Original Article

Possible Mechanism of Therapeutic Effect of 3-Methyl-1-phenyl-2-pyrazolin-5-one and Bone Marrow Stromal Cells Combination Treatment in Rat Ischemic Stroke Model

Li-Hua Shen[1], Jin Chen[1], Hua-Chao Shen[2], Min Ye[2], Xiao-Fei Liu[1], Wen-Sen Ding[1], Ya-Feng Sheng[1], Xin-Sheng Ding[2,3]

[1]Department of Neurology, The Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, China
[2]The BenQ Neurological Institute of Nanjing Medical University, Nanjing, Jiangsu 210029, China
[3]Department of Neurology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China

Abstract

Background: The functional improvement following bone marrow stromal cells (BMSCs) transplantation after stroke is directly related to the number of engrafted cells and neurogenesis in the injured brain. Here, we tried to evaluate whether 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186), a free radical scavenger, might influence BMSCs migration to ischemic brain, which could promote neurogenesis and thereby enhance treatment effects after stroke.

Methods: Rat transient middle cerebral artery occlusion (MCAO) model was established. Two separate MCAO groups were administered with either MCI-186 or phosphate-buffered saline (PBS) solution to evaluate the expression of stromal cell-derived factor-1 (SDF-1) in ischemic brain, and compared to that in sham group ($n = 5$/ group/time point[at 1, 3, and 7 days after operation]). The content of chemokine receptor-4 (CXCR4, a main receptor of SDF-1) at 7 days after operation was also observed on cultured BMSCs. Another four MCAO groups were intravenously administered with either PBS, MCI-186, BMSCs (2×10^6), or a combination of MCI-186 and BMSCs ($n = 10$/ group). 5-bromo-2-deoxyuridine (BrdU) and Nestin double-immunofluorescence staining was performed to identify the engrafted BMSCs and neuronal differentiation. Adhesive-removal test and foot-fault evaluation were used to test the neurological outcome.

Results: MCI-186 upregulated the expression of SDF-1 in ischemic brain and CXCR4 content in BMSCs was enhanced after hypoxic stimulation. When MCAO rats were treated with either MCI-186, BMSCs, or a combination of MCI-186 and BMSCs, the neurologic function was obviously recovered as compared to PBS control group ($P < 0.01$ or 0.05, respectively). Combination therapy represented a further restoration, increased the number of BMSCs and Nestin$^+$ cells in ischemic brain as compared with BMSCs monotherapy ($P < 0.01$). The number of engrafted-BMSCs was correlated with the density of neuronal cells in ischemic brain ($r = 0.72$, $P < 0.01$) and the improvement of foot-fault ($r = 0.70$, $P < 0.01$).

Conclusion: MCI-186 might promote BMSCs migration to the ischemic brain, amplify the neurogenesis, and improve the effects of cell therapy.

Key words: 3-Methyl-1-phenyl-2-pyrazolin-5-one; Bone Marrow Stromal Cells; Ischemic Stroke; Neurogenesis; Stromal Cell-derived Factor-1

Introduction

Stem cell therapy is a promising method to remodel the injured brain after ischemic stroke.[1] Bone marrow stromal cells (BMSCs) can be easily obtained from patients themselves without ethical or immunological problems. Interaction of BMSCs and ischemic brain might lead the stem cells to produce trophic factors and promote neurogenesis, those might contribute to function recover.[2,3] Therefore, BMSCs have emerged as a potential candidate for cell therapy after stroke. Stromal cell-derived factor-1/ chemokine receptor-4 (SDF-1/CXCR4) axis has been

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.183418

Address for correspondence: Prof. Xin-Sheng Ding, The BenQ Neurological Institute of Nanjing Medical University, 71 River Street, Nanjing, Jiangsu 210029, China
E-Mail: ding-xs@139.com

Received: 05-11-2015 **Edited by:** Peng Lyu
How to cite this article: Shen LH, Chen J, Shen HC, Ye M, Liu XF, Ding WS, Sheng YF, Ding XS. Possible Mechanism of Therapeutic Effect of 3-Methyl-1-phenyl-2-pyrazolin-5-one and Bone Marrow Stromal Cells Combination Treatment in Rat Ischemic Stroke Model. Chin Med J 2016;129:1471-6.

demonstrated to play an important role in BMSCs migration to injury tissue.[4,5] SDF-1 is presented around damaged central nervous system (CNS)[6,7] and its receptor CXCR4 is widely expressed on BMSCs. Therefore, the transplanted BMSCs have the potential to be guided toward ischemic brain, around which SDF-1 is expressed.[8]

