Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis

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Mesenchymal stem cells (MSCs) are pluripotent adult stem cells residing within the bone marrow microenvironment (11, 18). In contrast to their hematopoietic counterparts, MSCs have an adherent nature and are expandable in culture. MSCs can differentiate into not only osteoblasts, chondrocytes, neurons, and skeletal muscle cells but also vascular endothelial cells (19) and cardiomyocytes (23, 24). In vitro, MSCs have the potential to induce a neovascular response in murine Matrigel angiogenesis assay (2). In vivo, local MSC implantation induces therapeutic angiogenesis in a rat model of hindlimb ischemia (1). On the other hand, MSCs directly injected into the infarcted heart have been shown to induce myocardial regeneration and improve cardiac function (21). Stem or progenitor cells have been shown to circulate in peripheral blood and home to ischemic tissues (4). These results raise the possibility that intravenously administered MSCs participate in repair of the ischemic myocardium primarily by angiogenesis, which prevents apoptosis of native cardiomyocytes, and by direct regeneration of lost cardiomyocytes. However, little information is available regarding the therapeutic potential of systemically delivered MSCs for myocardial infarction.

Thus the purpose of this study was to investigate whether 1) intravenously administered MSCs are able to engraft in the ischemic myocardium, 2) transplanted MSCs induce angiogenesis and myogenesis after myocardial infarction, and 3) transplantation of MSCs decreases infarct size and improves cardiac function.

METHODS

Animals. Male Lewis rats (n = 70) weighing 220–250 g were used in this study. These isogenic rats (n = 8) served as donors and recipients of MSCs to simulate autologous implantation. The Animal Care Committee of the National Cardiovascular Center approved the experimental protocol.

Model of myocardial infarction and cell transplantation. Fifty-one rats underwent ligation of the left coronary artery to produce myocardial infarction, as described previously (15). Briefly, after rats were anesthetized by injection of pentobarbital sodium (30 mg/kg body wt), they were artificially ventilated using a volume-regulated respirator. The heart was exposed via a left thoracotomy, and the left coronary artery was ligated 2–3 mm from its origin between the pulmonary artery conus and the left atrium using a 6-0 Prolene suture.

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At 3 h after coronary ligation, 40 rats survived (78% survival rate); 30 were randomized to receive an intravenous injection of MSCs (MSC group, n = 14) or PBS (control group, n = 16), and 10 received fluorescence-labeled MSCs for examination of MSC differentiation (n = 5) and incorporation (n = 5). Eleven rats underwent a sham operation consisting of thoracotomy and cardiac exposure but without coronary artery ligation. At 3 h after coronary ligation, we administered 5 × 10⁶ MSCs/100μl in PBS or PBS alone through a catheter inserted into the left jugular vein in ~30 s. The subsequent mortality for 4 wk was 25% in the control group and 14% in the MSC group. This protocol resulted in the creation of three groups: normal rats given PBS (sham group, n = 11), myocardial infarction rats given PBS (control group, n = 12), and myocardial infarction rats given MSCs (MSC group, n = 12).

Expansion of bone marrow MSCs. MSC expansion was performed according to previously described methods (18). Briefly, we killed the male Lewis rats and harvested the bone marrow by flushing the cavity of the femurs and tibias with PBS. Bone marrow cells were introduced into 100-mm dishes and cultured in α-MEM supplemented with 10% FBS and antibiotics. A small number of cells developed visible symmetrical colonies by day 5–7. Nonadherent hematopoietic cells were removed, and the medium was replaced. The adherent, spindle-shaped MSC population expanded to >5 × 10⁶ cells by approximately four to five passages after the cells were first cultured.

