RESEARCH ARTICLE

Local injection of bone morphogenetic protein 7 promotes neuronal regeneration and motor function recovery after acute spinal cord injury

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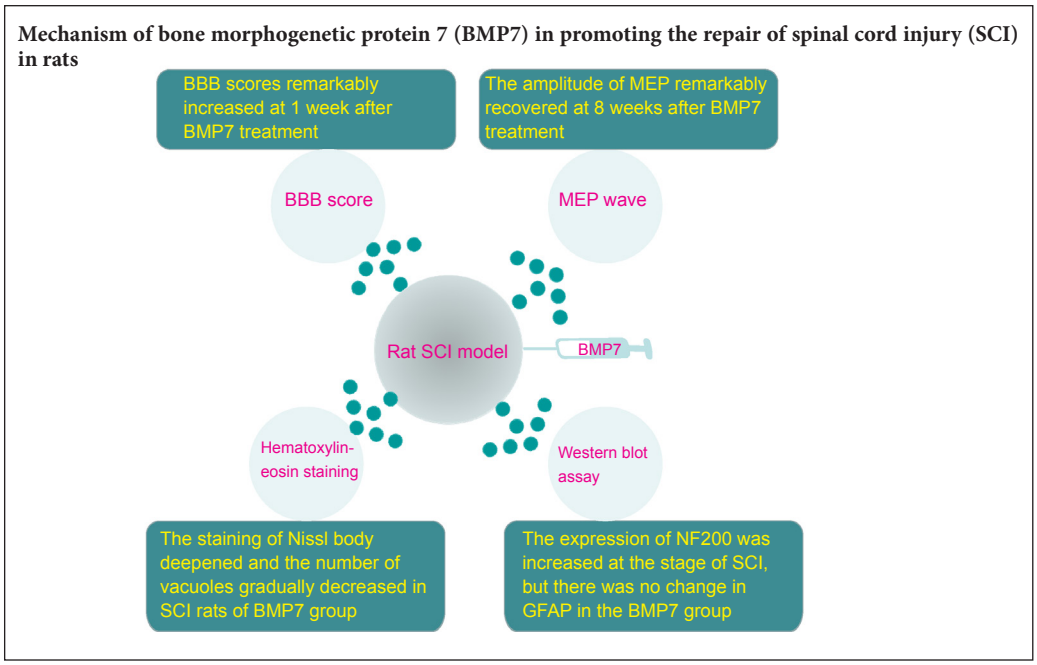
Graphical Abstract



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Abstract

After spinal cord injury, the number of glial cells and motor neurons expressing bone morphogenetic protein 7 (BMP7) increases, indicating that upregulation of BMP7 can promote nerve repair. We, therefore, tested whether direct injection of BMP7 into acutely injured rat spinal cord can affect neurological recovery. Allen's impactor was used to create spinal cord injury at T_{10}. The injury site was then injected with 50 ng BMP7 (BMP7 group) or physiological saline (control group) for 7 consecutive days. Electrophysiological examination showed that the amplitude of N1 in motor evoked potentials (MEP) decreased after spinal cord injury. At 8 weeks post-operation, the amplitude of N1 in the BMP7 group was remarkably higher than that at 1 week post-operation and was higher than that of the control group. Basso, Beattie, Bresnahan scale (BBB) scores, hematoxylin-eosin staining, and western blot assay showed that at 1, 2, 4 and 8 weeks post-operation, BBB scores were increased; Nissl body staining was stronger; the number of Nissl-stained bodies was increased; the number of vacuoles gradually decreased; the number of synapses was increased; and the expression of neuronal marker, neurofilament protein 200, was increased in the hind limbs of the BMP7 group compared with the control group. Western blot assay showed that the expression of GFAP protein in BMP7 group and control group did not change significantly and there was no significant difference between the BMP7 and control groups. These data confirmed that local injection of BMP7 can promote neuronal regeneration after spinal cord injury and promote recovery of motor function in rats.

Key Words: *nerve regeneration; behavior; Basso, Beattie, Bresnahan scale score; motor evoked potential wave; Nissl staining; neurons; glial cells; neurofilament protein 200; glial fibrillary acidic protein; neural regeneration*

Introduction

Spinal cord injury (SCI) is a severe traumatic disease of the central nervous system characterized by high incidence and high morbidity. SCI brings heavy economic and psychological burden to individuals and their families and to society (Uchida et al., 2016; Liu et al., 2017). SCI includes two stages: primary mechanical structural damage and secondary neurological dysfunction (Zhang et al., 2016; Ji et al., 2017; Yamasaki et al., 2017). Traditional surgical treatment can only restore the stability of the spine and has little effect on the secondary neurological dysfunction (Zhao et al., 2016; Han et al., 2017). Previous studies have focused on the use of

growth factors to alleviate secondary injury and to promote the recovery of axonal growth, limb movement and sensory function (Ding et al., 2014; Dhall et al., 2017; Fang et al., 2017; Farjah et al., 2017; Peng et al., 2017).

Bone morphogenetic proteins (BMPs) are a multifunctional family of growth factors that belongs to the transforming growth factor-β superfamily (Ruzicka et al., 2016; Yang et al., 2016). Numerous studies have shown that BMP7 is a potent factor in the treatment of neurological diseases (Lo et al., 2013; Celik et al., 2014; Bowers et al., 2016). After SCI, the expression of Bmp7 mRNA is remarkably increased (Chikuda et al., 2014), and the number of BMP7-expressing glial cells and motor neurons is dramatically increased (Yin et al., 2013; Furlan et al., 2016). Thus, the up-regulation of BMP7 after SCI may play an important role in nerve repair (Camassola et al., 2012; Ojo et al., 2017). The effects of direct injection of BMP7 into an injured spinal cord have not been investigated. Therefore, in this study, BMP7 was directly injected into rats with SCI, and we explored the role of BMP7 in repairing SCI. We provide a theoretical basis for the treatment of SCI with BMP7.

