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***In vitro* and *in vivo* effects of bone marrow stem cells on cardiac structure and function**

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Abstract

It is hypothesized that the protection of bone marrow stem cells (BMSCs) on ischemic myocardium might be related to the anti-apoptotic effect via paracrine mechanisms. In this study, a wide array of cytokines including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), stromal cell derived factor-1 (SDF-1) and insulin growth factor-1 (IGF-1) were detected in the BMSCs cultured medium by ELISA. Myocyte apoptosis was assayed by DNA fragmentation and annexin-V staining. Myocardial infarction model was produced by ligation of mouse left anterior descending coronary arteries (LAD). Before LAD ligation, mice were myoablated by irradiation and transplanted with bone marrow cells from transgenic green fluorescent protein (GFP) mice. After LAD ligation, animals were administered stem cell factor (SCF, 200 µg / day / kg, i.p.) or saline for 6 days. Animals were sacrificed on end of SCF treatment and four weeks later. Apoptotic cardiomyocytes were assayed after treatment finished by TUNEL. Myocardial function was analyzed by echocardiography and pressure-volume loop. Bcl-2 protein was analyzed by western blotting. Our results showed that cultured BMSCs released VEGF, bFGF, SDF-1 and IGF-1. Hypoxia induced cell apoptosis was diminished in cardiomyocytes co-cultured with BMSCs. Smaller LV dimension and increased LV ejection fraction were seen in SCF treated animals. SCF significantly reduced cardiomyocytes apoptosis within peri-infarct area and up-regulation expression of Bcl-2 in ischemic area. Moreover, conditioned medium from cultured BMSCs also induced up-regulation of Bcl-2 protein in cardiomyocytes. It is concluded that paracrine mediators secreted by BMSCs might be involved in early repair of ischemic heart by preventing cardiomyocytes apoptosis and improving cardiac function.

Keywords

Stem cells; Mobilization; Paracrine effect; Myocardial infarction; Apoptosis

1. Introduction

Tissue repair after ischemic injury is thought to involve the recruitment of bone marrow stem cells (BMSCs) or resident stem cells. Stem cells are capable of self-renewing and differentiation into multiple cell lineages. Most studies of adult stem cell therapy in animals [1,2] and in patients with acute myocardial infarction [3,4] have shown the improvement of

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cardiac function. A few clinical studies have shown spontaneous mobilization of BMSCs (CD 34⁺ cells) after acute myocardial infarction [5,6] and this spontaneous mobilization of BMSCs was associated with favourable LV remodeling after myocardium infarction (MI) [7]. Pharmacological mobilization of bone marrow stem cells is one of the strategies for cardiac repair. Several kinds of cytokines, including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), and stem cell factor (SCF), have been used to induce BMSC mobilization. Orlic and colleagues reported that a significant accelerated mobilization of BMSCs occurs after SCF + G-CSF administration resulting in enhanced myocardial regeneration in infarcted murine hearts [8]. The latter investigations have instead proposed myocardial protection of G-CSF through angiogenesis [9] and prevention of scar formation in the left ventricles (LV) [10,11]. It has been reported that BMSCs secrete a wide array of cytokines which may protect target cells [12,13]. How do these cytokines contribute to the ischemic protection is not clear. Our overall hypothesis is that immediate effect of BMSC is mediated by prevention of apoptosis and given the proper environment that lead to regeneration of the infarcted myocardium. This study is intended to determine the effect of BMSC on myocyte apoptosis, secretion of different cytokines and their effect on cardiac function following myocardial infarction.

2. Methods

All animals were treated in accordance with the *Guidelines for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health.

A. Induction of myocardial infarction

Wild type C57B6 recipient mice were sub-lethally irradiated with 12.0 Gy administered over two days prior to green bone marrow cell transplantation, using Cesium-137 radiation source (GAMMA Cell 40 Irradiator). GFP transgenic mice were used as bone marrow cell donors. Six weeks after bone marrow transplantation, myocardial infarction was created by permanent ligation of left anterior descending coronary artery (LAD). The animals were anesthetized with sodium pentobarbital (50 mg/Kg intraperitoneal) and mechanically ventilated. After a left sided mini thoracotomy, the heart was exposed and LAD was ligated by 7-0 ethicon suture at just below the atrioventricular border. The chest was closed and animals were weaned from ventilator and allowed to recover. The animals were divided into saline control (G-1) and SCF (200 µg / kg / day i.p) treated (G-2) groups. The treatments were started from 4 hours after MI and continued until 6 days. After 1 or 4 weeks following MI, 4 to 6 mice from each group were first analyzed for cardiac function and then sacrificed for immunohistostaining or TUNEL assay.

