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Panax notoginsenoside produces neuroprotective effects in rat model of acute spinal cord ischemia–reperfusion injury

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ABSTRACT

Ethnopharmacological relevance: Acute spinal cord ischemia–reperfusion injury (SCII) is associated with pathological changes, including inflammation, edema, and neuronal apoptosis. Panax notoginsenoside (PNS), an important traditional Chinese medicine, has shown a variety of beneficial effects, including homeostasis maintenance, anti-myocardial ischemia activities, and neuroprotective functions. However, whether it can produce neuroprotective effects in SCII and the underlying mechanisms remain largely elusive.

Aim of the study: In the present study, we investigated the effects of PNS on neurological and histopathological changes after SCII as well as the underlying mechanisms.

Materials and methods: Sixty-four adult rats were randomly assigned into one of the four groups: the sham group, the ischemic group, the PNS group, and the Methylprednisolone group. A rat model of SCII was adopted from a commonly used protocol that was initially proposed by Zivin. Neurological function was evaluated with the Basso, Beattie and Bresnahan (BBB) locomotor rating scale. Histopathological changes were examined with hematoxylin and eosin staining as well as Nissl staining. Immunohistochemistry and Western blot were conducted to compare the changes in tumor necrosis factor-α, interleukin-1β, interleukin-10, aquaporin-4 (AQP-4), member 6 of the TNF receptor superfamily (Fas), and Fas ligand (FasL) in the spinal cord. Finally, neuronal apoptosis was measured by electron microscopy.

Results: The BBB scores of the PNS-treated injured animals were significantly increased. The gross histopathological examination showed restored neuronal morphology and increased number of neurons after the PNS treatment. The PNS treatment decreased SCII-induced up-regulation of cytokine levels. In addition, PNS suppressed the increased expression of AQP-4 after SCII, suggesting an anti-edema effect. Finally, PNS treatment inhibited injury-induced apoptosis and reduced the expression levels of apoptosis-related proteins, Fas and FasL, confirming its anti-apoptosis effects against SCII.

Conclusion: The current findings suggest that PNS produces robust neuroprotective effects in spinal cord ischemia–reperfusion injury, and this role may be mediated by its anti-inflammation, anti-edema, and anti-apoptosis actions.

1. Introduction

Acute spinal cord ischemia–reperfusion injury (SCII), a serious and debilitating central nervous system injury, can induce an immediate or delayed paraplegia (Kuniyoshi et al., 2003). It is a major complication of surgeries in the thoracic and thoracoabdominal aneurysm with the incidence of 3–18% (MacArthur et al., 2005). Although many strategies, including temporary shunts or partial bypass, drainage of the cerebrospinal fluid, pharmacological measures, and hypothermia (McCullough et al., 1988; Svensson et al., 1993; Tabayashi et al., 1993), have been developed to increase ischemic tolerance in the spinal cord, the incidence of paraplegia remains high and consequently, this poses a persistent and devastating threat to patients (Etz et al., 2008).

Although the exact mechanism of SCII remains elusive, it is generally believed that inflammatory cytokines play a pivotal role in triggering a cascade of events which leads to cell apoptosis. The early accumulation of inflammatory cytokines in and around the microvessels at the ischemic zones has been widely reported (Clark et al., 1994; Jean et al., 1998; Fleming et al., 2006), which can be the cause of spinal cord edema and neuronal apoptosis (Samantaray et al., 2008).

Recent studies have suggested that traditional Chinese medicine, including tetramethylpyrazine (Fan et al., 2006) and resveratrol (Liu et al., 2011), can be very helpful in the treatment of SCII. Another effective medicine is Panax notoginseng (Burk) F.H. Chen (PNG), which belongs to the family of Araliaceae and has been used as a traditional Chinese herbal medicine for thousands

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of years. Panax notoginsenoside (PNS), a compound isolated from PNG, is the main effective ingredient of PNG. The main components of PNS are ginsenoside Rb1 (29.86%), Rg1 (20.46%), Rd (7.96%), Re (6.83%), and notoginsenoside R1 (2.74%) (Chen et al., 2008). The chemical structure of each component has also been determined recently (Yang et al., 2010). Extensive studies have demonstrated its broad physiological and pharmacological functions, including maintaining homeostasis, protecting against thrombotic events, and treating hyperlipidemia, atherosclerosis, coronary atherosclerotic heart disease, and cancer (Yang and Qin, 2003; He et al., 2007; Li et al., 2007). PNS also has pleiotropic benefits, such as anti-inflammation, anti-edema, anti-oxidation, and anti-apoptosis (Ng, 2006; Zheng et al., 2008). Particularly, PNS can protect neurons in animal models of cerebral ischemia–reperfusion injury (Li et al., 2009). However, to our knowledge, there is no study on its neuroprotective effects against SCII and more importantly, the underlying mechanism of its beneficial effects remains unknown.

In this study, we used a rat model of SCII and provided further evidence that PNS could produce neuroprotective effects after SCII through its roles in anti-inflammation, anti-edema, and anti-apoptosis.

2. Materials and methods

2.1. Animals and experimental groups

This study was approved by the ethic committee of College of Medicine, Xi'an Jiaotong University, and performed in accordance with the policies of Chinese animal research committees and guidelines from U.S. National Institute of Health (NIH Publication No. 96-23, revised 1996).

