuclear cell transplantation into the infarction area

Pts.	EF*		WMSI-1	WMSI-2	EDV		ESV		EF†
	H	3 m			H	3 m	H	3 m	
	%	%	H	H	(ml)	(ml)	(ml)	(ml)	(%)
No.1	34	45	1.75	1.125	135	141	89	78	39
No.2	32	33	2.12	1.81	128	147	90.6	99.0	29
No.3	35	—	1.75	1.25	115	—	68	—	26
No.4	34	—	1.825	1.625	197	—	146	—	34

EF* ejection fraction estimated by echocardiography, EF† ejection fraction estimated by nuclear ventriculography, WMSI-1/2 – wall motion score index before and at the peak of dobutamin infusion during echocardiography stress testing, H – in-hospital data

Radionuclide ventriculography, performed in all the patients at the end of the second week after the infarction, showed similar mean ejection fraction $32.0\% \pm 5.7\%$ (Table 2). We planned to repeat radionuclide ventriculography after 6 months, and to compare the results with the baseline values.

The mean value of CK-MB maximum after the infarction was $420.25 \text{ IU/L} \pm 103.30 \text{ IU/L}$, the mean CK-MB

ventricular beats), greatly enlarged 3 months later (6997 ectopic ventricular beats), without any other rhythm disturbances. The results of non-invasive electrophysiologic examination at discharge are shown in Table 3.

Inflammatory response varied between patients (Table 4). The mean maximum CRP value during 48 hours after infarction was $40.3 \text{ mg/L} \pm 17.3 \text{ mg/L}$, without significant changes the day after the transplantation $35.1 \text{ mg/L} \pm 21.8$

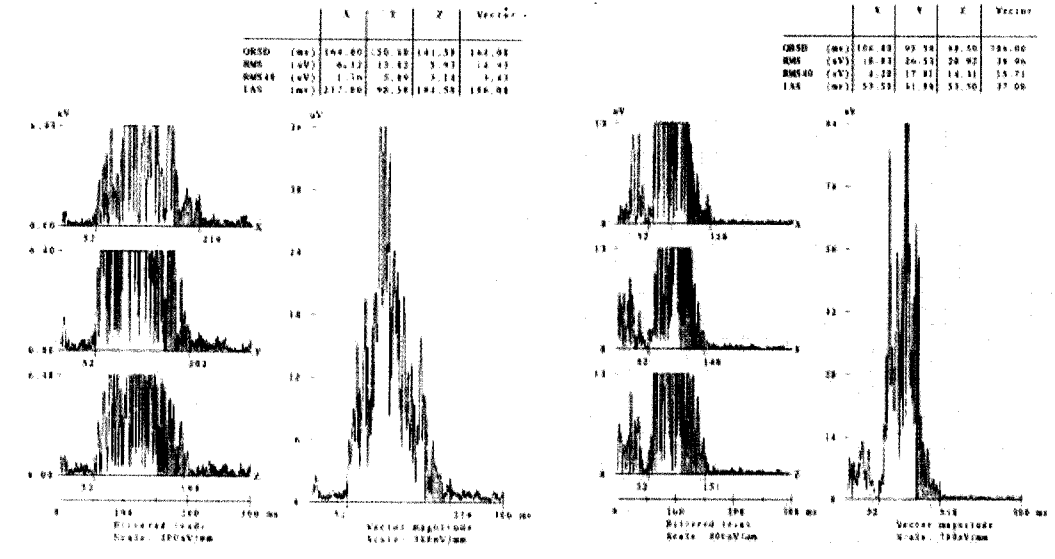


Fig. 3a and b.— Positive ate potentials in the second transplanted patient at discharge and its normalization after 3 months

Table 3

Late potentials and 24-hour Holter ECG monitoring at discharge		
Pts.	Late potentials	24-hour Holter ECG
No. 1	Negative	195 single ventricular ectopic beats
No. 2	Positive	55 single ventricular ectopic beats
No. 3	Negative	75 single ventricular ectopic beats
No. 4	Negative	164 single ventricular ectopic beats and 1 ventricular couplet

Table 4

C-reactive protein, 24 hrs after myocardial infarction, 24 hrs after stem cell transplantation, and 7 days after infarction

Pts.	CRP (mg/L)		
	24–48 hrs after AMI	24 hrs after cell transplantation	7 days after AMI
No. 1	16.9	13.2	26.9
No. 2	39.9	50.2	7.7
No. 3	48.7	20.1	4.3
No. 4	56.2	56.6	3.5

mg/L, and with a fast decrease in the following days, s mean value 10.6 mg/L ± 11.0 mg/L at day 7.

