Effect on Left Ventricular Function of Intracoronary Transplantation of Autologous Bone Marrow Mesenchymal Stem Cell in Patients With Acute Myocardial Infarction

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Sixty-nine patients who underwent primary percutaneous coronary intervention within 12 hours after onset of acute myocardial infarction were randomized to receive intracoronary injection of autologous bone marrow mesenchymal stem cell or standard saline. Several imaging techniques demonstrated that bone marrow mesenchymal stem cells significantly improved left ventricular function. ©2004 by Excerpta Medica, Inc.

Different methods have been used to repair infarcted myocardium after acute myocardial infarction.1 Of these, the most important is “biointerventional therapy,” which mainly involves stem cells derived from bone marrow. Bone marrow contains multipotent adult stem cells, which have a great capacity of directionally differentiating to myocardium.2 Although many cell types related to bone marrow contribute to organ repair in infarction models, bone marrow mesenchymal stem cells (BMSCs) have the greatest potential for repairing myocardia.3 BMSCs have long been considered “second-class citizens.” Recently, it became clear that a small population of BMSCs in the bone marrow includes putative adult stem cells with important functional features, such as the potential for rapid self-renewal, giving rise to a wide variety of connective tissues, including bone, cartilage, muscle, fat, hemopoiesis-supporting stroma, and perhaps neuronal cells.4,5 Several aspects of BMSCs have been elucidated. BMSCs occur at very low frequencies of 2 to 5/106 mononuclear cells in bone marrow harvests. However, rapid proliferation of these cells in vitro allows expansion of this population by a factor of 103 within 14 to 21 days of culture. The growth rate of BMSCs depends on the density of cells. Unless cultured for prolonged periods, BMSCs do not show senescence and apoptosis. Thus, BMSCs can be tremendously expanded within a reasonable period.

Several reports have demonstrated that implantation of cultured bone marrow mononuclear cells by intracoronary injection improves left ventricular function.3,6 Therefore, BMSCs may have a strong capability of penetrating the myocardium from the coronary artery and significantly regenerate the myocardium.8,9 We used a randomized study to investigate the effectiveness of intracoronary injection of BMSCs in patients with acute myocardial infarction. •

Seventy-eight patients with acute myocardial infarction according to World Health Organization criteria and within 12 hours of the onset of continuous chest pain were enrolled into the present study and underwent emergency angiography or angioplasty from November 2002 to May 2003. The mean time from the onset of acute myocardial infarction to angiography and angioplasty was 8 ± 3.7 hours.

The infarct-related artery was recanalized by balloon angioplasty only (n = 7) and then by stent deployment (n = 71) after catheterization of the right and left sides with residual stenosis of <20%. All patients were briefed in detail about the significance and exact procedure of BMSC implantation immediately after percutaneous coronary intervention (PCI). Formal consents from patients’ relatives were obtained, and the study protocol was approved by ethics committee of the local government and the Nanjing First Hospital (Nanjing, China). Inclusion criteria for BMSC implantation included age <70 years old, no cardiac shock or cardiac block, stable hemodynamics, and no severe co-morbidities. Sixty-nine of 78 patients were candidates for the BMSC procedure and were randomized to receive BMSCs (n = 34) or saline (PCI-only group, n = 35) as the control arm.

Sixty milliliters of autologous bone marrow was aspirated under local anesthesia from the ilia of all 69 patients in the morning 8 days after PCI and cultured for 10 days. BMSCs were cultured and harvested by the method of Jaiswal et al.5 BMSCs were harvested and washed 3 to 4 times with heparinized saline, and the BMSC suspension was mixed with heparin, filtrated, and prepared for implantation just 2 hours before implantation.

The infarct-related coronary artery was occluded just at the proximal edge of the previous angioplasty, as described by Strauer et al.7 Six milliliters of the BMSC suspension containing 8 to 10 × 109 cells/ml was injected directly into the target coronary artery.
through an inflated over-the-wire balloon catheter in the central lumen with high pressure (10 atm). The balloon remained inflated for ≥2 minutes to occlude anterior blood flow just before beginning the BMSC injection.

Six milliliters of standard saline rather than BMSC was injected through the coronary artery in the control group by using the same method described for the BMSC group, and the harvested BMSCs were collected for use in basic research only.

