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Early upregulation of myocardial CXCR4 expression is critical for dimethyloxalylglycine-induced cardiac improvement in acute myocardial infarction

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OUR PREVIOUS STUDIES and the data from several other laboratories demonstrate the importance of the stromal cell-derived factor-1 (SDF-1):CXCR4 axis in myocardial repair (2, 24, 33). SDF-1 is a major stem cell homing factor (3, 7, 33), and SDF-1 signaling is initiated by ligation of the chemokine with its G-protein-coupled receptor CXCR4 (5, 12). Acute ischemia upregulates SDF-1 expression (2, 26) and increased SDF-1 has the ability of recruiting CXCR4+ stem/progenitor cells to the ischemic tissue, including hematopoietic stem cells, endothelial progenitor cells (EPC), and cardiac progenitor cells (CPC) (3, 7). SDF-1 levels increase immediately post-myocardial infarction (MI) and decline after 4–7 days; however, myocardial CXCR4 expression occurs 2–3 days after MI and reaches a peak plateau at 4 days, late relative to myocardial SDF-1 expression. This temporal mismatch may in part explain why the heart has limited ability to repair itself (33). Manipulation of SDF-1 levels is a promising strategy to induce endogenous stem cell-based tissue repair (6). We have demonstrated that prolongation of SDF-1 expression reduces myocardial death in acute MI (7, 33). These findings have led us to propose a hypothesis that myocardial repair can be induced through the temporal alignment of myocardial SDF-1 expression and cardiac myocyte CXCR4 expression.

Dimethyloxalylglycine (DMOG) is a prolyl-4-hydroxylase inhibitor, which can stabilize hypoxia inducible factor-1α (HIF-1α) expression and is often used as a HIF-1 activator to induce ischemic preconditioning. Because of the unpredictable occurrence of acute coronary syndromes, ischemic preconditioning has not led to any therapy (one would have to know in advance of the upcoming event). In addition to increased HIF-1α expression, DMOG was reported to upregulate the expression of CXCR4 in bone marrow mesenchymal stromal cells (MSC) (29). In the current study, we aimed to determine the extent to which the effects of DMOG were mediated through the SDF-1:CXCR4 axis via early upregulation of CM-CXCR4 following MI.

METHODS

Experimental Animals

The animal work in this study was approved by the Institutional Animal Care and Use Committee of Northeast Ohio Medical University. We have recently published the generation and characterization of the CM-CXCR4 null mouse (1, 7). Briefly, it was generated by using a CXCR4f/f mouse (kind gift from Yong-Rui Zou, Feinstein Laboratory for Hematopoiesis, New York, NY) in a C57Bl/6 strain.
with the MerCreMer mouse (from The Jackson Laboratories) for inducible CM-CXCR4 null mouse. The MerCreMer mouse has Cre induced by a tamoxifen inducible cardiac-specific promoter and has been used extensively to induce recombination in cardiac myocytes and possibly cardiac stem cells (11, 31). The MerCreMer mice received tamoxifen (40 mg/kg) daily for 5 days starting 4 wk prior to left anterior descending (LAD) ligation to achieve cardiac-specific Cre expression. The cardiac-specific Cre expression leads to excision of Exon 2 of the CXCR4 gene, rendering the CM- CXCR4 null. The administration of tamoxifen led to a transient decline in cardiac function that was back to baseline 3 wk later (14). Therefore, all LAD ligations in this study were performed at least 3 wk after the tamoxifen injection and in animals with recovered cardiac function. Our results demonstrate that the administration of tamoxifen to these mice leads to an ~90% decrease in CXCR4 protein in infarct and infarct border zone (1). Wild-type control groups received tamoxifen as well. Mice were housed with free access to food and water in an American Association for Accreditation of Laboratory Animal Care-approved animal facility. The animal experiments were performed to conform with the NIH guidelines (Guide for the Care and Use of Laboratory Animals).

H9c2 Cell Culture

H9c2 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured at 37°C and 5% CO₂ in six well plates containing serum-free DMEM. H9c2 cells (2 × 10⁵ cells/well) were treated with DMOG (1 mmol/liter) for 0, 15, and 24 h at normoxic and hypoxic (1% oxygen) conditions.