Materials and Methods

Animals

One hundred specific-pathogen-free male Wistar rats aged 4 weeks and weighing approximately 110 g were provided by the Experimental Animal Center of Xinjiang Medical University of China [license No. SCXK (New) 2015-001]. The rats were placed in a standard environment: normal day/night lighting schedule, relative humidity 60 ± 10%, temperature 25 ± 1°C, free access to food and water. This experiment was reviewed and approved by the Ethics Committee of Xinjiang Production and Construction Corps Hospital, China (approval number: 2018[13]).

Acute SCI model

Wistar rats were divided into three groups: Sham operation, SCI control, and BMP7 (n = 30 per group); the remaining 10 rats were used to supplement groups if rats died. After preoperative shaving, rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (30 mg/100 g; Rongbo Biotechnology Co., Ltd., Shanghai, China). A laminectomy was performed at the T_{10} level of the spinal cord to expose the dura. In the control and BMP7 groups, Allen's impactor (provided by the Orthopedics Center, First Affiliated Hospital, Shihezi University School of Medicine, China) set at 15 g × 20 cm was used to injure the spinal cord at T_{10}. One end of a 0.1 mm polyethylene catheter was inserted into the subarachnoid space of the lesion, and the other end was sealed with a heparin cap. The dura mater, muscle and skin were then sutured layer by layer.

The acute SCI rat model was deemed to be successfully established in accordance with previous criteria (Jain et al., 2006; Chen et al., 2014; Rao et al., 2014; Hosseini et al., 2016): The local spinal cord tissue showed hemorrhage and edema, but the dura was intact, and spasmodic swing, retraction flutter and hind limb paralysis could be present.

During post-processing, the rats were placed in cages, allowed free access to food and water, and had bedding changed daily. All rats were intramuscularly injected with penicillin (Yu Bo Biotechnology Co., Ltd., Shanghai, China) (40 thousand U/ times) each day after the operation for 3 days. In the sham operation and BMP7 groups, auxiliary urination was performed by exerting bladder pressure twice a day until the recovery of autonomic urination. In the BMP7 group, 50 ng BMP7 protein (R&D Systems, Minneapolis, MN, USA) dissolved in normal saline was injected once per day into the injury site *via* the 0.1 mm polyethylene catheter for 7 consecutive days, starting 30 minutes after the operation. Rats in the control group were given an equal volume of 0.9% sodium chloride (Yu Bo Biotechnology Co., Ltd.) under the same administration regimen.

Behavioral assessment

The Basso, Beattie, Bresnahan (BBB) scale scores of rats were evaluated before operation, and at 6 hours, 3 days, 1, 2, 4 and 8 weeks after successful modeling to assess the functional recovery of hind limbs. A perfect score is 21. The higher the BBB score, the better the coordination function of the hind limbs and the higher the ability to perform fine hind limb movements, indicating good recovery of hind limb function (Yu et al., 2005; Celik et al., 2014).

Electrophysiology

Motor evoked potential (MEP) was measured using a biological signal acquisition and processing system MP150 (Yuyan Instrument Company, Shanghai, China) at 1 and 8 weeks post-operation. After anesthesia, a small hole 1 mm posterior to the anterior fontanel and the sagittal suture was opened. The stimulating electrode was placed into the small hole. The recording electrode was placed into the right posterior gastrocnemius, with the positive and negative poles 1 cm apart. The positive pole was at the proximal end, and the negative pole was at the distal end. The reference and recording electrodes were at the same level, and placed under the skin. Stimulus parameters: Coarse voltage, single stimulus, strength 3.00 V, gain G-2000, time constant T-0.01 s, filtering f = 1 kHz.

Hematoxylin-eosin staining

Rats in the control and BMP7 groups were anesthetized by intraperitoneal injection of 10% chloral hydrate at 6 hours, 3 days, 1, 2, 4 and 8 weeks after injury. The chest was opened to expose the heart, and 0.9% sodium chloride (containing heparin, 15 U/mL) was injected into the left ventricle until the body stiffened, followed by perfusion with 4% paraformaldehyde (Suobao Biotechnology Co., Ltd., Beijing, China). Spinal cord tissue of about 1 cm in length centered on the lesion was removed and placed in 4% paraformaldehyde for 24 hours. After paraffin embedding, six 6-μm-thick slices were cut for each rat using a microtome (Yuyan Instrument Company). The slices were placed in a 60°C oven for 30 minutes to melt the paraffin wax, deparaffinized in xylene (Rongbo Biotechnology Co., Ltd.), dehydrated in alcohol, and stained with hematoxylin (Junrui Biotechnology Co., Ltd., Shanghai,

China) for 5 minutes. These sections were then differentiated with 1% hydrochloric acid for 10 seconds, stained with eosin (Junrui Biotechnology Co., Ltd.) for 3 minutes, cleared in xylene, mounted with neutral resin, and finally observed under an optical microscope.

