

Hyperbaric oxygen improves functional recovery of rats after spinal cord injury via activating stromal cell-derived factor-1/CXC chemokine receptor 4 axis and promoting brain-derived neurotrophic factor expression

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Abstract

Background: Spinal cord injury (SCI) is a worldwide medical concern. This study aimed to elucidate the mechanism underlying the protective effect of hyperbaric oxygen (HBO) against SCI-induced neurologic defects in rats via exploring the stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) axis and expression of brain-derived neurotrophic factor (BDNF).

Methods: An acute SCI rat model was established in Sprague-Dawley rats using the Allen method. Sixty rats were divided into four groups ($n=15$ in each group): sham-operated, SCI, SCI treated with HBO (SCI+HBO), and SCI treated with both HBO and AMD3100 (an antagonist of CXCR4; SCI+HBO+AMD) groups. The rats were treated with HBO twice a day for 3 days and thereafter once a day after the surgery for up to 28 days. Following the surgery, neurologic assessments were performed with the Basso-Bettie-Bresnahan (BBB) scoring system on postoperative day (POD) 7, 14, 21, and 28. Spinal cord tissues were harvested to assess the expression of SDF-1, CXCR4, and BDNF at mRNA and protein levels, using quantitative real-time polymerase chain reaction, Western blot analysis, and histopathologic analysis.

Results: HBO treatment recovered SCI-induced descent of BBB scores on POD 14, (1.25 ± 0.75 vs. 1.03 ± 0.66 , $P < 0.05$), 21 (5.27 ± 0.89 vs. 2.56 ± 1.24 , $P < 0.05$), and 28 (11.35 ± 0.56 vs. 4.23 ± 1.20 , $P < 0.05$) compared with the SCI group. Significant differences were found in the mRNA levels of SDF-1 (mRNA: day 21, SCI+HBO vs. SCI+HBO+AMD, 2.89 ± 1.60 vs. 1.56 ± 0.98 , $P < 0.05$), CXCR4 (mRNA: day 7, SCI+HBO vs. SCI, 2.99 ± 1.60 vs. 1.31 ± 0.98 , $P < 0.05$; day 14, SCI+HBO vs. SCI+HBO+AMD, 4.18 ± 1.60 vs. 0.80 ± 0.34 , $P < 0.05$; day 21, SCI+HBO vs. SCI, 2.10 ± 1.01 vs. 1.15 ± 0.03 , $P < 0.05$), and BDNF (mRNA: day 7, SCI+HBO vs. SCI, 3.04 ± 0.41 vs. 2.75 ± 0.31 , $P < 0.05$; day 14, SCI+HBO vs. SCI, 3.88 ± 1.59 vs. 1.11 ± 0.40 , $P < 0.05$), indicating the involvement of SDF-1/CXCR4 axis in the protective effect of HBO.

Conclusions: HBO might promote the recovery of neurologic function after SCI in rats via activating the SDF-1/CXCR4 axis and promoting BDNF expression.

Keywords: Brain-derived neurotrophic factor; CXC chemokine receptor 4; Hyperbaric oxygen; Neurotrophic; Stromal cell-derived factor-1; Spinal cord injury

Introduction

Spinal cord injury (SCI) has a significant socioeconomic impact on society due to its associated substantial health care expenditures.^[1] The progression of SCI encompasses two phases: primary and secondary injuries. The primary injury is typically induced by the initial uncontrollable mechanical damage. The secondary injury occurs when a series of cellular and molecular “cascades” are initiated,

usually a few minutes after the primary damage, such as hemorrhage, edema, and inflammation. Nerve repair and regeneration may be hindered with excessive inflammatory responses.^[2] Numerous studies have been performed to improve the treatment of SCI by reducing secondary inflammatory responses.^[3,4] However, the SCI treatment is still a worldwide medical concern. Hyperbaric oxygen (HBO) therapy involves administration of 100% oxygen at a controlled pressure for a prescribed period of time

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(60–90 min).^[5] It is beneficial for neurologic recovery in acute and chronic SCI.^[6]

Effective SCI treatment targets two major pathologic channels: regulating inflammatory responses and promoting neuron regeneration. Stromal cell-derived factor-1 (SDF-1), also known as CXCL12, is the only known ligand for CXC chemokine receptor 4 (CXCR4).^[7] Further, the SDF-1/CXCR4 axis exerts a protective effect against inflammatory responses following SCI.^[8] Our previous study confirmed that HBO preconditioning promoted neovascularization via increasing the expression levels of SDF-1 and CXCR4 in transplanted skin flaps of rats.^[9] Brain-derived neurotrophic factor (BDNF) has a pivotal role in structural plasticity, learning, and memory.^[10] It prevents neurodegeneration and acts as one of the synaptic plasticity markers. The neuroprotective effect of BDNF correlates with its ability to modulate the expression of CXCR4 and SDF-1^[11–13] indicating the involvement of both SDF-1/CXCR4 axis and BDNF in the SCI pathophysiologic process. This study showed that HBO effectively improved SCI-induced neurologic functional defects in a rat model via the classical SDF-1/CXCR4 axis and promoting the expression of neurotrophic factor BDNF. The findings not only provided clinical guidance for effective and safe clinical treatment of SCI but also laid a solid foundation for future in-depth mechanistic investigations.

Methods

Ethical approval

The experiment protocol was approved by the Committee of the Ethics of Animal Experiments of Capital Medical University (Protocol ID: 2010-D-013). All surgeries were performed under chloral hydrate anesthesia with additional efforts made to ensure animal welfare.

Animal care

Healthy adult male Sprague-Dawley rats (250–300 g) were purchased from Beijing Vital River Laboratory Animal Technology Company (Beijing, China) and housed in individual cages in a temperature-controlled setting with 12-h light and dark cycles and free access to food and distilled water.

