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Insulin-like growth factor 1 improves the efficacy of mesenchymal stem cells transplantation in a rat model of myocardial infarction

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Abstract

Background Previous study demonstrated the improvement of cardiac function was proportional to the number of cells implanted. Therefore, increasing cell survival in the infarcted myocardium might contribute to the improvement of the functional benefit of cell transplantation. *Methods and results* MSCs were treated with IGF-1 in vitro and infused into the acute myocardial infarction rats via the tail vein. After treatment of MSCs with IGF-1 for 48 h, flow cytometric analysis showed marked enhancement of expression of CXCR4 in the cell surface. After 4 weeks of transplantation, we found 1) a greater number of engrafted MSCs arrived and survived in the peri-infarct region; 2) TnT protein expression and capillary density were enhanced; 3) LV cavitary dilation, transmural infarct thinning, deposition of total collagen in the peri-infarct region and cardiac dysfunction were attenuated. *Conclusion* 1) IGF-1 treatment has time-dependent and dose-dependent effects on CXCR4 expression in MSCs in vitro. 2) IGF-1 improves the efficacy of MSCs transplantation in a rat model of myocardial infarction mainly via enhancement of the number of cells attracted into the infarcted heart. These findings provide a novel stem cell therapeutic avenue against ischemic heart disease.

Introduction

Mesenchymal stem cells (MSCs) have been considered to be one of the potential cell sources for cellular cardiomyoplasty given their multipotency and immunomodulatory properties [1, 2]. MSCs directly injected into the infarcted heart have been shown to induce myocardial regeneration and improve cardiac function [3]. Since previous study demonstrated the improvement of cardiac function was proportional to the number of cells implanted [4], increasing cell survival in the infarcted myocardium might contribute to the improvement of myocardial functional. These findings provide a novel stem cell therapeutic avenue against ischemic heart disease.

CXCR4, the unique receptor of stromal derived factor-1 (SDF-1), has been considered to be a major determinant in the migration and repopulation capacity of stem cells [5, 6]. It has been reported that insulin-like growth factor 1 (IGF-1) promoted migration of endothelial cells and cardiac resident progenitor cells [7–9]. A recent report also revealed IGF-1 increased the expression of CXCR4 protein in vitro and the IGF-1-induced increased level of CXCR4 was attenuated by IGF-1 neutralizing antibody [10]. Little is known about the effect of pretreatment of MSCs with IGF-1 on the efficacy of cell transplantation therapy.

Whether the beneficial effect of MSCs transplantation therapy can be improved by pretreatment with

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IGF-1? Furthermore, in this study, we evaluated the effect of pretreatment with IGF-1 on MSCs transplantation in a rat model of myocardial infarction and explored its possible mechanism.

Methods

This study was approved by the Ethic Committee of Wuhan University School of Medicine. All animals received humane care according to the *Guide for the Care and Use of Laboratory Animals* published by National Institute of Health (NIH publication NO. 85-23, revised 1996).

Isolation and culture of bone marrow stromal cells (MSCs)

After rats were anesthetized with 20% sodium urethane (1.0 g/kg ip injection), bone marrow cells were extracted from the tibias and femurs of rats and were suspended in Dulbecco modified Eagle medium (DMEM; Invitrogen, Paisley, United Kingdom) with 20% heat-inactivated fetal calf serum (FCS, GibcoBRL), penicillin G (100 U/ml), and streptomycin (100 μg/ml). Cells were then introduced into 25 cm^2 flask (Corning, MA) and incubated with 95% air and 5% CO2 at 37°C. Medium was replaced every 4 days. Non attached cells were discarded and adherent cells were retained. Each primary culture was replated to three new flasks when MSCs grow to approximately 70–80% confluency.

Flow cytometric analysis

MSC were treated with IGF-1 (final concentration 0, 2.5, 5, and 10 ng/ml) for 4, 12, 24, and 48 h. Then MSCs (1×10^6) were incubated with anti-mouse CXCR4-Allophycocyanin (R&D Systems) for 30 min. The labeled cells were analyzed on a FACS Calibur using CellQuest software (Becton Dickinson, San Jose, CA).

Labeling of MSCs

Sterile 4,6-diamidino-2-phenylindole (DAPI) (final concentration 50 mg/ml) solution was added to the culture medium for 30 min. The MSCs were rinsed eight times in D-Hanks solution to remove all excess unbound DAPI.

