Propofol injection combined with bone marrow mesenchymal stem cell transplantation better improves electrophysiological function in the hindlimb of rats with spinal cord injury than monotherapy

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Abstract

The repair effects of bone marrow mesenchymal stem cell transplantation on nervous system damage are not satisfactory. Propofol has been shown to protect against spinal cord injury. Therefore, this study sought to explore the therapeutic effects of their combination on spinal cord injury. Rat models of spinal cord injury were established using the weight drop method. Rats were subjected to bone marrow mesenchymal stem cell transplantation *via* tail vein injection and/or propofol injection *via* tail vein using an infusion pump. Four weeks after cell transplantation and/or propofol treatment, the cavity within the spinal cord was reduced. The numbers of PKH-26-positive cells and horseradish peroxidase-positive nerve fibers apparently increased in the spinal cord. Latencies of somatosensory evoked potentials and motor evoked potentials in the hindlimb were noticeably shortened, amplitude was increased and hindlimb motor function was obviously improved. Moreover, the combined effects were better than cell transplantation or propofol injection alone. The above data suggest that the combination of propofol injection and bone marrow mesenchymal stem cell transplantation can effectively improve hindlimb electrophysiological function, promote the recovery of motor funtion, and play a neuroprotective role in spinal cord injury in rats.

Key Words: nerve regeneration; bone marrow mesenchymal stem cells; propofol; spinal cord injury; cell transplantation; electrophysiology; motor function; stem cells; neuroprotection; neural regeneration

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Introduction

Neurotrophic drugs and rehabilitation therapy are currently used to treat spinal cord injury (SCI) in the clinic, to save neurons on the verge of necrosis in ischemic penumbra, and to promote the recovery of neuronal function (Guan et al., 2008; Jiang et al., 2009b; Chen et al., 2013; Xiang et al., 2013). However, spinal cord neurons do not have the ability to self-repair, and cannot reach the desired clinical effect (Leman et al., 2000; Jacob et al., 2001; Yeoh et al., 2004; He et al., 2005). Under certain conditions, stem cells have the ability to self-renew, multi-differentiate and migrate (Hambly and Martin, 1998; Chen et al., 2000; Mizuno and Sugimoto, 2000; Xu et al., 2004). Stem cells are present in bone marrow, umbilical cord blood, placenta and peripheral blood, and can differentiate, be cultured and be amplified into genetic stability in multiple tissues and cells *in vitro* (Wallerstedt et al., 1998; Hsieh et al., 2007; Kahn et al., 2007; Yu et al.,

2011b). Stem cells can be used as ideal donors for neural transplantation (Kouchi et al., 1998; Zhao et al., 2003; Yao et al., 2007; Wang et al., 2009a). Transplanted bone marrow mesenchymal stem cells (BMSCs) can survive in the injured spinal cord, produce and release chemokines, secrete a variety of growth factors, inhibit the expression of inflammatory factors, induce microvascular regeneration in the injury region, lessen local secondary inflammatory response, differentiate into neurons and glial cells, promote neuronal regeneration and reconstruction, and treat SCI (Bolli et al., 2002; Weber et al., 2005; Huang et al., 2007; Li et al., 2013).

Propofol has been shown to play a protective effect on central nervous system injury. Moreover, propofol exerts effects rapidly, can be cleared rapidly, shows few adverse reactions, can reduce the metabolic rate of oxygen, inhibit cell apoptosis, and has been extensively used in the clinic (Monti et al., 2013). BMSCs can repair neurons under certain conditions,

but the repair effect of BMSC transplantation alone on nervous system injury is not satisfactory. Possible reasons are as follows: secondary injuries after SCI such as hemorrhage and ischemia as well as a series of biochemical, cytotoxic substances, metabolites, and free radicals cause nerve cell reperfusion injury, excitotoxicity, necrosis, apoptosis, and inflammatory response, which further results in difficult recovery from primary nerve damage and continued expansion of lesions (Arivazhagan and Ganesan, 2003; Brambilla et al., 2005; Dosenko et al., 2005). Therefore, we hypothesized that the combination of propofol and BMSC transplantation for treatment of SCI in rats might obtain better outcomes. This study was designed to observe the alterations in hindlimb movement and electrophysiological function in rats with SCI after propofol injection combined with BMSC transplantation.

