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Cell Therapy in Patients with Left Ventricular Dysfunction Due to Myocardial Infarction

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Objectives: The purpose of this study was to determine the impact of autologous transplantation of mononuclear bone marrow cells on myocardial function in patients with left ventricular (LV) dysfunction due to an acute myocardial infarction. Methods: The randomized study included 82 patients with a first acute myocardial infarction treated with a stent implantation. This presentation is a subanalysis of 47 patients with left ventricular dysfunction–EF (ejection fraction) ≤ 40%. Group H patients (n = 17) received higher number (100,000,000) of cells; Group L patients (n = 13) received lower number (10,000,000) of cells. The patients of control Group C (n = 17) were not treated with cells. The Doppler tissue imaging and single photon emission computed tomography were performed before cell transplantation and 3 months later. Results: At 3 months of follow-up, the baseline EF of 35%, 36%, 35% in Groups H, L, and C increased by 6% (P < 0.01 vs. baseline), 5% (P < 0.01 vs. baseline), and 4% (P = NS vs. baseline), respectively, as assessed by single photon emission computed tomography (P = NS between groups). The baseline number of akinetic segments of 6.9, 7.0, and 6.2 in H, L, and C groups decreased by 1.7 (P < 0.01 vs. baseline), 1.5 (P < 0.01 vs. baseline), and 0.7 (P = NS vs. baseline, P = NS between groups), respectively, as demonstrated by echocardiography. Conclusion: In our study, the statistically important effect of transplantation of mononuclear bone marrow cells on myocardial function was not found. Only an insignificant trend toward the improvement of global LV EF fraction was found at 3-month follow-up. (ECHOCARDIOGRAPHY, Volume 25, September 2008)

stem cells, *coronary artery disease*, *left ventricular dysfunction*

Postmyocardial infarction congestive heart failure remains to be a major clinical problem, despite advances in the medical and surgical treatment of acute coronary syndromes. Coronary artery disease accounts for approximately 50% of all cardiovascular deaths and is the leading cause of congestive heart failure. The 1-year mortality rate for patients diagnosed with congestive heart failure is about 20%, and from 1994 to 2004, deaths from heart failure increased 28%.[1,2] Development of heart failure in survivors of acute myocardial infarction involves myocyte loss in the area supplied by the infarct-related artery and subsequent formation of noncontractile fibrous tissue. To date, no therapeutic procedure like angioplasty or thrombolytic agents could reverse the

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irreversible myocardial injury completely. The recovery of contractile function after revascularization occurs only in the areas of hibernating myocardium. Heart transplantation may be an option in selected patients, but the donor supply is strictly limited.

Recent experimental and clinical studies suggest that cell transplantation into damaged myocardium may have the potential to restore myocardial viability and improve left ventricular function. Different cell types can be potentially used for transplantation. To avoid problems with donor availability, immunological rejection, arrhythmias, and ethical problems, autologous bone marrow cells appear particularly attractive. But in a majority of studies, only patients with almost normal function or only mild dysfunction of the left ventricle were studied.[3–12]

So the purpose of this study was to determine the impact of autologous transplantation of mononuclear bone marrow cells on myocardial function in patients with moderate-to-severe left ventricular dysfunction.

Materials and Methods

Study Population

The randomized study included patients with a first acute myocardial infarction treated with coronary angioplasty with a stent implantation. Only patients with successful recanalization of the infarct-related artery (TIMI flow grade 3) and the evidence of an irreversible damage of at least two akinetic or dyskinetic myocardial segments identified by dobutamine echocardiography, gated technetium-99 m sestamibi single photon emission computed tomography, and positron emission tomography (performed in only 73% of patients) were included. The exclusion criteria were: (1) age $>$ 70 years; (2) noncardiac disease adversely affecting prognosis; (3) another cardiac disease except coronary artery disease; (4) coagulopathy, thrombocytopenia, leucopenia; (5) absence of a significant increase in cardiac enzymes (creatine kinase over 20 μkat/l or creatine kinase-MB over 3 μkat/l or troponin I over 20 μg/l–normal upper limits in our laboratories are 2.85 μkat/l, 0.42 μkat/l, and 2.0 μg/l, respectively); (6) patient instability on days 3–7 after MI; and (7) need for coronary revascularization in the future for multivessel disease.

