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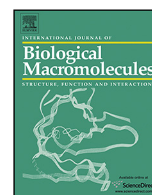

Neuroprotective effect of *Panax notoginseng* plysaccharides against focal cerebral ischemia reperfusion injury in rats

Dong Jia[a,1], Yangbin Deng[b,1], Junxian Gao[c], Xiaoquan Liu[b], Jianping Chu[b], Ya Shu[d,*]

[a] Department of Neurosurgery, Tangdu Hospital, The Fourth Military Medical University, Xi'an 710032, China
[b] Pediatric Intensive Care Unit, Xi'an Children's Hospital, Xi'an 710003, China
[c] Department of Neurology, the Ninth Hospital of Xi'an, Xi'an 710054, China
[d] Department of Anesthesiology, The First Affiliated Hospital of Xi'an Medical University, Xi'an 710077, China

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ABSTRACT

Our present study was conducted to investigate whether *Panax notoginseng* plysaccharides (PNPS) exerted a neuroprotective effect against focal cerebral ischemia/reperfusion (I/R) injury in rats. Before mice were subjected to middle cerebral artery occlusion (MCAO) for 2 h and reperfusion for 22 h, PNPS at the doses of 50, 100, and 200 mg/kg was administered once a day intragastrically for continuous 7 days. Oral administration of PNPS could significantly reduce the severity of neurological deficits, infarct volumes, cerebral edema, and neuronal death caused by MCAO in rats. Moreover, in the presence of PNPS, the Bcl-2/Bax ratio increased, but the level of cleaved caspase-3 reduced. Thus, these finding suggested that suppressing apoptosis through increasing Bcl-2/Bax ratio and evoking caspase-3 cascade should be potential mechanism by which PNPS exerts its neuroprotective function against focal cerebral I/R injury.

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1. Introduction

Ischemic stroke is one of the most frequent causes of death around the world and cerebral ischemia/reperfusion (I/R) injury often causes irreversible brain damage, and the cascade of events causing neuronal injury and death in brain ischemia includes interruption of the cerebral blood flow, the depletion of energy stores excitotoxicity, free radical-induced neuronal damage, inflammation, apoptosis, and necrosis [1,2]. The nerve cells damage caused by cerebral ischemia results in functional impairment and/or death. Accumulating evidence suggests that apoptosis plays a major role in delayed neuronal death following cerebral ischemia [3], thus repressing or blocking neuronal apoptosis could attenuate cerebral ischemia injury and reduce infarcted area. A variety of new neuroprotective therapeutic strategies for the prevention of brain injury in patients with cerebral ischemia have emerged, but many of these are still far from adequate [4]. At the present state of knowledge, natural products, especially medicinal plants, probably represent an ideal source to develop safe and effective agents for management of ischemic brain injury and deserve scientific investigation [5–7].

Panax notoginseng (Sanqi or Tienchi in Chinese), the root of *P. notoginseng* [(Burk.) F.H. Chen], is a well-known traditional Chinese herbal medicine found in the southwest of China and has been widely used for treatment of cardio- and cerebro-vascular disorders, such as stroke, ischemic heart and brain diseases [8–11]. Extensive studies have been conducted on the low-molecular-weight biologically active components, especially saponins (PNS) from this herb [12]. A wide range of pharmacological activities of PNS in cardiovascular, immune, endocrine and central nervous system had been reported previously [13–15]. Although the neuroprotective effect of PNS has been well studied, very limited information on this aspect was reported on the high-molecular-weight fraction (polysaccharides) of *P. notoginseng* roots (PNPS). Therefore, the present study was undertaken to evaluate the neuroprotective effect and potential molecular mechanisms of PNPS in middle cerebral artery occlusion (MCAO) occlusion (focal cerebral ischemia) induced cerebral ischemia in rats.

2. Materials and methods

2.1. Materials and chemicals

P. notoginseng was purchased from local drugstore in Xi'an, China. All other chemical reagents used were analytical grade.

* Corresponding author. Tel.: +86 029 84777435; fax: +86 029 84777435.
E-mail address: yashu_xamu@outlook.com (Y. Shu).
[1] Two authors contributed equally to this work.

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2.2. Extraction of the polysaccharide

The roots of *P. notoginseng* were dried at 60 °C in an oven for 24 h and then homogenized in a high speed disintegrator to obtain fine powder. Then the powder passed through a 60-mesh sieve was defatted by petroleum ether at 80 °C and pretreated twice with 95% ethanol to remove some colored materials, monosaccharides, oligosaccharides, and small-molecule materials. The insoluble residue was filtered and dried at 60 °C. Subsequently the dried residues were extracted with twenty volumes of deionized water at 60 °C for 90 min under continuous stirring, filtered through gauze and centrifuged to remove the insoluble materials. The supernatants were collected, concentrated to a proper volume under reduced pressure and mixed with four volumes of 95% ethanol and kept at 4 °C in refrigerator for 12 h. The precipitate was collected by centrifugation at 5000 rpm for 20 min and washed three times with absolute ethanol to a constant weight, affording *P. notoginseng* polysaccharides (PNPS).

