Functional and bioenergetic modulations in the infarct border zone following autologous mesenchymal stem cell transplantation

Julia Feygin,1,2 Abdul Mansoor,2 Peter Eckman,2 Cory Swingen,2 and Jianyi Zhang2
1Department of Biomedical Engineering and 2Department of Cardiology, University of Minnesota, Minneapolis, Minnesota

Submitted 26 February 2007; accepted in final form 11 June 2007

Feygin J, Mansoor A, Eckman P, Swingen C, Zhang J. Functional and bioenergetic modulations in the infarct border zone following autologous mesenchymal stem cell transplantation. Am J Physiol Heart Circ Physiol 293: H1772–H1780, 2007. First published June 15, 2007; doi:10.1152/ajpheart.00242.2007.—Preclinical and clinical studies have demonstrated that stem cell transplantation can improve the left ventricular (LV) contractile performance, yet the underlying mechanisms remain unknown. We examined whether mesenchymal stem cell (MSC) transplantation-induced beneficial effects are secondary to paracrine-associated improvements in LV contractile performance, wall stress, and myocardial bioenergetics in hearts with postinfarction LV remodeling. Myocardial contractile function and bioenergetics were compared 4 wk after acute myocardial infarction in normal pigs (n = 6), untreated pigs with myocardial infarction (MI group; n = 6), and pigs receiving autologous MSC transplantation (MI + MSC group; n = 5). A distal occlusion of the left anterior descending coronary artery instigated significant myocardial hypertrophy. Ejection fraction decreased from 55.3 ± 3.1% (normal) to 30.4 ± 2.3% (MI group; P < 0.01) and to 45.4 ± 3.1% (MI + MSC group; P < 0.01 vs. MI). Hearts in the MI group developed severe contractile dyskinesis in the infarct zone and border zone (BZ). MSC transplantation significantly improved contractile performance from dyskinesis to active contraction (P < 0.01 vs. MI). BZ systolic wall stress was severely increased in MI hearts but significantly improved after MSC transplantation (P < 0.01 vs. MI). The BZ demonstrated profound bioenergetic abnormalities in MI pigs; this was significantly improved after MSC transplantation (P < 0.01 vs. MI). Patchy spared myocytes were found in the infarct zone of hearts receiving MSC transplantation but not in control hearts. These data demonstrate that MSC transplantation into the BZ would result in improved LV contractile function and consequent significant reduction in BZ myocardial wall stress with improvement in BZ myocardial bioenergetics. We tested these hypotheses in an established porcine postinfarction LV remodeling model (13, 15, 18).

METHODS

All experiments were performed in accordance with the animal use guidelines of the University of Minnesota, and the experimental protocol was approved by the University of Minnesota Research Animal Resources Committee. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1985).

Bone marrow-derived MSC harvest, purification, and expansion. Methods for MSC isolation and expansion have previously been described in detail (15). Briefly, ~2 wk before induction of myocardial infarction, bone marrow was aspirated from the sternum into a syringe containing 6,000 U of heparin and diluted at a one-to-one ratio with Dulbecco’s PBS. Mononuclear cells were isolated by gradient density centrifugation. The bone marrow sample was carefully layered onto Ficoll-Paque 1077 (Sigma) in a 50-ml conical tube and centrifuged for 30 min at 400 g at room temperature. The mononuclear cells remain unclear but may be related to progressive expansion of contractile dysfunction from the region of viable myocardium that surrounds the infarct border zone (BZ) to the entire LV (13).

It has been demonstrated that the cardiac tissue in the infarct BZ experiences an increase in its radius of curvature and wall stress as a consequence of being tethered to the nonfunctional scar (11). We have recently reported that a remarkable bioenergetic heterogeneity exists between the BZ and the remote myocardial regions of swine hearts with compensated postinfarction LV remodeling (13). The abnormal myocardial bioenergetic state, as represented by high-energy phosphate content and the phosphocreatine (PCr)-to-ATP ratio, was markedly more severe in the BZ than in the remote myocardial regions in the same hearts (13). In addition, MRI-measured myocardial contractile function was most depressed in the BZ. The extent of reduction in myocardial energetics is known to be linearly related to the severity of LV contractile dysfunction in diseased hearts (36, 37). Together, these data suggest that the viable myocardium in the BZ has a severely reduced energetic capacity and operates at a very low energetic state and therefore is likely to be more vulnerable to oxidative stresses. Over time, the bioenergetic and contractile abnormalities of the BZ may extend laterally and eventually involve the entire LV, thereby leading to global LV dysfunction and the development of CHF.

Both preclinical and clinical studies have demonstrated that mesenchymal stem cell (MSC) transplantation can improve the LV contractile performance of hearts with postinfarction LV remodeling (1, 5, 15, 30, 34), yet the underlying mechanisms remain uncertain. We hypothesized that autologous MSC transplantation into the BZ would result in improved LV contractile function and consequent significant reduction in BZ myocardial wall stress with improvement in BZ myocardial bioenergetics. We tested these hypotheses in an established porcine postinfarction LV remodeling model (13, 15, 18).
were collected from the Ficoll-Paque-plasma interface, washed, and seeded on a T-150 flask previously coated with 10 ng/ml fibronectin. Cells were cultured in medium consisting of 60% low-glucose DMEM (GIBCO BRL), 40% MCDB-201 (Sigma), 1 x insulin transferrin selenium, 1 x linoleic acid-BSA, 2% FCS, 10 ng/ml PDGF, 10 ng/ml EGF, 10 U/ml penicillin, and 10 µg/ml streptomycin. After 3 days, the medium was replaced, and nonadherent cells were removed. By 5 days, large colonies of MSCs could be seen throughout the flask. The cells were trypsinized and replated at a density of 4,000–5,000 cells/cm². By ∼10–14 days, the primary MSC cultures had reached nearly 90% confluence and were ready for transfection with AdRSV-lacZ.