From a total number of 82 patients who completed the baseline and 3-month follow-up examination, 66 patients were analyzed in the previously published study.[12] This first 66 patients were randomized into three arms: (1) a group treated with a higher number of mononuclear bone marrow cells (defined as a mean number of 1×10^8 cells); (2) a group treated with a lower number of cells (defined as a mean number of 1×10^7 cells); and (3) a control group not treated with cell transplantation. Subsequent 16 patients were randomized into only two arms: higher-dose-treated group and control group. The reason for changing randomization schema was no significant effect of a lower-dose cells in the previous study. This presentation is a subanalysis of 47 (from all the 82) patients with significant left ventricular dysfunction—ejection fraction (EF) $\leq$ 40%. Forty-five patients underwent the primary angioplasty (within 12 hours of chest pain onset)and two patients were treated with angioplasty within the interval from 12 hours to 3 days after symptom onset.

Study Design

On day 3–6 after myocardial infarction, rest and dobutamine echocardiography was performed to evaluate the presence of akinetic or dyskinetic left ventricular segments without any contractile reserve. At the same time color Doppler tissue imaging was performed. Within the next 2 days patients underwent the gated technetium-99 m sestamibi single photon emission computed tomography and positron emission tomography. Patients with an evidence of an irreversible damage of at least two akinetic or dyskinetic myocardial segments proved by all methods were then randomized. Patients of cell groups underwent subsequently a bone marrow aspiration. Autologous bone marrow mononuclear cells were transplanted into the infarct-related artery 20–21 hours after the bone marrow aspiration, 5–9 days after myocardial infarction. Immediately before and 10 and 20 hours after the procedure, blood samples for cardiac enzymes (creatine kinase, creatine kinase–MB and troponin I) were acquired.

Three months after randomization, rest echocardiography with Doppler tissue imaging, single photon emission computed tomography, and coronary angiography were repeated. Patients of the control group underwent the same procedures and examinations, as did the transplanted patients except for bone marrow aspiration and cell transplantation.

In this subanalysis, the changes of following echocardiographic parameters were assessed: (1) the peak systolic velocity of the myocardium adjacent to mitral annulus of infarcted wall ($S_{infarct}$) (as a parameter of the regional longitudinal left ventricular systolic function); (2) the mean six-site systolic velocity of the myocardium adjacent to mitral annulus (as a parameter of the global longitudinal left ventricular systolic function), which was calculated as mean six-site $S = (S_{lateral} + S_{septal} + S_{anterior} + S_{inferior} + S_{anteroseptal} + S_{posterior})/6$; and (3) number of akinetic segments.

The changes of following parameters derived from single photon emission computed tomography were assessed: (1) left ventricle end-diastolic volume; (2) left ventricle end-systolic volume; (3) left ventricle ejection fraction; and (4) perfusion defect size.

The institutional ethics committee approved the study and written consent was obtained from each patient.

Echocardiography

Using commercially available equipment Vivid 7 (GE/Vingmed, Milwaukee, WI, USA) with an M3 S transducer, echocardiographic examinations were performed in one center. Two-dimensional and color Doppler tissue images of apical views (apical 4- and 2-chamber and apical long-axis views) were obtained and stored digitally for the subsequent offline quantitative analysis using a software incorporated in Vivid 7 (Echopac 7 version 1.3, GE/Vingmed). The wider-angle sector (60–70 degrees) was used to depict two-dimensional images for wall motion analysis. The narrow angle sector (30–45 degrees) was used to obtain color Doppler tissue images of individual left ventricular walls (septum, lateral, inferior, anterior, posterior, and anteroseptal walls) at the high frame rates of 172–234 frames per second.

