The Neuroprotective Effect of Puerarin in Acute Spinal Cord Injury Rats

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Abstract

Background: Acute spinal cord injury (SCI) leads to permanent disabilities. This study evaluated the neuroprotective effect of puerarin, a natural extract, in a rat model of SCI. Methods: Acute SCI models were established in rats using a modified Allen's method. Locomotor function was evaluated using the BBB test. The histological changes in the spinal cord were observed by H&E staining. Neuron survival and glial cells activation were evaluated by immunostaining. ELISA and realtime PCR were used to measure secretion and gene expression of cytokines. TUNEL staining was used to examine cell apoptosis and western blot analysis was used to detect protein expression. Results: Puerarin significantly increased BBB score in SCI rats, attenuated histological injury of spinal cord, decreased neuron loss, inhibited glial cells activation, alleviated inflammation, and inhibited cell apoptosis in the injured spinal cords. In addition, the downregulated PI3K and phospho-Akt protein expression were restored by puerarin. Conclusion: Puerarin accelerated locomotor function recovery and tissue repair of SCI rats, which is associated with its neuroprotection, glial cell activation suppression, anti-inflammatory and anti-apoptosis effects. These effects may be associated with the activation of PI3K/Akt signaling pathway.

Introduction

Acute spinal cord injury (SCI) is a traumatic injury resulted from mechanical insult on spinal cord tissue. It leads to permanent disabilities with high healthcare costs [1]. The initial mechanical insult leads to a neurological damage that is named “primary injury”. The mechanical injury leads to a cascade of biological changes, which is normally called “secondary injury”. The secondary injury occurs days to years after the initial of SCI and leads to further neurological damage. This secondary injury includes neuronal death, axonal degeneration, glial scar formation, as well as activation of microglia [2-5].
Traumatic injury in spinal cord initiates a massive immune response which may result in apoptosis of neurons and activation of glial cells, and finally the neurological dysfunction. Injury to the spinal cord leads to irreversible loss of neurons [6, 7] because that spinal cord lacks the ability to produce new neurons in adulthood [8, 9]. Astrocytes are distributed throughout the spinal cord [10]. When spinal cord is injured, they are activated and proliferate to form a glial scar that maintains the integrity of surrounding cells. However, besides forming a physical barrier, this scarring process is also associated with the increased release of chondroitin sulphate proteoglycans, which can further limit axon regeneration [11, 12]. Thus, although it is a defense mechanism, the persistence of a glial scar is detrimental to functional recovery of a damaged spinal cord. Microglia activation was found in injured spinal cord. It is considered to be responsible for much of the immune response at the lesion site and contribute to the loss of function after SCI [13-15]. Therefore, the development of strategies to help to restore neurons and control glial activation would be beneficial in the treatment of SCI.

Puerarin (8-(beta-d-glucopyranosyl-7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, C_{21}H_{20}O_{9}) is one of the main active ingredients of the traditional Chinese herb *Radix Puerariae lobata*. It has been widely used in treatment of various diseases such as cardiovascular disorder, neurodegenerative disease, cancer, diabetes and ischemic stroke [16]. Importantly, studies have demonstrated that puerarin had neuroprotective effect on spinal cord ischemia-reperfusion injury in rats [17, 18]. In addition, Heng LS et al. reported that puerarin could attenuate secondary injury in early SCI [19]. However, the available data are limited. Therefore, the present study aimed to investigate the neuroprotective effect of puerarin on SCI rats, including neuron survival and glial cells activation. The potential signaling pathway associated with the mechanisms was investigated as well.

**Material and Methods**

**SCI model**

Male Sprague Dawley rats weighing 300–350 g were obtained from Experimental Animal Centre of Harbin Medical University (Harbin, China) and housed under standard conditions with alternating 12 h light and dark cycles. Standard lab food and water was provided ad libitum. Rats were randomly divided into 5 groups: sham group, SCI group and SCI + puerarin 25, 50 and 100 mg/kg groups. All the experimental protocols were approved by the Ethics Committee of Harbin. Spinal cord injury was established using a modified Allen’s method [20]. Briefly, following intraperitoneal anesthesia with 10% chloral hydrate (3.5 mL/kg), a 2 cm midline incision was made on skin and subcutaneous tissues centered the eighth thoracic segment (T8) along the vertebrae. The spinal process and the vertebral lamina of T8 were removed (laminectomy). Rats in the SCI group were injured by dropping a 10 g rod from a distance of 5 cm onto the spinal cord and letting the rod rest on the lesion site for 3 min. Rats in the sham group received the laminectomy only. The surgical site was then seamed together in layers and 5 ml of physiological saline was injected intraperitoneal immediately after surgery. The rats received a daily intramuscular injection of 200,000 U penicillin for three consecutive days after surgery. Manual bladder evacuation was performed twice daily until micturition function returned. Rats in the SCI+ puerarin group received 50 mg/kg (i.p.) puerarin (>98%, Meilun, Dalian, China) immediately after surgery and once a day for 28 days. Puerarin 125 mg was dissolved in 1 ml DMSO and diluted to indicate concentration with distilled water. Rats in the sham and SCI groups received vehicle (10% DMSO in 0.9% NaCl) only.

