Original Article

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Transplantation of bone marrow mesenchymal stem cells pretreated with valproic acid in rats with an acute spinal cord injury

Lei Chen[1,2,*] , Xiaoyan Cui[1,*], Zhourui Wu[2], Long Jia[2], Yan Yu[2], Qiulian Zhou[3], Xiao Hu[2], Wei Xu[2], Dandan Luo[1], Jie Liu[1], Junjie Xiao[3], Qiao Yan[1], Liming Cheng[1,2,]**

[1] *Translational Center for Stem Cell Research, Tongji Hospital, Department of Regenerative Medicine, Tongji University School of Medicine, Shanghai, China;*
[2] *Department of Spinal Surgery, Tongji Hospital, Tongji University School of Medicine, Shanghai, China;*
[3] *College of Life Sciences, Shanghai University, Shanghai, China.*

Summary **This study aimed to investigate whether valproic acid (VPA) pretreatment enhances the therapeutic effectiveness of mesenchymal stem cells derived from bone marrow (BMSCs) transplanted into rats with an acute spinal cord injury (SCI). BMSCs were pretreated with VPA before transplantation and then intravenously injected 1 week after SCI. Before transplantation, levels of CXC chemokine receptor 4 (CXCR4) expression in BMSCs were tested using quantitative real-time PCR and Western blotting. Stromal derived factor-1 (SDF-1), the unique ligand of CXCR4, was quantified using RT-PCR and immunofluorescence. The locomotor function of rats with an SCI was evaluated using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale. Fluorescence microscopy and hematoxylin-eosin (HE) staining were also performed to evaluate pathophysiological changes after transplantation. On day 7 after SCI, the level of SDF-1 expression peaked. CXCR4 expression increased significantly in BMSCs pretreated with VPA. After intravenous transplantation, BrdU-labeled BMSCs were noted at the spinal injury site, and this was especially true for BMSCs pretreated with VPA. More significant functional improvement was observed in rats receiving BMSCs pretreated with VPA than in other groups of rats. AMD3100 partially inhibited improvement. This study demonstrates that pretreatment with VPA before transplantation enhances the therapeutic benefits of BMSCs in terms of greater cell migration and better neurological outcomes after traumatic SCI. The mechanism of this enhancement may be related to the SDF-1/CXCR4 axis. Therefore, pretreatment of BMSCs with VPA warrants further study in relation to the treatment of traumatic SCI.**

Keywords: Mesenchymal stem cells derived from bone marrow, spinal cord injury, valproic acid, stromal derived factor-1, CXC chemokine receptor 4

1. Introduction

Spinal cord injury (SCI) is a serious hazard to human health. It usually causes severe neurological dysfunction and disability. However, effective ways to treat SCI have not been available until now. One reason is because a complex series of pathophysiological changes, such as local inflammation and glutamate cytotoxicity, results in the apoptosis of neurons and oligodendrocyte glial cells and the reactivation of astrocytes that in turn lead respectively to disconnection of neuronal circuits, demyelination, and glial scar formation (*1*).

Over the past few decades, stem cell therapy has offered promise as a strategy to treat SCI. Mounting evidence has shown that mesenchymal stem cells derived from bone marrow (BMSCs) can improve

*These authors contributed equally to this works.
***Address correspondence to:*
Dr. Liming Cheng, Department of Spinal Surgery, Tongji Hospital, Tongji University School of Medicine, 389 Xincun Road, Shanghai 200065, China.
E-mail: chlm.d@163.com

recovery in SCI models by regulating host immunity, secreting neurotrophic factors, and differentiating into neurons (*1-3*). BMSC therapy has several advantages over therapy with other types of stem cells, including greater availability, less likelihood of immune rejection, and fewer ethical issues.

The systemic administration of BMSCs provides a therapeutic benefit in rats with a SCI (*4*). However, this treatment seems to have more limited therapeutic effectiveness than local direct injection because of the uneven distribution of BMSCs *in vivo* after intravenous injection (*5*). Ninety-nine percent of intravenously injected MSCs adhere to the lungs, while only 2-3% of adhering MSCs are released into the circulation (*6*). Numerous experiments have shown that BMSCs will preferentially migrate to sites of inflammation after intravenous administration (*4,7-9*). A way to promote the migration of exogenous BMSCs to the injury site has yet to be determined. One proven way is *via* chemotactic factors that mediate the homing of BMSCs. Stromal derived factor-1 (SDF-1) and its cellular receptor CXC chemokine receptor 4 (CXCR4) have been found to direct the migration of BMSCs in models of stroke, SCI, and cardiomyopathy (*7-9*).

Previous studies found that valproic acid (VPA) affected the structure of chromatin and gene expression as an inhibitor of histone deacetylase (HDAC) activity (*10*). Short-term (3 h) exposure of MSCs to a relatively high concentration (2.5 mM) of VPA markedly increased CXCR4 transcript and protein levels and it enhanced SDF-1-mediated MSCs migration (*11*). Thus, the current study sought to investigate whether BMSCs pretreated with VPA would enhance the migration of BMSCs to improve functional recovery in an experimental rat model of SCI.