Materials and Methods

Culture and identification of rat BMSCs

One Wistar rat aged 1 month was obtained from Hebei Experimental Animal Center in China (production license No. SCXK (Ji) 20080004). The protocols were approved by the Animal Ethics Committee, Hebei Medical University, China. After sacrifice, the rat was immersed in a 75% ethanol container for thorough disinfection for approximately 10 minutes. Bilateral tibia and femur of rats were obtained, and bilateral bone ends were removed. 1 mL L-DMEM complete medium (Gibco BRL, Gaithersburg, MD, USA) containing 5% fetal bovine serum (Hyclone, Logan, Utah, USA) was used to wash the marrow cavity from one side. Single-cell suspensions were made and incubated in 100 mL culture flasks. Cells at the concentration of 3×10^4/mL were cultured in an incubator of saturated humidity at 37°C and 5% CO_2 for 24 hours. The medium was completely replaced. From then on, the medium was replaced once every 3 days. Cells were subcultured at 1:2. Cell growth was observed under a light microscope (IX71; Olympus, Tokyo, Japan) every day. When cells were confluent at above 80%, cells were subcultured at 1:3. After repeated subculture amplification, BMSCs were gradually purified. Flow cytometry (BD FACSCalibur; Indianapolis, IN, USA) was used to detect surface antigens for identifying BMSCs.

PKH-26-labeled BMSCs

In the dark, 5 μL PKH-26 solution (Sigma, St. Louis, MO, USA) diluted by L-DMEM containing 5% fetal bovine serum was placed in a 1.5 mL Eppendorf tube. 1 mL of L-DMEM containing 5% fetal bovine serum was also added. After mixing, PKH-26 marking fluid was obtained. After removal of the medium, adherent BMSCs at 80% confluence were collected and washed three times with PBS. BMSCs were incubated with the above marking fluid at 40 μL/cm^2 in an incubator at 5% CO_2, saturated humidity and 37°C for 20 minutes. After removal of the marking fluid, 5 mL of 37°C L-DMEM containing 5% fetal bovine serum was added for 10 minutes of incubation. After removal of the above medium, BMSCs were washed three times with L-DMEM

containing 5% fetal bovine serum. The effects of labeling with PKH-26 and the morphology of labeled BMSCs were observed under a fluorescence microscope (IX71; Olympus).

Establishment of rat models of SCI

A total of 80 clean adult healthy female Wistar rats aged 4 weeks and weighing 200–250 g were obtained from Hebei Experimental Animal Center in China (production license No. SCXK (Ji) 20080004). After raising in the laboratory for 2 weeks, the rats were intraperitoneally anesthetized with 350 mg/kg 10% chloral hydrate, and then fixed on the experimental platform in a prone position. After the lower back was shaved, a median incision was made on the rat back taking the $T_{8–9}$ spinous process as a center to fully expose the $T_{7–10}$ spinous process and lamina. The $T_{8–9}$ spinous process and part of the lamina were removed. The complete dura mater was exposed and considered as the injury region. In accordance with the modified Allen's method (Jing et al., 2014; Liu et al., 2014), 10 g weight freely fell from a height of 2.5 cm and impacted the dura and spinal cord of the rats. Rat tail swing and spasm, and paralysis of the lower limbs, indicated successful model establishment. The wound was washed with hydrogen peroxide. The incision on the back was sutured layer by layer. After modeling, urine was squeezed twice or three times every day until the micturition reflex was restored in rats.

Combination of propofol injection and BMSC transplantation

Rat models were randomly divided into model, BMSC, propofol and combination groups. Each group contained 20 rats. At 6 hours after model establishment, rats in the model group were injected with 1 mL L-DMEM containing 5% fetal bovine serum *via* tail vein. Rats in the BMSC group were injected with 1 mL BMSC (3×10^6 cells) suspension *via* tail vein. Rats in the propofol group were injected with propofol (2 mL/kg per hour; Approval No. GYZZ H19990282; China Xi'an Libang Pharmaceutical Co., Ltd., Xi'an, Shaanxi Province, China) using a tail vein catheter pump for 4 hours. Rats in the combination group were injected with 1 mL BMSCs (3×10^6 cells) suspension *via* tail vein and propofol injection (2 mL/kg per hour) using the tail vein catheter pump for 4 hours.