**MSC transfection with AdRSV-lacZ.** To track MSCs after transplantation, cells were transfected with AdRSV-lacZ. AdRSV-lacZ is a replication-deficient recombinant adenovirus carrying the β-galactosidase reporter gene lacZ regulated by Rous sarcoma virus long-terminal repeat promoters. Viruses were purchased from the Gene Vector Core, University of Iowa. Cells were washed with serum-free modified DMEM and infected for 8–10 h with AdRSV-lacZ. After the transfection time elapsed, the supernatant was removed, and cells were washed two times with PBS. The cells were then put back into the regular MSC culture medium overnight. The next day, cells were washed with PBS and resuspended in 1 ml of normal saline.

**Induction of myocardial infarction.** Details of the animal model of postinfarction LV remodeling have been described previously (13, 15, 18). Briefly, young Yorkshire female swine (45 days old, ∼10 kg) were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and ventilated with a respirator and supplemental oxygen. A left thoracotomy was performed, and the 1st and 2nd diagonal branches of the left anterior descending coronary artery (LAD) were dissected free and permanently occluded with ligatures. Autologous MSCs (50 million) suspended in saline solution were delivered via a direct intramyocardial injection into five regions around the infarct BZ. Injection sites were marked with a suture to allow identification of the areas for histological evaluations (For enhancing the cell engraftment rate, MSCs were co-injected with the mouse-derived fibrin and growth factors, which were rejected shortly after the transplantation. Consequently, only the autologous MSCs were effectively examined). Animals in the MI group received a saline injection in five locations throughout the infarct BZ. Animals were observed in the open-chest state for 60 min. If ventricular fibrillation occurred, electrical defibrillation was performed immediately and was usually successful. The chest was then closed. Animals received standard postoperative care, including analgesia, until they ate normally and became active. Animals were returned to the laboratory for MRI and 31P-MR spectroscopic studies, respectively.

**MRI protocol.** MRI was performed ∼25 days after surgery on a 1.5-T clinical scanner (Siemens Sonata, Siemens Medical Systems, Islen, NJ) using a phased-array four-channel surface coil and ECG gating. Animals were anesthetized with 1% isoflurane and positioned in a supine position within the scanner. The protocol consisted of 1) localizing scouts to identify the long and short axis of the heart, 2) short- and long-axis cine for the measurement of global cardiac function, 3) short-axis imaging with myocardial tagging in three slices for the measurement of regional myocardial strain, and 4) delayed contrast enhancement for the assessment of scar size and position. Steady-state “True-FISP” cine imaging used the following MR parameters: repetition time (TR) = 3.1 ms, echo time (TE) = 1.6 ms, flip angle = 79°, matrix size = 256 x 120, field of view = 340 mm x 265 mm, and slice thickness = 6 mm (4-mm gap between slices); 16–20 phases were acquired across the cardiac cycle. Global function and regional wall thickness data were computed from the short-axis cine images using MASS (Medis Medical Imaging Systems, Leiden, The Netherlands) for the manual segmentation of the endocardial and epicardial surfaces at both end diastole and end systole from base to apex. The tagging preparation consisted of nonselective radiofrequency (RF) pulses separated by encoding gradients for spatial modulation of magnetization, resulting in a tag line separation of 6 mm. Three short-axis slices were prescribed at basal, midventricular, and apical levels identical to the cine image positions. At each slice location, two sets of cine images were acquired with tag lines in orthogonal directions with the following scan parameters: TR = 6.5 ms, TE = 2.1 ms, flip angle = 14°, matrix size = 256 x 128, field of view = 320 mm x 320 mm, slice thickness = 6 mm, and a minimum of 14 cardiac phases. Tagged images were acquired for resting conditions only. Tagging data were analyzed with the HARP analysis package (HARP version 2.0, Nael Osman, John Hopkins Medical School) as described elsewhere (24, 25). Two-dimensional myocardial strains were assessed offline in six circumferential myocardial segments per short-axis slice. Transmural strains were calculated between the reference end-diastolic and end-systolic state as the fractional change in length in the circumferential and radial directions.

After acquisition of the tagged images, a bolus of 0.2 mmol/kg gadopentetate dimeglumine (Magnevist, Berlex, Montville, NJ) was administered, and segmented inversion-recovery turbo fast low-angle shot (turboFLASH) images were acquired after a 10- to 15-min delay to accurately identify regions of myocardial scar. Short-axis turboFLASH imaging, from base to apex, used TR = 16 ms, TE = 4 ms, inversion time of ∼220 ms, flip angle = 30°, matrix size = 256 x 148, field of view = 320 mm x 185 mm, slice thickness = 6 mm (0-mm gap between slices), and two signal averages. The appropriate inversion time was chosen to adequately null the signal intensity of normal myocardium.