2. Materials and Methods

2.1. *Isolation, culture, identification, and differentiation of BMSCs*

The use of rats in this study was approved by the Ethical Committee of Tongji Hospital. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Pub, revised 2011).

BMSCs were isolated and identified as described previously (*4,7*). Briefly, primary BMSCs were harvested from tibias and femurs of 3-week-old Sprague-Dawley rats under aseptic conditions and then purified and passaged in dulbecco's modified eagle medium (DMEM, Gibco, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Gibco). Cells were maintained at 37°C in a humidified atmosphere with 5% carbon dioxide, and the medium was changed every 3 days. Upon reaching 80 to 90% confluence, adherent cells were detached with 0.25% Trypsin-EDTA (Gibco) and replated at a ratio of 1:3 in regular growth medium for continued passaging. Upon the third passage, the BMSCs were trypsinized and then filtered through a stainless steel mesh filter into single-cell suspensions. These suspensions were stained with fluorescein isothiocyanate-labeled antibodies for flow cytometric analysis, including rat anti-CD29, CD45, CD73, CD90, and CD106 (BD Pharmingen, San Diego, USA). BMSCs that had been passaged 4 times were harvested for the following experiments.

To ascertain differentiation, BMSCs that were passaged 4 times were divided into 3 groups: (*i*) untreated BMSCs; (*ii*) BMSCs pretreated with VPA, and (*iii*) BMSCs pretreated with VPA and AMD3100.

BMSCs were incubated with 2.5 mmol/L of VPA (Sigma-Aldrich, P4543-10G, St. Louis, MO, USA), a small molecule compound that is an histone deacetylase (HDAC) inhibitor, for 3 h and then washed with fresh medium to yield BMSCs pretreated with VPA. BMSCs pretreated with VPA were also treated with 20 umol/L AMD3100 (Sigma-Aldrich) for 6 h and then washed with fresh medium to yield BMSCs pretreated with VPA and AMD3100. All 3 groups of cells were plated in 6-well tissue culture plates and growth medium was replaced. Cells were then fed by completely replacing the medium with fresh BMSC osteogenic/adipogenic differentiation medium (Cyagen Biosciences Inc., California, USA) or growth medium supplemented with 10 ng/mL b-fibroblast growth factor (bFGF, Gibco) and 10 ng/mL epidermal growth factor (EGF, Gibco) (*12*). After differentiation, cells were fixed and subjected to Alizarin red staining, Oil red O staining, and immunofluorescence staining.

2.2. *VPA and AMD3100 pretreatment and BrdU labeling of BMSCs in vitro*

BMSCs that had been passaged 4 times were divided into 3 groups (1×10^6 cells in each group): (*i*) untreated BMSCs; (*ii*) BMSCs pretreated with VPA; and (*iii*) BMSCs pretreated with VPA and AMD3100. All groups of cells were labeled with 10 μmol/L of BrdU for 3 days. Cells were then harvested for subsequent experiments.

2.3. *Model of acute SCI*

Adult Sprague–Dawley rats weighing 180 to 200 g were chosen to create an SCI model. Rats were anesthetized with an intraperitoneal injection of 2% pentobarbital sodium (60 mg/kg). A laminectomy was performed at the T10 level using a micromanipulator, and then the spinal cord was exposed without scratching the dura mater. Rats were placed on the fixation plate of an IH-0400 impactor (Precision Systems and Instrumentation, Kentucky, USA), and then the vertebral bodies at T9 and T11 were grasped with 2 adjustable forceps

to immobilize the spine. The impact force of the impactor was set at 200 kDynes. Gentamycin (5 mg/kg) was injected *via* intraperitoneal injection in the first 3 days after SCI. Rats were left on heated pads until they awoke. Upon awakening, each rat's bladder was manually expressed twice a day, and the rat's locomotion was evaluated (*13,14*). To evaluate the expression of SDF-1 at the injury site on days 1 and 7 after SCI, RT-PCR and immunohistochemical analysis were performed on injured spinal cords and normal spinal cords.

2.4. *BMSC transplantation*

Rats were randomly divided into four groups: rats receiving DMEM (sham), rats receiving untreated BMSCs, rats receiving BMSCs pretreated with VPA, and rats receiving BMSCs pretreated with VPA and AMD3100. Cells were injected into the tail vein with a scalp vein needle. In rats receiving DMEM, only 0.5 mL of DMEM was injected intravenously into the tail vein 7 days after SCI. The other groups of rats were injected with 1×10^6 of the corresponding cells in 0.5 mL DMEM. After injection, a cotton ball soaked in alcohol was pressed on the wound to prevent bleeding.