Flow cytometry. Adherent cells were analyzed by fluorescence-activated cell sorting (FACS SCAN flow cytometer, Becton Dickinson). Cells were incubated for 30 min at 4°C with the FITC-conjugated mouse monoclonal antibodies against rat CD34 (clone ICO-115, Santa Cruz Biotechnology) and CD45 and CD90 (clones OX-1 and OX-7, respectively, Becton Dickinson). FITC-conjugated hamster anti-rat CD29 monoclonal antibody (clone Ha2/5, Becton Dickinson) and rabbit anti-rat c-Kit polyclonal antibody (clone C-19, Santa Cruz Biotechnology) were used. Isotype-identical antibodies served as controls.

Echocardiographic studies. Echocardiographic studies were performed by an investigator blinded to treatment allocation 4 wk after coronary ligation. Two-dimensional targeted M-mode traces were obtained at the level of the papillary muscles using an echocardiographic system equipped with a 7.5-MHz phased-array transducer (SONOS 5500, Hewlett-Packard, Andover, MA). Anterior and posterior end-diastolic wall thickness and left ventricular (LV) end-diastolic and end-systolic dimensions were measured by the American Society for Echocardiology leading-edge method from at least three consecutive cardiac cycles. LV fractional shortening was calculated as follows: \(\frac{LVD_d - LVD_s}{LVD_d} \times 100\), where \(LVD_d\) is LV diastolic dimension and \(LVD_s\) is LV systolic dimension. LV volume and ejection fraction were calculated on the basis of the Teicholtz formula.

Hemodynamic studies. Hemodynamic studies were performed 4 wk after coronary ligation. A 1.5-Fr micrometer-tipped catheter (Millar Instruments) was inserted in the right carotid artery for measurement of mean arterial pressure. Then the catheter was advanced into the LV for measurement of LV pressure. Hemodynamic variables were measured using a pressure transducer (model P23 ID, Gould) connected to a polygraph. After completion of these measurements, the left and right ventricles were excised and weighed. Infarction size was determined as a percentage of the entire LV area, as reported previously (8). Briefly, incisions were made in the LV, so that the tissue could be pressed flat. The circumference of the entire flat LV and the visualized infarcted area, as judged from the epicardial and endocardial sides, was outlined on a clear plastic sheet. The difference in weight between the two marked areas on the sheet was used to determine infarction size and was expressed as a percentage of LV surface area.

Histological examination. To detect fibrosis in cardiac muscle, the LV myocardium (n = 5 each group) was fixed in 10% formalin, cut transversely, embedded in paraffin, and stained with Masson’s trichrome. To detect capillary endothelial cells in the peri-infarct area, samples of the harvested muscle (n = 5 each) were embedded in OCT compound (Miles Scientific), snap frozen in liquid nitrogen, and cut into transverse sections. Tissue sections were stained for alkaline phosphatase with an indoxyltetrazolium method. The number of capillary vessels was counted in the peri-infarct area using a light microscope at ×200 magnification. The numbers in five high-power fields were averaged and expressed as the number of capillary vessels. These morphometric studies were performed by two examiners who were blinded to treatment.

An additional five rats were used to examine whether transplanted MSCs differentiated into cardiomyocytes or vascular endothelial cells. Suspended MSCs were labeled with fluorescent dyes with a PKH-26 red fluorescent cell linker kit (Sigma Chemical, St. Louis, MO) before implantation, as reported previously (13). Fluorescence-labeled MSCs were intravenously administered 3 h after coronary ligation. This subgroup of rats was killed 4 wk after coronary ligation. After the LV was excised and dissected free, muscle samples were embedded in OCT compound, snap frozen in liquid nitrogen, and cut into sections. Immunofluorescent staining for cardiac and endothelial cell markers was performed using monoclonal mouse antibodies (Dako), anti-cardiac troponin T (Novo), anticonnexin43 (Sigma Chemical), and polyclonal rabbit anti-von Willebrand factor (Dako). FITC-conjugated IgG antibody (BD Pharmingen and Molecular Probes) was used as a secondary antibody.

At 24 h after intravenous administration of PKH-26-labeled MSCs, cardiac muscle was embedded in OCT compound and snap frozen in liquid nitrogen. Then the cardiac muscle from base to apex was
transversely cut into 5-μm slices for calculation of the numbers of transplanted MSCs in the heart (n = 5).

