

*Full Length Research Paper*

# The effect of salidroside on inflammatory factors in rats with hemisection-induced spinal cord injury

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**The effect of salidroside on inflammatory factors in rats with hemisection-induced spinal cord injury (SCI) was studied. The Sprague-Dawley (SD) rats were divided into six groups: Sham control group, SCI model group, the methylprednisolone sodium succinate (MPSS) treatment group, the salidroside-low dosage treatment group, the salidroside-moderate dosage treatment group and the salidroside-high dosage treatment group. The C2 hemisection method was developed to establish SCI models. 24 h after operation, different dosage of salidroside treatment inhibited some inflammatory factors such as, interleukin-1 beta (IL-1 $\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ ) and Intercellular adhesion molecule 1 (ICAM-1). These results suggested that salidroside can significantly inhibit the increased inflammatory factors in acute SCI.**

**Key words:** Salidroside, spinal cord injury (SCI), inflammatory factors.

## INTRODUCTION

Salidroside (p-hydroxyphenethyl-b-D-glucoside, C<sub>14</sub>H<sub>20</sub>O<sub>7</sub>, structure shown in Figure 1) is a major active constituent in *Rhodiola crenulata*. It has been reported to possess several pharmacological properties including anti-inflammation, antioxidative, antifatigue, neuro-protective, hepatoprotective and cardioprotective effects (Diaz Lanza et al., 2001; Ma et al., 2009; Nan et al., 2003; Wang et al., 2004; Wang et al., 2009; Zhang et al., 2007).

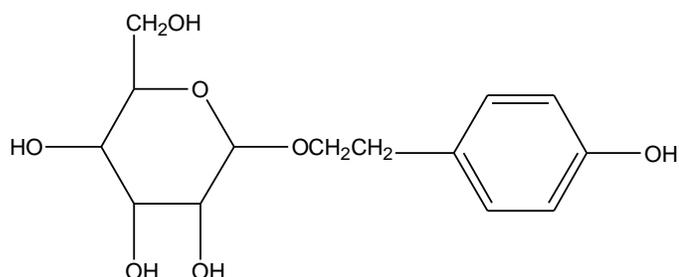
Spinal cord injury (SCI) is a complex process which causes destruction of nerve tissue (Aoki et al., 2010). Potentially toxic substances are activated and released in injured spinal cords, including free radicals, inflammatory cytokines, phospholipases and lipid peroxidases, which lead to oxidative stress damage, thereby resulting in neuronal necrosis or apoptosis and progressive secondary nerve tissue destruction (Zhou et al., 2011). There are

various agents for treatment of the SCI, such as anti-inflammatory, antioxidants, anti-apoptosis agents and myelin-associated growth inhibitors (Mallei et al., 2005; Yang and Piao, 2002; Festoff et al., 2006; Tian et al., 2007).

Methylprednisolone sodium succinate (MPSS) is the only Food and Drug Administration (FDA) approved and clinically used agent for the treatment of acute SCI (Bracken et al., 1998). It remains controversial for systemic high-dose MPSS applications in acute SCI due to the risks of serious side effects (Qian et al., 2005). Obviously, some novel agents for treating SCI are urgently needed. Previous studies have shown that salidroside plays an important role in some inflammation-mediated diseases. Therefore, we propose that salidroside may have some effects on hemisection-induced SCI in rats.

In the present work, we established rat models of SCI by C2 hemisection and attempted to investigate the effects of salidroside on hemisection-induced SCI in rats. The interleukin-1 beta (IL-1 $\beta$ ), Tumor necrosis factor alpha (TNF- $\alpha$ ) and Intercellular adhesion molecule 1 (ICAM-1) levels were evaluated.

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**Figure 1.** The chemical structure of salidroside.

## MATERIALS AND METHODS

Salidroside was obtained from China Pharmaceutical and Biological Products Inspection (Lot: 110818-201005). MPSS was purchased from Pharmacia and Upjohn Company (Belgium). IL-1 $\beta$ , TNF- $\alpha$  and ICAM-1 enzyme linked immunosorbent assay (ELISA) Kits were purchased from ADL Company (San Diego, CA, USA).

### Animals

A total of 72 healthy, female, Sprague-Dawley (SD) rats, aged 2 months, weighing 180 to 220 g, were purchased from the Laboratory Animal Centre of Lanzhou University. Animal care and experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Lanzhou University.

### Establishment of SCI models

Adult female SD rats (retired breeders, 180 to 220 g) were anesthetized with 70 mg/kg of ketamine and 7 mg/kg of xylazine solution administered intraperitoneally (i.p.). After the rats reached a surgical plane of anesthesia, the animals were prepared for surgery by shaving the dorsal surface of the neck area and scrubbing with betadine and 70% of ethyl alcohol. A midline incision of approximately 4 cm was made and the paravertebral muscles retracted. A multi-level laminectomy was performed to expose the upper cervical segments of the spinal cord. The dura was cut, and with a microblade a hemisection was made on the animal's left spinal cord caudal to the C2 dorsal roots starting at the midline and extending to the lateral most extent of the spinal cord. Sham operation group received all procedures but the lesion (Alilain et al., 2011).

