Allogeneic Mesenchymal Stem Cell Transplantation in Postinfarcted Rat Myocardium
Short- and Long-Term Effects

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Background—Mesenchymal stem cells (MSCs) have the potential to replace infarct scar, but the long-term effects are unknown. We studied short- and long-term effects of MSC transplantation on left ventricular (LV) function in a rat myocardial infarction model.

Methods and Results—Saline (n = 46) or MSCs labeled with 1,1’-dioctadecyl-3,3,3’3’-testramethylindocarbocyanine perchlorate (DiI; n = 49, 2 × 10^6 cells each) were injected into the scar of a 1-week-old myocardial infarction in Fischer rats. The presence and differentiation of engrafted cells and their effect on LV ejection fraction was assessed. At 4 weeks, LV stroke volume was significantly greater in the MSC-treated group (145 ± 9 μL) than in the saline group (122 ± 3 μL, P = 0.032), and LV ejection fraction was significantly greater in MSC-treated animals (43.8 ± 1.0%) than in the saline group (38.8 ± 1.1%, P = 0.0027). However, at 6 months, these benefits of MSC treatment were lost. DiI-positive cells were observed in the MSC group at 2 weeks and at 3 and 6 months. Expression of the muscle-specific markers α-actinin, myosin heavy chain, phospholamban, and tropomyosin was not observed at 2 weeks in DiI-positive cells. At 3 and 6 months, the DiI-positive cells were observed to express the above muscle-specific markers, but they did not fully evolve into an adult cardiac phenotype. Some of the DiI-positive cells expressed von Willebrand factor.

Conclusions—Allogeneic MSCs survive in infarcted myocardium as long as 6 months and express markers that suggest muscle and endothelium phenotypes. MSCs improved global LV function at 4 weeks; however, this benefit was transient, which suggests a possible early paracrine effect. (Circulation. 2005;112:214-223.)

Key Words: cardiomyoplasty ■ stem cells, mesenchymal ■ myocardial infarction

Cellular cardiomyoplasty may be a promising approach to improve cardiac regeneration or vascularization after myocardial infarction.1 Our group has reported that transplantation of fetal or neonatal cardiomyocytes can increase the thickness of the infarct wall and left ventricular (LV) stroke volume, decrease LV end-systolic volume, and improve LV ejection fraction in a rat model of myocardial infarction2,3 and induce neoangiogenesis.4 However, transplanted allogenic cardiomyocytes can cause immune rejection in recipient hearts, and sources of these immature cardiomyocytes are likely to be limited.5 Therefore, the beneficial effects of fetal or neonatal cardiomyocyte transplantation in clinical use are limited.

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In clinical practice, bone marrow cells are easily obtained from bone marrow aspirate drawn through the skin, and marrow contains multipotential progenitor cells, which can differentiate into various kinds of cells, including myogenic cells.1 Furthermore, mesenchymal stem cells (MSCs) from bone marrow are thought to be immune-privileged and have been successfully transplanted into hearts without immunosuppression (for review, see Pittenger and Martin6). Autologous or allogenic MSCs are considered to be one of the potential cell sources for cellular cardiomyoplasty; however, few studies have investigated the long-term effects of this type of therapy. In the present study, we investigated the engraftment, survival, and differentiation of allogenic MSCs obtained from a commercial source and their short- and long-term effects on LV function in a rat myocardial infarction model.

Methods

The Heart Institute at Good Samaritan Hospital is accredited by the American Association for Accreditation of Laboratory Animal Care. All procedures were approved by the Institutional Animal Care and Use Committee and performed in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 85-23, National Academy Press, Washington, DC, revised 1996).
Bone Marrow Harvest, MSC Isolation, Expansion, and Labeling