Evaluation of hindlimb motor function

Motor function was assessed before model establishment at 1 and 3 days, and 1, 2, 3 and 4 weeks after model establishment. Assessment items included modified inclined plate test, Tarlov score and Basso Beattie Bresnahan (BBB) score.

Modified inclined plate test: Rats were placed on a smooth wood plate. The body axis was parallel to the vertical axis of the plate. The plate angle increased by 5° every trial. The maximum angle that rat could stay for 5 seconds was considered as its function value (Zi et al., 2006).

Tarlov score: grade 0, no activity, cannot load; grade 1, activity, cannot load; grade 2, active or powerful activities, cannot load; grade 3, hindlimb can support body weight,

Table 1 Effects of propofol injection combined with BMSC transplantation on motor function of hindlimb of rats with spinal cord injury

Group	Before injury	After injury					
		1 day	3 days	1 week	2 weeks	3 weeks	4 weeks
BBB score							
Model	21.00±0.00	0.00±0.00	1.23±0.05	2.45±0.67	8.41±1.52[*]	11.12±1.30[*]	13.79±0.68[*]
BMSC	21.00±0.00	0.00±0.00	2.42±0.06	3.91±1.02[*]	10.31±1.46[*]	12.70±1.42[*]	15.14±0.17[*]
Propofol	21.00±0.00	0.00±0.00	2.44±0.05	3.94±1.05[*]	10.27±1.10[*]	12.10±1.42[*]	15.24±0.12
Combination	21.00±0.00	0.00±0.00	3.43±0.06	6.12±1.00[**#†]	12.31±1.18[**#†]	14.76±1.33[**#†]	17.57±0.23[**#†]
Inclined plate test							
Model	42.51±1.63	15.84±1.53	16.51±1.22	21.84±2.15	23.12±2.00	26.19±2.14	28.21±1.36
BMSC	42.52±1.72	16.77±2.21	18.89±1.24[*]	24.61±2.14[*]	30.31±2.24	34.55±2.03[*]	38.72±2.10[*]
Propofol	42.51±2.13	16.79±1.46	18.79±1.32[*]	24.71±1.52[*]	30.29±2.13[*]	34.27±2.36[*]	38.32±2.30[*]
Combination	42.51±2.24	18.48±1.36	21.61±2.13[**#†]	25.45±2.24[*#†]	33.52±2.41[**#†]	38.49±2.49[**#†]	41.50±2.30[**#†]
Modified Tarlov score							
Model	5.00 ±0.00	0.00±0.00	0.40± 0.12	0.81±0.14[*]	1.64±0.29	2.46±0.17	2.65±0.34[*]
BMSC	5.00 ±0.00	0.00±0.00	0.71± 0.17	1.60±0.18	2.80± 0.31[*]	3.62±0.23[*]	3.91±0.34
Propofol	5.00 ±0.00	0.00±0.00	0.72± 0.18	1.61±0.19	2.82± 0.47[*]	3.62±0.18[*]	3.89±0.22[*]
Combination	5.00 ±0.00	0.00 ±0.00	0.83 ±0.14	1.83±0.17	3.04±0.42[**#†]	3.46±0.24[**]	4.16±0.23[**#†]

Data are expressed as the mean ± SD, with eight rats in each group. Intergroup comparison was done using one-way analysis of variance and the least significant difference. *$P < 0.05$, **$P < 0.01$, *vs.* model group; #$P < 0.05$, *vs.* BMSC group; †$P < 0.05$, *vs.* propofol group. BMSC: Bone marrow mesenchymal stem cell; BBB: Basso Beattie Bresnahan.

Table 2 Effects of propofol injection combined with BMSC transplantation on somatosensory evoked potential and motor evoked potential in hindlimb of rats with spinal cord injury

Group	Somatosensory evoked potential		Motor evoked potential	
	Latency (ms)	Amplitude (μV)	Latency (ms)	Amplitude (mV)
Model	35.652±1.014	1.315±0.122	15.932±0.360	1.582±0.142
BMSC	26.753±1.001[*]	1.724±0.116[*]	12.152±0.142[*]	2.351±0.146[*]
Propofol	26.746±1.012[*]	1.722±0.102[*]	12.148±0.167[*]	2.340±0.218[*]
Combination	15.014±0.752[**#†]	2.011±0.134[**#†]	7.951±0.185[**#†]	4.161±0.202[**#†]

Data are expressed as the mean ± SD, with eight rats in each group. Intergroup comparison was done using one-way analysis of variance and the least significant difference. *$P < 0.05$, **$P < 0.01$, *vs.* model group; #$P < 0.05$, *vs.* BMSC group; †$P < 0.05$, *vs.* propofol group. BMSC: Bone marrow mesenchymal stem cell.

can walk 1 to 2 steps; grade 4, can walk, only mild disorders; grade 5, normal walking (Zhang et al., 2008).