Western Blot Analysis of CXCR4 Expression

H9c2 cells were collected and sonicated in a lysis buffer containing 20 mmol/liter Tris (pH 7.4), 150 mmol/liter NaCl, 1 mmol/liter EDTA, 1 mmol/L EGTA, 1% Triton, 0.1% sodium dodecyl sulfate (SDS), and protease inhibitor cocktail. Equal amounts of protein lysates (50 mg/lane) were separated on 10% SDS-polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad) and transferred to nitrocellulose membranes (0.2 mM). The membranes were blocked in 5% nonfat milk in Tris-buffered saline-T buffer, and then incubated with anti-CXCR4 (1:500, Abcam) at 4°C overnight. After incubation in 5% nonfat milk in PBS for 60 min to suppress nonspecific binding of IgG, the membranes were incubated again with a murine monoclonal anticardiac myosin heavy chain (Abcam, San Francisco, CA) to specifically recognize cardiac tissue for analyses.

Immunostaining

Mice were anesthetized and perfusion fixed with 10% phosphate-buffered formalin at physiological pressures 15 h and 21 days after LAD ligation. Fixed hearts were embedded in paraffin and serially cut at 4 μm from the apex to the level just below the coronary artery ligation site. Antigen retrieval was performed with 10 mmol/liter sodium citrate buffer (pH 6.0) and incubated with 1% normal blocking serum in PBS for 60 min to suppress nonspecific binding of IgG. Tissue were stained for CXCR4 (NB100-74396, Novus Biologicals, Littleton, CO) and mouse monoclonal antibody to recognize cardiac myosin heavy chain (Abcam, San Francisco, CA) 15 h post-MI. Three weeks post-MI, tissue was stained to measure percent cardiac myosin-positive area in the infarct zone normalized to total infarct area. Negative control with no fluorescent probe was used in immunostaining to control the autofluorescence.

Echocardiographic Assessment of Heart Function

Two-dimensional echocardiography was performed with a 15-MHz linear array transducer interfaced with a Sequoia C256 (Acuson) as previously described (8). Briefly, baseline before LAD ligation as well as 3 and 21 d after LAD ligation, left ventricle (LV) dimensions were quantified by digitally recorded 2D clips and M-mode images from the mid-LV just below the papillary muscles to allow for consistent measurements from the same anatomical location in different mice. Ejection fraction (EF), fractional shortening, diastolic thicknesses of the LV posterior wall (LVPWD), systolic thicknesses of the LV posterior wall (LVPSWS), diastolic LV internal dimensions (LVIDD), and systolic LV internal dimensions (LVIDS) were measured. Echocardiographic measurements were performed and analyzed by investigators who were blinded to the treatment and identity of the mouse.

CXCR4 mRNA Expression

Fifteen hours after LAD ligation and DMOG injection, the hearts were perfused with saline and infarcted left ventricles were cut at a level just below the ligation. The RNA was harvested with Trizol and cDNA was synthesized with SuperScript VILO cDNA synthesis kit from Invitrogen. The real-time PCR reaction for CXCR4 and GAPDH was carried out with TaqMan primers on Applied Biosystems 7500 Real-Time PCR System. CXCR4 primer was purchased from Invitrogen for real-time PCR.

TUNEL Assay for Assessment of Cell Apoptosis

Mice were anesthetized and perfusion fixed with 10% phosphate-buffered formalin at physiological pressures 4 days after LAD ligation. Fixed hearts were embedded in paraffin and serially cut at 4 μm from the apex to the level just below the coronary artery ligation site. Terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) was performed to detect apoptotic nuclei according to the manufacturer’s protocol (Roche, Indianapolis, IN). The sections were incubated again with a murine monoclonal anti-tumor necrosis factor (TNF)-α antibody (Abcam) and mouse monoclonal antibody to recognize cardiac myosin heavy chain (Abcam, San Francisco, CA) to specifically recognize apoptotic cardiomyocytes. The fluorescence staining was viewed with a confocal laser scanning microscope. The number of apoptotic cells with TUNEL-positive nuclei was counted by two independent observers blinded to treatment group and expressed as a percentage of total myocyte population. TUNEL-positive nuclei in 1,000 nuclei within 4–5 cells from the infarct border zone and the remote zone (4,000–5,000 nuclei total) were obtained from the LV.
Percent Cardiac Myosin-Positive Area Measurement

Three weeks post-MI, tissue was prepared as described above for immunostaining. Slides were stained with the mouse anti-CM primary antibody and then with goat antimouse IgG Alexa Fluor 594 (Molecular Probes, Carlsbad, CA) and mounted with aqueous mounting medium (Vectashield Mounting Medium with DAPI, H-1200, Vector Laboratories, Burlingame, CA). Random fields in the infarct zone for each sample were selected and measured by an observer blinded to treatment group with ImagePro Plus software (Media Cybernetics) as previously described (7, 33).