B. Flow cytometric (FACS) analysis of mobilized stem cells

Mice were bled via tail vein to collect blood samples for analysis of mobilized cells. Approximately 20 µl peripheral blood was collected after 1 week following MI into heparinized micro-hematocrit capillary tubes (Fisher Scientific) and diluted with 100 µl of FACS wash buffer (1 % BSA in PBS). Cells were labeled for FACS analysis with anti-c-kit and anti-Sca-1 antibodies conjugated with phycoerythrin (PE) and allophycocyanin (APC) (BD Bioscience). FACS analysis was performed using a BD FACSCalibur and quantified by using CellQuest Pro software.

C. Echocardiography

Heart function of the animals was assessed by transthoracic echocardiography which was performed at 4 weeks after MI, using HDI 5000 SonoCT (Phillips) with 15-Mhz probe [14]. Mice were anesthetized with 100 mg/kg ketamine and 5 mg/kg xylazine. Two-dimensional

image were obtained with mice orientated on a heating pad in a left lateral decubitus or supine position. LV parameters were obtained from M-mode interrogation in long-axis view: Interventricular septum thickness (IVST), LV posterior wall thickness (LVPWT), LV internal diastolic diameter (LVIDd), LV internal systolic diameter (LVIDs). LV percent fractional shortening (LV%FS) and LV ejection fraction (LVEF) were calculated as: $LV\%FS = (LVIDd - LVIDs) / LVIDd \times 100$; $LVEF = [(LVIDd)^3 - (LVIDs)^3] / (LVIDd)^3 \times 100$. All echocardiographic measurements were averaged from at least 3 separate cardiac cycles.

D. Pressure-Volume loop

Mice were anesthetized with a sodium pentobarbital (40mg/kg, i.p.) and right carotid artery was cannulated with a 1.4 F catheter (SPR-839, Millar Instruments) connected to MPCU-200 P-V conductance system which provided analog outputs of the time-varying ventricular pressure and volume signals for data acquisition. The inferior vena cava (IVC) was exposed and IVC occlusion was performed by external compression for pressure-volume (P-V) loops. Hemodynamic and P-V loops were recorded during steady state. LV systolic and diastolic function was evaluated as previously described [15]. Hemodynamic parameter analysis was carried out using Millar's PVAN software (Version 3.2).

E. Immunohistostaining and TUNEL analysis

For detecting GFP positive cells as well as GFP⁺ cardiomyocytes and vessels, fluorescent immunostaining for α -smooth muscle actin (SMA), and desmin (Sigma) were performed. Nuclei were stained with 4', 6-diamino-2-phenylindole (DAPI). Fluorescent images were obtained with an Olympus BX 41 microscope equipped with digital camera (Olympus). Apoptotic cardiomyocytes in the infarcted heart were evaluated by TUNEL assay in serial paraffin and cryo-sections with an ApopTag kit (Chemicon). Tissue sections were examined microscopically and at least 100 myocytes were counted in eight randomly chosen fields. Four fields each were selected in non-infarction and peri-infarct areas. The percentage of apoptotic myocyte was termed the apoptotic index.

F. Culture of BMSCs and cardiomyocytes

Isolation and purification of BMSCs from transgenic GFP mice were performed as described previously [16]. In brief, the femora and tibia were removed after euthanasia of animals with overdose of anesthesia. The bone-marrow plugs of the femora and tibia were flushed with phosphate-buffered saline solution (PBS). Cells were cultured with complete medium (Iscove's Modified Dulbecco's medium containing 20 % fetal bovine serum, 100 U/ml of penicillin, and 100 μ g/ml streptomycin) at 37°C in humid air with 5% CO₂. The cells adherent to the culture flask were maintained for propagation and non-attached cells were discarded by 4 changes of medium.

Cardiomyocytes were isolated from the ventricles of neonatal rats (1 to 3 days old) using commercially available neonatal cardiomyocyte isolation kit (Worthington Biochemical Co) as described by supplier's protocol. Myocytes were co-cultured with BMSCs at different ratios in dual-set system in which myocytes and BMSCs shared same medium but were separated by semi-permeable membrane. Hypoxia was induced by incubating the cells at 37°C in hypoxic incubator (Sanyo, CO₂/O₂ incubator-MCO-18M) and adjusted 5% CO₂ and 1% O₂.

