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Neuroprotective effect of salidroside on hemisection-induced spinal cord injury in rats

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The effect of salidroside on hemisection-induced spinal cord injury (SCI) in rats was investigated. The Sprague Dawley (SD) rats were randomly divided into six groups: Sham control group, SCI model group, the methylprednisolone sodium succinate (MPSS) group, the salidroside-low dosage group, the salidroside-moderate dosage group and the salidroside-high dosage group. The SD rats were hemisected at spinal cord at the T8 vertebra to establish SCI models. 24 h after operation, different dosage of salidroside increased the superoxide anion (O^{2-}) level and superoxide dismutase (SOD) activity, and reduced malondialdehyde (MDA) content. Noticeably, salidroside at the 100 mg/kg dosage exhibited similar effects as MPSS, which has been frequently used for clinical acute SCI. These results suggested that salidroside can significantly suppress oxidation in acute SCI.

Key words: Salidroside, spinal cord injury (SCI), oxidation.

INTRODUCTION

Salidroside (p-hydroxyphenethyl-b-D-glucoside, $C_{14}H_{20}O_7$, structure is as shown in Figure 1) is one of the major active constituents in *Rhodiola Crenulata*. It has been reported to possess various pharmacological properties, including resisting antiinflammation, antioxidative, antifatigue, neuroprotective, hepatoprotective and cardioprotective effects (Diaz Lanza et al., 2001; Ma et al., 2009; Nan et al., 2003; Wang et al., 2004, 2009; Zhang et al., 2007).

Spinal cord injury (SCI) is a complex process which causes destruction of nerve tissue (Yazihan et al., 2008). 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 (Bao et al., 2006). There are also various agents for treatment of SCI, such as anti-inflammatory, antioxidants, antipoptosis agents and myelin-associated growth inhibitors (Mallei et al., 2005; Yang et al., 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). Nevertheless, 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 urgely needed. Previous studies have shown that salidroside plays an important role in free radical-mediated diseases. Therefore, we proposed that salidroside might have some effects on hemisection-induced SCI in rats.

In our current work, we established rat models of SCI

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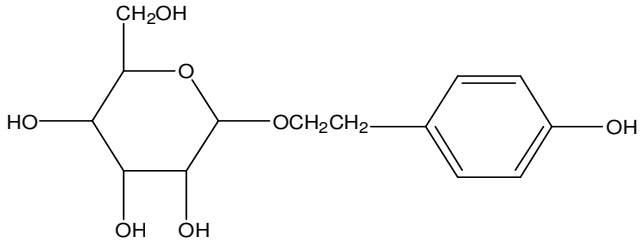

Figure 1. The chemical structure of salidroside.

by hemisecting the spinal cord at the T8 vertebra and attempted to investigate the effects of salidroside on hemisection-induced SCI in rats. The superoxide anion (O^{2-}) level, superoxide dismutase (SOD) activity and malondialdehyde (MDA) content were measured.

MATERIALS AND METHODS

Salidroside was purchased from China pharmaceutical and biological products inspection (Lot: 110818-201005). MPSS was obtained from Pharmacia and Upjohn Company, (Belgium), O^{2-} level, SOD activity and MDA content kits were purchased from Jiancheng Company (Nanjing, China).

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 The Third Military Medical University of China PLA. Animal care and experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of The Third Military Medical University of China PLA.

Establishment of SCI model

The SD rats were anesthetized with chloral hydrate, and placed in a prone position on a heating pad to maintain a constant body temperature. A longitudinal incision was made at the midline of the back and the paravertebral muscles were exposed. These muscles were dissected and thoracic level 7 to 11 (T7-11) vertebrae were exposed. A laminectomy at T7-11 was performed. Acute SCI was induced by hemisection at the T8 vertebra to a depth of 0.5 cm. The wound was sutured layer-by-layer (Kim et al., 2009). Rats in the Sham operation group experienced the same procedures, while without the hemisection, was at the T8 vertebra. Following spinal cord hemisection, the rat tails swung spastically, and the affected hind limbs exhibited flaccid paralysis after several spastic seizures. Rats were sacrificed 24 h after administration of salidroside or 0.9% normal saline (NS).

Drug treatment and sample preparation

The Sham operation group underwent laminectomy to expose the spinal cord without hemisection, and received 0.9% NS (2 ml/kg). The SCI model group underwent laminectomy followed by SCI, and received 0.9% NS (2 ml/kg). The MPSS group, positive control group underwent laminectomy followed by SCI, and was administered 100 mg/kg single dose of MPSS (2 ml/kg, i.p.) 5 min after hemisection. The 25, 50 and 100 mg/kg salidroside 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. Nine animals were used for biochemical analyses of O^{2-}, SOD and MDA in spinal cord tissues. 24 h after administration, the rats were anesthetized with chloral hydrate (0.3 g/kg), transcardially perfused with 150 ml of 0.9% NS and 200 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.2). Approximately, 2 cm of spinal cord segments between the T7 and T11 levels were obtained and cryopreserved at -70°C for measurements of O^{2-} level, SOD activity and MDA content.

