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Original Article

Intravenous administration of atorvastatin-pretreated mesenchymal stem cells improves cardiac performance after acute myocardial infarction: role of CXCR4

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Abstract: Background: The interaction between stromal cell-derived factor 1 (SDF-1) and its receptor CXC chemokine receptor 4 (CXCR4) plays an important role in mesenchymal stem cells (MSCs) migration and engraftment. Statins can increase the survival of MSCs. However, whether statins could enhance MSCs migration and engraftment is still unknown. Therefore, we designed the study to investigate whether atorvastatin (ATV) could enhance CXCR4 expression of MSCs and promote them homing toward the injured myocardium. Methods and results: Expression of CXCR4 was evaluated by flow cytometry and real time PCR. A transwell system was used to assess MSCs migration ability. Recruitment of systematically delivered MSCs to the infarcted heart was evaluated in Sprague-Dawley rats with acute myocardial infarction (AMI). ATV pretreatment enhanced the expression of CXCR4 and stimulated MSCs migration in vitro. However, the effect was largely abolished by CXCR4 neutralizing antibody. In AMI models, we found much more ATV-pretreated MSCs homing toward the infarcted myocardium than non-treated cells and this was accompanied by improved cardiac performance. Conclusions: ATV increases the migration ability of MSCs and improves cardiac performance due to up-regulated expression of CXCR4. These results suggest that ATV pretreatment of donor MSCs is an effective way to promote cell therapeutic potential for AMI.

Keywords: Acute myocardial infarction, cell migration, CXCR4, mesenchymal stem cells, atorvastatin

Introduction

Bone marrow-derived mesenchymal stem cells (MSCs) represent an ideal candidate for cell therapy because they are easily obtained, possess multilineage potential and immunologic advantage [1, 2]. Moreover, transplantation of cultured MSCs into the infarcted heart has emerged as a promising therapy for myocardial repair [3, 4]. However, poor engraftment of donor MSCs limits reparative capability of the therapy.

Recruitment of more MSCs to the ischemic myocardium is an effective strategy to increase the number of the engrafted cells. Mobilization and migration of MSCs are mainly controlled by stromal cell-derived factor 1 (SDF-1) and its receptor CXC chemokine receptor 4 (CXCR4) [5, 6]. SDF-1 is up-regulated at sites of injury, and the interaction between SDF-1 and CXCR4 triggers MSCs migration toward the injured area [7-9]. Moreover, only cell-surface CXCR4 can respond to an outside SDF-1 gradient. Unfortunately, MSCs progressively down-regulate CXCR4 expression after ex vivo expansion and lose their ability to migrate toward SDF-1 [10, 11]. Although only a small number of MSCs express CXCR4 on the cell surface, most cells have a high intracellular storage [10]. Therefore, strategies to mobilize the internalized receptor and increase CXCR4 expression are critical to improve the engraftment of MSCs.

Previous studies from our group and others have demonstrated that the combined therapy with MSCs and statins, a kind of popular prescription drugs for patients with coronary heart

disease, significantly improved cardiac function after acute myocardial infarction (AMI), and the mechanism was that statins improved the harsh infarcted microenvironment and decreased apoptosis of transplanted MSCs [12-17]. So, statins are an effective method to increase MSCs survival. However, whether statins could enhance MSCs migration and engraftment is still unknown.

Therefore, we designed the study to investigate: (1) whether atorvastatin (ATV) enhanced the cell surface expression of CXCR4 in MSCs in vitro; (2) whether ATV-pretreated MSCs could home to ischemic heart tissue and further improve cardiac performance after AMI in vivo.

Materials and methods

Animals

Sprague-Dawley rats were used in the study. MSCs were obtained from male Sprague-Dawley rats (60-80 g), and AMI models were created in female Sprague-Dawley rats (200-220 g). All animals received humane care and the experimental protocol was approved by the Care of Experimental Animals Committee of Fuwai Hospital.

Cell culture

Briefly, bone marrow was harvested from the tibia and femur of Sprague-Dawley rats and seeded into cell culture flasks with Iscove's Modified Dulbecco's Medium (IMDM, Gibco, USA) containing 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA) in an incubator set at 37°C under a 5% CO_2 environment. When the cells reached 70-80% confluence, they were detached using 0.25% trypsin-EDTA (Gibco, USA) and subcultured at the ratio of 1:2. All cells used in the experiment were passage 3.

Cell treatment

To select the optimal concentration of ATV treatment, MSCs were pretreated with different concentrations of ATV (Sigma, USA) (control, 0.01 μM, 0.1 μM, 1 μM, and 10 μM) for 6 h. To determine the optimal ATV treatment time, a series of lengths of time (control, 1 h, 3 h, 6 h, 12 h, 24 h, 36 h, 48 h) were detected. All cells were under 37°C and 5% CO_2 environment.

Flow cytometric analysis

To analyze CXCR4 expression at the cell surface of MSCs, the cells were stained with rabbit polyclonal anti-rat CXCR4 antibody (Alomone labs, Israel). Briefly, MSCs were removed from the plate and washed with phosphate-buffered saline (PBS) twice, then stained with 1 mg/mL CXCR4 for 20 min at room temperature. Finally, the cells were collected and resuspended in 300 μL PBS. Isotype controls were given to enable correct compensation and confirm antibody specificity. Stained cells were analyzed using a FACSCalibur flow cytometer and Cell Quest software (BD, USA).

Real time PCR

Real time PCR was performed to analyze the expression of CXCR4 mRNA. Total RNA was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. cDNA was produced from the total RNA using TIANScript RT Kit (Qiagen, German). Real time PCR was performed with SYBR FAST qPCR Kit Master Mix (2×) Universal (KAPA, USA). β-actin was chosen as housekeeping gene for normalization. Reactions were as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 3 s, and 63°C for 20 s. Primers were as follows:

CXCR4-F, 5'-CTCTGAGGCGTTTGGTGCTC; CXCR4-R, 5'-CGGAAGCAGGGTTCCTTGTT; β-actin-F, 5'-GCACCATGAAGATCAAGATCATT; and β-actin-R, 5'-TAACAGTCCGCCTAGAAGCATT.