Rat model of acute SCI

A baseline behavioral assessment was conducted before the surgery. Acute SCI surgeries were performed using the modified Allen test, as previously described.^[14] During the surgery, the rats were anesthetized with the intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g) following the 12-h preoperative fasting of food and water. A dorsal laminectomy was performed on the center of T9 spinous process to expose the spinal cord under sterile conditions. Subsequently, each rat sustained a contusive SCI from a Spinal Cord Impactor (W.M. Keck Center for Collaborative Neuroscience, Rutgers, NJ), with a 10-g impact rod vertically dropped from a height of 5 cm, which then impinged on the spinal cord in a circular zone with a 2-mm

diameter. The dwell time for each injury was 10 s, which was sufficient to cause a moderate contusion. The release weight, height of drop, and velocity of each SCI were determined using the Spinal Cord Impactor software (version 7.5). The signs of the successful infliction of SCI were as follows: spastic swinging of rat tails, retraction of the lower limbs and a torso-like flutter, and flaccid paralysis of both hind extremities. In the sham-operated group, the spinal cord was exposed in the same manner as described earlier, but without the contusive SCI procedure.

Experimental grouping

Sixty rats were randomly assigned to one of the following four groups ($n=15$ in each group) according to random number table: (1) sham-operated (SH); (2) SCI; (3) spinal cord injury+hyperbaric oxygen treatment (SCI+HBO); and (4) spinal cord injury+chemokine receptor antagonist (AMD3100)+hyperbaric oxygen treatment (SCI+HBO+AMD). The rats in each group were then randomly divided into five subgroups for harvesting at various endpoints of postoperative day (POD) 0, 7, 14, 21, and 28. For the sham (SH) group, only a laminectomy was performed without either SCI procedure or HBO treatment. For the SCI group, no treatment was given. For the SCI+HBO group, HBO was performed after the surgery following a detailed method described later. For the SCI+HBO+AMD group, the rats were treated with AMD3100 (5 mg/kg; Pfizer, Inc., NY, USA) using a microsyringe (Hamilton), immediately after the SCI procedure and before HBO treatment. The AMD3100 dosage was selected based on previous findings.^[15]

Hyperbaric oxygen intervention

For HBO treatment, both SCI+HBO and SCI+HBO+AMD groups were exposed to 100% O₂ at 2.0 ATA for 1 h in a hyperbaric chamber (701 Space Research Institute, Beijing, China) twice a day at 12-h intervals for three consecutive days and thereafter once a day, starting from POD 0 to POD 28. Compression and decompression of gas were carried out at a rate of 0.2 kg·cm⁻²·min⁻¹. During the HBO exposure, oxygen and carbon dioxide contents were continuously monitored and maintained at ≥98% and ≤0.03%, respectively. The temperature of the chamber was maintained at a range of 22°C to 25°C. After exposure to HBO, the rats were maintained in a normoxic environment. For the sham group, the rats were treated with normobaric air at 1.0 ATA in 21% O₂ at an ambient temperature of 22°C to 25°C.

Histological assessment

At desired time points (POD 0, 7, 14, 21, and 28), the animals were euthanized in a CO₂ chamber. The spinal cord tissues containing an SCI-injured epicenter were dissected, fixed in 10% neutral buffered formaldehyde at 4°C for 1 week, and embedded in paraffin. Each block was serially sectioned horizontally at 5 μm. The sections were stained with hematoxylin and eosin (H&E). For immunohistochemical staining analysis, the slides were

deparaffinized, rehydrated, and subjected to antigen retrieval with EDTA solution (pH 8.0). The tissue sections were blocked in 2% goat serum and incubated with various primary antibodies, including SDF-1 (Abcam, Cambridge, MA, USA) at dilution 1:200, BDNF (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at dilution 1:100, and CXCR4 (Abcam) at dilution 1:500 overnight at 4°C. The horseradish peroxidase-labeled mouse/rabbit secondary antibody (Zhongshan Golden Bridge, Beijing, China) was used to detect both the primary antibodies. The standard 3, 3'-diaminobenzidine procedure was adopted to visualize the signal. Hematoxylin was used for counterstaining. The immunoglobulin G control was performed following identical procedures, excluding the primary antibody.

Western blot analysis

At various time points, freshly isolated spinal cord samples were homogenized using radioimmunoprecipitation lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) and then centrifuged at $12,000 \times g$ for 15 min. The supernatant containing 50 µg total protein was subjected to electrophoresis on a 10% sodium dodecyl sulfate gel (Beyotime Institute of Biotechnology) and then transferred onto polyvinylidene fluoride (PVDF; Millipore, MA) membranes. The PVDF membranes were blocked with Tris-buffered saline with Tween-20 (Biosciences, Shanghai, China) and incubated with primary antibodies, including anti-CXCR4 (1:200; Abcam), anti-SDF-1 antibody (1:100; Abcam), BDNF (1:200; Santa Cruz), or rabbit beta-actin antibody (1:2000; Xianzhi Biological Co. Ltd., Hangzhou, China) at 4°C overnight, followed by incubation with horseradish peroxidase-labeled goat anti-rabbit secondary antibody (A00098; 1:50,000; Santa Cruz Biotechnology, Inc.) at room temperature for 2 h. Blots were developed with enhanced chemiluminescence agents (ECL Plus; Beijing Sunbio Biotech Co. Ltd., Beijing, China) prior to X-ray exposure. For quantification, Western blotting films were scanned using a Minolta scanner (Konica Minolta, Inc.) and the Adobe Photoshop software (Adobe Systems Inc., San Jose, CA). The labeling density was quantitated using the LabWorks software (UVP Inc., Upland, CA). The relative expression of SDF-1, CXCR4, and BDNF was obtained via dividing target band density by actin density.

Real-time polymerase chain reaction

Total RNA was extracted from frozen spinal cord tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and RNA kit (Sangon, Shanghai, China). RNA was then reverse transcribed to synthesize cDNA. Quantitative real-time polymerase chain reaction (PCR) was performed using a Line-Gen sequence detector (ABI, Vernon, CA, USA). PCRs were detected by incorporating SYBR green using a BioEasy SYBR Green I real-time PCR kit (Biomed, Bioer, Hangzhou, China) and authenticated by an amplification curve. PCR amplification was performed for 40 cycles of 20 s at 95°C, 25 s at 60°C, and 30 s at 72°C. Data were analyzed using the $2^{-\Delta\Delta C_t}$ method. The sequences of primers are illustrated in Supplementary Table 1, <http://links.lww.com/CM9/A16>.