**Behavioral tests**

Basso, Beattie, Bresnahan (BBB) scale was performed to evaluate the effect of puerarin on locomotor function in rats subjected to SCI. Eight rats selected at random from each group were used for this test on Days 1, 4, 7, 14, 21 and 28 after the surgery. The test was performed as described previously [21]. Briefly, the rats were allowed to walk around freely in an open field for 4 min and the movements of the hindlimbs were closely observed and scored by three observers who blinded to the treatment group. The final score for each rat were averaged by the three scores.

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Tissue preparation

Six rats selected at random in each group were sacrificed after treatment of puerarin for 3 days for inflammatory cytokines assay, and another 6 rats were randomly selected and sacrificed at day 7 for apoptosis examination. The other 12 rats in each group were sacrificed after treatment of puerarin for 4 weeks for other examinations. For histological examinations, rats were perfused transcardially with 0.1 mol/L phosphate-buffered saline (PBS) and then with 4% paraformaldehyde (PFA) to pre-fix for 10 min. The lesion site of spinal cord was dissected and post-fixed in 4% PFA for 24 h. After being dehydrated using a series of ethanol washes, spinal cord tissues were embedded in paraffin and cut into 5-μm sections. Sections were deparaffinized in xylene, hydrated using a series of ethanol washes before being stained. For molecular biological assay, the spinal cord tissues of the lesion site were harvested and immediately frozen in liquid nitrogen and stored in -80°C until use.

Haematoxylin & eosin and Nissl staining

Spinal cord sections were stained with haematoxylin & eosin (H&E) or thionine (Solarbio Science & Technology, Beijing, China) using the standard protocol and mounted using Permount (Sinopharm, Beijing, China). The sections were visualized under an optic microscope (DP73; Olympus, Tokyo, Japan). Three sections from each animal and three fields within each slide were randomly selected for neuronal counts.

Enzyme-linked immunosorbent assay (ELISA)

Concentrations of inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 in the lesion site of the spinal cord were assessed using ELISA kits specific for rats according to the manufacturers’ instructions (USCN Life Science, Wuhan, China). The cytokine contents are given in pg/mg protein.

RNA extraction and Real-time PCR

Total RNA from injury site of the spinal cord tissue was extracted using a RNA simple Total RNA Kit (Tiangen, Beijing, China) according to the manufacturer’s protocol. The concentration and purity of the RNA were determined by calculating the ratio of the absorbance at 260 and 280 nm. Complementary DNA was synthesized with RNA and oligonucleotide primer using super Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV) (BioTeke, Beijing, China). Quantitative real-time PCR reactions were performed in 20 μl reaction mixture containing 10 μl of SYBR-Green Master Mix (Tiangen Biotechnology Co., Ltd.), 1 μl cDNA and 10 μM of forward and reverse primers on an Exicycler™ 96 real-time quantitative thermal block (Bioneer, Daejeon, Korea). The sequences of primers are as follows: TNF-α, forward: 5’- TGGCGTGTTCATCCGTTCT-3’, reverse: 5’- CCACACTCTAGGCTCTCTGTT-3’; IL-1β, forward: 5’- TCCAGTACGGCTCTCTGTG-3’, reverse: 5’- CGAGATGCTGCTGTGAGATT-3’; IL-6, forward: 5’- GGAGATTACTGCCCTGGCTCCTAGC-3’, reverse: 5’- GGCCGGACTCATGCTACTCTCCTGCT-3’. Relative mRNA levels were normalized to those of β-actin and are calculated as $2 \Delta \Delta Ct$.

