Methylprednisolone inhibits the proliferation of endogenous neural stem cells in nonhuman primates with spinal cord injury

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OBJECTIVE The aim of this work was to investigate the effects of methylprednisolone on the proliferation of endogenous neural stem cells (ENSCs) in nonhuman primates with spinal cord injury (SCI).

METHODS A total of 14 healthy cynomolgus monkeys (*Macaca fascicularis*) (4–5 years of age) were randomly divided into 3 groups: the control group (n = 6), SCI group (n = 6), and methylprednisolone therapy group (n = 2). Only laminectomy was performed in the control animals at T-10. SCI was induced in monkeys using Allen's weight-drop method (50 mm × 50 g) to injure the posterior portion of the spinal cord at T-10. In the methylprednisolone therapy group, monkeys were intravenously infused with methylprednisolone (30 mg/kg) immediately after SCI. All animals were intravenously infused with 5-bromo-2-deoxyuridine (BrdU) (50 mg/kg/day) for 3 days prior to study end point. The small intestine was dissected for immunohistochemical examination. After 3, 7, and 14 days, the spinal cord segments of the control and SCI groups were dissected to prepare frozen and paraffin sections. The proliferation of ENSCs was evaluated using BrdU and nestin immunofluorescence staining.

RESULTS Histological examination showed that a larger number of mucosa epithelial cells in the small intestine of all groups were BrdU positive. Nestin-positive ependymal cells are increased around the central canal after SCI. After 3, 7, and 14 days of SCI, BrdU-positive ependymal cells in the SCI group were significantly increased compared with the control group, and the percentage of BrdU-positive cells in the left/right ventral horns and dorsal horn was significantly higher than that of the control group. Seven days after SCI, the percentages of both BrdU-positive ependymal cells around the central canal and BrdU- and nestin-double positive cells in the left/right ventral horns and dorsal horn were significantly lower in the methylprednisolone therapy group than in the SCI group.

CONCLUSIONS While ENSCs proliferate significantly after SCI in nonhuman primates, methylprednisolone can inhibit the proliferation of ependymal cells after SCI.

<https://thejns.org/doi/abs/10.3171/2017.12.SPINE17669>

KEYWORDS spinal cord injury; nonhuman primates; endogenous neural stem cells; methylprednisolone; proliferation

IN recent years, stem cell transplantation has been widely used for the treatment of CNS injury. Stem cells have the potential to cure many CNS diseases as they are not yet specialized and have the potential to become a variety of specialized cells in the human body.²⁴ Neurons and glia could be successfully generated from stem cells

such as embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), neural stem/progenitor cells (NSPCs), and induced pluripotent stem cells (iPSCs).^{6,7,27,39,43} NSCs present attractive targets in stem cell transplantation therapy due to their self-replicating ability and multidirectional differentiation potential.¹⁸ Many stud-

ABBREVIATIONS BrdU = 5-bromo-2-deoxyuridine; ENSC = endogenous neural stem cell; ESC = embryonic stem cell; MP = methylprednisolone; NPC = neural precursor cell; NSC = neural stem cell; PBS = phosphate-buffered saline; SCI = spinal cord injury.

SUBMITTED June 15, 2017. **ACCEPTED** December 12, 2017.

INCLUDE WHEN CITING Published online May 18, 2018; DOI: 10.3171/2017.12.SPINE17669.