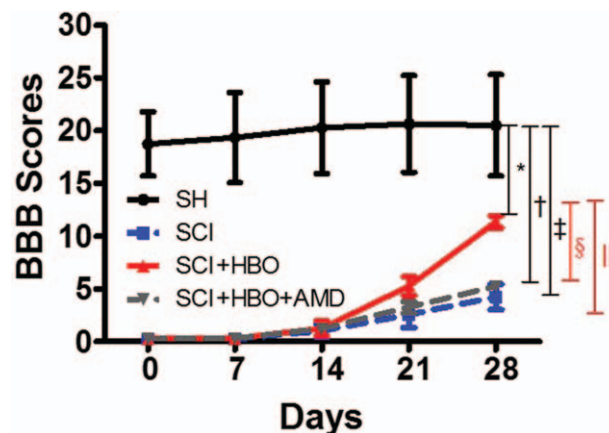


Figure 1: Bass-Bettie-Bresnahan (BBB) score as a functional evaluation of all animal groups ($n=15$ in each group). At early time points POD Day 7 and Day 14, the spinal cord injury (SCI), SCI + hyperbaric oxygen treatment (HBO), and SCI + HBO + AMD groups showed significantly lower BBB scores compared with the sham-operated (SH) group. HBO treatment significantly improved animal functional recovery, as suggested by a gradual elevation of BBB score in the SCI + HBO group to a sham baseline level, on both POD 21 and 28. *SH vs. SCI (Day 0, Day 7, Day 14, Day 21, Day 28, $P < 0.05$), †SH vs. SCI + HBO (Day 0, Day 7, Day 14, Day 21, Day 28, $P < 0.05$), ‡SH vs. SCI + HBO + AMD (Day 0, Day 7, Day 14, Day 21, Day 28, $P < 0.05$), §SCI vs. SCI + HBO (Day 14, Day 21, Day 28, $P < 0.05$), ||SCI + HBO vs. SCI + HBO + AMD (Day 14, Day 21, Day 28, $P < 0.05$). SCI group: SCI procedure, without HBO treatment; SCI + HBO group: HBO was performed after SCI procedure; SCI + HBO + AMD group: Treated with AMD3100 (5 mg/kg) immediately after SCI procedure and before HBO treatment; SH group: Sham-operated, without either SCI procedure or HBO treatment. AMD: Chemokine receptor antagonist; BBB: Bass-Bettie-Bresnahan; HBO: Hyperbaric oxygen; POD: Postoperative day; SCI: Spinal cord injury.

Statistical analysis

Data were statistically analyzed using the GraphPad Prism software, version 5.0 (GraphPad Software Inc., USA). Quantitative data were expressed as the mean \pm standard deviation (SD). Two-way analysis of variance (ANOVA) or t test was used to test the differences in the levels of mRNA or proteins of SDF-1, CXCR4, and BDNF. A P value < 0.05 was considered to be statistically significant.

Results

SCI model and Basso-Bettie-Bresnahan score

Basso-Bettie-Bresnahan (BBB) scale is a valid and predictive measure of locomotor recovery. It can distinguish behavioral outcomes due to different injuries and predict anatomical alterations at the lesion center. BBB scores for the SH, SCI, SCI+HBO, and SCI+HBO+AMD groups were assessed at various time points after the surgery to elucidate the effect of HBO on functional recovery in SCI-injured rats. Totally, HBO treatment recovered SCI-induced descent of BBB scores on POD 14 (1.25 ± 0.75 vs. 1.03 ± 0.66 , $P < 0.05$), 21 (5.27 ± 0.89 vs. 2.56 ± 1.24 , $P < 0.05$), and 28 (11.35 ± 0.56 vs. 4.23 ± 1.20 , $P < 0.05$) compared with the SCI group. As shown in Figure 1, the sham (SH) group demonstrated a baseline BBB score (≥ 20 points) before and after the surgery, while all SCI-injured (with or without treatment) groups showed complete paralysis of both lower extremities with a BBB score of 0 to 3 at early time points (Day 7 and Day 14), suggesting SCI-induced neurologic damage. Intriguingly, at later time points (Day 21 and Day 28), unlike SCI only and SCI+