2.5. *Behavioral test*

Hind limb locomotion was evaluated using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale on days 1, 3, 7, 14, and 28 after SCI (*12*). During the evaluation, rats were placed in an open field and observed for 5 minutes at each time point by 2 observers who were blind to the experimental protocol. The BBB locomotor rating scale ranges from 0 to 21 points, with 0 points indicating no spontaneous hind limb movement and 21 points indicating normal hind limb movement. Observers completed their ratings independently.

2.6. *q-PCR analysis*

Total RNA was isolated from cells and injured spinal cords with TRIzol reagent (Gibco-Invitrogen, Carlsbad, CA, USA). Injured spinal cord segments were first homogenized. RNA concentrations were quantified using ultraviolet spectroscopy. As outlined in the instruction manual, reverse transcription was performed in a 20 μL reaction system with 4 mg of total RNA treated by RNase-free DNase I (TaKaRa Bio, Inc., Otsu, Japan). The PCR primers were synthesized from Invitrogen as follows: β-Actin forward primer, CACCCGCGAGTACAACCTTC; β-Actin reverse primer, CCCATACCCACCATCACACC; rCXCR4 forward primer: CCTTCTGGGCAGTGGACG; and CXCR4-reverse primer, GGACAATGGCAAGGTAGCG. Reverse transcription was performed with a SYBR Premix Ex Taq Kit (TaKaRa Bio) in a real-time fluorescence quantitative PCR system (ABI 7800, USA). The reaction mixture was a 20-μL mixture containing 10 μL of SYBR Premix Ex Taq TM (Takara Bio), 0.4 μL of each primer, and 2 μL of cDNA. In accordance with the instruction manual, the cycling conditions were as follows: an initial cycle of heating at 95°C for 15 sec, followed by 40 cycles at 95°C for 5 sec and 40 cycles at 60°C for 31 sec. After PCR, the RT-PCR system automatically analyzed the fluorescence signal and converted it to the cycle threshold (Ct) value. Each sample was analyzed in triplicate.

2.7. *Western blot analysis of CXCR4 in BMSCs*

All groups of BMSCs were cleaved in radio-immunoprecipitation assay (RIPA, Sigma-Aldrich) buffer supplemented with a protease inhibitor and phosphatase inhibitor cocktail (Sigma) for 30 min at 4°C. Protein homogenates were then centrifuged at 12,000 g for 15 min at 4°C to collect the supernatant. The protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit and Bio-Rad spectrophotometer. The samples were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% albumin from bovine serum (BSA, Sigma-Aldrich) and incubated with goat anti-CXCR4 polyclonal antibody (1:1,000; Abcam, London, England). Finally, blots were incubated with rabbit anti-goat IgG (1:2,000; Jackson, Lancaster, USA) conjugated with horseradish peroxidase. Protein signals were detected in an X-ray developing darkroom. The results were expressed as relative integrated intensity compared to β-actin.

2.8. *Immunohistochemical analysis*

To label and track BMSCs, the rate of BrdU labeling *in vitro* was analyzed and the distribution of grafted MSCs in the spinal cord was determined using immunofluorescence. The spinal cord, dissected 5 days after the transplantation of BMSCs, was frozen and sectioned before staining. Sections or cells were fixed with 4% paraformaldehyde (PFA) for 20 min. After rinsing with tris-buffered saline (TBS), samples were permeabilized with 0.3% Triton in TBS for 15 min at room temperature (RT). They were then rinsed 3 times with TBS for 30 min at RT. Samples were incubated in 2 N HCl for 30 min at 37°C followed by 0.1 M sodium tetraborate (pH 8.5) for 10 min at 37°C. After they were rinsed 3 times with TBS, the samples were incubated in a blocking buffer (1% BSA and 10% goat normal serum in 0.3% Triton X-100-TBS) for 1 h at RT. Samples were then incubated in rat anti-BrdU (1:200) diluted with blocking buffer overnight at 4°C. After they were rinsed 3 times with TBS, sections were incubated for 1

h at RT with Goat Anti-Rat IgG Cy3 (1/1,000, Abcam). Sections were then rinsed 3 times in TBS-0.05% Tween 20. Sections or cells were finally coverslipped with 4',6-diamidino-2-phenylindole (DAPI) mounting medium (Sigma-Aldrich). Each spinal sample was cut into 3 slices, and results represented the average for the slices.

2.9. *Hematoxylin and eosin (HE) staining to estimate the volume of injured tissue*

Four weeks after SCI, all rats were anesthetized and transcardially perfused with PBS followed by 4% PFA until blood had been removed. Six spinal cords were dissected from 6 rats in each group and these spinal cords were immersed in 4% PFA for 2 days until they sank. Each spinal cord was embedded in paraffin and sectioned into 3 slices. Cross-sections (5 μm) were cut and stained with HE. A cross-section of the injured spinal cord was obtained using a microscope camera, and its area was measured using the image analysis software NIH Image. The injured area consisted of all meaningful (> 100 μm) cavities present at the injury site. The total area of the spinal cord was divided by the area of injured tissue to yield a ratio of the injured area/entire area. This ratio represented the final result.