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ies have reported that NSC transplantation promoted the recovery of nerve function after spinal cord injury (SCI) in both rodent and nonhuman primate SCI models. For example, McDonald et al. transplanted neural differentiated mouse ESCs into rat spinal cords 9 days after traumatic injury. The authors observed that transplanted ESCs differentiated into astrocytes, oligodendrocytes, and neurons, thus promoting recovery in the injured rat spinal cords.²⁶ In addition, it has been reported that transplanted NSCs can secrete various neurotrophic factors and cytokines to promote axonal regeneration and myelination.^{23,46} However, there are still a series of key problems in exogenous NSC transplantation for the treatment of SCI, including cell compatibility and rejection, incomplete differentiation of transplanted NSCs due to altered microenvironment in injured spinal cords, and abnormal pain.¹² Therefore, experimental strategies designed to enhance the reactivation and mobilization of endogenous neural stem cells (ENSCs) have appeared and may lead to novel therapeutic approaches to treat CNS diseases.³² It is well established that ENSCs exist in the cerebral cortex, lateral ventricle, hippocampus, striatum, and central ependymal region of the spinal cord in both mammalian embryos and adult animals.^{21,31} ENSCs have the same biological characteristics as all NSCs, including the ability for self-renewal and differentiation into neurons and astrocytes.

Methylprednisolone (MP) is one of the steroids currently approved by the US Food and Drug Administration for the treatment of acute SCI in patients.¹ It has been shown that MP inhibits the activation and proliferation of various inflammatory cell types in animal models of SCI by reducing the production of inflammatory cytokines/chemokines and free radicals, as well as inhibiting lipid peroxidation.⁷ It has also been shown that MP inhibits the proliferation and migration of ENSCs and oligodendrocytes after SCI.²⁷ However, most of these studies were conducted in rats or mice, or other rodents, or with cells isolated from these animals. Due to the fact that there are significant differences in CNS, physiology, immune system, and molecular mechanisms of inflammation between rodents and primates, it is essential to investigate the effects of MP on SCI in nonhuman primate models.

In the present study, we first established the nonhuman primate SCI model in cynomolgus monkeys. Then, we evaluated the proliferation of ENSCs after SCI, and the effects of MP treatment on the proliferation of ENSCs in the spinal cords, to verify the effects of MP therapy on SCI in a nonhuman primate model.

Methods

Ethics Statement

All animal studies were approved by the Research Institute for Spinal Cord Injury at Sun Yat-sen University (Guangzhou, Guangdong, China) and were performed in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki and its later amendments.

Establishment of the SCI Model in Cynomolgus Monkeys

A total of 14 healthy cynomolgus monkeys (*Macaca fascicularis*) (male/female ratio 1:1; age 4–5 years; and

weight 4.8–5.6 kg) were purchased from the Blue Island Biotechnology Co., Ltd. Animal breeding and surgery were conducted in the experimental base of Blue Island Biotechnology Co., Ltd. The animals were housed in a room maintained at 24°C ± 4°C and a relative humidity of 50% ± 10%, with a 12-hour/12-hour light-dark cycle. Throughout the study, the animals were fed a commercial diet twice daily at 9:00 AM and 2:00 PM and were given drinking water *ad libitum*.

Eight cynomolgus monkeys were anesthetized by intraperitoneal injection of 10% pentobarbital (300 mg/kg). Ten minutes after induction of anesthesia, the animals were fixed on a board to remove back hair. After conventional iodine disinfection of the skin, thoracic laminectomy at the T-10 level was conducted through an incision in the back. The T-10 lamina was removed using microscissors to expose the dura. The T-10 spinal cord was injured using an NYU MASCIS impactor (50 mm × 50 g). Signs of the successful establishment of the SCI model are as follows: spastic swing of the tail, retraction and fluttering of the hindlimbs and body, and subsequent spastic paralysis of the hindlimbs. After the surgery, the animals were placed in individual cages at 22°C and the body was warmed using a heating pad. Animals received intravenous injection of penicillin (20,000 U/kg) twice a day in the first 3 days after the surgery to prevent infections. Bladders were gently massaged, and an increased dose of antibiotics was used for animals with urethral hemorrhage.

