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Polydatin attenuates spinal cord injury in rats by inhibiting oxidative stress and microglia apoptosis via Nrf2/HO-1 pathway

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ABSTRACT

Aims: Spinal cord injury (SCI) is one of the most devastating central lesions, resulting in serious locomotor deficit. Polydatin is a glucoside of resveratrol with proven anti-cardiovascular, anti-inflammatory and anti-oxidative properties. The main purpose of this study was to investigate whether polydatin could alleviate SCI in rats and explore the underlying mechanisms.

Materials and methods: SCI rats induced by a weight-drop device were treated with intraperitoneal injection of 20 or 40 mg/kg polydatin. Then the locomotor function of SCI rats was evaluated by the Basso, Beattie and Bresnahan locomotor rating scale, spinal cord edema was measured by the wet/dry weight method, oxidative stress markers were detected by commercial kits and cell apoptosis status was measured by TUNEL staining. In addition, reactive oxygen species (ROS) generation, lactate dehydrogenase (LDH) production and apoptosis status were detected in murine microglia BV2 cells treated with 100 ng/ml lipopolysaccharides (LPS) and 4.0 μM polydatin. The expression of apoptosis-related proteins involved in nuclear factor E2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway was measured by western blot.

Key findings: Our data showed that polydatin treatment improved locomotor performance of SCI rats, as well as reduced oxidative stress and inhibited apoptosis by enhancing Nrf2/HO-1 signaling. In addition, polydatin was found to up-regulate Nrf2 activity and the inhibitory effects of polydatin on oxidative stress and apoptosis in LPS-stimulated BV2 microglia was neutralized by silencing Nrf2 using specific siRNA.

Significance: We demonstrate that polydatin may protect the spinal cord from SCI by suppression of oxidative stress and apoptosis via improving Nrf2/HO-1 signaling in microglia.

1. Introduction

Spinal cord injury (SCI) is a spinal nerve dysfunction caused by trauma. The majority of SCI-induced spinal cord impairments, accompanied by physiological, biochemical, and structural abnormities, are destructive and irreversible [1]. It is estimated that around 2.5 million people live with SCI and > 130,000 new reports come out each year in the worldwide [1,2]. Although the accurate statistical data on SCI cases in China is unavailable, the situation is serious in regard of the huge population. There is no FDA-approved pharmacotherapy for SCI currently, despite the development of various therapeutic methods for the treatment of SCI. Among current therapeutics, methylprednisolone is the common treatment for SCI patients in adulthood, whereas this therapy is controversial due to its limited efficacy and side effects [3,4]. Therefore, it is pressing to develop efficient therapeutics for SCI and explore the underlying mechanisms.

Recent studies showed that multiple types of cells in the central nervous system (CNS) participated in the development of lesions following SCI. Microglia is one of the main immune effector cells to maintain CNS homeostasis [5–7]. Under normal conditions, microglia are responsible for shaping neuronal synapses and enhancing synaptic transmissions. Nevertheless, microglia immediately take part in phagocytosis, elimination of causal agents, as well as the release of diversified pro-inflammatory cytokines in the case of injury [8,9]. It is well described that SCI contains two injury phases: primary and secondary. The primary injury is the crucial element that directly causes tissue damages leading to irreversible necrotic cell loss at the lesion site [10]. In contrast, the secondary injury cannot be ignored, since it participates in a series of complex pathological changes characterized by abundant recruited microglia, excessive apoptosis as well as

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Abbreviations

ARE	anti-oxidant response element
CNS	central nervous system
GSH	glutathione
HO-1	heme oxygenase-1
LPS	lipopolysaccharides
MDA	malondialdehyde
Nrf2	nuclear factor E2-related factor 2
ROS	reactive oxygen species
SCI	spinal cord injury
SOD	superoxide dismutase

anabatic oxidative stress and inflammatory response [11,12]. In these processes, aggravated oxidative stress in microglia not only affects the synaptic repair function but also promotes apoptosis that further triggers the secondary spinal cord injury. Herein, we speculate that suppressing oxidative stress may be a potent strategy for therapeutic intervention of SCI, thereby inhibiting apoptosis in microglia.

Nuclear factor E2-related factor 2 (Nrf2), belonging to the cap 'n' collar subfamily of basic region-leucine zipper transcription factors, is responsible for regulating oxidative stress-related molecules, such as superoxide dismutase (SOD), glutathione (GSH) and reactive oxygen species (ROS) [13–15]. The activity of Nrf2 is negatively regulated by the cytoplasmic Keap1. Once cells are exposed to oxidative stress or chemopreventive factors, Nrf2 is dissociated from Keap1, translocated into nucleus, and thereby regulating the transcription of anti-oxidant genes [16,17]. Recent studies found that Nrf2 activation in neurons or asctrocytes ameliorated spinal cord ischemia-reperfusion injury by promoting neuronal anti-oxidant, anti-apoptotic and survival abilities [18,19]. Likewise, the neurologic deficits in the spinal cord tissues were exacerbated after SCI surgery in Nrf2 knockout mice [20]. Hence, it is possible to restrain oxidative stress and subsequently block apoptotic cascades by activating Nrf2 pathway in damaged spinal cord.

Polydatin, mainly found in the roots of *Polygonum cuspidatum*, is a glucoside of resveratrol [21,22]. Recent studies have showed that polydatin possesses various pharmacological activities, including anti-cardiovascular, anti-inflammatory and anti-oxidative effects [23,24]. Moreover, a recent experiment indicated that polydatin treatment attenuated d-galactose-induced liver and brain impairments by hindering inflammatory response, oxidative stress and apoptosis in mice [25]. Although the protective properties of polydatin have been well demonstrated, few studies focus on its effects on SCI and the associated mechanism. Thus, we aim to investigate the effects of polydatin on oxidative stress, apoptosis and Nrf2 pathway in SCI rats and explore the underlying mechanism in this study.