Allogeneic MSCs were isolated, characterized, cultured, and labeled according to established methods at Osiris Therapeutics, Inc (Baltimore, Md).6–8 Briefly, 25 male ACI rats (weight 250 to 300 g) were used in this study as MSC donors. The MSCs were isolated from the femoral and tibial bones of donor ACI rats as described previously.7,8 MSCs were expanded to passage 3 before infusion to recipient animals. At this time, cultures were 95% homogenous for rat MSCs. The multipotentiality of the resulting cells was verified with the use of in vitro assays to differentiate MSCs into osteogenic (alkaline phosphatase activity), adipogenic (oil red O staining), and chondrogenic (type II collagen staining) lineages.8

To label cells with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), 0.25 µg of DiI per microliter of dimethylsulfoxide (DMSO; Sigma) stock solution was added to MSC suspensions at 1 × 10^6 cells/mL in Plasmalyte A (Baxter) to yield a final concentration of 1 µg of DiI solution/mL. This suspension was incubated at 37°C for 5 minutes, then at 4°C for 15 minutes with occasional mixing. MSCs labeled with DiI were washed 3 times with DPBS before preparation for frozen storage in freezing medium containing 90% fetal bovine serum (Valley Biomedical)/10% DMSO. All cells were kept frozen until use. MSC viability on thawing was routinely measured to range from 90% to 95%.

Model of Myocardial Infarction and Injection of MSCs

Myocardial infarction was performed in female Fischer CDF rats as described previously.2 Briefly, rats were anesthetized with ketamine (75 mg/kg IP) and xylazine (5 mg/kg IP). After endotracheal intubation and initiation of ventilation (room air, rate 60 cycles/min, tidal volume 1 mL per 100 g of body weight, Harvard Apparatus Rodent Ventilator, model 683), the heart was exposed through a left thoracotomy, and the proximal left coronary artery was ligated. The rats were allowed to recover under care. One week later, rats were reanesthetized and hearts reexposed (as described above) for the injection of MSCs or saline. Saline (~ 70 µL, n=46) or MSCs (2 × 10^6 cells in ~ 70 µL of saline, n=49; Osiris Therapeutics Inc) were injected directly into the infarct area with a 28-gauge needle attached to an insulin syringe. Successful injection was typified by the formation of a bleb covering the infarct zone.

Assessment of Survival and Differentiation of MSCs in Myocardial Infarction

Some rats were euthanized (KCl 2 mEq IV to arrest the heart in diastole) under deep anesthesia at 2 weeks, 3 months, and 6 months after saline or stem cell injection. Hearts were harvested, frozen, and sent to Osiris to blindly examine the survival and differentiation of the cells in the infarction. The survival of the transplanted MSCs was demonstrated by the presence of DiI-labeled cells. Immunohistochemical staining with antibodies against α-actinin (Sigma, 1:200), MF-20 (myosin heavy chain; Developmental Studies Hybridoma Bank at University of Iowa, 1:2), phospholamban (Affinity BioReagents, 1:100), and tropomyosin (Sigma, 1:100) was performed to show muscle-specific marker expression. Endothelial cells were identified by the expression of von Willebrand factor (Dako, 1:100). Colocalization of the DiI label and muscle-specific marker expression were examined with a confocal microscope (Nikon Eclipse TE 300, Simple PCI image software).

The number of blood vessels positive for von Willebrand factor was assessed in the scar area at 6 months after MSC or saline treatment. Five fields on the slide representing infarcted area from each heart were randomly chosen for counting stained blood vessels. The slides were first examined at ×100 magnification to identify the infarcted area, and then the number of blood vessels was counted at ×400 magnification. All stained vessels oriented with the lumen cut transversely were counted. The blood vessel density was expressed as vessel number/field ×400.

Figure 1. Confocal microscopy of fluorescent immunohistochemical staining of DiI-labeled cells in infarcted myocardium for α-actinin at 6 months. A, DiI-labeled MSCs appear red (white arrows). B, Sections stained with antibody to muscle marker α-actinin appear green. White arrows point to MSC transplant. C, Merged image of A and B shows DiI-labeled MSCs express α-actinin (yellow cells; white arrows). Original magnification ×400.
LV Angiography
At 4 weeks and 6 months after treatment, rats were anesthetized and a catheter was inserted into the left jugular vein. LV contrast angiography was performed after injection of 1 mL of nonionic contrast into the left jugular vein with a XiScan 1000 C-arm x-ray system (XiTec, Inc; 3-inch field of view). Video images of anterior-posterior and lateral projections were acquired on half-inch super-VHS videotape at 30 frames per second under constant fluoroscopy. LV volumes in systole and diastole were calculated blindly from the video images. All parameters were averaged over 3 consecutive cycles in both projections. Ejection fraction (%) was calculated as [100 \times (volume in diastole - volume in systole)/volume in diastole] and averaged over both projections.