Transwell migration assay

Migration assays were performed in a 24-well polycarbonate transwell with 8 μm pores (Corning Costar, USA). MSCs were pretreated with or without 1 μM ATV for 12 h under 37°C and 5% CO_2 environment. For inhibitor studies, MSCs treated with or without ATV were then incubated for 1 h with 0.5 mg/mL anti-CXCR4 neutralizing antibody (Biovision, USA). MSCs were detached from the culture dish and adjusted to a concentration of 1×10^6 cells/mL in IMEM medium. 100 μL of MSCs were placed in the upper chamber, and the lower chamber contained 600 μL medium with 50 ng/mL SDF-1 (PeproTech, USA). The chambers were incubated at 37°C and 5% CO_2 for 6 h, then the upper surface of the membrane was scraped gently to remove non-migrated cells and

washed with PBS. The membrane was then fixed in 4% paraformaldehyde for 15 min and stained in 0.5% crystal violet for 20 min. The number of migrated cells was determined by counting 5 random fields per well under the microscope at ×200 (Leica, Germany). Experiments were performed four times for each group [18].

AMI model and cell transplantation

AMI models in female Sprague-Dawley rats were established as described previously [14, 19]. Briefly, the left anterior descending coronary artery (LAD) was permanently ligated with a 6-0 polyester suture 1-2 mm from the tip of the left atrial appendage. Successful ligation of the LAD was verified by myocardial blanching and abnormal movement of the anterior wall. Sham operations were performed by passing a suture around the LAD without ligation. Groups were as follows: Sham operation (Sham); AMI + PBS (PBS); AMI + MSCs (MSCs); AMI + ATV-pretreated-MSCs (ATV-MSCs).

Prior to transplantation, MSCs were labeled with CellTracker CM-DiI (Molecular Probe, invitrogen, USA). 24 h after AMI, 2.0×10^6 CM-DiI-labeled MSCs, CM-DiI-labeled ATV-MSCs, or PBS were injected in a total volume of 500 μL through the tail vein.

MSCs homing and survival

3 days after cell infusion, rats from the MSCs (n = 4) and ATV-MSCs (n = 4) groups were sacrificed and hearts were harvested to examine MSCs homing. In addition, rats from the PBS group (n = 2) were sacrificed and worked as the negative control. To explore the 30 days survival of systematically infused cells in infarcted myocardium, rats from the MSCs (n = 4) and ATV-MSCs (n = 4) groups were sacrificed and hearts were harvested. Hearts were embedded in Tissue-Tek OCT compound (Sakura) and cut into 4 μm frozen sections at the mid-LV level. The sections were analyzed by a laser scanning confocal microscope FV1000 (Olympus). The number of labeled MSCs was quantified in the infarct zone by an independent blinded researcher in 10 randomly chosen high-power fields (×600) in 2 sections from per animal.

Echocardiographic assessment

Cardiac function and ventricular dimensions were measured by transthoracic echocardiography (Sonos 7500; Phillips; equipped with a 12-MHz phased-array transducer) 3 days after cell transplantation (baseline) and 30 days after cell transplantation (endpoint) as previously described [14]. The hearts (n = 5 per group) were recorded in 2D and M-mode from the parasternal long-axis view at the papillary muscle level. Left ventricular end-diastolic dimension (LVEDd) and end-systolic dimension (LVESd) were measured for at least 3 consecutive cardiac cycles. Left ventricular fractional shortening (LVFS) was calculated as [(LVEDd-LVESd)/LVEDd] × 100% and left ventricular ejection fraction (LVEF) were determined as $[(LVEDd)^3-(LVESd)^3]/(LVEDd)^3] \times 100\%$. All measurements were made by an independent blinded sonographer.

Histological analysis

All rats were sacrificed after endpoint echocardiography measurements being recorded, and then the hearts were removed and fixed in 10% formalin. The hearts were cut into 4 μm paraffin sections at the the mid-LV level, and Masson's Trichrome and Hematoxylin-Eosin (HE) stains were performed. Masson's trichrome stain was used to quantify the extent of fibrosis and infarct size in the left ventricular (LV). The lengths of the infarcted surfaces and the total LV circumferences, involving both epicardial and endocardial regions, were measured using Image-Pro Plus 6.0 software (Media Cybernetics Inc., USA). The percentage of the fibrotic area was calculated as (fibrotic length/LV circumference) × 100%. HE stain was used to evaluate the degree of inflammatory cells infiltration. At least 3 sections from each heart were stained.

Western blot analysis

The expression of inflammatory cytokines IL-6 and TNF-α were detected by western blot 3 days after cell transplantation and 30 days after cell transplantation. Tissues were extracted from infarcted and peri-infarcted regions of myocardium. The concentrations of proteins were determined by BCA protein assay. After electrophoresis, transmembrane and blocking, the membranes were incubated overnight at 4°C with primary antibodies. The primary antibodies used were as follows: polyclonal goat anti-IL6 (Santa Cruz, USA, 1:300), polyclonal goat anti-TNF-α (Santa Cruz, USA, 1:300). Next day, the membranes were incubated with peroxidase-conjugated secondary antibodies.