BBB score: 22 grades in total; grade 0, hindlimb paralysis; grade 21, normal function. Main outcome measures included the number of joint activities, range of motion, loading degree, coordination of forelimb and hindlimb, activities of forepaws, hind paws and tail (Ding et al., 2011).

Pathological observation

At 4 weeks after model establishment, five rats were obtained from each group, and anesthetized with 10% chloral hydrate (350 mg/kg). The chest was opened to completely expose the heart. The right auricle was incised and washed with physiological saline through ascending aortic cannulation, followed by fixation with 4% paraformaldehyde. Approximately 1 cm of complete spinal cord was obtained from the lesion site, dehydrated through a graded alcohol series, and longitudinally sectioned into 20 μm-thick frozen slices. These slices were stained with hematoxylin for 5 minutes, washed with running water, treated with ethanol hydrochloride for 10

seconds, washed with running water for 10 minutes, stained with eosin for 7 minutes, washed with running water, dehydrated through a graded alcohol series, permeabilized with xylene, mounted with neutral resin, and observed with a microscope. An additional spinal cord sample at the injury region was sliced into frozen sections. Ten fields of each frozen section were observed directly under the fluorescence microscope at 200× magnification. The number of PKH-26-positive cells was calculated in each field, and the average was obtained.

Detection of somatosensory evoked potential and motor evoked potential

At 4 weeks after model establishment, six rats were obtained from each group. In accordance with a previous method (Yeoh et al., 2004), KEYPOINT 4 evoked potential instrument (Beijing Weidi Kangtai Medical Instrument Co., Ltd., Beijing, China) was applied to determine the somatosensory and motor evoked potentials in the hindlimbs. The rat was intraperitoneally anesthetized with 10% chloral hydrate, and

placed on the horizontal plane. The hindlimb was connected to the stimulating electrode. The recording electrode was placed under the scalp at the intersection of the healing line of the coronal and sagittal suture (*i.e.*, the cortical sensory area of the hindlimb). The reference electrode was placed at 0.5 cm posterior to the recording electrode. Direct-current square wave electrical pulse stimulation was given at a current intensity of 5–15 mA, wave width of 0.2 ms, frequency of 3 Hz and superposition times of 50–60 times. Slight twitch of the hindlimb was appropriate. Changes in the latency and amplitude of the somatosensory evoked potential were recorded. Detection of motor evoked potential: after anesthesia, the acicular stimulating electrode was placed below the scalp 2 mm anterior to the coronal suture and 2 mm lateral to the sagittal suture (*i.e.*, motor cortex) at a stimulus intensity of 40 mA, wave width of 0.1 ms, frequency of 1 Hz, superposition of 300–500 times, scanning speed of 5 ms/D and sensitivity of 5 μV/D. Changes in the latency and amplitude of the motion evoked potential were observed and recorded.

Horseradish peroxidase (HRP) retrograde nerve tracing

At 4 weeks after model establishment, four rats were randomly obtained from each group. After anesthesia, the spinal cord was exposed. The needle was inserted at 1 mm left and right of the median vein of the T_{12} spinal cord on the dorsal side at a depth of 1.5 mm. 50% horseradish peroxidase 1 μL (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was injected at 0.1 μL/10 minutes. The needle was maintained in place for 15 minutes. After being raised for 3 days, the rats were anesthetized with chloral hydrate. The heart was perfused with 4% paraformaldehyde. Rat $T_{3–11}$ spinal cord was immersed in 30% sucrose solution at 4°C for 20 hours, and sliced into 5 μm-thick frozen sections. These sections were treated with 3,3′-diaminobenzidine. The number of HRP-positive nerve fiber bundles on the cross-section of the spinal cord was quantified using a light microscope (Olympus).

