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A study on the mechanism of PP2A in the recovery of SCI in rats through downregulation of MMP-9 *via* MAPK signaling pathway

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Abstract. – OBJECTIVE: The aim of this study was to investigate the mechanism of action of protein phosphatase 2A (PP2A) in the recovery of spinal cord injury (SCI) in rats by downregulating matrix metalloproteinase 9 (MMP-9) via the mitogen-activated protein kinase (MAPK) signaling pathway.

MATERIALS AND METHODS: A model of SCI was first successfully established in rats. A total of three groups were set, including: sham operation group (A group), SCI group (B group) and PP2A group (C group). The Basso, Beattie and Bresnahan (BBB) motor function score and inclined plane test were adopted to evaluate the motor ability and limb muscle strength of rats in each group. The water content in spinal cord tissues was detected as well. Quantitative Polymerase Chain Reaction (qPCR) assay was performed to analyze the messenger ribonucleic acid (mRNA) expression levels of MAPK, MMP-2, and MMP-9 in spinal cord tissues. The expressions of inflammatory factors tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 in each group of rats were determined via enzyme-linked immunosorbent assay (ELISA). Western blotting (WB) was employed to measure the protein expression levels of MAPK, MMP-2 and MMP-9 in each group of rats. Additionally, the apoptosis of nerve cells in spinal cord tissues was analyzed through terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay.

RESULTS: The BBB score was 8.8 points in C group at 5 d after operation, which was significantly different from that in B group ($p<0.05$). The slope in B and C groups was clearly lower than that in A group at each time point ($p<0.001$). Meanwhile, it was significantly higher in C group than that in B group at 5, 7 and 9 d ($p<0.05$). The edema rate rose notably in B group compared with A group ($p<0.001$). However, spinal cord edema was remarkably relieved after treatment with FRY720 ($p<0.01$), suggesting that PP2A agonist could treat SCI in rats. The levels of cytokines TNF-α, IL-1β and IL-6 were markedly higher in B group than those in A group ($p<0.01$). However, they were significantly reduced after treatment with PP2A agonist ($p<0.01$). In comparison with A group, B group exhibited remarkably decreased mRNA expression of MAPK and elevated mRNA expressions of MMP-2 and MMP-9 ($p<0.01$). However, C group exhibited an upregulated mRNA expression of MAPK ($p<0.05$), a downregulated mRNA expression of MMP-9 ($p<0.01$), and an undifferentiated mRNA expression of MMP-2 ($p>0.05$). Compared with B group, the protein expression level of MAPK significantly increased ($p<0.05$), while that of MMP-9 evidently decreased in C group ($p<0.05$). Besides, no statistically significant difference was observed in the protein expression level of MMP-2 between C group and B group ($p>0.05$). Compared with that in A group, the apoptosis rate significantly increased in B group ($p<0.001$). In addition, the apoptosis rate was significantly lower in C group than that in B group, showing a statistically significant difference ($p<0.01$).

CONCLUSIONS: PP2A downregulates MMP-9 through the MAPK signaling pathway, thereby conducing to the recovery of SCI in rats.

Key Words:
PP2A, MAPK signaling pathway, MMP-9, Recovery of spinal cord injury.

Introduction

Spinal cord injury (SCI), a traumatic injury to the cervical cord, involves hypoxia, lipid perox-

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idation and apoptosis. SCI can be classified into primary SCI and secondary SCI[1]. As a disease of the nervous system, SCI is characterized by non-recoverable physical disability and nervous system. Clinically, SCI leads to physical and psychological damages through extracellular matrix metabolic disorders and inflammatory responses. Excessive inflammatory responses seriously damage the repair and regeneration of nerves[2]. Currently, treatment approaches, such as drugs, surgery and hyperbaric oxygen intervention, are able to effectively alleviate SCI. However, side effects and secondary injuries still bother SCI patients[3]. For this reason, the exploration of effective therapeutic methods for SCI remains necessary.

Stem cell transplantation is one of the major therapeutic methods for SCI. In patients, the proliferation and differentiation abilities of stem cells decrease with age[4]. The mitogen-activated protein kinase (MAPK) signaling pathway is one of the important signaling pathways for the proliferation and differentiation of bone marrow mesenchymal stem cells into osteogenic cells, including extracellular signal-regulated kinase (ERK) 1/2 and p38 MAPK[5].