Statistical analysis. Numerical values were expressed as means ± SE unless otherwise indicated. Comparisons of parameters among the three groups were made using one-way analysis of variance (ANOVA) followed by Scheffé’s multiple comparison test. Comparisons of parameters between two groups were made by unpaired Student’s t-test. P < 0.05 was considered significant.

RESULTS

Characterization of cultured MSCs. Most of cultured adherent cells expressed CD29 and CD90 (Fig. 1). In contrast, a majority of adherent cells were negative for CD34 and CD45. A small fraction of the adherent cells expressed c-Kit. Thus we confirmed that the major population of adherent cells was MSCs.

Reduction of myocardial infarct size after MSC transplantation. Moderate-to-large infarcts were observed in Masson’s trichrome-stained myocardial sections 4 wk after coronary ligation (control group; Fig. 2A). However, MSC transplantation markedly decreased the infarct size after myocardial infarction (MSC group). Quantitative analysis also demonstrated that cardiac infarct size was significantly smaller in the MSC than in the control group: 24 ± 2 vs. 33 ± 2% (n = 12 each, P < 0.05; Fig. 2B).

Hemodynamic effects of MSC transplantation. At 4 wk after coronary ligation, hemodynamic studies were performed in the sham (n = 11), control (n = 12), and MSC (n = 12) groups. LV end-diastolic pressure showed a marked elevation in the control group (18 ± 1 mmHg); the elevation was significantly attenuated in the MSC group (13 ± 1 mmHg, P < 0.05; Fig. 3A). LV maximum dP/dt was significantly higher in the MSC than in the control group (Fig. 3B). LV minimum dP/dt tended to be lower in the MSC than in the control group (Fig. 3C). Although mean arterial pressure was significantly lower in the control than in the sham group, no decrease was observed in the MSC group (Table 1). Heart rate did not significantly differ among the three groups.

LV diastolic dimension was significantly smaller in the MSC than in the control group (Table 2). Fractional shortening was significantly greater in the MSC than in the control group (Fig. 3D). LV ejection fraction was also higher in the MSC than in
the control group (Table 2). Diastolic anterior wall thickness was significantly attenuated in the MSC group compared with the control group.

**Myogenesis and angiogenesis induced by MSCs.** Red fluorescence-labeled MSCs were intravenously administered 3 h after coronary ligation \((n/1005)\). Semiquantitative analysis demonstrated that \(3\%\) of the transplanted MSCs were incorporated into the heart 24 h after transplantation. At \(4\) wk after transplantation \((n/1005)\), MSCs were incorporated predominantly into the border zone of infarcts (Fig. 4), whereas few MSCs were detected in the noninfarcted myocardium. Immunofluorescence analyses demonstrated that the engrafted MSCs were positive for desmin (Fig. 4), cardiac troponin T (Fig. 5A), and connexin43 (Fig. 5B). These results suggest the ability of MSCs to engrafe in the ischemic myocardium and differentiate into cardiomyocytes. On the other hand, some of the transplanted MSCs were positive for von Willebrand factor and formed vascular structures (Fig. 6). Alkaline phosphatase staining of the ischemic myocardium showed marked augmentation of neovascularization in the MSC group (Fig. 7A). Quantitative analysis demonstrated that capillary density was significantly higher in the MSC than in the control group \((n = 5\) each; Fig. 7B).

**DISCUSSION**

In the present study, we demonstrated that intravenously administered MSCs were capable of engraftment in the ischemic myocardium and that the engrafted MSCs differentiated into cardiomyocytes and vascular endothelial cells, resulting in myogenesis and angiogenesis. We also demonstrated that MSC transplantation decreased myocardial infarct size and improved cardiac function after acute myocardial infarction in rats.