Dobutamine echocardiography was performed in all patients with starting dose of 5 μg/kg per min. The dose was increased at 5-minute intervals to 10, and 20 μg/kg per min. The parasternal long-axis and three apical views were digitally stored at rest and at the last minute of all doses of dobutamine for a subsequent wall motion analysis. A 16-segment model was used for regional wall motion analysis.[13] The akinetic and dyskinetic segments with no improvement in thickening after any dose of dobutamine were regarded as irreversibly damaged. A good interobserver and intraobserver variability in scoring dysfunctional segments (agreement 93% and 96%, respectively) and in determining the contractile reserve (agreement 92% and 95%, respectively) has already been described.[14]

The regional longitudinal systolic function was evaluated from the color Doppler tissue imaging.[15,16] Peak systolic velocities (S) were determined for the basal myocardium of each wall adjacent to the mitral annulus ($S_{lateral}$, S_{septal}, $S_{anterior}$, $S_{inferior}$, $S_{anteroseptal}$, and $S_{posterior}$). The results were obtained as a mean from three consecutive heart cycles. Two experienced echocardiographers who were blinded to the patient treatment performed the analyses. The reproducibility of estimation of S values of individual walls was evaluated in our initial 3-month project.[12] For all S values, the estimated 95% confidence limits for differences between intraobserver (JM) pairs of measurement revealed repeated results to vary in a range of $\pm$ 10.6% as based on the mean primary values and similarly $\pm$ 11.5% for the interobserver variability (JM and RP). The sufficient interobserver reproducibility was also proved in applied pairwise ANOVA models: only 4.8% of overall variability could be attributed to the differences among observers and the interobserver effect was unambiguously not significant ($P = 0.963$).

Gated Technetium-99 m Sestamibi Single Photon Emission Computed Tomography

Seven hundred forty MBq technetium-99 m sestamibi was injected at rest. Gated single photon emission computed tomography imaging acquisition (64 projections from the 45° right anterior oblique projection to the 45° left posterior oblique projection) began 1 hour after sestamibi injection using a 2-detector gamma camera (ecam, Siemens, Erlangen, Germany) equipped with a low-energy, high-resolution parallel-hole collimators. The MIBI uptake was analyzed visually and quantitatively on computer-generated polar maps by an experienced nuclear cardiologist who was unaware of the patients treatment. Pixels with a sestamibi activity $>$ 2.5 SD below the corresponding normal mean values were considered abnormal. The computer automatically expressed a perfusion defect as the number of abnormal pixels divided by the total number of left ventricle pixels $\times$ 100 project.[17] In the viability analysis, the myocardial region with the maximum sestamibi uptake was used as a reference

region. The tracer uptake in other myocardial regions was then expressed as a percentage of the activity measured in the reference region. Nonviable myocardium was defined as that having sestamibi uptake below the threshold of 50% of the maximum project.[18] Gated single photon emission computed tomography rest left ventricular ejection fractions and left ventricular end-diastolic/end-systolic volumes were obtained using automated, commercially available software four-dimensional-MSPECT (University of Michigan, Ann Arbor, MI, USA).

Positron Emission Tomography

To assess myocardial viability, F-18-fluorodeoxyglucose-positron emission tomography was performed with a whole-body positron emission tomography scanner (ECAT ACCEL, Siemens, Knoxville, TN, USA). Acquisition was started 50 minutes after the administration of fluorodeoxyglucose (200–250 MBq intravenously) and images of glucose utilization were acquired for 15–20 minutes in a 3D mode. The metabolic defects were analyzed on computer-generated polar maps. The myocardial fluorodeoxyglucose uptake for each part of the left ventricle was normalized to a myocardial region with the maximum fluorodeoxyglucose uptake. A nonviable myocardium was defined as that having less than 50% of the maximum fluorodeoxyglucose uptake.[18]

Bone Marrow Aspiration and Preparation

The target volume of bone marrow blood (100 ml for the lower cell dose, 150 ml for the higher cell dose) was obtained from iliac crests under local anesthesia and moderate sedation with midazolam, mixed with 4% human albumin and 5,000 IU of heparin, and centrifuged (15 minutes, 240 g) to receive buffy-coat. Mononuclear cells were collected using density gradient centrifugation of the buffy-coat (20 minutes, 1,200 g, Histopaque 1077, Sigma-Aldrich, St. Louis, MO, USA), washed, and resuspended. One hundred twenty-five percent of the target amount of mononuclear cells was added to the CellGro serum-free medium (CellGenix, Freiburg, Germany) to reach 0.3–1.0 $\times$ 106 cells/ml. After an overnight cultivation (37 °C, 5% CO2) in a teflon bag (VueLife, CellGenix), 105% of the target number of mononuclear cells was withdrawn, washed, and resuspended in the Hank's salt solution (Sigma-Aldrich) with 4% human albumin and 1,000 IU of heparin into a total volume of 22 ml.