Myocardial infarction models preparation MSCs implantation

Rats underwent myocardial ischemia by occlusion of the left coronary artery. Briefly, rats were anesthetized with 20% sodium urethane (1.0 g/kg ip injection). The chest was opened and the ribs were gently spread. The heart was quickly expressed out of the thoracic cavity. Ligation of the LAD was performed 1–2 mm distal to the line between the left border of the pulmonary conus and the right border of left atrial appendage. Then the heart was repositioned to the chest. MI was assessed by electrocardiograph.

MSCs implantation

Experimental animals were randomized (n = 8/group) for MSCs pretreated with IGF-1 (10 ng/ml, 48 h) transplantation group, MSCs transplantation group and MI control group. The transplantation was performed at 1 h after induction of MI. 100 μl DMEM basal medium without cells (MI control group) or containing 1×10^7 MSCs treated with or without IGF-1 (MSCs group and IGF-1-treated MSCs group) were infused into the MI rats via the tail vein.

Left ventricular function measurements

After 4 weeks of MSCs transplantation, hemodynamics was measured with lead 2000, B type, multichannel physiologic recorder (Jinjiang Tongyong Industry Limited Company, Sichuan, China). The cannula was inserted through the right carotid artery into the left ventricle to monitor the left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP) and left ventricular +dp/dt (maximum rate of pressure rise).

Evaluation of myocardial infarct size

The LV chamber was filled with fixative at a pressure equal to the in vivo measured end diastolic pressure. The LV intracavitary axis was measured. The hearts were cut into three sections from the base to the apex of the left ventricle. The mid section was used to measure LV thickness. The lengths of the endocardial and epicardial surfaces delimiting the infarcted region, and the

endocardium and epicardium of the entire LV were measured in each section. Subsequently, their quotients were computed to yield the average infarct size in each case. Tissue samples in peri-infarct region were collected, parts of them embedded in paraffin, and cut into slices of 5 μm thick for Masson's trichome stain and other immunohistochemistry. Collagen volume density fraction (CVF) was determined by measuring the area as a proportion of the total area under observation.

Evaluation of survival of engrafted MSCs

The tissues were soaked in 30% sucrose in PBS overnight at 4°C and then embedded in Tissue-Tak OCT (Sakura). Serial 8 um thick sections were cut at −20°C and placed on poly-L-lysine-coated microscopic slide. DAPI-positive cells were observed in frozen sections, which indicate survival of engrafted MSCs in infarcted myocardium. Fluorescence imaging was performed with an Olympus BX51 microscopy equipped with fluorescence and CCD camera. From each section, 10 light microscopic fields were used to identify DAPI-positive cells.

Immunohistochemistry for von Willebrand factor (vWF)

The number of vessels was counted using immunohistochemistry for von Willebrand factor (rabbit polyclonal, Santa cruz). The secondary antibodies, TRITC conjugated anti-rabbit IgG (1:200 dilution, Molecular probes) was applied, and incubated for 60 min at room temperature. Immunoreactivity was evaluated under the microscope with computer-assisted image analysis system (Image-Pro Plus 4.0).

Western blot of Troponin T protein

Samples containing 30 μg of total protein in infarcted zone were subjected to electrophoresis using a 10% SDS–Tris–Glycine polyacrylamide gel (Invitrogen, San Diego, CA). Protein were electrophoretically transferred to nitrocellulose membranes, Then blocked with 5% defatted milk for 2 h at 37°C. Protein expression was detected by using a 1:500 dilution of Troponin T (mouse monoclonal, Santa cruz) as the first antibody. A 1:5000 dilution of horseradishperoxidase-conjugated rabbit anti mouse IgG (Santa Cruz) was then used as the second antibody and developed with the enhanced chemiluminescence western blotting detection system (Pierce Company Product).

Statistical analysis

Results are presented as mean ± SEM. Data were statistically analyzed by student's t test. A value of $P < 0.05$ was considered significant.

Results

Elevation of CXCR4 expression

Figure 1 indicated that CXCR4 expression was significantly enhanced in MSCs treated with IGF-1 (10 ng/ml) for 48 h group compared with IGF-1 (10 ng/ml) for 4, 12, 24 h groups ($*P < 0.01$). CXCR4 expression was also markedly increased in MSCs treated with IGF-1 (10 ng/ml) for 24 h group compared with IGF-1 (10 ng/ml) for 4, 12 h groups ($\#P < 0.01$).