2.10. *Statistical analysis*

Data were statistically analyzed and expressed as mean values ± S.D. Differences between the means of different groups were assessed with one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Scores on the BBB locomotor rating scale on different days were analyzed using repeated-measures ANOVA. A p value < 0.05 was considered statistically significant. All analyses were performed using SPSS 16.0 software.

3. Results

3.1. *Isolation, culture, characterization, and differentiation of rat BMSCs*

About 7-10 days after isolation, BMSCs cultured as plastic adherent cells proliferated consistently and were passaged to cover 80-90% of the plate. Cells passaged 3 times had consistent morphological features of being flat and spindle-shaped (Figure 1A). The proliferative activity of BMSCs decreased with passaging. Flow cytometry analysis indicated that BMSCs passaged 3 times expressed CD29, CD73, and CD90 but not CD45 or CD106 (Figure 1B). Upon induction, BMSCs consistently differentiated into osteogenic/adipogenic/neurogenic cells, indicating that the BMSCs were multipotent. Certain concentrations of VPA and AMD3100 did not markedly affect the differentiation of BMSCs into other cell types (Figures 1D, 1E, and 1F).

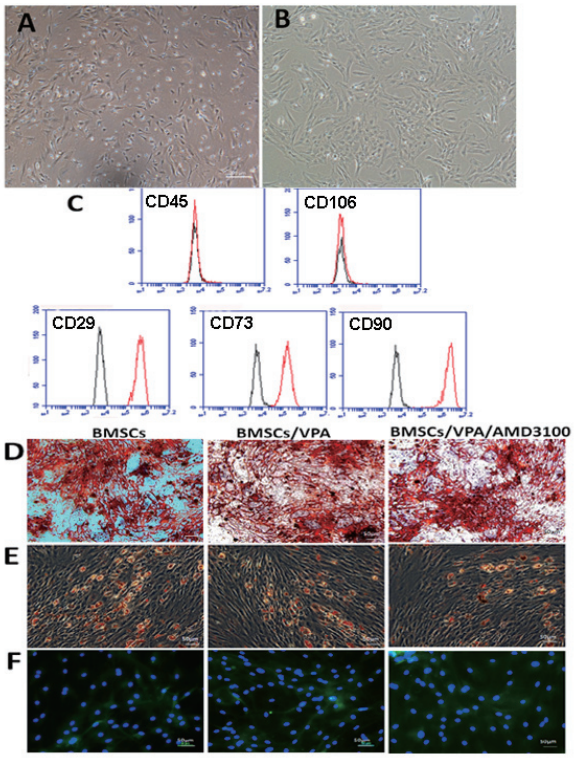

Figure 1. Morphology, phenotypic characterization, and differentiation of rat BMSCs. (A) Primary BMSCs after 7 days of culturing (magnification: ×100, bar = 200 μm). **(B)** BMSCs passaged 3 times (magnification: ×100, bar = 200 μm). **(C)** Flow cytometric analysis of cultured BMSCs with the cell surface markers CD29, CD45, CD73, CD90, and CD106. **(D)** Analysis of osteogenic differentiation using Alizarin red staining. **(E)** Analysis of adipogenic differentiation using Oil red O staining. **(F)** Neural cell differentiation indicated by immunostaining. The figure shows the staining of GFAP since NeuN was negative (data not shown). [magnification: ×200, bar = 50 μm].

3.2. *VPA and AMD3100 affected CXCR4 expression and did not affect BrdU labeling of BMSCs*

Pretreating BMSCs with 2.5 mmol/L of VPA and 20 μmol/L of AMD3100 had no apparent negative effect on their morphology, such as nuclear shrinkage or formation of cytoplasmic vacuoles. RT-PCR results suggested that VPA increased the expression of CXCR4 in BMSCs about 2.25 ± 0.20-fold while VPA and AMD3100 increased the expression of CXCR4 1.44 ± 0.12-fold (Figure 2A). BMSCs pretreated with VPA had significant greater ($p < 0.05$) expression of CXCR4 than other groups of cells, and the same was true according to the results of Western blotting (Figure 2B).

BrdU-labeled BMSCs had proliferative activity at a rate of 80-90%. VPA and AMD3100 did not significantly affect BMSC proliferation and BrdU labeling (Figure 2C).