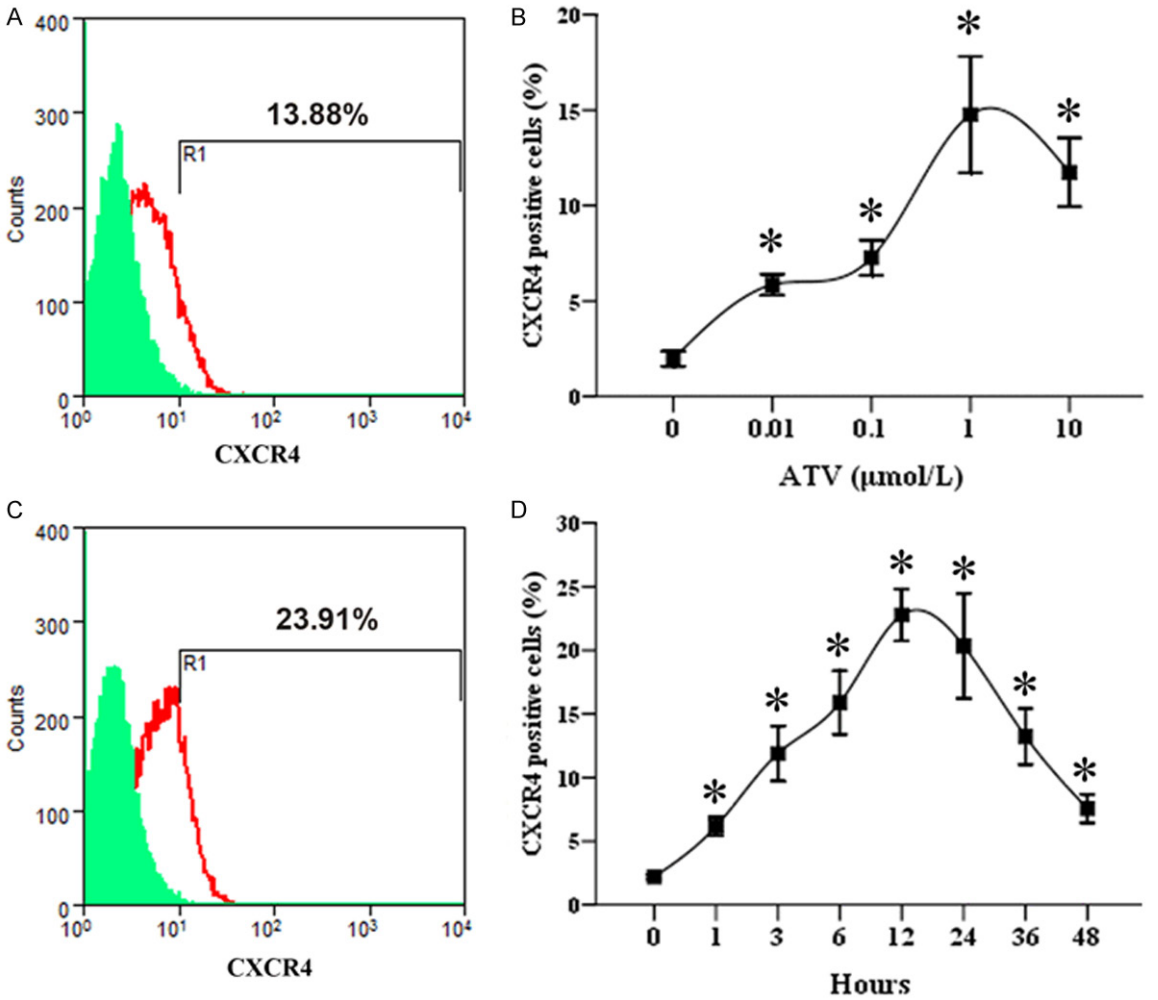

Figure 1. ATV enhanced MSCs surface expression of CXCR4 assessed by flow cytometry. (A) Representative histogram of MSCs treated by 1 μM ATV for 6 h. Green: Isotype control; Red: CXCR4 staining of ATV-treated cells. (B) Dose- dependent effect of ATV on CXCR4 expression harvested 6 h after treatment. Data are mean ± SD; $^{*}P < 0.05$ vs. the normal group (n = 4). (C) Representative histogram of MSCs treated by 1 μM ATV for 12 h. Green: Isotype control; Red: CXCR4 staining of ATV-treated cells. (D) Kinetic expression of CXCR4 on MSCs treated by 1 μM ATV. Data are mean ± SD; $^{*}P < 0.05$ vs. the normal group (n = 4). ATV, atorvastatin; CXCR4, CXC chemokine receptor 4; MSCs, mesenchymal stem cells.

Finally, bands were visualized by enhanced Chemiluminescence Detection Kit (Pierce). Target signals were normalized to the β-actin (1:2000, Protein Tech) signal and analyzed semiquantitatively with Quantity One system.

Statistical analysis

Statistical analysis was performed with SPSS 16.0 software. Data was expressed as mean ± SD. Comparisons between two groups were evaluated using Student's t-test. Comparisons among three or more groups were performed with one-way ANOVA. Two-sided *P* value less than 0.05 was considered statistically significant.

Results

ATV pretreatment increased CXCR4 expression in MSCs

Cell surface expression of CXCR4 assessed by flow cytometry showed that ATV enhanced CXCR4 expression in a dose-dependent manner, especially in the 1 μM ATV group (**Figure 1A**, **1B**). Then the time-course experiments at 1 μM ATV concentration revealed that, compared with the control group, CXCR4 expression was significantly increased with ATV treatment (1 to 48 h), peaking at 12 h (22.77 ± 2.03% vs. 2.20 ± 0.18%, $P < 0.001$) and maintaining at a high level within 24 h (20.34 ± 4.13 vs. 2.20 ± 0.18,