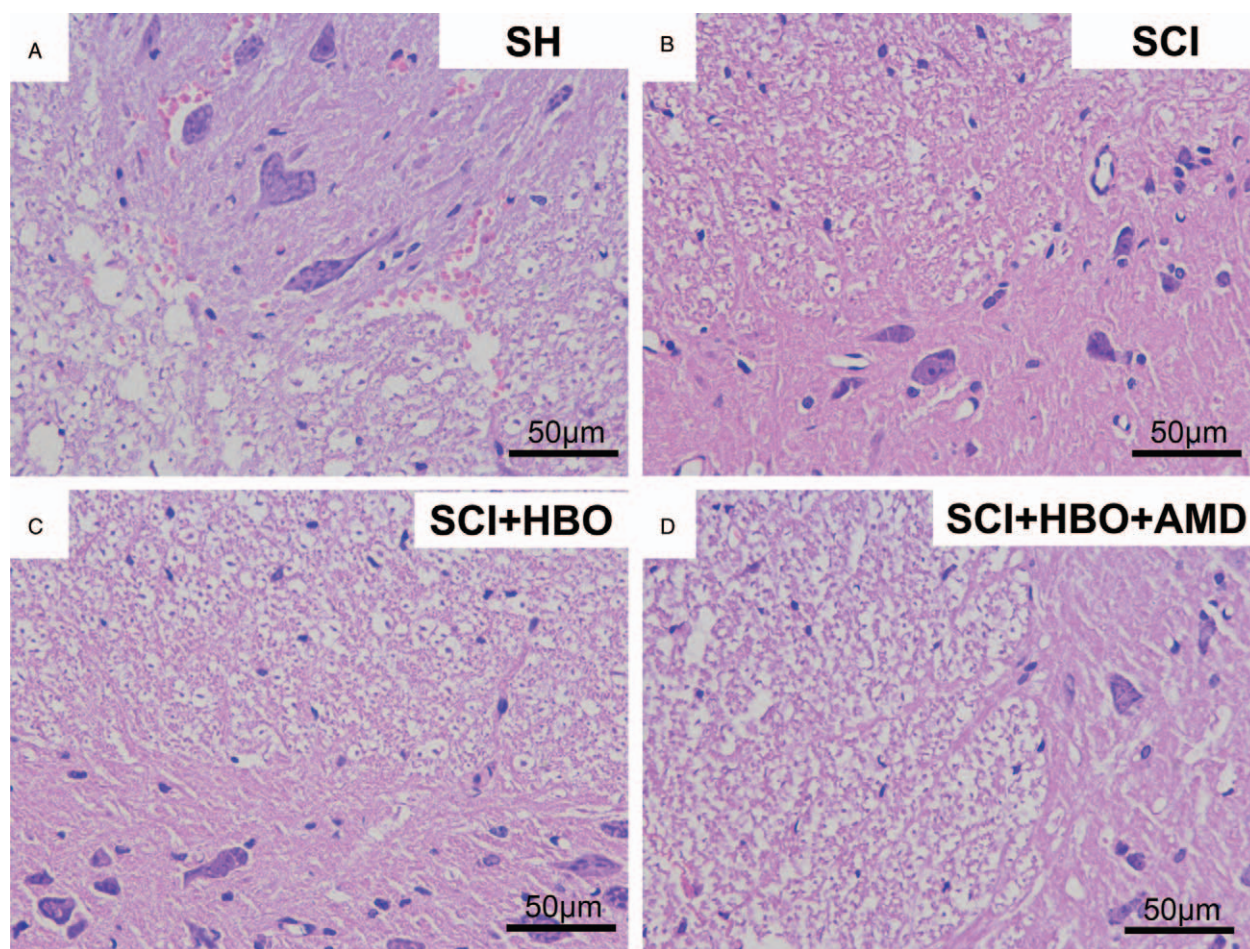


Figure 2: Spinal cord pathologic section in groups on the seventh day after the surgery (hematoxylin and eosin staining, $\times 400$). (A) Sham (SH) group, (B) SCI group, (C) SCI + hyperbaric oxygen (HBO) group, (D) HBO + SCI + AMD group. SCI group: SCI procedure, without HBO treatment; SCI + HBO group: HBO was performed after SCI procedure; SCI + HBO + AMD group: Treated with AMD3100 (5 mg/kg) immediately after SCI procedure and before HBO treatment; SH group: Sham-operated, without either SCI procedure or HBO treatment. AMD: Chemokine receptor antagonist; HBO: Hyperbaric oxygen; SCI: Spinal cord injury.

HBO + AMD groups, the animals in the SCI + HBO group demonstrated a significantly improved BBB score over time and close to the baseline level of SH group ($P < 0.05$ vs. SCI only and SCI + HBO + AMD groups). Such gradual functional recovery initiated solely by HBO at later time points further validated the protective effect of HBO in the chronic phase following SCI injury. Interestingly, co-treatment with AMD3100, a well-established SDF-1/CXCR4 axis inhibitor, significantly inhibited the protective recovery of BBB scores by HBO at all time points after SCI injury (gray line) ($P < 0.05$), further revealing mechanistic evidence that the SDF-1/CXCR4 axis might be a major pathway in the therapeutic effect of HBO.

HBO promoted tissue recovery after SCI

The HE sections revealed histologic characteristics after treatment in the four groups. As expected, the SH and SCI + HBO groups showed better histologic characteristics [Figure 2A and 2C]. In contrast, the SCI group showed prominent edema, hemorrhage, neutrophil infiltration, and disordered tissue structure [Figure 2B]. The pathologic features of tissues from animals in the HBO + AMD group were similar to those in the SCI group [Figure 2D].

HBO treatment increased mRNA expression levels of SDF-1, CXCR4, and BDNF

Quantitative real-time PCR results revealed that significant differences were found in the mRNA levels of SDF-1 (day 21, SCI + HBO vs. SCI + HBO + AMD, 2.89 ± 1.60 vs. 1.56 ± 0.98 , $P = 0.0019$), CXCR4 (day 7, SCI + HBO vs. SCI, 2.99 ± 1.60 vs. 1.31 ± 0.98 , $P = 0.0181$; day 14, SCI + HBO vs. SCI + HBO + AMD, 4.18 ± 1.60 vs. 0.80 ± 0.34 , $P = 0.0365$; day 21, SCI + HBO vs. SCI, 2.10 ± 1.01 vs. 1.15 ± 0.03 , $P = 0.0367$), and BDNF (day 7, SCI + HBO vs. SCI, 3.04 ± 0.41 vs. 2.75 ± 0.31 , $P = 0.0127$; day 14, SCI + HBO vs. SCI, 3.88 ± 1.59 vs. 1.11 ± 0.40 , $P = 0.0352$) [Figure 3]. The results showed that at most time points, mRNA expression levels of SDF-1, CXCR4, and BDNF in the SCI group were not significantly different from those in the SH group, but dramatically lower than those in the SCI + HBO group [Figure 3], consistently with the behavioral results in Figure 1. The chemokine receptor antagonist AMD3100 co-administered with HBO, effectively inhibited HBO-induced activation of SDF-1 and CXCR4, as shown by a relatively low level in the SCI + HBO + AMD group [Figure 3A and 3B]. These findings indicated the mechanistic involvement of SDF-1, CXCR4,

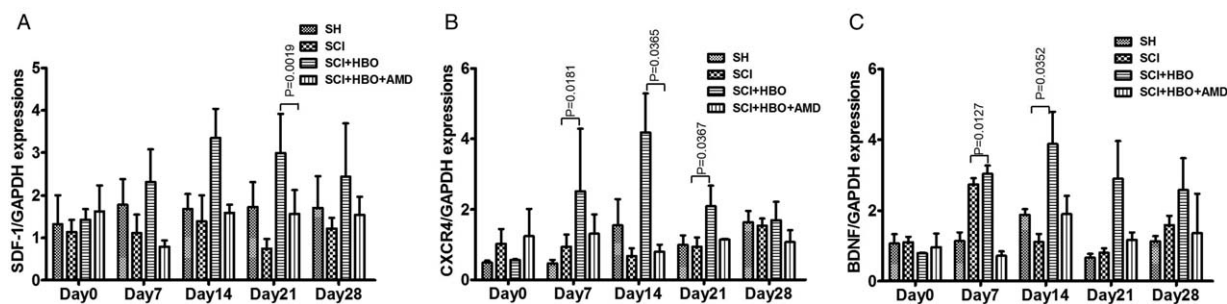


Figure 3: Quantitative real-time polymerase chain reaction results showing the changes in mRNA levels of SDF-1, CXCR4, and BDNF in each group (repeat for three times) at various time points after the surgery. Data are presented as the mean \pm standard deviation. SCI group: SCI procedure, without HBO treatment; SCI+HBO group: HBO was performed after SCI procedure; SCI+HBO+AMD group: Treated with AMD3100 (5 mg/kg) immediately after SCI procedure and before HBO treatment; SH group: Sham-operated, without either SCI procedure or HBO treatment. AMD: Chemokine receptor antagonist; BDNF: Brain-derived neurotrophic factor; CXCR4: CXC chemokine receptor 4; HBO: Hyperbaric oxygen; SCI: Spinal cord injury; SDF-1: Stromal cell-derived factor-1.

and BDNF at the mRNA level in the therapeutic effect of HBO on SCI.