Discussion

The results of this pilot trial demonstrated transplantation of adult progenitor cells by intracoronary infusion as a feasible and safe method in the treatment of patients with AMI. It is well known that prompt reperfusion during AMI combined with the state-of-art medical therapy, which includes ACE inhibitors and beta-blocking agents (46) beneficially affects LV remodeling processes after AMI. However, two recently published larger trials (47, 48) as well as large single centers' registry (49), which used stent implan-

AMI reported increases in LV ejection fraction after 6 months in the range of 3–4.1%. However, these numbers are significantly less, compared to the increase of the left ventricular ejection fraction (5–10%), 3–6 months after the infarction in 4 published trials on bone marrow-derived stem cell infusion into the infarct related artery in the sub-acute AMI phase (27–30). Two German studies (27, 28) about the use of sophisticated cardiac magnetic resonance imaging and F-18-Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) demonstrated significant decrease of the infarction area and increase of myocardial viability in the infarction zone. In all these clinical trials with a total of 83 patients, there were no reported major adverse cardiac events, including death, reinfarction, and symptomatic heart failure. However, Magic trial (30), the only trial that in-

cluded control coronarography in study protocol after 6 months, showed very high incidence in stent restenosis (5 of 7 patients) in the group of patients under cell therapy. But, in this trial granulocyte colony stimulating factor (G-CSF) was used for the mobilization of bone marrow progenitors to peripheral blood, and this fact might be the reason for the high incidence of in-stent restenosis (50). Thus, we decided to perform control coronarography in all the patients enrolled in our study after six months. The use of drug-eluting stents in primary angioplasty probably has an advantage of reducing restenosis (51) but it highly raises the costs of this therapy, since the majority of early detected in-stent restenosis could be easily solved with balloon angioplasty.

Trials with bone marrow-derived stem cell transplantation have not yet reported the increase propensity to arrhythmias (27–34), but we registered a large number of single ventricular ectopic beats in one of our patients.

There is still a number of open questions considering progenitor cell therapy in the treatment of ischemic heart disease. What is the exact mechanism and benefit of bone mar-

row-derived progenitor cells infusion into the infarct related artery? Do we need some special cell compartment, and some selection of bone marrow cells before the application into the myocardium? Do we need cytokine preparation of bone marrow and myocardium to accelerate the number of the required progenitors and to improve homing and engraftment of these cells into the ischemic myocardium? First clinical trials showed encouraging results of this therapy, with the possibility of enhancing regeneration of damage myocardial tissue, which seemed impossible just a few years ago.

In conclusion, we need a lot of basic research and randomized clinical trials to define the exact role of this probably revolutionary therapy for ischemic heart disease. This article is a preliminary report with the basic aim to present the study protocol of stem cell transplantation for the treatment of acute myocardial infarction to our medical public. We had 4 patients till now, without the sufficient follow-up period, but we think it is worthwhile to show the early results without any preliminary conclusion.

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Apstrakt

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AUTOLOGNA TRANSPLANTACIJA PROGENITORNIH ČELIJA POREKLOM IZ KOSTNE SRŽI U CILJU REGENERACIJE MIOKARDA NAKON AKUTNOG INFARKTA

Uvod. Eksperimentna istraživanja i prve kliničke studije ukazuju na mogućnost da intrakoronarna infuzija progenitornih ćelija poreklom iz kostne srži u infarktnu zonu može da spreči remodelovanje i proširenje leve komore nakon infarkta miokarda. **Cilj.** Ova pilot studija ukazuje na izvodljivost i bezbednost primene autologne transplantacije progenitornih ćelija poreklom iz kostne srži u cilju brže regeneracije miokarda nakon infarkta. **Metode i rezultati.** Kod 4 bolesnika (starosti između 47 i 68 godina) sa dijagnostikovanim prvim akutnim infarktom miokarda (AIM) sa ST elevacijom prednjeg zida, koji su lečeni primarnom angioplastikom, od 3 do 5 dana posle infarkta primenjena je intrakoronarna infuzija mononukleusnih ćelija poreklom iz kostne srži. Kostna srž bolesnika je prikupljena višestrukim aspiracijama iz zadnjih ilijačnih krista u opštoj anesteziji i u aseptičkim uslovima. Nakon toga ćelije su

filtrirane kroz specijalne filtere, centrifugovane su, dodat im je specijalni medijum i tri sata nakon toga ćelijska suspenzija je u 8 jednakih bolusa od 20 ml preko kate-tera data u infarktnu arteriju. Vijabilnost miokarda je potvrđenja dobutamin stres testom i perfuzionom scintigrafijom miokarda između 10. i 14. dana posle infarkta. Kod jednog bolesnika je došlo do rane tromboze stenta neposredno pre infuzije progenitornih ćelija, što je uspešno rešeno sekundarnom angioplastikom. Kod jednog bolesnika su registrovani pozitivni kasni potencijali pred otpust koji su se zatim tri meseca kasnije normalizovali, dok je kod drugog tokom 24-časovnog Holter monitoringa registrovan veliki broj pojedinačnih ekstrasistola. Nisu registrovani značajniji kardiološki neželjeni događaji u daljem periodu praćenja (od 30 do 120 dana posle infakta). Kontrolna scintigrafija miokarda nakon 4 meseca kod dva bolesnika je pokazala značajno poboljšanje perfuzije tkiva u infarktnoj zoni. Ehokardiografski parametri kod ova dva bolesnika takođe ukazuju i na značajan oporavak sistolne funkcije 3 meseca posle infarkta. **Zaključak.** Naši preliminarni rezultati pokazuju da je primena autologne transplantacije ćelija poreklom iz kostne srži u infarktnu zonu svega nekoliko dana posle infarkta miokarda bezbedna i lako izvodljiva. Dalje praćenje će pokazati da li ova terapija pozitivno utiče na remodelovanje srčanog mišića i da li postoje neke kasne komplikacije (aritmogenost i sklonost ka restenozi).

K l j u č n e r e č i : infarkt miokarda; transplantacija matičnih ćelija; transplantacija, autologna; srce, remodelovanje komore; regeneracija.