Matrix metalloproteinases (MMPs) exert a dual effect on the central nervous system. On the one hand, these proteolytic enzymes promote the occurrence of diseases, including Alzheimer's disease, malignant glioma, and stroke[6]. On the other hand, they are involved in the recovery of the central nervous system by removing debris after injury, reconstructing extracellular matrix or stimulating angiogenesis[7]. MMPs belong to the zinc-dependent and calcium-dependent endopeptidase family, with the ability to degrade all components of extracellular matrix. Based on their structural similarity and substrate specificity, MMPs can be classified into such subclasses as collagenase, gelatinase, and matrix protease[8]. Romanic et al[9] have suggested that MMPs, especially MMP-2 (gelatinase A) and MMP-9 (gelatinase B), are upregulated in the case of stroke and central nervous system injury. This suggests that they participate in blood-brain barrier breakdown and post-traumatic secondary injury. Meanwhile, inhibition of MMPs can increase the functional recovery after central nervous system injury. In mice with MMP-9 knockout, the breakdown of the blood-spinal cord barrier and infiltration of neutrophils are significantly alleviated at 24 h after SCI, eventually promoting motor recovery[10].

In the present study, we found that protein phosphatase 2A (PP2A) downregulated MMP-9 through the MAPK signaling pathway, thus facilitating the recovery of SCI in rats. PP2A, a heterotrimeric serine/threonine phosphatase, is able to regulate the RAF-MEK-ERK/MAPK pathway in mammals and *Caenorhabditis elegans*. However, no report has elucidated the effect of PP2A on SCI in rats and its underlying mechanism. All our findings reveal that PP2A may be an effective therapeutic target for SCI, providing new opportunities for the treatment of SCI.

Materials and Methods

Materials

A nitrocellulose membrane was purchased from Bio-Rad (Hercules, CA, USA). Hypersensitive luminescent solution was bought from Thermo Fisher Scientific (Waltham, MA, USA). Transfection cloning vectors were provided by Shanghai Sangon Biotechnology (Shanghai, China). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA). Phosphate-buffered saline (PBS) was bought from Beyotime (Shanghai, China). TRIzol was provided by Invitrogen (Carlsbad, CA, USA). Male Sprague-Dawley (SD) rats weighing 180-220 g were purchased from the Animal Center of Shanghai, Chinese Academy of Sciences (Shanghai, China). Animal experiments were conducted in the Experimental Animal Center. This investigation was approved by the Animal Ethics Committee of Southwest Medical University Animal Center.

Western Blotting (WB)

Sodium dodecyl sulphate (SDS) polyacrylamide gels were first prepared, and a comb was inserted. Secondly, the concentration of extracted proteins was measured *via* the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). 30-50 μg of protein samples was loaded, and the loading volume was calculated. Protein samples were added with 1× loading buffer and heated for 5 min at 100°C for protein denaturation. Proteins were subjected to electrophoresis at 120 V for about 2 h and transferred onto membranes at constant voltage of 100 V for 60 min. The membrane was blocked on a shaker at room temperature for 60 min. The membrane was incubated with primary antibodies on a shaker at a low speed at 4°C for 14 h. On the next day, the membrane was washed with 1×phosphate-buffered saline and tween (PBST) on a shaker for 3 times. Then, the membrane was incubated with corresponding sec-

ondary antibody on a shaker at room temperature for 60 min. Finally, immuno-reactive bands were exposed by a chemiluminescence instrument, and the expression of proteins was finally calculated.

Modeling of SCI in Rats

In this experiment, 24 healthy male SD rats with strong exercise ability and a weight of 180-220 g were selected. 4% isoflurane was used to induce anesthesia that was maintained with 98% O_2/2% isoflurane. The degree of sedation throughout the experiment was assessed by monitoring whether there was no response to toe pressing. Next, laminectomy was performed at the level of the 10th thoracic vertebrae in a 37.5°C incubator[11]. After the rats were completely recovered from anesthesia, they were housed separately. A modified Allen's weight-drop device (vertical height: 40 mm, weight: 8 g × 40 mm) was employed to induce moderate contusion injury to the spinal cord. In sham operation group (A group), the rats only underwent laminectomy. After that, the muscles were sutured layer by layer, and skin incisions were sutured with a silk thread. A total of 3 experimental groups were set (n=8), namely A group (with the same exposure area, without injury), B group (PP2A control + SCI) and C group (PP2A agonist + SCI).