**Surgical preparation for open-chest magnetic resonance spectroscopy studies.** Animals were anesthetized with pentobarbital sodium (loading dose of 30 mg/kg iv, maintenance dose of 4 mg·kg⁻¹·h⁻¹), intubated, and mechanically ventilated with oxygen-supplemented air to maintain arterial blood gases within the physiological range. Polyvinyl chloride catheters (3 mm OD) were inserted into the ascending aorta, inferior vena cava, and LV. The ascending aorta catheter was placed via the left internal carotid artery. Two intravenous catheters were placed through the left external jugular vein. The heart was exposed via a sternotomy and suspended in a pericardial cradle. The LV catheter was introduced through the apical dimple. A 28-mm-diameter single loop transmit/receive RF coil for magnetic resonance spectroscopy was sutured onto the anterior wall of the LV such that most of the coil was directly over the scar and a small portion of the coil was over the infarct BZ. Because the LV scar tissue does not contain high-energy phosphates, the entire high-energy phosphate NMR signal was obtained from the myocardial tissue in the BZ. The pericardial cradle was released to restore the heart to its normal position, and the animal was placed into a 4.7-T superconducting magnet.

**Hemodynamic measurements.** Aortic and LV pressures were measured by pressure transducers positioned at mid-chest level and recorded on an eight-channel recorder (18, 32, 36, 37). Ventilation rate, volume, and inspired oxygen content were adjusted to maintain physiological values for arterial P0₂, P CO₂, and pH. Aortic and LV pressures were monitored continuously throughout the study (18, 32, 36, 37). Hemodynamic measurements were acquired simultaneously with the spectra.

**Spatially localized 31P-MR technique.** Spatially localized 31P-NMR spectroscopy was performed by the adiabatic plane-rotation pulses for phase modulation (RAPP)-image-selected in vivo spectroscopy (ISIS)/Fourier series window (FSW) method (rotating-frame experiment using RAPP-ISIS/FSW method) (18, 32, 36, 37). Detailed experiments documenting voxel profiles, voxel volumes, and spatial resolution attained by this method have been published previously. In this application of RAPP-ISIS/FSW, the signal origin was first restricted to a 12 x 12-mm two-dimensional column perpendicular to the LV wall. The signal was later localized into three well-resolved and five partially resolved layers along the column and hence across.
the LV wall. Localization along the column was based on B1 phase encoding and employed a nine-term FSW as previously described (18, 32, 36, 37). Whole wall spectra were obtained with the ISIS technique, defining a column 12 × 12-mm² perpendicular to the heart wall. The calibration of spectroscopic parameters was facilitated by placing a polyethylene capillary filled with 15 μl of 3 M phosphonoacetic acid into the inner diameter of the surface coil. This phosphonoacetic acid standard was used only for calculating the 90° pulse length of the RAPP-ISIS method (18, 32, 36, 37). The position of the voxels relative to the coil was set according to the B1 strength at the coil center, which was experimentally determined in each case by measuring the 90° pulse length for the phosphonoacetic acid standard contained in the reference capillary at the coil center. NMR data acquisition was gated to the cardiac and respiratory cycles using the cardiac cycle as the master clock to drive both the respirator and the NMR spectrometer as previously described (18, 32, 36, 37). The LV was opened at the lateral wall from base to apex, exploring. The LV was opened at the lateral wall from base to apex, and the LV structure was assessed in three horizontal short-axis rings from the central portion of the LV. Each ring was divided into six regions according to the dynamics variables were not significantly different between the groups. Infarct size was calculated using the concentration of free creatine and ATP values obtained from the spectra of the present study were calibrated using ATP and total creatine values chemically determined from biopsies taken during previous studies in the same animal model (13). [Cr]free was calculated by subtracting the PCr values from total creatine measured in biopsies.

Infarct size. After completion of the open-chest magnetic resonance spectroscopy (MRS) and hemodynamic measurements, animals were killed by an overdose of pentobarbital sodium, and the heart was explanted. The LV was opened at the lateral wall from base to apex, and a photograph was taken for infarct size measurement. Infarct size was determined by an image analysis system (NIH image J program, http://rsb.info.nih.gov/ij) and expressed as a percentage of LV surface area.

Evaluation of engraftment. One transverse LV ring (~6 mm in thickness), which contained both the infarcted area and one cell injection site, was selected and cut into 12 small pieces along the circumferential direction. Two of the 12 pieces were from the infarct zone (IZ), and 2 more were from the infarct border zone (BZ). Each of the 12 pieces of cardiac tissue was stained by X-gal (Invitrogen) and embedded in Tissue-Tek OCT (Fisher Scientific). Serial cryostat sections (10 μm in thickness) were obtained from the whole transverse ring. β-Galactosidase-positive cells were counted from 1 of every 10 consecutive cryostat sections to calculate the percent engraftment. Assuming that the number of cells migrating into the ring from neighboring LV rings is equal to the number of cells migrating out from the given ring, the engraftment was calculated with the following equation: %engraftment = 100% × total number of β-galactosidase-positive cells counted × 10/million.

Data analysis. Data were analyzed with one-way ANOVA for repeated measurements. A value of P < 0.05 was considered significant. When significant results were found, individual comparisons were made with the Bonferroni correction. Data analyses were not blinded.