All 69 patients underwent positron emission tomography with F-18-2 fluoro-2-deoxyglucose, cardiac catheterization, and cardiac echocardiography on the day of BMSC transplantation and 3 and 6 months after transplantation. Myocardial viability and cardiac function were recorded as the basal index. The contractility index was calculated by dividing left ventricular systolic pressure by end-systolic volume. Perfusion defect was calculated by the scintigraphic bull’s-eye technique. To quantify infarction wall movement velocity by cardiac echocardiography, 5 axes were placed perpendicular to the long axis in the main akinetic or dyskinetic segment of the ventricular wall. Relative systolic and diastolic lengths were measured, and the mean difference was divided by the systolic duration.

Electromechanical mapping (EMM) system was performed for 15 patients in the BMSC group and 8 patients in the saline group the day before and 3 months after transplantation. The EMM system has been previously described in detail. Briefly, a 45-cm, 8Fr sheath was inserted into the right femoral artery. Systemic heparin (100 U/kg) was administered through the side arm of the sheath. The 8Fr electromagnetic mapping catheter was inserted into the sheath and directly onto the aortic valve after standard ventriculography at a right oblique angle of 30° and a left oblique angle of 30°. The operator advanced the handle to ease the distal mapping catheter into the left ventricle, and points were acquired when the catheter tip was stable on the endocardium; this occurred after documentation of stability for local activation time, location, loop, and cycle length. An interpolation threshold of 40 mm was set between adjacent points. Continuous mapping of the left ventricle was done at a right oblique angle of 30° and a left oblique angle of 30°. Linear shortening maps and color codes were generated for each patient. EMM images were divided into 14 segments corresponding to positron emission tomographic images. The long axis was defined as the line connecting the apex and the center of the mass. Unipolar voltage was recorded automatically at the line connecting the apex and the center of the mass. The percentage of hypokinetic, akinetic, and dyskinetic segments decreased significantly in the BMSC group than in the control group (Table 1).

All patients received care for 24 hours after intracoronary injection and the EMM procedure and underwent clinical follow-up for ≥6 months. The primary end point was cardiac death. Cardiac echocardiography was recorded once a month, and positron emission tomography was performed 3 and 6 months after implantation in all patients. Electrocardiographic monitoring for 24 hours was also recorded 3 months after the procedure. Repeat electromechanical mapping was performed only if the patient wanted the procedure. All indexes were collected and analyzed by 3 statisticians who had no knowledge of the study.

All data are presented as mean ± SD, and p < 0.05 was considered statistically significant. Discrete variables were compared as rates, and comparisons were done by chi-square analysis. Intra-individual comparison of continuous variables at baseline with those at follow-up was performed with the paired t test. Comparison of nonparametric data between groups was performed with the Wilcoxon test and the Mann-Whitney test. Statistical analysis was performed with SPSS 10.1 for Windows (SPSS, Inc., Chicago, Illinois).

Baseline clinical characteristics between groups did not differ significantly. The level of creatinine kinase-MB was slightly but not significantly higher in the BMSC group than in the control group (Table 1). No deaths occurred during the 6-month follow-up.

Left ventricular dynamics according to a left ventriculogram demonstrated several differences (Table 2). The percentage of hypokinetic, akinetic, and dyskinetic segments decreased significantly in BMSC patients after 3 months compared with that before BMSC transplantation (13 ± 5% vs 32 ± 11%). This percentage was also smaller in the control group after 3 months (13 ± 5% vs 28 ± 10%). Wall movement velocity over the infarcted region increased significantly in the BMSC group (2.17 ± 1.3 cm/s vs 4.2 ± 2.5 cm/s) but not in the control group (2.19 ± 1.5 cm/s vs 2.7 ± 1.7 cm/s). Left ventricular ejection fraction 3 months after transplantation in the BMSC group increased significantly compared with that before implantation and with the control group 3 months after injection (67 ± 11% vs 49 ± 9% and 53 ± 8%). Left ventricular ejection fraction showed no change 3 months versus 6 months after transplantation in the BMSC group.

Perfusion defects detected by positron emission tomography decreased significantly in the BMSC group after 3 months compared with those in the control group (134 ± 66 cm² vs 185 ± 87 cm²). Left ventricular end-diastolic volume (162 ± 27 ml vs 136