G. Detection of apoptosis (DNA laddering and Annexin V staining)

DNA laddering—After incubation for 48 hours with or without hypoxia under serum-free medium, 2×10^6 culture cells were resuspended in PBS and homogenized in buffer containing proteinase K and RNase. After 15 minutes of incubation at 37°C, NaI solution was added. Cell lysates were incubated at 50°C for 30 minutes and isopropanol was added. DNA was

precipitated by centrifugation and washed by 70% ethanol. DNA (8 µg) was then analyzed using 1.2% agarose gel electrophoresis and visualized under a UV (302 nm) transilluminator. Annexin V staining was performed with a commercially available kit (Roche) according to the manufacturer's protocols and annexin V positive cells were counted by FACS.

H. Electroimmunoblotting

In a dual-set culture system, cardiomyocytes were seeded in the lower layer and BMSCs were seeded in the upper layer. In control group, upper layer was seeded with cardiomyocytes. Twenty-four hours before the experiment, standard culture medium was replaced by serum-free medium. After 24 hours, cardiomyocytes seeded in lower layer were homogenized in buffer (20 mM Tris [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1 mM Na₃VO₄) containing protease inhibitors cocktail. The protein content of samples was determined according to the Bradford method. Denatured protein (25 µg) was then analyzed using 12 % sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) and transferred to the nitrocellulose membranes (Bio-Rad). Membranes were immunoblotted overnight at 4°C with polyclonal antibodies including Bcl-2 (1:1000, Cell Signaling) and β-actin (1:5000; Sigma). Densitometric analysis for the blots was performed with NIH image software.

I. ELISA for VEGF, bFGF, IGF-1 and SDF-1 analysis

Second passage BMSCs and primary cultured cardiomyocytes were plated on 60 mm dishes with 2×10^5 cells per dish. After incubation in serum free culture medium for 24 hours, media were collected and centrifuged at 10,000g at 4°C for 5 min. Supernatants were stored at -20°C. Level of cytokine expression was measured with enzyme-linked immunosorbent assay (ELISA) kits (R&D systems) according to the instructions of the manufacturer. Specific antibodies used were: VEGF, bFGF, IGF-1 and SDF-1. ELISA values were calibrated for total cell protein.

J. Statistical analysis

Data are expressed as mean ± SEM. For the comparison of *in vitro* data, ultrasound parameters and histological data, the unpaired Student's *t* test was used for continuous data. Mann-Whitney U-test was performed for nonparametric continuous data. Differences with a *p*-value of <0.05 were regarded as statistically significant.

3. Results

A. *In vitro* studies

(1) Do BMSCs secrete various cytokines?—In order to explain the beneficial effects of BMSCs, first we determined whether these cells secrete various prosurvival factors and cytokines. BMSCs were cultured with serum-free medium for 24 hours. Media from cultured BMSCs were used to measure various cytokines. VEGF, bFGF, SDF and IGF-1 were significantly higher in the medium from cultured BMSCs than in the medium from cultured cardiomyocytes (Figure 1).

(2) BMSCs prevent cardiomyocyte apoptosis—We hypothesized that the immediate effect of myocyte protection was due to alleviation of apoptosis of myocytes by BMSC. The anti-apoptotic effect of BMSCs was confirmed by *in vitro* study (Figure 2). After 48 hours hypoxia, DNA prepared from cardiomyocytes displayed the typical nucleosome spacing ladder upon agarose gel electrophoresis. Co-culture with BMSCs, in a ratio of 10: 1 completely inhibited DNA fragmentation induced by hypoxia for 48 hours. Staining apoptotic myocytes with annexin V positive cardiomyocytes indicated that the percentage of apoptotic cell was

significantly reduced in co-cultured cells compared with cardiomyocytes alone after exposed to hypoxia for 24 hours ($24.8 \pm 2.8\%$ vs $8.9 \pm 0.9\%$, $p < 0.01$).

The next evidence of antiapoptotic effect of BMSC was obtained by measuring anti-apoptotic protein Bcl-2 in myocytes by BMSCs. A slight increase of Bcl-2 protein was observed in cultured cardiomyocytes (Figure 3) while there was a 2.9-fold increase in cardiomyocytes when they were incubated together with BMSCs. These data suggest that reduction of cardiomyocyte apoptosis by BMSCs is also related to Bcl-2 protein over-expression in myocytes.