Effects of HBO treatment on the protein expression levels of SDF-1, CXCR4, and BDNF

Subsequently, the expression of SDF-1, CXCR4, and BDNF was explored in spinal cord tissues at the protein level. The normalized protein expression level of SDF-1/ β -actin was the highest in the SCI+HBO group at most time points, such as POD 7, 14, and 21, compared with the SH and SCI+HBO+AMD groups. This phenomenon was more prominent for CXCR4/ β -actin expression when HBO promoted the expression of CXCR4 at all time points after SCI compared with the other groups. The coherent trending between CXCR4 and SDF-1 further supported the involvement of SDF-1/CXCR4 axis. In contrast, the co-treatment with chemokine receptor antagonist (AMD3100) led to decreased expression levels of CXCR4 and SDF-1 in the SCI+HBO+AMD group compared with the SCI+HBO group at all time points after the surgery. Similar to SDF-1 and CXCR4, the Western blot analysis demonstrated a moderate elevation of the expression of BDNF in the SCI+HBO group compared with the SCI group, but an increasing trend was observed compared with the SCI+HBO+AMD group. As expected, HBO promoted the expression of BDNF after HBO treatment, benefiting the regeneration and neurologic recovery after SCI [Supplementary Figure 1, <http://links.lww.com/CM9/A16>].

Effects of HBO treatment on the expression of SDF-1, CXCR4, and BDNF in spinal cord tissues

Immunohistochemical staining was used to determine the expression of SDF-1, CXCR4, and BDNF in the spinal cord tissues from SH rats. SDF-1 was located in white and gray matters [Figure 4A]. Meanwhile, BDNF-positive cells were identified primarily in the white matter [Figure 4A]. Moreover, CXCR4, a chemokine receptor, was found in the inflammatory cells scattered in spinal cord tissues with less abundance [Figure 4A]. Besides, BDNF and SDF-1 were stained to compare their levels in spinal cord tissues from the rats subjected to both SCI and HBO treatments on POD 14, 21, and 28. It was visually distinctive that

HBO treatment increased the expression of SDF-1 in white matter, consistent with the findings at mRNA and protein levels [Figure 4B]. In addition, HBO treatment also increased the expression of BDNF [Figure 4C].

Discussion

This study evaluated the effects of HBO on improving functional recovery following the injury by establishing a rat SCI model. The results indicated that HBO treatment efficiently enhanced the recovery following SCI. Further effects were used to explore the changes in the expression of SDF-1 and CXCR4. HBO treatment significantly increased the levels of SDF-1 and CXCR4 in rat models. More importantly, HBO increased the levels of neurotrophic BDNF, one of the major mediators of neuroplasticity. These findings provided a clue that HBO promoted SCI recovery by upregulating the expression of SDF-1/CXCR4 and BDNF in the spinal cord.

The SCI is a serious medical problem with high mortality and disability rates.^[16] HBO is a medical treatment in which patients breath 100% oxygen under increased atmospheric pressure.^[17] Many studies on rodents and humans have demonstrated HBO as a beneficial treatment for patients with acute and chronic SCI.^[18,19] In recent years, HBO therapy has gained increased attention in reducing secondary injury.^[20,21] A clinical study revealed that HBO therapy in an early stage of acute SCI benefited recovery through observing functional scores, magnetic resonance imaging, and electrophysiology.^[22] In accordance with these results, the present study demonstrated that consecutive days of HBO treatment greatly enhanced BBB scores in rats. The locomotor functions of the rats significantly increased with HBO treatment compared with the SCI group, indicating that HBO benefited the recovery after SCI.

This study revealed that the SDF-1/CXCR4 axis and BDNF were activated by HBO treatment. As mentioned earlier, SDF-1 acts as a chemotactic factor for many cell types and CXCR4 is its receptor expressed on the surface of different kinds of stem cells.^[23] The SDF-1/CXCR4 axis was proved to promote the derivation of bone marrow from mesenchymal stem cells after SCI.^[24] Multiple roles