Western blot assay

At 6 hours, 3 days, 1, 2, 4 and 8 weeks after injury, the expression of neurofilament protein 200 (NF200) and glial fibrillary acidic protein (GFAP) was detected by western blot assay. After homogenization of spinal cord specimens from control group and BMP7 groups, RIPA lysate (Wako Pure Chemical Industries, Tokyo, Japan) was added and incubated for 30 minutes. Samples were then centrifuged at 7200 ×*g* and 4°C for 5 minutes and the supernatants collected. Protein concentration was determined using the bicinchoninic acid assay and the same amount of protein from each sample was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Subsequently, proteins were transferred onto polyvinylidene fluoride membranes. Membranes were blocked in 5% bovine serum albumin 1H solution at room temperature. Internal reference β-actin was monitored with mouse anti-β-actin monoclonal antibody. Mouse monoclonal NF-200 (neuron marker) and GFAP (astrocyte marker) (1:400; Boster Biological Technology, Wuhan, China) primary antibodies were added and incubated overnight at 4°C. After washing with Tris-buffered saline Tween-20 three times, a goat anti-mouse secondary antibody (1:10,000; Boster Biological Technology) was added and incubated at 37°C in the dark for 1 hour. The samples were visualized by ECL luminescence. The target protein bands were compared by grayscale scan analysis.

Statistical analysis

Data were analyzed using SPSS 19.0 statistical software (IBM, Armonk, NY, USA). Normally distributed data were expressed as the mean ± SD. The mean between groups was analyzed by one-way analysis of variance, and the Student-Newman-Keuls test. A value of $P < 0.05$ was considered statistically significant.

Results

Ninety-six rats were included in the study. During the experiment, three deaths occurred in the BMP7 group; two deaths occurred in the control group; and one death occurred in the sham operation group. All dead rats were supplemented with new ones. A total of 90 rats were included in the results.

Effects of local BMP7 injection on rat hind limb motor function

Two rats in the control group and one in the BMP7 group had died at 3 days after injury. After 1 week, one further rat in each of the control and BMP7 groups had died. Both groups were supplemented with new rats. The results of BBB showed that the hind limb motor function was not affected in the sham operation group. In the control and BMP7 groups, hind limb motor function was completely lost after model establishment, and gradually recovered over 3 days. The BBB scores for the BMP7 group were significantly higher than those for the control group at 1, 2, 4 and 8 weeks ($P < 0.05$; **Table 1**).

Effects of local BMP7 injection on electrophysiological parameters in rats

The MEP waveform at the T_{10} level was normal in the sham operation group, *i.e.*, P1-N1-P2. The upward N1 wave was the most stable among the three groups (**Figure 1A**). At 1 week after injury, the N1 wave in the MEP wave of control and BMP7 groups was lower than that of the normal N1 waveform (**Figure 1B, C**). At 8 weeks after injury, the MEP waveforms in the control and BMP7 groups were mostly M-shaped bimodal waves, and their amplitudes were significantly higher compared with those at 1 week. The amplitude was higher in the BMP7 group compared with that of the control group (**Figure 1D, E**).

Effects of local BMP7 injection on injured tissue of rats

As shown in **Figure 2**, Nissl body staining in the control and BMP7 groups was weak; the number of Nissl bodies was reduced; the number of neuronal cells was decreased; and the number of neuronal nuclei was reduced; the nuclei of neuronal cells became pyknotic; and many cavities appeared after liquefaction of spinal cord tissue. Histological changes were similar between the control and BMP7 groups at 6 hours and 3 days after surgery. At 1–8 weeks after BMP7 treatment, the number of Nissl bodies at the injury site was higher in the BMP7 group than in the control group, and the BMP7 group had fewer holes than the control group.

Effects of local BMP7 injection on the levels of NF200 and GFAP at the injury site of the spinal cord

The levels of NF200 and GFAP were different between the control and BMP7 groups (**Figure 3A**). The gray-scale scanning results of each band showed no significant changes in NF200 levels at different time points in the sham operation group. The level of NF200 in the BMP7 group was remarkably increased at 3 days, and was highest at 4 weeks after injury. At 1, 2, 4, and 8 weeks after injury (**Figure 3B**), NF200 levels were higher in the BMP7 group than in the control group ($P < 0.05$). The levels of GFAP at the injury site reached a peak after 2 weeks in both control and BMP7 groups, and then decreased, but there was no significant difference between groups (**Figure 3C**).

Discussion

BMP7, a member of the BMP family, was first shown to have an osteogenic effect; however, subsequent studies showed that BMP7 has a neuroprotective function at the early stage of SCI (Wilson et al., 2012; Jia et al., 2014; Wang et al., 2014; Luan et al., 2015). Wang et al. (2016) found that BMP7 can inhibit oligodendrocyte apoptosis induced by tumor necrosis factor-α, reduce demyelination, and protect nerve function after SCI. Hu (2014) intravenously injected BMP7 into Sprague-Dawley SCI model rats, and found that GFAP expression increased in the injured spinal cord segment. This finding indicated that BMP7 promoted the proliferation of

Table 1 BBB scores at different time points

Group	Pre-operation	Post-operation					
		6 hours	3 days	1 week	2 weeks	4 weeks	8 weeks
Control	21.00±0.00	0.00±0.00	0.00±0.00	0.33±0.02	3.00±0.19	4.33±0.52	4.41±0.56
Sham operation	21.00±0.00	20.00±0.02*	20.35±0.05*	20.86±0.04*	21.00±0.00*	21.00±0.00*	21.00±0.00*
BMP7	21.00±0.00	0.00±0.00	0.00±0.00	0.50±0.05*	5.50±0.15*	8.00±0.63*	8.20±0.34*

*$P < 0.05$, *vs.* control group (mean ± SD, $n = 5$; one-way analysis of variance and Newman-Keuls multiple comparison test). SCI: Spinal cord injury.

astrocytes, reduced glial scaring and inhibited inflammation, thereby protecting the nerves and reducing the occurrence of secondary damage. Therefore, this study investigated the effect of BMP7 protein on the recovery of motor function after SCI, and further explored its mechanism of action to provide new ideas for the treatment of acute SCI.