Assessment of Regional Wall Motion by LV Angiography
Regional wall motion after myocardial infarction was also assessed by LV angiography. Tracings of the LV circumference during end diastole and end systole of the same cardiac cycle were superimposed on transparent film in both the anterior-posterior and lateral views with the base of the heart as a reference. If the tracing from end systole was not confined within that of end diastole, it was defined as bulging. Akinesis was evident when the tracing from end systole was superimposable on the tracing for end diastole. The size of paradoxical LV systolic bulging (dyskinesis) or akinetic motion was calculated by measuring the length of total LV diastolic circumference and circumferential length of the bulging or akinetic segment with computerized planimetry, expressed as % of total LV diastolic circumference.

Hemodynamics
To record arterial and LV hemodynamic parameters, a 2F high-fidelity, catheter-tipped micromanometer (model SPR-869, Millar, Inc) was advanced into the ascending aorta and into the LV through the right carotid artery.

Regional Myocardial Blood Flow
To measure regional myocardial blood flow (RMBF), 103Ru-labeled radioactive microspheres (\textasciitilde 500 000) were injected directly into the LV, and a reference blood sample was withdrawn simultaneously from an arterial catheter (0.361 mL/min) for 1 minute. Radioactivity in the scar tissue, the noninfarcted myocardium, and the reference blood sample was measured in a multichannel pulse-height analyzer (model ND62, Nuclear Data). After correction for background, RMBF was calculated as the ratio of counts in the tissue and the reference blood sample multiplied by pump flow (0.361 mL/min) and divided by the weight of the tissue.

Postmortem LV Volume, Wall Thickness, and Infarct Size
After intravenous injection of 0.6 mL of 50% Unisperse blue, a suspension of blue particles obtained from Ciba Geigy was performed for confirmation of a perfusion defect of the scar area, the rats were euthanized (KCl 2 mEq IV to arrest the heart in diastole) under deep anesthesia at 4 weeks (n=12 in each group) and 6 months (n=21 in each group). The hearts were excised and pressure-fixed with formalin (pressure equal to 13 cm H2O column). Postmortem LV volumes were measured by filling the cavity with water and weighing, which was repeated 3 times.

The hearts were cut into 3 transverse slices after LV volume measurement. The middle slice was embedded in paraffin and processed for histology, and the other 2 slices were cut for RMBF measurement of the infarct and noninfarct areas. Sections (5-\textmu m thickness) of the paraffin-embedded tissue were stained with hematoxylin and eosin, as well as picrosirius red. The density of arterioles and small arteries in the scar area (but excluding capillaries) also was calculated on hematoxylin and eosin–stained slides (expressed as vessel number/mm²). Computerized planimetry of the histological images of the stained sections was used to measure and calculate (1) scar thickness (average of 5 equidistant measurements) and septum thickness (average of 3 equidistant measurements); (2) epicardial and endocardial circumference and circumference occupied by infarcted

Figure 2. Confocal microscopy of fluorescent immunohistochemical staining of Dil-labeled cells in infarcted myocardium for MF-20 at 6 months. A, Dil-labeled MSCs appear red (white arrows). B, Sections stained with antibody to muscle marker MF-20 appear green. White arrows point to MSC transplant. C, Merged image of A and B shows Dil-labeled MSCs express MF-20 (yellow cells; white arrows). Original magnification \times 400.
wall (infarct size was expressed as percentage of total LV circumference); (3) expansion index, as defined by Hochman and Choo, which is expressed as [LV cavity area/total LV area \times \text{septum thickness}/\text{scar thickness}].