3-Methyl-1-phenyl-2-pyrazolin-5-one (MCI-186), a free radical scavenger, can enhance the number of endogenous neural stem cells (NSCs) in the ischemic brain after a stroke.[9] However, there are no reports to evaluate whether MCI-186 can influence the migration of transplanted BMSCs to ischemic brain and thus amplify the neurogenesis and improve the effects of cell therapy. In the present study, we aimed to examine the hypothesis. *In vitro*, CXCR4 expression on normal and hypoxic BMSCs was examined using immunostaining and enzyme-linked immunosorbent assay (ELISA). *In vivo*, MCI-186 was injected into the right middle cerebral artery occlusion (MCAO) rats to monitor the level of SDF-1 in ischemic brain. Furthermore, we treated MCAO rats with BMSCs, MCI-186, or a combination of MCI-186 and BMSCs to determine the donor BMSCs migration toward ischemic brain and neurogenesis and to assess whether this combination treatment could induce additional functional improvement after a stroke.

Methods

All experimental procedures were approved by the Nantong University Administrative Panel on Laboratory Animal Care (No: NTU200805-14) and conformed to international guidelines on the ethical use of animals.

Isolation and characterization of bone marrow stromal cells

The BMSCs were harvested from male Sprague-Dawley rats (3–4 weeks old). The procedures were reported previously.[10] The adherent cells were passed 4 times. At passage 4 (P4), cells were harvested for experiments. Some BMSCs were incubated at 37°C in flasks to identify the localization of CXCR4 on BMSCs. A rabbit polyclonal antibody CXCR4 (1:200, Abcam, Hong Kong, China) was used for cellular fluorescence labeling. Hoechst 33342 (2 μg/ml, Sigma, USA) was used for staining cell nuclei. The rest cells were labeled with bromodeoxyuridine (5-bromo-2-deoxyuridine [BrdU], 30 μg/ml, Sigma, St. Louis, MO, USA) for 3 days before intravenous administration to identify the cell migration in the ischemic brain, and then the labeled cells were washed and digested for transplantation.

Enzyme-linked immunosorbent assay for chemokine receptor-4 in cultured bone marrow stromal cells

Cultured BMSCs (P4) were incubated under normoxic (21% O_2) or hypoxic (1% O_2) conditions for 24 h, then the culture mediums were withdrawn and the protein extract was obtained from BMSCs. CXCR4 was measured in these samples with an ELISA kit (Abcam, Hong Kong, China) for rat CXCR4 according to the manufacturer's instructions.

Fluorescent staining in cultured bone marrow stromal cells

Some normoxic (21% O_2) and hypoxic (1% O_2) BMSCs grown on coverslips were fixed with 4% formaldehyde for 30 min, subsequently treated with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 5 min and blocked with PBS containing 3% normal goat serum for 1 h. Then, the cells were incubated overnight at 4°C with anti-CXCR4 antibodies, washed 3 times with PBS, and incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (1:100, Sigma, USA) for 1 h. After additional washes, the slides were incubated with Hoechst 33342 for 3 min and mounted with an antifade solution. Cells were examined under a Leica fluorescence microscope (Leica DM4000B, Wetzlar, Germany).

Animal transient middle cerebral artery occlusion model and experimental groups

Adult male Sprague-Dawley rats weighing 240–270 g served as subjects, the animals were randomly allocated into different groups. All surgical procedures were conducted under aseptic conditions. MCAO rats were subjected to 2 h right MCAO via the intraluminal filament technique followed by reperfusion.[11] The sham group received the same surgical procedures except that the right-sided MCA was not occluded. During the operation, rectal temperature was monitored in all animals and temperature was maintained at constant (37.0 ± 0.5°C) with a heating pad and an overhead lamp. Rats in vehicle-treated MCAO control group and the sham-operated group received an injection of PBS via the tail vein 1 time. Rats in MCI-186-treated group received intraperitoneal injections of MCI-186 (3 mg/kg, Promega, Madison, WI, USA) immediately after MCAO. Because of the short half-life of MCI-186, we applied the free radical scavenger twice a day until day 7. Rats in BMSCs-treated group received an intravenous injection of BMSCs (2×10^6) via the tail vein 24 h after MCAO. Rats in combination therapy group received MCI-186 and BMSCs after MCAO. First, we observed the effects of MCI-186 on the expression of SDF-1 in ischemic brain. Animals from the sham-operated group, MCAO control group, and MCI-186 treated group were sacrificed at 1, 3, and 7 days after operation for reverse transcriptase-polymerase chain reaction (RT-PCR) (n = 5/group/time point), and immunofluorescence staining was performed at 7 days after operation for SDF-1 (n = 5/group). Second, animals from MCAO control group, MCI-186-treated group, BMSCs treated group, and combination therapy group (n = 10/group) were sacrificed at 7 days after operation for immunohistochemical staining to identify engrafted-BMSCs and evaluate neurogenesis. Neurological function tests were performed at each time point.