In the present study, the SCI model was established according to Allen's method and BMP7 was applied to the injury site. The effect of BMP7 on the recovery of motor function after SCI was determined by BBB scores and neurophysiological examination. The BBB score of the BMP7 group was higher than that of the control group at 1 week after injury. At 8 weeks, the BBB score was also higher in the BMP7 group compared with the control group, indicating that BMP7 promoted the recovery of motor function after SCI in rats. Park et al. (2013) used agmatine in SCI rats and found that elevated expression of BMP7 resulted in obvious improvement of histological findings and motor function, neuroprotection, and reduction of local glial scarring. Their results suggest that BMP7 favors the recovery of neurological function after SCI. Our study involved application of BMP7 directly at the injury site. This produced a higher BBB score in the BMP7 group than in the sham operation group, consistent with the results of Park et al (2013).

MEP is an electrical signal recorded in the central nervous system and in the distal spinal cord, peripheral nerve or muscle, that can directly reflect the functional status of the descending spinal cord or a peripheral motor nerve (Gage et al., 2000; Lo et al., 2013; Zhang et al., 2014; Yılmaz et al., 2015). The MEP waveform and amplitude changes can verify whether BMP7 treatment promoted spinal motor conduction recovery after SCI. The results showed that the MEP waveforms in the BMP7 group at 8 weeks after injury were mostly M-shaped bimodal waves; the amplitude in the BMP7 group was dramatically higher at 8 weeks than that at 1 week, and the amplitude was higher in the BMP7 group compared with the control group, suggesting that the motor conduction pathway in the BMP7 group was partially repaired and that motor function was improved at 8 weeks. In this experiment, morphological observation and neurophysiology showed that BMP7 promotes recovery of motor function in SCI rats.

We also observed histological changes by hematoxylin-eosin staining. After BMP7 treatment, Nissl body staining of rat spinal cord lesions became stronger; the number of vacuoles was gradually decreased; and the number of synapses was increased. Nissl bodies indicate the main sites of protein synthesis in neurons (Haubruck et al., 2016; Westhauser et al., 2016; Dong et al., 2017; Yang et al., 2017). When neuronal cells are over-stimulated, such as after mechanical damage to the spinal cord, Nissl bodies can reduce in size or disappear; when the damaging agent disappears, Nissl bodies can be restored with simultaneous restoration of protein synthesis and cell metabolism. Therefore, Nissl bodies can be used to indicate neuronal status (Fehlings et al., 2012; Chikuda et al., 2014; Cui et al., 2015; North et al., 2015; Holland et al., 2016; Okuda et al., 2017). Our results show that, after BMP7 treatment, Nissl body staining at the injury site became stronger, suggesting that after BMP7 treatment, the metabolic function of neurons was gradually restored, and neuronal function was partially repaired in SCI rats.

NF200, a specific marker of neurons, mainly exists in the cytoplasm and axons of neurons. The expression level of NF200 reflects the number and function of neurons (Chen et al., 2015; García-Álvarez et al., 2015; Yin et al., 2015; Manthou et al., 2017). GFAP is a cytoskeletal component specifically expressed in astrocytes. GFAP levels can reflect the degree of astrocyte proliferation and necrosis (Perron et al., 2011; Martinez et al., 2013; Namsolleck et al., 2013; Kanno et al., 2014). In this experiment, the expression of NF200 and GFAP in the injured spinal cord at different time points was detected by western blotting. In the BMP7 group, NF200 expression in the injured segment was significantly increased after 1 week and reached a peak level at 4 weeks. The amount of GFAP was not remarkably different from that in the sham operation group. The increased level of NF200 in the injured segment indicated that the number of local neuronal cells was increased and that neuronal cell function was enhanced. We conclude that BMP7 can promote the differentiation of local neural progenitor cells into neuronal cells, thus increasing the number of neuronal cells and promoting the repair of motion conduction pathways after SCI. The expression of GFAP in the injured segment was not obvious, indicating that BMP7 had little effect on astrocytes. However, this result is in contrast to the conclusion of Hu et al (2014) who showed that BMP7 can promote GFAP expression after SCI. Differences between this study and that of Hu et al., may explain these differences; for example: (1) Hu et al. (2014) modeled SCI using the cerclage method, whereas we established the SCI model using Allen's method. The mechanism of the two methods and the degree of SCI were different, thus the specific mechanism of spinal cord repair was dif-