2. Materials and methods

2.1. Spinal cord injury and post-surgical schedule

Adult male Sprague-Dawley rats weighting 220–250 g were purchased from Changsheng biotechnology Co., Ltd. (License number: SCXK (Liaoning, China) 2015-0001) for the study. All the animal care, surgical procedure and post-operative intervention were performed in accordance with the Guide for Care and Use of Laboratory Animals. All laboratory procedures were approved by Shengjing Hospital of China Medical University.

Rats were randomly divided into 4 groups: (1) Sham; (2) SCI; (3) SCI + 20 mg/kg polydatin (Polydatin-L); (4) SCI + 40 mg/kg polydatin (Polydatin-H). After being anesthetized with 30 mg/kg pentobarbital sodium solution, the rat dorsal surface was shaved and sterilized. The skin was longitudinally dissected and the laminectomy was conducted at the T8 level exposing the spinal cord beneath. Afterwards, a self-made impactor weighting 10 g was vertically dropped from a height of 5 cm onto the exposed surface of the spinal cord, resulting in a weight-drop injury. Rats in the control sham group only received T8 laminectomy without the weight-drop impact. Afterwards, the incision was closed and all the post-operative care followed the procedures of the previous description [26]. Rats were subjected to a single intraperitoneal injection of polydatin (20, 40 mg/kg, Aladdin, No: P109978) or equal volume of saline 30 min post-surgery according to previous studies [27,28].

For each group, eight rats were used for detecting locomotor function, eight rats for determining the spinal cord edema, eight rats for histopathological and immunohistochemical analysis, eight rats for evaluating physiological changes and six rats for western blot.

2.2. Evaluation of locomotor function of SCI rats

Based on the Basso, Beattie and Bresnahan (BBB) locomotor rating scale, the locomotor ability of rats was assessed at day 1, 4, 7, 10 and 14 post-surgery. The BBB scoring system ranged from 0 to 21, score 21 represented the normal movement and the lower score suggested the damages to locomotor ability [29].

2.3. Detection of spinal cord edema in SCI rats

Rats were anesthetized with 100 mg/kg pentobarbital sodium solution 24 h after SCI surgery and the water contents of spinal cord were measured as previously reported [30]. Simply, the separated spinal cord was weighed on an electronic balance to obtain the wet weight and the corresponding dry weight was measured after being baked at 80 °C for 48 h. The ratio of the wet weight to dry weight of the spinal cord tissues indicated the degree of spinal cord edema.

2.4. Assay of oxidative stress markers in spinal cord of SCI rats

The isolated spinal cord tissue was homogenized with phosphate buffer saline (PBS) and centrifuged at 421 ×*g* for 10 min. The supernatant was then separated to determine the levels of superoxide dismutase (SOD) and malondialdehyde (MDA) according to the manufacturer's instruction (Jiancheng Bioengineering Institute, China).

2.5. Histological analysis and TUNEL staining

Rats were perfused 24 h after SCI surgery. The spinal cord tissues were carefully harvested, fixed in 4% paraformaldehyde, and then embedded and dissected in paraffin blocks. For the histological analysis, the spinal cord slides were stained with hematoxylin and eosin, whereafter, the microstructural changes in the spinal cord were observed under a light microscopy at 200× and 400× magnification. Terminal deoxynucleotidyl transferase-mediated dUTP (2-deoxyuridine 5-triphosphate) nick-end labeling (TUNEL) assay was conducted to detect cell apoptosis in spinal cord tissues using the In Situ Cell Death Detection Kit (Roche, Switzerland). Cell apoptosis was observed under a light microscopy at 400× magnification.

2.6. Cell culture and treatment

Murine microglia BV2 cells were cultured in DMEM medium (Gibco, USA) containing 10% fetal bovine serum (FBS, BI, USA) at 37 °C with 5% CO_2 in a humidified incubator. BV2 microglia were transfected with Nrf2-specific small interfering RNA (siRNA) or control siRNA, and then incubated in fresh medium for 20 h. Afterwards, lipopolysaccharides (LPS) at the final concentration of 100 ng/ml was added to DMEM medium and the BV2 cells were treated with 4.0 μM polydatin for 24 h.

The sense sequences:
Nrf2 siRNA sense 5′-GCCCAUUGAUGUUUCUGAUTT-3′
antisense 5′-AUCAGAAACAUCAAUGGGCTT-3′
Control siRNA sense 5′-UUCUCCGAACGUGUCACGUTT-3′
antisense 5′-ACGUGACACGUUCGGAGAATT-3′

2.7. ROS generation

The levels of intracellular ROS in murine microglia BV2 cells were detected by specific fluorescent probe dye DCFH-DA according to the instructions of ROS detection kit (Jiancheng Bioengineering Institute). The fluorescence intensity of ROS at excitation and emission wavelengths of 500 and 525 nm was read by a fluorescence microplate reader (TECAN, Switzerland).

2.8. LDH production

After incubation with 4.0 μM polydatin for 24 h, the BV2 microglia were harvested and lysed in an ultrasonic cell shredder, and then centrifuged at 600 ×*g* for 10 min to obtain the supernatants. The protein concentrations of the supernatant were assessed by the BCA protein quantification kit (Beyotime Institute of Biotechnology, China). The levels of lactate dehydrogenase (LDH) in BV2 microglia were quantified by the specialized kit (Jiancheng Bioengineering Institute). The data of LDH were shown as U per gram protein (U/g prot).

2.9. Hoechst staining

After LPS and polydatin treatment, the BV2 cells were washed twice with cold PBS before being fixed, and incubated with Hoechst 33258 (Beyotime Institute of Biotechnology) for 5 min. The BV2 microglia were observed and photographed under a fluorescence microscope at 400× magnification. The photos were randomly obtained. Both apoptotic and total cell numbers were counted in three random fields, the ratio of apoptotic cell number to total cell number represented the apoptotic levels.

2.10. Western blotting analysis

According to the experimental procedure of western blotting, the nuclear and total proteins were separately extracted from spinal cord tissues and stimulated BV2 cells, and quantified by the BCA kit (Beyotime Institute of Biotechnology). After the separation of sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes, which were subsequently incubated with the diluted primary antibodies overnight at 4 °C. Then, the membranes were subjected to horseradish peroxidase (HRP) labelled secondary antibody (Beyotime Institute of Biotechnology) for 45 min at 37 °C. The protein bands were finally visualized by chemiluminescence (ECL) kits (Beyotime Institute of Biotechnology) and the corresponding integrated intensity was calculated by Gel-Pro-Analyzer.