Early studies showed that MSCs directly injected into the myocardium or those injected into coronary arteries improve cardiac function after myocardial infarction. However, little information is available regarding the therapeutic potential of systemically delivered MSCs for myocardial infarction. This study demonstrated that intravenous administration of MSCs

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**Table 1. Characterization of animals**

<table>
<thead>
<tr>
<th></th>
<th>Sham ((n = 11))</th>
<th>Control ((n = 12))</th>
<th>MSC ((n = 12))</th>
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</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>331(\pm)4</td>
<td>301(\pm)7*</td>
<td>321(\pm)7†</td>
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<td>LV wt/body wt, g/kg</td>
<td>1.83(\pm)0.11</td>
<td>2.22(\pm)0.10*</td>
<td>2.17(\pm)0.09*</td>
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<td>RV wt/body wt, g/kg</td>
<td>0.55(\pm)0.02</td>
<td>0.83(\pm)0.04*</td>
<td>0.71(\pm)0.03†</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>404(\pm)15</td>
<td>428(\pm)17</td>
<td>418(\pm)15</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>128(\pm)2</td>
<td>113(\pm)4*</td>
<td>119(\pm)3</td>
</tr>
</tbody>
</table>

*Values are means \(\pm\) SE. Sham, sham-operated rats given vehicle; control, myocardial infarction rats given vehicle; MSC, myocardial infarction rats given mesenchymal stem cells; LV, left ventricle; RV, right ventricle. *\(P < 0.05\) vs. sham. †\(P < 0.05\) vs. control.

**Table 2. Echocardiographic data**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Control</th>
<th>MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDd, mm</td>
<td>6.3(\pm)0.1</td>
<td>8.6(\pm)0.2*</td>
<td>7.5(\pm)0.3†</td>
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<tr>
<td>LVDs, mm</td>
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<td>6.9(\pm)0.3*</td>
<td>5.5(\pm)0.5†</td>
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<td>%FS, %</td>
<td>37(\pm)1</td>
<td>20(\pm)2*</td>
<td>29(\pm)3†</td>
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<tr>
<td>LVEF, %</td>
<td>65(\pm)1</td>
<td>39(\pm)3*</td>
<td>53(\pm)5†</td>
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<td>AWT diastole, mm</td>
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<td>1.1(\pm)0.1*</td>
<td>1.4(\pm)0.1†</td>
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<tr>
<td>PWT diastole, mm</td>
<td>1.6(\pm)0.1</td>
<td>1.7(\pm)0.1</td>
<td>1.7(\pm)0.1</td>
</tr>
</tbody>
</table>

*Values are means \(\pm\) SE. LVDd, LV diastolic dimension; LVDs, LV systolic dimension; %FS, LV fractional shortening; LVEF, LV ejection fraction; AWT, anterior wall thickness; PWT, posterior wall thickness. *\(P < 0.05\) vs. sham. †\(P < 0.05\) vs. control.
improves cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis in the ischemic myocardium.

Earlier studies showed that endothelial progenitor cells are mobilized from bone marrow into the peripheral blood in response to tissue ischemia and home to and incorporate into sites of neovascularization (21). Similar to epithelial progenitor cells, in the present study, transplanted MSCs were preferentially attracted to and retained in the border zone of infarcts. This is consistent with recent findings in the ischemic heart (5).

![Fig. 5](image_url) Differentiation of transplanted MSCs in ischemic myocardium. Engrafted MSCs were positive (arrows) for cardiac troponin T (A) and connexin43 (B). Magnification ×400.

![Fig. 6](image_url) Transplanted MSCs were positive for von Willebrand factor (vWF) and formed vascular structures. Magnification ×400.
or brain (7). Although the underlying mechanisms remain unclear, ischemic tissue may express specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to ischemic sites.