Measurement of O^{2-} level, MDA content and SOD activity

O^{2-} level, SOD activity and MDA content in spinal cord tissues were measured by using commercial kits. Fresh spinal cord tissues were weighed and homogenized, 1:9 (w/v) in 0.86% (w/v) normal saline on ice cubes. The homogenates were centrifuged at 2000 rpm for 15 min at 4°C. The supernatant was respectively used for spectrophotometric assay of O^{2-} level, SOD activity (xanthine oxidase assay) and MDA content (thiobarbituric acid assay) according to the manufacturer's instructions. Total protein concentration was determined using the Coomassie blue method (Van Duijnhoven et al., 2010).

Statistical analysis

Data were expressed as mean ± standard deviation (SD), and analyzed by one-way analysis of variance followed by least significance difference multiple comparison or Dunnett's multiple comparison tests using Statistical Package for Social Sciences (SPSS) 16.0 software (SPSS, Chicago, IL, USA). Multiple comparison tests were used when appropriate. A P value of 0.05 was considered statistically significant.

RESULTS

Determination of O^{2-} level following SCI

24 h following SCI, the suppression of O^{2-} level in spinal cord tissue significantly decreased ($P<0.01$) (Table 1). These changes in O^{2-} level were significantly reversed by salidroside treatments ($P<0.05$ or $P<0.01$, respectively). In particular, the effect of salidroside at the 100 mg/kg dose was equivalent to MPSS (Table 1).

Determination of SOD activity following SCI

24 h following SCI, the suppression of SOD activity in spinal cord tissue significantly decreased ($P<0.01$) (Table 2). These changes in SOD activity were significantly reversed by salidroside treatments ($P< 0.05$ or $P<0.01$, respectively). In particular, the effect of salidroside at the 100 mg/kg dose was equivalent to MPSS (Table 2).

Determination of MDA content following SCI

24 h following SCI, the MDA content significantly increased ($P<0.01$) (Table 3). These changes in MDA

Table 1. O^{2-} level of rats among different groups.

Group	O^{2-} level (n=9, U/gprot)
Sham operation	110.96 ± 7.65
SCI model	59.63 ± 4.75[b]
MPSS	102.35 ± 6.74[c]
25 mg/kg of salidroside	77.64 ± 7.25[df]
50 mg/kg of salidroside	79.32 ± 6.35[de]
100 mg/kg of salidroside	103.43 ± 7.82[c]

VS Sham: [a]P<0.05, [b]P<0.01; VS SCI: [c]P<0.05, [d]P<0.01; VS MPSS: [e]P<0.05, [f]P<0.01.

Table 2. SOD activity of rats among different groups.

Group	SOD activity (n=9, U/gprot)
Sham operation	140.82 ± 8.74
SCI model	99.21 ± 8.62[b]
MPSS	132.35 ± 9.53[c]
25 mg/kg of salidroside	127.65 ± 9.25[df]
50 mg/kg of salidroside	129.12 ± 8.85[de]
100 mg/kg of salidroside	133.43 ± 9.42[c]

VS Sham: [a]P<0.05, [b] P<0.01; VS SCI: [c]P<0.05, [d]P<0.01; VS MPSS: [e]P<0.05, [f]P<0.01.

Table 3. MDA content of rats among different groups.

Group	MDA content(n=9, nmol/gprot)
Sham operation	5.96 ± 0.35
SCI model	15.63 ± 1.05[b]
MPSS	6.35 ± 0.14[c]
25 mg/kg of salidroside	6.14 ± 0.32[d]
50 mg/kg of salidroside	6.32 ± 0.35[d]
100 mg/kg of salidroside	7.43 ± 0.42[c]

VS Sham: [a]P<0.05, [b] P<0.01; VS SCI: [c]P<0.05, [d]P<0.01; VS MPSS: [e]P<0.05, [f]P<0.01.

content were significantly reversed by salidroside treatment (P<0.05 or P<0.01, respectively). In particular, the effect of salidroside at the 25, 50 and 100 mg/kg dose was equivalent to MPSS (Table 3).

DISCUSSION

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 patho-physiological 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 hemisection injury model was used to investigate the effect of salidroside on SCI. In addition, as a female rat possesses a short urethra, this reduces the incidence of urinary tract infection, edema and urethral obstruction, and is beneficial for post-injury recovery. Therefore, we selected female rats as the subject.

In the present study, a significant reduction of reactive oxygen species (ROS) was detected at 24 h after SCI and salidroside treatments. In SCI models, ROS are activated and released in injured spinal cords, which induce an oxidative stress reaction, that ultimately cause necrotic/apoptotic death of neurons, and progressive secondary neuronal tissue destruction. However, ROS can be also scavenged by SOD, and other antioxidant enzymes in cells. In addition, ROS can induce the production of MDA, which is a marker for oxidative damage to the plasma membrane (Xiao et al., 2008).

In our present study, rat models of SCI were established by hemisecting the spinal cord at the T8 vertebra. Hemisection SCI could reduce O^{2-} and SOD activity, and increase MDA levels in rats. Salidroside could enhance O^{2-} and SOD activity, and depress the level of MDA. However, the real mechanisms of its neuroprotective effect are still unclear and require more research.

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