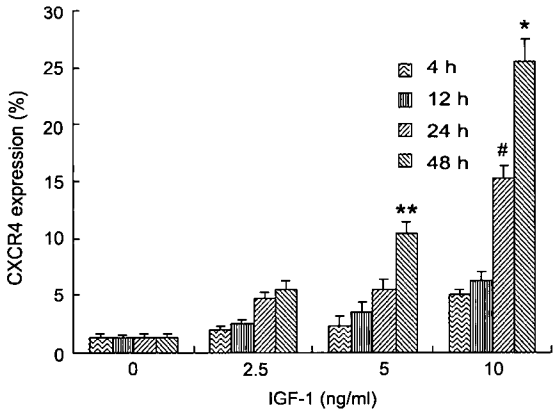

Figure 1. Effects of IGF-1 treatment (final concentration 0, 2.5, 5, and 10 ng/ml for 4, 12, 24, and 48 h) on CXCR4 expression evaluated by flow cytometric analysis. CXCR4 expression was significantly enhanced in MSCs treated with IGF-1 (10 ng/ml) for 48 h group compared with IGF-1 (10 ng/ml) for 4, 12, 24 h groups ($*P < 0.01$). And CXCR4 expression was also markedly enhanced in MSCs treated with IGF-1 (10 ng/ml) for 24 h group compared with IGF-1 (10 ng/ml) for 4, 12 h groups ($\#P < 0.01$). $**P < 0.05$ compared with IGF-1 (5 ng/ml) for 4, 12, 24 h groups. Similar results were obtained in four other experiments.

Improvement of left ventricular function

We analyzed parameters of left ventricular function after 4 weeks of MSCs transplantion. When IGF-1-treated MSCs were transplanted, LVESP and dp/dtmax were increased ($P < 0.01$), while LVEDP was decreased ($P < 0.05$) (Table 1).

Histologic changes

The left ventricular free wall thickness in IGF-1-treated MSCs group was thicker than that of MSCs group ($P < 0.05$). On the other hand, the transmural infarct area was decreased in the IGF-1-treated MSCs group compared with MSCs group ($P < 0.05$) (Table 2).

Decrease of total collagen in peri-infarct area

CVF in peri-infarct area was markedly decreased in MSCs group compared with MI group (4.6 ± 0.6 vs. 6.7 ± 0.8, respectively, $P < 0.01$). Furthermore, CVF in peri-infarct area was markedly decreased in IGF-1-treated MSCs group compared with MSCs group (3.5 ± 0.4 vs. 4.6 ± 0.6, $P < 0.05$) (Figure 2)

Enhancement of engrafted cells homing and survival

Figure 3 showed that DAPI-positive cells were seen in the peri-infarct region. DAPI-positive cells indicates that engrafted MSCs survive successfully. After 4 weeks of IGF-1-treated MSCs transplantion via the tail vein, the number of DAPI-positive nuclei staining was increased significantly in the peri-infarct region.

Table 2. Comparison of thickness of LVFW (left ventricular free wall) and transmural infarct area between groups.

Group	MI group	MSCs group	IGF-1-MSCs group
Thickness of LVFW (cm)	1.5 ± 0.3	1.9 ± 0.4^{a}	2.2 ± 0.3^{ab}
Transmural infarct area (%)	41 ± 4.0	33 ± 5.0^{a}	25 ± 3.0^{ab}

Values represent means ± s.e.m ($n = 8$/group)
$^{a}P < 0.05$ versus MI group
$^{b}P < 0.05$ versus MSCs group.

Increase of vascular density

Semi-quantitative analysis showed the capillary density was significantly greater in IGF-1-treated MSCs group compared with MSCs group and MI control group (Figure 4).

Increase of TnT protein production

We investigated the effects of IGF-1-treated MSCs transplantation on TnT production in infarcted myocardium. Western blotting analysis showed that TnT protein production was significantly up-regulated in IGF-1-treated MSCs transplantation group compared with MSCs group and MI control group (Figure 5).

Discussion

The main findings of this study are 1) in vitro pretreatment of MSCs with IGF-1 enhanced expression of CXCR4 in cell surface; 2) a greater

Table 1. Effect of MSC transplantation on cardiac function parameter.

Group	MI group	MSCs group	IGF-1-MSCs group
LVESP (mm Hg)	90.500 ± 5.200	115.30 ± 3.600^{a}	121.8 ± 3.200^{ab}
LVEDP (mm Hg)	15.200 ± 0.350	11.400 ± 0.500^{a}	10.200 ± 0.600^{ac}
dp/dtmax (mm Hg/s)	4369.500 ± 230.500	5800.200 ± 220.700^{a}	$6200.500 \pm 200.600^{ab}$

Values represent means ± s.e.m ($n = 8$/group)
$^{a}P < 0.01$ versus MI control group. $^{b}P < 0.01$ and $^{c}P < 0.05$ versus MSCs group
LVESP: left ventricular end-systolic pressure, LVEDP: left ventricular end-diastolic pressure, dp/dtmax: left ventricular maximum rate of pressure rise.