**Statistical Analysis**

All data are presented as mean±SEM. Comparisons between groups were made by Student t test or Fisher’s exact test, where appropriate. Results were considered statistically significant if *P*<0.05.

**Results**

Of the 95 successful rats (46 in the saline group and 49 in the MSC group), 29 hearts were sent to Osiris for assessment of survival and differentiation of MSCs (8 at 2 weeks, 9 at 3 months, and 12 at 6 months). Twenty-four hearts at 4 weeks (12 in each group) and 42 hearts at 6 months (21 in each group) were processed for postmortem analysis, and 2 rats in the saline group and 1 rat in the MSC group were excluded because of small infarct sizes (<20% of LV circumference) at 6 months. Successful LV angiographic images were obtained in 24 rats at 4 weeks and 49 rats at 6 months.

**Survival and Differentiation of MSCs in Myocardial Infarction**

At 2 weeks postimplantation, 3 of 4 hearts in the cell-treated group but none of the 4 hearts in the saline group showed the presence of grafted MSCs with DiI labeling in the infarct zone. In the 3 DiI-labeled–positive hearts in the cell-treated group, the muscle-specific markers α-actinin, myosin heavy chain, phospholamban, and tropomyosin staining were negative in the DiI-positive cells observed by confocal microscopy. At 3 months, 4 of 5 hearts in the cell group but none of the 4 hearts in the saline group showed the presence of grafted DiI-labeled MSCs in the infarct zone. In 3 of 4 hearts with DiI-labeled MSCs, colocalization of the DiI label and the marker α-actinin was observed. In only 1 heart, DiI-positive cells also expressed myosin heavy chain, phospholamban, and tropomyosin. At 6 months, 7 of 7 hearts in the cell group but none of the 5 hearts in the saline group contained DiI-labeled MSCs. DiI-labeled cells in all 7 hearts in the cell group also expressed the muscle-specific markers α-actinin, myosin heavy chain, phospholamban, and tropomyosin (Figures 1, 2, 3, and 4). Although DiI-positive cells could be observed in both the center of the scar area and the border zone, it was easier to identify DiI-positive cells in the center of the scar area. The percentage of DiI-positive cells that expressed muscle markers was 46±5% for α-actinin, 40±2% for myosin heavy chain, 42±5% for phospholamban, and 38±5% for tropomyosin, respectively. Although MSCs expressed the cardiac cell markers, differentiation was incomplete, and myofibril organization was immature (Figure 5). In addition, all of these hearts at 6 months were found to have MSCs that expressed von Willebrand factor (Figure 6), which indicates a role for MSCs in angiogenesis.

**LV Stroke Volume and Ejection Fraction by Angiography**

At 4 weeks after transplantation, LV stroke volume was 122±3 μL and ejection fraction was 38.8±1.1% in the saline group. Stroke volume was significantly higher (145±9 μL, *P*=0.032) and ejection fraction was significantly greater (43.8±1.0%, *P*=0.0027; Figure 7) in the MSC-treated group (Table 1). At 6 months, LV stroke volume and ejection fraction were comparable between the 2 groups (Table 1; Figure 7).
Figure 4. Confocal microscopy of fluorescent immunohistochemical staining of Dil-labeled cells in infarcted myocardium for tropomyosin at 6 months. A, Dil-labeled MSCs appear red (white arrows). B, Sections stained with antibody to muscle marker tropomyosin appear green. White arrows point to MSC transplant. C, Merged image of A and B shows Dil-labeled MSCs express tropomyosin (yellow cells; white arrows). Original magnification \( \times 400 \).