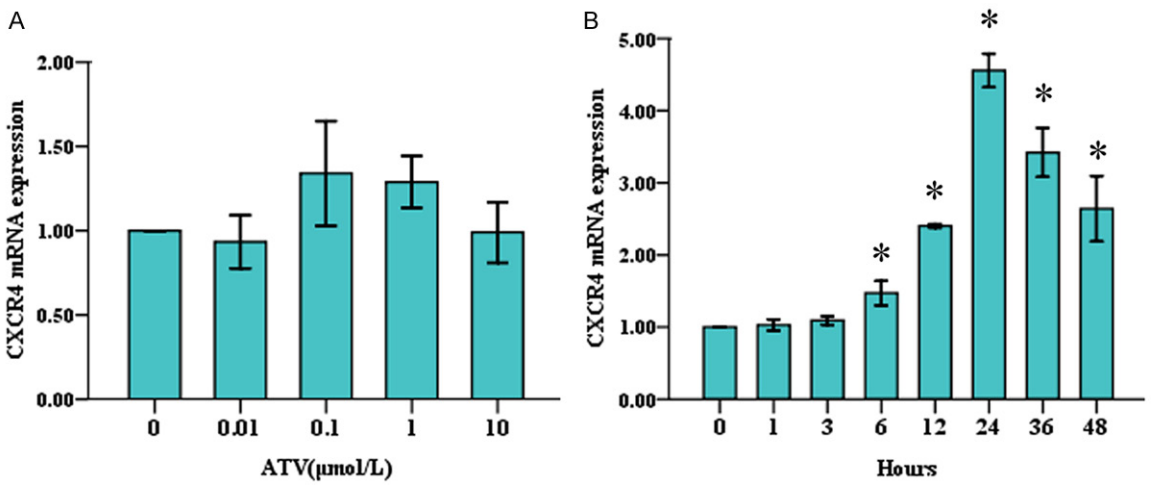

Figure 2. ATV increased CXCR4 mRNA expression in MSCs analyzed by real time PCR. A. Dose-dependent effect of ATV on CXCR4 mRNA expression for 6 h treatment. Expression values were normalized to β-actin and to untreated cells. Data are mean ± SD. There were no significant differences among groups (n = 3). B. Kinetic expression of CXCR4 mRNA in MSCs treated by 1 μM ATV. Data are mean ± SD; $^{*}P < 0.05$ vs. control (n = 3). ATV, atorvastatin; CXCR4, CXC chemokine receptor 4; MSCs, mesenchymal stem cells.

$P < 0.001$) (**Figure 1C**, **1D**). The results indicated that ATV treatment increased cell surface expression of CXCR4 in MSCs. The optimal concentration (1 μM) and time point (12 h) of ATV treatment were applied for subsequent study.

To further investigate the regulation of CXCR4 expression by ATV, we analyzed the CXCR4 mRNA expression level. We observed the same tendency as the cell surface expression in each group (**Figure 2**), though there were no significant differences among different concentration groups. However, time-course experiments revealed the maximal effect of ATV at 24 h (4.56-fold, $P < 0.001$) (**Figure 2B**). Together, these data showed that cell surface and mRNA levels of CXCR4 in MSCs were significantly up-regulated by ATV.

ATV pretreatment enhanced MSCs migration in vitro

Our flow cytometric analysis indicated that ATV increased cell surface expression of CXCR4 in MSCs. Further, cell surface CXCR4 is critical for cell migration. Thus, a transwell system was used to determine whether ATV enhanced MSCs migration ability accordingly. As expected, MSCs pretreated with ATV showed enhanced migration ability demonstrated by the increased number of cells migrating toward SDF-1 compared with untreated MSCs (24.65 ± 5.57 vs. 12.70 ± 2.40, $P < 0.001$). However, the enhanced migration ability was inhibited when the cells were pre-incubated with anti-CXCR4 neutralizing antibody (**Figure 3**). These data demonstrated that ATV enhanced MSCs migration by up-regulating the expression of CXCR4.

ATV pretreatment improved homing and survival of systemically infused MSCs in vivo

To explore the role of ATV on MSCs homing following intravenous infusion, the hearts were harvested 3 days after cell injection, and 4 μm frozen sections of hearts were analyzed using a confocal microscope. As shown in **Figure 4A**, **4B**, both ATV untreated and treated MSCs migrated to the infarcted myocardium. However, CM-DiI positive cells in the ATV-MSCs group were markedly increased compared with that in the MSCs group (41.68 ± 10.80 vs. 65.30 ± 13.37, $P < 0.05$). In addition, almost no CM-DiI positive cells were detected in the non-infarcted myocardium. As shown in **Figure 4C**, the result revealed that at 30 days after cell injection, the survival rate in both ATV-MSCs and MSCs groups were low, and fewer cells can be seen in the MSCs group (10.47 ± 2.37 vs. 5.23 ± 1.55, $P < 0.05$).

ATV-pretreated MSCs improved cardiac function

As shown in **Table 1**, the baseline data of cardiac function parameters at 3 days after stem

