ELSEVIER

Monocyte chemotactic protein 1 increases homing of mesenchymal stem cell to injured myocardium and neovascularization following myocardial infarction

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Abstract

Objective: To investigate the effect of MCP-1 on mesenchymal stem cells(MSCs) homing to injured myocardium in a rat myocardial infarction(MI) model. **Methods:**Rat myocardial infarction model was established by permanent left anterior descending branch ligation. Mesenchymal stem cells from donor rats were cultured in IMDM and labeled with BrdU. The Rats were divided into two groups. Monocyte chemotactic protein 1(MCP-1) expression were measured by in situ hybridization and immunohistochemistry in the sham operated or infarcted hearts at 1, 2, 4, 7, 14 and 28 days post operation in MCP-1 detection group. The rats were injected with MCP-1, anti-MCP-1 antibody or saline 4 days after myocardial infarction in intervention group. Then, a total of 5×10^6 cells in 2.5 ml of PBS were injected through the tail vein. The number of the labeled MSCs in the infarcted hearts was counted 3 days post injection. Cardiac function and blood vessel density were assessed 28 days post injection. **Results:**Self-generating MCP-1 expression was increased at the first day, peaked at the 7th day and decreased thereafter post MI and remained unchanged in sham operated hearts. The MSCs enrichment in the host hearts were more abundant in the MI groups than that in the non-MI group($P = 0.000$), the MSCs enrichment in the host hearts were more abundant in the MCP-1 injected group than that in the anti-MCP-1 antibody and saline injected groups ($P = 0.000$). Cardiac function was improved more in MCP-1 injected group than anti-MCP-1 antibody and saline injected groups($P = 0.000$). Neovascularization in MCP-1 injected group significantly increased compared with that of other groups($P = 0.000$). **Conclusion:** Myocardial MCP-1 expression was increased only in the early phase post MI. MCP-1 may enhance MSCs homing to the injured heart and improve cardiac function by promoting neovascularization.

Keywords: mesenchymal stem cells; homing; myocardial infarction; cardiac function; monocyte chemotactic protein 1

INTRODUCTION

Heart failure is a major health problem, and its mobidity is still increasing. Its most common etiologic origin is related to ischemic cardiomyopathy due to previous myocardial infarction(MI). At the infarct site, a transmural MI involves the loss of necrotic cardiomyocytes and extracelluar matrix, coronary vasculature and nerves. Subsequent tissue repair leads to scar formation, restoring structural integrity to the infarcted heart, but ventricular function may be compromised. The potential for mesenchymal stem cells(MSCs) to rebuild heart function has been suggested. Lu et al[1] found that MCP-1 increased in myocardial infarct site in the early stage after MI. Wang et al[2] reported that CCR2(CC-chemokine receptor 2)-receptor of monocyte chemotactic protein 1 (MCP-1) is expressed on the surface of MSCs, and MCP-1 might induce the homing of MSCs by conjugating to CCR2. So we hypothesized that MCP-1 may play an important role in the repairing process after MI.

Using an experimental rat MI model, created by permanent left anterior descending branch ligation, we determined the temporal and spatial expression of MCP-

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1 at the infarct site from the 1st day to 28th day post MI. And we investigated the effect of MCP-1 on MSCs homing to myocardial infarction site by using an MCP-1 and anti-MCP-1 antibody.

MATERIALS AND METHODS

Animal and material

Adult male Fischer344 rats(240 ± 20 g) were purchased from Shanghai Animal Administration Center. All animal experimental protocols were approved by the Animal Care and Use Committee of Nanjing Medical University, and were in compliance with "Guidelines for the Care and Use of Laboratory Animals", as published by the National Academy Press (NIH Publication No. 85-3, revised 1996).

Isolation and expansion of MSCs

MSCs were isolated and harvested as previously described [3, 4]. Shortly, bone marrow was collected from the femur and tibia of the rats. Bone-marrow mononuclear cells were isolated through Ficoll Hypaque gradient centrifugation and resuspended in the IMDM (Hyclone, Logan, UT, USA) with 10% fetal bovine serum(Gbico, USA), 2 mmol/L L-glutamine, penicillin (100 U/ml) and streptomycin (100 μ g/ml). Cells were incubated in 95% air and 5% CO_2 at 37℃ for 24 hours, and the adherent cells were washed twice consecutively in phosphate-buffered saline(PBS). The cultures were depleted of erythroid progenitor cells through the removal of cells that did not adhere to the culture dish at medium change. At 80% confluence, cells were harvested with 0.25% trypsin and passaged at a ratio of 1: 2. The medium was changed 3~4 days, by which almost all the hematopoietic stem cells were washed away. MSCs were harvested after being cultured for 21~28 days.