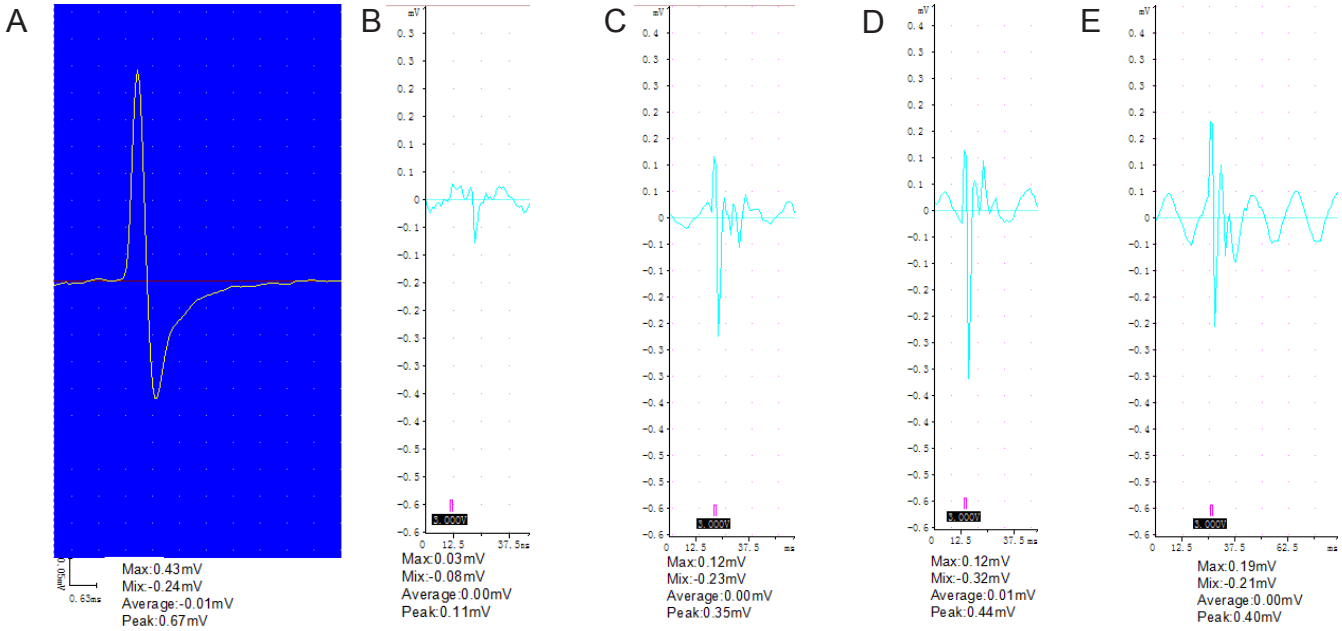

Figure 1 Effects of local injection of BMP7 on MEP waveforms at different time points in each group.
(A–C) MEP waveforms of sham operation (A) control (B), and BMP7 groups (C) at 1 week. (D, E) MEP waveforms of control (D) and BMP7 groups (E) at 8 weeks. Abscissa: Recording time (ms); ordinate: nerve voltage (mV); BMP7: bone morphogenetic protein 7; MEP: motor evoked potential.

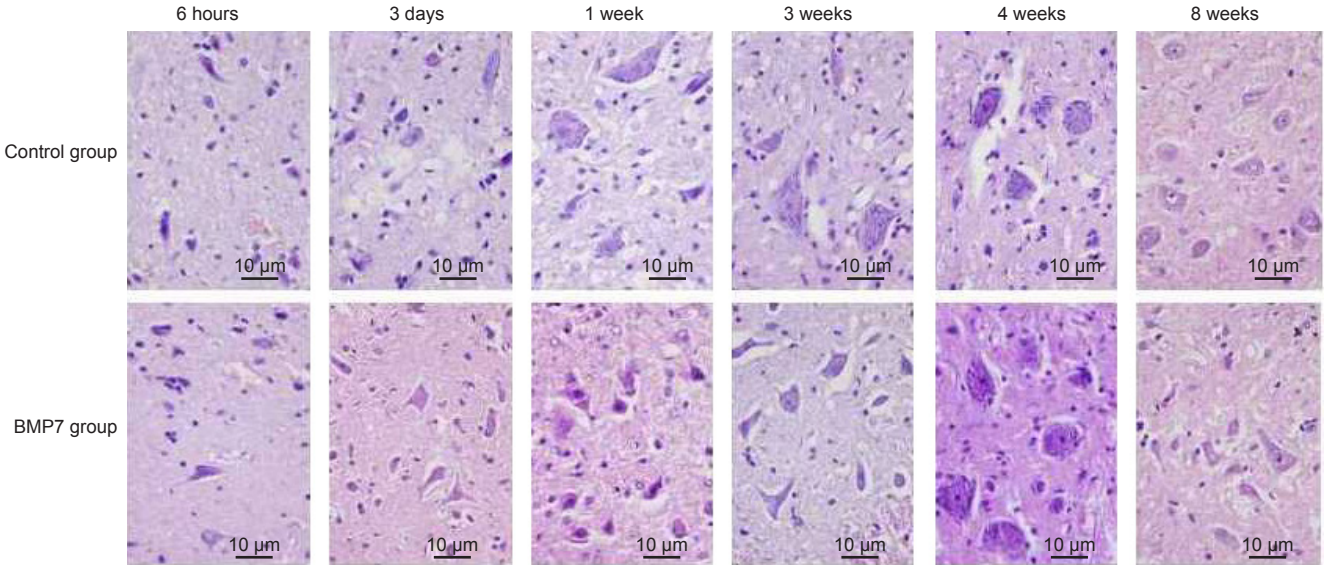

Figure 2 Effects of local BMP7 injection on injured tissue (hematoxylin-eosin staining).
At 1–8 weeks after BMP7 treatment, the Nissl staining was stronger in the BMP7 group compared with the control group, and there were more Nissl bodies in the BMP7 group than in the control group at each corresponding time point. Scale bars: 10 μm. Original magnification, 400×. BMP7: Bone morphogenetic protein 7.

ferent. (2) Hu et al. treated SCI by intravenous injection of BMP7. In this study, the BMP7 protein was directly injected onto the injury site through the subarachnoid catheter. Different injection protocols can cause different local BMP7 protein concentrations, and different drug concentrations stimulate different repair mechanisms.