Immunohistochemistry

After being boiled in 0.1 M sodium citrate buffer for 10 min to perform the heating antigen retrieval, the spinal cord sections were incubated in 3% H2O2 for 15 min to quench the endogenous peroxidase activity, and then boiled with goat serum (Solarbio) for 15 min at room temperature. The sections were incubated overnight at 4°C with NeuN (1: 100, bs-10394R, Bioss, Beijing, China) and growth associated protein-43 (GAP-43) (1:100, orb14656, Biorbyt, Cambs, UK) antibodies. After a washing stage using 1×PBS, the sections were incubated with horseradish peroxidase (HRP)-labeled streptavidin (Beyotime) for 30 min at 37°C. The staining was visualized using diaminobenzidine (Beyotime). After being co-stained with haematoxylin, the sections were mounted and observed under an optic microscope (DP73; Olympus).

Immunofluorescence

The sections were incubated in Tris-Buffered saline (PBS) containing 0.1% Triton X-100 for 30 min and blocked with goat serum (Solarbio) for 30 min at room temperature. After a washing stage with PBS, the sections were incubated with primary antibodies against mouse monoclonal glial fibrillary acidic protein (GFAP) (1: 50, sc-33673, Santa Cruz Biotechnology, Inc, Dallas, TX, USA) or mouse monoclonal OX-
42 (1: 50, sc-53086, anta Cruz Biotechnology, Inc.) at 4°C overnight. For primary antibody detection, Cy3-conjugated secondary antibodies (A0521, goat anti-mouse, 1:200; Beyotime) were applied for an hour at 37°C in darkness. Fluorescent labeling was visualized and captured using a fluorescence microscope (BX53, Olympus, Tokyo, Japan)

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining

TUNEL staining was used to assess apoptotic status in the spinal cord at day 7 after the surgery. The spinal cord sections were stained using a TUNEL apoptosis detection kit (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China) according to the manufacturer’s instruction. Subsequently, the sections were co-stained with haematoxylin (Solarbio) and observed at 400× magnification under an optic microscope (DP73, Olympus). TUNEL-positive cells were counted in three fields of view per section.

Western blotting analysis

The spinal cord tissue of injury site was homogenized in cooled RIPA buffer supplement with phenylmethanesulfonylfluoride (Beyotime) on ice. After centrifugation at 15,000 g for 10 min at 4°C, the supernatants were collected and the protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Beyotime). Equivalent amounts of protein (40 μg) were separated by 12% (w/v) sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA) using a wet transfer method. The membranes were blocked with 5% (w/v) non-fat milk for 1 h at room temperature and then incubated with primary antibodies against GFAP (1: 200, mouse monoclonal, sc-33673, Santa Cruz Biotechnology, Inc.), OX-42 (1: 200, mouse monoclonal, sc-53086, Santa Cruz Biotechnology, Inc.), Bax (1:400, rabbit polyclonal, BA0412, Boster, Whuan, China), Bcl-2 (1:400, rabbit polyclonal, BA0315, Boster), cleaved-caspase 3 (1:1000, rabbit polyclonal, ab2302, Abcam, Cambridge, MA, USA), PI3K (1: 400, rabbit polyclonal, Boster), Akt (1:200, sc-8312, rabbit polyclonal, Santa Cruz Biotechnology, Inc.), p-Aktser473 (1:200, sc-135651, rabbit polyclonal, Santa Cruz Biotechnology, Inc.) and β-actin (1: 1000, mouse monoclonal, sc-47778, Santa Cruz Biotechnology, Inc.) in PBST overnight at 4°C. After being incubated at 37°C for 45 min with HRP conjugated secondary antibodies (1: 5000; Beyotime), the blots were visualized using an enhanced chemiluminescence system (7 Sea Pharmtech, Shanghai, China) and exposed on Fuji Rx 100 X-ray film (Fuji Photo Film, Tokyo, Japan). Quantification of the band density was carried out with Gel-Pro-Analyzer (Media Cybernetics, Bethesda, MD, USA). Protein expression levels were normalized to internal β-actin.

Statistical analysis

Data were analyzed by SPSS 19.0 (IBM, New York, NY, USA) and represented as means ± standard deviation (SD). Samples from each group were compared with one-way analysis of variance followed by Fisher’s least significant difference (LSD) test. A p value < 0.05 was considered statistical significance.