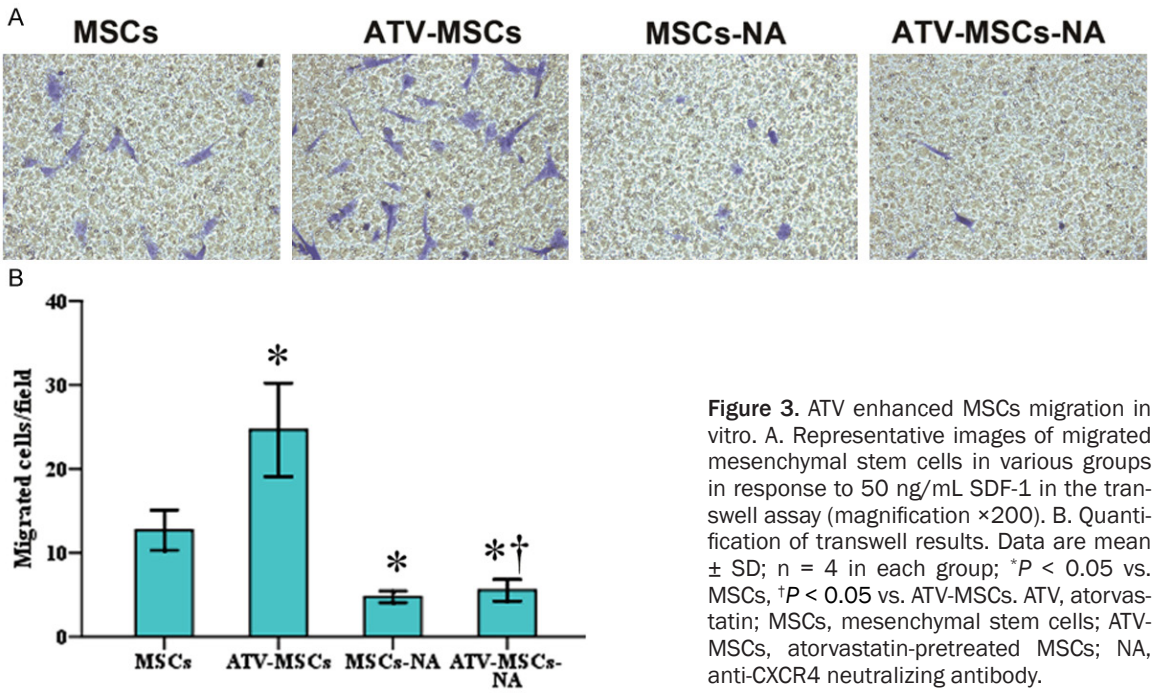

Figure 3. ATV enhanced MSCs migration in vitro. A. Representative images of migrated mesenchymal stem cells in various groups in response to 50 ng/mL SDF-1 in the transwell assay (magnification ×200). B. Quantification of transwell results. Data are mean ± SD; n = 4 in each group; $^{*}P < 0.05$ vs. MSCs, $^{\dagger}P < 0.05$ vs. ATV-MSCs. ATV, atorvastatin; MSCs, mesenchymal stem cells; ATV-MSCs, atorvastatin-pretreated MSCs; NA, anti-CXCR4 neutralizing antibody.

cell transplantation (4 days after AMI model was established) indicated that, compared with the sham group, AMI model was successful. Moreover, there was no significant difference among AMI models in different groups, indicating the model was stable. The endpoint data at 30 days after MSCs transplantation showed that, LVEDd and LVESd were markedly increased in the PBS group, and there was a moderate improvement of LVESd in the MSCs group, and LV chamber enlargement was further prevented in the ATV-MSCs group. LVEF was moderately enhanced in the MSCs group versus the PBS group, though the improvement of LVFS was not statistically significant. LVEF and LVFS showed dramatic improvement in the ATV-MSCs group compared with the PBS group and the MSCs group. These results suggested that the ATV-pretreated MSCs significantly promoted functional recovery of the heart and inhibited cardiac remodeling.

ATV-pretreated MSCs decreased infarct size, fibrosis and inflammation

Histological analysis was performed 30 days after cell transplantation. As shown in **Figure 5**, Masson's trichrome staining indicated transmural infarction in all groups, and there were thinning anterior wall, dilated LV chamber, severe fibrosis and large infarct size in the PBS- and MSCs-treated rats. Whereas, anterior wall thinning, chamber dilation, fibrosis and infarct size were significantly reduced in the ATV-MSCs group.

HE staining was used to assess the degree of inflammation in the infarcted hearts. And we found decreased inflammatory cells infiltration in the ATV-MSCs group compared with the other groups (**Figure 5**). In consistent with HE staining, western blot analysis of inflammatory cytokines IL-6 and TNF-α indicated that at 3 days after cell transplantation the expression of IL-6 and TNF-α was decreased in the ATV-MSCs group compared with other groups, and the inflammation level was further lowered at 30 days after cell transplantation (**Figure 6**).

Discussion

In the present study, the effect and the underlying mechanism of ATV pretreatment on the migration ability of MSCs were investigated. First, we found that ATV enhanced the expression of CXCR4 and stimulated MSCs migration in vitro. However, the effect was largely abolished by CXCR4 neutralizing antibody, indicating that the benefit was mediated by CXCR4 expression. Second, we demonstrated that ATV-pretreated MSCs possessed increased homing ability to the infarcted myocardium in vivo and this was accompanied by improved cardiac performance. To the best of our knowl-