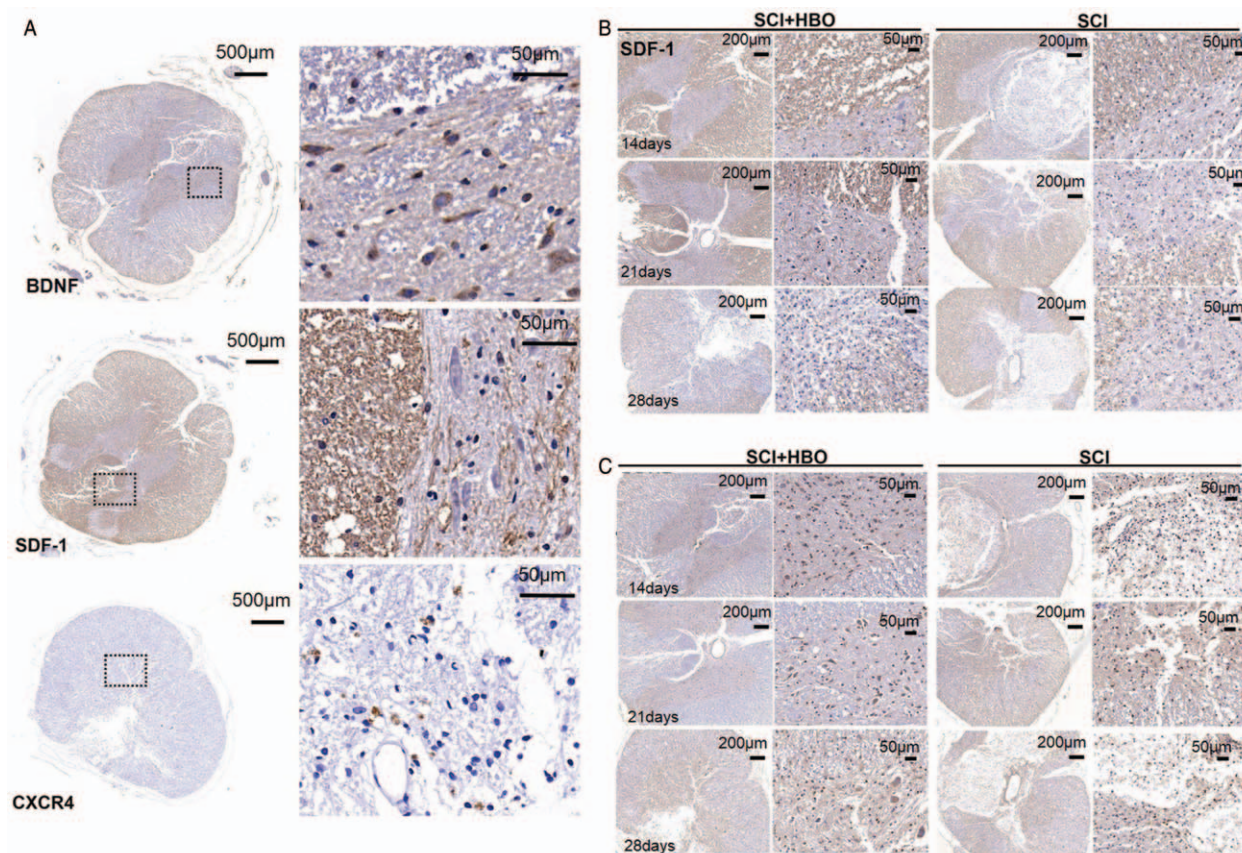


Figure 4: Immunohistochemistry staining of SDF-1, CXCR4, and BDNF in spinal cord tissues in the SH group. (A) General anatomical localization of immuno-positive SDF-1, CXCR4, and BDNF signals suggested varied distribution and expression patterns. Varied expression levels of SDF-1 (B) and BDNF (C) were visualized in spinal cord tissues in the SCI and SCI + HBO groups on Day 14, Day 21, and Day 28. SCI group: SCI procedure, without HBO treatment; SCI + HBO group: HBO was performed after SCI procedure; SCI + HBO + AMD group: Treated with AMD3100 (5 mg/kg) immediately after SCI procedure and before HBO treatment; SH group: Sham-operated, without either SCI procedure or HBO treatment. AMD: Chemokine receptor antagonist; BDNF: Brain-derived neurotrophic factor; CXCR4: CXCR4 chemokine receptor 4; HBO: Hyperbaric oxygen; SCI: Spinal cord injury; SDF-1: Stromal cell-derived factor-1.

of SDF-1/CXCR4 were further demonstrated by other studies, including regulation of cell migration.^[25] Normally, the level of CXCR4 was low in normal tissues, but it significantly increased in the injured spinal cord.^[26] Consistently, the levels of CXCR4 increased after HBO treatment in the present study. The activation in the SDF-1/CXCR4 axis was related to the maintenance of neural stem cells and worked as a salvage signaling pathway for initiating endogenous stem cell-based tissue repair.^[27] During repair, CXCR4-positive macrophages were recruited around wound tissues and were important for regulating the inflammatory response after SCI.^[26] Besides, SDF-1/CXCR4 showed a positive role in increasing neovascularization in tumor tissues.^[28] Rats with SCI were treated with HBO, showing increases in the levels of both SDF-1 and CXCR4. This was consistent with the roles of SDF-1/CXCR4 in regulating repair. The expression of BDNF was also tested in the present study, which is recognized as a synaptic plasticity marker.^[10] BDNF is widely expressed in the cerebral cortex, striatum, and basal forebrain. It is an important determinant of induced pluripotent stem cells.^[29] BDNF bound TrkB receptors to trigger PI3K/Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase pathways.^[30] The overexpression of BDNF increased the proliferation of spinal cord neurons, supporting the concept that HBO

promoted the recovery by enhancing the expression of BDNF after SCI.

The rats were treated with AMD3100 to investigate the potential effect of SDF-1/CXCR4/BDNF expression after SCI and treatment with HBO. AMD3100 is a specific antagonist to the CXCR4 receptor.^[31,32] AMD3100 could inhibit the migration of BMSCs during the repair of ischemic kidneys.^[27] In the present study, AMD3100 decreased the levels of CXCR4, SDF-1, and BDNF in the SCI + HBO + AMD group compared with the SCI group. A previous study showed that inflammation improved the expression of SDF-1.^[33] Besides, increasing evidence supported that SCI induced a robust immune response characterized by the production of chemokines and cytokines.^[34] Most of them enhanced inflammation and functional recovery.^[34] Neuronal cell death following SCI was an important contributor to neurologic deficits.^[35] It might be an effective way to improve recovery by promoting neuronal survival via attenuating inflammation after SCI.^[36] The present study not only provided a clue that HBO might benefit recovery after SCI but also elucidated mechanistic support that this protective function might be mediated through activating the SDF-1/CXCR4 axis and promoting neurotrophic elements such as

BDNF. Therefore, it was presumed that HBO might synergistically inhibit neurodegeneration. The dynamic and longitudinal changes in the expression of these proteins (SDF-1/CXCR4/BDNF) at various time points after SCI and HBO treatment not only confirmed the hypothesis in supporting the translational therapeutic function of HBO but also led to interesting scientific questions for future investigations.

This study provided fundamental behavioral and mechanistic support for HBO treatment. However, the detailed molecular mechanism underlying drug action and SCI pathogenesis needs further clarification. Several studies demonstrated the involvement of TGF- β , Akt, and Wnt signaling pathways in the effect of SDF-1/CXCR4/BDNF on cell migration or differentiation.^[37,38] SDF-1/CXCR4/BDNF had been confirmed to benefit recovery after SCI. Future studies should focus on monitoring the levels of SDF-1 or CXCR4 or BDNF in the peripheral blood from patients with SCI and assisting the clinicians to predict the outcome of HBO treatment.