Basso, Beattie and Bresnahan (BBB) Motor Function Score

The motor function of rats in each group was assessed using BBB scoring method at 1, 3, 5, 7 and 9 d after SCI, respectively[12]. Each rat was observed for exercise for 4 min by two independent and well-trained testers. Then, the motor function was scored according to the BBB scale by two-person method and double-blind method. Combining the advantages of the two methods, the scores obtained were averaged and recorded.

HE Staining Assay

The spinal cord specimens fixed in 10% formalin and embedded in paraffin were sliced into 5 μm-thick sections. The cytoplasm and nucleus were stained with eosin and hematoxylin, respectively. After washing with running water, the sections were mounted for microscopic examination.

Water Content Measurement

Spinal cord edema was evaluated by measuring the water content in spinal cord tissues. Briefly, at 72 h after SCI, the spinal cord was dried at 80°C for 48 h. Next, dry weight was measured. The water content in the spinal cord was calculated based on the following formula: spinal cord water content (%)=(wet weight - dry weight)/wet weight ×100%.

Measurement of Levels of Serum Inflammatory Factors Via Enzyme-Linked Immunosorbent Assay (ELISA)

Rats were killed by cold PBS perfusion *via* hepatic artery to remove ribonucleic acids (RNAs) and proteins expressed by blood cells. Next, the spinal cord was immediately dissected on ice. Thereafter, the spinal cord segment containing the injury center was resected as soon as possible. The levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 in rats of each group at 72 h after SCI were measured by ELISA. All tests were performed repeatedly.

Inclined Plane Test in Each Group of Rats

A modified inclined plane test was carried out to assess the limb muscle strength of rats in each group at 0, 1, 3, 5, 7 and 9 d after SCI, respectively[13].

RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR) Analysis

Rats were sacrificed and quick-frozen in liquid nitrogen. Total RNAs were extracted using TRIzol reagent. Chloroform was then added, followed by centrifugation at 12,000 rpm for 10 min. Thereafter, the supernatant was collected, added with an equal volume of isopropanol and mixed, followed by standing at room temperature for 10 min. After that, the mixture was centrifuged at 12,000 rpm for 10 min. Next, the supernatant was discarded, and the precipitate was washed with 75% ethanol twice. The precipitate was dissolved in nuclease-free water. The concentration and purity of RNAs were determined, followed by reverse transcription. Finally, the resulting cDNAs were subjected to qPCR analysis using LightCycler 480. The pimer sequences used in this study were shown in Table I.

Extraction of Nerve Cells from Spinal Cord Tissues in Rats of Each Group

Nerve cells were extracted from spinal cord tissues of rats in each group as follows. Briefly, the spinal meninges adhering to the spinal cord were discarded. The spinal cord of rats was taken out and quickly washed with PBS for three times. Next, the spinal cord was cut into 1 mm^3 blocks

Table I. Primer sequences.

Primer name	Primer sequence
GAPDH-F	5'-GGTGAAGGTCGGTGTGAACG-3'
GAPDH-R	5'-CTCGCTCCTGGAAGATGGTG-3'
MAPK-F	5'-TCTCCCGCACAAAAATAAGG-3'
MAPK-R	5'-TCGTCCAACTCCATGTCAAA-3'
MMP-9-F	5'-TTGACAGCGACAAGAAGTGG-3'
MMP-9-R	5'-GCCATTCACGTCGTCCTTAT-3'
MMP-2-F	5'-GGCAAACCCCAGGCCAC-3'
MMP-2-R	5'-GGTATTGCACTGCCAACTCTT-3'

with surgical scissors and digested with 10 mL of 0.25% trypsin in a constant temperature shaker (37°C) for 60 min. During digestion, the centrifuge tube was taken out for tissue pipetting every 10 min. Then, 10 mL of complete medium was added to terminate the digestion, and the tissues were repeatedly pipetted to form a single-cell suspension. Undigested tissues were removed using a 100-mesh screen. After centrifugation in a centrifuge at 1,000 rpm for 10 min, cell pellets were collected. All cells were cultured in DMEM containing 11.0% fetal bovine serum (FBS) and 1.0% penicillin-streptomycin in a constant temperature incubator at 37°C and 5% CO_2 (volume ratio). On the next day, 2.5 μg/mL cytarabine was added to the culture dish for 24 h of reaction to limit the proliferation of non-nerve cells.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL) Analysis