The primary antibodies were as follows: Bax (BOSTER, dilution 1:500), Bcl-2 (BOSTER, dilution 1:500), cleaved-caspase-3 (Cell Signaling Technology, dilution 1:500), Nrf-2 (Cell Signaling Technology, dilution 1:1000), HO-1 (Abcam, dilution 1:1000), β-actin (KeyGen, dilution 1:5000) and Histone H3 (Bioss, dilution 1:5000).

2.11. Statistical analysis

Data were expressed as mean ± S.D. and analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for intergroup comparisons. The value of $p < 0.05$ was deemed to be statistically significant.

3. Results

3.1. Polydatin improved the locomotor function of SCI rats

To explore whether polydatin could enhance motor function of SCI rats, the behavioral performance graded by the BBB score system was observed at 1, 4, 7, 10 and 14 day following SCI surgery. As shown in Fig. 1, rats only exposed to the T8 laminectomy did not suffer locomotor impairment, while the SCI rats in other three groups showed severe locomotor deficit and their locomotor ability was gradually recovered throughout the experiment. Additionally, the SCI rats treated with polydatin (20, 40 mg/kg, $p < 0.05$) had higher scores than untreated SCI rats and the significant difference in BBB scores between polydatin-treated and untreated SCI rats was observed since the fourth day post-surgery. The data indicated that polydatin treatment relieved the spinal cord impairment and improved the mobility of SCI rats.

3.2. Polydatin alleviated spinal cord edema of SCI rats

SCI-induced edema that is closely related to the locomotor performance covers from the center of the lesion site to the edge, additionally, the hemorrhagic and necrotic tissues usually present in the center of the lesion. We detected the effects of polydatin treatment on spinal cord

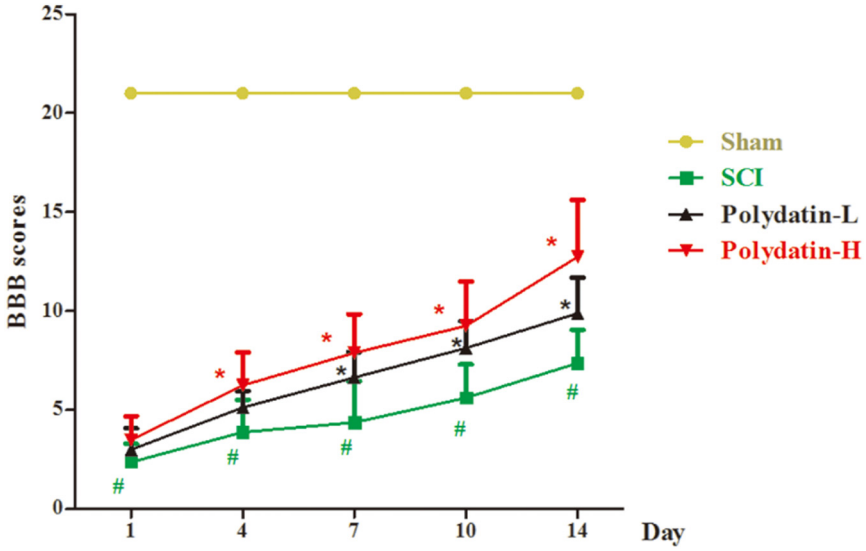

Fig. 1. Polydatin improved the locomotor function of SCI rats. BBB scores were recorded at day 1, 4, 7, 10 and 14 after SCI surgery. SCI: spinal cord injury group; Polydatin-L: SCI + 20 mg/kg polydatin group; Polydatin-H: SCI + 40 mg/kg polydatin group. Data were reported as mean ± S.D. (n = 8), # $p < 0.05$ vs. Sham group and * $p < 0.05$ vs. SCI group. Data were analyzed with one-way ANOVA test followed by Newman–Keuls test.

edema of SCI rats. As described in Fig. 2A, we found that the wet/dry weight ratio of the spinal cord tissues was observably increased after SCI surgery, which was reversed by polydatin administration (20, 40 mg/kg, $p < 0.05$). Additionally, histopathological changes in spinal cord tissues were examined by H&E staining. The congestion, edema and structural damages in the spinal cord of SCI rats were showed to be notably relieved after polydatin treatment (20, 40 mg/kg, $p < 0.05$, Fig. 2B).

3.3. Polydatin suppressed oxidative damage in spinal cord of SCI rats

To evaluate the effects of polydatin on oxidative stress in spinal cord tissues of SCI rats, SOD activity and MDA content were assessed. As shown in Fig. 3A and B, SOD activity was decreased, while MDA level was increased in SCI model rats compared to those in the control sham group ($p < 0.05$). Whereas, the single injection of polydatin (20, 40 mg/kg, $p < 0.05$) up-regulated SOD activity and down-regulated MDA level in spinal cord tissues, suggesting that the SCI-induced oxidative damage was attenuated by polydatin treatment.

3.4. Polydatin inhibited apoptosis in spinal cord of SCI rats

As described in Fig. 4A, TUNEL staining showed that SCI surgery greatly increased TUNEL-positive cells in the spinal cord of rats compared to the control sham group ($p < 0.05$). These changes were restored by polydatin treatment with significantly less positive staining in spinal cord tissues of SCI rats than that of untreated SCI rats (20, 40 mg/kg, $p < 0.05$). Consistently, the expressions of cleaved caspase-3 and Bax were greatly increased and Bcl-2 was visibly decreased in spinal cord tissues of SCI model rats ($p < 0.05$, Fig. 4B–D). Whereas the administration of polydatin (20, 40 mg/kg, $p < 0.05$) almost returned these apoptosis-associated proteins to the normal levels. All these data verified that polydatin attenuated apoptosis in the spinal cord of SCI rats.