Reverse transcriptase-polymerase chain reaction assay of stromal cell-derived factor-1

Total RNA was isolated from the ischemic boundary zone (IBZ, between the infarct core area and noninfarct area) using TRIzol reagent. RT-PCR was performed using Promega Revert Aid First Strand cDNA Synthesis Kit (Madison, WI, USA) according to

the manufacturer's instructions. cDNA was synthesized with oligo-dT primers, and PCR was performed with designed primers using a master mix purchased from Promega. PCR products (5 μl) were analyzed in 2% agarose electrophoretic gels stained with ethidium bromide and visualized by ultraviolet transillumination. The density of each band was quantified with the Image 1.59 program (Vilber Lourmat, France). All results represented the average density of positive bands obtained from five independent experiments using different reaction cycles. The PCR primers used in this study were designed using online software Applied Biosystems, USA. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. The sequences of the primers were as follows:

- SDF-1: Forward: 5-GGTCTGGAGACTATGA CTCCA-3; Reverse: 5-GTGCTGGAACTG GAACACCA-3
- GAPDH: Forward: 5-GTTCCAGTAT GACTCTACCC-3; Reverse: 5-AGTCTTCT GAGTGGCAGTGA TGGC-3.

For quantitative results, all samples were normalized against the GAPDH control band, and the results were expressed as the relative fold change of SDF-1/GAPDH.

Immunofluorescence staining of brain sections of rats

The brains were fixed in 4% paraformaldehyde and embedded in paraffin, and a series of 5 μm thick coronal sections were cut from a standard paraffin block obtained from the center of the lesion (Bregma −1 to +1 mm) for immunostaining. Some sections were treated with the primary antibody of rabbit polyclonal anti-SDF-1 (1:100, Chemicon, USA) at room temperature for 2 h. After incubation, sections were washed in PBS and placed for 2 h in FITC-conjugated goat anti-rabbit IgG (1:100, Sigma, USA) for SDF-1 staining.

Double-immunofluorescence staining was employed to visualize the cellular colocalization of BrdU and Nestin for engrafted BMSCs and neuronal stem cells. Sections were incubated in 2 mol/L HCl at 37°C for 30 min to denature DNA and exposed BrdU, rinsed thoroughly in PBS and incubated in a mixture of mouse anti-BrdU (1:100, Sigma, USA) and rabbit anti-Nestin (1:100, Sigma, USA) at room temperature for 2 h. After incubation, sections were washed in PBS and placed for 2 h in tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse IgG (1:100, Sigma, USA) for BrdU and FITC-conjugated goat anti-rabbit IgG (1:100, Sigma, USA) for Nestin. All sections were visualized with a fluorescence microscope (Leica DM4000B, Wetzlar, Germany). Immunoreactive cells were photographed using a 3-CCD color video camera (DXC-970MD, Sony, Japan) interfaced with Micro Computer Imaging Device software (Imaging Research, Leica, Germany).

For quantitative measurements of BrdU and Nestin positive cells, five sections from each block, each containing eight fields consisting of cortex and stratum from the IBZ[12] was digitized. Cell counts were averaged for each animal and expressed as cells/mm^2. All analyses were performed by observers who were blinded to the experimental groups.

Neurological behavioral tests

BMSCs treated, MCI-186 treated, and combination treated MCAO rats (n = 10/group) received an adhesive-removal test and foot-fault evaluation[13,14] and were compared to the MCAO control group. Before MCAO and at 1, 3, and 7 days after MCAO, two investigators who were blinded to the experimental groups performed the assessment.

Statistical analysis

All measurements were performed blinded. Results were presented as a mean ± standard deviation (SD). Statistical analyses were performed using SPSS 16.0 software (IBM, Chicago, IL, USA). The statistical differences between different groups were evaluated with one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant. Correlation analysis was used to discuss the relationship.