The 8 cynomolgus monkeys with SCI were randomly divided into 2 groups: the SCI group (n = 6) and the MP group (n = 2). The 2 animals in the MP group received intravenous infusion of MP (30 mg/kg) immediately after SCI. Intravenous infusion of MP was completed within 30 minutes. Another 6 cynomolgus monkeys, which only received thoracic laminectomy at the T-10 level and vehicle infusion, were used as the controls. All animals received intravenous infusion of 5-bromo-2-deoxyuridine (BrdU) (50 mg/kg/day) for 3 days prior to sacrifice. The experiments were conducted at two stages because there were a limited number of animals. First, the proliferation of ependymal cells in spinal cords of the SCI group were evaluated 3, 7, and 14 days after SCI to identify the time when ependymal cells exhibited the highest rate of proliferation. To evaluate the proliferation of ependymal cells in the MP group, the ependymal cells in spinal cords were dissected when ependymal cells were at the highest rate of proliferation.

Histopathology

After anesthesia was induced with intraperitoneal injection of 10% pentobarbital (300 mg/kg), the animals were perfused with saline (500 ml) followed by 4% paraformaldehyde (200 ml) via the heart. The injured spinal cord segment along with 3 mm of the upper and 3 mm of the lower sections was dissected and fixed in 4% paraformaldehyde (20 ml) for 24 hours. Then, the spinal cord segments were transferred to 30% sucrose solution for incubation overnight at 4°C. Paraffin-embedded tissues were transversely sliced in 5-μm thickness to prepare sections. Tissue sections were stained with H & E and examined under light microscopy (Nikon). The small intestine was also dissected for immunohistochemical staining assay.

FIG. 1. The histopathology of spinal cords in control and SCI animals. Dense and normal spinal cord structures were observed in the control animals. The damage to the spinal cord of the SCI group was located on the dorsal side on the center of the spinal cord. H & E, original magnification $\times 100$. Figure is available in color online only.

Immunohistochemical Staining

Paraffin sections were dried in an incubator at 58°C overnight. After conventional xylene dewaxing and gradient alcohol dehydration, the tissue sections were incubated in 3% H_2O_2 at 37°C for 10 minutes and rinsed 3 times with phosphate-buffered saline (PBS) for 5 minutes. The tissues were denatured using 2 mol/L HCl at room temperature for 30 minutes, dried with filter paper, and neutralized in 0.1 mol/L sodium borate (pH 8.5) for 10 minutes. After drying with filter paper, the tissue sections were washed 3 times with PBS for 10 minutes. Then, 0.5% Triton X-100 was used to penetrate cell membranes for 30 minutes. The tissue sections were blocked in goat serum at 37°C for 10 minutes, incubated in the primary antibody in dark at 4°C overnight, and washed 3 times with PBS for 5 minutes. Then, the tissue sections were incubated with biotinylated secondary antibody at 37°C for 30 minutes and washed 3 times with PBS for 5 minutes. After washing with distilled water, the tissue sections were stained with H & E, dehydrated, dried, and sealed. The tissues were examined under a microscope and photographed. The proliferation of BrdU-positive cells was evaluated under a microscope (Nikon; magnification $\times 100$) every fifth slice.

Immunofluorescence Staining

The tissue sections were fixed in cold acetone for 20 minutes, washed 3 times with PBS for 5 minutes, and treated with 3% hydrogen peroxide. The tissue sections were blocked in goat serum at 37°C for 30 minutes, incubated in 50 μL of the primary antibody nestin (1:200) in dark at 4°C overnight, and washed 3 times with PBS for 5 minutes. PBS was used to replace the first antibody for a negative control. The tissue sections were incubated with goat anti-mouse IgG-FITC (1:100) at 37°C for 30 minutes and washed 3 times with PBS for 5 minutes. The tissue samples were examined under a microscope (magnification $\times 400$) and photographed. Nestin-positive cells were counted on five slides. Image-Pro Plus 6.0 (Media Cyber-

netics) was used to determine the integral optical density values of nestin in ependymal cells around the central canal ($n = 2, 5$ slides/animal).