3.5. Polydatin improved Nrf2/HO-1 activation in spinal cord of SCI rats

To investigate the possible mechanism of polydatin on SCI, we assessed the expressions of Nrf2 and HO-1 in spinal cord tissues of SCI rats. As described in Fig. 5A and B, the levels of nuclear Nrf2 and cytoplasmic HO-1 were both slightly increased in spinal cord tissues of SCI rats compared to that in the sham group. The slight increase in Nrf2 expression was probably due to the stress response to severe stimulation. Furthermore, nuclear Nrf2 and cytoplasmic HO-1 levels were found to be significantly elevated with polydatin treatment (20, 40 mg/kg, $p < 0.05$), indicating that polydatin might reduce oxidative stress via Nrf2/HO-1 pathway in spinal cord tissues.

3.6. Polydatin restored the anti-oxidant system in BV2 cells

Because Nrf2 expression was found to be significantly increased in the spinal cord after the administration of polydatin, we inferred that Nrf2 might be the latent target of polydatin. Hence, the effects of polydatin in LPS-treated BV2 cells with Nrf2 knockdown were examined in the subsequent experiments. As described in Fig. 6A, the expressions of Nrf2 were notably diminished in BV2 cells transfected

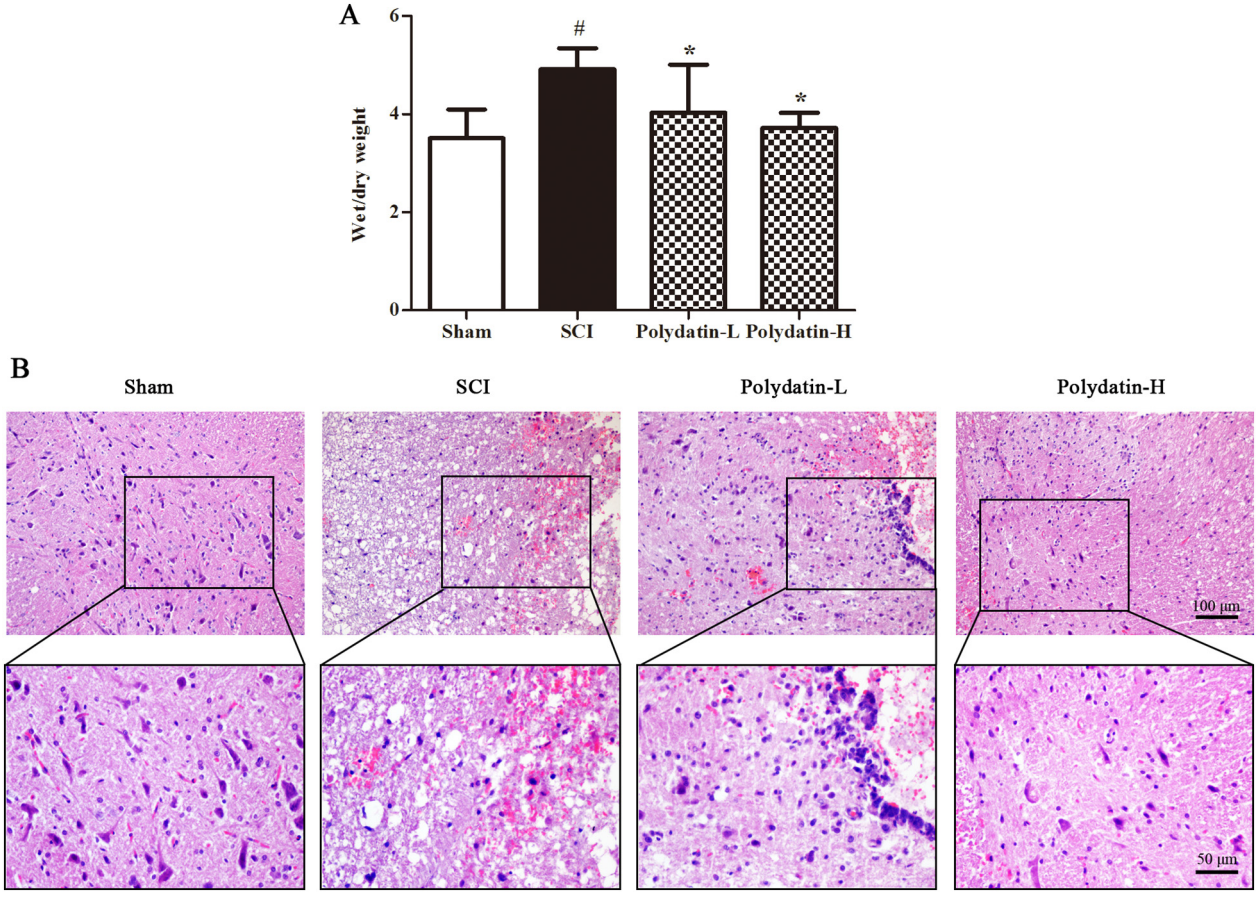

Fig. 2. Polydatin alleviated spinal cord edema of SCI rats. (A) Ratio of wet to dry spinal cord weight. (B) Representative images of H&E staining at 200× and 400× magnification in spinal cord tissues. SCI: spinal cord injury group; Polydatin-L: SCI + 20 mg/kg polydatin group; Polydatin-H: SCI + 40 mg/kg polydatin group. Data were reported as mean ± S.D. (n = 8), $^{\#}p < 0.05$ vs. Sham group and $^{*}p < 0.05$ vs. SCI group. Data were analyzed with one-way ANOVA test followed by Newman–Keuls test.