RESULTS

Anatomic data. Table 1 summarizes the anatomic data from six normal animals, six animals with postinfarction LV remodeling (MI group), and five animals receiving autologous MSC transplantation (MI + MSC group). The LV weight-to-body weight ratio (LV/BW) was increased in both groups of postinfarction animals, indicating the onset of LV hypertrophy (P < 0.05; Table 1). Infarct size was expressed as the ratio of scar surface area to LV surface area. LAD occlusion resulted in a 13 ± 3% infarct in the MI group and a 14 ± 2% infarct in the MI + MSC group (Table 1). Infarct size, LV weight/BW, and RV weight/BW were not affected by MSC transplantation.

LV ejection fraction and hemodynamics. LV ejection fraction (EF) was measured from CINE MRI images. Four weeks after LAD ligation, LV EF decreased from 55.3 ± 3.1% (normal group) to 30.4 ± 2.3% in hearts with postinfarction LV remodeling without MSC therapy (MI group), suggesting significant LV dysfunction. LV EF was significantly greater in animals receiving MSC transplantation (45.4 ± 3.1%; P < 0.01 vs. MI group; Table 2 and see Fig. 2A), Systemic hemodynamics variables were not significantly different between the three groups of animals, suggesting that the hearts had compensated postinfarction LV remodeling 4 wk after MI.

MRI analysis of LV structure. LV structure was assessed in three horizontal short-axis rings from the central portion of the LV. Each ring was divided into six regions according to the coronary artery perfusion pattern, as depicted in Fig. 1. The scar and BZ were localized in the anterior region (anterior wall and anterior papillary region, Fig. 1) of the LV. Short-axis cine MRI

Table 1. Anatomic data

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, kg</th>
<th>LV, g</th>
<th>RV, g</th>
<th>LV/BW, g/kg</th>
<th>RV/BW, g/kg</th>
<th>Scar Size (SSA/LVSA), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 8)</td>
<td>45±5</td>
<td>118±16</td>
<td>40±3</td>
<td>2.6±0.1</td>
<td>0.9±0.1</td>
<td>NA</td>
</tr>
<tr>
<td>MI (n = 8)</td>
<td>44±8</td>
<td>146±9*</td>
<td>51±6</td>
<td>3.5±0.3*</td>
<td>1.2±0.1</td>
<td>13±3</td>
</tr>
<tr>
<td>MI + MSC (n = 5)</td>
<td>40±8</td>
<td>144±11*</td>
<td>46±4</td>
<td>3.6±0.3*</td>
<td>1.1±0.1</td>
<td>14±2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of pigs. BW, body weight; LV, left ventricular weight; MI group, untreated pigs with myocardial infarction; MI + MSC group, pigs with myocardial infarction receiving autologous mesenchymal stem cell transplantation; NA, not available; RV, right ventricular weight; SSA, scar surface area; LVSA, LV surface area (by postmortem examination). *P < 0.05 vs. normal group.
analyses revealed that animals in the MI group developed significant diastolic wall thinning in the anterior region (Table 3; \( P < 0.01 \) vs. normal group). Systolic wall thickness was decreased throughout the entire LV ring; however, reduction was most severe in the anterior wall (Table 3; \( P < 0.01 \) vs. normal group). Anterior wall thickness was substantially preserved in MSC-treated animals during both systole and diastole (\( P < 0.05 \) vs. MI group; Table 3).

**MRI-measured LV function.** Regional myocardial contractile function was assessed by calculating the radial thickening fraction (CINE MRI) and circumferential shortening fraction (tagging MRI) in the six different segments from three horizontal short-axis rings in the central portion of the LV (Fig. 1). The radial thickening fraction significantly decreased throughout the entire LV ring in the MI group; however, the most significant reduction occurred in the anterior wall (normal: 38.5 \( \pm \) 3.9\%; MI: 1.0 \( \pm \) 1.6\%; \( P < 0.01 \); Table 4 and Fig. 2B). The negative radial thickening fraction values were observed in some animals in the MI group, indicating that the myocardial tissue in the infarct BZ bulged outward from not withstanding the high intraventricular systolic pressure. MSC transplantation significantly improved contractile performance in the IZ-BZ from systolic “bulging” to active thickening (27.2 \( \pm \) 0.3\%, \( P < 0.01 \) vs. MI; Table 4 and Fig. 2B). The circumferential shortening fraction was severely reduced in the MI group (\( P < 0.01 \); Table 4 and Fig. 2C) and significantly improved in response to MSC transplantation (Table 4 and Fig. 2C).