Statistical Analyses

Statistical analyses were conducted using SPSS version 19.0 statistical software (IBM Corp.). All data are expressed as the mean $\pm$ SD. The one-way ANOVA test followed by a Tukey-Kramer multiple comparisons post-test was used to analyze differences among the 3 groups, and differences between 2 groups were compared using an independent samples t-test. A $p < 0.05$ was considered statistically significant.

Results

Histopathological Changes After SCI

The histopathological findings of spinal cord specimens from control and SCI animals were shown in Fig. 1. Dense and normal spinal cord tissue was observed in the control animals (Fig. 1 left). The damage to the spinal cord in the SCI group was located on the dorsal side on the center of the spinal cord. The unaffected white matter was confined to the lateral and ventral side. The spinal cord tissue was slightly damaged, with structural destruction and necrotic cysts (Fig. 1 right).

BrdU-Positive Mucosal Epithelial Cells in the Small Intestine

The small intestine tissues of SCI animals were dissected 3 days after intravenous injection of BrdU (50 mg/kg). Most ($> 80\%$) of the mucosal epithelial cells in the small intestine were BrdU positive, with brown nuclei staining (Fig. 2).

Nestin-Positive Ependymal Cells in Spinal Cords

The spinal cords of the control and SCI animals were dissected for nestin immunofluorescence staining. As shown in Fig. 3, nestin-positive ependymal cells around the

FIG. 2. BrdU immunohistochemical staining of small intestinal mucosal epithelia of SCI animals. Most (> 80%) of the mucosal epithelial cells in the small intestine were BrdU positive with brown nuclei staining. Original magnification $\times 100$. Figure is available in color online only.

central canal were observed under a light microscope. Ependymal cells in both control and SCI animals are ENSCs or neural precursor cells (NPCs). However, ependymal cells in the SCI animals exhibited stronger nestin staining than did ependymal cells in the control animals. The integral optical density values of the SCI animals were $32,328 \pm 2291.3$, $40,812 \pm 1428.4$, and $34,736 \pm 2128.5$ 3, 7, and 14 days after SCI, respectively, and these were significantly higher than that ($24,091 \pm 2240.2$) seen in normal control animals ($p < 0.05$).

BrdU-Positive Ependymal Cells of SCI Animals

BrdU staining was confined to the nucleus of ependymal cells. Scattered BrdU-positive ependymal cells were observed around the central canal of the spinal cord in

the control animals. More BrdU-positive ependymal cells were observed around the central canal of the spinal cords in the SCI animals 3, 7, and 14 days after SCI (Fig. 4). There were significantly higher numbers of BrdU-positive ependymal cells around the central canal on days 3 (11.5 ± 6.4), 7 (16.3 ± 8.4), and 14 (9.4 ± 5.4) after SCI than were seen (3.2 ± 1.2) in the control animals.

Nestin/BrdU-Positive Cells in the Ventral and Dorsal Horns of Spinal Cord Gray Matter

The numbers of nestin-positive cells in the right ventral (28.5 ± 5.8 , 29.7 ± 10.3 , and 20.9 ± 10.1) and dorsal (45.9 ± 20.9 , 51.5 ± 10.3 , and 48.6 ± 7.2) horns of the SCI group on the 3rd, 7th, and 14th days after SCI were significantly higher than were seen in the ventral (15.5 ± 7.3) and dorsal

FIG. 3. Hoechst staining and nestin immunofluorescent staining of ependymal cells in control (A–C) and SCI (D–F) animals. Nestin-positive ependymal cells around the central canal were observed under a light microscope. Ependymal cells in both the control and SCI animals are ENSCs or NPCs. However, ependymal cells in the SCI animals exhibited stronger nestin staining than those of the control animals. Original magnification $\times 400$. Figure is available in color online only.