Cell Implantation

Autologous mononuclear bone marrow cells were transplanted 5–9 days after the infarction onset using a modification of the method described previously by Strauer et al.[19] Cells were implanted intracoronary via a percutaneous transluminal catheter into the infarct-related coronary artery. A total of seven balloon inflations at the place of previous stent implantation lasting for 3 minutes were carried out with 3-minute intervals of balloon deflation. At the beginning of each balloon inflation, 3 ml of cell suspension was slowly injected into the artery. All patients were on daily doses of 75 mg of clopidogrel and 100 mg of aspirin and, in addition, a bolus of 100 units/kg of body weight of heparin was administered immediately before the procedure to minimize the risk of thrombotic complications.

Statistical Analysis

Standard descriptive statistics were used to summarize the sample distribution of individual variables (means, standard errors, confidence limits). A univariate *t*-test for two independent samples was applied to compare values of parameters between the groups. A paired *t*-test was applied to compare changes in values prior and after the treatment. All parametric tests were performed with the verified assumption of normal distribution (Shapiro–Wilk's W-test). Two independent samples were mutually compared on the basis of proved homogeneity of variance (Variance ratio F-test). The correlation analysis was based on Pearson's correlation coefficient. A $P < 0.05$ was considered statistically significant.

Repeated measures ANOVA model was used to test the results obtained by different observers (measured in all patients included in the reproducibility test). The pairwise design included overall F-test of the main effects (i.e., differences among different observers) and then estimation of within-observer variability.

Results

This subanalysis contains 47 patients. Thirty of them were treated with mononuclear bone

TABLE I

Characteristics of the Study Population

Parameter	Control (C) Group (n = 17)	Lower Cell Dose (L) Group (n = 13)	Higher Cell Dose (H) Group (n = 17)
Age (years)	52 (2)	55 (2)	55 (5)
Men	15 (88%)	12 (92%)	15 (88%)
Hypertension	9 (53%)	5 (39%)	5 (29%)
Hyperlipidemia	6 (35%)	9 (69%)	7 (41%)
Diabetes mellitus	4 (24%)	1 (8%)	3 (18%)
Single-vessel disease	11 (65%)	9 (69%)	13 (76%)
Double-vessel disease	6 (35%)	3 (23%)	4 (24%)
Triple-vessel disease	0%	1 (8%)	0%
IRA: LAD	16 (94%)	12 (92%)	16 (94%)
IRA: LCX	0%	0%	0%
IRA: RCA	1 (6%)	1 (8%)	1 (6%)
Maximum CK (ukat/l)	80.2 (11.1)	80.2 (9.4)	68.9 (7.2)
Maximum CK-MB (ukat/l)	7.4 (0.6)	7.6 (0.9)	6.8 (0.7)
Time from infarct onset to reperfusion (min)	507 (240)	263 (53)	484 (192)
Time from infarct onset to cell transplantation (days)	–	7 (0.4)	7 (0.3)
Dobutamine echo			
No. of irreversibly damaged segments	6 (0.7)	7 (0.4)	7 (0.7)
Medication on hospital discharge			
Aspirin	17 (100%)	13 (100%)	17 (100%)
Clopidogrel	15 (88%)	13 (100%)	17 (100%)
ACE inhibitor	17 (100%)	13 (100%)	17 (100%)
Beta blocker	17 (100%)	13 (100%)	17 (100%)
Statin	17 (100%)	13 (100%)	17 (100%)

The values are expressed as the mean supplied by standard error (in parentheses) or number (%) of subjects. ACE = angiotensin-converting enzyme; CK = creatine kinase; echo = echocardiography; IRA = infarct-related artery; LAD = left anterior descending coronary artery; LCX = left circumflex artery; No = number; RCA = right coronary artery.

marrow cell implantation–17 patients in the Group H with higher cell doses, while 13 in the Group L with lower cell doses, and 17 of them served as a control Group C. The baseline characteristics are presented in Table I. There were no significant differences among the groups.