3.3. *Changes in SDF-1 levels at the spinal injury site*

Compared to normal spines, injured spines had 2.01 ± 0.29 times more expression of SDF-1 on day 1 post-

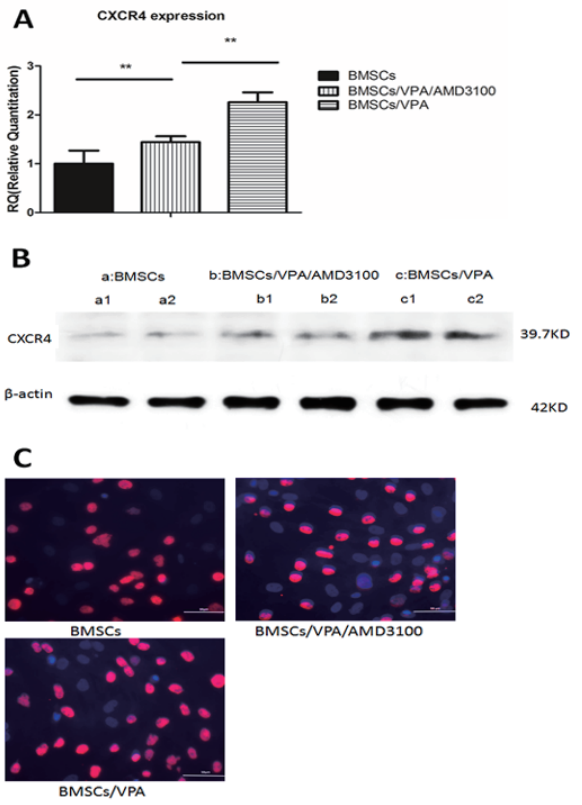

Figure 2. CXCR4 expression and BrdU immunofluorescence. **(A)** mRNA expression of CXCR4 in each group. Data are the mean ± S.D. (n = 6). BMSCs pretreated with VPA had significantly greater CXCR4 expression than other groups. ** $p < 0.01$ indicates significant differences between groups (one-way ANOVA). **(B)** CXCR4 and β-actin in each group (n = 6) were detected with Western blotting. The blots were 2 representative examples from 6 experiments per group. **(C)** BrdU (Cy3)-DAPI staining indicated no significant differences in BrdU-labeled BMSCs among the groups (magnification: ×400, bar = 50 μm).

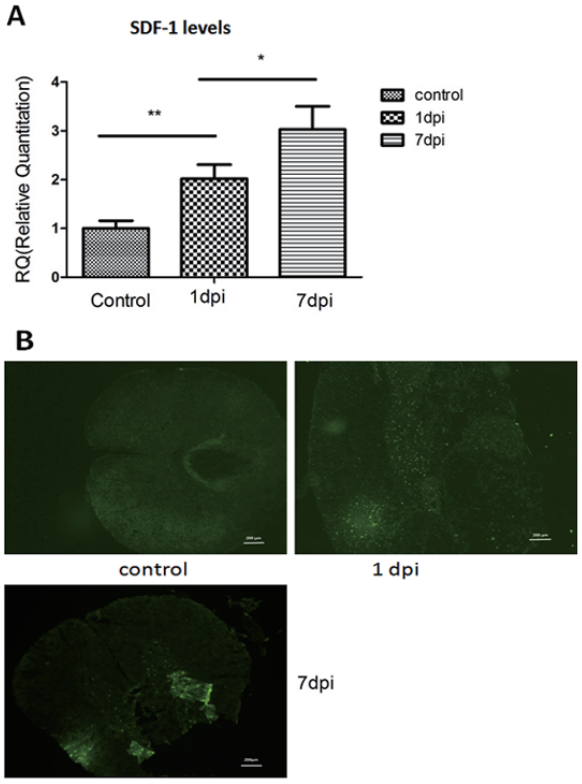

Figure 3. RT-PCR and immunofluorescence of SDF-1 at the spinal injury site. **(A)** RT-PCR. About 3 centimeters of the injured spinal cord was removed from each rat on days 1 and 7 after SCI, and these injured spinal cords were compared to normal spinal cords (n = 6 in each group). ** $p < 0.01$ indicates significant differences between groups, * $p < 0.05$ for day 1 post-injury versus day 7 post-injury (one-way ANOVA). **(B)** Immunofluorescent staining. Normal spinal cords had slight expression of SDF-1 that increased on day 1 post-injury and peaked on day 7 post-injury (magnification: ×40, bar = 200 μm).

injury and 3.03 ± 0.47 times more expression on day 7 post-injury (Figure 3A). Immunofluorescent staining of spinal cord sections indicated that normal spinals expressed a slight amount of SDF-1 although expression was marked on day 7 post-injury (Figure 3B). Fluorescence was more marked in gray matter, which may be related to astrocyte activation.

3.4. *Pretreatment with VPA enhanced the migration of BMSCs to the spinal injury site*

Five days after intravenous transplantation, BMSCs that were grafted to the injured spinal cord were tracked using BrdU immunostaining (Figure 4). The number of labeled cells at the injury site was as follows: no labeled cells in the control group, 12.67 ± 2.08 cells per slice in rats receiving untreated BMSCs, 33.33 ± 6.1 cells per slice in rats receiving BMSCs pretreated with VPA, and 14 ± 2.65 cells per slice in rats receiving BMSCs pretreated with VPA and AMD3100. There were significant differences ($p < 0.05$) in the number of cells in rats receiving BMACs pretreated with VPA and rats receiving untreated BMSCs and there were significant differences ($p < 0.05$) in the number of cells in rats receiving BMSCs pretreated with VPA and rats receiving BMSCs pretreated with VPA and AMD3100.