FIG. 4. BrdU immunohistochemical staining of ependymal cells in control animals (A) and SCI animals on days 3 (B), 7 (C), and 14 (D). This staining is confined to the nucleus of ependymal cells. Scattered BrdU-positive ependymal cells were observed around the central canal of the spinal cord of the control animals. More BrdU-positive ependymal cells were observed around the central canal of the spinal cord of the SCI animals on days 3, 7, and 14 after SCI. Original magnification $\times 400$. Figure is available in color online only.

TABLE 1. The number of nestin-positive cells in the gray matter of ventral and dorsal horns in control and SCI group monkeys

Gray Matter	Control Group	SCI Group		
		Day 3	Day 7	Day 14
Ventral horn	15.5 ± 7.3	$28.5 \pm 5.8^*$	$29.7 \pm 10.3^*$	20.9 ± 10.1
Dorsal horn	34.6 ± 11.5	45.9 ± 20.9	$51.5 \pm 10.3^*$	$48.6 \pm 7.2^*$

* $p < 0.05$ compared with control group (ANOVA followed by a Tukey-Kramer multiple comparisons post-test).

(34.6 ± 11.5) horns of control animals (Fig. 5 and Table 1). Similarly, the numbers of BrdU-positive cells in the ventral (31.5 ± 9.82 , 29.3 ± 12.65 , and 26.4 ± 8.08) and dorsal (55.9 ± 15.9 , 61.5 ± 9.6 , and 53.6 ± 15.2) horns of the SCI group on the 3rd, 7th, and 14th days after SCI were also significantly higher than the number in the ventral (19.5 ± 8.1) and dorsal (41.5 ± 13.5) horns of control animals (Fig. 6 and Table 2).

Nestin/BrdU-Positive Cells in Ventral and Dorsal Horns in SCI and MP Groups

To evaluate the proliferation of ependymal cells in the MP group, the ependymal cells in spinal cords were dissected when ependymal cells had the highest rate of proliferation. Based on the results mentioned above, we compared nestin/BrdU-double positive cells between the SCI and MP groups on the 7th day after SCI. Our data suggested MP infusion significantly reduced the number of nestin/BrdU-positive cells in the ventral and dorsal horns (Fig. 7).

FIG. 5. Nestin-positive cells in the ventral and dorsal horns of spinal cord gray matter. The number of nestin-positive cells in the right ventral and dorsal horns of the SCI group (C and D) on day 7 after SCI was significantly higher than that in the ventral and dorsal horns of control animals (A and B). Original magnification $\times 100$. Figure is available in color online only.

FIG. 6. BrdU-positive cells in the ventral and dorsal horns of spinal cord gray matter. The number of BrdU-positive cells in the right ventral and dorsal horns of the SCI group (**C and D**) on day 7 after SCI was significantly higher than that in the ventral and dorsal horns of control animals (**A and B**). Original magnification $\times 100$. Figure is available in color online only.

Discussion

Spinal cord injury poses big challenges for public health because of its high incidence and high morbidity rate, and there are no satisfactory treatments available to date.¹⁷ Various approaches using stem cell transplantation have been attempted in the treatment of SCI. The role of ENSCs in the repair of CNS injury has been extensively studied in recent years.^{9,45} It is well established that SCI is accompanied by significant cell apoptosis and altered gene transcription.⁴ In higher vertebrates, such as monkeys and humans, axonal regeneration is very limited, and SCI usually results in permanent paralysis below the lesion. In contrast, lower vertebrates, such as mice and rats, display robust axonal regeneration and recovery of function within a few weeks.²⁵ There are several reasons for this phenomenon: 1) the regenerative capability of neurons formed by NSCs is rather limited;² 2) several factors are produced by SCI that inhibit the growth of neurons;²² 3) following SCI, many glial cell types are recruited to the lesion sites, forming the glial scar, which is an inhibitory component of axon growth.³⁴ Unfortunately, no effective therapy has been introduced for treating SCI in higher primates. A recent study has demonstrated that rhesus monkey NSCs, as a subpopulation of stem cells present in the brain sub-ventricular zone, can survive, differentiate to neurons, and promote functional recovery after SCI;²⁹ another study has suggested that NSC-derived neurons may be efficient in the functional recovery through remyelination of axons and that they compensate for dead neurons.¹¹ These findings show that NSCs exhibit the multipotential characteristic of differentiating into neurons and glial cells and that transplantation of NSCs into an injured spinal cord may provide a new therapeutic approach to treating SCI.