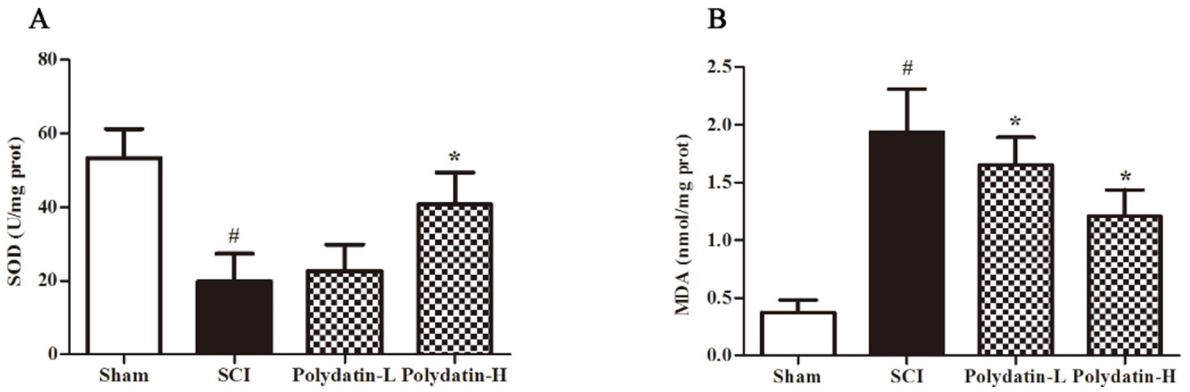

Fig. 3. Polydatin suppressed oxidative damage in spinal cord of SCI rats. (A) The activities of SOD in spinal cord tissues. (B) The concentrations of MDA in spinal cord tissues. SCI: spinal cord injury group; Polydatin-L: SCI + 20 mg/kg polydatin group; Polydatin-H: SCI + 40 mg/kg polydatin group. Data were reported as mean ± S.D. (n = 8), $^{\#}p < 0.05$ vs. Sham group and $^{*}p < 0.05$ vs. SCI group. Data were analyzed with one-way ANOVA test followed by Newman–Keuls test.

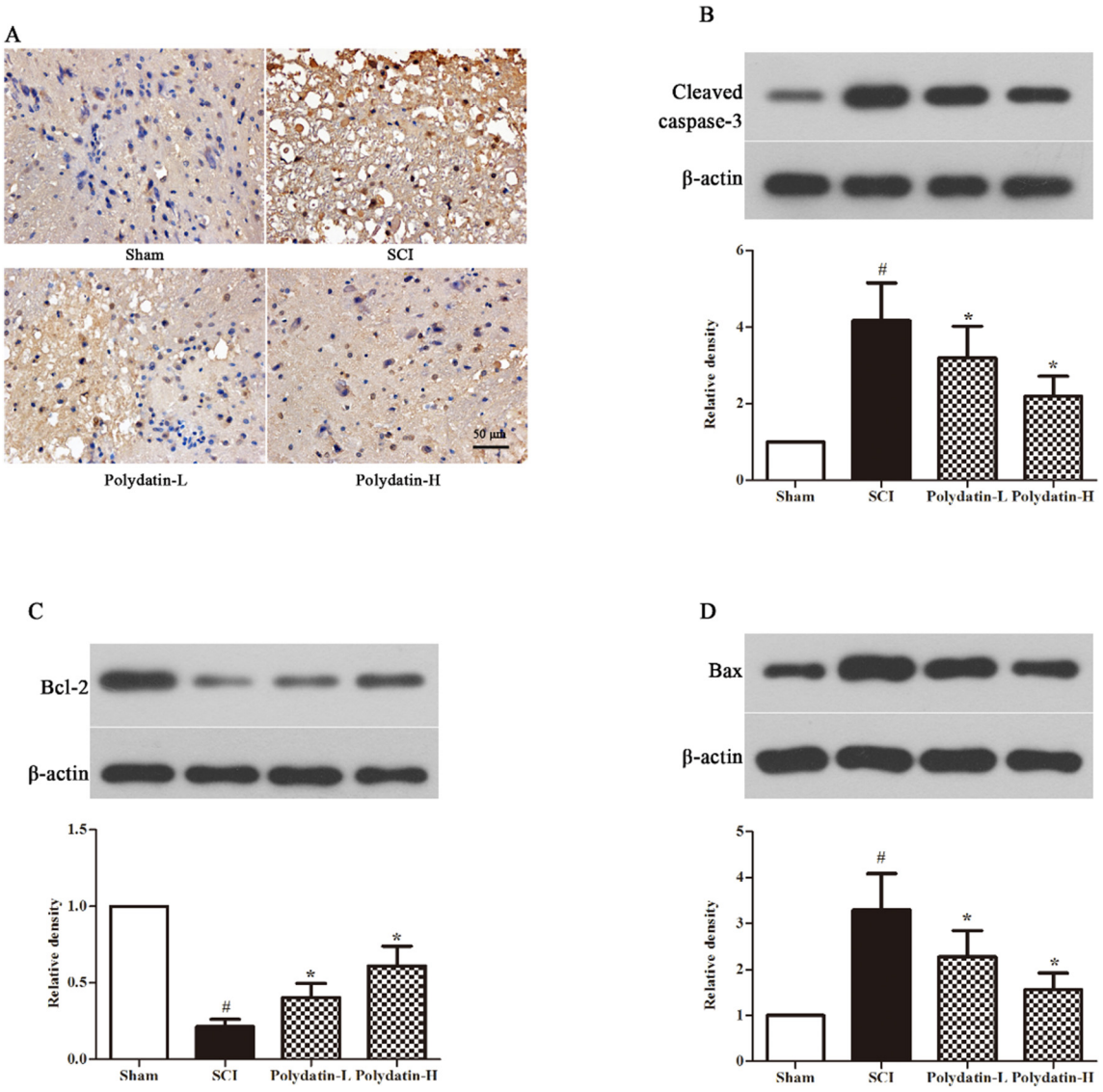

Fig. 4. Polydatin inhibited apoptosis in spinal cord of SCI rats. (A) Apoptosis in spinal cord tissues was detected by TUNEL staining and observed at 400× magnification. (B) Western blotting for cleaved caspase-3 in spinal cord tissues. (C) Western blotting for Bcl-2 in spinal cord tissues. (D) Western blotting for Bax in spinal cord tissues. SCI: spinal cord injury group; Polydatin-L: SCI + 20 mg/kg polydatin group; Polydatin-H: SCI + 40 mg/kg polydatin group. Data were reported as mean ± S.D. (n = 6/8), $^{\#}p < 0.05$ vs. Sham group and $^{*}p < 0.05$ vs. SCI group. Data were analyzed with one-way ANOVA test followed by Newman–Keuls test.