Results

Chemokine receptor-4 expression on bone marrow stromal cells

CXCR4 expression on normoxic and hypoxic BMSCs was evaluated using immunostaining [Figure 1a] and ELISA [Figure 1b], respectively. Immunostaining showed

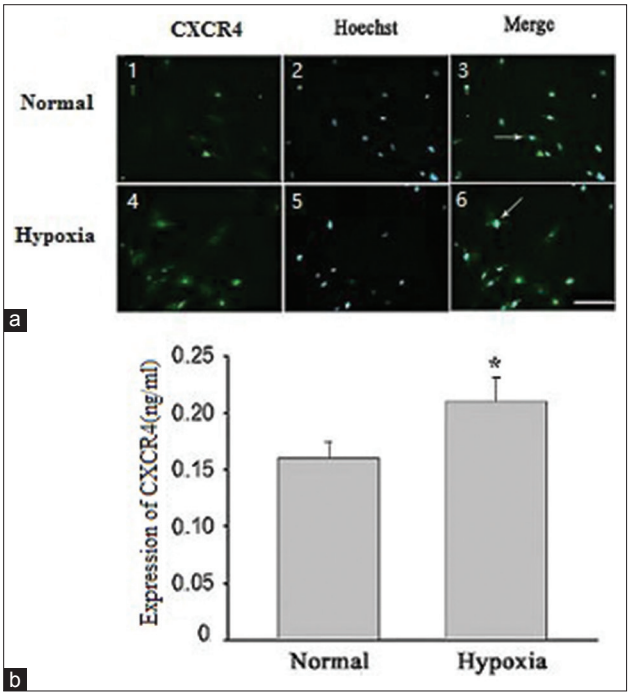

Figure 1: Analysis of the expression of CXCR4 in BMSCs. (a) Immunofluorescence staining for CXCR4 and Hoechst 33342 in two BMSC groups. CXCR4 (green, 1) and Hoechst 33342 (blue, 2) in normal BMSC group, CXCR4 (green, 4) and Hoechst 33342 (blue, 5) in hypoxic BMSC group. Panels 3 and 6 are the merged images, respectively. Arrows represent the double-immunofluorescence cells. Scale bar indicates 100 μm in both normal and hypoxic groups. (b) ELISA revealed that the content of CXCR4 protein in hypoxic BMSCs was higher than that in normoxic culture cells (n = 5). *$P < 0.05$ versus normoxic cultured BMSCs. CXCR4: Chemokine receptor-4; BMSCs: Bone marrow stromal cells; ELISA: Enzyme-linked immunosorbent assay.

the cultured BMSCs under normoxic condition just expressed a marginal CXCR4, but when cells were stimulated by hypoxia, the expression of CXCR4 was obviously upregulated. ELISA also revealed that the content of CXCR4 protein in hypoxic BMSCs was higher than that in normoxic culture cells ($P < 0.05$).

3-Methyl-1-phenyl-2-pyrazolin-5-one upregulated stromal cell-derived factor-1 expression in ischemic brain

The expression of SDF-1 in ischemic brain was monitored using immunostaining and RT-PCR. Immunofluorescent staining revealed that MCAO substantially increased the density of SDF-1 positive cells in the IBZ [Figure 2a]. RT-PCR also found that SDF-1 mRNA [Figure 2b and 2c] began to increase on day 1, peaked on day 3, and remained high on day 7 in the IBZ ($P < 0.01$, respectively) after MCAO as compared to sham. MCI-186 upregulated the expression of SDF-1 after ischemic stroke and postponed their decline ($P < 0.05$ vs. MCAO control group, $P < 0.01$ vs. MCAO control group, respectively).

Combination therapy significantly improved neurological outcome

Figure 3 shows that either MCI-186 or BMSCs monotherapy caused an improvement on adhesive-removal [Figure 3a] and foot-fault [Figure 3b] tests at 7 days after MCAO ($P < 0.01$ vs. MCAO control group, respectively). However, the two functional outcome tests showed an additive effect on combination therapy in comparison with either MCI-186 or BMSCs monotherapy at the same time ($P < 0.01$ or 0.05, respectively) after MCAO.