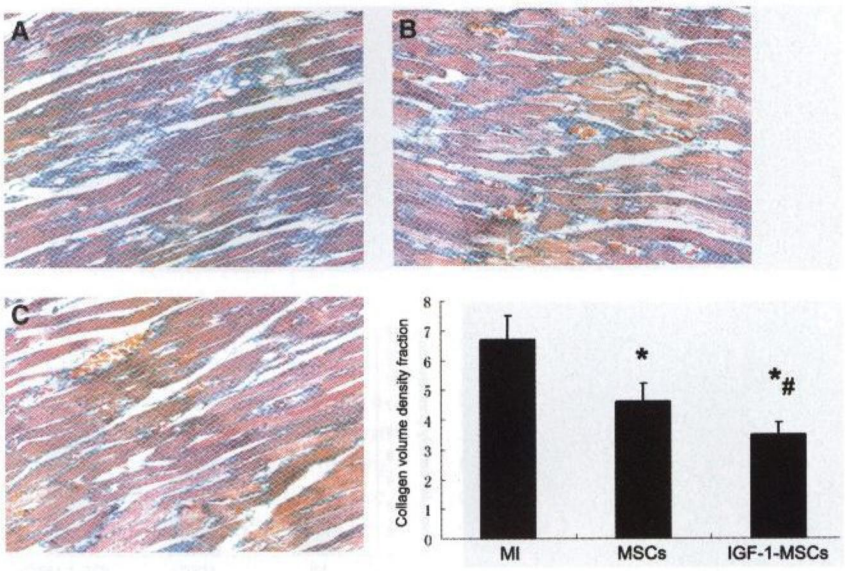

Figure 2. A (MI group), B (MSCs group) and C (IGF-1-MSCs group) showed Masson's trichome stained for total collagen in peri-infarct region of left ventricle. Magnification: 200×. The blue represents total collagen. The red represents myocardium. $*P < 0.01$ compared with MI group, $\#P < 0.05$ compared with MSCs group. Similar results were obtained in four other experiments.

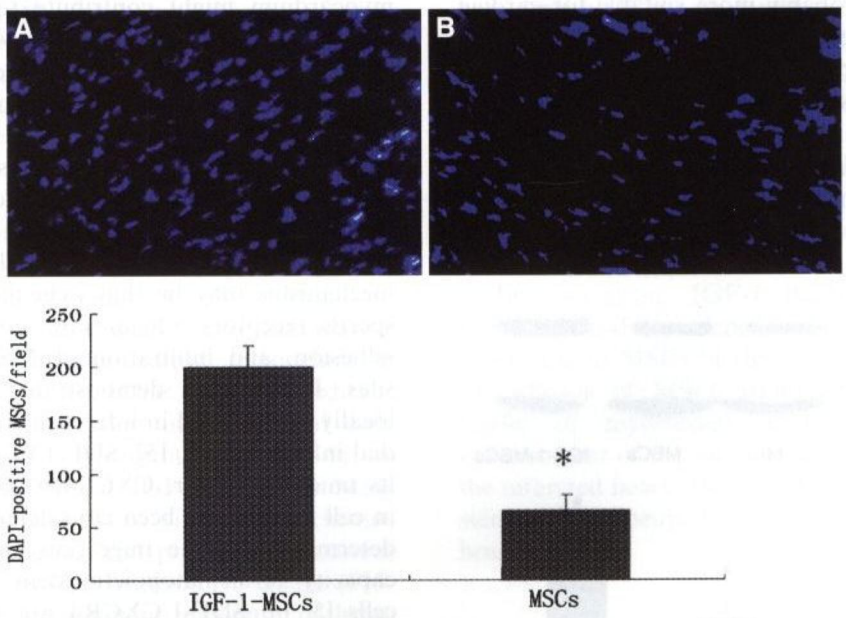

Figure 3. A (IGF-1-MSCs group) and B (MSCs group) showed that immunofluroscence micrographs of engrafted DAPI positive MSCs in the peri-infarct region. Magnification: 100×. Quantitative analysis of DAPI positive MSCs per field was measured. $*P < 0.05$ compared with MSCs group. Similar results were obtained in four other experiments.

number of engrafted MSCs arrived and survived in the peri-infarct region in IGF-1-treated MSCs group; 3) IGF-1-treated MSCs transplantation promoted TnT protein expression and capillary density; 4) IGF-1-treated MSCs transplantation attenuated LV cavitary dilation, transmural infarct thinning and deposition of total collagen in the peri-infarct region, thus prevent myocardial remodeling and cardiac dysfunction after myocardial infarction.