An *in situ* cell death detection kit (Roche, Basel, Switzerland) was used to verify the apoptosis of spinal nerve cells in each group of rats. Briefly, cells (5×10^4) were fixed with 4% PFA at room temperature for 30 min, and washed with PBS (pH =7.0) twice. Then, the cells were treated with 3% hydrogen peroxide at room temperature for 10 min and 0.1% Triton X-100 at 4°C for 2 min. After incubation with the TUNEL reaction mixture at 37°C for 1 h, the cells were stained with 4',6-diamidino-2-phenylindole (DAPI) (1:100) at room temperature for 10 min. Cell apoptosis was observed under an inverted fluorescence microscope (×40, Nikon, Tokyo, Japan). The percentages of TUNEL-positive cells and apoptotic nuclei were determined in three independent samples and quantified using Image J 1.8.0 (National Health Service). The apoptosis index (AI) was calculated as follows: AI = (number of apoptotic cells/total number of cells) ×100%.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Experimental data were expressed as mean ± standard deviation. Univariate analysis was applied to compare the differences between groups. $p<0.05$ was considered statistically significant.

Results

BBB Motor Function Score of Rats in Different Groups

In three groups (A group, B group and C group), the model of SCI was first established. The BBB scoring was performed for the first time after the rats were recovered from anesthesia. The results (Figure 1) showed that after recovery from anesthesia, normal motion was observed in rats in A group, whereas paralysis of hind limbs was found in rats in B and C groups. Subsequently, the rats in C group were intraperitoneally injected with FRY720, a PP2A agonist, for 9 consecutive days. Meanwhile, rats in A and B groups were given the same volume of solvent. Before SCI modeling, the BBB score was 21 points in the three groups. Rats in A group showed no paralysis of hind limbs after operation, with a BBB score of 19-21 points. However, paralysis of hind limbs was observed in both B and C groups after SCI, with a BBB score of 0 point. The BBB score was 8.8 points in C group at 5 d after operation, which was significantly different from that in the B group ($p<0.05$). Moreover, it was 16.3 points in C group and 7.2 points in B group at 9 d after operation, showing an extremely significant difference ($p<0.001$).

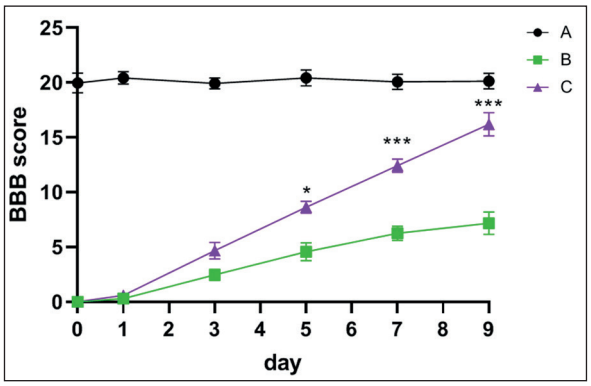

Figure 1. Changes in BBB score in the three groups (*$p<0.05$, ***$p<0.01$).

Score in Inclined Plane Test in Different Groups

The effect of PP2A on SCI rats was evaluated *via* inclined plane test in each group. It was revealed that in A group, the hind limbs of rats were non-paralytic, and there was no significant difference in slope at each time point ($p>0.05$) (Figure 2). The slope was significantly lower in B and C groups than that in A group at each time point ($p<0.001$). Meanwhile, it was significantly higher in C group than that in B group at 5, 7 and 9 d ($p<0.05$). It can be inferred that PP2A exerts a certain repair effect on the motor function of SCI rats.

Spinal Cord Edema Rate Evaluated Based on the Water Content in Spinal Cord Tissues

At 72 h after SCI, the dry weight and wet weight of spinal cord tissues were measured in the three groups, so as to calculate the spinal cord edema rate. As shown in Figure 3, the edema rate notably rose in B group compared with that in A group ($p<0.001$). However, spinal cord edema was remarkably relieved after treatment with FRY720 ($p<0.01$), suggesting that PP2A agonist could treat SCI in rats.