Isolation of CPCs from Mouse Hearts

CPCs were isolated from mouse hearts in different groups 7 days post-MI as described previously (7). Briefly, infarct zone of hearts were digested with 0.1% collagenase B and 2.4 U/ml dispase II (Roche Molecular Biochemicals) containing 2.5 mmol/liter CaCl2 at 37°C for 45 min. Cells then were filtered through 30-μm filters, washed in Hank’s balanced salt solution (Invitrogen) containing 2% fetal calf serum and 10 mmol/liter HEPES, and were lysed with red blood cell lysing buffer. After washing with PBS, cells were resuspended in 2 × 10^6 nucleated cells/ml. Cells were fixed with 4% paraformaldehyde and then incubated with antibodies against the mouse CD16/32, Allophycocyanin rat-antimouse CD117, and R-phycocerythrin rat-antimouse CD45 (BD Biosciences). Flow cytometry was performed on a FACS Calibur (BD Biosciences) (8). Data were analyzed with FlowJo software. The percentage of c-kit+/CD45− cells in the infarction zone 7 d post-MI was calculated.

Measurement of Area at Risk and Infarct

We measured the area at risk and infarct as described previously (4, 18, 22). Briefly, mouse hearts were harvested 4 days post-MI, and the area of the LV at risk for ischemic injury was determined by 2% Evans blue staining of the myocardium via a cannula inserted into the proximal ascending aorta. Then the hearts were cut into transverse slices 2-mm thick with a cutting block. The slices were then placed in 2,3,5-triphenyltetrazolium chloride solution (TTC, 1% in sodium phosphate buffer at 37°C, pH 7.4) for 20 min to delineate infarcted myocardium. The slices were then placed in 10% neutral buffered formalin for 20 min to increase the contrast between stained and unstained tissue. This differentiated infarcted area (Evans blue and TTC unstained, white) from risk area (Evans blue stained and unstained tissue). This differentiated infarcted area (Evans blue and TTC unstained, white) from risk area (Evans blue stained, TTC stained, red). The healthy area was identified as Evans blue stained (blue). Both sides of all slices were photographed for measurement of area at risk and infarct (ImageJ software). The volume of myocardium at risk and infarcted myocardium was calculated from the measured areas and slice thickness. Infarct size was normalized as a fraction of the area at risk. Samples were blinded and randomized prior to analysis.

Hematoxylin and Eosin Staining

To measure myocytes cell size, cardiac tissue sections were stained with hematoxylin and eosin. Three weeks post-MI, heart tissue was embedded in paraffin, cut in 5-μm sections, stained with hematoxylin and eosin, and photographed with a digital camera under a microscope at a magnification of ×40. Three to five images showing cardiomyocytes in cross section were taken from the remote zone of the ventricular wall in each sample. Cardiac myocyte surface area was quantified on at least 60 myocytes per section (at least 180 cardiomyocytes per heart, n = 7–9, ImageJ software). The volume of myocardium at risk and infarcted myocardium was calculated from the measured areas and slice thickness. Infarct size was normalized as a fraction of the area at risk. Samples were blinded and randomized prior to analysis.

Statistical Analysis

Data are presented as means ± SE. Comparisons among multiple groups were made with two-way ANOVA followed by the Tukey post hoc analysis. All differences were considered statistically significant at P < 0.05 levels.

RESULTS

Role of DMOG on CXCR4 Expression

DMOG increases CM-CXCR4 expression in H9c2 cells in vitro. The effects of the HIF hydroxylase inhibitor DMOG (1 mmol/liter) on CXCR4 expression was tested in H9c2 cells in both normoxic and hypoxic conditions. Protein expression of CXCR4 was quantified by Western blot. We found that in the presence of hypoxia, DMOG (1 mmol/liter) treatment upregulated CXCR4 expression of H9c2 cells by 29 and 42% at 15 and 24 h, respectively (Fig. 1, A and B).