In summary, BMP7 can promote motor function recovery after SCI in rats. Nevertheless, its precise mechanism of action requires further study. We speculate that BMP7 can promote the differentiation of local neural progenitor cells into neuronal cells, thereby increasing the number of neurons and promoting repair of the motor conduction pathway. Taken together, our results provide a theoretical basis for the clinical application of BMP7, and provide a new perspective for the treatment of acute SCI.

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Author contributions: *KXL designed the study. CC, GCB and KL per-*

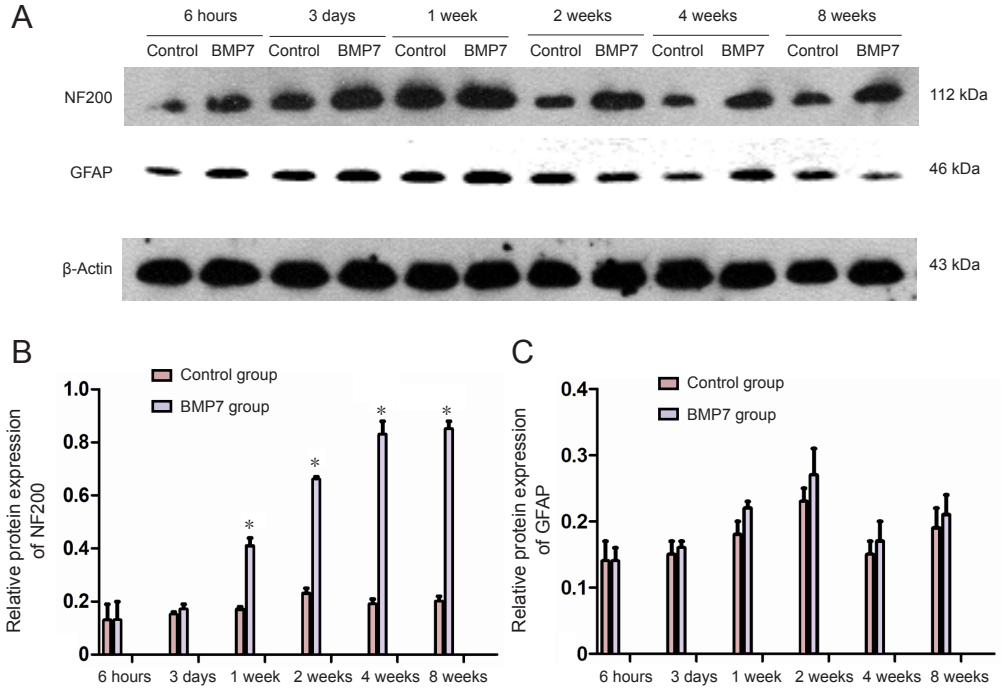

Figure 3 Effects of local BMP7 injection on the expression of NF200 and GFAP at various time points in the control and BMP7 groups.
(A) NF200 and GFAP at the injury site at different time points. (B, C) The grayscale ratios of NF200 and GFAP bands relative to β-actin for control and BMP7 groups at different time points. *$P < 0.05$, *vs.* control group (mean ± SD, $n = 5$; one-way analysis of variance and Newman-Keuls multiple comparison test). BMP7: Bone morphogenetic protein 7; NF200: neurofilament protein 200; GFAP: glial fibrillary acidic protein.

formed experiments. CC and GCB analyzed data and wrote the paper. All authors approved the final version of the paper.

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References

Bowers CA, Kundu B, Hawryluk GWJ (2016) Methylprednisolone for acute spinal cord injury: an increasingly philosophical debate. Neural Regen Res 11:882-885.

Camassola M, Chagastelles PC, Nardi NB (2012) Methodology, biology and clinical applications of human mesenchymal stem cells. Methods Mol Biol 879:4281-4298.

Celik B, Ones K, Celik EC, Bugdayci DS, Paker N, Avci C, Ince N (2014) The effects of using the Internet on the health-related quality of life in people with spinal cord injury: a controlled study. Spinal Cord 52:388-391.

Chen J, Zhang Z, Liu J, Zhou R, Zheng X, Chen T, Wang L, Huang M, Yang C, Li Z, Yang C, Bai X, Jin D (2014) Acellular spinal cord scaffold seeded with bone marrow stromal cells protects tissue and promotes functional recovery in spinal cord-injured rats. J Neurosci Res 92:307-317.

Chen WF, Chen CH, Chen NF, Sung CS, Wen ZH (2015) Neuroprotective effects of direct intrathecal administration of granulocyte colony-stimulating factor in rats with spinal cord injury. CNS Neurosci Ther 21:698-707.

Chikuda H, Yasunaga H, Takeshita K, Horiguchi H, Kawaguchi H, Ohe K, Fushimi K, Tanaka S (2014) Mortality and morbidity after high-dose methylprednisolone treatment in patients with acute cervical spinal cord injury: a propensity-matched analysis using a nationwide administrative database. Emerg Med J 31:201-206.

Cui ZS, Zhao P, Jia CX, Liu HJ, Qi R, Cui JW, Cui JH, Peng Q, Lin B, Rao YJ (2015) Local expression and role of BMP-2/4 in injured spinal cord. Genet Mol Res 14:9109-117.

Dong L, Dong G, Cao J, Zhang J (2017) Association of α2-HS glycoprotein with neurogenic heterotopic ossification in patients with spinal cord injury. Med Sci 23:5382-5388.