Levels of TNF-α, IL-1β and IL-6 in Spinal Cord Tissues Detected Through ELISA

The expressions of inflammatory cytokines TNF-α, IL-1β and IL-6 in spinal cord tissues were detected through ELISA at 72 h after SCI. The results (Figure 4) uncovered that the expression levels of TNF-α, IL-1β and IL-6 were significantly higher in B group than those in A group ($p<0.01$). However, they were evidently reduced after treatment with FRY720 ($p<0.01$). These findings imply that PP2A agonist FRY720 is able to prominently repress inflammation after SCI.

MRNA Expressions of MAPK, MMP-2 and MMP-9 Determined Via qPCR

The mechanism of action of PP2A in the recovery of SCI in rats was explored in this study. Previous studies have indicated that MMP-2 and MMP-9, members of MMPs, are upregulated in the case of stroke and central nervous system injury. Meanwhile, they are involved in the breakdown of the blood-brain barrier and secondary injury after trauma. Inhibition of MMPs can also increase functional recovery after central nervous system injury. Therefore, it was supposed that PP2A might downregulate MMPs through the MAPK signaling pathway to attenuate SCI in rats. The messenger RNA (mRNA) expression levels of MAPK, MMP-2 and MMP-9 were measured by qPCR in A group, B group and C group. It was found that B group exhibited significantly decreased mRNA expression of MAPK and elevated mRNA expressions of MMP-2 and MMP-9 in comparison with A group ($p<0.01$). However, C group exhibited an upregulated expression of MAPK ($p<0.05$), decreased mRNA expression of MMP-9 ($p<0.01$), and undifferentiated mRNA expression of MMP-2 in comparison with B group (Figure 5). This suggests that the PP2A/MAPK signaling pathway suppresses MMP-9 instead of MMP-2 to relieve SCI.

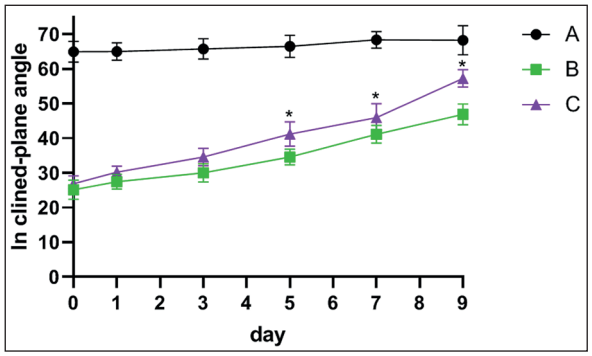

Figure 2. Changes in inclined plane test score in three groups of rats with time (*$p<0.05$).

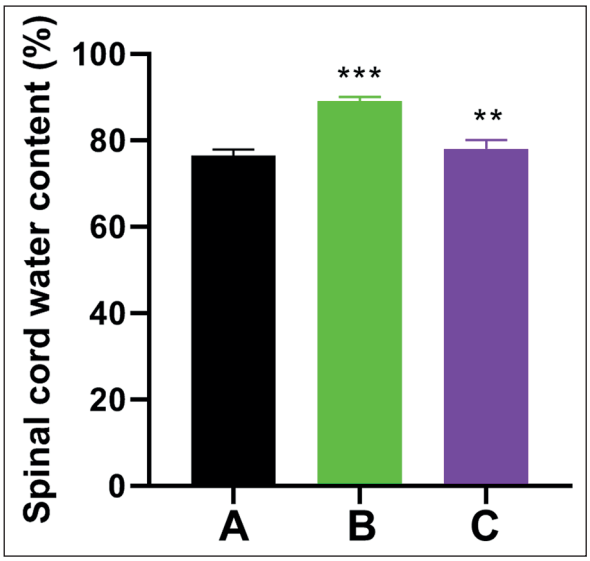

Figure 3. Spinal cord edema rate in the three groups (**$p<0.01$, ***$p<0.001$).