DMOG increases CM-CXCR4 expression in vivo. Mice were randomly selected to receive injection of 100 mg/kg DMOG (DMOG group) or saline (Saline group) into the border zone immediately after LAD ligation. Protein and mRNA expression of CM-CXCR4 in CM-CXCR4 and control mice 15 h after DMOG injection were measured by immunofluorescent staining and qPCR. We observed no CM-CXCR4 expression in Saline group (both control and CM-CXCR4 null mice) 15 h post-MI. DMOG treatment induced CM-CXCR4 expression at 15 h post-MI in control mice but not in CM-CXCR4 null mice in border zone. No CM-CXCR4 expression in remote zone was found (Fig. 2, A–C).

Role of DMOG on Cardiac Myocyte Preservation and Infarct Size Following MI

To investigate role of DMOG on cardiac myocyte preservation following MI, we performed TUNEL to measure CM apoptosis. The number of TUNEL+ cardiac myocytes in the infarct border zone and remote zone 4 days after MI was...
In the Saline treatment group, there was no difference in the number of TUNEL+/H11001 cardiac myocytes in mice with or without CM-CXCR4 expression. Interestingly, DMOG led to a significant 33% reduction in TUNEL+/H11001 cardiac myocytes in mice with CM-CXCR4 expression (P < 0.05 compared with the control group). However, in the absence of CM-CXCR4 expression, the decrease in cardiac myocyte apoptosis was blunted by 54% (Fig. 3, A and B). There was no significant difference in the number of TUNEL+ cardiac myocytes in mice with or without CM-CXCR4 expression.
after DMOG treatment in the remote zone (Fig. 3C). Consistent with a decrease in cardiac myocyte apoptosis, we observed a significant decrease in infarct/risk ratio after DMOG treatment in control mice 4 days after MI, but no significant decrease in CM-CXCR4 null mice (Fig. 4C). We also found the cardiac myosin-positive area within the infarct zone by immunofluorescence 21 days after MI was significantly increased in the control animals treated with DMOG (36.5%, \( P < 0.05 \)). However, in CM-CXCR4 null mice DMOG treatment did not change the cardiac myocyte positive area (Fig. 4, A and B).

**Role of DMOG on Cardiac Function Following DMOG Treatment**

CM-CXCR4 expression does not alter LV function and LV remodeling 21 d after MI in the absence of DMOG treatment (Table 1 and Fig. 5), which is consistent with our previous findings (1, 7). The delivery of DMOG led to a significant improvement in EF (62%, \( P = 0.02 \)) in control mice 21 days after MI without change to the LVISD, LVIDD, LVPWS, and LVPWD in control mice; however, in the absence of CM-CXCR4 expression there was no significant improvement in cardiac function or remodeling (EF, LVISD, LVIDD, LVPWS, and LVPWD) in response to DMOG injection (Table 1 and Fig. 5). These data demonstrate that CM-CXCR4 plays a critical role in post-MI cardiac function following DMOG injection.

**Role of DMOG on CPC Recruitment Following Cell Therapy**

We quantified CPC recruitment following MI with and without DMOG infusion. We found that DMOG injection led to a significant increase of CPC recruitment (155.3%, \( P < 0.01 \)) in the heart of control mice 7 days after MI; CPC recruitment in response to DMOG was significantly decreased to 57.5% in CM-CXCR4 null mice (Fig. 6, A and B).

**Role of DMOG on Cardiac Myocyte Hypertrophy Following MI**

We additionally evaluated the effects of DMOG on cardiac myocyte size in the remote area of the infarction. In the Saline group, there was no difference in the cardiac myocyte size in mice with or without CM-CXCR4 expression. Interestingly, DMOG led to a significant 20% reduction in cardiac myocytes surface area in control mice but not in CM-CXCR4 null mice 21 days after MI (Fig. 7, A and B).