In the present study, we established an SCI model in cynomolgus monkeys to study the effects of MP on the proliferation of ENSCs in SCI. We counted the numbers of nestin-positive ependymal cells around the central canal in both the control and SCI groups. We observed that nestin-positive ependymal cells around the central canal in the SCI group exhibited significantly stronger nestin staining. Ependymal cells, which are original neuroepithelial cells including NPCs/NSCs, are columnar epithelia distributed in the ventricle and the central lumen of the spinal cord.³ In adult mammals, ependymal cells of the spinal cord have a limited capacity for proliferation.¹³ It has been shown that SCI induces ependymal cell proliferation,⁴⁴ and proliferative ependymal cells exhibit characteristics of NPCs/NSCs in SCI.^{37,42} In adult rodents with SCI, elevated proliferation of ependymal cells has been observed; the cells can migrate to surrounding tissues and differentiate into astrocytes.¹⁶ These data indicate that NSCs in the central ventricle of the spinal cord play essential roles in SCI recovery. We also observed more BrdU-positive ependymal cells in the SCI group on the 3rd, 7th, and 14th days after SCI than we did in the control group. In addition, BrdU/nestin-double positive cells in the right ventral and dorsal horns in the SCI group increased on the 3rd, 7th, and 14th days after SCI, a finding consistent with previous studies conducted in rodent SCI models.^{8,35} Therefore, regarding ependymal cells, including NPCs or NSCs, our results confirm that the proliferation of ependymal cells was increased after SCI in the nonhuman primate model.

One study reported that SCI induced proliferation of ENSCs in adult mice and that the isolated ENSCs formed typical neurospheres and differentiated into neurons, oligodendrocytes, and astrocytes.³⁸ However, most NSCs differentiate into astrocytes after SCI, which are not use-

TABLE 2. The number of BrdU-positive cells in the gray matter of ventral and dorsal horns in control and SCI group monkeys

Gray Matter	Control Group	SCI Group		
		Day 3	Day 7	Day 14
Ventral horn	19.5 ± 8.1	31.5 ± 9.8*	29.3 ± 12.7*	26.4 ± 8.1*
Dorsal horn	41.5 ± 13.5	55.9 ± 15.9*	61.5 ± 9.6*	53.6 ± 15.2*

* $p < 0.05$ compared with control group (ANOVA followed by a Tukey-Kramer multiple comparisons post-test).

ful for axillary myelination, and may lead to nerve scars, thus preventing the reconstruction of nerve bundles.¹⁵ Neurons in the adult mammalian CNS do not spontaneously regenerate axons after SCI due to CNS myelin and other inhibitory mechanisms.¹⁰ Neurons are involved in the formation of synapses and the delivery of neurotransmitters, and they display neuroprotective effects following SCI;^{19,41} however, the effects of astrocytes on the nervous system are more complex. On the one hand, they secrete neurotrophic and antiinflammatory factors to promote the survival of neurons; on the other hand, they also secrete some proinflammatory factors to accelerate the apoptosis of neurons.⁴⁰ Piechota et al. reported that glial cells might be the primary targets of the transcriptional action of steroids in the CNS,³³ and recent findings suggest that endogenous neurosteroids such as allopregnanolone can protect neurons from insults such as stroke and cerebral trauma.^{5,14} However, the effects of steroids on NSC differentiation are incompletely understood. Therefore, understanding the molecular mechanisms underlying ENSC proliferation and differentiation after steroid treatment is important for the management of SCI.