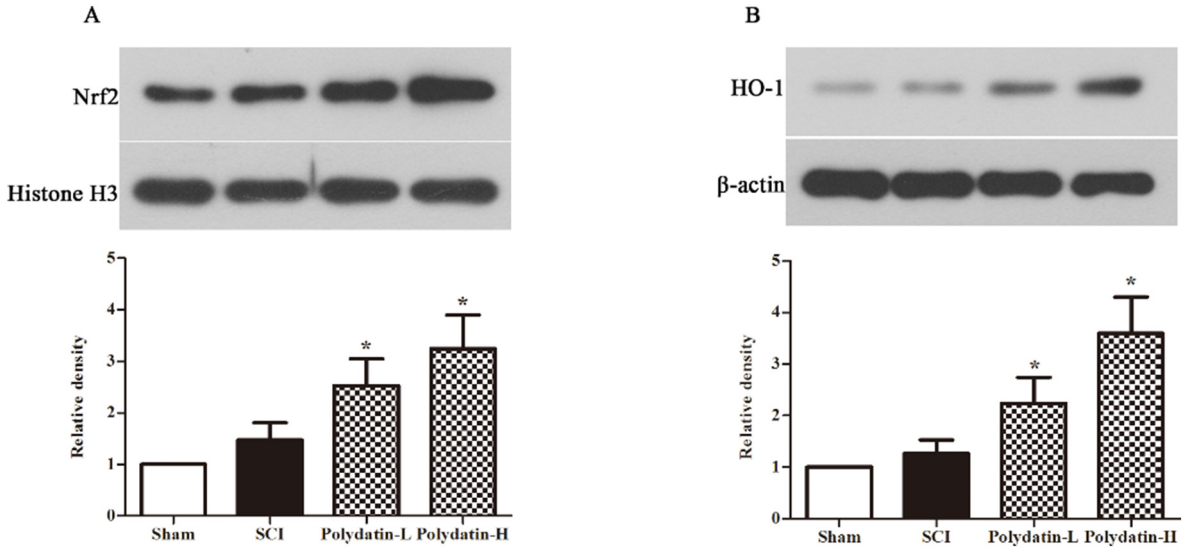

Fig. 5. Polydatin improved Nrf2/HO-1 activation in spinal cord of SCI rats. (A) Western blotting for nuclear Nrf2 in spinal cord tissues. (B) Western blotting for HO-1 in spinal cord tissues. SCI: spinal cord injury group; Polydatin-L: SCI + 20 mg/kg polydatin group; Polydatin-H: SCI + 40 mg/kg polydatin group. Data were reported as mean ± S.D. (n = 6), $^{\#}p < 0.05$ vs. Sham group and $^{*}p < 0.05$ vs. SCI group. Data were analyzed with one-way ANOVA test followed by Newman–Keuls test.