In conclusion, this study indicated that HBO treatment, a clinically translational approach, significantly improved functional recovery after SCI in rats for up to 28 days. In addition, it also demonstrated that this promising effect of HBO might be associated with the activated SDF-1/CXCR4 axis and promoted BDNF (synaptic plasticity markers) expression at mRNA, protein, and tissue levels. These data laid a solid preclinical foundation for translating HBO into a safe and effective clinical treatment of SCI, warranting further in-depth investigations.

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Conflicts of interest

None.

References

- Martirosyan NL, Turner GH, Kaufman J, Patel AA, Belykh E, Kalani MY, *et al.* Manganese-enhanced MRI Offers Correlation with Severity of Spinal Cord Injury in Experimental Models. *Open Neuroimag J* 2016;10:139–147. doi: 10.2174/1874440001610010139.
- Sutherland TC, Mathews KJ, Mao Y, Nguyen T, Gorrie CA. Differences in the cellular response to acute spinal cord injury between developing and mature rats highlights the potential significance of the inflammatory response. *Front Cell Neurosci* 2016;10:310. doi: 10.3389/fncel.2016.00310.
- Truffandier K, Beaumont E, Charbonney E, Maghni K, de Marchie M, Spahija J. Mechanical ventilation modulates pro-inflammatory cytokine expression in spinal cord tissue after injury in rats. *Neurosci Lett* 2018;671:13–18. doi: 10.1016/j.neulet.2018.01.028.
- Noller CM, Groah SL, Nash MS. Inflammatory stress effects on health and function after spinal cord injury. *Top Spinal Cord Inj Rehabil* 2017;23:207–217. doi: 10.1310/sci2303-207.
- Dayan K, Keser A, Konyalioglu S, Erturk M, Aydin F, Sengul G, *et al.* The effect of hyperbaric oxygen on neuroregeneration following acute thoracic spinal cord injury. *Life Sci* 2012;90:360–364. doi: 10.1016/j.lfs.2011.12.005.
- Nie H, Xiong L, Lao N, Chen S, Xu N, Zhu Z. Hyperbaric oxygen preconditioning induces tolerance against spinal cord ischemia by upregulation of antioxidant enzymes in rabbits. *J Cereb Blood Flow Metab* 2006;26:666–674. doi: 10.1038/sj.jcbfm.9600221.
- Hao H, Hu S, Chen H, Bu D, Zhu L, Xu C, *et al.* Loss of endothelial CXCR7 impairs vascular homeostasis and cardiac remodeling after myocardial infarction: implications for cardiovascular drug discovery. *Circulation* 2017;135:1253–1264. doi: 10.1161/circulationaha.116.023027.
- Marquez-Curtis LA, Janowska-Wieczorek A. Enhancing the migration ability of mesenchymal stromal cells by targeting the SDF-1/CXCR4 axis. *Biomed Res Int* 2013;2013:561098. doi: 10.1155/2013/561098.
- Liu X, Yang J, Li Z, Yang L, Wang C, Gao C, *et al.* Hyperbaric oxygen preconditioning promotes neovascularization of transplanted skin flaps in rats. *Int J Clin Exp Pathol* 2014;7:4734–4744.
- Geist PA, Dulka BN, Barnes A, Totty M, Datta S. BDNF heterozygosity is associated with memory deficits and alterations in cortical and hippocampal EEG power. *Behav Brain Res* 2017;332:154–163. doi: 10.1016/j.bbr.2017.05.039.
- Ahmed F, Tessarollo L, Thiele C, Mocchetti I. Brain-derived neurotrophic factor modulates expression of chemokine receptors in the brain. *Brain Res* 2008;1227:1–11. doi: 10.1016/j.brainres.2008.05.086.
- Bachis A, Major EO, Mocchetti I. Brain-derived neurotrophic factor inhibits human immunodeficiency virus-1/gp120-mediated cerebellar granule cell death by preventing gp120 internalization. *J Neurosci* 2003;23:5715–5722. doi: 10.1523/jneurosci.23-13-05715.2003.
- Azoulay D, Herishanu Y, Shapiro M, Brandshaft Y, Suriu C, Akria L, *et al.* Elevated serum BDNF levels are associated with favorable outcome in CLL patients: Possible link to CXCR4 down-regulation. *Exp Hematol* 2018;63:17–21.e1. doi: 10.1016/j.exphem.2018.04.005.
- Yang J, Liu X, Zhou Y, Wang G, Gao C, Su Q. Hyperbaric oxygen alleviates experimental (spinal cord) injury by downregulating HMGB1/NF-kappaB expression. *Spine (Phila Pa 1976)* 2013;38:E1641–E1648. doi: 10.1097/brs.0000000000000005.
- Li J, Guo W, Xiong M, Han H, Chen J, Mao D, *et al.* Effect of SDF-1/CXCR4 axis on the migration of transplanted bone mesenchymal stem cells mobilized by erythropoietin toward lesion sites following spinal cord injury. *Int J Mol Med* 2015;36:1205–1214. doi: 10.3892/ijmm.2015.2344.
- Tan J, Zhang F, Liang F, Wang Y, Li Z, Yang J, *et al.* Protective effects of hyperbaric oxygen treatment against spinal cord injury in rats via toll-like receptor 2/nuclear factor-kappaB signaling. *Int J Clin Exp Pathol* 2014;7:1911–1919.
- Zhao B, Pan Y, Xu H, Song X. Hyperbaric oxygen attenuates neuropathic pain and reverses inflammatory signaling likely via the Kindlin-1/Wnt-10a signaling pathway in the chronic pain injury model in rats. *J Headache Pain* 2017;18:1. doi: 10.1186/s10194-016-0713-y.
- Zhao BS, Meng LX, Ding YY, Cao YY. Hyperbaric oxygen treatment produces an antinociceptive response phase and inhibits astrocyte activation and inflammatory response in a rat model of neuropathic pain. *J Mol Neurosci* 2014;53:251–261. doi: 10.1007/s12031-013-0213-3.
- Gibbons CR, Liu S, Zhang Y, Sayre CL, Levitch BR, Moehlmann SB, *et al.* Involvement of brain opioid receptors in the anti-allodynic effect of hyperbaric oxygen in rats with sciatic nerve crush-induced neuropathic pain. *Brain Res* 2013;1537:111–116. doi: 10.1016/j.brainres.2013.08.050.
- Banerjee N, Javed A, Deepak D, Pawar M, Chattopadhyay TK. Hyperbaric oxygen therapy: an adjuvant treatment modality for chemo-radiation induced hemorrhagic gastritis. *Trop Gastroenterol* 2011;32:248–250.
- Herfarth AE, Martinez-Ramos D, Nomdedeu-Guinot J, Salvador-Sanchis JL. Hyperbaric oxygen therapy as an adjuvant treatment for pyoderma gangrenosum (in Spanish). *Cir Esp* 2009;85:319–321. doi: 10.1016/j.ciresp.2008.09.015.
- Tan JW, Zhang F, Liu HJ, Li Z. Hyperbaric oxygen ameliorated the lesion scope and nerve function in acute spinal cord injury patients: a retrospective study. *Clin Biochem* 2018;53:1–7. doi: 10.1016/j.clinbiochem.2017.12.001.
- Kucia M, Jankowski K, Reza R, Wysoczynski M, Bandura L, Allendorf DJ, *et al.* CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. *J Mol Histol* 2004;35:233–245. doi: 10.1023/b:hijo.0000032355.66152.b8.