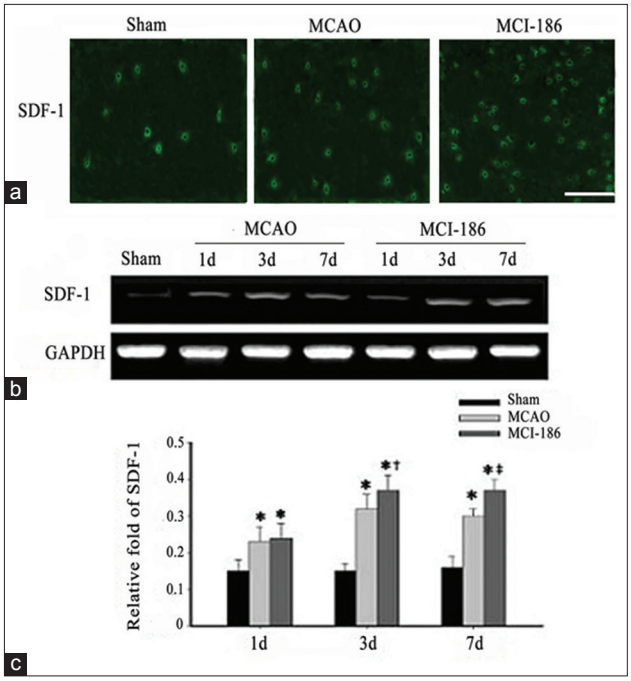

Figure 2: Effects of MCI-186 on SDF-1 expression in ischemic brain. (a) SDF-1 expression (measured by immunofluorescence staining) in three groups at day 7 after operation. Scale bar indicates 200 μm. (b) SDF-1 expression (measured by reverse transcriptase-polymerase chain reaction) in three groups at 3 time points. (c) Relative fold change in SDF-1 in the three groups. *$P < 0.01$ versus sham operation group; †$P < 0.05$ versus MCAO control group; ‡$P < 0.01$ versus MCAO control group. MCI-186: 3-methyl-1-phenyl-2-pyrazolin-5-one; SDF-1: Stromal cell-derived factor-1; MCAO: Middle cerebral artery occlusion.

Combination therapy significantly enhanced the number of engrafted bone marrow stromal cells and neurogenesis in ischemic brain

BrdU and Nestin double-immunofluorescence staining was performed to identify the engrafted BMSCs and neuronal differentiation [Figure 4a] at 7 days after MCAO. BrdU-positive cell primarily aggregated in IBZ, and the number of BMSC was significantly higher after combination therapy than that of BMSCs monotherapy [Figure 4b, $P < 0.01$, $n = 10$/group], the number of engrafted-BMSCs in ischemic brain was correlated with improvement of foot-fault ($r = 0.70$, $P < 0.01$). In addition, some $BrdU^+$-BMSCs (red) were double-labeled with Nestin [a marker of neural progenitors; Figure 4a, green] in ischemic brain, Figure 4b shows the statistic difference of $Nestin^+$ cell numbers between the two groups ($P < 0.01$). There was a close correlation between the density of neuronal cells and the number of engrafted-BMSCs in the ischemic brain ($r = 0.72$, $P < 0.01$).

Discussion

Transplanted BMSCs can migrate selectively into ischemic brain area and present a promising recovery from stroke[15] SDF-1/CXCR4 axis has been identified as a critical mediator for stem cell migration.[16] SDF-1 is a small molecule with alternatively spliced forms, belonging to the CXC chemokine family.[17] In CNS, SDF-1 is triggered in the impaired tissue subsequent to ischemic attack,[18] presents a significant role in orchestrating cells and promotes axon outgrowth and neurogenesis.[8,19] CXCR4 is widely expressed on BMSCs.[4] The interaction of CXCR4 and SDF-1 directs BMSCs migrating toward ischemic brain, around which SDF-1 is presented abundantly,[20] and inhibition of CXCR4 expression decreases BMSCs movement.[21]

In the present study, SDF-1 expression was upregulated in IBZ from day 1, and peaked at day 3 after stroke, correlating well with previous reports.[22] MCI-186 amplified the effects and kept the high level to day 7. MCI-186 has shown a strong inhibition of DNA damage after brain injury.[23] Both human and animal experiments revealed that MCI-186 could protect NSCs from irradiation and increase their proliferation around the brain lesion.[24-26] In the previous study, we also observed that MCI-186 significantly reduced the consumption of antioxidase superoxide dismutase and the oxidative product of malondialdehyde,[27] the mechanism of its improving SDF-1 expression needs further explore.