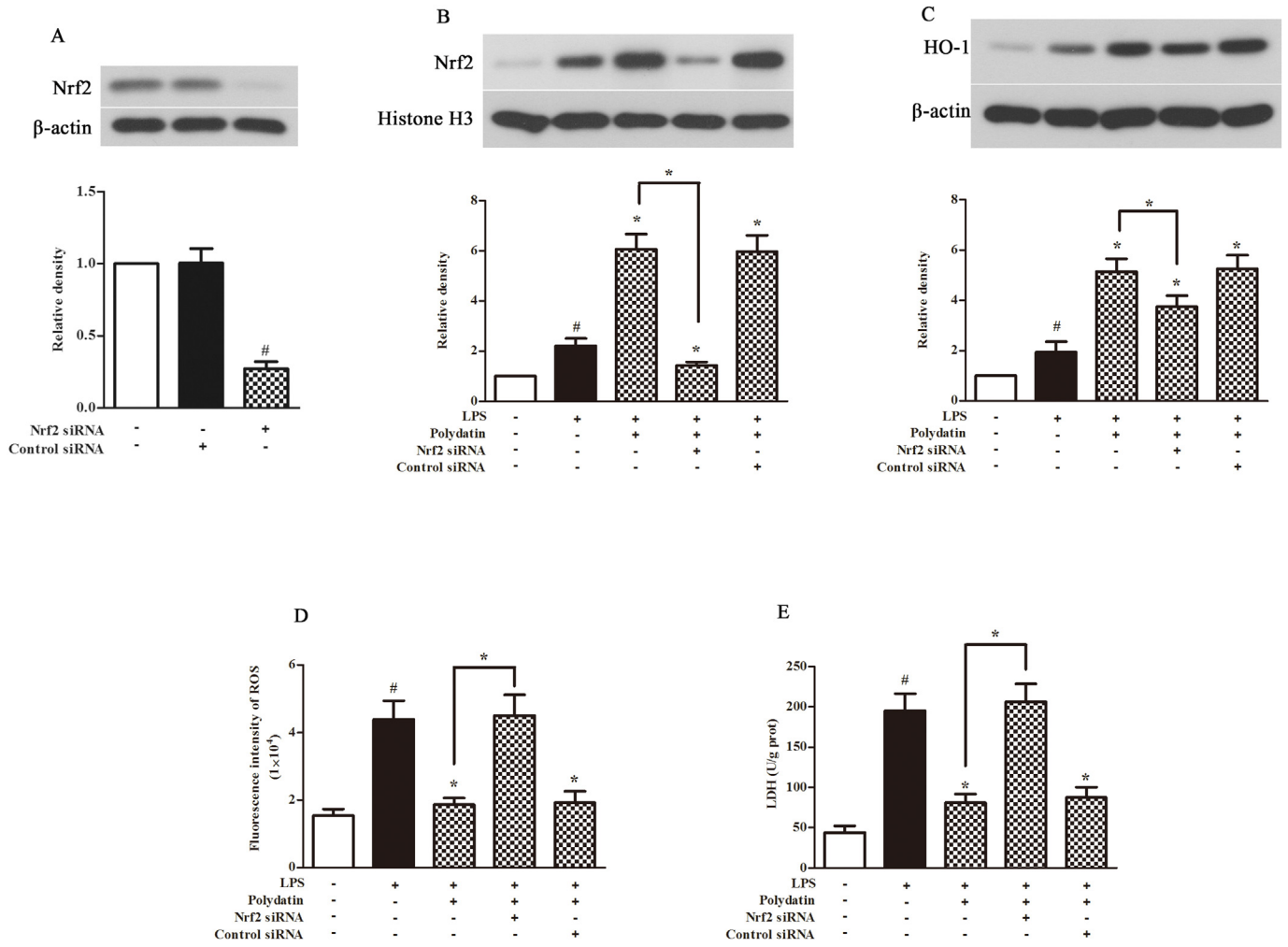

Fig. 6. Polydatin restored the anti-oxidant system in BV2 cells. (A) Western blotting for total Nrf2 in BV2 cells with Nrf2 specific siRNA treatment. (B) Western blotting for nuclear Nrf2 in LPS-induced BV2 cells. (C) Western blotting for HO-1 in LPS-induced BV2 cells. (D) The generation of ROS in LPS-induced BV2 cells. (E) The production of LDH in LPS-induced BV2 cells. Data were reported as mean ± S.D. (n = 3), $^{\#}p < 0.05$ vs. blank and $^{*}p < 0.05$ vs. control or indicated group. Data were analyzed with one-way ANOVA test followed by Newman–Keuls test.

with Nrf2 specific siRNA ($p < 0.05$), indicating that Nrf2 was successfully knocked down in BV2 cells. Furthermore, as shown in Fig. 6B–E, polydatin (4 μM, $p < 0.05$) markedly inhibited LPS-induced intracellular ROS and LDH over-production accompanied by the improved Nrf2/HO-1 pathway, whereas the anti-oxidative effect of polydatin could be offset by Nrf2 knockdown. These results suggested that polydatin might perform inhibitory effects on oxidative stress by activating Nrf2/HO-1 pathway.

3.7. Polydatin prevented apoptosis in BV2 cells

Apoptosis in LPS-treated BV2 cells was verified by Hoechst 33258 staining presented in Fig. 7A. The results showed that LPS treatment significantly increased apoptosis in BV2 cells, which could be down-regulated by the intervention of polydatin (4 μM, $p < 0.05$). In addition, the reductions of cleaved caspase-3 and Bax, along with elevated Bcl-2 level were detected in LPS-induced BV2 cells with polydatin treatment (4 μM, $p < 0.05$) (Fig. 7B–D). However, knockdown of Nrf2 diminished the anti-apoptotic effect of polydatin on LPS-treated BV2 cells. The results demonstrated that the effects of polydatin on apoptosis reduction were closely related to Nrf2 expression.

4. Discussion

Increasing cases have proved that SCI mostly occurs after strong shock or trauma and could cause unpredictable and destructive complications, including locomotor deficits, paraplegia and even quadriplegia [31]. In our study, we investigated the beneficial effects of polydatin on SCI and explored the possible mechanisms by using a rat model of SCI. We found that polydatin treatment improved the motor ability of SCI rats, attenuated oxidative stress and prevented apoptosis in spinal cord tissues of SCI rats. In addition, polydatin administration up-regulated the expression of Nrf2 and downstream HO-1, diminished the production of ROS and LDH, and thereby inhibiting the apoptosis of microglia. Hence, our results manifested that polydatin could be effective in the protection of spinal cord against SCI by ameliorating microglia apoptosis via Nrf2/HO-1 pathway in rats.

The SCI rat model was developed by a weight-drop contusion method, which was well admitted to evaluate the effectiveness of treatment to SCI and its associated mechanisms [32]. In our study, the BBB scoring system was used to assess the locomotion of SCI rats and we found that SCI surgery sharply damaged the locomotor function of rats, which was in accordance with the previous research [33]. The behavioral performance of SCI rats was improved by the administration

