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Neuroprotective effect of *Buyang Huanwu Decoction* on spinal ischemia/reperfusion injury in rats

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ABSTRACT

Aim of the study: The aim of this study was to investigate the protective effect of *Buyang Huanwu Decoction*, a traditional Chinese medicine formula, on spinal ischemia/reperfusion injury and explore the possible mechanism of the protective effect.

Materials and methods: The spinal ischemia/reperfusion injury model was conducted in male Sprague–Dawley rats, and 40 g/kg *Buyang Huanwu Decoction* was administered by introgastric infusion. Motor function of hind limbs and apoptosis index were measured 72 h after reperfusion was started. The expression of thioredoxin and thioredoxin reductase was examined at 6 h and at 24 h after reperfusion.

Results: Motor function scores and apoptosis indices were significantly improved in the *Buyang Huanwu Decoction* group, as compared to the saline-infused control group. Spinal ischemia/reperfusion injury resulted in a decrease in the expression of thioredoxin, while *Buyang Huanwu Decoction* administration greatly elevated the expression of thioredoxin-1/thioredoxin-2 mRNA and thioredoxin reductase-1/thioredoxin reductase-2 mRNA.

Conclusions: Our results suggest that administration of *Buyang Huanwu Decoction* may reduce spinal ischemia/reperfusion damage. This neuroprotective effect may be mediated, in part, by an increase in the transcription of thioredoxin.

1. Introduction

Approximately 11–40% of paraplegia cases result from spinal ischemia/perfusion injury, as complications of spinal orthopedic surgery and thoracoabdominal aneurymectomy (Crawford et al., 1986). Many protective therapies have been tested in animal models, and have yielded significantly effective results for spinal cord injury, but translating these experimental therapies to humans is enormously challenging (Bradbury and McMahon, 2006).

Buyang Huanwu Decoction (*BYHWD*) is a well-known traditional Chinese medicine formula, and is composed of Radix Astragali (Huangqi), Radix Angelicae Sinensis (Danggui), Radix Paeoniae Rubra (Chishao), Rhizoma Ligustici Chuanxiong (Chuanxiong), Semen Persicae (Taoren), Flos Carthami (Honghua), and Lumbricus (Dilong). *BYHWD* has been applied in the treatment of stroke and paralysis for hundreds of years in Oriental medicine (Wang, 2005), and has increasingly gained attention due to its significant neuroprotective properties. A number of studies of the effects of *BYHWD* on ischemic brain injury have been documented, but only one study of spinal ischemia/reperfusion injury is reported (Fan et al., 2006). Therefore, the protective effects of *BYHWD* on spinal ischemia/reperfusion injury and the mechanism of the protective effect need to be explored.

Previous studies have shown that *BYHWD* promotes growth and differentiation of neural cells (Sun et al., 2007; Chen et al., 2008), and inhibits apoptosis of nerve cells (Li et al., 2003). One critical mechanism of spinal ischemia/reperfusion injury involves the thioredoxin (Trx) system (Mustacich and Powis, 2000). Trx are members of an evolutionarily conserved family of redox-active proteins, and have been shown to regulate the state of cellular reduction/oxidation and cellular proliferation (Hirota et al., 2002). Trx exist in several forms, with the cytosolic (Trx-1) and mitochondrial (Trx-2) forms being the most prevalent (Watson et al., 2004). Multiple thioredoxin reductases (TrxR) are also present, with a predominant cytosolic form, TrxR-1, and a mitochondrial form, TrxR-2. Numerous activities, including exerting significant control over apoptosis, have been identified for Trx-1 and Trx-2 (Watson et al., 2004).

Therefore, the present study was designed to confirm the therapeutic effects of *BYHWD*, using an animal model of spinal ischemia/reperfusion injury, and to elucidate the role of the Trx system in the underlying therapeutic mechanism.

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2. Materials and methods

2.1. Composition and preparation of BYHWD

BYHWD is composed of *Radix Astragali* (120 g), *Radix Angelicae Sinensis* (6 g), *Radix Paeoniae Rubra* (6 g), *Rhizoma Chuanxiong* (3 g), *Semen Persicae* (3 g), *Flos Carthami* (3 g) and *Lumbricus* (3 g). All herbs were purchased from Jiangsu Province Pharmacy Company (Nanjing, China). The herbs were decocted by boiling in distilled water for 30 min. The solution was then freeze-dried under vacuum, and made into a powder. The powder was dissolved in distilled water to a final concentration of 5 g/ml (equivalent to dry weight of raw materials).

2.2. Preparation of spinal ischemia model

All animal care complied with the US guidelines for laboratory animals (NIH publication #85-23, revised in 1985), and the study was approved by the Ethics Committee of Chongqing University, China.