**Wall stress.** LV wall stress in different LV segments was calculated according the Laplace law, as previously described, using the following equation: wall stress = \( PR / (2T) \) (27), where \( P \) corresponds to LV pressure measured with a pressure transducer in a fluid-filled catheter, \( R \) corresponds to chamber radius, and \( T \) corresponds to wall thickness measured from CINE MRI images in the particular region of the LV. Systolic wall stress was significantly increased throughout the LV ring in both groups of postinfarction animals (\( P < 0.05 \) vs. normal; Table 5 and Fig. 2D). This increase was most severe in the IZ-BZ (anterior wall) of animals in the MI group, being almost double that of normal (normal group: 157.0 \( \pm \) 10.7 mmHg, MI group: 297.4 \( \pm \) 16.6 mmHg; \( P < 0.01 \); Table 5 and Fig. 2D). MSC transplantation reduced the systolic wall stress in the IZ-BZ by \( \sim 24\% \) to 227.8 \( \pm \) 9.1 mmHg (\( P < 0.01 \) vs. MI; Table 5 and Fig. 2D). MSC transplantation reduced the elevated wall stress by 23\% in the anterior wall (\( P < 0.01 \) vs. MI; Table 5 and Fig. 2D). Diastolic wall stress was increased twofold throughout the entire LV ring in the MI group (\( P < 0.01 \) vs. normal; Table 5 and Fig. 2E); however, the increase was most dramatic in the anterior wall (IZ-BZ) (normal group: 11.0 \( \pm \) 0.9 mmHg, MI group: 25.3 \( \pm \) 2.7 mmHg). MSC transplantation reduced the diastolic wall stress in the IZ-BZ by \( \sim 42\% \) to 14.7 \( \pm \) 1.3 mmHg (\( P < 0.01 \) vs. MI group; Table 5 and Fig. 2E).

**Myocardial bioenergetics.** The bioenergetic state of the myocardium was assessed by the PCr-to-ATP ratio. PCr/ATP was severely decreased in the BZ myocardium in the MI group (normal group: 2.20 \( \pm \) 0.10, MI group: 1.15 \( \pm \) 0.10; \( P < 0.01 \), Table 6). MSC transplantation was associated with a significant improvement in the BZ PCr/ATP (PCr/ATP: 1.80 \( \pm \) 0.40; \( P < 0.01 \) vs. MI group; Table 6).

Under basal conditions, no deoxymyoglobin resonance peak was detected in normal hearts, in the BZ of MI hearts, or in the

---

**Table 2. LV EF and hemodynamics**

<table>
<thead>
<tr>
<th>Group</th>
<th>EDV, ml</th>
<th>ESV, ml</th>
<th>EF, %</th>
<th>HR, beats/min</th>
<th>Ao-S, mmHg</th>
<th>Ao-D, mmHg</th>
<th>LVSP, mmHg</th>
<th>LVEDP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>63.3 ( \pm ) 3.3</td>
<td>28.4 ( \pm ) 1.9</td>
<td>55.3 ( \pm ) 3.1</td>
<td>110 ( \pm ) 5</td>
<td>110 ( \pm ) 9</td>
<td>76 ( \pm ) 12</td>
<td>114 ( \pm ) 12</td>
<td>4.2 ( \pm ) 1.2</td>
</tr>
<tr>
<td>MI</td>
<td>83.6 ( \pm ) 9.8</td>
<td>54.3 ( \pm ) 6.9</td>
<td>30.4 ( \pm ) 2.3</td>
<td>103 ( \pm ) 8</td>
<td>108 ( \pm ) 5</td>
<td>78 ( \pm ) 5</td>
<td>109 ( \pm ) 5</td>
<td>6.2 ( \pm ) 1.5</td>
</tr>
<tr>
<td>MI + MSC</td>
<td>69.3 ( \pm ) 4.6</td>
<td>38.5 ( \pm ) 3.6 ( \dagger )</td>
<td>45.4 ( \pm ) 3.1 ( \ddagger )</td>
<td>102 ( \pm ) 5</td>
<td>102 ( \pm ) 9</td>
<td>67 ( \pm ) 8</td>
<td>119 ( \pm ) 8</td>
<td>5.4 ( \pm ) 0.9</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. EDV, end diastolic volume; ESV, end systolic volume; HR, heart rate; Ao-S, aortic systolic pressure; Ao-D, aortic diastolic pressure; LVSP, LV systolic pressure; LVEDP, LV diastolic pressure. \(* P < 0.01 \) vs. normal group; \( \ddagger P < 0.05 \) vs. normal group; \( \dagger P < 0.01 \) vs. MI group; \( \ddagger P < 0.05 \) vs. MI group.

---

**Fig. 1. Regional analysis by MRI.** A: delayed hyperenhancement CINE MRI (apical view) was used to localize the scar on the anterior region of the left ventricle (LV) in the middle region of the heart. LV structure and function were assessed from horizontal short-axis images. For regional analysis, the myocardium was divided into 6 segments according to the coronary artery perfusion pattern. AN, anterior wall; AP, anterior papillary region; LAT, lateral wall; PP, posterior papillary region; PO, posterior wall; SP, septal wall. B: tagged MRI analysis was used to calculate the circumferential shortening fraction (SF) in the different regions of the myocardium. The myocardial tissue was magnetized with a spatial modulation of magnetization pattern to superimpose lines onto the myocardium, and an annular mesh was defined around the LV. The deformation of the mesh was tracked throughout the cardiac cycle to assess the circumferential SF in the same 6 myocardial segments as depicted in A.
Table 4. Myocardial regional contractile function