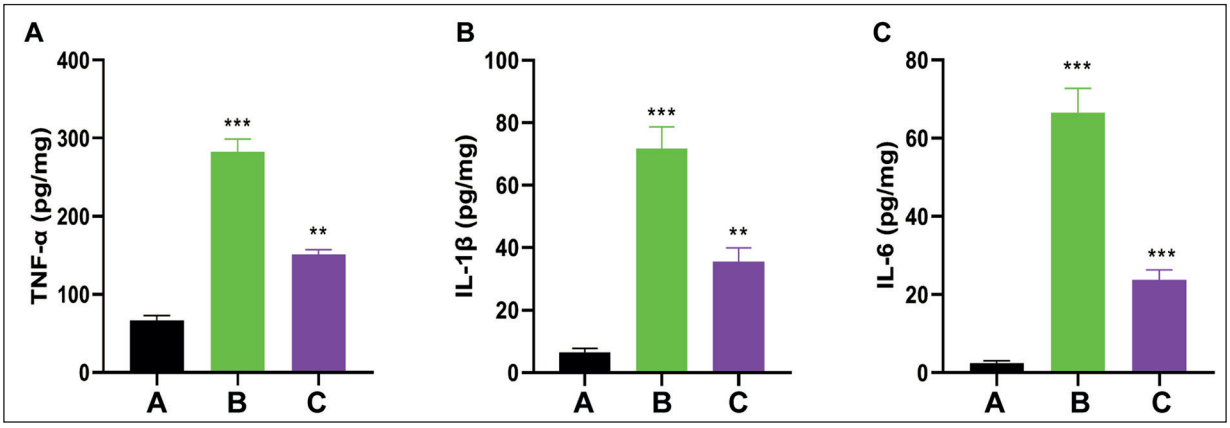

Figure 4. Levels of TNF-α, IL-1β and IL-6 in spinal cord tissues detected through ELISA (**p<0.01, ***p<0.001).

Protein Expression Levels of MAPK, MMP-2 and MMP-9 in Spinal Cord Tissues Measured by WB

As shown in Figure 6, the protein expression level of MAPK was distinctly lower in B and C groups than that in A group (p<0.05). Meanwhile, it was clearly higher in C group than that in B group (p<0.05). Both B and C groups exhibited a considerably up-regulated protein expression level of MMP-2 in comparison with A group (p<0.05). However, no significant difference was observed in the protein expression level of MMP-2 between C group and B group (p>0.05). Compared with A group, the protein expression level of MMP-9 was distinctly higher in B and C groups (p<0.01). The protein expression of MMP-9 was evidently lower in C group than that in B group (p<0.05). It can be seen that PP2A agonist can upregulate the MAPK signaling pathway to inhibit MMP-9 protein expression, thereby mitigating SCI in rats.

Apoptosis of Nerve Cells in Spinal Cord Tissues in Each Group Analyzed Through TUNEL Assay

The apoptosis of nerve cells in A group, B group and C group was determined *via* TUNEL assay, based on which AI was calculated. The results manifested that the apoptosis rate was significantly higher in B group than that in A group (p<0.001). However, it was remarkably lower in C group than that in B group, showing a statistically significant difference (p<0.01) (Figure 7). These results indicate that PP2A agonist can reduce apoptotic nerve cells in spinal cord tissues of rats probably by alleviating SCI through the MAPK/MMP-9 pathway.

Discussion

As a traumatic injury to the cervical cord, SCI involves hypoxia, lipid peroxidation and apop-

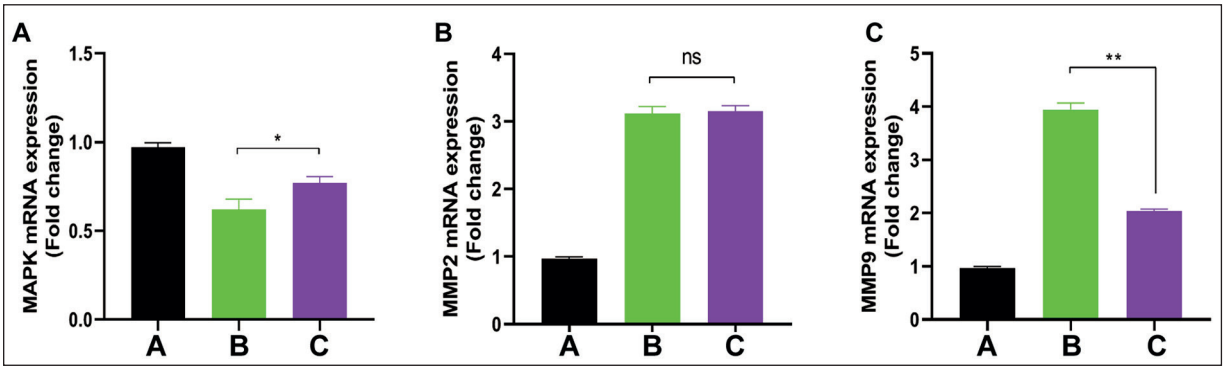

Figure 5. MRNA expression levels of MAPK, MMP-2 and MMP-9 measured by qPCR (*p<0.05, **p<0.01).