In parallel, *in vitro* experiments showed that CXCR4 content in BMSCs was elevated under hypoxia as compared to normal cultured condition, so it might be possible that the hypoxia brain environment might also stimulate the

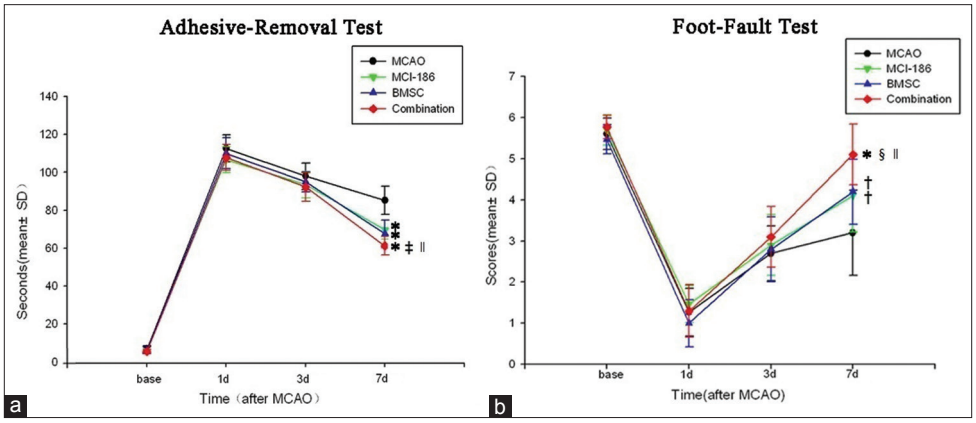

Figure 3: Combination therapy significantly improved restoration. Combination therapy and BMSCs or MCI-186 monotherapy improved functional outcome (adhesive-removal and foot-fault evaluation tests) in MCAO rats (a and b). *$P < 0.01$ versus MCAO control group; †$P < 0.05$ versus MCAO control group; ‡$P < 0.01$ versus MCI-186 group; §$P < 0.05$ versus MCI-186 group; ||$P < 0.05$ versus BMSCs group. BMSCs: Bone marrow stromal cells; MCI-186: 3-methyl-1-phenyl-2-pyrazolin-5-on; MCAO: Middle cerebral artery occlusion; SD: Standard deviation.

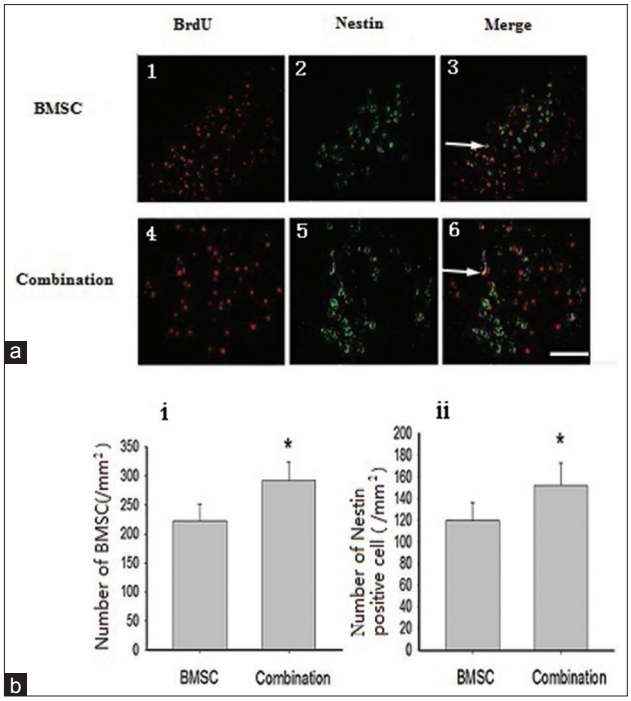

Figure 4: Combination therapy improved the transplantation and differentiation of BMSCs in ischemic brain. (a) Immunofluorescence staining for BrdU and Nestin positive cells at day 7 after MCAO. BrdU (red, 1) and Nestin (green, 2) in BMSCs monotherapy group, BrdU (red, 4) and Nestin (green, 5) in the combination group. Panels 3 and 6 are the merged images, respectively. Arrows represent the double-immunofluorescence cells. Scale bar indicates 200 μm in BMSC and combination group. (b) The number of engrafted-BMSCs (i) and Nestin positive cells (ii) in BMSCs monotherapy group and combination group. *$P < 0.01$ versus BMSCs monotherapy group. BMSCs: Bone marrow stromal cells; BrdU: 5-bromo-2-deoxyuridine; MCAO: Middle cerebral artery occlusion.

expression of CXCR4 on BMSCs and contribute to cell migration.

When transplantation of BMSCs in MCAO rats, our study showed the combination therapy of BMSCs and MCI-186 obviously increased the number of engrafted-BMSCs in ischemic brain and induced additional functional improvement, there was a tight correlation between them. Our data suggested that MCI-186 promoting the trafficking of BMSCs to ischemic brain might be connected with the upregulated expression of SDF-1 in IBZ, and the efficiency of the cell therapy might be correlated with the number of BMSC retained in ischemic brain.