**DISCUSSION**

Several studies have suggested that ischemic preconditioning with DMOG can protect heart tissue from ischemic injury (9, 16, 21, 23, 34). However, this is the first study that demonstrated that the benefit of DMOG treatment or the benefit of upregulation of HIF-1α in the heart requires cardiac CXCR4 expression. Our results further indicate that early upregulation of CM-CXCR4 is a strategy for improving car-
diac repair after MI. Results from this study demonstrated that DMOG treatment induces early myocardial CXCR4 expression. DMOG is a prolyl-4-hydroxylase inhibitor, which stabilizes HIF-1α by inhibiting HIF-1α hydroxylases, thereby preventing degradation (10, 25). Some literature suggests that HIF-1α plays an important role in cardioprotection. Specifically, it was shown that the constitutive overexpression of HIF-1α in the murine heart attenuated infarct size and improved cardiac function following ligation of the LAD (13). Furthermore, systemic DMOG treatment given prior to ischemia attenuated the infarct size in myocardial ischemia-reperfusion (9, 16, 21). We would like to add that our results and approach are different in that we give DMOG after MI, not an intervention given before the ischemic insult. Although HIF-1α modulates expression of many genes, and DMOG significantly increases HIF-1α stability and levels, a criticism of our study is that the effects of DMOG are not unique to the SDF-1: CXCR4 axis. We do not believe this argument has merit since DMOG did not influence ischemic injury in the CXCR4 null mice. Indeed, our results demonstrate that the effects of DMOG are mediated through the SDF-1:CXCR4 axis by inducing CXCR4 expression in vitro and in vivo, and the DMOG-induced CXCR4 expression is earlier and greater than that observed in hypoxic conditions alone. These data indicate

Table 1. left ventricle function at baseline and 21-d post-myocardial infarction

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<th></th>
<th>LVDD, mm</th>
<th>LVPWD, mm</th>
<th>LVISD, mm</th>
<th>LVPWS, mm</th>
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<th>FS, %</th>
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<td>Control</td>
<td>2.92 ± 0.21</td>
<td>0.91 ± 0.08</td>
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<td>CM-CXCR4−/−</td>
<td>3.06 ± 0.23</td>
<td>1.07 ± 0.12</td>
<td>1.27 ± 0.2</td>
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<td>90.3 ± 2.6</td>
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<td>DMOG</td>
<td>3.1 ± 0.24</td>
<td>1.07 ± 0.09</td>
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<td>90.4 ± 2.14</td>
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<td>21 D post-MI saline</td>
<td>4.79 ± 0.34</td>
<td>0.97 ± 0.1</td>
<td>4.28 ± 0.38</td>
<td>1.11 ± 0.13</td>
<td>26.8 ± 3.9</td>
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<td>Control</td>
<td>4.82 ± 0.28</td>
<td>0.89 ± 0.10</td>
<td>4.17 ± 0.25</td>
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<td>CM-CXCR4−/−</td>
<td>4.75 ± 0.18</td>
<td>0.84 ± 0.08</td>
<td>3.36 ± 0.17</td>
<td>1.20 ± 0.07</td>
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<td>DMOG</td>
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<td>1.42 ± 0.21</td>
<td>34.0 ± 6.0</td>
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Data are expressed as means ± SE, n = 7–8 in each group. LVDD, diastolic left ventricle internal dimension; LVPWD, diastolic thicknesses of the LV posterior wall; LVISD, systolic LV internal dimension; LVPWS, systolic thicknesses of the LV posterior wall; EF, ejection fraction; FS, fractional shortening; CM-CXCR4, cardiomyocyte CXCR4; DMOG, dimethyloxalylglycine; MI, myocardial infarction. *P < 0.05 vs. corresponding saline treatment group.
that DMOG can be used as a CXCR4 inducer to induce upregulation of CM-CXCR4 at an earlier point in time (than occurring normally in ischemia) to coincide with the expression of its ligand, SDF-1.