### Drug treatment and sample preparation

The Sham operation group underwent laminectomy to expose the spinal cord without hemisection, and received 0.9% normal saline (NS, 2 ml/kg). The SCI model group underwent laminectomy followed by SCI, and received 0.9% NS (2 ml/kg). The MPSS treatment group underwent laminectomy followed by SCI, and was administered 100 mg/kg dose of MPSS (2 mL/kg, i.p.) 5 min after C2 hemisection. The 25, 50, and 100 mg/kg salidroside treatment groups underwent laminectomy followed by SCI, and were given a single dose of 25, 50 or 100 mg/kg of salidroside (dissolved in 0.9% NS, 2 ml/kg, i.p.) 5 min after hemisection. Blood (4 ml) was harvested by orbital plexus puncture (removing the eyeballs), for measurements of IL-1 $\beta$ , TNF- $\alpha$  and ICAM-1 levels.

### Determination of serum IL-1 $\beta$ level by ELISA

The blood samples were allowed to clot at room temperature (RT) for 1 h and at 4°C overnight, and centrifuged at 1000 rpm for 10 min at 4°C. The supernatant was collected and stored at -70°C. ELISA assay was performed by using a commercial kit according to the manufacturer's instructions. The standards and serum samples were added to the wells, 100  $\mu$ l per well. 0.01 M of phosphate buffered saline (PBS) solution was added to the control well. 50  $\mu$ l of enzyme conjugate reagent was added into each well, gently shaken for 15 s, incubated at 37°C for 1 h and rinsed. The microtiter wells were rinsed 5 times with deionized water. 50  $\mu$ l of 3,3',5,5'-tetramethylbenzidine (TMB) A and TMB B reagents were added into each well and incubated at 37°C for 15 min. The reaction was terminated by adding 50  $\mu$ l of the stop solution to each well until the original blue colour changed completely to yellow. A Model 680 Microplate Reader (Bio-Rad, Hercules, CA, USA) was used to read the absorbance at 450 nm within 20 min.

### Determination of serum TNF- $\alpha$ level by ELISA

The blood samples were allowed to clot at RT for 1 h and at 4°C overnight, and centrifuged at 1000 rpm for 10 min at 4°C. The supernatant was collected and stored at -70°C. ELISA assay was performed by using a commercial kit according to the manufacturer's instructions. The standards and tested serum samples were added to the wells, 100  $\mu$ l per well. 0.01 M of PBS solution was added to the control well. 50  $\mu$ l of enzyme conjugate reagent was added into each well, gently shaken for 15 s, incubated at 37°C for 1 h and rinsed. The microtiter wells were rinsed 5 times with deionized water. 50  $\mu$ l of TMB A and TMB B reagents were added into each well and incubated at 37°C for 15 min. The reaction was terminated by adding 50  $\mu$ l of the stop solution to each well until the original blue colour changed completely to yellow. A Model 680 Microplate Reader was used to read the absorbance at 450 nm within 20 min.

### Determination of serum ICAM-1 level by ELISA

The blood samples were allowed to clot at RT for 1 h and at 4°C overnight, and centrifuged at 1000 rpm for 10 min at 4°C. The supernatant was collected and stored at -70°C. ELISA assay was performed by using a commercial kit according to the manufacturer's instructions. The standards and tested serum samples were added to the wells, 100  $\mu$ l per well. 0.01 M of PBS solution was added to the control well. 50  $\mu$ l of enzyme conjugate reagent was added into each well, gently shaken for 15 s, incubated at 37°C for 1 h and rinsed. The microtiter wells were rinsed 5 times with deionized water. 50  $\mu$ l of TMB A and TMB B reagents were added into each well and incubated at 37°C for 15 min. The reaction was terminated by adding 50  $\mu$ l of the stop solution to each well until the original blue colour changed completely to yellow. A Model 680 Microplate Reader was used to read the absorbance at 450 nm within 20 min.

### Statistical analysis

Data were expressed as Mean  $\pm$  SD, and analyzed by one-way analysis of variance followed by least significance difference multiple comparison or Dunnett's multiple comparison tests using SPSS16.0 software. Multiple comparison tests were used when appropriate. A *P*-value of 0.05 was considered statistically significant.

**Table 1.** Serum IL- $\beta$  level of rats among different groups.

Group	IL- $\beta$ level (n = 12, pg/ml)
Sham operation	110.34 $\pm$ 6.76
SCI model	245.62 $\pm$ 12.45 <sup>b</sup>
MPSS	142.45 $\pm$ 9.72 <sup>b,c</sup>
25 mg/kg of salidroside	143.34 $\pm$ 10.25 <sup>b,c</sup>
50 mg/kg of salidroside	129.36 $\pm$ 9.35 <sup>b,c,e</sup>
100 mg/kg of salidroside	123.33 $\pm$ 8.62 <sup>b,c,e</sup>

(VS Sham: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ ; VS SCI: <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ ; VS MPSS: <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ ).