The neuroprotective effects of BMSCs arise not only from their ability to provide trophic support to neurons at risk near the infarct area but also from their differentiation to neurotization when they are engrafted into damaged CNS.[28] The intermediate filament protein Nestin is a widely accepted molecular marker for NSCs[29] and has the potential to develop into neuroectodermal, endodermal, and mesodermal lineages. It is reported in adult, the maximal level of Nestin expression after brain ischemia was on day 7 and continued to 4 weeks. However, the number of endogenous NSCs is too small to meet the need of damage. Here, we found some transplanted BMSCs ($BrdU^+$ cells) co-located with $Nestin^+$ cells and revealed that the engrafted-BMSCs could differentiate into neural lines and improve neurogenesis. Thus, the increased number of engrafted-BMSCs and NSCs in modified ischemic brain mediated by combination therapy might improve the efficiency of cell transplantation.

There are several limitations in this study. First, the mechanism of MCI-186 up-regulating the expression of SDF-1 in ischemic brain is not very clear. Second, it is better to add a group of using hypoxic BMSCs to treat ischemic rats to see whether the therapeutic effect is better. Third, it should be better to use whole brain coronal sections for immunostaining and to add a dynamic observation, such as days 1, 3, and 7.

In summary, our data demonstrated that MCI-186 could promote transplanted BMSCs migrating to the ischemic brain by stimulating SDF-1/CXCR4 axis, thereby enhance

neurotization and produce additive effects on BMSCs transplantation in rat ischemic stroke model.

Financial support and sponsorship

This work was supported by grants from National Natural Science Foundation of China (No. 30971022) and Natural Science Foundation of Jiangsu Province (No. BK2008471).

Conflicts of interest

There are no conflicts of interest.