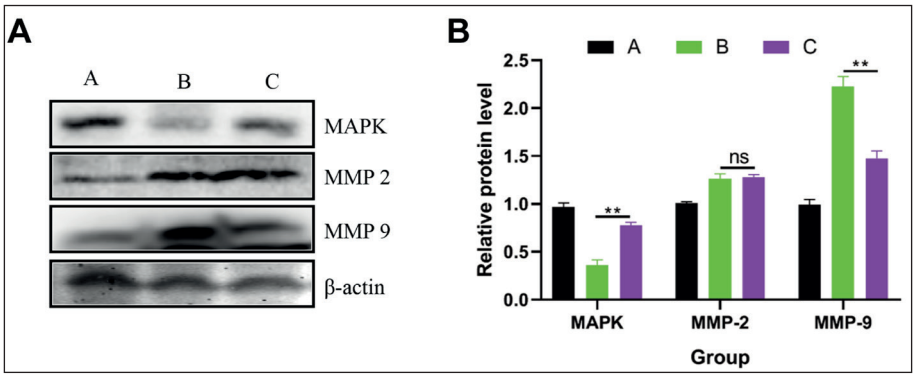

Figure 6. Protein expression levels of MAPK, MMP-2 and MMP-9 in spinal cord tissues measured by WB (*p<0.05, **p<0.01).

tosis. It is classified into two types, including: primary SCI and secondary SCI. In terms of treatment, drugs, surgery and hyperbaric oxygen intervention are mainly adopted. However, there is still no effective treatment method that can completely restore the motor function and injured nerves of SCI patients at present. Therefore, the exploration of the mechanism in treating SCI and restoring nerve function have become hotspots in the academic field.

PP2A is a heterotrimeric serine/threonine phosphatase. PP2A-related pathways have been found interfered in various diseases. The common pathological mechanisms in cancers and neurodegeneration include activated kinase signaling pathways and downregulated PP2A activity[14,15]. In the case of neurodegenerative disorders, PP2A dysfunction leads to hyperphosphorylation of tau protein[16]. Tau protein usually stabilizes microtubules, and over-phosphorylated tau protein results in the formation of neurofibrillary tangles, which plays a vital role in the etiology of Alzheimer's disease[17]. Thus, we tried to speculate that PPA2 could play some roles in the recovery of SCI. In this study, the model of SCI was first successfully established in A group, B group and C group. BBB scoring was conducted after rats resuscitated from anesthesia. Next, PP2A agonist was intraperitoneally injected into the rats in C group for 9 consecutive days. It was found that PP2A agonist significantly enhanced the motor function of SCI rats. Besides, the spinal cord edema rate was overtly reduced. In addition, PP2A agonist also evidently downregulated the expressions of inflammatory factors IL-1β, IL-6 and TNF-α. These results indicate that PP2A is capable of facilitating the recovery after SCI in rats.

MMPs exerts a dual effect on the central nervous system. MMPs are members of the zinc-dependent and calcium-dependent endopeptidase family and can degrade all components of extracellular matrix[18]. Evidence[19] has shown that