Sprague-Dawley rats were purchased from the Experimental Animal Facilities of Xi'an Jiaotong University. Sixty-four adult rats (260–320 g) were randomly assigned into one of the four groups (n = 16 per group): Group A (the sham group), Group B (the ischemic group), Group C (the PNS group), and Group D (the Methylprednisolone group). Methylprednisolone, a standard clinical drug in the treatment of acute spinal cord injury, was used as a positive control in this study. Rats in Group A were exposed to the operational area without injury; rats in Group B had spinal cord ischemia–reperfusion injury and 0.9% saline intraperitoneal injection 30 min before aortic clamping and reperfusion; and rats in Groups C and D received intraperitoneal injection of PNS (30 mg/kg, Kunming Pharmaceutical Group Corporation Ltd., China; approval number: GYZZ Z53020662; catalog number: 08FL03) and Methylprednisolone (30 mg/kg, Pfizer, Belgium, dissolved in 0.9% saline), respectively, 30 min before aortic clamping and reperfusion. All animals were housed in separated cages with free access to food and water. Room temperature was set at 25 ± 3 °C with standard 12 h light/dark cycle.

2.2. Rat model of acute SCII

The rat model of SCII was adopted from a commonly used protocol (Zivin and Degirolami, 1980). The SCII model proposed by Zivin et al. has been developed into a mature and reliable model. Although initially developed in rabbits, many following studies have proved its effectiveness in rats (Usul et al., 2004; Wang and Jiang, 2009; Tian et al., 2011). All animals were prohibited from drinking during the morning of the surgery. Animals were anesthetized with chloral hydrate (40 mg/kg, intraperitoneal injection, Paini Chemical, China) and placed in the supine position. After a 3- to 4-cm medial incision, the abdominal aorta was exposed at the level of the left renal artery. Four hundred units of heparin were administered 5 min before the aortic occlusion, and spinal cord ischemia was induced with the aorta clamped by a bulldog clamp just below the left renal artery. After the occlusion, the pulsation of the femoral artery disappeared. The blood flow was obstructed for 30 min. Then the bulldog clamp was removed, and the abdominal wall was closed with a sterile 6-0 silk suture. Ampicillin (Shuangye Pharmaceutics, China) was injected to the lower limb muscles once a day for 3 days postoperatively to prevent infection. Body temperatures were closely monitored. All the rats were housed individually with free access to food. Bedding in each cage was changed every day to keep it dry. After the injury, bladder massage was performed twice a day to stimulate autonomic urinary reflex. Rats were sacrificed 3 days after the surgery.

2.3. Evaluation of neurological function

Locomotor recovery after SCII was scored in an open field test according to the Basso, Beattie and Bresnahan (BBB) locomotor rating scale from 0 (complete paralysis) to 21 (normal locomotion) (Basso et al., 1996). BBB scores measured a combination of rat hind limb movement, joint movement, weight support, fore/hind limb coordination, trunk position and stability, stepping, paw placement, toe clearance, and tail position, which represents the sequential recovery stages that rats usually attained after SCII. Rats were allowed to move freely for 4 min. Locomotion activity of the hind limb was evaluated at 24, 48 and 72 h postoperatively. Scoring standard was detailed as follows: the first part evaluated the activity of the hind limb joints, the second part evaluated the pace and coordination of the hind limbs, and the third part evaluated the delicate activity of the paws during locomotion. The locomotion was scored by two independent observers blind to the design of the experiment.

2.4. Hematoxylin and eosin (HE) and Nissl staining

Three days after the surgery, the rats (n = 6 for each group) were transcardially perfused with 0.1 mol/L phosphate buffered saline (PBS) and then with 4% paraformaldehyde (PFA) in PBS for 30 min. Spinal cords were dissected and kept in 4% PFA for post-fixation overnight. After dehydration, the spinal cords were embedded with paraffin and serial coronal sections with the thickness of 5 μm were collected. To assess the histopathological changes, the sections were further subjected to HE and Nissl staining using well-established protocols.

2.5. Immunohistochemistry

Sections obtained in 2.4 were used for immunohistochemistry. Horseradish peroxidase (HRP)-labeled Streptavidin kit (Bosen, China) was used for immunohistochemical studies for tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-10 (IL-10), aquaporin-4 (AQP-4), membrane 6 of the TNF receptor superfamily (Fas), and Fas ligand (FasL). The following primary antibodies were used: rabbit anti-TNF-α (1:200), rabbit anti-IL-1β (1:200), rabbit anti-IL-10 (1:200), rabbit anti-AQP-4 (1:200), rabbit anti-Fas (1:200), and rabbit anti-FasL (1:200) (all from Bosen, China). HRP-conjugated goat anti-rabbit secondary antibody (Santa Cruz, USA) was used for 3,3′-diaminobenzidine staining using well-established protocols. Images were acquired with a biological imaging microscope (Olympus, Japan), and analyzed with Image-ProPlus (MediaCybernetics, USA).

2.6. Western blot

For Western blot, the rats (n = 6 for each group) were immediately sacrificed and the spinal cord was taken out. Proteins were extracted with RIPA lysis buffer kit (Bio-Tek, USA) and the protein