References

1. Yan T, Venkat P, Chopp M, Zacharek A, Ning R, Cui Y, *et al.* Neurorestorative therapy of stroke in type 2 diabetes mellitus rats treated with human umbilical cord blood cells. Stroke 2015;46:2599-606. doi: 10.1161/STROKEAHA.115.009870.
2. Qi X, Shao M, Peng H, Bi Z, Su Z, Li H. *In vitro* differentiation of bone marrow stromal cells into neurons and glial cells and differential protein expression in a two-compartment bone marrow stromal cell/neuron co-culture system. J Clin Neurosci 2010;17:908-13. doi: 10.1016/j.jocn.2009.10.013.
3. Tsai MJ, Tsai SK, Hu BR, Liou DY, Huang SL, Huang MC, *et al.* Recovery of neurological function of ischemic stroke by application of conditioned medium of bone marrow mesenchymal stem cells derived from normal and cerebral ischemia rats. J Biomed Sci 2014;21:5. doi: 10.1186/1423-0127-21-5.
4. Cui X, Chen J, Zacharek A, Roberts C, Yang Y, Chopp M. Nitric oxide donor up-regulation of SDF1/CXCR4 and Ang1/Tie2 promotes neuroblast cell migration after stroke. J Neurosci Res 2009;87:86-95. doi: 10.1002/jnr.21836.
5. Wang Y, Fu W, Zhang S, He X, Liu Z, Gao D, *et al.* CXCR-7 receptor promotes SDF-1α-induced migration of bone marrow mesenchymal stem cells in the transient cerebral ischemia/reperfusion rat hippocampus. Brain Res 2014;1575:78-86. doi: 10.1016/j.brainres.2014.05.035.
6. Xu X, Zhu F, Zhang M, Zeng D, Luo D, Liu G, *et al.* Stromal cell-derived factor-1 enhances wound healing through recruiting bone marrow-derived mesenchymal stem cells to the wound area and promoting neovascularization. Cells Tissues Organs 2013;197:103-13. doi: 10.1159/000342921.
7. Wang Y, Deng Y, Zhou GQ. SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. Brain Res 2008;1195:104-12. doi: 10.1016/j.brainres.2007.11.068.
8. Lanfranconi S, Locatelli F, Corti S, Candelise L, Comi GP, Baron PL, *et al.* Growth factors in ischemic stroke. J Cell Mol Med 2011;15:1645-87. doi: 10.1111/j.1582-4934.2009.00987.x.
9. Han Q, Shen LH, Zhang C, Wu EB, Liu XF. Effects of edaravne on endogenous neural stem cells in cerebral ischemic rats. J Clin Rehabil Tissue Eng Res 2011;15:5962-66. doi: 10.3969/j.issn.1673-8225.2011.32.016.
10. Deng W, Bivalacqua TJ, Chattergoon NN, Jeter JR Jr., Kadowitz PJ. Engineering *ex vivo*-expanded marrow stromal cells to secrete calcitonin gene-related peptide using adenoviral vector. Stem Cells 2004;22:1279-91. doi: 10.1634/stemcells.2004-0032.
11. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989;20:84-91. doi: 10.1161/01.STR.20.1.84.
12. Chen J, Cui X, Zacharek A, Chopp M. Increasing Ang1/Tie2 expression by simvastatin treatment induces vascular stabilization and neuroblast migration after stroke. J Cell Mol Med 2009;13:1348-57. doi: 10.1111/j.1582-4934.2008.00380.x.
13. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, *et al.* Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 2003;73:778-86. doi: 10.1002/jnr.10691.
14. Chen YL, Hsieh CL, Wu PH, Lin JG. Effect of *Polygala tenuifolia* root on behavioral disorders by lesioning nucleus basalis magnocellularis in rat. J Ethnopharmacol 2004;95:47-55. doi: 10.1016/j.jep.2004.06.015.
15. Deng YB, Ye WB, Hu ZZ, Yan Y, Wang Y, Takon BF, *et al.* Intravenously administered BMSCs reduce neuronal apoptosis and promote neuronal proliferation through the release of VEGF after stroke in rats. Neurol Res 2010;32:148-56. doi: 10.1179/174313209X414434.
16. Liu H, Liu S, Li Y, Wang X, Xue W, Ge G, *et al.* The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. PLoS One 2012;7:e34608. doi: 10.1371/journal.pone.0034608.
17. Cencioni C, Capogrossi MC, Napolitano M. The SDF-1/CXCR4 axis in stem cell preconditioning. Cardiovasc Res 2012;94:400-7. doi: 10.1093/cvr/cvs132.
18. Jaerve A, Bosse F, Müller HW. SDF-1/CXCL12: Its role in spinal cord injury. Int J Biochem Cell Biol 2012;44:452-6. doi: 10.1016/j.biocel.
19. Opatz J, Küry P, Schiwy N, Järve A, Estrada V, Brazda N, *et al.* SDF-1 stimulates neurite growth on inhibitory CNS myelin. Mol Cell Neurosci 2009;40:293-300. doi: 10.1016/j.mcn.
20. Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, *et al.* Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A 2004;101:18117-22. doi: 10.1073/pnas.0408258102.
21. Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A, *et al.* One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. Stroke 2007;38:2150-6. doi: 10.1161/strokeaha.106.481218.
22. Shyu WC, Lee YJ, Liu DD, Lin SZ, Li H. Homing genes, cell therapy and stroke. Front Biosci 2006;11:899-907. doi: 10.2741.
23. Takizawa Y, Miyazawa T, Nonoyama S, Goto Y, Itoh M. Edaravone inhibits DNA peroxidation and neuronal cell death in neonatal hypoxic-ischemic encephalopathy model rat. Pediatr Res 2009;65:636-41. doi: 10.1203/PDR.0b013e3181a16a9f.
24. Ishii J, Natsume A, Wakabayashi T, Takeuchi H, Hasegawa H, Kim SU, *et al*. The free-radical scavenger edaravone restores the differentiation of human neural precursor cells after radiation-induced oxidative stress. Neurosci Lett 2007;423:225-30. doi: 10.1016/j.neulet.2007.07.029.
25. Itoh T, Satou T, Nishida S, Tsubaki M, Hashimoto S, Ito H. The novel free radical scavenger, edaravone, increases neural stem cell number around the area of damage following rat traumatic brain injury. Neurotox Res 2009;16:378-89. doi: 10.1007/s12640-009-9081-6.
26. Itoh T, Satou T, Nishida S, Tsubaki M, Imano M, Hashimoto S, *et al.* Edaravone protects against apoptotic neuronal cell death and improves cerebral function after traumatic brain injury in rats. Neurochem Res 2010;35:348-55. doi: 10.1007/s11064-009-0061-2.
27. Shen LH, Ye M, Ding XS, Han Q, Zhang C, Liu XF, *et al.* Protective effects of MCI-186 on transplantation of bone marrow stromal cells in rat ischemic stroke model. Neuroscience 2012;223:315-24. doi: 10.1016/j.neuroscience.
28. Vaquero J, Zurita M. Bone marrow stromal cells for spinal cord repair: A challenge for contemporary neurobiology. Histol Histopathol 2009;24:107-16.
29. Gilyarov AV. Nestin in central nervous system cells. Neurosci Behav Physiol 2008;38:165-9. doi: 10.1007/s11055-008-0025-z.