B. *In vivo* studies

(1) Cytokines prevent cardiomyocyte apoptosis—Since cytokines are known to attenuate apoptosis under *in vitro* condition [17,18], we hypothesized that anti-apoptotic effect of cytokines can be mimicked *in vivo*. TUNEL assay was employed to confirm anti-apoptosis effect of SCF in animals after LAD ligation for 1 week. The percentage of TUNEL-positive cells in the peri-infarct region was significantly lower after SCF treatment than that in the control tissue (Apoptotic cardiomyocyte index was 1.17 ± 0.18 in SCF vs 2.95 ± 0.25 in control) ($p < 0.01$) (Figure 4).

(2) Cytokines up-regulate Bcl-2 protein in cardiomyocytes—It is widely believed that Bcl-2 acts as an anti-apoptotic protein [19,20]. We investigated whether cytokines which are released by BMSCs prevent cardiomyocyte apoptosis by up-regulation of Bcl-2. Electroimmunoblotting analysis revealed 2.1-fold increase in expression of Bcl-2 protein in peri-infarct heart tissue obtained from SCF treated animals (Figure 5).

(3) SCF treatment preserves ischemic cardiac function—Four weeks after MI, LV internal dimensions measured by echocardiography (at both diastole and systole) were significantly decreased in SCF treated animals as compared with control (Table 1 and Figure 6). Hemodynamic parameters measured by pressure-volume conductance system in two groups have been shown in Figure 7. Heart function in the SCF treated group significantly improved contractility as compared with that in the vehicle treated control. Ventricular end-systolic elastance (Ees) of SCF-treated mice (B) was 2-fold greater than control group (A). Besides, end-systolic volume (Ves) and end-diastole volume (Ved) of SCF treated mice were obviously less shifted rightward which supports the observation that LV contractility of SCF-treated group was significantly improved. In SCF treated mice, LV systolic (Pmax, E-max) and diastolic functions (Pmin, tau-W) were significantly better than that in control group (C).

To further confirm SCF treatment induced mobilization, BMSCs in circulation were counted by FACS. Both Sca-1⁺/c-kit⁺ cells was considered. Supplement of SCF significantly increased Sca-1⁺/c-kit⁺ cells in peripheral blood ($3.14 \pm 1.21\%$ of nucleated cells) as compared with saline control ($0.51 \pm 0.40\%$ of nucleated cells; $p < 0.05$). SCF not only increased mobilization, but also enhanced the mobilized BMSCs homing to the ischemic myocardium. To investigate the effect of SCF on homing of BMSCs, hearts were removed to perform immunohistostaining after cardiac function measurements. GFP-positive cells were observed 20 times more in the SCF treated hearts as compared with control hearts. Interestingly enough, the propensity of their localization was mostly in peri-infarct area. A higher number of GFP positive cardiomyocytes were observed in the myocardium of SCF treated animals (4.8 ± 1.7 cells / 4×10^4 cardiomyocytes) as compared with control mice (0.43 ± 0.54 cells / 4×10^4 cardiomyocytes) ($p < 0.05$) (Figure 8). Some BMSCs were positive for desmin and SMA staining, which suggested that mobilized BMSCs participated in myogenesis and angiogenesis.

4. Discussion

Recent clinical studies have reported that spontaneous mobilization of BMSCs after AMI contributes to the progression of LV remodeling [7], and that the level of circulating progenitor cells can predict the occurrence of cardiovascular events and death in patients with coronary artery disease [21,22]. Experimental animal studies also suggest that mobilized BMSCs participate in neomyogenesis and angiogenesis [8]. Recent studies suggest that BMSC attenuate LV remodeling not only by their differentiation into cardiomyocytes and vascular cells but also by their ability to release large amounts of angiogenic, antiapoptotic, and mitogenic factors [23]. The findings of this study demonstrate that BMSC *in vitro* secrete cytokines and prosurvival proteins which prevent apoptosis of myocytes in early stage and improve cardiac function in later stage.