Figure 5. Higher-power magnification (\( >600 \)) of confocal microscopy of fluorescent immunohistochemical staining of Dil-labeled cells in infarcted myocardium for \( \alpha \)-actinin at 6 months. A, Dil-labeled MSCs appear red (white arrows). B, Sections stained with antibody to muscle marker \( \alpha \)-actinin appear green. White arrows point to MSC transplant without typical cross striation. Red arrows point to host cardiac cells with cross striations. C, Merged image of A and B shows Dil-labeled MSCs express \( \alpha \)-actinin without typical cross striation (yellow cells; white arrows).
Regional Wall Motion After Myocardial Infarction

All of the hearts had regions of akinetic wall motion. At 4 weeks, for rats that received MSCs, LV ejection fraction was 43.8±1.0%; for rats that received saline, LV ejection fraction (38.8±1.1%) was significantly lower than that in MSC-treated group (P=0.0027). At 6 months, LV ejection fraction was comparable between saline (42.0±1.0%) and MSC group (41.8±1.1%, P=0.89).

Hemodynamics

At 4 weeks after treatment, heart rate was significantly lower and systolic blood pressure was significantly higher in the MSC group than in the saline group, but diastolic blood pressure, +dP/dt (LV positive change in pressure over time) and −dP/dt (LV negative change in pressure over time) were comparable between the groups (Table 2). At 6 months, heart rate, systolic and diastolic blood pressure, and +dP/dt and −dP/dt were similar in the 2 groups (Table 2).

Table 1 shows the parameters of LV function and wall motion by angiography. The extent of dyskinetic plus akinetic motion was similar at 6 months in the 2 groups.

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*Statistically significant.
RMBF and Blood Vessel Density in Infarcts

RMBF in the scar tissue and noninfarct tissue was comparable between the 2 groups at both 4 weeks and 6 months after treatment (Table 3). Although at 6 months after MSC or saline treatment, the total blood vessel density that stained positive for von Willebrand factor (including capillaries) in the scar area was significantly greater in the MSC group (21.4±6.7, n=7) than in the saline group (17.4±6.0, n=5;  \( P=0.047 \)), the density of arterioles and small arteries in the scar area was similar in the MSC group (6.8±0.6, n=19) and in the saline group (7.9±1.0, n=19;  \( P=0.4 \)).

**Postmortem LV Volumes, Infarct LV Wall Sizes, Scar Wall Thickness, Septum Thickness, Expansion Index, and Histology**

At 4 weeks or 6 months, there were nonsignificant trends for smaller postmortem LV volume in the MSC-treated group versus saline-treated group; however, infarct LV wall sizes, scar wall thickness, septum thickness, and expansion index were comparable between the saline- and MSC-treated groups (Table 4). Hematoxylin and eosin staining and picrosirius red staining showed that the scars were transmural and thin, composed of collagenous tissues with a thin discontinuous layer of subendocardial cardiac myocytes in both the saline and MSC groups (Figure 8). In the MSC group, no graft appearing to “bulk up” the infarcted wall with new muscle was seen, compared with our previous observations after fetal or neonatal cardiac cell transplantation.\(^2,3\) No evidence of an inflammatory response was observed in the MSC transplantation area.

**Discussion**

This study demonstrated that allogeneic MSCs survived in the infarcted myocardium as long as 6 months. The transplanted MSCs did not express muscle-specific markers at 2 weeks when assessed by confocal microscopy. Although expression of some markers was observed at 3 months, further expression of muscle markers had occurred by 6 months. MSCs improved global LV function at 4 weeks, at a time when muscle markers were not fully expressed in the transplanted MSCs. However, although the transplanted MSCs further expressed muscle-specific markers at 6 months after transplantation, the benefit of improved LV function was no longer present. These results suggest that a paracrine effect of the transplanted MSCs might play a role in improving LV function at 4 weeks after transplantation, because improvement in function occurred before full expression of muscle markers (which was incomplete even at 3 months but complete at 6 months) and also because at neither 4 weeks or 6 months did we observe “bulking up” of the scar with new muscle. In contrast, in our previous work with neonatal and fetal cardiac cell transplantation into a scar, improved function was associated with a clear-cut increase in muscle mass of the scar. The fact that function was improved at 4 weeks in the present study of MSCs, without thickening of the infarct scar with muscle, favors the concept that these cells may have produced humoral substances that beneficially affected surrounding viable muscle cells in some fashion, a so-called paracrine effect.