Statistical analysis

Data are expressed as the mean ± SD, and analyzed using SPSS 17.0 software (SPSS, Chicago, IL, USA). Intergroup comparison was done using one-way analysis of variance and the least significant difference test. A value of $P < 0.05$ was considered statistically significant.

Results

Morphology and identification results of BMSCs

Five days after culture, the numbers of BMSCs and colonies had apparently increased. BMSC proliferation at passages 1, 2 and 3 was active. After subculturing, most cells were adherent and monostratal, mostly fusiform, large polygonal or triangular. BMSC morphology gradually became similar, mainly fusiform. BMSCs had strong refraction, with the presence of two or more processes, nucleus and nucleolus (**Figure 1**). Flow cytometry results revealed that BMSCs were positive for CD29, CD105, CD44, CD166, and negative for

CD34, CD86 and CD80. The homogenicity of BMSCs was good, and the purity of BMSCs was above 95%.

Combination of propofol injection and BMSC transplantation improved hindlimb motor function in rats with SCI

The scores of the modified inclined plate test, the Tarlov scores and the BBB scores were similar in each group before model establishment ($P > 0.05$). At 2–4 weeks after SCI, compared with the model group, the BBB, modified inclined plate test and Tarlov scores were significantly higher in the BMSC, propofol and combination groups ($P < 0.05$ or $P < 0.01$). Moreover, the above scores were higher in the combination group than in the BMSC and propofol groups ($P < 0.05$; **Table 1**).

Combination of propofol injection and BMSC transplantation improved spinal cord morphology and promoted BMSC survival in rats with SCI

Hematoxylin-eosin staining results demonstrated that by 4 weeks after model establishment, the spinal cord broke, the scar connected, and an apparent cavity formed in the model group. The cavity nearly disappeared in the lesion site in the combination group. The cavities in the propofol and BMSC groups were bigger than those in the combination group, but smaller than those in the model group (**Figure 2**).

Under the fluorescence microscope, PKH-26-positive cells were scattered in the BMSC and combination groups. The number of PKH-26-positive cells was significantly greater in the combination group than in the BMSC group ($P < 0.01$; **Figure 3**).

Combination of propofol injection and BMSC transplantation improved somatosensory and motor evoked potentials in the hindlimb of rats with SCI

The somatosensory and motor evoked potentials in the hindlimb completely disappeared in each group after SCI. At 4 weeks, the somatosensory and motor evoked potentials were slightly restored in the model group. Compared with the model group, the latency of the somatosensory and motor evoked potentials in the hindlimb was shorter and their amplitude was larger in the BMSC, propofol and combination groups ($P < 0.05$ or $P < 0.01$). Compared with the BMSC and propofol groups, the latency of the somatosensory and motor evoked potentials in the lower extremity was shorter and their amplitude was larger in the combination group ($P < 0.05$; **Table 2**).

Combination of propofol injection and BMSC transplantation increased the number of nerve fibers in the injured spinal cord of rats