One significant finding of our study is that BMSCs *in vitro* secreted a wide array of cytokines such as VEGF, bFGF, SDF-1 and IGF-1. These cytokines appear to protect cardiomyocytes under hypoxia as evidenced by enhanced expression of survival protein, Bcl₂, in cardiomyocytes. Our study clearly shows that hypoxia induced apoptosis was significantly abolished in cardiomyocytes co-cultured with BMSCs. Paracrine mediators released from BMSCs directly up-regulated Bcl-2 protein in cardiomyocytes. Homodimers of Bcl-2 may stabilize the mitochondrial membrane and prevent the activation of downstream apoptotic signaling. It is widely believed that activation of caspase 3 leads to nucleosomal fragmentation of DNA, a hallmark of apoptosis. The activation of caspase 3 can be regulated by the Bcl-2 protein family. It has been demonstrated that the death of cardiac fibroblasts during hypoxia was associated with loss of Bcl-2. Knockdown of Bcl-2 expression by siRNA in cardiac fibroblasts increased their apoptotic response to staurosporine, serum, and glucose deprivation and to simulated ischemia [24]. Our study indicates that BMSCs induced over-expression of Bcl-2 in myocytes, which increased their resistance to oxidative stress. The cytokines released from BMSCs result in the over-expression of Bcl-2 in cardiomyocytes. IGF-1 is one of the key ligand to regulate the Bcl-2 protein family in cardiomyocytes [25]. VEGF is a well known endothelial cell-specific angiogenic factor. Previous reports suggested VEGF induced expression of Bcl-2 which eventually functions to enhance the survival of endothelial cells in the toxic, oxygen-deficient environment [26]. This report points out that enhanced level of VEGF may have some role in the inhibition of cardiomyocyte and endothelial cell apoptosis. VEGF could activate the myocardial PI-3K pathway and decrease myocardial infarct size [27]. Basic fibroblast growth factor also plays an important role in cardioprotection against myocardial cell death and arrhythmias in acute myocardial infarction (AMI). Administration of bFGF prevented ischemia-induced myocardial apoptosis, cell death and arrhythmias as well as induced a greater expression of Bcl-2 [28,29]. SDF-1 exerts an antiapoptotic effect on CD34⁺ cells through an autocrine/paracrine regulatory loop, although no evidence for SDF-1 directly induce the expression of Bcl-2 [30].

SCF is another important hematopoietic growth factor for the growth and proliferation of primitive progenitor cells. Prior studies showed that SCF at high dose, 100 ~ 200 µg/kg, caused 10 ~ 100-fold increase of circulating progenitor cells in circulation and this effect remained for 7 to 14 days after termination of SCF administration [31,32]. Our study is in agreement with this conclusion that high dose of SCF mobilizes progenitor cells from their bone marrow niches to the peripheral blood. How these mobilized BMSCs play a role in cardiac repair remains contentious. Since the pioneering work of Orlic and colleagues showed that mobilized BMSCs homed into the injured myocardium and regenerated new cardiomyocytes [8], recent reports have shown inability of implanted BMSCs to cross lineage restriction to form cardiomyocytes [33,34]. Fukuhara and colleagues have shown that cytokine mobilized BMSCs contribute very little towards regeneration [35]. Our *in vivo* study tends to support the notion that immediate effect of mobilized BMSCs is mediated through secretion of cytoprotective

proteins to prevent cardiomyocyte apoptosis. Our study also gives evidence that SCF activated the expression of Bcl-2 in vivo. Furthermore, our in vitro results indicate that BMSCs also secrete VEGF and bFGF which are important angiogenic growth factors. Mobilization of bone marrow stem cells due to SCF treatment resulted in a higher vessel density (data not shown here). Therefore, improvement of cardiac perfusion due to angiogenesis may alleviate deleterious effects of ischemia.

Conclusion

The underlying mechanisms by which BMSCs ameliorate cardiac dysfunction involve cardiomyocytes survival and prevention of cardiomyocytes apoptosis by paracrine mediators.

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Figure 1. BMSCs secreted cytokines under *in vitro* conditions. A, A significant amount of VEGF, bFGF, SDF-1 and IGF-1 was released from BMSCs (n=8). MC = cardiomyocytes

Figure 2.