Cell labeling

MSCs were stained in 3 μmol/L bromodeoxyuridine (BrdU, Sigma-Aldrich, St. Louis, MO, USA) for 24 hours. Cells were resuspended in PBS at a density of 2×10^6 cells/ml and then kept on ice for transplantation.

Myocardial infarction model, cell transplantation and study protocol

Left ventricular anterior transmural MI was created by permanent ligation of the left coronary artery with silk ligature[5]. Rats were anesthetized with ketamine (75~100 mg/kg), intubated and ventilated with a rodent respirator. After left thoracotomy and pericardiotomy, the heart was rapidly exteriorized and 6-0 silk suture was placed around the left anterior descending coronary artery. The vessel was ligated and the heart was returned to the chest. The chest was closed and lungs were reinflated using positive end-expiratory pressure. Coronary artery occlusion with MI was confirmed microscopically by evidence of myocyte necrosis and grossly visible scarring of the anterior left ventricular free wall. Sham operation rats received only chest open surgery.

Two groups of rats were studied:① MCP-1 detection group($n = 65$). MI rats were sacrificed at 1, 2, 4, 7, 14 and 28 days post-infarction. Non-MI control rats were sacrificed at pre-infarction, 1, 2, 4, 7, 14 and 28 days after operation. Hearts were removed, rinsed in 10% formalin for MCP-1 mRNA and protein detection. ② Intervention group($n = 64$). Rats were divided into four subgroups(16 rats in each subgroup):MCP-1(20 ng in 50 μl PBS injected into peri-infarcted zone evenly) + MI group, anti-MCP-1 antibody(2 μg in 1ml PBS injected through tail vein) + MI group, Saline(50 μl injected into peri-infarcted zone evenly) + MI group, Saline(50 μl injected into anterior left ventricular free wall evenly) group. In each subgroup, MSCs 5×10^6 in 2.5 ml PBS were injected through tail vein one hour later. Three days post-transplantation, 8 animals were sacrificed for the cell count of MSCs homing into the host hearts. The remaining 8 rats were studied 28 days post-transplantation by using echocardiography and analyzing blood vessel density.

In situ hybridization.

The localization and optical density of MCP-1 mRNA was detected by semi-quantitative in situ hybridization. In situ hybridization analysis was performed on a series of left ventricular tissue paraffin sections. The sections were hybridized with MCP-1 mRNA probe(Haoyang Biological, Tianjin, China). The probes' sequences were as follows:1; 5' -TGA GTA GCA GCA GGT GAG TGG GGC ATT A-3'; 5' -CTG CTG CTG G TG ATT CTC TTG TAG TTC TC-3'

Immunohistochemistry

Immunohistochemical analysis was performed on a series of left ventricular tissue paraffin sections. The stained sections were digitally imaged using a computerized image-analysis system. In order to examine the MCP-1 protein surrounding the infarction zone, transversal left ventricular sections were stained primarily with American Hamster anti-MCP-1 monoclonal antibody(eBioscience, San Diego, CA, USA), secondarily with peroxidase-conjugated goat anti-Hamster IgG (Jackson Immuno Research Laboratories, Inc., Pennsylvania, CA, USA). For the homing assessment of transplanted MSCs in the infracted myocardium, transversal left ventricular sections were stained with sheep anti-bromodeoxyuridine monoclonal antibody (USBiological, Swampscott, MA, USA), secondarily

with HRP-conjugated donkey anti-sheep IgG(Bethyl Laboratories, Inc., Montgomery, TX, USA). The number of BrdU^{+} cells was evaluated by counting ten selected high-power(200 ×) fields randomly. For the blood vessel density, transversal left ventricular sections were stained with rabbit anti-factor Ⅷ polyclonal antibody (Zymed Laboratories, South San Francisco, CA, USA), secondarily with peroxidase-conjugated goat antirabbit IgG(Jackson Immuno Research Laboratories, Inc., Pennsylvania, CA, USA). Appropriate immunohistological controls were performed to as-sess specificity, including exclusion of primary antibody and use of sheep, goat and rabbit sera isotype in place of the antibodies.