24. Wang GD, Liu YX, Wang X, Zhang YL, Zhang YD, Xue F. The SDF-1/CXCR4 axis promotes recovery after spinal cord injury by mediating bone marrow-derived from mesenchymal stem cells. *Oncotarget* 2017;8:11629–11640. doi: 10.18632/oncotarget.14619.
25. Li M, Sun X, Ma L, Jin L, Zhang W, Xiao M, *et al.* SDF-1/CXCR4 axis induces human dental pulp stem cell migration through FAK/PI3K/Akt and GSK3beta/beta-catenin pathways. *Sci Rep* 2017;7:40161. doi: 10.1038/srep40161.
26. Tysseling VM, Mithal D, Sahni V, Birch D, Jung H, Belmadani A, *et al.* SDF1 in the dorsal corticospinal tract promotes CXCR4+ cell migration after spinal cord injury. *J Neuroinflammation* 2011;8:16. doi: 10.1186/1742-2094-8-16.
27. Li M, Hale JS, Rich JN, Ransohoff RM, Lathia JD. Chemokine CXCL12 in neurodegenerative diseases: an SOS signal for stem cell-based repair. *Trends Neurosci* 2012;35:619–628. doi: 10.1016/j.tins.2012.06.003.
28. Benslimane-Ahmim Z, Pereira J, Lokajczyk A, Dizier B, Galy-Fauroux I, Fischer AM, *et al.* Osteoprotegerin regulates cancer cell migration through SDF-1/CXCR4 axis and promotes tumour development by increasing neovascularization. *Cancer Lett* 2017;395:11–19. doi: 10.1016/j.canlet.2017.02.032.
29. Ramalho AF, Bombassaro B, Dragano NR, Solon C, Morari J, Fioravante M, *et al.* Dietary fats promote functional and structural changes in the median eminence blood/spinal fluid interface-the protective role for BDNF. *J Neuroinflammation* 2018;15:10. doi: 10.1186/s12974-017-1046-8.
30. Nasrolahi A, Mahmoudi J, Akbarzadeh A, Karimipour M, Sadigh-Eteghad S, Salehi R, *et al.* Neurotrophic factors hold promise for the future of Parkinson's disease treatment: is there a light at the end of the tunnel? *Rev Neurosci* 2018;29:475–489. doi: 10.1515/revneuro-2017-0040.
31. Liu X, Mao J, Han C, Peng S, Li C, Jin T, *et al.* CXCR4 antagonist AMD3100 ameliorates thyroid damage in autoimmune thyroiditis in NOD.H2h(4) mice. *Mol Med Rep* 2016;13:3604–3612. doi: 10.3892/mmr.2016.4965.
32. Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E, *et al.* Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. *Antimicrob Agents Chemother* 2000;44:1667–1673. doi: 10.1128/AAC.44.6.1667-1673.2000.
33. Mills SC, Goh PH, Kudatsih J, Ncube S, Gurung R, Maxwell W, *et al.* Cell migration towards CXCL12 in leukemic cells compared to breast cancer cells. *Cell Signal* 2016;28:316–324. doi: 10.1016/j.cell-sig.2016.01.006.
34. Yan X, Huang G, Liu Q, Zheng J, Chen H, Huang Q, *et al.* Withaferin A protects against spinal cord injury by inhibiting apoptosis and inflammation in mice. *Pharm Biol* 2017;55:1171–1176. doi: 10.1080/13880209.2017.1288262.
35. Wang PF, Xu DY, Zhang Y, Liu XB, Xia Y, Zhou PY, *et al.* Deletion of mammalian sterile 20-like kinase 1 attenuates neuronal loss and improves locomotor function in a mouse model of spinal cord trauma. *Mol Cell Biochem* 2017;431:11–20. doi: 10.1007/s11010-017-2969-1.
36. Chen H, Ji H, Zhang M, Liu Z, Lao L, Deng C, *et al.* An agonist of the protective factor SIRT1 improves functional recovery and promotes neuronal survival by attenuating inflammation after spinal cord injury. *J Neurosci* 2017;37:2916–2930. doi: 10.1523/jneurosci.3046-16.2017.
37. Zhang SJ, Song XY, He M, Yu SB. Effect of TGF-beta1/SDF-1/CXCR4 signal on BM-MSCs homing in rat heart of ischemia/perfusion injury. *Eur Rev Med Pharmacol Sci* 2016;20:899–905.
38. Liu MH, Bian BS, Cui X, Liu LT, Liu H, Huang B, *et al.* Mesenchymal stem cells regulate mechanical properties of human degenerated nucleus pulposus cells through SDF-1/CXCR4/AKT axis. *Biochim Biophys Acta* 2016;1863:1961–1968. doi: 10.1016/j.bbamcr.2016.05.004.

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