Consistent with previous studies (9, 16, 21), we found that DMOG treatment improved ejection fraction in control mice following an MI. Several mechanisms that may responsible for DMOG-induced ischemic preconditioning have been reported (19, 30). DMOG treatment upregulated HIF-1α and VEGF expression in the heart (21, 28). DMOG can attenuate post-ischemic cardiac injury through induction of endoplasmic reticulum stress genes (19). DMOG can also inhibit prolyl hydroxylase domain protein 3, prevent activation of the ATR/CHK1/p53 pathway, and decrease apoptosis induced by DNA damage (30). It has been reported that DMOG is able to inhibit proinflammatory cytokines, reduce neutrophil sequestration, and upregulate hemeoxygenase-1 (20). A recent study showed that DMOG pretreatment significantly enhances angiogenesis by increasing circulating EPCs and bone marrow progenitor cells (27). Importantly, we would emphasize that in our study, the actions of DMOG were dependent on CXCR4 expression in that the drug produced no significant effect in CXCR4 null mice. This is not to say the other mechanisms are not important or do not contribute, only that the SDF-1:CXCR4 axis is critical to HIF-1α signaling in post-MI cardiac repair. Moreover, to more decisively show the role of CXCR4 in this effect, our analyses revealed that DMOG led to a significant reduction in TUNEL+/cardiac myocytes and infarct size, increased CSC recruitment, significant increase in surviving myocardium and an improvement in ejection fraction in control mice. Importantly, none of these benefits of DMOG treatment were observed in CM-CXCR4 null mice. Our previous study showed that CXCR4 signaling did not enhance cardiomyocyte hypertrophy (1). In this study, we found that DMOG led to a significant reduction in cardiac myocytes surface area in control mice but not in CM-CXCR4 null mice 21 days after MI.

In addition to the effects of cardiomyocyte preservation, our previous study demonstrated that CM-CXCR4 expression plays an important role on CPC recruitment, following cell therapy for MI (7). DMOG significantly enhances angiogenesis by increasing circulating EPCs and bone marrow progenitor cells (27). In our current study, we investigated the influence of DMOG treatment on CPC recruitment following MI and found

![Fig. 5. Role of CM-CXCR4 expression on left ventricle function following DMOG injection. Ejection fraction at baseline and 21 days after LAD ligation in control (open and light grey bars) and CM-CXCR4 null (black and dark grey) mice. Mice were infused with saline (open and black bars) or DMOG (light and dark grey bars) right after LAD ligation. Data are means ± SE, n = 7 to 8 per group. *P < 0.05 vs. strain matched control.](http://ajpheart.physiology.org/)

![Fig. 6. Cardiac progenitor cell recruitment following MI with and without DMOG injection. A: representative flow cytometry analysis of isolated cells within the infarct zone 7 days after LAD ligation from groups. B: percent c-Kit+/CD45− cells within the infarct zone 7 days after LAD ligation. Data represent means ± SE, n = 5 per group. *P < 0.05 vs. control group.](http://ajpheart.physiology.org/)
an increase of CPC in the infarct zone in control mice after DMOG injection, but no significant increase of CPC number was found in the infarct zone in CM-CXCR4−/− mice. Our data are consistent with the previous results that hypoxic preconditioning of c-kit+ CPCs notably increased migration toward SDF-1 (32), and suggests that DMOG induces CPC migration to the infarct zone via a CXCR4-dependent pathway. DMOG is found to be able to decrease the apoptosis of MSC (17). Our data also showed that DMOG increased CPC migration to the infarct zone, which means DMOG also plays an important role in stem cell therapy following cardiac injury by improving stem cell survival and migration. Taken together, these findings indicate that DMOG-induced benefits in MI is due, at least in part, to the early and increased expression of CM-CXCR4. These data also indicate that early upregulation of CM-CXCR4 is a strategy for improving cardiac repair after MI.

In summary, our study revealed that the early upregulation expression of CM-CXCR4 leads to a significant increase in cardiac myocyte survival and an improvement in cardiac function after MI. We demonstrated a critical role for cardiac myocyte CXCR4 expression on cardiac repair and proved that the engagement of the SDF-1:CXCR4 axis through the early upregulation of CM-CXCR4 is a strategy for improving cardiac repair after MI.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.M., M.K., P.S., F.F., K.W., and F.D. performed experiments; M.M., M.K., P.S., F.F., K.W., W.M.C., M.S.P., and F.D. analyzed data; M.M., M.K., P.S., F.F., K.W., W.M.C., M.S.P., and F.D. approved final version of manuscript; W.M.C., M.S.P., and F.D. conception and design of research; W.M.C., M.S.P., and F.D. edited and revised manuscript; M.S.P. and F.D. interpreted results of experiments; F.D. prepared figures; F.D. drafted manuscript.

Fig. 7. Representative hematoxylin and eosin-stained sections of the remote area of the MI in animals (A) after 21 days with quantitative measurements of cardiomyocytes (B). Data represent means ± SE, n = 7–9 per group. *P < 0.01 compared with corresponding sham groups; #P < 0.01 compared with Control-saline group; $P < 0.01 compared with CXCR4 null sham group.
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