Echocardiography

Echocardiography was performed using a 11.5-MHz phased-array transducer(HP-SONOS 5500, Hewlett-Packard, MA, USA) at pre-infarction, pre-transplantation and 28 days post-transplantation in each group; fractional shortening(FS) and ejection fraction(EF) were measured as previously described[6]. All measurements were averaged for three consecutive cardiac cycles and were carried out by three experienced technicians who were unaware of the identities of the respective experimental groups.

Statistical analysis

Data (mean ± SD) was analyzed by a two-way repeated measures ANOVA with the SPSS13.0 statistical program. The P value < 0.05 was considered statistically significant.

RESULTS

MCP-1 expression in the infarcted heart

Fig 1 and *2* show the quantitative analyses of MCP-1 mRNA and protein expressions in MI and sham operation groups. MCP-1 expression were significant higher within 14 days post operation in the MI group than in the SHAM group and peaked at the 7th day post MI. Both the mRNA and protein expressions of MCP-1 decreased continuously thereafter and MCP-1 mRNA and protein expressions in the SHAM group were similar at various time points.

Myocardial homing of transplanted MSCs

Examined at the third day post transplantation, MSCs homed to the infarcted hearts significantly more in the MI groups than those in the non-MI group, MSCs homed
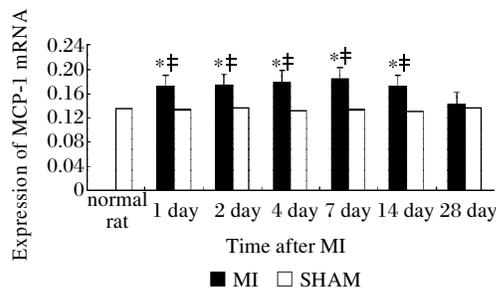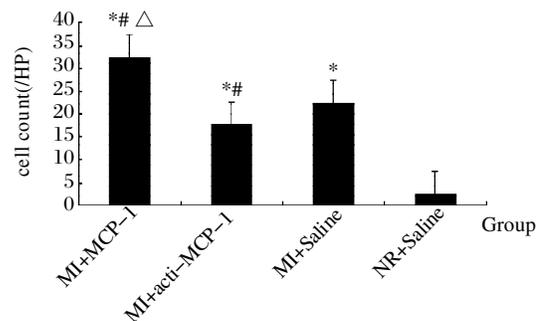

compared with normal rat, *$P = 0.000$; compared with SHAM,‡$P < 0.01$

**Fig 1** Time course of myocardial MCP-1 mRNA expression post myocardial infarction or sham operation.
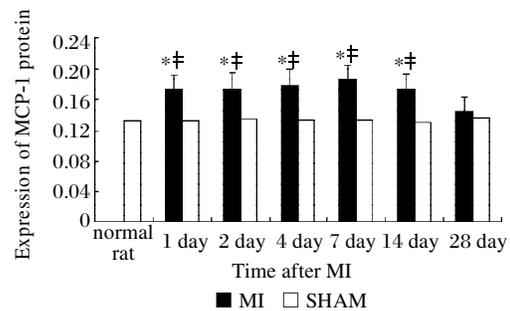
compared with normal rat, *$P = 0.000$; compared with SHAM,‡$P < 0.01$

Fig 2 Time course of myocardial MCP-1 protein expressions post myocardial infarction or sham operation.

to the infarcted hearts were significantly more in the MCP-1 injected group than those in the anti-MCP-1 antibody and saline injected groups (*Fig 3*). Representative immunohistochemical micrographs of transversal left ventricular sections around the infarction zone 3 days post-transplantation stained with anti-BrdU antibody were shown in *Fig 4A*(MCP-1 + MI), *Fig 4B*(anti-MCP-1 antibody+MI),*Fig 4C*(Saline+MI) and *Fig 4D* (Saline+normal rat), respectively. To exclude the nonspecificity BrdU labeling, sections were stained with mouse sera isotype in place of the BrdU antibody, and the BrdU-positive cells were not observed in these sections (data not shown).

compared with NR+Saline, *$P = 0.000$; compared with MI+Saline, #$P = 0.000$; compared with MI+anti-MCP-1, △$P = 0.000$; (NR, normal rat; HP, 200 ×).