Twenty-four male Sprague–Dawley rats, weighing 300–350 g, were randomly divided into three groups with eight rats in each group: sham operation, *BYHWD* treatment, and saline treatment control groups. All animals were anesthetized by the inhalation of 2% oxygen-isoflurane through a face mask. Body temperature was maintained at 37 °C with an infrared heat lamp and a heating pad, and monitored with a rectal probe. The femoral artery was cannulated with a polyethylene tube (PE-50) to facilitate continuous monitoring of heart rate and arterial blood pressure, and for collecting blood samples for the analysis of blood gases and blood pH. Laser-Doppler flowimetry was recorded continuously during surgery using a method described previously (Westergren et al., 2001).

Spinal cord ischemia/reperfusion was induced using the previously described method (Zivin and DeGirolami, 1980). Ischemia of the lumbar spinal cord was produced by occlusion of the abdominal aorta 0.5 cm below the left renal artery for 60 min, followed by 72 h of reperfusion. Sham operation rats underwent the same procedure, but no occlusion of the aorta was performed.

2.3. Therapeutic effect of BYHWD

A *BYHWD* introgastric infusion treatment was used as previously described (Fan et al., 2006). Rats in the saline treatment and *BYHWD* treatment groups were administered 8 ml of saline and 40 g/kg of *BYHWD*, respectively, by introgastric infusion, starting at reperfusion, and the same dose was infused every 24 h for 72 h. After 72 h of reperfusion, all of the animals were evaluated for the motor function of the hind limbs. The animals were then sacrificed and the spinal cords were quickly removed to determine apoptosis.

The dosage of 40 g/kg of *BYHWD* was chosen after preliminary experimental results with 20 and 30 g/kg of *BYHWD* infusion showed no protective function on spinal damage after reperfusion (data not shown).

2.4. Evaluation of motor function of hind limbs

An independent observer, who was blinded to the protocol and group assignments, performed the motor function assessments on rats. Grading of neurological function was performed using previously published criteria (Johnson et al., 1993). The motor functions of the hind limbs were graded as: 0, complete paralysis of the hind limbs; 1, severe incomplete paralysis of the hind limbs; 2, the hind limbs could move but could not jump; 3, the hind limbs could jump but with obvious instability; 4, the hind limbs could jump but with slight instability; 5, the hind limbs had normal motor function.

2.5. Determination of apoptosis indices

After the evaluation of motor function, the rats were sacrificed, and the L_{2-3} section of the lumbar spinal cord was taken. A TUNEL staining technique was performed on spinal cord sections using an in situ apoptosis detection kit (Intergen Company, USA). The total number of TUNEL-positive cells on each section was counted, and expressed as the TUNEL index.

2.6. Expression of Trx and TrxR

Forty-eight male Sprague–Dawley rats, weighing 300–350 g, were randomly divided into sham operation, saline treatment and *BYHWD* treatment groups. After occlusion of the abdominal aorta 0.5 cm below the left renal artery for 60 min, commencing at reperfusion, animals in the saline and *BYHWD* groups were administered 8 ml of saline and *BYHWD* at 40 g/kg, respectively, by introgastric infusion. Sham operation rats underwent the same procedure, but no occlusion of the aorta was performed. Animals were sacrificed at 6 h and at 24 h after reperfusion, and the expression levels of Trx-1/Trx-2 and TrxR-1/TrxR-2 mRNA were detected.

2.7. Statistical analysis

Data are presented as mean ± SEM. The Mann–Whitney non-parametric test was used to analyze motor function scores. Comparisons of the number of TUNEL-positive cells, and comparisons of the expression levels of Trx and TrxR at different time points were performed with two-way ANOVA analyses and Scheffe post hoc comparisons. Experimental data were analyzed using a statistical software package, SPSS11.0.

3. Results

Heart rate, arterial blood gases and pH, blood pressure, and body temperature were stable and similar in all groups before, during and after the procedure. Two rats died due to pulmonary insufficiency caused by an anesthetic overdose during surgery, and were excluded from the study analyses. All surviving animals completed the study.

3.1. Effects of BYHWD on motor function of hind limbs

The motor functions of hind limbs in sham-operated animals were normal. A clear distinction was observed between the *BYHWD* and saline groups in motor function. Spinal ischemia/reperfusion resulted in a significant neurological dysfunction in the saline group, but *BYHWD* administration greatly improved function (Table 1).

Table 1
Scores of motor function of hind limbs after spinal ischemia/reperfusion.

Groups	Scores of motor function					
	0	1	2	3	4	5
Sham (n)	0	0	0	0	0	8
Saline (n)	0	1	4	2	1	0[a]
BYHWD (n)	0	0	1	3	4	0[b]

Scores of motor function of hind limbs after reperfusion ($n = 8$). Scores of motor function of hind limbs were normal in sham-operated group. A low score was seen in the group with saline infusion, but the score was significantly increased in the group with *BYHWD* administration. Sham group: sham-operated group; saline group: spinal ischemia/reperfusion group with saline infusion; *BYHWD* group: spinal ischemia/reperfusion group with *BYHWD* infusion; n: rat number for each score; *BYHWD: Buyang Huanwu Decoction*.

[a] $F = 1.71$, $P = 0.03$ compared to the sham group.

[b] $F = 1.71$, $P = 0.03$ compared to the saline group.