In the present study, some of the engrafted MSCs were stained by cardiac proteins such as desmin and cardiac troponin T. Transplanted MSCs also expressed connexin43, a gap junction protein, at contact points with native cardiomyocytes. These results suggest that MSCs differentiated into cardiomyocytes in the ischemic myocardium and formed connections with native cardiomyocytes. In contrast to skeletal myoblasts, which have been used as a tool for myocardial repair, MSCs may have the capacity for electromechanical coupling. Earlier studies demonstrated the importance of the microenvironment for cardiomyogenic differentiation. Possible factors might include direct cell-cell contact (9), electrical and mechanical stimulation (10), and unknown growth factors. On the other hand, recent studies showed that stem cells may fuse with existing native cells (22, 25). Although the mechanisms by which MSCs develop into cardiomyocyte-like cells remain unclear, it is possible that the direct attachment with host cardiomyocytes in the ischemic myocardium contributes to the cardiogenic differentiation of transplanted MSCs. Further studies are necessary to investigate whether engrafted MSCs are actually becoming contractile.

In the present study, some of the transplanted MSCs were positive for an endothelial cell marker and participated in vessel formation. MSC transplantation significantly increased the capillary density in ischemic myocardium. The recently reported phenotypic plasticity of MSCs to transform into endothelial-like cells provides a rationale for their potential role in neovascularization. Hypoxia has been shown to induce MSC migration and capillary-like structure formation by upregulation of membrane type 1 matrix metalloproteinase (3). MSC implantation has been shown to induce therapeutic angiogenesis in a rat model of chronic hindlimb ischemia (1). These findings support the theory that intravenously administered MSCs are able to differentiate into vascular endothelial cells in the ischemic myocardium. Interestingly, MSCs enhance angiogenesis partly by increasing endogenous levels of vascular endothelial growth factor and vascular endothelial growth factor type 2 receptor (7). Together, these findings suggest that MSCs may contribute to neovascularization in the ischemic myocardium not only through their ability to generate capillary-like structures and but also through growth factor-mediated paracrine regulation.

The present study showed that MSC transplantation significantly reduced infarct size and attenuated wall thinning after acute myocardial infarction. Cardiomyocyte apoptosis during ischemia is one of the major contributors to the development of myocardial infarcts (16, 20). It is possible that newly formed vessels after MSC transplantation improve tissue perfusion around the ischemic boundary zone, resulting in functional recovery after acute myocardial infarction. We also demonstrated that transplanted

![Fig. 7.](http://ajpheart.physiology.org/)

A: representative samples of alkaline phosphatase staining in peri-infarct area. Magnification ×200. B: quantitative analysis of capillary density in peri-infarct area. Values are means ± SE. *P < 0.05 vs. sham. †P < 0.05 vs. control.
MSCs differentiated into cardiomyocytes in the ischemic myocardium. These results suggest that the decrease in infarct size and the increase in wall thickness may be attributable not only to MSC-induced neovascularization but also to myocardial regeneration. In the present study, MSC transplantation improved cardiac function after acute myocardial infarction, as indicated by a significant decrease in LV end-diastolic pressure, a tendency for an increase in maximum LV dP/dt, and a decrease in minimum LV dP/dt. Thus MSC-induced angiogenesis and myogenesis and the resultant reduced infarct size may have contributed to the hemodynamic improvement after acute myocardial infarction.

The low percentage of MSC migration to the heart is in agreement with some previous studies (5, 14). The present study also showed that only a small percentage of transfused MSCs were incorporated into the heart. This may be explained by MSC apoptosis (12), tracking in the lung (5), and a dilution of the fluorescent dyes as the cells reproduce. Nevertheless, when MSCs were intravenously administered in an acute phase of myocardial infarction, MSCs induced angiogenesis and myogenesis and modestly, but significantly, improved cardiac function. Thus systemic delivery of MSCs may be beneficial for the treatment of myocardial infarction.

A limitation of this study is that the cell population may be mixed, rather than limited to MSCs, although cell surface markers of cultured cells were consistent with those of previously reported MSCs (12, 18).

In conclusion, intravenously administered MSCs were preferentially attracted to the infarcted myocardium and differentiated into vascular endothelial cells and cardiomyocytes. MSC transplantation decreased the infarct size and improved cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis. Thus MSC transplantation may be a new therapeutic strategy for the treatment of myocardial infarction.

GRANTS
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REFERENCES