Fig 3 The number of MSCs homed to host hearts 3 days post-transplantation in various groups.
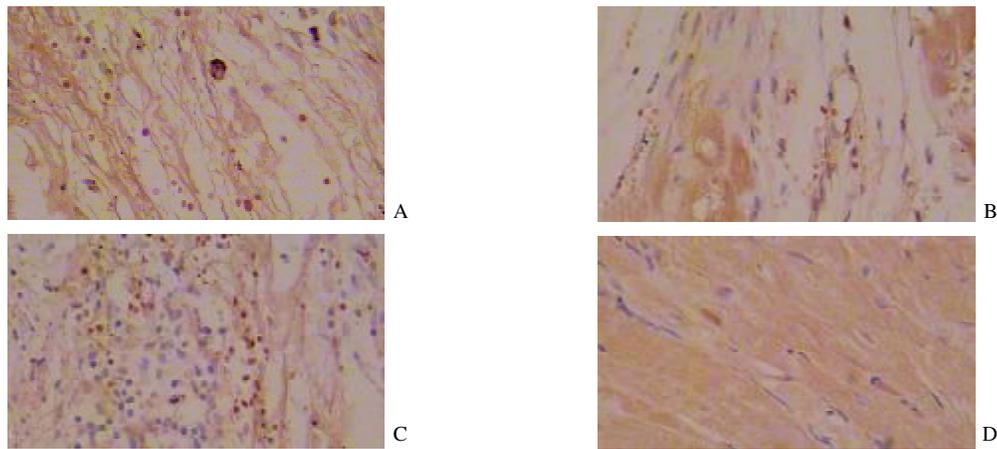
A:MI+MCP-1, B:MI+anti-MCP-1, C:MI+saline, D: NR+saline. The nuclei of transplanted MSCs prelabeled with BrdU were stained as brown.

Fig 4 The left ventricular sections around the infarct border zone stained with anti-BrdU antibody 3 days post-transplantation various groups(Immunohistochemistry, × 200).

Blood vessel density

Examined at the 28th day post transplantation, blood vessel density was significant higher in the MI groups than in the non-MI group, and higher in MCP-1 injected group than in anti-MCP-1 antibody and saline injected group(***Fig 5***).
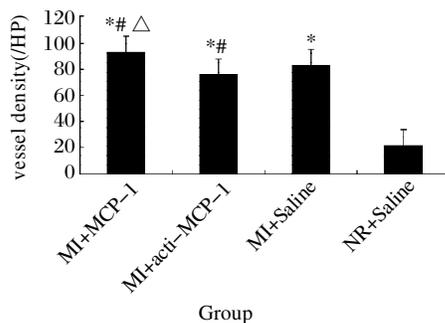
compared with NR+Saline, *$P= 0.000$; compared with MI+Saline, #$P= 0.000$; compared with MI+anti-SDF-1, △$P= 0.000$; (NR, normal rat; HP, 200×).

Fig 5 Quantitative analyses of vessel density by staining with factor Ⅷ.

Cardiac function

Tab 1 shows the changes of EF and FS post infarction and transplantation in various groups. EF and FS were reduced post infarction in all groups and not affected by Saline. 28 days after transplantation, EF and FS were improved in MCP-1+MI injected group, anti-MCP-1 antibody+MI injected group and saline+MI injected group while there was no change in the saline group. Heart rate and body weight were not different among the groups(data not shown).

DISCUSSION

The major findings of this study are as follows: ①self-generating MCP-1 expression were significantly higher within 14 days post operation in the MI groups than in the SHAM groups and peaked at 7th day post MI. ②There were more MSCs homed to injured hearts examined 3 days post transplantation in the MI groups, especially in the MCP-1 injected group. ③ There was more new-formed blood vessels in the MI groups, especially in the MCP-1 injected group examined 28 days post transplantation. ④ Similarly, an increase of car-

Tab 1 Cardiac function(EF and FS) assessments

	Group			
	MI+MCP-1	MI+anti-MCP-1	MI+Saline	NR+Saline
EF(%)				
baseline	95.30 ± 2.65	95.39 ± 2.53	95.53 ± 2.21	95.26 ± 2.01
Pre-trans	43.99 ± 2.05*△	44.08 ± 2.34*△	44.05 ± 2.13*△	95.20 ± 2.09
28days	72.94 ± 3.45*#△	50.71 ± 3.41*#△□	53.16 ± 3.20*#△□	95.23 ± 2.16
FS(%)				
baseline	65.34 ± 2.05	65.41 ± 1.99	65.64 ± 1.93	65.55 ± 2.14
Pre-trans	15.81 ± 2.21*△	15.74 ± 2.15*△	15.84 ± 2.07*△	65.46 ± 2.15
28days	34.65 ± 3.6*#△	21.34 ± 2.83*#△□	23.25 ± 2.77*#△□	65.45 ± 2.42

compared with baseline, *$P= 0.000$; compared with pre-trans, #$P= 0.000$; compared with NR+Saline, △$P= 0.000$; compared with MI+MCP-1, □$P= 0.000$

EF: left ventricular ejection fraction, FS: shortening fraction, Pre-trans: pre-transplantation.

diac function improvement were found in all the MI groups, most significant in the MCP-1 injected group examined 28 days post transplantation.