**Table 2.** Serum TNF- $\alpha$  level of rats among different groups.

Group	TNF- $\alpha$ level (n = 12, pg/ml)
Sham operation	57.34 $\pm$ 6.76
SCI model	146.72 $\pm$ 11.32 <sup>b</sup>
MPSS	121.54 $\pm$ 8.63 <sup>b,c</sup>
25 mg/kg of salidroside	123.34 $\pm$ 9.42 <sup>b,c</sup>
50 mg/kg of salidroside	103.67 $\pm$ 8.43 <sup>b,c,e</sup>
100 mg/kg of salidroside	93.43 $\pm$ 7.92 <sup>b,c,e</sup>

(VS Sham: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ ; VS SCI: <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ ; VS MPSS: <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ ).

**Table 3.** Serum ICAM-1 level of rats among different groups.

Group	ICAM-1 level (n = 12, pg/ml)
Sham operation	15.34 $\pm$ 2.35
SCI model	36.48 $\pm$ 4.22 <sup>b</sup>
MPSS	19.46 $\pm$ 2.63 <sup>b,c</sup>
25 mg/kg of salidroside	19.34 $\pm$ 2.42 <sup>b,c</sup>
50 mg/kg of salidroside	17.65 $\pm$ 1.92 <sup>b,c,e</sup>
100 mg/kg of salidroside	16.43 $\pm$ 1.84 <sup>b,c,e</sup>

(VS Sham: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ ; VS SCI: <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ ; VS MPSS: <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ ).

## RESULTS

### Determination of serum IL-1 $\beta$ level following SCI

24 h following SCI, the serum IL-1 $\beta$  level in SCI rats significantly increased ( $P < 0.01$ ; Table 1). These changes in IL-1 $\beta$  level were significantly reversed by either salidroside or MPSS treatment ( $P < 0.05$  and  $P < 0.01$ , respectively). In particular, the effect of salidroside at the 25 mg/kg dose was equivalent to MPSS, while the effect of salidroside at 50 and 100 mg/kg dose was much higher than MPSS (Table 1).

### Determination of serum TNF- $\alpha$ activity following SCI

24 h following SCI, the serum TNF- $\alpha$  level in SCI rats

significantly increased ( $P < 0.01$ ; Table 2). These changes in TNF- $\alpha$  level were significantly reversed by either salidroside or MPSS treatment ( $P < 0.05$  and  $P < 0.01$ , respectively). In particular, the effect of salidroside at the 25 mg/kg dose was equivalent to MPSS and at 50 and 100 mg/kg doses the effect was much higher than MPSS (Table 2).

### Determination of serum ICAM-1 level following SCI

24 h following SCI, the serum ICAM-1 level in SCI rats significantly increased ( $P < 0.01$ ; Table 3). These changes in ICAM-1 level were significantly reversed by either salidroside or MPSS treatment ( $P < 0.05$  and  $P < 0.01$ , respectively). In particular, the effect of salidroside at the 25 mg/kg dose was equivalent to MPSS, while the effect of salidroside at 50 and 100 mg/kg dose were superior to MPSS (Table 3).

## DISCUSSION

As female rat possesses a short urethra, it could reduce the incidence of urinary tract infection, edema, urethral obstruction, and is beneficial for post-injury recovery. Therefore, the female rats were chosen as the subject.

Experimental models of SCI mainly include contusive injury, clip compressive injury, transection/partial section injury, ischemic and chemical injury (Robins et al., 2008). The contusive injury model is similar to the pathological characteristics seen in humans. However, the contusive injury model often varies considerably, and lateral migration leads to poor reproducibility and high rates of animal mortality (Kontogeorgakos et al., 2009). The partial section injury model can provide valuable information for studies regarding the magnitude of axon regeneration, and allow direct comparison to control tissues on the normal side. Furthermore, the survival rate of SCI animals is also improved (Bavetta et al., 1999). Therefore, the C2 hemisection injury model was used to investigate the effect of salidroside on inflammatory factors in SCI rats.

SCI can induce a significant increase in the serum levels of IL-1 $\beta$ , TNF- $\alpha$ , and ICAM-1 (Chen et al., 2008; Koopmans et al., 2009 and Oruckaptan et al., 2009). These proinflammatory cytokines could activate microglia and induce neuron and myelin damage. The therapeutic values that mitigate either IL-1 $\beta$  or TNF- $\alpha$  production or signaling have been demonstrated in other models of central nervous system diseases, including ischemia, traumatic brain injury, and SCI (Lucas et al., 2006).

Therefore, the inflammatory factors were investigated in this study. In the present study, a rat model of SCI was established by C2 hemisection. C2 hemisection SCI increased the levels of IL-1 $\beta$ , TNF- $\alpha$  and ICAM-1 in SCI rats. Salidroside was found to reduce the levels of IL-1 $\beta$ , TNF- $\alpha$  and ICAM-1, which may indicate that the protective

effect of salidroside on the injured spinal cord is likely related to its anti-inflammatory action.

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