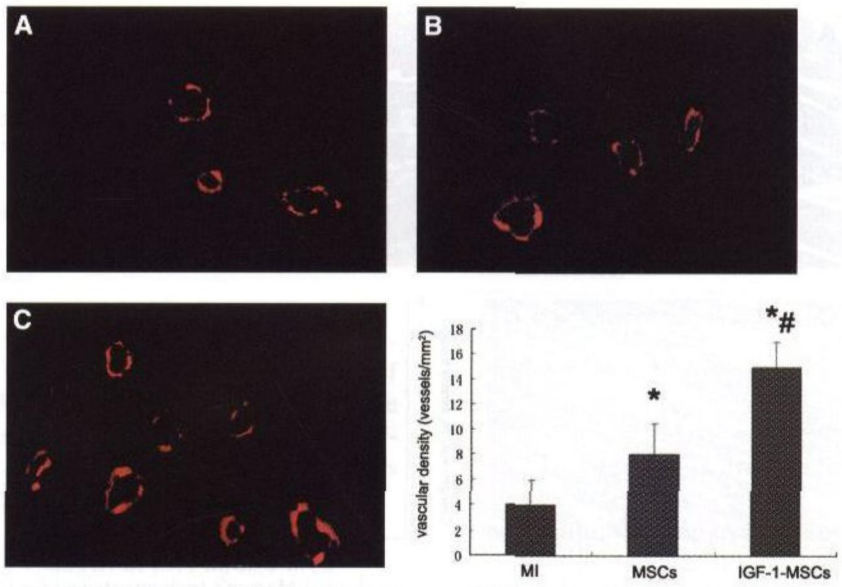

Figure 4. A (MI group), B (MSCs group) and C (IGF-1-MSCs group) showed the capillary density in the peri-infarct hearts. Magnification: 200×. $*P < 0.01$ compared with MI group, $\#P < 0.05$ compared with MSCs group. Similar results were obtained in four other experiments.

MSCs are probably more suitable for cardiac cell therapy given their multipotency and immunomodulatory properties [1–2]. Autologous or allogeneic MSCs have been considered to be one of the potential cell sources for cellular cardiomyoplasty. While enhancement of the number of cells successfully homed and survived in infarcted myocardium might contribute to the replenishment of the lost cardiomyocytes [4].

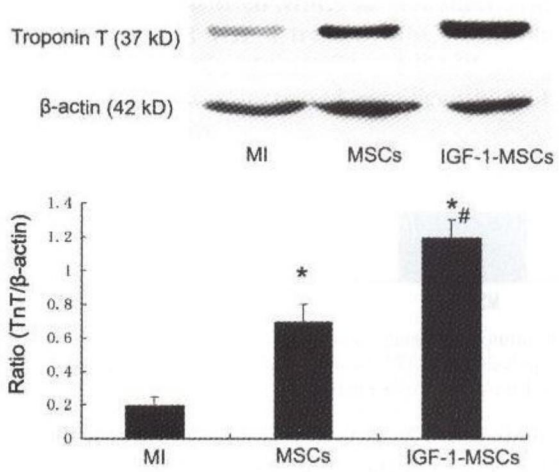

Figure 5. TnT protein production in infarcted myocardium evaluated by western blot. $*P < 0.01$ compared with MI group, $\#P < 0.05$ compared with MSCs group. Similar results were obtained in four other experiments.

In the present study, MSCs were transplanted by intravenous administration instead of direct intramyocardial injection because intramyocardial injection has practical limitations to widespread clinical use. Recent study found intravenously administered MSCs are attracted to and retained in the border zone of infarcts [11–13]. The possible mechanisms may be that ischemic tissue express specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to ischemic sites. It has been demonstrated that SDF-1 is locally upregulated in infarct region after myocardial infarction [14, 15]. SDF-1 exerts its effect via its unique receptor, CXCR4. CXCR4 expression in cell surface has been considered to be a major determinant in the migration and repopulation capacity of hematopoietic stem and progenitor cells [5, 6]. SDF-1/CXCR4 are involved in the chemoattraction of BM-derived cardiac progenitor cells after myocardial infarction [16, 17].