Myocardial infarction leads to the loss of cardiomyocytes, followed by pathological LV remodeling and progression to heart failure. The goals of cellular cardiomyoplasty are to replace cardiomyocytes lost after ischemia, induce revascularization of the injured region, and prevent deleterious pathological remodeling after myocardial infarction. Our group previously reported that fetal or neonatal cardiac cell transplantation can reach these goals. Because allogeneic fetal or neonatal cardiac cells can

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**TABLE 2. Hemodynamics**

<table>
<thead>
<tr>
<th>Time</th>
<th>Saline Group</th>
<th>Stem Cell Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 4 weeks</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>256±8</td>
<td>227±9</td>
<td>0.018*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>132±5</td>
<td>155±7</td>
<td>0.017*</td>
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<td>Diastolic blood pressure, mm Hg</td>
<td>102±3</td>
<td>110±4</td>
<td>0.15</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td>4779±896</td>
<td>4042±548</td>
<td>0.49</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>3258±576</td>
<td>2637±407</td>
<td>0.39</td>
</tr>
<tr>
<td>At 6 months</td>
<td>24</td>
<td>26</td>
<td></td>
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<tr>
<td>Heart rate</td>
<td>235±5</td>
<td>236±6</td>
<td>0.97</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>133±5</td>
<td>134±4</td>
<td>0.98</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>96±2</td>
<td>98±1</td>
<td>0.55</td>
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<td>+dP/dt, mm Hg/s</td>
<td>4000±503</td>
<td>3894±444</td>
<td>0.96</td>
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<tr>
<td>−dP/dt, mm Hg/s</td>
<td>2607±317</td>
<td>2463±285</td>
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*Statistically significant.

**TABLE 3. Regional Myocardial Blood Flow**

<table>
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<th>Time</th>
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<th>Stem Cell Group</th>
<th>P</th>
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<tr>
<td>At 4 weeks</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Scar tissue, mL.min(^{-1}).g(^{-1})</td>
<td>0.7±0.13</td>
<td>0.63±0.12</td>
<td>0.71</td>
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<tr>
<td>Noninfarct tissue, mL.min(^{-1}).g(^{-1})</td>
<td>2.16±0.32</td>
<td>2.01±0.28</td>
<td>0.71</td>
</tr>
<tr>
<td>Scar/noninfarct tissue, %</td>
<td>37.4±7.2</td>
<td>35.2±8.0</td>
<td>0.84</td>
</tr>
<tr>
<td>At 6 months</td>
<td>16</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Scar tissue, mL.min(^{-1}).g(^{-1})</td>
<td>0.41±0.06</td>
<td>0.50±0.12</td>
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<td>Noninfarct tissue, mL.min(^{-1}).g(^{-1})</td>
<td>1.48±0.13</td>
<td>1.46±0.26</td>
<td>0.94</td>
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<tr>
<td>Scar/noninfarct tissue, %</td>
<td>28.8±4.3</td>
<td>33.8±4.5</td>
<td>0.43</td>
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**TABLE 4. Parameters of Postmortem Morphometry**