Results

Effects of puerarin on locomotor function of SCI rats

Evaluation of locomotor function demonstrated that BBB scores in the sham group were approximately 21 at every time point during the experiment (Fig. 1). Immediately after the surgery, the BBB score of every rat was approximately 0, which indicated the success of the SCI model establishment. Seven days after, the BBB score of every rat was increased, which indicated the recovery of locomotor function of rats. However, BBB scores of rats treated with puerarin 50 and 100 mg/kg were significantly higher than that of the SCI rats. In the following days, the rats treated with puerarin 50 and 100 mg/kg exhibited more rapid recovery of locomotor function, represented by higher BBB scores than the SCI rats (P < 0.01). Although the BBB score in the 100 mg/kg puerarin group was higher than that in the 50 mg/kg puerarin group in day 4 and 7, they showed no significant difference after day 14. Puerarin 25 mg/kg has not changed the BBB score of SCI rats. The results indicated that puerarin 50 and 100 mg/kg promoted locomotor function recovery after SCI.
Effects of puerarin on SCI-induced spinal cord tissue damage

Histopathological alterations in the spinal cord after injury were examined using H&E staining at day 28. The results illustrated that neurons in the sham operated rats exhibited normal morphology. No neurons apoptosis or glial proliferation was found in the sham group. In the SCI group, obvious neuron loss and glial proliferation were observed. Neurons exhibited abnormal morphology, dissolved membrane, and condensed nuclei. The extent of

Fig. 1. Puerarin improved locomotor function recovery after SCI. BBB scores for puerarin treated rats were significantly higher at 7, 14, 21, and 28 days post injury. (●), sham rats; (▲), SCI rats; (▲▲), puerarin-treated SCI rats. Data are the means±SD (n=8). **P<0.01 vs. the sham rats, ##P<0.01 vs. the SCI rats.

Fig. 2. Puerarin improved histological alterations after SCI. HE staining showed normal neural and glial morphology in the sham group. In the SCI group, injured spinal cord exhibited glial proliferation and neural loss with dissolved membrane and condensed nuclei. After treatment of puerarin 50 mg/kg for 28 days, more remained neurons with normal morphology and mild glial proliferation were found. Typical necrotic cells were indicated by arrows. Scale bar: 100 μm.

Fig. 3. Puerarin increased neuron survival in lesion site of spinal cord of SCI rats. Nissl staining showed normal morphology and function of neurons in the sham group. Neurons in the SCI group showed lighter staining and less number. Puerarin 50 and 100 mg/kg treatment for 28 days restored these changes. NeuN immunohistochemistry staining illustrated less NeuN-positive cells (neurons) in the SCI group and puerarin 50 and 100 mg/kg treatment significantly increased the number of neurons. Scale bar: 50 μm. Data are the means±SD (n=6). **P<0.01 vs. the sham rats, ##P<0.01 vs. the SCI rats.
histopathological changes in the puerarin group was evidently attenuated compared with SCI group. Although still some proliferation of glial cells existed, many neurons remained and showed normal morphology (Fig. 2).

**Effects of puerarin on neuron survival in the spinal cord of SCI rats**

Neurons in the sham group showed integrative and clear Nissl staining, indicating the normal function of neuronal nutrients and energy supply. In the SCI group, the plasma of neurons was less stained and the number of Nissl bodies was remarkably lower than that in the sham group, which indicated the death of neurons and neuronal dysfunction. Treatment of puerarin 50 and 100 mg/kg for 28 days rescued neurons from SCI though some were still lighter stained (Fig. 3). In line with this result, NeuN immunohistochemistry staining reflected that the number of neurons was reduced after SCI and was retained after puerarin administration. The therapeutic effect of 25 mg/kg puerarin was not significant. These results showed that puerarin had a capacity of neuron protection in SCI rats.

**Effects of puerarin on neuron regeneration in the spinal cord of SCI rats**

GAP-43 protein expression was measured as an indicator of axon regeneration. As shown in Figure 4, following SCI, expression of GAP-43 was increased in the spinal cord, which indicated that the regeneration of neurons was triggered. After treatment of puerarin 50 and 100 mg/kg for 28 days, the expression of GAP-43 was further increased, which indicated that puerarin promoted the regeneration of neurons.

**Effects of puerarin on glial cell activation in the spinal cord of SCI rats**

As illustrated in Figure 5, immunofluorescence staining and western blotting analysis showed that the expressions of GFAP, an astrocytic marker, and OX-42, a microglial activation marker, were markedly increased in the spinal cord of SCI rats, which suggested the activation of astrocytes and microglia. In puerarin 50 and 100 mg/kg groups, significant decreases of the two proteins were observed, which indicated that treatment of puerarin for 28 days suppressed astrocyte and microglia activation in the spinal cord of SCI rats.