2.3. Characterization of polysaccharide

The total carbohydrate content was determined by the phenol–H_2SO_4 method, with glucose as the standard [16]. Protein content was quantified according to the Bradford's method [17]. Total uronic acid contents were measured by m-hydroxydiphenyl method using galacturonic acid or glucuronic acid as the standard [18].

2.4. Animals

Male Wistar rats (weighing 250–300 g) purchased from Animal Center of the Forth Military Medical University (Shannxi, China), were used in this study. All animal were maintained in polypropylene cages in air conditioned room with a 12-h light cycle at a controlled temperature (23 ± 2 °C) and allowed free access to pellet diet and water ad libitum. All animal experiments and care were performed in accordance with the procedure approved by the Animal Ethics Committee of the Forth Military Medical University.

2.5. Experimental protocol

The rats were randomly divided into the following 5 groups of 8 rats each for the experiments of focal cerebral ischemia: sham control group, ischemia model group, low dose PNPS group (50 mg/kg), medium dose PNPS group (100 mg/kg) and high dose PNPS group (200 mg/kg). All rats were treated by gavage once a day for 7 days prior to prior to ischemia.

2.6. Induction of transient focal cerebral ischemia (MCAO)

After 7 days of pretreatment with PNPS, rats were subjected to 2 h right MCAO via the intraluminal filament technique and 22 h reperfusion as described in detail previously [19,20]. Before surgery, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital at a dose of 100 mg/kg. Briefly, the right common carotid artery was exposed at the level of the external and internal carotid artery bifurcation. A 4-0 nylon suture with a blunted tip was inserted into the internal carotid artery and advanced into the anterior cerebral artery to occlude the middle cerebral artery (MCA). After occluding the MCA for 2 h, the operator carefully removed the suture to restore blood flow and then sutured the skin. Animals were then returned to their cages and closely monitored for 22 h. In sham-operated group, the external carotid artery was surgically prepared for insertion of the filament, but the filament was not inserted.

2.7. Evaluation of neurological deficits

A neurological examination was blindly performed by a single examiner at 22 h after reperfusion (before killing) according to the method described by Longa et al. [20]. The neurological findings were scored using a 5-point scale: rats with no neurologic deficit scored 0, rats with (failure to extend left forepaw fully) a mild focal neurologic deficit scored 1, rats with (circling to the contralateral side) a moderate focal neurologic deficit scored 2, rats with (falling to the left or no spontaneous motor activity) a severe focal deficit scored 3, rats with a score of 4 do not walk spontaneously and have a depressed level of consciousness.

2.8. Determination of water content in the brain

The brain water content was determined as the measure of cerebral edema after 2 h of ischemia and 22 h of reperfusion. Rats were decapitated and brains were quickly removed. The wet weight was measured and then the brain was dried at 110 °C for 24 h to obtain the dry weight. The following formula was used to calculate the water of the brain: water content of brain (%) = (wet weight of brain − dry weight of brain)/wet weight of brain × 100%.

2.9. Assessment of cerebral infarct volume by TTC staining

After reperfusion for 22 h, rats were decapitated under anesthesia with chloral hydrate (300 mg/kg), and the brains was kept at −20 °C for 40 min. Frozen brain was sliced into uniform coronal sections of approximately 2 mm thickness each. Brain slices were incubated with 2% 2,3,5-triphenyltetrazolium chloride (TTC) in 0.2 mol/L phosphate buffer (pH 7.4) at 37.5 °C for 30 min and fixed in 10% neutral buffered formalin for overnight [21]. The unstained areas of the fixed brain sections were defined as infracted. After 24 h of fixation, the slices were photographed on each side. The areas of infarct and both hemisphere for each slice were measured using an image analysis system (IBAS 2.0, Contron, Munich, Germany). The total infarct volume was determined by measuring the infarct area in each slice section and integrating the values of all slices. Infarct volumes were presented as percentage of the contralateral hemisphere volume [22].

2.10. Brain sample preparation and Western blot analysis

After the behavioral studies, the rats were anaesthetized by chloral hydrate followed by decapitation and their brains were removed. The brain tissue surrounding the cortical contusion site was dissected and were immediately stored at 4 °C until analyzed, when they were first homogenized and quantified. The homogenates (in 150 mM NaCl, 25 mM Tris–HCl, 1 m MEGTA, 1 Mm EDTA, pH 7.4, 1% Triton X-100, 1 mM PMSF) were used for western blot analysis. Total protein samples (20 μg/lane) were separated by 10% SDS–PAGE gel electrophoresis, and then transferred to nitrocellulose membranes. The membranes were blocked overnight at 4 °C in a buffer containing PBS, 0.1% Tween 20, and 5% low fat milk powder and then incubated with the Bcl-2 antibody (1:500), Bax antibody (1:500), cleaved caspase-3 antibody (1:500), and β-actin antibody (1:1000), respectively. After thoroughly being washed, the membranes were incubated with a horseradish peroxidase conjugated secondary antibody for 1 h at room temperature and processed for visualization by enzyme-linked chemiluminescence. Relative intensities of the bands were quantified by densitometric analysis.

2.11. Tunel staining

The neuron apoptosis was labeled in paraffin embedded tissue sections by the terminal deoxynucleotidyl-transferase (TDT)

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