MP can inhibit the activation and proliferation of various inflammatory cell types, thereby reducing the production of inflammatory factors and free radicals, which are necessary to maintain a balanced microenvironment for axonal growth and regeneration.²⁸ In our study, we evaluated the effects of MP on the proliferation of ependymal cells on the 7th day after SCI when BrdU-positive cells reached their highest level. We found fewer nestin/BrdU-positive ependymal cells in the ventral and dorsal horns of the spinal cords on the 7th day after SCI in the MP group compared with the SCI group. Yu et al. found that glucocorticoid induced the apoptosis of ENSCs in the dentate gyrus and immature neurons in neonatal mice, affecting the development of the dentate gyrus in neonatal mice.⁴⁷ High-dose MP treatment in a mouse model of SCI has been shown to inhibit the proliferation of ENSCs and oligodendrocyte progenitor cells in spinal cords.³⁶ As the main structure of myelin in the spinal cord, oligodendrocytes can induce remyelination of unmyelinated nerve axons. After SCI, NSCs preferentially differentiate into astrocytes, contributing to glial scarring, which is the most important inhibitory factor for neuroregeneration after SCI.²⁰ The change in the local microenvironment produced by SCI may be the main cause of this phenomenon, in which a large number of inflammatory factors was secreted, thereby promoting the differentiation of astrocytes and induction of NSCs to differentiate into glial cells.³⁰

FIG. 7. Nestin/BrdU-double positive ependymal cells in ventral and dorsal horns of spinal cord in SCI and MP animals. The number of nestin/BrdU-double positive ependymal cells was significantly decreased in the MP group compared with the SCI group. * $p < 0.05$ versus SCI group.

Limitations

The major limitation of our study is the relatively small sample size, especially in the MP treatment group. Therefore, studies based on a larger sample size of the SCI model in nonhuman primates should be conducted in the future. In addition, labeling ENSCs in vivo in nonhuman primate SCI models and investigating the proliferation, migration, and differentiation of ENSCs in vivo will be useful for understanding the molecular mechanism underlying the inhibitory effects of MP on ENSC proliferation.

Conclusions

Based on current and previous findings, we speculate that MP inhibits the proliferation of ENSCs and oligodendrocyte progenitor cells. We demonstrated increased ependymal cell proliferation in a nonhuman primate SCI model and observed that MP inhibited the proliferation of ependymal cells. Although NSC transplantation has been widely used to explore strategies for functional recovery after SCI in rodent models, there is still a ways to go before the clinical application, for several reasons, such as the difficulty of the separation and enrichment of NSCs, expensive surgical costs, and potential tumorigenicity after NSC transplantation.

Acknowledgments

This study was supported by National Natural Science Foundation of China (no. U1301223); Science and Technology Planning Project of Guangzhou, China (no. 201707010273); Science and Technology Planning Project of Guangdong Province, China (nos. 2016A020225001 and 2017A020213024); Guangdong Provincial Medical Science and Technology Research Fund (no. A2016233);

and Established Project of Guangdong Provincial Bureau of Traditional Chinese Medicine (no. 20161233).

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Shen, Ye. Acquisition of data: Ye, Qin, Ma, Chen. Analysis and interpretation of data: Wang. Drafting the article: Shen, Ye, Qin, Wang. Critically revising the article: Shen, Tang, Ma, Huang, Yang, Wu. Reviewed submitted version of manuscript: Shen, Qin, Tang, Huang, Yang, Chen. Approved the final version of the manuscript on behalf of all authors: Shen. Statistical analysis: Shen, Ma, Wang, Chen, Chai, Wu. Administrative/technical/material support: Tang, Wang, Huang, Yang, Chai. Study supervision: Qin, Tang, Ma, Huang, Yang, Chai, Wu.

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