However, recent studies showed SDF-1α mRNA expression in the heart was only upregulated within 3 days after coronary artery ligation [17–19]. Ma et al. found intravenous MSCs administration at the early phase of MI increased myocardial homing, angiogenesis and improved

cardiac function [19]. Therefore, we speculate intravenously administered MSCs at every time points within 3 days post MI should have positive effects on MSCs homing in spite of different effects at different time points.

Previous studies have shown that intravenous injection into mice of an adenoviral vector encoding SDF-1α contributed to the increase of mobilization of hematopoeitic stem cells [20, 21]. Elmadbouh et al. also demonstrated that ex vivo SDF-1α transgene delivery promoted stem and progenitor cell migration to the heart, activated cell survival signaling and enhanced angiomyogenesis in the infarcted heart [22]. In contrast, stem cell mobilization was inhibited by neutralizing CXCR4 or SDF-1 antibodies [23]. Furthermore, Kahn et al. also demonstrated CXCR4 overexpression for improved definitive human stem cell motility, retention, and multilineage repopulation, which could be beneficial for in vivo navigation and expansion of hematopoietic progenitors [24]. Some evidence showed that transduction of MSCs by CXCR4 migrate rapidly toward a gradient of SDF-1 in vitro [25].

In this study, our research demonstrated that IGF-1 significantly elevated CXCR4 protein production in cell surface after pretreatment of MSCs with IGF-1 for 48 h in vitro. Furthermore, we observed that a greater number of engrafted MSCs arrived and survived in the infarcted myocardium in IGF-1-treated MSCs group. IGF-1 has been considered as a potent growth hormone capable of inducing cell proliferation, limiting apoptotic cell death, and attenuating maladaptive extracellular matrix remodeling in the failing heart [26–33]. It has been reported that IGF-1 promotes migration of endothelial cells and cardiac resident progenitor cells [7–9]. A recent report also found IGF-1 increases MSC migratory responses in vitro via CXCR4 chemokine receptor signaling and provided substantial evidence that phosphatidylinositol 3-kinase (PI3-kinase)/Akt is the dominant signaling pathway underlying IGF-1 enhanced MSC migration [10]. Kofidis and his colleagues found insulin-like growth factor promotes engraftment, differentiation, and functional improvement after transfer of embryonic stem cells for myocardial restoration [34]. In that study, they speculated the beneficial effects might be attributable to the expression of cardiomyocyte phenotype in ESCs promoted by IGF-1 in vivo, which is different from our findings.

We found IGF-1-treated MSCs promoted cardiac specific protein TnT expression and capillary density in infarcted heart after 4 weeks of MSCs transplantation. Although the detailed mechanism of the elevation of TnT expression and capillary density was not explored in our study, these beneficial effects may be considered to be the following factors: 1) the myogenesis and angiogenesis actions of MSCs; 2) the effective role of some angiogenic cytokines such as VEGF or other beneficial cytokines secreted by engrafted MSCs; 3) the decrease of cardiomyocytes apoptosis. All these results are attributable to the enhancement of the number of implanted cells homed and survived in the infarcted heart.

A limitation in our experiment is that the specific antagonist of CXCR4 such as AMD 3100 is not used to investigate whether the effect of IGF-1 increased MSC migration is due to CXCR4 dependent. So the key role played by CXCR4 in cell transplantation therapy couldn't be established here directly. While we found the time-dependent and dose-dependent effects of IGF-1 treatment on the CXCR4 expression in MSCs in vitro. And recent research also found that IGF-1 enhanced migration of MSCs was inhibited by pretreatment with blocking anti-CXCR4 antibody in vitro [10]. Thus we could speculate that the elevation of CXCR4 expression in MSCs might be attributable to cell migration in vivo.

In conclusion, IGF-1 treatment has time-dependent and dose-dependent effects on CXCR4 expression in MSCs in vitro and IGF-1 improves the efficacy of MSCs transplantation in a rat model of myocardial infarction mainly via enhancement of the number of cells attracted into the infarcted heart. These findings provide a novel stem cell therapeutic intervention against ischemic heart disease.