<table>
<thead>
<tr>
<th>Group</th>
<th>AN, %</th>
<th>AP, %</th>
<th>LAT, %</th>
<th>PP, %</th>
<th>PO, %</th>
<th>SP, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>45.7±2.8</td>
<td>45.7±2.8</td>
<td>45.9±2.3</td>
<td>35.8±3.3</td>
<td>39.4±5.1</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>26.1±0.7*</td>
<td>29.2±0.6*</td>
<td>25.5±1.2†</td>
<td>20.1±2.2†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI + MSC</td>
<td>32.7±3.3‡</td>
<td>32.6±4.9‡</td>
<td>29.4±1.8§</td>
<td>30.3±2.3§</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study, autologous MSCs were injected throughout the infarct BZ. The main findings are that autologous MSC transplantation was associated with a significant improvement in myocardial contractile performance in the BZ and MBZ, which is evidenced by the prevention of LV bulging. This, in turn, resulted in the reduction of regional myocardial wall stress that consequently induced a significant improvement in BZ myocardial bioenergetics. Because the cell engraftment is so low, a direct structural contribution and myocyte regeneration by transdifferentiated MSCs is unlikely. The exact mechanisms by which the functionally beneficial effects occur are not known but may be related to MSC-mediated stimulation of endogenous repair mechanisms and paracrine-induced trophic effects that spare IZ-BZ cardiomyocytes from necrosis and apoptosis and signal for the recruitment of endogenous cardiac progenitors to repair and regenerate the site of injury.

BZ myocardial energetics. Using the same porcine model of postinfarction LV remodeling, we were recently surprised to find a substantially more severe bioenergetic abnormality in the infarct BZ than in areas remote from the scar (13). In fact, the reduction in bioenergetic efficiency in the BZ was even more severe than in failing hearts with concentric LV hypertrophy (33, 37) or in the remote zone of failing hearts with postinfarction LV remodeling (17, 36). In the present study, the BZ myocardial bioenergetic efficiency was assessed by the PCr/ATP ratio.

PCr/ATP reflects the mitochondrial oxidative phosphorylation regulation (7), myocardial energy efficiency (12), and LV

Table 3. Wall thickness

<table>
<thead>
<tr>
<th>Group</th>
<th>AN, mm</th>
<th>AP, mm</th>
<th>LAT, mm</th>
<th>PP, mm</th>
<th>PO, mm</th>
<th>SP, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.5±0.7</td>
<td>7.7±0.7</td>
<td>7.7±0.6</td>
<td>8.1±0.6</td>
<td>8.3±0.6</td>
<td>8.1±0.7</td>
</tr>
<tr>
<td>MI</td>
<td>4.4±0.1*</td>
<td>5.3±0.2*</td>
<td>6.7±0.3†</td>
<td>6.7±0.2†</td>
<td>7.0±0.1†</td>
<td>6.2±0.2*</td>
</tr>
<tr>
<td>MI + MSC</td>
<td>6.4±0.4‡</td>
<td>6.2±0.4‡</td>
<td>7.4±0.3§</td>
<td>7.3±0.3§</td>
<td>7.4±0.2</td>
<td>6.9±0.3§</td>
</tr>
</tbody>
</table>

End systolic wall thickness

<table>
<thead>
<tr>
<th>Group</th>
<th>AN, mm</th>
<th>AP, mm</th>
<th>LAT, mm</th>
<th>PP, mm</th>
<th>PO, mm</th>
<th>SP, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.6±0.6</td>
<td>5.5±0.6</td>
<td>5.3±0.4</td>
<td>5.6±0.4</td>
<td>6.3±0.5</td>
<td>6.5±0.6</td>
</tr>
<tr>
<td>MI</td>
<td>4.3±0.1†</td>
<td>4.8±0.2</td>
<td>5.2±0.2</td>
<td>5.2±0.1</td>
<td>5.4±0.1</td>
<td>4.9±0.1†</td>
</tr>
<tr>
<td>MI + MSC</td>
<td>5.1±0.3§</td>
<td>4.7±0.1</td>
<td>5.3±0.2</td>
<td>5.4±0.3</td>
<td>5.4±0.3</td>
<td>5.3±0.4</td>
</tr>
</tbody>
</table>

End diastolic wall thickness

Values are means ± SE. AN, anterior wall; AP, anterior papillary region; LAT, lateral wall; PP, posterior papillary region; PO, posterior wall; SP, septal wall. *P < 0.01 vs. normal group; †P < 0.05 vs. normal group; ‡P < 0.01 vs. MI group; §P < 0.05 vs. MI group.

BZ of hearts receiving MSC transplantation. From our previously reported (6) signal-to-noise ratio of Mb-δ during partial and complete LAD occlusions, the resonance peak should be recognized when there is >10% myoglobin desaturation. These data indicate that the BZ bioenergetic abnormality in hearts with postinfarction LV remodeling was not the result of persistent, severe myocardial ischemia.

Using the same swine model of postinfarction LV remodeling, we reported a significant reduction in ATP concentration within the BZ (13). The myocardial free ADP concentration (Table 6) was determined with the use of mean values of myocardial ATP and creatine concentrations reported in our previous study (13) and the PCr/ATP from the present study. These data indicate that myocardial free ADP increased in MI myocardial ATP and creatine concentrations reported in our previous study (13) and the PCr/ATP from the present study. These data indicate that myocardial free ADP increased in MI but was reduced after MSC transplantation.

Figure 3 illustrates the correlation between PCr/ATP and contractile indexes, namely, the radial thickening fraction and circumferential shortening fraction. When each PCr/ATP was plotted against the respective IZ-BZ contractile index, it was apparent that abnormal contractile performance was associated with abnormal myocardial bioenergetics. MSC transplantation resulted in significantly improved contractile performance, which in turn was associated with remarkably improved BZ bioenergetics.