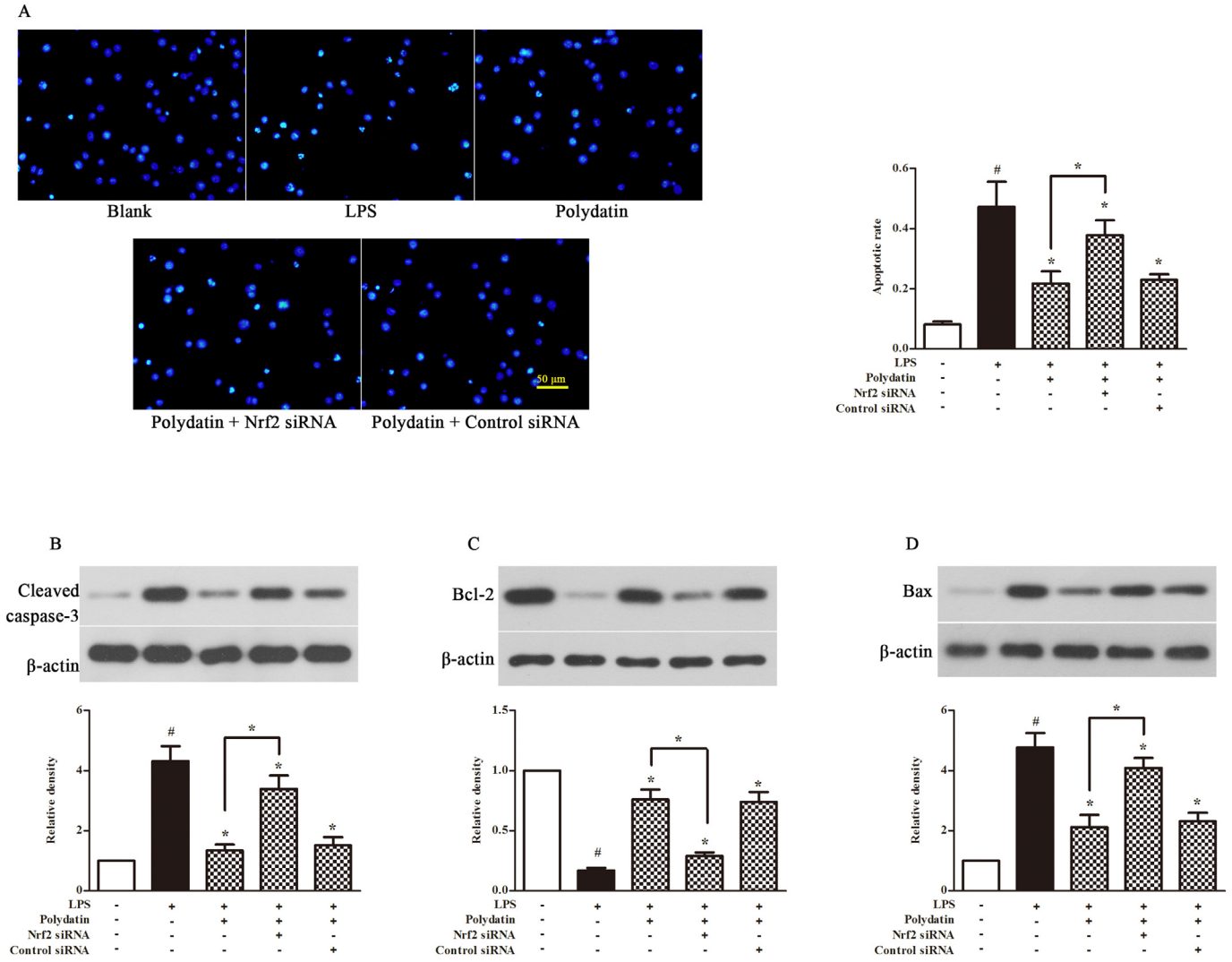

Fig. 7. Polydatin prevented apoptosis in BV2 cells. (A) Representative images of Hoechst 33258 staining at 400× magnification and apoptosis rate in LPS-induced BV2 cells. (B) Western blotting for cleaved caspase-3 in LPS-induced BV2 cells. (C) Western blotting for Bcl-2 in LPS-induced BV2 cells. (D) Western blotting for Bax in LPS-induced BV2 cells. Data were reported as mean ± S.D. (n = 3), $^{\#}p < 0.05$ vs. blank and $^{*}p < 0.05$ vs. control or indicated group. Data were analyzed with one-way ANOVA test followed by Newman–Keuls test.

of polydatin as indicated by the gradually increased BBB scores, suggesting that polydatin could alleviate SCI-induced locomotor dysfunction in rats.

It is well understood that structural and functional normality of spinal cord is obligatory to maintain limb movement. A series of complicated alterations, including myelin loss, ongoing apoptosis and interrupted axonal tracts, were reported to develop at the lesion site when the structure of spinal cord was radically disrupted [34,35]. The role of aggravated oxidative stress in these pathological changes could not be ignored. Emerging evidences have demonstrated that there was a strong relationship between excessive oxidative stress and abundant apoptosis [36]. It was generally accepted that increased oxidative stress, along with enhanced apoptosis and inhibited neurogenesis, emerged at the edema areas in traumatic brain injury, cerebral ischemia and SCI models [37]. Therefore, modifying cell morphology and improving cell survival may contribute to alleviate SCI. In our study, we found that SCI surgery caused spinal cord edema, aggravated oxidative stress and apoptosis in spinal cord tissues of SCI rats. Nevertheless, the single injection of polydatin largely suppressed the occurrence of edema and reversed the up-regulation of oxidative stress and apoptosis in SCI rats. These data indicated that polydatin exerted anti-oxidant and anti-apoptotic activity in spinal cord tissues of SCI rats.

Since many signaling pathways have been showed to be involved in maintaining normal function of spinal cord, the pathway we studied was associated with the anti-oxidant activity of polydatin. The activity of Nrf2 is crucial to adjust intracellular oxidative stress status [38], and cytoplasmic Nrf2 activity is restricted by its negative regulator Keap1. Briefly, Nrf2 transfers into the nucleus after Keap1 degradation and binds to anti-oxidant response element (ARE), exerting anti-oxidation through promoting the transcription of its downstream effector molecules [39]. We observed that polydatin administration increased the expressions of nuclear Nrf2 and the downstream HO-1 in spinal cord tissues of SCI rats. Therefore, we preliminarily concluded that polydatin could reduce oxidative stress in the spinal cord by elevating Nrf2 activity; however, it was unclear whether Nrf2 was the target of polydatin. To verify the regulatory role of polydatin on Nrf2 activity, Nrf2 was knocked down using specific siRNA in BV2 microglia in the present study. Given that microglia, the central immune effector cells in CNS, are responsible for monitoring and eliminating danger signals. Once the danger signals were detected, microglia are immediately activated and recruited [40]. Recent studies have focused on the toxicity of hyperactivated microglia around the lesion site [41]. However, a large number of recruited microglia undergo apoptosis due to the altered microenvironment, which worsen the secondary spinal cord damage. Therefore, it is essential to investigate the effects of polydatin on lipopolysaccharides (LPS)-stimulated BV2 microglia. Consistent with the results in spinal cord tissues of SCI rats, Nrf2 activity was increased with polydatin treatment in LPS-stimulated BV2 cells. Additionally, polydatin administration significantly suppressed the production of ROS and LDH, and inhibited BV2 cell apoptosis by regulating apoptosis-related protein expression. The therapeutic effects of polydatin on antioxidant and anti-apoptosis were counteracted by the knockdown of Nrf2 in LPS-induced BV2 cells. Thus, our data verified that the beneficial effects of polydatin are strongly related to the increased Nrf2 activity.

In conclusion, our study showed that polydatin could improve SCI-induced locomotor dysfunction, which was attributed to its effects on anti-oxidant and anti-apoptosis via regulation of Nrf2 signaling in microglia. Therefore, we demonstrate that polydatin is effective in alleviating SCI via Nrf2/HO-1 pathway which provides an insight into therapeutics for the treatment of SCI.

Conflict of interest

The authors declared no conflict of interest.

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