A few HRP-positive nerve fibers were observed in segments above T_8 in the model group at 4 weeks after injury. The number of HRP-positive nerve fibers was greater in the BMSC and propofol groups than in the model group ($P < 0.05$). The number of HRP-positive nerve fibers was greater in the combination group than in the model, BMSC and propofol groups ($P < 0.01$; **Figure 4**).
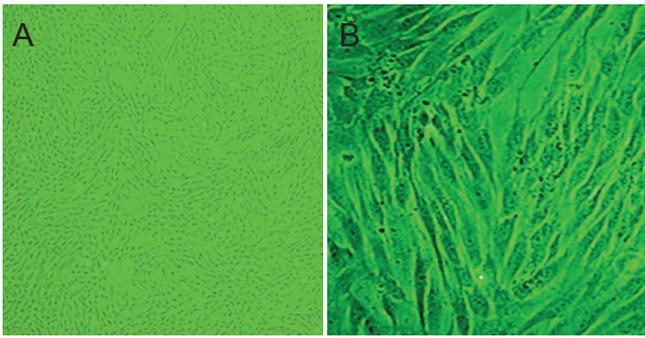
Figure 1 Morphology of cultured rat BMSCs (× 200).
(A) Primary cultured BMSCs show swirling arrangement; (B) third passage BMSCs are fusiform. BMSCs: Bone marrow mesenchymal stem cells.
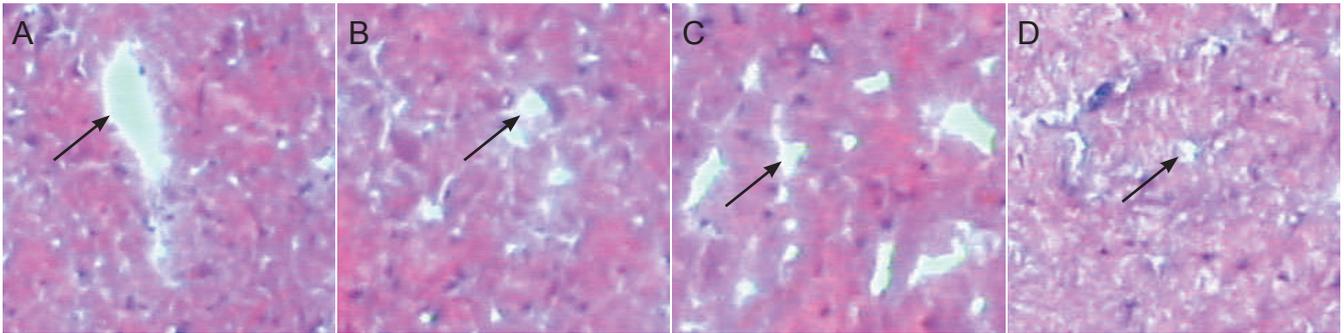
Figure 2 Effects of propofol injection combined with BMSC transplantation on morphology of the spinal cord of rats at 4 weeks after injury (hematoxylin-eosin staining, × 40).
(A) Noticeable cavity in the rat spinal cord in the model group; (B–D) cavity became small in the propofol group (B), BMSC group (C) and combination group (D). The therapeutic effects were best in the combination group. Arrows show cavity. BMSC: Bone marrow mesenchymal stem cell.
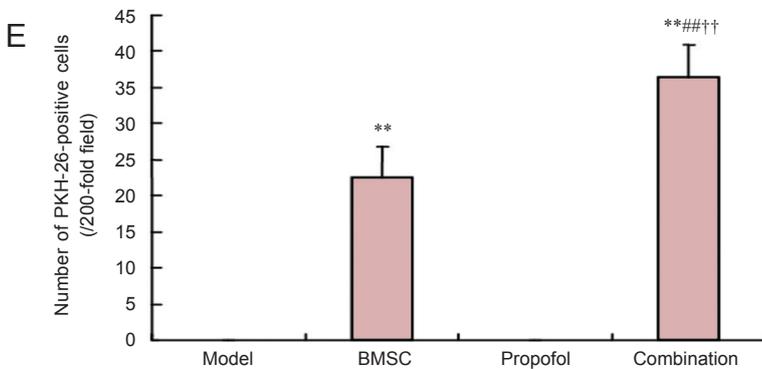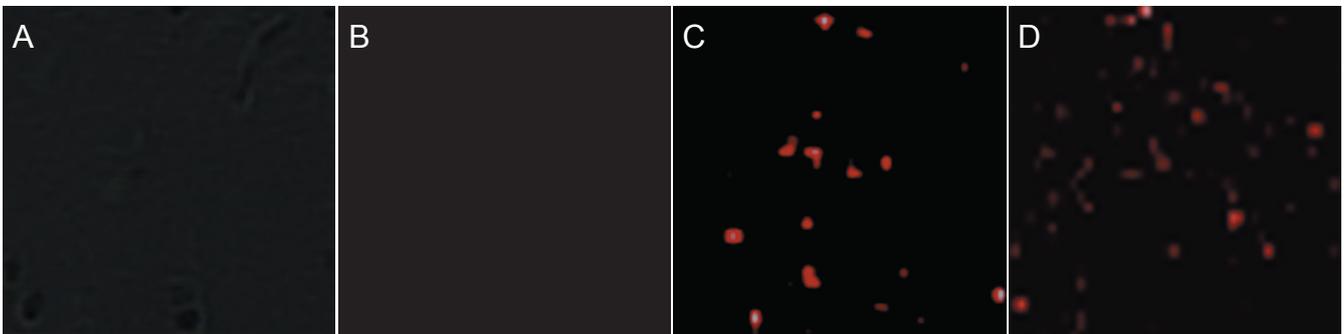
Figure 3 Effects of propofol injection combined with BMSC transplantation on BMSC survival in the spinal cord of rats at 4 weeks after injury.