<table>
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<th>Time</th>
<th>Saline Group</th>
<th>Stem Cell Group</th>
<th>P</th>
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<tbody>
<tr>
<td>Postmortem analysis at 4 weeks</td>
<td>12</td>
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<tr>
<td>LV volume, g</td>
<td>338±14</td>
<td>310±14</td>
<td>0.16</td>
</tr>
<tr>
<td>Infarct size, % of LV circumference</td>
<td>42.4±2.4</td>
<td>45.9±2.2</td>
<td>0.29</td>
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<tr>
<td>Scar thickness, μm</td>
<td>446±42</td>
<td>475±20</td>
<td>0.54</td>
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<tr>
<td>Septum thickness, μm</td>
<td>900±39</td>
<td>885±42</td>
<td>0.79</td>
</tr>
<tr>
<td>Infarct expansion index</td>
<td>1.37±0.17</td>
<td>1.15±0.04</td>
<td>0.25</td>
</tr>
<tr>
<td>Postmortem analysis at 6 months</td>
<td>19</td>
<td>20</td>
<td></td>
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<tr>
<td>LV volume, g</td>
<td>436±24</td>
<td>398±16</td>
<td>0.20</td>
</tr>
<tr>
<td>Infarct size, % of LV circumference</td>
<td>51.4±2.3</td>
<td>52.7±1.6</td>
<td>0.63</td>
</tr>
<tr>
<td>Scar thickness, μm</td>
<td>413±20</td>
<td>407±21</td>
<td>0.85</td>
</tr>
<tr>
<td>Septum thickness, μm</td>
<td>932±38</td>
<td>965±40</td>
<td>0.56</td>
</tr>
<tr>
<td>Infarct expansion index</td>
<td>1.52±0.15</td>
<td>1.51±0.08</td>
<td>0.98</td>
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</table>
induce immunorejection, and sources of these cells may be difficult to obtain in the clinical realm, alternative cell sources for cellular cardiomyoplasty are needed. Tomita et al\(^9\) transplanted bone marrow cells directly into the LV at 3 weeks after cryoinjury in rats. The transplanted bone marrow cells could be identified and were found to express muscle-specific proteins 8 weeks later, and LV function was improved in these rats. Orlic et al\(^{10}\) reported that hematopoietic stem cells, which were injected directly into infarcts, could differentiate into cardiac myocytes and improve LV function in mice. In contrast, Agbulut et al\(^{11}\) could not find transplanted bone marrow–derived hematopoietic stem cells at 1 month after transplantation in rats, although improvement of LV function was observed. Balsam et al\(^{12}\) also reported that 30 days after injection into the ischemic myocardium in mice, implanted hematopoietic stem cells could not be found, but LV function was improved. These results suggest that the mechanism that accounts for functional improvement after bone marrow cell transplantation is likely due to the release of soluble

Figure 8. Representative picrosirius red-stained sections of myocardial infarction show thin-walled, transmural collagenous scars. Cardiac muscle stains yellow, collagen stains red, and blue staining shows blood vessels. No grafts appearing to bulk up infarcted wall with new cardiac muscle (yellow) were observed. A (4 weeks) and E (6 months) represent heart after saline treatment; C (4 weeks) and G (6 months) represent heart after MSC treatment. B, D, F, and H are higher magnification (×100) of boxed area in A, C, E, and G, respectively.
factors (paracrine mechanisms) of transplanted cells and not to the replacement of lost cardiomyocytes. The present study also suggested that the paracrine effect of transplanted MSCs played a role in the improvement of cardiac function. In the present study, MSC transplantation improved LV function but did not cause complete myogenic differentiation at 4 weeks after transplantation into the myocardial infarction in rats and did not result in visible replacement of scar with sheets of muscle cells. Although transplanted MSCs expressed muscle-specific markers at 6 months after transplantation, LV function was comparable between the 2 groups. Although MSC treatment increased total blood vessel density in the scar area, RMBF in the scar tissue and noninfarct tissue was comparable between the 2 groups. The explanation may be that MSC treatment did not increase the density of arterioles and small arteries in the scar, and the increase of capillary density was not enough to increase RMBF. Compared with fetal or neonatal cardiac cells in which discrete sheets of new muscle could be identified, as well as thickening of the infarct wall, the roles of transplanted stem cells in myocardial infarction are different. Methods to obtain the maximum therapeutic potential of stem cells are needed to optimize this form of therapy.