The Effect of Cell Transplantation on Myocardial Function and Left Ventricle Remodeling

The results of echocardiographic examinations and single photon emission computed tomography data are demonstrated in Table II.

There was a trend toward the prevention of the left ventricle dilatation (end-diastolic volume) and the improvement of the left ventricle ejection fraction in transplanted patients. Patients of the high-doses group significantly improved the regional systolic function ($S_{infarct}$) after 3-month follow-up. We proved significant improvement in these parameters (left ventricle ejection fraction, end-systolic volume, peak systolic velocity of infarcted myocardium and number of akinetic segments) in cell therapy patients, as it is documented through significant results of within-group testing. However, there were no statistically differences among the groups.

The side effects have already been published.[12]

Phenotype of Transplanted Cells

The samples were analyzed from 29 patients (in one patient a small sample size did not allow adequate analysis). The transplanted leukocytes contained in the mean 43.4% CD3+ cells, 2.9% CD16+ cells, 11.0% CD19+ cells, 0.4% CD33+ cells, and 1.1% CD34+ cells, respectively. The viability of mononuclear cells was evaluated after the cultivation. In all cases, the viability exceeded 95%.

TABLE II

Comparison of Baseline and 3-Month Follow-Up Echocardiographic and Single Photon Emission Computed Tomography Results for the Treatment and Control Groups

Parameter	C Group (n = 17)	L Group (n = 13)	H Group (n = 17)	Mutual Comparison (P-Values)†		
				C vs. L	C vs. H	L vs. H
Echocardiography						
Mean 6-site S (cm/s)	4.9 (0.2)	5.1 (0.3)	5.2 (0.2)	0.821	0.416	0.594
Baseline	5.2 (0.3)	4.9 (0.3)	5.0 (0.2)	0.485	0.611	0.822
Follow-up	0.3 (0.2)	−0.2 (0.3)	−0.2 (0.2)	0.298	0.153	0.813
Change‡	0.393	0.625	0.193			
P-value‡						
$S_{infarct}$ (cm/s)						
Baseline	4.5 (0.2)	4.2 (0.3)	4.3 (0.2)	0.691	0.975	0.704
Follow-up	4.8 (0.3)	4.4 (0.3)	4.7 (0.3)	0.283	0.432	0.728
Change‡	0.3 (0.2)	0.2 (0.2)	0.4 (0.1)	0.261	0.342	0.215
P-value‡	0.153	0.337	0.013			
No. of akinetic s*						
Baseline	6.2 (0.6)	7.0 (0.4)	6.9 (0.6)	0.366	0.411	0.889
Follow-up	5.5 (0.7)	5.5 (0.6)	5.2 (0.7)	0.995	0.744	0.768
Change‡	−0.7 (0.4)	−1.5 (0.5)	−1.7 (0.5)	0.242	0.128	0.798
P-value‡	0.062	<0.001	<0.001			
SPECT						
EDV (ml)	171 (9)	176 (12)	178 (13)	0.786	0.677	0.907
Baseline	183 (13)	180 (12)	181 (12)	0.841	0.876	0.957
Follow-up	12 (8)	4 (10)	3 (8)	0.509	0.431	0.941
Change‡	0.153	0.696	0.713			
P-value‡						
ESV (ml)						
Baseline	112 (7)	112 (9)	117 (10)	0.998	0.674	0.694
Follow-up	115 (11)	106 (9)	107 (9)	0.555	0.572	0.949
Change‡	3 (8)	−6 (7)	−10 (4)	0.402	0.094	0.706
P-value‡	0.713	0.408	0.023			
LV EF (%)						
Baseline	35 (1)	36 (1)	35 (1)	0.343	0.939	0.308
Follow-up	39 (2)	41 (2)	41 (2)	0.284	0.324	0.897
Change‡	4 (2)	5 (1)	6 (2)	0.609	0.262	0.589
P-value‡	0.062	<0.001	<0.001			
Perfusion defect (%)						
Baseline	52 (4)	51 (4)	53 (4)	0.880	0.911	0.799
Follow-up	41 (4)	41 (5)	43 (4)	0.872	0.657	0.800
Change‡	−11 (3)	−10 (2)	−10 (2)	0.601	0.537	0.958
P-value‡	<0.001	<0.001	<0.001			