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References

1. Toma C., Pittenger M.F., Cahill K.S., Byrne B.J. and Kessler P.D., Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105: 93–98, 2002.
2. Wang J.S., Shum-Tim D., Galipeau J., Chedrawy E., Eliopoulos N. and Chiu R.C., Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J. Thorac. Cardiovasc. Surg. 120: 999–1005, 2000.
3. Shake J.G., Gruber P.J., Baumgartner W.A., Senechal G., Meyers J., Redmond J.M., Pittenger M.F. and Martin B.J., Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann. Thorac. Surg. 73: 1919–1925, 2002.
4. Pouzet B., Vilquin J.T., Hagege A.A., Scorsin M., Messas E., Fiszman M., Schwartz K. and Menasche P., Factors affecting functional outcome after autologous skeletal myoblast transplantation. Ann. Thorac. Surg. 71: 844–850, 2001.
5. Kollet O., Spiegel A., Peled A., Petit I., Byk T., Hershkoviz R., Guetta E., Barkai G., Nagler A. and Lapidot T., Rapid and efficient homing of human CD34(+)CD38(−/low) CXCR4(+) stem and progenitor cells to the bone marrow and spleen of NOD/SCID and NOD/ SCID/B2m(null) mice. Blood. 97: 3283–3291, 2001.
6. Peled A., Petit I., Kollet O., Magid M., Ponomaryov T., Byk T., Nagler A., Ben-Hur H., Many A., Shultz L., Lider O., Alon R., Zipori D. and Lapidot T., Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science. 283: 845–848, 1999.
7. Urbich C., Aicher A., Heeschen C., Dernbach E., Hofmann W.K., Zeiher A.M. and Dimmeler S., Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. J. Mol. Cell. Cardiol. 39: 733–742, 2005.
8. Xu M., Uemura R., Dai Y., Wang Y., Pasha Z. and Ashraf M., In vitro and in vivo effects of bone marrow stem cells on cardiac structure and function. J. Mol Cell Cardiol. 42: 441–448, 2007.
9. Su E.J., Cioffi C.L., Stefansson S., Mittereder N., Garay M., Hreniuk D. and Liau G., Gene therapy vector-mediated expression of insulin-like growth factors protects cardiomyocytes from apoptosis and enhances neovascularization. Am. J. Physiol. Heart Circ. Physiol. 284: H1429–H1440, 2003.
10. Li Y., Yu X., Lin S., Li X., Zhang S. and Song Y.H., Insulin-like growth factor 1 enhances the migratory capacity of mesenchymal stem cells. Biochem. Biophys. Res. Commun. 356: 780–784, 2007.
11. Nagaya N., Fujii T., Iwase T., Ohgushi H., Itoh T., Uematsu M., Yamagishi M., Mori H., Kangawa K. and Kitamura S., Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. Am. J. Physiol. Heart Circ. Physiol. 287: H2670–H2676, 2004.
12. Barbash I.M., Chouraqui P., Baron J., Feinberg M.S., Etzion S., Tessone A., Miller L., Guetta E., Zipori D., Kedes L.H., Kloner R.A. and Leor J., Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation. 108: 863–868, 2003.
13. Chen J., Zhang Z.G., Li Y., Wang L., Xu Y.X., Gautam S.C., Lu M., Zhu Z. and Chopp M., Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ. Res. 92: 692–699, 2003.
14. Askari A.T., Unzek S., Popovic Z.B., Goldman C.K., Forudi F., Kiedrowski M., Rovner A., Ellis S.G., Thomas J.D., DiCorleto P.E., Topol E.J. and Penn M.S., Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 362: 697–703, 2003.
15. Pillarisetti K. and Gupta S.K., Cloning and relative expression analysis of rat stromal cell derived factor-1 (SDF-1)1: SDF-1 alpha mRNA is selectively induced in rat model of myocardial infarction. Inflammation 25: 293–300, 2001.
16. Hiasa K., Ishibashi M., Ohtani K., Inoue S., Zhao Q., Kitamoto S., Sata M., Ichiki T., Takeshita A. and Egashira K., Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. Circulation 109: 2454–2461, 2004.
17. Askari A.T., Unzek S., Popovic Z.B., Goldman C.K., Forudi F., Kiedrowski M., Rovner A., Ellis S.G., Thomas J.D., DiCorleto P.E., Topol E.J. and Penn M.S., Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 362: 697–703, 2003.
18. Abbott J.D., Huang Y., Liu D., Hickey R., Krause D.S. and Giordano F.J., Stromal cell-derived factor-1alpha plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. Circulation 110: 3300–3305, 2004.
19. Ma J., Ge J., Zhang S., Sun A., Shen J., Chen L., Wang K. and Zou Y., Time course of myocardial stromal cell-derived factor 1 expression and beneficial effects of intravenously administered bone marrow stem cells in rats with experimental myocardial infarction. Basic Res Cardiol. 100: 217–223, 2005.
20. Hattori K., Heissig B., Tashiro K., Honjo T., Tateno M., Shieh J.H., Hackett N.R., Quitoriano M.S., Crystal R.G., Rafii S. and Moore M.A., Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. Blood 97: 3354–3360, 2001.
21. Moore M.A., Hattori K., Heissig B., Shieh J.H., Dias S., Crystal R.G. and Rafii S., Mobilization of endothelial and hematopoietic stem and progenitor cells by adenovector-mediated elevation of serum levels of SDF-1, VEGF, and angiopoietin-1. Ann. N.Y. Acad. Sci. 938: 36–45, 2001.
22. Elmadbouh I., Haider H.Kh., Jiang S., Idris N.M., Lu G. and Ashraf M., Ex vivo delivered stromal cell-derived factor-1α promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. J. Mol. Cell. Cardiol. 42: 792–803, 2007.