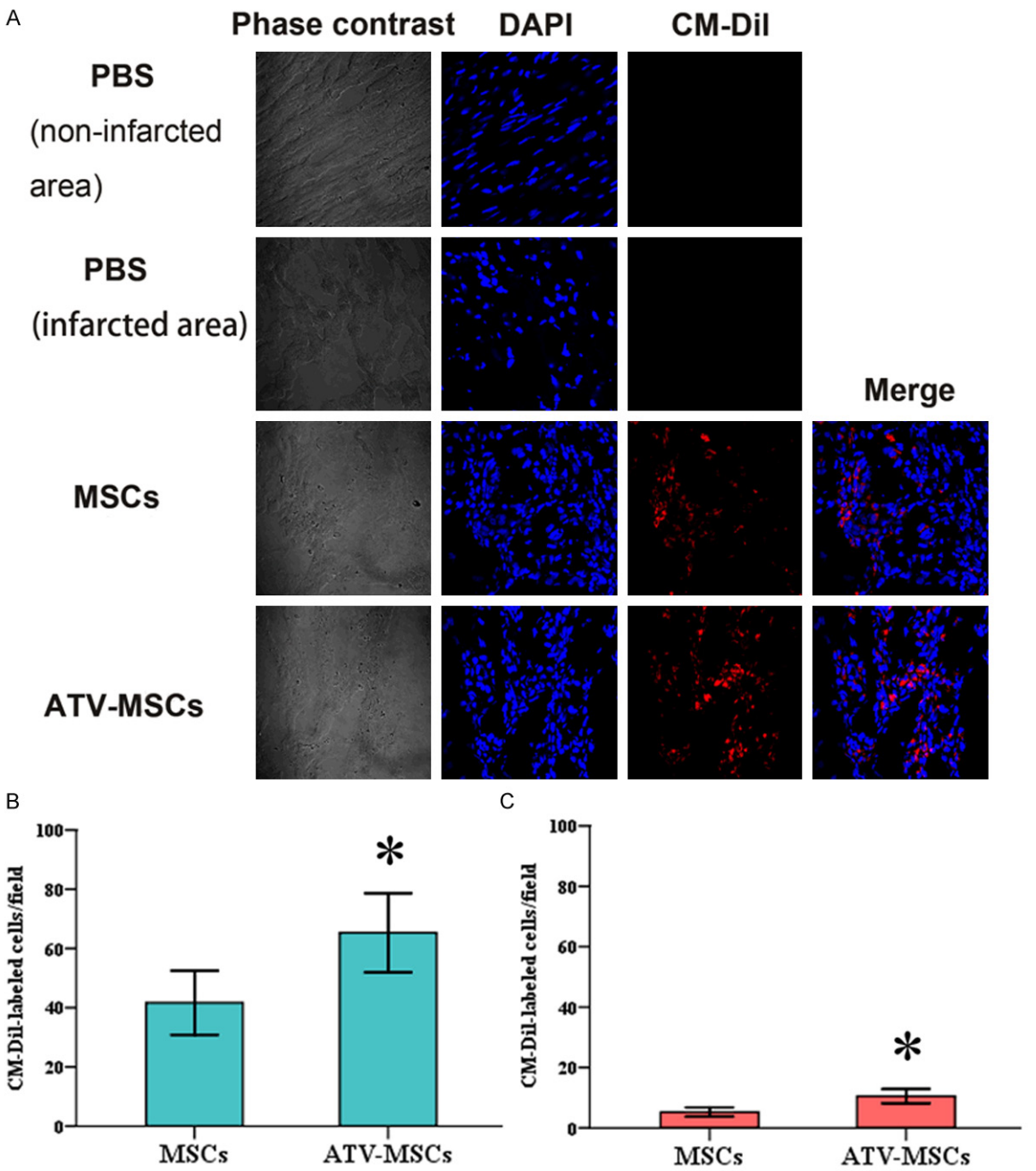

Figure 4. Homing and survival of CM-DiI-labeled MSCs after systemic infusion. A. Homing of CM-DiI-labeled cells (red) in the infarcted myocardium sections from each group at 3 days after systemic infusion. Nuclei were stained with DAPI (blue). Sections from the PBS group worked as the negative control (magnification ×600). B. Quantification of CM-DiI-labeled cells homing in each group at 3 days after cell transplantation. C. Quantification of CM-DiI-labeled cells survival in each group at 30 days after cell transplantation. Data are mean ± SD; $^{*}P < 0.05$ vs. MSCs (n = 4). MSCs, mesenchymal stem cells; ATV-MSCs, atorvastatin-pretreated MSCs.

edge, this is the first study to investigate the pro-migratory effect of ATV on MSCs.

In recent years, stem cell therapy has attracted much attention as a future approach to treat AMI, and many clinical trials have been conducted [20]. However, poor engraftment rate of transplanted MSCs in the infracted myocardium hinders the therapeutic effect [21]. Enormous work has done to identify the best cell delivery approach to transplant ample cells into ischemic heart tissue and to achieve maxi-

Table 1. LV dimensions and function at baseline and endpoint

	LVEDd (mm)	LVESd (mm)	LVFS (%)	LVEF (%)
Baseline				
Sham	5.95 ± 0.37	3.81 ± 0.44	36.13 ± 3.59	73.75 ± 4.50
PBS	6.75 ± 0.53*	5.37 ± 0.39*	20.38 ± 4.22*	49.19 ± 8.06*
MSCs	6.98 ± 0.59*	5.56 ± 0.58*	20.45 ± 2.90*	49.50 ± 5.74*
ATV-MSCs	7.11 ± 0.35*	5.69 ± 0.27*	19.96 ± 2.80*	48.58 ± 5.45*
Endpoint				
Sham	6.03 ± 0.32	3.77 ± 0.37	37.55 ± 3.32	75.48 ± 3.97
PBS	9.13 ± 1.04*	7.46 ± 0.65*	18.06 ± 3.23*	44.77 ± 6.54*
MSCs	8.41 ± 0.90*	6.52 ± 0.46*,†	22.27 ± 3.84*	52.77 ± 7.05*,†
ATV-MSCs	7.22 ± 1.14†	5.23 ± 0.93*,†,‡	27.80 ± 2.16*,†,‡	62.28 ± 3.27*,†,‡

All values are expressed as mean ± SD (n = 5 in each group). Baseline = 3 days after stem cell transplantation; Endpoint = 30 days after stem cell transplantation. LV, left ventricle; LVEDd, left ventricular end-diastolic dimension; LVESd, left ventricular end-systolic dimension; LVFS, left ventricular fractional shortening; LVEF, left ventricular ejection fraction. *$P < 0.05$ vs. Sham; †$P < 0.05$ vs. PBS; ‡$P < 0.05$ vs. MSCs.

mum retention there [22-24]. Among all the delivery methods, local intramyocardial injection was most effective [25, 26]. However, it is technically more demanding and more invasive, thus limits its widespread clinical use. In contrast, intravenous infusion is simple and least invasive, and allows multiple intermittent infusion treatments. Nevertheless, it is considered to be less effective because most of the infused cells are lost in the lung, spleen and lymphatic tissues, and only a small fraction home in toward the ischemic myocardium [26, 27]. Actually, if efficacious, the intravenous infusion has definite practical advantages [28]. So the mechanism about cardiac homing and engraftment of MSCs should be further studied to achieve better therapeutic effect.

The SDF-1/CXCR4 axis is crucial in homing and engraftment of MSCs [5, 6]. SDF-1 increased after myocardial infarction in the heart providing a signal to which CXCR4-positive stem cells can be recruited [29-31]. CXCR4, the specific chemokine receptor of SDF-1, is highly expressed in MSCs in the bone marrow. However, CXCR4 expression is markedly reduced during ex vivo expansion of the cells, leading to decreased migration toward the SDF-1 gradient [10, 11]. Studies proved that methods to increase the expression of CXCR4 can enhance the migration ability of MSCs. Cheng et al. [18] found that retrovirally transduced MSCs with over-expressed CXCR4 increased engraftment in the infarcted myocardium and improved cardiac performance. Zhang et al. [32] demonstrated that CXCR4 overexpression with adenoviral transduction led to enhanced mobilization and engraftment of MSCs into ischemic myocardium and alleviated left ventricular remodeling. Similarly, Xie et al. [33] reported that TanshinoneIIA-and astragaloside IV-stimulated MSCs showed enhanced ability to home to ischemic myocardium via promotion of the CXCR4 expression. In addition, human MSCs transduced with a retroviral vector containing CXCR4 exhibited enhanced migration ability [9]. In consistent with these studies, we found that, increased CXCR4 expression with ATV pretreatment enhanced the migration of MSCs toward SDF-1 in vitro transwell assay, and promoted the homing of MSCs toward ischemic myocardium following intravenous administration in vivo.

