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Neuroprotective effect of Buyang Huanwu Decoction against focal cerebral ischemia/reperfusion injury in rats – Time window and mechanism

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ABSTRACT

Ethnopharmacological relevance: Buyang Huanwu Decoction, a traditional Chinese medicine, consists of different herbal medicines, and has been traditionally used for centuries to treat paralysis and stroke. However, its optimal therapeutic time window and the mechanism are still unclear.
Aim of the study: This study was designed to explore the therapeutic time window and mechanism of Buyang Huanwu Decoction on transient focal cerebral ischemia/reperfusion injury.
Materials and methods: Middle cerebral artery occlusion was conducted in male Sprague-Dawley rats, and 40 g/kg of Buyang Huanwu Decoction was intragastrically infused at different time points, and the same dose was infused every 24 h for 3 days. The level of glutamate in cerebrospinal fluid and the expression of metabotropic glutamate receptor-1 RNA in striatum were detected before, during, and after ischemia/reperfusion. Neurological deficit scores and brain infarction volumes were measured at 72 h after reperfusion.
Result: Cerebral ischemia/reperfusion resulted in significant neurological deficit and extensive cerebral infarct volume, associated with a large amount of glutamate in cerebrospinal fluid and elevation of metabotropic glutamate receptor-1 RNA expression. Buyang Huanwu Decoction significantly suppressed the release of glutamate, and reduced the expression of metabotropic glutamate receptor-1 RNA. The neurological defect score and infarction volume were significantly improved by administration of Buyang Huanwu Decoction, when compared with the Ischemia group.
Conclusions: Administration of Buyang Huanwu Decoction, within 4 h of post-transient focal stroke, reduced significant cerebral ischemia/reperfusion damage. The neuroprotective mechanism of Buyang Huanwu Decoction is, in part, associated with the down-regulation of metabotropic glutamate receptor-1 RNA and inhibition of glutamate release resulting from cerebral ischemia.

1. Introduction

Buyang Huanwu Decoction (BYHWD) is a Chinese herbal medicine, and consists of extracts of Astragalus root (The Latin name is *Astragalus Membranaceous*, and the Chinese name is Huangqi, from the family Fabaceae); Chinese Angelica root (*Angelica Archangelica* in Latin, and Danggui in Chinese, from the family Apiaceae); Red Peony root (*Paeonia Lactiflora* in Latin, and Chishao in Chinese, from the family Paeoniaceae); Szechuan Lovage root (*Rhizoma Ligustici Chuanxiong* in Latin, and Chuanxiong in Chinese, from the family Umbelliferae); Peach Seed (*Semen Persicum* in Latin, and Taoren in Chinese, from the family Rosaceae); Safflower (*Gnecos* in Latin, and Honghua in Chinese, from the family Asteraceae); and Earthworm (*Lumbricus* in Latin, and Dilong in Chinese, from the family Lumbricidae). The decoction has been used for centuries to treat a variety of disorders, including paralysis (Wang and Jiang, 2009) and stroke (Sun et al., 2007). From a viewpoint of traditional Chinese medicine, it is used to invigorate the body, enhance blood circulation, and activate Qi flow through energy meridians (Johnston, 2011). The ancient wisdom for centuries has demonstrated significant effects on the clinical treatments, and modern science has shown that BYHWD exerts neuroprotective effects.

Although BYHWD has been demonstrated in vivo to improve recovery of neurological function, stimulate neural proliferation (Cai et al., 2007), and decrease cerebral ischemic damage (Li et al., 2003a,b), its optimal therapeutic time window and mechanism are still unclear. For the neuroprotective mechanism, BYHWD has been demonstrated in vitro to promote growth and differentiation of neural cells (Sun et al., 2007), and to inhibit apoptosis of nerve cells (Li et al., 2003a,b). Some of those neuroprotective effects

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have indicated that BYHWD reduces ischemia/reperfusion damage via inhibition of the thioredoxin system (Wang and Jiang, 2009), [Ca^{2+}] (Sun et al., 2007), apoptosis formation (Li et al., 2003a,b), and free radical scavenger activity (Choi et al., 2011), suggesting that neuroprotective effects of BYHWD on brain ischemia are through multiple mechanisms. One of the mechanisms of cerebral ischemia/reperfusion injury involves glutamate.

Glutamate is the most common excitatory neurotransmitter in the mammalian central nervous system, and has a role in long-term neuronal potentiation, as a proposed molecular substrate for learning and memory. Glutamate may also be a potent neurotoxin, and glutamate excitotoxicity has been implicated in the pathogenesis of many devastating human neurological diseases such as stroke, amyotrophic lateral sclerosis and epilepsy (Eweka et al., 2010). In the normal condition, glutamate is largely confined inside the cells (Danbolt, 2001), while it is released in huge amount following cerebral ischemia/reperfusion injury (Ponce et al., 2008). The uncontrolled release of glutamate leads to a large amount of positive charge to flow into the neurons, which results in the death of nerve cells (Bonde et al., 2005). Glutamate can act as an endogenous agonist to metabotropic glutamate receptors (mGluRs). It has been shown that the activation of metabotropic glutamate receptor-1 (mGluR-1) aggravates nerve injury resulting from cerebral ischemia (Henrich-Noack et al., 2000), and administration of mGluR-1 antagonist reduces the brain injury owing to cerebral artery occlusion (Bonde et al., 2005). These were confirmed by the in vivo research evidence revealing that the over-expression of mGluR-1 RNA was observed after cerebral ischemia/reperfusion, and that mGluR-1 RNA was involved in the process of ischemia/reperfusion injury (Zhang et al., 2005). In addition, Planells-Cases et al. found that sustained activation of glutamate receptors is a casuistic phenomenon leading to the neuronal death underlying neurodegeneration, and that the glutamate receptor antagonist acts as key therapeutic targets of neurodegenerative diseases (Planells-Cases et al., 2006).