(A–D) Survival of transplanted BMSCs in the spinal cord (fluorescence microscope, × 200). PKH-26-positive cells were not detected in the rat spinal cord in the model group (A) and propofol group (B). (C) PKH-26-positive cells were visible in the BMSC group. (D) PKH-26-positive cells were numerous in the combination group. PKH-26-labeled cells present red. (E) Number of transplanted BMSCs in the spinal cord of rats with spinal cord injury. Data are expressed as the mean ± SD, with eight rats in each group. Intergroup comparison was done using one-way analysis of variance and the least significant difference test. **$P < 0.01$, *vs.* model group; ##$P < 0.01$, *vs.* BMSC group; ††$P < 0.01$, *vs.* propofol group. BMSC: Bone marrow mesenchymal stem cell.
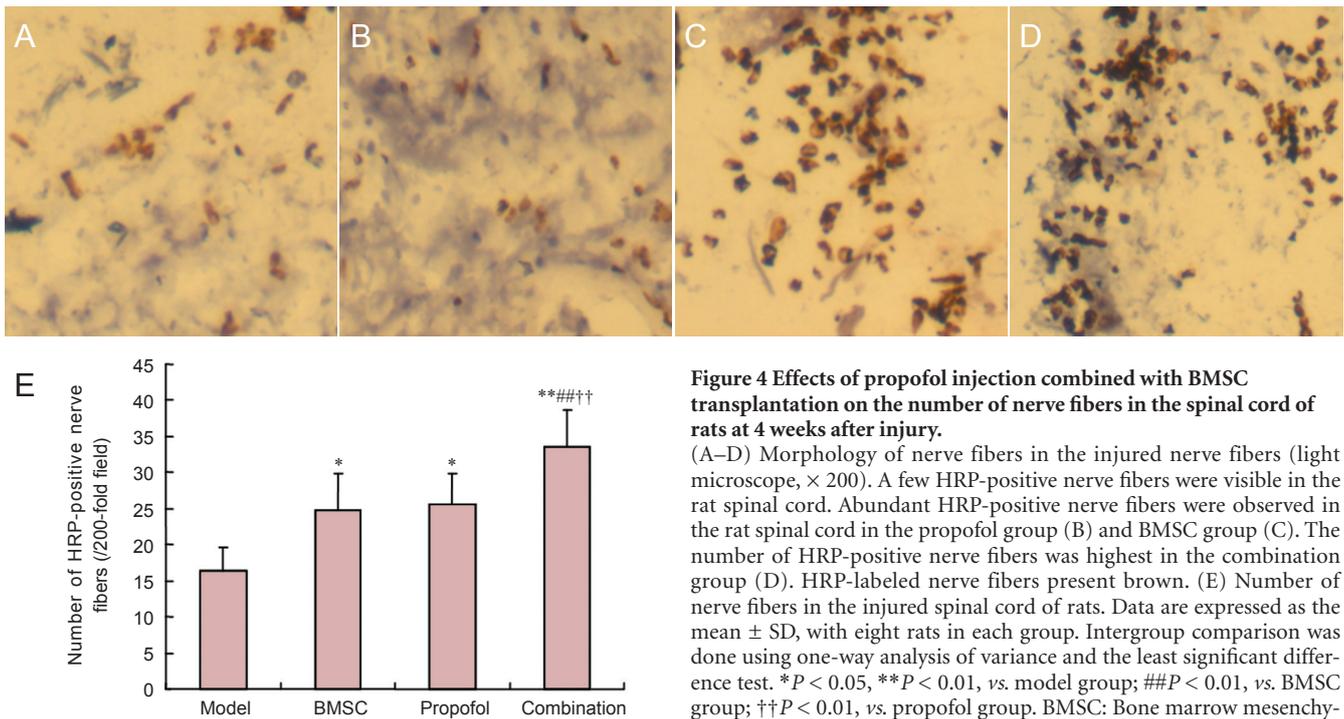
Figure 4 **Effects of propofol injection combined with BMSC transplantation on the number of nerve fibers in the spinal cord of rats at 4 weeks after injury.**
(A–D) Morphology of nerve fibers in the injured nerve fibers (light microscope, × 200). A few HRP-positive nerve fibers were visible in the rat spinal cord. Abundant HRP-positive nerve fibers were observed in the rat spinal cord in the propofol group (B) and BMSC group (C). The number of HRP-positive nerve fibers was highest in the combination group (D). HRP-labeled nerve fibers present brown. (E) Number of nerve fibers in the injured spinal cord of rats. Data are expressed as the mean ± SD, with eight rats in each group. Intergroup comparison was done using one-way analysis of variance and the least significant difference test. $*P < 0.05$, $**P < 0.01$, *vs.* model group; $\#\#P < 0.01$, *vs.* BMSC group; $\dagger\dagger P < 0.01$, *vs.* propofol group. BMSC: Bone marrow mesenchymal stem cell; HRP: horseradish peroxidase.