We further confirmed that, in MSCs, ATV pretreatment not only enhanced the expression of CXCR4 in cell surface, but also increased CXCR4 mRNA expression level. The cell surface expression of CXCR4 increased from 1 h of ATV pretreatment and peaked at 12 h, while the expression of CXCR4 mRNA increased from 6 h and peaked at 24 h. So, there was a time lag between CXCR4 cell surface expression and CXCR4 mRNA expression. The reason may be that at early stage of ATV pretreatment, CXCR4 cell surface expression was mainly depended on mobilizing the internalized receptor in MSCs, and with consumption of intracellular storage, CXCR4 mRNA expression was motivated as a feedback.

Moreover, we found that, intravenous infusion of ATV-pretreated MSCs at 24 h after AMI reduced infarct size and improved cardiac function after 30 days of cell transplantation. And we provided additional evidence about the therapeutic efficacy of systemically delivered MSCs in post-infarction cardiac repair. For example, Ma et al. [30] demonstrated that SDF-1 was peaked at 1 day after AMI in a rat model. With intravenous delivery, more MSCs homed in

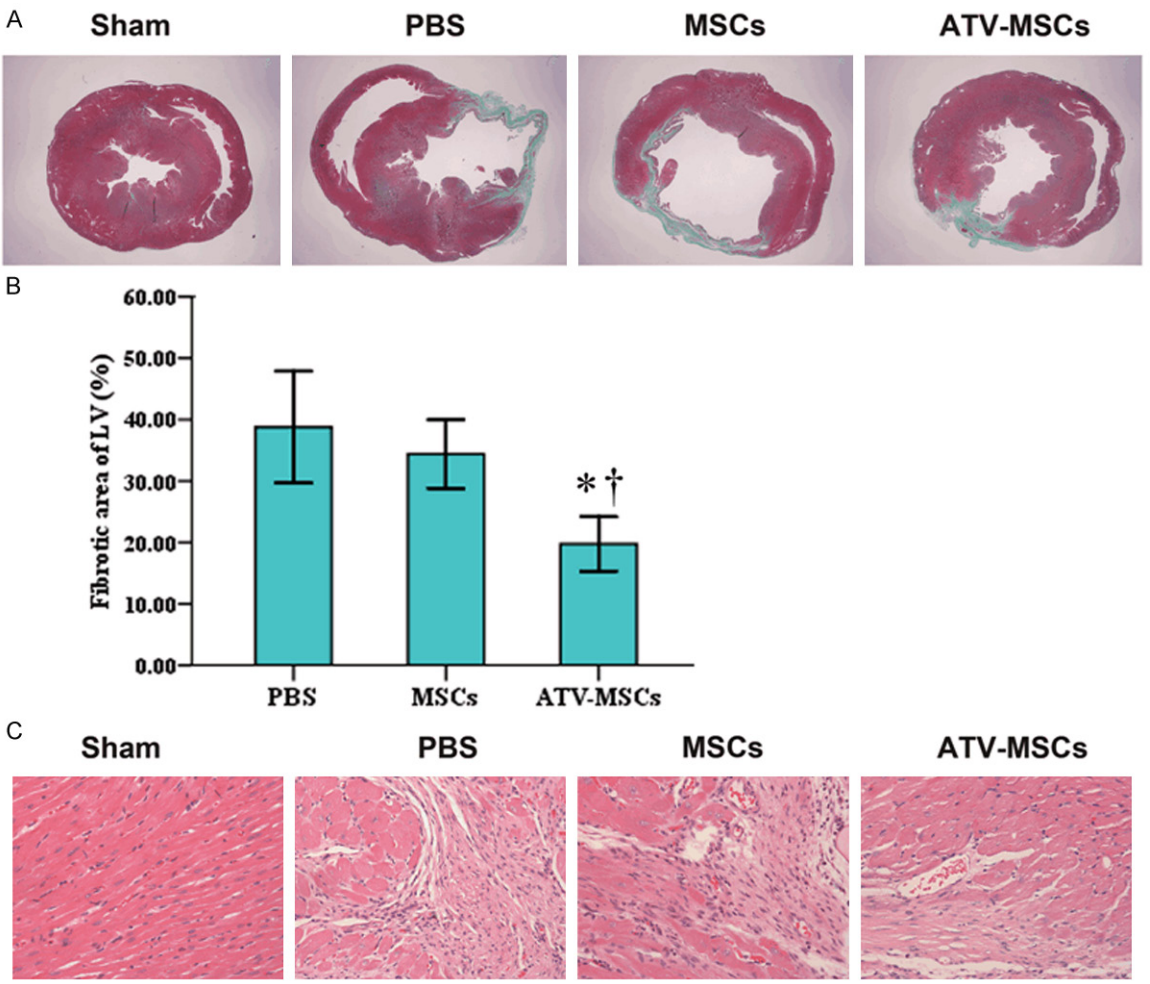

Figure 5. Masson's Trichome staining and Hematoxylin-Eosin staining. A. Representative Masson's Trichome staining images in each group (magnification ×12.5), B. quantitative data for the LV fibrotic area. C. Inflammatory cells infiltration of heart tissue in the peri-infarct area (magnification ×400). Data are mean ± SD; n = 5 in each group; $^{*}P < 0.05$ vs. PBS, $^{\dagger}P < 0.05$ vs. MSCs. LV, left ventricle; MSCs, mesenchymal stem cells; ATV-MSCs, atorvastatin-pretreated MSCs.

toward the infarcted hearts when infused at 1 day after MI than other time points and improved cardiac function. Cheng et al. [18] reported that intravenous infusion of MSCs with highly-expressed CXCR4 at 24 h after AMI resulted in significant recruitment of MSCs in the infarct region and improvement of LV function, and only a few of MSCs homed to liver, spleen and lung. This may be because the CXCR4 highly-expressed cells owned increased ability to respond to homing signals emanating from the ischemic myocardium. Therefore, intravenous delivery of MSCs with high CXCR4 expression at 24 h after AMI to coincide with the peak of SDF-1 expression is a promising method for cell therapy.