BMSCs enhanced cardiomyocyte survival under hypoxia. Panel A and B: Representative photographs show typical of Annexin-V positive cells. Red fluorescence shows apoptotic cells stained with PE-labeled annexin-V. Nuclei were counterstained with DAPI (blue). A: cardiomyocytes; B: co-culture. Panel C and D: Representative FACS of count Annexin-V-PE positive cells in cardiomyocytes (C) and co-culture (D), respectively. Panel E: quantitation of annexin-V positive cells. Data are shown as mean $\pm$ SEM. * $p < 0.05$ vs cardiomyocytes culture alone. Panel F: DNA fragmentation in cardiomyocytes alone or co-cultured with BMSCs following hypoxia [lane 1: DNA marker; lane 2: cardiomyocyte (normoxia); lane 3: co-culture

(normoxia); lane 4: cardiomyocytes (hypoxia); lane 5: co-culture (hypoxia); lane 6: positive control].

Figure 3. Representative western blotting of Bcl-2 protein in dual-set system cultured cardiomyocytes exposed to hypoxia for 24 hours (panel A). Panel B: Bcl-2 was quantified by western blots in cultured cardiomyocytes. Data are shown as mean $\pm$ SEM. * $p < 0.01$ vs cardiomyocytes culture alone.

Figure 4. Apoptosis of cardiomyocytes after 1 week of MI. A-B, Representative photomicrographs showing TUNEL staining in peri-infarct area. Arrow indicates TUNEL positive cardiomyocytes (A: saline control mice; B: SCF treated mice) ($\times 400$). Asterisks show infarct area. C, Percentage of TUNEL positive cardiomyocytes in both groups ($n = 4$). * $p < 0.05$ vs saline control.

Figure 5.

Representative western blotting of Bcl-2 protein within peri-infarct myocardium 1 week after MI (panel A). Panel B: Bcl-2 was quantified by western blots (n = 4) in peri-infarcted myocardium. Data are shown as mean $\pm$ SEM. * $p < 0.01$ vs saline control.

Figure 6.

LV function assessed by echocardiography. Shown are representative LV M-mode echocardiographic recordings in both SCF treated and saline treated mice at 4 weeks after MI. IVS, interventricular septum; LVPW, left ventricular posterior wall; LVID, left ventricular internal dimensions.

Figure 7.

Representative LV Pressure-Volume Relationships obtained 4 weeks after LAD occlusion. Slope of the regression line (end-systolic pressure volume relationship, red color) represents ventricular end-systolic elastance (Ees). Ees of SCF-treated mice (B) was 2-fold greater than control group (A). Besides, Ves and Ved of SCF treated mice were obviously less shifted rightward which supports the observation that LV contractility of SCF-treated group was significantly improved. In SCF treated mice, LV systolic (Pmax, E-max) and diastolic functions (Pmin, tau-W) were significantly better than the control group (C). Data are mean $\pm$ SEM, n = 6 in each group, * p < 0.05 vs. control. Pmax indicates maximum pressure; Pmin, minimum pressure; Emax, maximal elastance; Tau-W, Tau-Weiss method.

Figure 8.

Incorporation of green BMSCs into cardiomyocytes and smooth muscle cells. Representative fluorescence micrographs of peri-infarct area at 4 weeks after MI. A-B, GFP-positive cells (arrow) is shown as a desmin-positive cardiomyocytes (red). Yellow in (B) indicates cardiomyocytes derived from GFP-positive cells ($\times 1000$). C-D, GFP-positive cells expressed SMA (arrow). Yellow labeling indicates vascular smooth muscle cells derived from GFP-positive cells ($\times 400$). E. Percentage of GFP-positive cardiomyocytes. Total 15 sections were examined in both groups ($n = 6$). Data are shown as mean $\pm$ SEM. * $p < 0.05$. Bar scale, 20 μ m.

Table 1

Echo parameters after 4 week of MI

	No cytokine (n=6)	CSF (n=6)	p
IVSTd (mm)	0.41 ± 0.07	0.42 ± 0.08	0.89
LVPWTd (mm)	0.89 ± 0.10	0.87 ± 0.05	0.66
LVIDd (mm)	5.87 ± 0.34	5.43 ± 0.28	<0.05
LVIDs (mm)	5.30 ± 0.35	4.78 ± 0.26	<0.01
% FS	9.7 ± 2.5	11.9 ± 2.5	0.12
EF (%)	26.2 ± 6.1	31.6 ± 5.8	0.12

IVSTd, interventricular septum thickness; LVPWTd, left ventricular posterior wall thickness; LVIDd & LVIDs, left ventricular internal dimensions at end diastole and end systole; FS, fractional shortening; EF, ejection fraction