23. Petit I., Szyper-Kravitz M., Nagler A., Lahav M., Peled A., Habler L., Ponomaryov T., Taichman R.S., Arenzana-Seisdedos F., Fujii N., Sandbank J., Zipori D. and Lapidot T., G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat. Immunol. 3: 687–694, 2002.
24. Kahn J., Byk T., Jansson-Sjostrand L., Petit I., Shivtiel S., Nagler A., Hardan I., Deutsch V., Gazit Z., Gazit D., Karlsson S. and Lapidot T., Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. Blood 103: 2942–2949, 2004.
25. Bhakta S., Hong P. and Koc O., The surface adhesion molecule CXCR4 stimulates mesenchymal stem cell migration to stromal cell-derived factor-1 in vitro but does not decrease apoptosis under serum deprivation. Cardiovasc. Revasc. Med. 7: 19–24, 2006.
26. Ambler G.R., Johnston B.M., Maxwell L., Gavin J.B. and Gluckman P.D., Improvement of doxorubicin induced cardiomyopathy in rats treated with insulin-like growth factor I. Cardiovasc. Res. 27: 1368–1373, 1993.
27. Duerr R.L., McKirnan M.D., Gim R.D., Clark R.G., Chien K.R. and Ross J. Jr., Cardiovascular effects of insulin-like growth factor-1 and growth hormone in chronic left ventricular failure in the rat. Circulation 93: 2188–2196, 1996.
28. Kinugawa S., Tsutsui H., Ide T., Nakamura R., Arimura K., Egashira K. and Takeshita A., Positive inotropic effect of insulin-like growth factor-1 on normal and failing cardiac myocytes. Cardiovasc. Res. 43: 157–164, 1999.
29. Li G., Borger M.A., Williams W.G., Weisel R.D., Mickle D.A., Wigle E.D. and Li R.K., Regional overexpression of insulin-like growth factor-I and transforming growth factor-beta1 in the myocardium of patients with hypertrophic obstructive cardiomyopathy. J. Thorac. Cardiovasc. Surg. 123: 89–95, 2002.
30. Li Q., Li B., Wang X., Leri A., Jana K.P., Liu Y., Kajstura J., Baserga R. and Anversa P., Overexpression of insulin-like growth factor-1 in mice protects from myocyte death after infarction, attenuating ventricular dilation, wall stress, and cardiac hypertrophy. J. Clin. Invest. 100: 1991–1999, 1997.
31. Redaelli G., Malhotra A., Li B., Li P., Sonnenblick E.H., Hofmann P.A. and Anversa P., Effects of constitutive overexpression of insulin-like growth factor-1 on the mechanical characteristics and molecular properties of ventricular myocytes. Circ. Res. 82: 594–603, 1998.
32. Ross J. Jr., Growth hormone, cardiomyocyte contractile reserve, and heart failure. Circulation 99: 15–17, 1999.
33. Welch S., Plank D., Witt S., Glascock B., Schaefer E., Chimenti S., Andreoli A.M., Limana F., Leri A., Kajstura J., Anversa P. and Sussman M.A., Cardiac-specific IGF-1 expression attenuates dilated cardiomyopathy in tropomodulin-overexpressing transgenic mice. Circ. Res. 90: 641–648, 2002.
34. Kofidis T., de Bruin J.L., Yamane T., Balsam L.B., Lebl D.R., Swijnenburg R.J., Tanaka M., Weissman I.L. and Robbins R.C., Insulin-like growth factor promotes engraftment, differentiation, and functional improvement after transfer of embryonic stem cells for myocardial restoration. Stem Cells 22: 1239–1245, 2004.