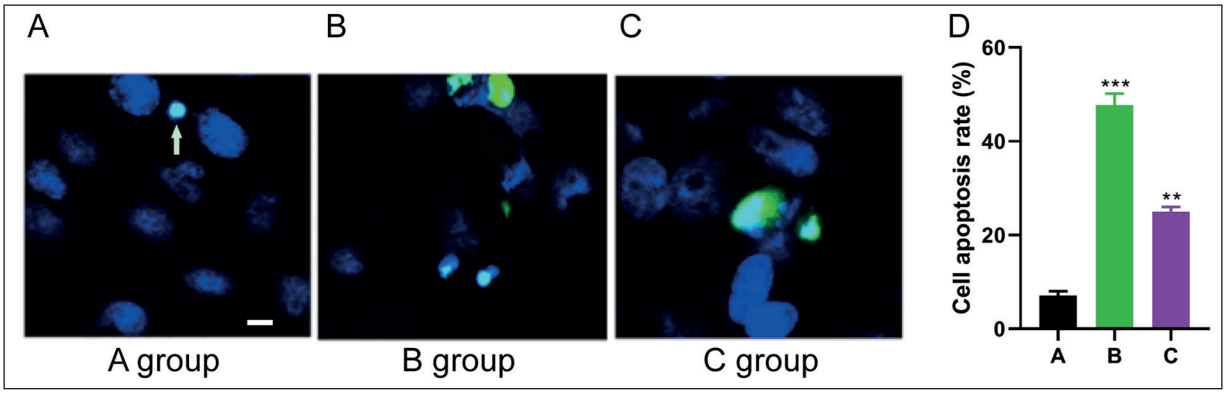

Figure 7. Apoptosis of nerve cells in spinal cord tissues in each group analyzed through TUNEL assay (magnification: 400×) (**p<0.01, ***p<0.001).

MMPs are elevated in the case of stroke and central nervous system injury, suggesting that they participate in blood-brain barrier breakdown and post-traumatic secondary injury. MMP-9 is upregulated by leukocytes stimulated by inflammation in vitro, promoting their trans-endothelial migration[20]. Besides, the upregulation of MMP activity in vascular endothelial cells of the central nervous system promotes the degradation of extracellular matrix components and facilitates the infiltration of inflammatory cells. The activity of MMP-1, MMP-2 and MMP-9 also increases at the site of angiogenesis[21]. MMPs are proteolytic enzymes able to trigger invasion of endothelial cells to the surrounding matrix, known as the chemotaxis of endothelial cells[22]. Based on relevant data, the permeability of the blood-spinal cord barrier is enhanced in the early stage of SCI, resulting in strong activation of MMPs[23]. Multiple studies have emphasized the importance of barrier breakdown in the pathogenesis of secondary SCI, namely the exposure of the spinal cord to toxic effect of inflammatory cells. These inflammatory cells are closely associated with delayed neuronal death and demyelination[24]. However, MMPs are conducive to the release of inflammation-regulated growth factors and cytokines, as well as the maturation of TNFα precursors[25]. There is other evidence proving that MMPs is beneficial for injured spinal cord by promoting angiogenesis and regeneration. MMPs can accelerate the restoration of blood supply, which is essential for the healing of the spinal cord. They are also conducive to the recovery of the spinal cord and the regeneration of axons. Furthermore, MMPs can promote the regeneration of axons by removing the myelin sheath and nerve fragments from lesions[26]. In this study, the mechanism of action of PP2A in the recovery after SCI in rats was explored. The mRNA expression levels of MAPK, MMP-2, and MMP-9 were detected in A group, B group, and C group, respectively. The results showed that the PP2A/MAPK signaling pathway inhibited MMP-9 rather than MMP-2 to relieve SCI. The protein expression level of MAPK was distinctly lower in B and C group than that in A group ($p<0.05$). Meanwhile, it was remarkably higher in C group than that in B group ($p<0.05$). Both B and C groups exhibited a considerably upregulated protein expression level of MMP-2 in comparison with A group ($p<0.05$). There was no significant difference in the protein expression level of MMP-2 between C group and B group ($p>0.05$). Compared with A group, the protein expression level of MMP-9 increased significantly in B and C groups ($p<0.01$). However, it was evidently lower in C group than that in B group ($p<0.05$). It can be seen that PP2A agonist can upregulate the MAPK signaling pathway to repress the protein expression of MMP-9 and the apoptosis of nerve cells in the spinal cord, eventually mitigating SCI in rats.

Conclusions

PP2A downregulates MMP-9 through the MAPK signaling pathway to facilitate the recovery of SCI in rats. At present, there is still no report on the role of PP2A in SCI rats and its underlying mechanism. The novelty of this study was that PP2A may be an effective therapeutic target for SCI, providing new opportunities for the treatment of SCI.

Conflicts of interest

The authors declare no conflicts of interest.

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