3.5. *Spared tissue after SCI*

The injured portion of the spinal cord was embedded in paraffin and stained with HE on day 28 post-injury. Slices were then analyzed using the methods described previously (*15*). The ratio of the injured area/total area was calculated and the results were expressed as a mean value. Staining indicated that rats receiving BMSCs pretreated with VPA had relatively smaller cavities and scar tissue formation compared to rats receiving BMSCs and rats receiving BMSCs pretreated with VPA and AMD3100 (Figure 5).

3.6. *Neurological outcomes*

The recovery of hind limb function in all rats was assessed in an open field test using the BBB Locomotor Rating Scale. Wild-type (WT) rats had a score of 21 points during evaluation. Injured rats had complete hind

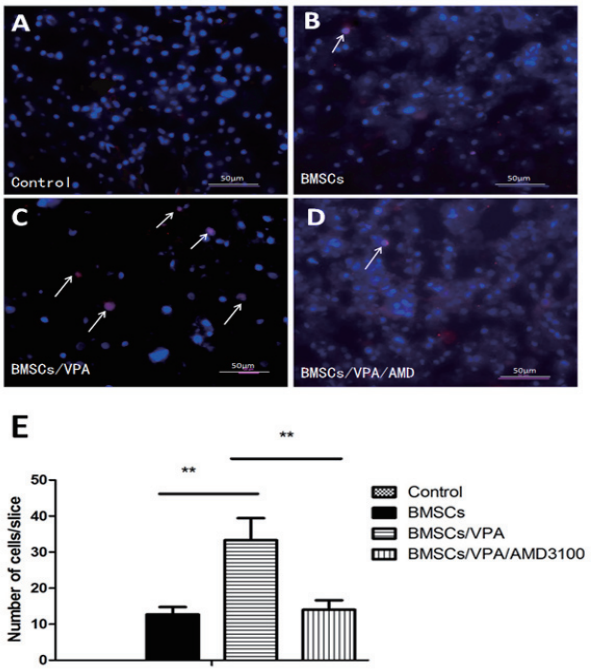

Figure 4. BrdU immunostaining of BMSCs grafted to the spinal injury site. (A) Control group. **(B)** Rats receiving untreated BMSCs. **(C)** Rats receiving BMSCs pretreated with VPA. **(D)** Rats receiving BMSCs pretreated with VPA and AMD3100 (magnification: ×400, bar = 50 μm). BrdU-labeled BMSCs were stained red by Cy3 and blue by DAPI. Rats receiving BMSCs pretreated with VPA had significantly more BMSCs than other groups of rats. **(E)** Calculation of the average number of BMSCs at the spinal injury site. ** $p < 0.01$ indicates significant differences between groups (one-way ANOVA) ($n = 6$).

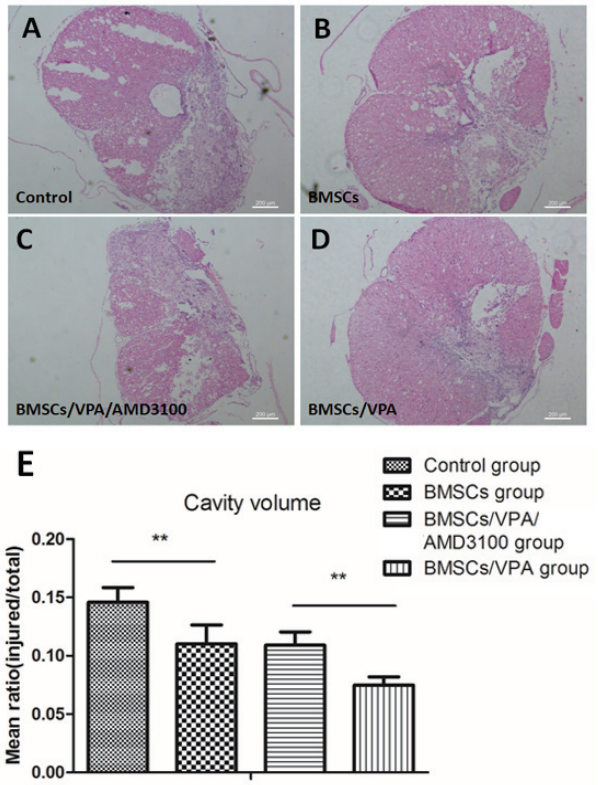

Figure 5. HE staining to estimate the volume of injured tissue and spared tissue. (A) Control group. **(B)** Rats receiving untreated BMSCs. **(C)** Rats receiving BMSCs pretreated with VPA and AMD3100. **(D)** Rats receiving BMSCs pretreated with VPA (magnification: ×40, bar = 50 μm). **(E)** The mean ratio of the injured area/total area for all groups of rats. Compared to the control group, other groups of rats had a cavity with a smaller volume. ** $p < 0.01$ indicates significant differences between groups (one-way ANOVA) ($n = 6$).