**Effects of puerarin on SCI-induced inflammatory responses in the spinal cord of SCI rats**

To evaluate a possible mechanism by which puerarin exerts neuroprotective effects, mRNA expression and secretion of pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 were determined using real-time PCR and ELISA (Fig. 6). Because the inflammatory responses occur within a short period after the injury, we detected the cytokines at day 3 after the SCI. Puerarin reduced the mRNA expression levels...
Fig. 5. Puerarin attenuated the activation of glial cells in the spinal cord of SCI rats. (A) Immunofluorescence staining showed that numbers of GFAP and OX-42-positive cells in the spinal cord were increased in the SCI rats and decreased after treatment of puerarin. (B) Western blotting analysis results also showed the significant decrease of GFAP and OX-42 expression levels in spinal cords after treatment of puerarin 50 and 100 mg/kg for 28 days. Scale bar: 50 μm. Data are the means±SD (n=6). **P<0.01 vs. the sham rats, ##P<0.01 vs. the SCI rats.

Fig. 6. Puerarin attenuated acute inflammatory response in spinal cord of SCI rats. Secretion and mRNA expression of TNF-α (A and D), IL-1β (B and E) and IL-6 (C and F) were decreased in puerarin-treated rats compared with the SCI rats at day 3. Data are the means±SD (n=6). **P<0.01 vs. the sham rats, ##P<0.01 vs. the SCI rats.
and the production of TNF-α, IL-1β and IL-6 at the lesion site after SCI (P < 0.01 compared with SCI group). These results indicated that puerarin attenuated inflammatory responses in the spinal cord of SCI rats.

**Effects of puerarin on cell apoptosis in the spinal cord of SCI rats**

The effect of puerarin on cell apoptosis was evaluated using TUNEL staining at day 7. There was seldom TUNEL-positive cells in the spinal cord of the sham rats. While in the SCI
rats, a number of TUNEL-positive cells were found in the lesion site of spinal cord. Puerarin treatment obviously reduced the number of apoptotic cells. The effect of 50 and 100 mg/kg puerarin was better than that in 25 mg/kg dosage (Fig. 7A). The expression of apoptosis-associated proteins Bax, Bcl-2 and cleaved-caspase 3 were examined using western blotting analysis. Markedly diminished Bcl-2 expression and pronounced Bax and cleaved-caspase 3 expressions were found in the spinal cord of SCI rats (Figure 7B). In line with the results in TUNEL staining, puerarin restored these changes and exhibited effective anti-apoptotic effect.

**Effects of puerarin on PI3K/Akt signaling pathway in the spinal cord of SCI rats**

To further investigate the molecular mechanisms of the neuronal protective effects of puerarin in the injured spinal cord, its effect on phosphoinositide 3-kinase (PI3K)/Akt signaling pathway was evaluated. The results demonstrated that protein expression of PI3K was diminished in the spinal cord tissue of the SCI rats compared with the sham rats (P < 0.01), as well as the phosphorylation of Akt (Fig. 8). Puerarin administration restored the PI3K and phospho-Akt\text{ser}473 expression. These data suggested that the neuronal protective effect of puerarin in the injured spinal cord may be associated with the activation of PI3K/Akt signaling pathway.

**Discussion**

An appropriate animal model is necessary for basic studies. In the present study, a classical SCI model establishment method which has been first reported by Allen et al. in 1914 was used in rats. The changes of locomotor functional and histological features of the SCI rat model used in this study were similar to that in previous studies [22, 23], which indicated the success of the model establishment.

Assessment of neurologic function is essential for recovery evaluation of SCI. In the present study, BBB test, a well accepted behavioral test, was used to assess the locomotor function of SCI rats. We observed that puerarin significantly improved locomotor function recovery in the SCI rats. However, we have not observed a platform period of the locomotor function recovery, which indicates that the recovery may continue in the following days. The long-term effect of puerarin on SCI should be studied in the future. Anyhow, these data suggest that puerarin treatment can accelerate the locomotor function recovery in SCI rats.

Neuronal damage plays an important role in the progress and prognosis of acute SCI. In the present study, Nissl staining showed a significant reduction in Nissl bodies in rats with SCI. Nissl bodies are rough endoplasmic reticulum where proteins are synthesized. They are widely used to reflect neural structure of brain and spinal cord and are considered to be related to the nutritional condition of neurons. They may dissolve and even disappear under pathological conditions. The decreased Nissl bodies observed in our study indicated a neuronal damage in the spinal cord after SCI. This observation was parallel with the decreased density of NeuN-positive cells, which indicated the loss of neurons after SCI. Moreover, in this study, evidently increased expression of GAP-43, a commonly used indicator of neuronal growth propensity [24], was observed in the spinal cord after SCI. Similar findings were reported by Cizkova’s and Wang’s groups [25, 26]. The upregulated GAP-43 and decreased number of neurons indicated the auto-compensation could not restore the neuron injury. After puerarin administration, neuron loss was prevented and neuronal regeneration was promoted, as representing by the increased numbers of Nissl bodies and NeuN-positive cells and the more enhanced expression of GAP-43. These results indicate that puerarin has neuroprotective effect in SCI rats, and this effect may contribute to the recovery of the locomotor function.