MSC engraftment in vivo. Only 0.75 ± 0.05% of 50 million injected MSCs could be detected 4 wk after transplantation. About ~70% of these cells were found at areas near the injection sites in the BZ, whereas ~30% migrated into the IZ. Patches of spared myocytes were observed in the IZ of hearts that received MSC therapy. Such patches of spared myocytes were not observed in the IZ of hearts that did not receive MSC therapy.
chamber dysfunction (20, 36). In the in vivo heart, mitochondrial oxidative phosphorylation is regulated by NADH, O2, ADP, and Pi. Among these four factors, the myocardial free ADP concentration has the lowest \( K_m \) value and therefore is most likely contributing to the regulation of mitochondrial oxidative phosphorylation during different cardiac work states or disease conditions (10).

Because the creatine kinase reaction is nearly in equilibrium in the in vivo heart, a lower PCr/ATP indicates elevated levels of myocardial free ADP. The phosphorylation potential for hydrolysis of ATP is proportional to the ratio of ATP to ADP and Pi; therefore, elevated levels of ADP result in a significantly lower phosphorylation potential; consequently, less energy is made available for each unit of ATP that is utilized (12). As a result, hearts with lower a PCr/ATP are energetically less efficient (12).

It is also worth noting that the PCr/ATP is strongly influenced by the total creatine level because the enzyme creatine kinase catalyzes the phosphorylation of approximately two-thirds of the total creatine pool (20). Creatine is produced in the

---

Table 5. Wall stress

<table>
<thead>
<tr>
<th>Group</th>
<th>AN, mmHg</th>
<th>AP, mmHg</th>
<th>LAT, mmHg</th>
<th>PP, mmHg</th>
<th>PO, mmHg</th>
<th>SP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic wall stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>157.0±10.7</td>
<td>153.0±10.5</td>
<td>155.0±11.4</td>
<td>146.2±8.5</td>
<td>140.5±6.9</td>
<td>144.1±8.8</td>
</tr>
<tr>
<td>MI</td>
<td>297.4±16.6*</td>
<td>247.9±14.7*</td>
<td>206.2±14.6*</td>
<td>196.0±9.0*</td>
<td>186.1±7.1*</td>
<td>210.8±8.5*</td>
</tr>
<tr>
<td>MI+MSC</td>
<td>227.8±9.1†‡</td>
<td>235.9±14.3*</td>
<td>198.7±14.2†</td>
<td>202.1±15.4*</td>
<td>201.3±10.3*</td>
<td>211.5±14.0*</td>
</tr>
<tr>
<td><strong>Diastolic wall stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>11.0±0.9</td>
<td>11.2±1.0</td>
<td>11.3±0.7</td>
<td>10.7±0.5</td>
<td>9.5±0.6</td>
<td>9.3±0.8</td>
</tr>
<tr>
<td>MI</td>
<td>25.3±2.7*</td>
<td>23.1±2.5*</td>
<td>21.1±2.2*</td>
<td>20.8±1.9*</td>
<td>20.3±2.0*</td>
<td>22.2±2.1*</td>
</tr>
<tr>
<td>MI+MSC</td>
<td>14.7±1.3†‡</td>
<td>15.7±1.2*§</td>
<td>13.9±1.2†‡</td>
<td>13.8±1.1†‡</td>
<td>13.7±1.2*§</td>
<td>14.1±1.3*‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.01 vs. normal group; †P < 0.05 vs. normal group; ‡P < 0.01 vs. MI group; §P < 0.05 vs. MI group.

Fig. 2. Myocardial contractile function. A: global myocardial function was assessed by the ejection fraction (EF). Regional myocardial function was assessed throughout the LV ring by measuring radial thickening fraction (TF%; B), circumferential SF (SF%; C), systolic wall stress (D), and diastolic wall stress (E). Myocardial infraction (MI) caused significant abnormalities in both global and regional myocardial functions. These abnormalities were improved after mesenchymal stem cell (MSC) transplantation. *P < 0.05 vs. normal; †P < 0.05 vs. MI.
Table 6. Myocardial bioenergetics

<table>
<thead>
<tr>
<th>Group</th>
<th>PCr/ATP</th>
<th>[ADP]free, μmol/g Dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.20±0.10</td>
<td>0.27±0.03</td>
</tr>
<tr>
<td>MI (BZ)</td>
<td>1.15±0.10*</td>
<td>0.48±0.05*</td>
</tr>
<tr>
<td>MI + MSC (BZ)</td>
<td>1.80±0.40†</td>
<td>0.29±0.04†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BZ, border zone; PCr, phosphocreatine; [ADP]free, free cytosolic ADP concentration. *P < 0.01 vs. normal; †P < 0.01 vs. MI group (BZ).

liver and kidneys and taken up by myocytes from the circulation via the creatine transporter protein. The protein expression level of the creatine transporter was observed to be significantly decreased in failing hearts; this was associated with a reduction in the myocardial PCr/ATP (21). Therefore, in the in vivo failing heart, the reduced PCr/ATP is likely the result of both mitochondrial ATP machinery dysfunction and the reduction in expression of the creatine transporter.

Using a porcine model of cardiac hypertrophy and CHF, we reported that the reduction of the myocardial PCr/ATP is linearly related to the protein expression levels of mitochondrial creatine kinase isoforms (32) and mitochondrial ATPase subunits (16). Therefore, it is likely that a lower PCr/ATP reflects the severity of mitochondrial dysfunction. However, in the in vivo heart, the mitochondrial ATP machinery almost never operates at its maximum capacity (3, 10). Thus the in vivo experimental results do not demonstrate a direct causal relationship between the mitochondrial dysfunction and myocardial ATP concentration.