<table>
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<th>TABLE 1</th>
<th>Baseline Characteristics of 69 Patients</th>
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<td>Parameters</td>
<td>BMSC Group (n = 34)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>58 ± 7</td>
</tr>
<tr>
<td>Men/women</td>
<td>32/2</td>
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<tr>
<td>Creatinine kinase-MB (U/L)</td>
<td>98 ± 54</td>
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<tr>
<td>Time</td>
<td></td>
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<tr>
<td>Onset to PCI (h)</td>
<td>8.3 ± 3.8</td>
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<tr>
<td>PCI to injection* (d)</td>
<td>18.4 ± 0.5</td>
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<tr>
<td>Coronary angiography</td>
<td></td>
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<tr>
<td>No. of diseased coronary arteries</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>AMI-related vessel: LAD/IC*/right</td>
<td>20/6/8</td>
</tr>
<tr>
<td>Stents deployed (n=)</td>
<td>36</td>
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*Intracoronary injection of BMSC or saline.
AMI = acute myocardial infarction; LAD = left anterior descending; IC = left circumflex.
In recent years, the understanding that regenerative processes exist at the level of the myocardium has placed stem cell research at the center stage of cardiology. The concept of “growing” heart muscle and vascular tissue has revolutionized the approach to treating myocardial infarction. Autologous bone marrow stem cells, which are not rejected by the immune system, have the ability to differentiate into myocardium and play a central role in modern “cell therapy” of myocardial infarction. However, use of stem cells has posed more questions than answers. Which kind of stem cell is suitable for patients? When is it best to transplant? The most crucial questions we had to address before designing this trial were the following. (1) Which kind of cell should be delivered? (2) When should cells be transplanted? (3) How should the viability of transplanted cells be monitored?

The microenvironment plays a fundamental role in the transdifferentiation of stem cells. Use of skeletal myoblasts has invariably succeeded in reconstituting the structure of heart muscle, that is, the myocardium and coronary vessels. However, this kind of cell has several disadvantages, such as being too large to inject through the coronary artery, thromboembolism (which requires open chest surgery), and documenting cardiac improvement until 5 months after the procedure. Therefore, much more attention has been paid to BMSCs.

Intracoronary injection of BMSCs is the simplest clinical approach, as previously reported. Theoretically, BMSCs have the greatest potential to adhere to the vessel wall and may infiltrate into the infarct zone even after balloon deflation and restoration of anterior blood flow. We found experimentally that anterior blood flow took up the BMSCs very quickly and that a large amount of these cells infiltrated the small distal infarcted zone compared with injected BMSCs. The EMM system confirmed this phenomenon clinically and showed that BMSCs were transplanted into the infarcted zone along the vessel from the proximal edge at the site of injection. This contributed to the growth of BMSCs in the heart and significant improvement of cardiac function. These were the reasons we chose BMSCs as the source of stem cells for transplantation in the present study.

Wakitani et al first reported that BMSCs could...
differentiate into skeletal muscle, which supplied the basis for clinical research. Fukuda et al\textsuperscript{12} carried out animal research with cultured BMSCs preconditioned with 5-azacytidine and transplanted those cells into hearts. The results showed BMSCs can differentiate into myocardium. This process of differentiation was characterized as automatic contraction within 1 week and myocardial special gene expression as expressing atrial natriuretic peptide and troponin T. However, the time window was the key problem for transplantation of BMSCs in patients with myocardial infarction and remained unclear. Strauer et al\textsuperscript{7} found that the best time for transplantation was 7 to 14 days after acute myocardial infarction. The results indicated that more aggressive prevention of left ventricular remodeling by cell therapy during the late dilation phase was practical because cell therapy was not possible at an early phase of remodeling. Hence, we investigated the effect of relatively delayed BMSC transplantation on infarction after 3 weeks. The interval was nearly 18 days from PCI to BMSC transplantation, which definitely excluded the effect of PCI on outcomes of BMSC transplantation. The final results of the present study demonstrated that BMSC implantation significantly improves cardiac function and thus confirms that BMSC improves remodeling of the left ventricle by supplying viable cardiomyocytes.

Nuclear perfusion imaging, including positron emission tomography, has been considered a golden standard for the detection of viable myocardium.\textsuperscript{13} From clinical and practical viewpoints, a real-time detecting device should be the best because it is “real-time in the catheter laboratory.” For example, we need immediate and accurate answers about the viability of the myocardium in an area perfused by a long occluded coronary artery during angiography. Cardiac electromechanical mapping resolved this problem and correlated significantly with positron emission tomographic results reported by other investigators.\textsuperscript{14} This is the first study to follow and detect the viability of BMSCs and cardiac function with cardiac electromechanical mapping. The results showed that BMSCs 3 months after transplantation were viable, with high left line local shortening and unipolar voltage in the infarcted area and increased cardiac functional indexes as demonstrated by cardiac echocardiography, which encouraged our further study. The results clinically resolved the assessment of viability of implanted BMSCs and confirmed that BMSCs function with host cardiomyocytes.

Serial cardiac echocardiographic monitoring demonstrated improvement of cardiac function 1 to 3 months after implantation of BMSCs, and improvement was maintained nearly 6 months after the procedure.