limb paraplegia immediately after SCI. The recovery of motor function was most pronounced 1-2 weeks after SCI, and especially on days 10-14 post-injury. All of the groups of rats had relatively few changes in their score on the BBB Locomotor Rating Scale from day 14 post-injury to day 28 post-injury (Figure 6A). On day 28 post-injury, the control group had a score of 4.17 ± 0.75 points on the BBB Locomotor Rating Scale, rats receiving untreated BMSCs had a score of 6.33 ± 0.82 points, rats receiving BMSCs pretreated with VPA had a score of 9.5 ± 1.05 points, and rats receiving BMSCs pretreated with VPA and AMD3100 had a score of 7 ± 0.89 points. Rats receiving BMSCs pretreated with VPA had a more marked recovery than other groups of rats ($p < 0.05$). Moreover, there were significant differences ($p < 0.05$) between rats receiving untreated BMSCs or BMSCs pretreated with VPA and AMD3100 and the control group.

4. Discussion

BMSC transplantation *via* the tail vein is a useful way to treat SCI (*4,16*). However, the uneven distribution of exogenous BMSCs *in vivo* has seriously hindered further research. Recent studies have found that systemic infusion of BMSCs resulted in their distribution to a wide range of tissues in nonhuman primates and that these BMSCs may migrate to the site of an SCI (*17*). The current study confirmed that exogenous BMSCs migrated to the spinal injury site and that pretreatment with VPA enhanced the therapeutic benefits of BMSCs in terms of greater cell migration and improved neurological recovery. These results also indicate that SDF-1/CXCR4 plays an important role in the migration of BMSCs to the spinal injury site.

BMSCs can be harvested, cultured, and grown *in vitro* and exhibit low immunogenicity (*4*). BMSCs were isolated from the tibias and femurs of rats and passaged 3 times, resulting in a consistent morphology and phenotype. Cells passaged 3 times had consistent morphological features of being flat and spindle-shaped. The results of flow cytometry indicated that BMSCs were positive for CD29, CD73, and CD90 and negative for CD45 and CD106, and these findings agree with the results of previous studies (*7,18*). Moreover, an attempt was made to analyze the multipotency of BMSCs by inducing osteogenic and adipogenic cell differentiation. As noted in other sources, BMSCs were able to differentiate into osteoblasts and adipocytes with a high level of efficiency (*15*).

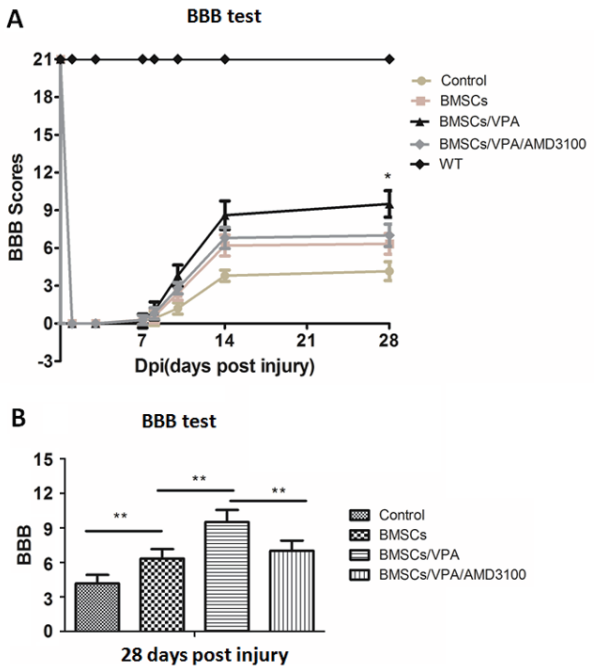

Figure 6. Evaluation of the recovery of hind limb function using the BBB Locomotor Rating Scale. (A) Functional recovery was similar for all of the groups of rats. Rats receiving BMSCs pretreated with VPA had a better recovery than did other groups of rats. Significant differences were noted between the control group and rats receiving BMSCs pretreated with VPA ($p < 0.0001$), and significant differences ($p < 0.0001$) were also noted between rats receiving BMSCs pretreated with VPA and rats receiving BMSCs pretreated with VPA and AMD3100. **(B)** On day 28 post-injury, the score on the BBB Locomotor Rating Scale peaked for all of the groups of rats. Rats receiving BMSCs/VPA had a significantly higher score than rats receiving BMSCs and rats receiving BMSCs pretreated with VPA and AMD3100. Rats receiving untreated BMSCs had a significantly higher score than the control group. ** $p < 0.01$ indicates significant differences between groups.