Fehlings MG, Vaccaro A, Wilson JR, Singh A, W Cadotte D, Harrop JS, Aarabi B, Shaffrey C, Dvorak M, Fisher C, Arnold P, Massicotte EM, Lewis S, Rampersaud R (2012) Early versus delayed decompression for traumatic cervical spinal cord injury: results of the surgical timing in acute spinal cord injury study (STASCIS). PLoS One 7:e32037.

Furlan JC, Craven BC, Massicotte EM, Fehlings MG (2016) Early versus delayed surgical decompression of spinal cord after traumatic cervical spinal cord injury: a cost-utility analysis. World Neurosurg 88:166-174.

Gage FH (2000) Mammalian neural stem cells. Science 287:1433-1438.

García-Álvarez I, Fernández-Mayoralas A, Moreno-Lillo S (2015) Inhibition of glial proliferation, promotion of axonal growth and myelin production by synthetic glycolipid: a new approach for spinal cord injury treatment. Restor Neurol Neurosci 28:159-163.

Han D, Chen S, Fang S, Liu S, Jin M, Guo Z, Yuan Y, Wang Y, Liu C, Mei X (2017) The neuroprotective effects of muscle-derived stem cells via brain-derived neurotrophic factor in spinal cord injury model. Biomed Res Int 1:1-11.

Haubruck P, Kammerer A, Korff S, Apitz P, Xiao K, Büchler A, Biglari B, Zimmermann G, Daniel V, Schmidmaier G, Moghaddam A (2016) The treatment of nonunions with application of BMP-7 increases