Astrocytes are the most abundant type of glial cells in the central nervous system (CNS), and are actively involved in neuropathological responses. Astrogliosis is a typical inflammatory
reactive response of astrocytes after SCI. After prolonged injury, astrocytes migrate to lesion site and form around the injury cavity. On one hand, it restrict inflammatory responses [27], on the other hand, it prevent axonal regrowth by secreting chondroitin sulfate proteoglycans [28]. The expression of GFAP indicates activation of astrocytes after neurological insults. In the present study, marked activation of astrocytes was observed in the lesion site of spinal cord, which was revealed by immunostaining for GFAP, and puerarin treatment effectively abolished astrocytes activation. This may contribute to the neuron regeneration and improve the locomotor function of rats.

Among all the mechanisms of secondary injury of SCI, inflammation is the most important because it can directly or indirectly control the sequelae. The activation of resident microglial cells is closely related to the inflammatory responses to the injury. Activated microglia has been found in the spinal cord between 12 and 24 hours post-injury, the two peaks appeared at 4 – 8 days and 60 days post-injury, respectively [29-31]. In SCI, microglia secret pro-inflammatory cytokines, induce astrocyte activation and promote gliosis formation, damage oligodendrocytes that are responsible for the myelination of multiple axons, and induce axonal dieback [29, 32-35]. Inhibition of microglia has been shown to improve recovery of locomotive function in SCI rats [36]. In the present study, the activation of microglia was detected using OX-42 immunostaining. Treatment with puerarin attenuates microgliosis and pro-inflammatory cytokines secretion in injured spinal cord, which suggests that the effect of puerarin on the recovery of SCI rats may be associated with the microglia inhibition and anti-inflammation.

Apoptosis is another prominent character in the spinal cord after SCI. Apoptosis of neurons and oligodendrocytes may be associated with the paralysis of SCI patients [37, 38]. In rodents with SCI, caspase activation and apoptosis were also observed in neurons and oligodendrocytes [39, 40]. In the present study, puerarin significantly upregulated expression of anti-apoptotic protein Bcl-2 and downregulated expressions of pro-apoptotic proteins Bax and cleaved-caspase 3. Decreased number of TUNEL-positive cells evidenced the attenuated apoptosis in puerarin-treated rats. The anti-apoptotic effect of puerarin we observed here is consistent with previous studies which were focused on other diseases [41-43] and it may promote the recovery of rats from SCI.

Activation of the PI3K/Akt signaling pathway is implicated in various mechanisms of neuroprotection including anti-oxidant, anti-apoptosis, and axonal outgrowth promotion [44, 45]. In SCI model, activation of the PI3K/Akt has also been demonstrated to mediate recovery of motor function [46]. Numerous bioactivities of puerarin were mediated through regulating the PI3K/Akt signaling pathway, including protection of pancreatic β-cell [47], prevention of osteoporosis [48], attenuation of hepatic fibrosis [49], alleviation of cardiac hypertrophy [50], as well as neuroprotection [51-53]. Interestingly, the majority of studies demonstrated that puerarin exerted bioactivities via activating the PI3K/Akt pathway, whereas some of the studies reported that the therapeutic effects of puerarin were mediated via blocking PI3K/Akt [49, 50]. These evidences suggest that puerarin may be a bidirectional regulatory factor of PI3K/Akt. In the present study, the PI3K/Akt signaling pathway was suppressed in the lesion site of spinal cord, and puerarin treatment restored the activity of this signaling pathway, which indicates that the neuroprotective effect of puerarin on SCI rats may mediated by the PI3K/Akt signaling pathway.

Conclusion

In conclusion, the present study provides evidence that puerarin has neuroprotective effect against SCI in rats. This effect may be associated with the activation of the PI3K/Akt signaling pathway. This study provides a candidate agent for the development of therapeutic strategy of SCI.
Disclosure Statement

The authors declare no conflicts of interest.

References

Zhang et al. The Neuroprotective Effect of Puerarin