The values are expressed as the mean supplied by standard error (in parentheses). *Identified as nonviable on pretransplant dobutamine echocardiography. †Mutual significance "between groups" tested by *t*-test for two independent samples. ‡Pairwise calculated "within group" change of values tested by *t*-test for two-paired samples.
EDV = end-diastolic volume; ESV = end-systolic volume; LV EF = left ventricular ejection fraction; m = myocardium; S = peak systolic velocity of basal myocardium adjacent to mitral annulus; $S_{infarct}$ = peak systolic velocity of the infarcted wall; Mean 6-site S = ($S_{lateral}$ + S_{septal} + $S_{anterior}$ + $S_{inferior}$ + $S_{anteroseptal}$ + $S_{posterior}$)/6; s = segments; SPECT = single photon emission computed tomography; other abbreviations as in Table I.

Discussion

Potential Effect of Cell Therapy

Bone marrow contains a great number of primitive cells that are able to differentiate into specialized cells, for example into endothelial cells or myocytes.[20–24] Some of these primitive cells produce different growth factors,[21] for example vascular endothelial growth factor, basic fibroblast growth factor, and cytokines with proangiogenetic effect. For these reasons, many experimental studies were performed and

proved the possibility of cell therapy to improve perfusion or/and function of dysfunctional myocardium.[20–26]

Despite numerous unresolved questions concerning the cell transplantation, these first hopeful experimental studies were immediately followed by clinical trials, mostly in patients with acute myocardial infarction. The numbers of patients included are relatively small. Many of these studies are not randomized. The type and amount of cells that are necessary to implant to really regenerate damaged myocardium are not known.

At present, we do not know the mechanism of action of the implanted cells in studies that found improvement in myocardial function or perfusion following the cell therapy. Recently, several experimental projects described no or only negligible transdifferentiation of adult stem cells into the myocytes.[27–30] The benefit of cell transplantation may be induced by the paracrine stem cell effect.[31,32]

Studies in Patients with Acute Myocardial Infarctions

Transplantation of mononuclear bone marrow cells into the region of infarcted myocardium has been previously suggested as a promising alternative treatment for left ventricle dysfunction. Nevertheless, the results of randomized studies are controversial.[3,4,7–9] Some of them indicated that patients with the most depressed left ventricular contractile function had the greatest improvement in contractile function after intracoronary administration of bone marrow cells. For example, REPAIR-AMI,[33] so far the largest randomized multicenter "cell study," showed significantly greater increase in the global left ejection fraction in the bone marrow cell group (5.5% vs. 3.0% in the control group) at 4 months follow-up. Higher impact of cells was found among patients with a baseline left ventricle ejection fraction below the median value (48.9%). In these patients, the absolute increase in ejection fraction was three times higher that in the placebo group (7.5% as compared with 2.5%; absolute difference: 5.0%). Among patients with a baseline ejection fraction above median, the absolute difference between groups was only 0.3% (4.0% vs. 3.7%). Similar observations were previously described in TOPCARE-AMI trial,[34] in which baseline left ventricle ejection fraction was the only significant predictor of improvement in ejection fraction during the 4-months follow-up.

In the randomized, double-blind, placebo-controlled study of Janssens group,[35] in 67 patients with ST-elevation myocardial infarction treated with coronary intervention, no effect of autologous bone marrow-derived stem cell transfer on left ventricle ejection fraction was found. However, the treatment was associated with a significant reduction in myocardial infarct size and better recovery of regional systolic function. The effect of treatment on the probability of improvement in regional function showed a predominant interaction in the most severely affected segments. In addition to that, on positron emission tomography examination, patients with larger myocardial infarction had a greater increase in metabolic activity after cell therapy than after placebo infusion.