VPA has been widely used as an anticonvulsant for over 40 years and is believed to act *via* g-aminobutyric acid (GABA) metabolism. A study has indicated that VPA inhibits HDAC, altering the structure of chromatin and neuronal gene expression (*10*). In previous studies, VPA increased the expression of glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) in astrocytes to protect neurons (*19*). A recent study showed that VPA promoted the BDNF and GDNF expression of BMSCs and that it also improved the ability of BMSCs to migrate (*20*). Studies have indicated that VPA increases the expression of CXCR4 by inhibiting HDAC, thus improving the ability of BMSCs to migrate. Results of one study indicated that short-term (3 h) exposure of MSCs to a relatively high concentration (2.5 mM) of VPA markedly increased CXCR4 transcript and protein levels (*11*). The current study found that pretreatment with 2.5 mM of VPA for 3 h significantly increased the CXCR4 expression of BMSCs. This study also examined the effects of certain concentrations of VPA on the rate of BrdU labeling of BMSCs. Results indicated that the rate of labeling differed little among 3 groups of cells, thus ruling out the effect of different drugs on the BrdU labeling of MSCs.

Various chemokines and receptors are involved in the migration of BMSCs, and the involvement of SDF-1 and its cognate receptor CXCR4 in BMSC migration to injured tissues is well documented (*8,21*). Though high levels of SDF-1 were expressed by meningeal cells in both intact and injured spinal cords, the level of SDF-1 was elevated at the site following SCI and a gradient arose to guide immune and stem cells to the injury site. The current study found little expression of SDF-1 in the normal spinal cord, an increased level on day 1 post-injury, and the highest level on day 7 post-injury. This agrees with the results of a previous study (*22*). Only a small proportion of BMSCs expresses functionally active CXCR4 receptor on their surface and this expression diminished with passage, but CXCR4 is key to mediating specific migration of these cells (*23,24*). Overexpression of CXCR4 by genetic modification enhances the ability of MSCs to respond to SDF-1-induced chemotaxis *in vitro* and promotes MSC recruitment around the ischemic core *in vivo* (*25*).

To explore the hypothesis that VPA promotes the migration of MSCs, BMSC transplantation *via* tail vein injection was performed on rats with an SCI 7 days after injury. The transplantation consisted of 1×10^6 BrdU-labeled BMSCs, and the injured segments of the spinal cord were analyzed using immunofluorescence and HE staining. BrdU immunostaining indicated that rats receiving BMSCs pretreated with VPA or BMSCs pretreated with VPA and AMD3100 had relatively more MSCs at the injury site than rats receiving untreated BMSCs. Pretreatment with VPA and AMD3100 largely suppressed the homing ability of BMSCs, indicating that SDF-1/CXCR4 played an important role in the migration of BMSCs. In rats receiving untreated BMSCs, few BMSCs were present at the injury site. This may be related to the low level of CXCR4 expression on the surface of BMSCs.

BMSCs had more of a therapeutic effect on motor recovery of rats with an SCI with a greater number of BMSCs at the injury site. The BBB Locomotor Rating Scale is a general method of evaluating behavior and can accurately assess changes in behavior after SCI. HE staining also indicated that injured rats with better motor recovery had a smaller cavity in the spinal cord. Pretreatment of BMSCs with VPA may enhance the therapeutic effects of BMSCs in SCI by increasing BMSC migration, which is closely related to SDF-1/CXCR4. However, the mechanism of action of BMSCs in SCI therapy has yet to be fully elucidated (*1-3*). One possible mechanism is *via* the secretion of growth factors, like BDNF and GDNF, by BMSCs to reduce lesion volume and promote axonal regrowth of the injured spinal cord (*26*). More BMSCs may maintain an effective concentration of growth factors. Another

possible mechanism is *via* the differentiation of BMSCs into neurons, which may serve to rebuild neural circuits (*27,28*). Moreover, VPA may promote the secretion of TGF-β, BDNF, and bFGF by BMSCs (*20*). TGF-β plays an important role in immunosuppression by inhibiting the activity of T cells (CD4, CD8) and dendritic cells (*29*). Previous studies also indicated that BDNF and bFGF were factors that induced BMSCs to transform into neural cells (*30*). Thus, an increase in the aforementioned autocrine factors of MSCs might result in enhanced immunosuppression and transformation of BMSCs into neural cells that help to repair SCI. Compared to a study by Cho *et al*. (*20*), the current study used a relatively low concentration of VPA. This may have a limited effect on the aforementioned factors secreted by MSCs. A high concentration of VPA could result in apoptosis of BMSCs (*11*).

In summary, the current study indicates that BMSC transplantation *via* the tail vein is a minimally invasive and useful way to provide SCI therapy. However, the therapeutic effectiveness of BMCSs is limited. Pretreatment with VPA can enhance the therapeutic benefits of BMSCs in terms of greater cell migration and better neurological outcomes after traumatic SCI. The mechanism of this enhancement may be related to growth factors, axons, and neuron transdifferentiation from BMSCs at the injury site (*31*). Therefore, pretreatment of BMSCs with VPA warrants further study in relation to the treatment of traumatic SCI.

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