Utilizing an in vivo canine model of severe LV hypertrophy, we reported that severity of the reduction in PCr/ATP is linearly related to the severity of LV hypertrophy and that failing hearts had the lowest PCr/ATP (37). We also found that a reduced PCr/ATP is linearly related to the decrease in LV EF in hearts with postinfarction LV remodeling (36). In the perfused heart, the reduction of myocardial PCr/ATP reflects the energetic efficiency, which is linearly related to the severity of LV dysfunction (28). In the present study, the myocardial bioenergetic efficiency in the infarct BZ, as reflected by the PCr/ATP, was severely reduced in hearts without MSC transplantation (Fig. 3 and Table 6), which was remarkably improved in response to MSC transplantation (Fig. 3 and Table 6).

Because <1% of transplanted MSCs engrafted and survived in the heart, the bioenergetic improvements are most likely secondary to a trophic effect that is mediated by the action of cytokines, which are released from transplanted MSCs. Although the particular cytokines contributing to this trophic effect remain to be defined, the list most likely includes VEGF, HGF, and FGF as reported earlier (9, 23, 26). In addition, the exact mechanisms governing this trophic effect remain unclear. Likely mechanisms include 1) cytokine-induced recruitment of endogenous cardiac progenitors to the site of injury for regeneration of the damaged myocardium (2, 31); 2) activation of an antiapoptosis signaling system at the infarct BZ that effectively protects ischemia-threatened myocytes from apoptosis (2); and 3) neovascularization in the ischemia-threatened BZ myocardium for enhanced oxygen delivery (15). All of these factors could potentially improve the IZ-BZ contractile function and contribute to the reduction in LV bulging, decreased wall stress, and improved myocardial bioenergetics. Each of the observed functional improvements likely affected the other aspects of myocardial performance causing a “positive feedback cycle” that allowed for the improvement in myocardial function.

MRI assessments of contractile performance in the infarct BZ. We have reported that swine hearts without cell transplantation have persistent dyskinesis in the infarct region and hypokinesis in the infarct BZ at ~4 wk after MI (13). In the present study, MRI data demonstrated that stem cell transplantation significantly improved myocardial contractile performance in the infarct BZ (Fig. 2). In addition, the LV EF also significantly increased. These functional improvements are in agreement with previous reports (1, 5, 15, 30). It is interesting to note that the patches of spared myocytes inside the infarct
region were only found in hearts with MSC transplantation. This is likely the result of the aforementioned trophic effects. These spared myocytes may have played a significant structural role in preventing the LV dyskinesis that was observed in infarcted hearts without MSC transplantation and in effectively reducing the LV wall stress in the infarct BZ.

Several studies published over the past few years have suggested that bone marrow-derived stem cells can differentiate into cardiomyocytes on transplantation into an infarcted myocardium and thereby improve cardiac function (22, 29). On the other hand, a number of studies have also suggested that improved cardiac function may be independent of stem cell differentiation-associated myocardial regeneration (4, 19). In the absence of evidence for significant MSC engraftment and myocyte regeneration, the improved cardiac function observed in the present study likely resulted from the paracrine effects of MSCs on native cardiomyocytes, increased angiogenesis, and/or repair of by endogenous cardiac progenitors that were recruited into the infarct site (30).

MSC-induced antiapoptosis effect. Both in vitro and in vivo animal models of postinfarction ventricular remodeling have revealed that MSC transplantation is associated with a significant antiapoptosis effect that results from cytokine release by stem cells (8, 9, 35). It is interesting to note that patchy spared myocytes were only observed in hearts that received MSC transplantation. These spared myocytes likely provide the structural basis for improved IZ and BZ myocardial contractile performance, reduced LV wall stress, and normalized myocardial bioenergetics in the infarct BZ (Figs. 2 and 3).

Increased neovascularization in response to MSC therapy. Increased neovascularization has consistently been observed after stem cell transplantation, and myocardial revascularization has been considered to be an important mechanism for the beneficial effects of stem cell transplantation (15, 30, 31). However, Dzau’s group (8, 9) has recently reported that the beneficial effects occurred as early as 3 days after cell transplantation; it is likely the result of the aforementioned trophic effects. These additional biochemical enzymes [e.g., Krebs cycle enzymes and the electron transport chain (ETC)] were not measured. These additional biochemical measurements that could address some significant mechanistic issues should be done in future studies. The autologous MSCs were coinfected with mouse-derived fibrin and mouse-derived growth factors that were rejected shortly after the transplantation. However, this could be a confounding factor.

Conclusions. In conclusion, these data suggest that stem cell transplantation into the BZ of hearts with postinfarction LV remodeling results in a significant improvement of myocardial contractile performance, effective reduction of myocardial wall stress in the IZ and BZ areas, and consequent improvements in myocardial bioenergetics.

GRANTS

This work was supported by National Heart Lung and Blood Institute Grants HL-50470, HL-61353, and HL-67828 (to J. Zhang) and American Heart Association predoctoral fellowship 0610077Z (to J. Feygin).

REFERENCES


8. Gnecehi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noisecs X, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 11: 367–368, 2005.