As is well known, the microenvironment of infarcted myocardium is hostile, such as inflammation and oxidative stress, which may lead cell death and impair the therapeutic potential [13, 34, 35]. Zhang et al. [16] showed that most of the implanted adipose-derived MSCs died in the infarcted myocardium within 21 days. And we demonstrated that few cells can be seen in the infarcted myocardium at 30 days after cell injection in both ATV-MSCs and MSCs groups.

As shown in H & E staining, less inflammation cells can be seen in the ATV-MSCs group compared with the other groups. Moreover, the western blot analysis indicated that at 3 days after cell transplantation the expression of IL-6

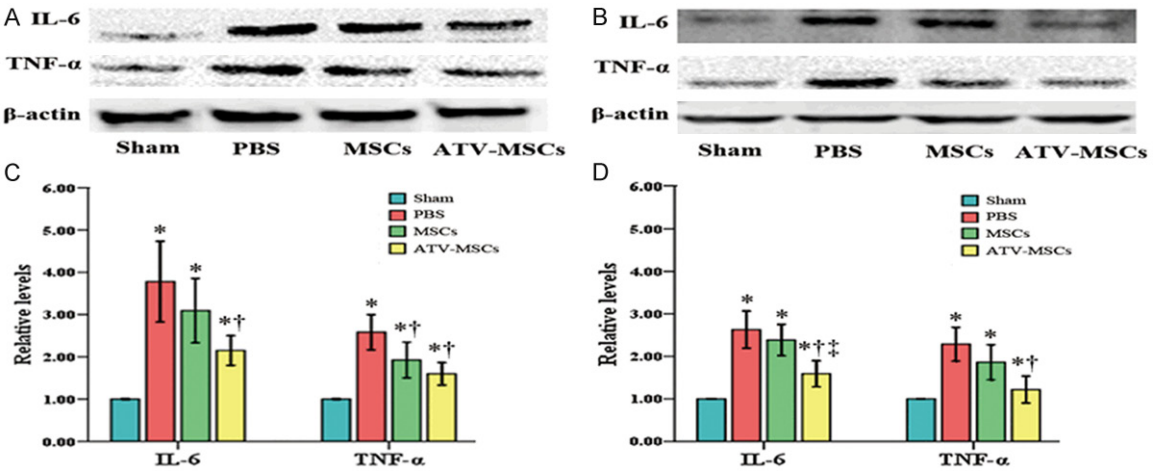

Figure 6. Inflammatory cytokines expression in infarcted heart. A, C. The expression of IL-6 and TNF-α at 3 days after cell transplantation were analyzed by western blot, and semiquantitative analysis of the western blots was conducted. B, D. The expression of IL-6 and TNF-α at 30 days after cell transplantation were analyzed by western blot, and semiquantitative analysis of the western blots was conducted. Data are mean ± SD; n = 4 in each group; $^{*}P < 0.05$ vs. Sham; $^{\dagger}P < 0.05$ vs. PBS; $^{\ddagger}P < 0.05$ vs. MSCs.

and TNF-α was decreased in the ATV-MSCs group, and the inflammation level was further lowered at 30 days after cell transplantation. Previous studies have demonstrated that MSCs release some growth factors and cytokines, which can suppress inflammation [36, 37]. Moreover, the coupling of SDF-1 with CXCR4 can exert robust anti-inflammatory effect [38]. Reduced inflammation may be one reason why more cells can be seen in ATV-MSCs group than MSCs group at 3 days and 30 days after cell transplantation, and why though not so many MSCs were identified in the myocardium after intravenous injection, the cardiac function was still improved.

Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, have many biological activities independent of their lipid-lowering ability, such as anti-thrombotic, anti-inflammatory, anti-oxidant activity and so on [39-42]. Previous studies have confirmed that statins decreased apoptosis and increased survival of MSCs [12-16, 43, 44], and combination therapy with statins and MSCs showed more obvious improvement in myocardial function after MI than MSCs administration alone [12-16]. Based on our results, we speculated that the increased homing capacity of MSCs toward infarcted myocardium, aside from decreased apoptosis, might contribute to cell survival and cardiac performance improvement. This is a novel point of view to elucidate the underlying mechanism of statin treatment.

Genetic approach is an effective method to increase the expression of CXCR4 in MSCs, which effectively promote MSCs migration and accelerate injured organ repair [8, 18, 32, 45]. However, it seems infeasible in clinical practice at present time. Unlike genetically manipulative approaches, ATV pretreatment is a simple and clinically feasible approach. Therefore, our findings have obvious translational implications for the treatment of patients with AMI. To our knowledge, there has been rare study to explore this kind of effect with commonly used medications.

In the future, the pro-migratory effect of statins pretreatment on MSCs will be testified in human-originated ones, and provide evidence for the clinical use. Moreover, whether cardiac performance can be further improved by combined therapy with statins and ATV-pretreated MSCs will be studied.

In conclusion, ATV increases the migration ability of MSCs and improves cardiac performance due to up-regulated expression of CXCR4. This research may deepen our understanding of the protective effect of ATV on MSCs. And these results suggest that ATV pretreatment of donor MSCs is an effective way to promote cell therapeutic potential for AMI.

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Disclosure of conflict of interest

None.

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