Stem cells have the capability to self-renew and can form 1 or more differentiated cell types. Adult bone marrow contains different kinds of multipotential stem cells, such as MSCs and hematopoietic stem cells. A number of studies have demonstrated that bone marrow stem cells can survive and differentiate into cardiac myocytes, endothelial cells, and vascular cells in the myocardium in various animal models.13 Orlic et al10 injected hematopoietic stem cells (Lin− c-kit+) directly into infarcts within 5 hours after coronary occlusion in mice. Nine days later, the transplanted eGFP+ cells differentiated into cardiac myocytes. However, recently, Murry et al14 transplanted Lin− c-kit+ cells into infarcts 5 hours after coronary occlusion in MHC-nLAC mice. The transplanted cells did not differentiate into cardiac myocytes 1 to 4 weeks after transplantation. Thoelen et al15 transplanted MSCs into the infarcted area at 4 hours after coronary occlusion in sheep. One month later, immunohistochemical staining for troponin I and cardiac-specific myosin of transplanted MSCs was negative. Thus, whether the bone marrow stem cells could differentiate into cardiac myocytes remains controversial. In the present study, transplanted MSCs in infarcts survived as long as 6 months. Implanted MSCs did not express muscle-specific markers at 2 weeks as assessed by confocal microscopy. Expression of muscle markers appeared in some rats at 3 months and in all rats at 6 months. These results suggest that the time needed for differentiation of stem cells in myocardial infarction may be longer than expected, and this phenomenon should be kept in mind when considering whether transplanted stem cells could differentiate into myogenic cells in infarcts after myocardial infarction. How to enhance the differentiation of stem cells into myogenic cells will play a crucial role in the regeneration of myocardium by stem cell transplantation.

MSCs represent only 0.001% to 0.01% of nucleated cells in bone marrow, but they can be cloned and expanded in culture. Compared with other stem cells, MSCs are easy to obtain and handle. Moreover, MSCs are suggested to be immune-privileged, so that allogeneic MSCs can be prepared in advance for the patient at the needed time. Allogeneic MSCs are not rejected even after they differentiate after transplantation in vivo. In the present study, transplanted allogeneic MSCs survived 6 months, differentiated into myogenic phenotype cells and endothelial cells, and did not induce immunorejection. But recently, Grinnemo et al16 transplanted human MSCs into myocardial infarction in rats. One week later, human MSCs could not be detected, and massive macrophage infiltration was observed in rats without immunosuppression. The results suggest that the implanted human MSCs were rejected by the host rats. This discrepancy may be because the transplanted MSCs were derived from a different species.16

In conclusion, the results of the present study suggest that allogeneic MSCs can be transplanted into infarcted tissue, survive, gradually express muscle markers, cause a transient but not long-lasting improvement in LV function, and incorporate as endothelial cells into the vasculature. The time needed for expression of muscle markers by MSCs in myocardium infarction may be longer than previously expected. Unfortunately, the MSCs did not appear to fully take on a cardiomyocyte phenotype, nor did they replace scar tissue significantly. The mechanisms whereby transplantation of MSCs only transiently improves LV function at 4 weeks after transplantation remains unclear and needs to be further investigated but may represent a transient paracrine mechanism. A very recent study17 suggested that paracrine effects were responsible for cardioprotection of Akt-modified mesenchymal stem cells. Methods to enhance MSC differentiation and continued contribution to contraction are needed to optimize this form of therapy.

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References

CLINICAL PERSPECTIVE

Heart attack causes irreversible loss of heart muscle cells leading to a thin fibrotic scar that cannot contribute to heart function. Cellular cardiomyoplasty provides a possible approach to the treatment of heart failure after heart attack. The basic concept of cellular cardiomyoplasty is to increase the number of functional heart muscle cells by injecting immature heart muscle cells directly into the wall of the damaged heart. Many kinds of cells have been investigated for regenerating heart muscle. Bone marrow–derived stem cells might be an ideal cell source for cardiac regeneration because they are easily obtained by routine bone marrow aspiration and expansion of cells in culture. In the present study, we investigated the fate and effect of bone marrow–derived mesenchymal stem cells (MSCs) after transplantation into the scar area in a rat heart attack model. The results demonstrated that MSCs survived, gradually expressed muscle markers, and caused a transient but not long-lasting improvement in left ventricular function; some MSCs were incorporated into the inner lining of blood vessels. The mechanisms whereby transplantation of MSCs only transiently improves left ventricular function at 4 weeks after transplantation remain unclear and need to be further investigated; however, this phenomenon may represent a mechanism involving humoral factors expressed by the transplanted cells that cause other cells to function better. Methods to enhance MSC differentiation and continued contribution to contraction are needed to optimize this form of therapy.
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