On the other hand, BOOST trial [36,37] did not describe the inverse relation between baseline left ventricular ejection fraction and absolute improvement of the left ventricle function after implantation of the bone marrow cells into the infarcted myocardium. At 6-month follow-up, patients in a control group of this study improved their ejection fraction from 51.3% to 52% (0.7% absolute change), while the bone marrow cell group from 50.0% to 56.7% (6.7% absolute change). The bone marrow cell subgroup patients with ejection fraction of the left ventricle $> 52\%$ increased their ejection by 8.0%, but patients with ejection fraction $\leq 52\%$ only by 4.5%.

The main limitation of these studies is the fact, that patients with only mild left ventricular dysfunction were included.

Studies in Patients with Moderate-to-Severe Left Ventricular Dysfunction

There are only few trials studying cell therapy in patients with moderate-to-severe left ventricular dysfunction and their results are controversial too. Bartunek et al.[3] described improvement of the left ventricular performance and increased myocardial perfusion and viability among patients with acute myocardial infarction treated with stenting and intracoronary administration of $CD133^+$ progenitor cells. The left ventricular ejection fraction increased from 45.0% to 52.1%.

Controversially, ASTAMI trial [11] did not find any significant difference between 47 patients treated with cell transplantations and 50 patients in the control group. The left ventricular

ejection fraction and end-diastolic volume were assessed by single photon emission computed tomography, echocardiography, and magnetic resonance. Improvement versus baseline values was found in both groups, but they did not significantly differ. Results were consistent for all the three methods. No improvement in cardiac function was also found in Kuethe et al.[38] in their study with five patients with a large acute anterior myocardial infarction and intracoronary mononuclear bone marrow cell implantation.

In our previous study,[12] the significant and dose-related improvement was found in the regional systolic function of the infarcted wall after cell transplantation. As compared to controls, a higher cell dose significantly improved global LV systolic function. Both cell doses prevented the left ventricle from the dilation, while the end-diastolic volume significantly increased in the control group. Because patients with the greatest damage to their myocardium are the ones who need treatment most, the substudy of these patients was performed. In this substudy the statistically important effect of autologous transplantation of mononuclear bone marrow cells on myocardial function was not found in patients with moderate-to-severe left ventricular dysfunction. Only an insignificant trend toward the prevention of the left ventricular dilatation and improvement of global left ventricle ejection fraction was found at 3-month follow-up.

Study Limitations

Except the fact that our study is subanalysis, the major limitations of our study are the small number of patients enrolled. However, to this moment it is one of the studies with the highest number of patients with more severe left ventricular dysfunction ever published. Compared to other studies, the very rigorous myocardial viability assessment was performed before inclusion to this study.

The groups differ slightly in time from onset of infarction to reperfusion. The differences were not statistically significant. The heterogeneity was caused by the inclusion of two patients with delayed coronary angioplasty (one patient in the Group H and one patient in the Group C). In our previous study the biggest effect of cell transplantations was found between higher dose and control groups. In this study, the difference in time from infarct onset to reperfusion between Groups H and C was just 23 minutes. So this difference has not been supposed to affect the results.

Because of ethical consideration, the patients included into the control group did not undergo the identical procedures, as did the bone marrow cell patients, being excluded from the bone marrow aspiration and coronary angiography with the sham cell transplantation. For technical reasons, the positron emission tomography (PET) was not performed in all our patients.

In addition to the limited study population, another explanation of our results could be the very severe myocardial damage with almost no surviving myocytes. In these conditions, there is no suitable milieu for catching implanted cells and their differentiation into cardiomyocytes. Also the severe destruction of microcirculation could make the cell homing more difficult compared to patients with less severe myocardial damage.

Conclusion

The important thing is the fact that the selection of cells and the whole method of cell therapy are just at the beginning of the way. Probably, it is not realistic to expect some greater changes of left ventricle function in this manner of treatment. It is necessary to look for the best cell type, an optimal way and time of cell delivery, and the help of some cytokines. For solving these clinical questions, we must also better understand the mechanisms of potential positive effect of the cell therapy.

Taking together, the results of trials show that there is still work to be done to understand a lot of questions related to the cell therapy. Further studies, including larger numbers of patients, are needed to resolve all these tasks.

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