Discussion

BMSCs are characterized by an abundant source, convenient collection and autotransplantation, and provide a new therapeutic tool for nervous system injury (Adembri et al., 2006; Iijima et al., 2006; Yu et al., 2011a; Zhang et al., 2013). BMSCs in the injured spinal cord can differentiate into neurons and glial cells, and possibly form synaptic connections to nervous processes at both ends of the injury region (Sufianova et al., 2002; Kitz et al., 2006; Choi et al., 2007; Jiang et al., 2009a). Cell transplantation is an effective method to repair the injured spinal cord (Feng et al., 2013; Gao et al., 2013; Liu et al., 2013). BMSCs can divide and proliferate in the transplanted region, and differentiate into corresponding cells under local microenvironment so as to replace injured cells (Sayin et al., 2002; Gaweł et al., 2004; Shen et al., 2009). Many scholars believe that BMSCs should be first cultured *in vitro* and differentiated into neural precursor cells, whose transplantation in the injury region contributes to cell survival and forming cells with corresponding functions (Miyanji et al., 2007; Jing et al., 2008; Zhang et al., 2010). The repair effect of neural stem cell transplantation alone on the injured spinal cord is not satisfactory, but it can be combined with drugs and biological engineering materials for comprehensive treatment (Kumagai et al., 2006; Wang et al., 2009b; Li et al., 2010).

The early application of propofol could reduce serum S100β protein content, total calcium content, and water content in the injured spinal cord (Wang et al., 2009a; Morizane et al., 2012), suppress free radical generation, prevent free radical chain reactions, resist oxidation activity, and inhibit lipid peroxidation. Therefore, propofol can diminish the metabolic rate of oxygen, mediate specific cellular pathways, and thus show neuroprotective effects (Feng et al., 2005). Propofol has been shown to decrease nitric oxide synthase activity and ET-1 synthesis, to regulate vasomotor function, and to improve blood flow in ischemic tissue. Endothelin existing in the vascular endothelial cells is the strongest vasoconstrictor. SCI completely destroys vascular endothelial cells, and endothelin increases in the injury region (Chen and Wang, 2004; Wang et al., 2006). Propofol acts on vasodilation, causes hypotension, reduces hemoperfusion in local tissue, and further aggravates ischemic injury. Propofol administration, ischemic postconditioning in early reperfusion and early restoration of blood supply in the injured spinal cord play a great protective effect on spinal cord ischemia/reperfusion injury.

Results from the present study demonstrated that the numbers of PKH-26-positive cells and HRP-positive nerve fibers apparently increased, the latencies of somatosensory evoked potentials and motor evoked potentials obviously shortened, amplitudes increased, and motor function in the lower extremities improved significantly after BMSC transplantation and/or propofol injection in rats with SCI. Moreover, the numbers of PKH-26-positive cells and HRP-positive nerve fibers, somatosensory evoked potentials and motor evoked potentials, and motor function in the hindlimbs were better in the combination group than in the BMSC group and propofol group. The above data indicate that the combination of propofol injection and BMSC transplantation for treating SCI in rats could effectively promote the regeneration of synapses and improve motor function and electrophysiological function in the hindlimb.

Author contributions: *YXW designed the study and wrote the paper. YXW and JJS performed experiments. JJS and MZ*

evaluated the study. XHH and JH collected data. YJZ and ZYZ performed statistical analysis. ZYZ was in charge of manuscript authorization. All authors approved the final version of the paper.

Conflicts of interest: *None declared.*

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