the expression pattern for angiogenic and inflammable cytokines: a matched pair analysis. J Inflamm Res 9:155-165.
Holland CM, Kebriaei MA, Wrubel DM (2016) Posterior cervical spinal fusion in a 3-week-old infant with a severe subaxial distraction injury. J Neurosurg Pediatr 17:353-356.
Hosseini SM, Sharafkhah A, Koohi-Hosseinabadi O, Semsar-Kazerooni M (2016) Transplantation of neural stem cells cultured in alginate scaffold for spinal cord injury in rats. Asian Spine J 10:611-618.
Hu LX (2014) Effect of BMP-7 on the expression of gfap after acute spinal cord injury in rats. Wuhu: Wannan Medical College. 2014.
Jain A, Kim YT, McKeon RJ, Bellamkonda RV (2006) In situ gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury. Biomaterials 27:497-504.
Ji WC, Zhang XW, Qiu YS (2016) Selected suitable seed cell, scaffold and growth factor could maximize the repair effect using tissue engineering method in spinal cord injury. World J Exp Med 6:58-62.
Jia Y, Wu D, Zhang R, Shuang W, Sun J, Hao H, An Q, Liu Q (2014) Bone marrow-derived mesenchymal stem cells expressing the Shh transgene promotes functional recovery after spinal cord injury in rats. Neurosci Lett 24:46-51.
Kanno H, Pressman Y, Moody A, Berg R, Muir EM, Rogers JH, Ozawa H, Itoi E, Pearse DD, Bunge MB (2014) Combination of engineered schwann cell grafts to secrete neurotrophin and chondroitinase promotes axonal regeneration and locomotion after spinal cord injury. J Neurosci 34:1838-1855.
Liu S, Sandner B, Schackel T, Nicholson L, Chtarto A, Tenenbaum L, Puttagunta R, Müller R, Weidner N, Blesch A (2017) Regulated viral BDNF delivery in combination with Schwann cells promotes axonal regeneration through capillary alginate hydrogels after spinal cord injury. Acta Biomater 60:167-180.
Lo V, Esquenazi Y, Han MK, Lee K (2013) Critical care management of patients with acute spinal cord injury. J Neurosurg Sci 57:281-292.
Luan L, Yang X, Zhou C, Wang K, Qin L (2015) Post-hypoxic and ischemic neuroprotection of BMP-7 in the cerebral cortex and caudate-putamen tissue of rat. Acta Histochem 117:148-154.
Manthou M, Abdulla DS, Pavlov SP, Jansen R, Bendella H, Nohroudi K, Stein G, Meyer C, Ozsoy O, Ozsoy U, Behram Kandemir Y, Sarikcioglu L, Semler O, Schoenau E, Dunlop S, Angelov DN (2017) Whole body vibration (WBV) following spinal cord injury (SCI) in rats: timing of intervention. Restor Neurol Neurosci 35:185-216.
Martinez M, Delivetmongrain H, Rossignol S (2013) Treadmill training promotes spinal changes leading to locomotor recovery after partial spinal cord injury in cats. J Neurophysiol 109:2909-2922.
Namsolleck P, Boato F, Schwengel K, Paulis L, Matho KS, Geurts N, Thöne-Reineke C, Lucht K, Seidel K, Hallberg A, Dahlöf B, Unger T, Hendrix S, Steckelings UM (2013) AT2-receptor stimulation enhances axonal plasticity after spinal cord injury by upregulating BDNF expression. Neurobiol Dis 51:177-191.
North HA, Pan L, McGuire TL, Brooker S, Kessler JA (2015) β1-Integrin alters ependymal stem cell BMP receptor localization and attenuates astrogliosis after spinal cord injury. J Neurosci 35:3725-3733.
Ojo OA, Poluyi EO, Owolabi BS, Kanu OO, Popoola MO (2017) Surgical decompression for traumatic spinal cord injury in a tertiary center. Niger J Clin Pract 20:1455-1460.
Okuda A, Horii-Hayashi N, Sasagawa T, Shimizu T, Shigematsu H, Iwata E, Morimoto Y, Masuda K, Koizumi M, Akahane M, Nishi M, Tanaka Y (2017) Bone marrow stromal cell sheets may promote axonal regeneration and functional recovery with suppression of glial scar formation after spinal cord transection injury in rats. J Neurosurg Spine 26:388-395.
Park YM, Lee WT, Bokara KK, Seo SK, Park SH, Kim JH, Yenari MA, Park KA, Lee JE (2013) The multifaceted effects of agmatine on functional recovery after spinal cord injury through modulations of BMP-2/4/7 expressions in neurons and glial cells. PLoS One 8:e53911.
Perron J C, Dodd J (2011) Inductive specification and axonal orientation of spinal neurons mediated by divergent bone morphogenetic protein signaling pathways. Neural Dev 6:1-16.
Rao YJ, Zhu WX, Du ZQ, Jia CX, Du TX, Zhao QA, Cao XY, Wang YJ (2014) Effectiveness of olfactory ensheathing cell transplantation for treatment of spinal cord injury. Genet Mol Res 13:4124-4129.
Ruzicka J, Machova-Urdzikova L, Gillick J, Amemori T, Romanyuk N, Karova K, Zaviskova K, Dubisova J, Kubinova S, Murali R, Sykova E, Jhanwar-Uniyal M, Jendelova P (2016) A comparative study of three different types of stem cells for treatment of rat spinal cord injury. Cell Transplant 26:585-603.
Uchida S, Hayakawa K, Ogata T, Tanaka S, Kataoka K, Itaka K (2016) Treatment of spinal cord injury by an advanced cell transplantation technology using brain-derived neurotrophic factor-transfected mesenchymal stem cell spheroids. Biomaterials 109:1-11.
Wang LJ, Zhang RP, Li JD (2014) Transplantation of neurotrophin-3-expressing bone mesenchymal stem cells improves recovery in a rat model of spinal cord injury. Acta Neurochirurgica 156:1409-1418.
Wang X, Xu JM, Wang YP, Yang L, Li ZJ (2016) Protective effects of BMP-7 against tumor necrosis factor α-induced oligodendrocyte apoptosis. Int J Dev Neurosci 53:10-17.
Westhauser F, Höllig M, Reible B, Xiao K, Schmidmaier G, Moghaddam A (2016) Bone formation of human mesenchymal stem cells harvested from reaming debris is stimulated by low-dose bone morphogenetic protein-7 application in vivo. J Orthop 13:404-408.
Wilson JR, Singh A, Craven C, Verrier MC, Drew B, Ahn H, Ford M, Fehlings MG (2012) Early versus late surgery for traumatic spinal cord injury: the results of a prospective Canadian cohort study. Spinal Cord 50:840-843.
Yamasaki A, Kasai A, Toi A, Kurita M, Kimoto S, Hayata-Takano A, Nakazawa T, Nagayasu K, Shintani N, Hashimoto R, Ito A, Meltzer HY, Ago Y, Waschek JA, Onaka Y, Matsuda T, Baba A, Hashimoto H (2015) Identification of the role of bone morphogenetic protein (BMP) and transforming growth factor-β (TGF-β) signaling in the trajectory of serotonergic differentiation in a rapid assay in mouse embryonic stem cells in vitro. J Neurochem 132:418-428.
Yang Y, Yi L, Yeerzhati Hajiaheman, Telieke Kanzhale, Jin GL (2016) Stromal cell derived factor-1/chemokine receptor 4 signaling pathway is involved in bone morphogenetic protein 2-induced migration of bone marrow mesenchymal stem cells. Zhongguo Zuzhi Gongcheng Yanjiu 20:6098-6104.
Yang Y, Guo C, Liao B, Cao J, Liang C, He X (2017) BAMBI inhibits inflammation through the activation of autophagy in experimental spinal cord injury. Int J Mol Med 39:423-429.
Yılmaz T, Kaptanoğlu E (2015) Current and future medical therapeutic strategies for the functional repair of spinal cord injury. World J Orthop 6:42-55.
Yin Y, Sun W, Li Z, Zhang B, Cui H, Deng L, Xie P, Xiang J, Zou J (2013) Effects of combining methylprednisolone with rolipram on functional recovery in adult rats following spinal cord injury. Neurochem Int 62:903-912.
Yin YM, Lu Y, Zhang LX, Zhang GP, Zhang ZQ (2015) Bone marrow stromal cells transplantation combined with ultrashortwave therapy promotes functional recovery on spinal cord injury in rats. Synapse 69:139-147.
Yu TT, Shoichet MS (2005) Guided cell adhesion and outgrowth in peptide-modified channels for neural tissue engineering. Biomaterials 26:1507-1514.
Zhang DX, Ding HZ, Jiang S, Zeng YM, Tang QF (2014) An in vitro study of the neuroprotective effect of propofol on hypoxic hippocampal slice. Brain Inj 28:1-8.
Zhang R, Pei H, Ru L, Li H, Liu G (2013) Bone morphogenetic protein 7 upregulates the expression of nestin and glial fibrillary acidic protein in rats with cerebral ischemia-reperfusion injury. Biomed Rep 1:895-900.
Zhao Y, Zuo Y, Jiang J, Yan H, Wang X, Huo H, Xiao Y (2016) Neural stem cell transplantation combined with erythropoietin for the treatment of spinal cord injury in rats. Exp Ther Med 12:2688-2694.

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