The above-mentioned findings leads to the speculation that the suppressed expression of mGluR RNA may partly explain the mechanism behind neuroprotection of BYHWD against cerebral ischemia/reperfusion damage (Sommer et al., 2000; Kohara et al., 2008). Therefore, the present study was designed to explore the therapeutic time window of BYHWD, by using an animal model of cerebral ischemia/reperfusion injury, in an attempt to elucidate the role of glutamate in the underlying therapeutic mechanism.

2. Materials and methods

All male Sprague-Dawley rats, weighing 250–280 g, were obtained from the Laboratory Animal Center of Xuzhou Medical College. The principles of laboratory animal care were followed, and the study was approved by the Ethics Committee of Xuzhou Medical College, China.

2.1. Preparation of BYHWD

Dried crude drugs were purchased from Nanjing Pharm (Nanjing, China). According to the original prescription from the "Yi Lin Gai Cuo" (Correction of Errors in Medical Classics), the decoction comprised *Astragalus Membranaceous* (extracted from Astragalus root, Voucher Ref. No. 2008-203S); *Angelica Archangelica* (from Chinese Angelica root, Voucher Ref. No. 2007-198R); *Paeonia Lactiflora* (Red Peony root, Voucher Ref. No. 2008-197E); *Rhizoma Ligustici Chuanxiong* (Szechuan Lovage root, Voucher Ref. No. 2009-102S); *Semen Persicum* (Peach Seed, Voucher Ref. No. 2008-202D); *Gnecos* (Safflower, Voucher Ref. No. 2007-136F) and *Lumbricus* (Earthworm, Voucher Ref. No. 2008-128E). They were identified by the Department of Pharmacology, Southeast University at Nanjing, China, and were mixed in the ratio of 120:6:6:3:13:3:3 (dry weight). To maintain the consistency of the herbal chemical ingredients, all the components were obtained from the original sources and extracted according to the standard in the National Pharmacopoeia of China. The herbs were cut, mixed, and decocted by boiling in distilled water at 100 °C for 30 min. The solution was then freeze-dried under vacuum, and made into drug powder. The powder was dissolved in distilled water to a final concentration of 5 g/ml.

2.2. Cerebral ischemia model

A total of 98 adult male Sprague-Dawley rats, weighing 250–280 g, were used in the study. All rats were maintained under anaesthesia with chloral hydrate (350 mg/kg) administered intraperitoneally. The femoral artery was cannulated with a polyethylene tube (Winsum, The Netherlands) for monitoring of the heart rate and arterial blood pressure, and for collecting blood samples for the analysis of blood gasses and blood pH. Body temperature was maintained at 37 °C with an infrared heat lamp and a heating pad. Transient focal cerebral ischemia was produced by right middle cerebral artery occlusion for 90 min followed by 72-h reperfusion, as previously described (Zea-Longa et al., 1989). The rats in the Control group underwent the same procedure, but no arteries were occluded.

2.3. Determination of therapeutic time window of BYHWD

Seven rats in the Control group and 35 rats receiving BYHWD were intragastrically administered 8 ml of saline and 40 g/kg of BYHWD, respectively, starting at reperfusion, 1, 2, 4, and 6 h after reperfusion, and the same dose was infused every 24 h for 3 days. The samples were taken at 30, 60, 120, 240, and 360 min after reperfusion, with seven rats in each time-point.

The dosage of 40 g/kg of BYHWD was chosen after our preliminary experimental results with 20 and 30 g/kg of BYHWD infusion showed no function on inhibition of glutamate release after brain injury (data not shown in this study).

2.4. Determination of neurological defect score

After 72 h of reperfusion, all animals were evaluated for a neurological deficit score. An independent observer performed the assessment of neurological defect score on the rats, based on the previously published criteria (Zea-Longa et al., 1989): 0, no neurological deficit; 1, failure to fully extend left forepaw; 2, circling to the left; 3, falling to the left; 4, loss of spontaneous walking with a depressed level of consciousness; and 5, dead.

2.5. Measurement of brain ischemic infarction volume

After assessment of neurological deficit score following 72 h of reperfusion, the animals were sacrificed, and the brains were quickly removed to measure the infarct volume. The brains were cut into 2-mm coronal sections, stained with 2% triphenyltetrazolium chloride, and then fixed in PBS. The infarction volume was measured by using image analyzer software (Media Cybernetics, MD, USA).

2.6. Determination of glutamate level in cerebrospinal fluid

Fifty-six male Sprague-Dawley rats, weighing 250–280 g, underwent the same procedure of focal cerebral ischemia/reperfusion and intragastric infusion of BYHWD. The animals were maintained under anesthesia, and body temperature was maintained at 37 °C with a heating pad. The level of glutamate in cerebrospinal fluid