Our results found that the myocardial MCP-1 expression increased in the 1st day, peaked at 7th day (post MI) and decreased thereafter. Lu et al[1] also found the MCP-1expression increased at the 3rd day, peaked at 7th day post MI. These findings indicate there is a time course of MCP-1 expression. If we intend to assist the MI using MCP-1, the therapeutic strategy should be taken timely, unless the expression was reestablished.

In the present study, we found intravenously transplanted MSCs successfully homed to infarcted hearts. In line with our observation, most MSCs were found homed to acute injured brain tissue after intravenous administration[7]. Similarly, Wang et al[2] discovered that MCP-1 could increase MSCs migration *in vitro*. Although Freyman and co-workers[8] found that MSCs infused through venous could not home to the myocardial infarct site, we could consider that MSCs infused through venous injection could home to the myocardial infarct site and achieve a therapeutical effect. Of course, the effect of different cell delivery methods maybe different, these need further investigate. The best outcome of cell transplantation is the transplanted cells homing to the myocardial infarct area, but we saw that transplanted MSCs mainly homed to peri-infarct zone. Aicher et al[9] also found that transplanted endothelial progenitor cells home to the peri-infarct zone. Thereby suggesting that there is some disadvantages with intravenous instillation and other effective methods should be adopted.

Compared with other groups, the highest increase of homed MSCs, blood vessel density and cardiac function was seen in MCP-1 injected group. This suggests that exogenous MCP-1 could induce homing of MSCs and improve cardiac function. Compared with MCP-1 antibody injected group, the increase of homed MSCs, blood vessel density and cardiac function was seen in the saline injected group. This suggests that endogenous MCP-1 could enhance homing of MSCs. All of which indicates that there may be other chemokines that have the same chemotactic effect of MCP-1. Ma [10] and Askari [11] found that another chemokine stromal cell derived factor 1(SDF-1) also induced homing of MSCs. Askari et al[11] even found that expression of SDF-1 downregulated to normal level 56 days post MI, and reestablished the expression of SDF-1 inducing the homing of CD117+ stem cells into injured myocardium. Similarly, we found the expression of MCP-1 also downregulated to normal level 28 days after MI, and MCP-1 injected into myocardial infarcted zone could induce homing of MSCs. It seemed that MSCs could be used to repair the infracted heart through establishing the expression of chemokines, including MCP-1.

Myocardial MCP-1 expression in non-MI rats was low, and remained unchanged at various time points after operation. Accordingly, MSCs homing account was also very low. We still saw few MSCs homed to the heart in non-MI group, it may be elucidated that the hearts were injured by saline injection, this caused subsequently inflammation that induced the MSCs transplantation.

The results of the study show that more blood vessels are counted when more MSCs are homed to the hearts, which indicates that MSCs may promote neovascularization. New formed vessels may enhance blood flow in and around the infarcted site, which could improve hibernate and stunned myocardium, reduce cell apoptosis and assist cardiac function. The present study showed that increase of cardiac function in MCP-1 injected group is higher than that in other groups.

Wang et al[2] reported that 44.6% MSCs express CCR2-receptor of MCP-1, homing of MSCs is induced by the conjugating of MCP-1 and CCR2. These results suggested MCP-1 and CCR2 could be two elements for MSCs homing. It may be verified by using anti-CCR2 antibody in the future.

In contrast, Hayashidani et al[12] found that inhibiting MCP-1 signaling pathway can attenuate heart failure after MI. The reason may be as follows:In the early stage after MI, inflammatory response induced by MCP-1/ CCR2 is acute, which may interfere the MSCs' chemotactic potency. And that MSCs mobilized autologously after MI, showed fewer than those injected in the present study, as may also contribute to the disrepair of the infarcted heart. This indicates that MSCs transplantation combining with inflammatory modulation therapy may be a feasible way to treat MI.

In conclusion, myocardial expression of MCP-1 in the host infarcted hearts increased early post MI. MCP-1 may enhance MSCs homing to the injured heart and improved cardiac function by promoting neovascularization.

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