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# Rutin attenuates neuroinflammation in spinal cord injury rats

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ABSTRACT

*Background:* Neuroinflammatory responses involve the activation of the interleukin (IL) -1β and IL-18. Processing and activation of the pro-inflammatory IL require NLRP3 inflammasome activation. Rutin can protect spinal cord against damage, but the potential mechanisms underlying remain unknown. Here, we investigated the molecular mechanisms of rutin-mediated neuroprotection in a rat model of spinal cord injury (SCI).

*Materials and methods:* One hundred twenty female Sprague–Dawley rats were randomly assigned to four groups: sham group, SCI group, SCI + Rutin50 group, and the SCI + Rutin100 group. The influences of rutin on inflammatory marker levels, histologic alterations, and locomotion scale were analyzed.

*Results:* SCI significantly increased the expression of the NLRP3, ASC, IL-1β, IL-18, and tumor necrosis factor-alpha. Rutin significantly reduced the levels of reactive oxygen species, malondialdehyde, NLRP3, ASC, caspase-1, IL-1β, IL-18, and tumor necrosis factor-alpha. Furthermore, rutin administration significantly attenuated histologic alteration and improved locomotion recovery.

*Conclusions:* Our data provide clear evidence that rutin attenuates tissue damage and improves locomotion recovery, and the mechanism may be related to the alleviation of inflammation and oxidative stress.



## Introduction

Traumatic spinal cord injury (SCI) is a devastating condition that leads to a progressive state of nerve degeneration with accompanying physiological, biochemical, and structural changes.[1-3] The pathophysiology of SCI consists of two major mechanisms: primary injury and secondary injury, induced by diverse pathophysiological mechanisms including inflammation and apoptosis.[4-6] Previous research has demonstrated that targeting the inflammatory response can improve nerve functional recovery in rat models of SCI.[7]

Neuroinflammatory responses include maturation and secretion of pro-inflammatory cytokines interleukin (IL)-1β and IL-18, which induce cell death.[8] The maturation and secretion of pro-IL-1β and pro-IL-18 require the activation of proteolytic enzyme caspase-1, which is mediated by the activation of nucleotide-binding domain-like receptor protein 3 (NLRP3) and subsequently the recruitment of

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apoptosis-associated speck-like protein (ASC).[9,10] The NLRP3 inflammasome, a kind of cytosolic protein signaling complex, consists of NLRP3, ASC, and caspase-1 and is assembled after endogenous "danger".[11,12] The NLRP3 inflammasome can be activated by a variety of stimulating factors including reactive oxygen species (ROS).[13] The NLRP3 inflammasome regulates the maturation and release of IL-1β and IL-18, and targeting of the NLRP3 inflammasome can exert neuroprotection in SCI rats.[8]

Rutin, a flavonoid obtained from foods and plants, is one of important compounds and has been shown to be effective in some other disease conditions involving inflammation[14] and oxidative stress.[15] For example, in rats, rutin has been shown to reduce neural damage after intracerebral hemorrhage in rats.[16] Previous study has shown that rutin can exert neuroprotective effects through enhancing of the neurotrophy in SCI rats.[17] There are other studies that show the rutin-mediated effect, which further inhibits NLRP3 inflammasome activation.[17,18] However, this mechanism of inhibition of NLRP3 inflammasome remains unknown in the case of SCI.

Based on aforementioned considerations, we investigated whether rutin could inhibit NLRP3 inflammasome activation with neuroprotection in a rat model of SCI.

## Materials and methods

### *Animal preparation and grouping*

Adult female Sprague–Dawley rats weighing 250-300 g were purchased from Beijing Haidian Thriving Experimental Animal Centre (Beijing, China). All procedures for these experiments complied with the guidelines of the Animal Ethics Committee of Hangzhou First People's Hospital (Hangzhou, China). The rats were housed in a standard animal room with a 12 h light/dark cycle.

One hundred twenty rats were randomly assigned to four equal groups via a random number table: (1) sham group, where the rats only underwent laminectomy and intraperitoneal injection with 1 mL of dimethyl sulfoxide (DMSO) immediately after SCI; (2) SCI group, where rats underwent SCI and intraperitoneal injection with 1 mL of DMSO immediately after SCI; (3) SCI + Rutin50 group, where 50 mg/kg of rutin (Sigma-Aldrich, St. Louis, MO) in 1 mL of DMSO was intraperitoneally injected immediately after SCI; (4) SCI + Rutin100 group, where 100 mg/kg of rutin in 1 mL of DMSO was intraperitoneally injected immediately after SCI. All animals in the four groups underwent an intraperitoneal injection daily for 3 d (3 times totally). The dose and timing of rutin were based on previous studies.[18]

### *Establishment of the SCI model*

SCI was induced by a model described by Farsi *et al.*[19] Rats were anesthetized with an intraperitoneal injection of 3.0 mL/kg of 10% chloral hydrate. Laminectomy was performed to expose the spinal cord at the vertebral T9-T11 segment without damage to the dura. The spinal cord at the vertebral T10 segment (spinal T9) underwent a 1-min compression with an aneurysm clip, horizontally. Rats were administered an intramuscular injections of penicillin (400,000 unit/animal/d) and buprenorphine to prevent infection and relieve pain postoperatively. In addition, rats underwent manual bladder emptying twice a day.

### *Evaluation of locomotor deficit*

Locomotion deficit was evaluated using the Basso–Beattie–Bresnahan (BBB) locomotion rating scale, as previously described.[8] The rating scale was from 0 to 21 (0 = complete paralysis, 21 = normal). The rats ($n = 5$ for each group) were assessed with this scale at days 1, 3, 7, and 14 after SCI by two independent investigators who were blinded to group assignment.

### *Histologic study*

Seventy-two hours after injury, rats ($n = 5$ in each group) were perfused with 0.9% saline and subsequently with 4% paraformaldehyde. For the histologic analyses, some paraffin spinal cord sections were stained with hematoxylin–eosin reagent. Histologic scoring was on the basis of (1) edema, (2) neutrophil infiltration, and (3) hemorrhage. The score representing severity of SCI was recorded as follows: 0, none or minor; 1, limited; 2, intermediate; 3, prominent; and 4, widespread.[20]

### *Biochemical analysis*

After the spinal cord samples at the damaged area (10 mm, $n = 5$ for each group) were removed 72 h after injury, they were immediately homogenized in phosphate-buffered saline and centrifuged at 1000 rpm for 15 min at 4°C. IL-1β, IL-18, and tumor necrosis factor-alpha (TNF-a) concentrations in the collected supernatants were determined through enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN).

### *Protein extraction and Western blot analysis*

The spinal cord samples at the damaged area (10 mm, $n = 5$ for each group) were removed 24 h after injury and stored at −80°C until further use. Specimens were homogenized in radioimmunoprecipitation assay buffer and then centrifuged at 12,000 rpm for 30 min at 4°C. Protein concentration in the supernatant was quantified via the bicinchonininc acid method. Total protein (20 μg) was separated with 10% sodium dodecyl sulfate polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were blocked with 5% skimmed milk and subsequently incubated with specific primary antibodies overnight at 4°C. Primary antibodies contained anti-NLRP3, anti-ASC, anti-caspase-1, and anti- β-actin (all 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA). After washing with phosphate buffered saline with tween, the membranes were incubated with a horseradish peroxidase–coupled secondary antibody (1:1000; Millipore). Detection of proteins was performed using the enhanced chemiluminescence kit (Thermo Scientific, Rockford, IL). Protein levels were analyzed via imaging software (Quantity One; Bio-Rad Co Ltd, Hercules, CA).