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Protective effect of Sheng-Nao-Kang decoction on focal cerebral ischemia-reperfusion injury in rats

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ABSTRACT

Ethnopharmacological relevance: Sheng-Nao-Kang decoction (SNK), a modified traditional Chinese medicine (TCM), has been used clinically for the treatment of acute and chronic cerebrovascular related diseases. To evaluate the protective effect of SNK on focal cerebral ischemia-reperfusion (I/R) injury in rats and investigate the underlying mechanisms.

Materials and methods: Focal cerebral I/R injury in rats was induced by middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion for 24 h. Adult male Sprague-Dawley (SD) rats were randomly divided into six kinds of groups: Sham group; I/R group; SNK-treated groups at doses of 0.7 g/kg, 1.4 g/kg and 2.8 g/kg; and nimodipine (NMP)-treated group. The recoveries of neurological function in rats were estimated by neurological defect scoring and 2,3,5-triphenyltetrazolium chloride (TTC) staining after 24 h reperfusion. Various biochemical indexes in serum were assayed by colorimetry, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), inducible nitric oxide synthase (iNOS) and total nitric oxide synthase (TNOS). Histological structures of the brain in rats were observed by hematoxylin and eosin (H&E) staining. Immunohistochemistry was performed to detect the caspase-3 protein content in rats.

Results: SNK administration significantly reduced the neurological defect scores and lessened the cerebral infarction volume. The treatment of SNK lowered MDA content, up-regulated SOD and GSH-Px levels, down-regulated iNOS and TNOS levels in serum. Furthermore, histological examination indicated that dense neuropil and largely surviving neurons were seen in SNK-treated rats. SNK administration restrained the expression of caspase-3 positive protein significantly.

Conclusion: The results suggest that SNK demonstrates a strong and ameliorative effect on cerebral I/R damage in rats. The protective mechanisms of SNK are associated with its properties of anti-apoptosis and anti-oxidation as well as regulation of iNOS and TNOS.

1. Introduction

Stroke is one of the major diseases which threaten human life and health because of its high rates of disability and mortality (Sun et al., 2010; Zhang et al., 2006). With the development of shock therapy and application of advanced intravascular procedures and thrombolytic agents, neurological defects caused by cerebral ischemia can be effectively treated in most cases. However, reperfusion after cerebral ischemia generally leads to more brain damage, including mitochondrial dysfunction, cerebral edema, cerebral hemorrhage, neuronal death and toxic effect of dopamine (Uzar et al., 2012). Chemical drugs such as neuroprotective agents and radical scavengers as well as calcium ion antagonist have been used for the treatment of cerebral ischemia-reperfusion (I/R) injury. Nevertheless, side effects such as cerebral hemorrhage,

Abbreviations: ANOVA, one-way analysis of variance; CCA, common carotid artery; ECA, external carotid artery; GSH-Px, glutathione peroxidase; H&E, hematoxylin and eosin; HPLC, high performance liquid chromatography; ICA, internal carotid artery; iNOS, inducible nitric oxide synthase; IPP, Image-Pro Plus; I/R, ischemia-reperfusion; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; NMP, nimodipine; NO, nitric oxide; p.o., per os; RNS, reactive nitrogen radicals; ROS, reactive oxygen species; SD, Sprague-Dawley; S.E.M., standard error of mean; SNK, Sheng-Nao-Kang decoction; SOD, superoxide dismutase; TCM, traditional Chinese medicine; TNOS, total nitric oxide synthase; TTC, 2,3,5-triphenyltetrazolium chloride

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Table 1
The Chinese medicines contained in SNK.

Latin name (Chinese name)	The part used	Place of production	Voucher numbers	Weight (g)
Salvia miltiorrhiza Bge. (Dan Sen)	Root	Sichuan	20110010101	12
Ligusticum chuanxiong Hort. (Chuan Xiong)	Root	Shaanxi	20110010102	12
Astragalus membranaceus (Fisch.) Bge. (Huang Qi)	Root	Shanxi	20110010103	12
Pueraria lobata (Willd.) Ohwi (Ge Gen)	Root	Henan	20110010104	12
Paeonia lactiflora Pall. (Chi Shao)	Root	Shaanxi	20110010105	9
Leonurus japonicus Houtt. (Yi Mu Cao)	Whole herb	Shaanxi	20110010106	9
Uncaria rhynchophylla (Miq.) Jacks (Gou Teng)	Branch	Shaanxi	20110010107	9
Sophora flavescens Ait. (Ku Shen)	Root	Shaanxi	20110010108	9
Gastrodia elata Bl. (Tian Ma)	Tuber	Shaanxi	20110010109	7
Panax notoginseng (Burk.) F. H. Chen (San Qi)	Root	Yunnan	20110010110	3
Whitmania pigra Whitman (Shui Zhi)	Whole animal	Shandong	20110010111	9
Pheretima aspergillum (E. Perrier) (Di Long)	Whole animal	Guangxi	20110010112	9
Eupolyphaga sinensis Walker (Tu Bie Chong)	Whole animal	Henan	20110010113	9
Bombyx mori Linnaeus (Jiang Can)	Whole animal	Shaanxi	20110010114	9
Scolopendra subspinipes mutilans L. Koch (Wu Gong)	Whole animal	Henan	20110010115	7

resistance to drugs and gastrointestinal irritation may exceed the clinical benefits for long-term therapy. Furthermore, chemical drugs are generally difficult to achieve significant therapeutic results used alone because of its single therapeutic target (Wang et al., 2012).

In recent years, experimental reports and clinical applications of traditional Chinese medicines (TCMs) against cerebral I/R injury have been ascendant due to its effects of multiple targeting and multi-directional regulations (He et al., 2012; Lin et al., 2008; Zhao et al., 2012). Sheng-Nao-Kang decoction (SNK), containing fifteen traditional Chinese medicines as specified in Table 1, was originated from classical Chinese prescriptions with the effects of blood-invigorating and stasis-removing, such as Bu-Yang-Huan-Wu decoction recorded in *Yilin Gaicuo*, a famous TCM canon in China's Qing Dynasty (A.D. 1616–1911). SNK has been used clinically about 10 years for the treatment of acute and chronic cerebrovascular diseases due to its traditional Chinese medicine effects of activating blood circulation and dissipating blood stasis, dredging meridians and collaterals (Song et al., 2011). In clinical practice, SNK could significantly increase cerebral blood flow and effectively ameliorate neurological function in stroke patients (Wen et al., 2013). Besides, the active constituents of SNK, such as tanshinone IIA, puerarin, ferulic acid and astragaloside, have already been reported to improve neurological deficits and ischemia-induced cerebral injury (Li et al., 2012; Sung et al., 2012; Tang et al., 2007; Tian et al., 2011). However, the underlying therapeutic mechanisms of SNK on cerebrovascular diseases are still unclear even though it has shown to provide good clinical application.

Therefore, in the present study, we investigated the protective effect and potential mechanisms of SNK on cerebral I/R injury induced by middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion for 24 h, in order to decipher the clinical efficacy and make better application of SNK.

2. Materials and methods

2.1. Experimental animals

Male Sprague-Dawley (SD) rats (Certificate no. SCXK 2012-0001) weighing 280–320 g, were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). They were maintained in animal laboratory of specific pathogen free (Certificate no. SYXK 2010-004) controlled at constant temperature of 23 ± 2 °C with a relative humidity of $45 \pm 5\%$ and a 12 h light/dark cycle. All animal procedures were approved by the Institutional Animal Care and Use Ethical Committee of the Northwest University, Xi'an, China.

2.2. Reagents and chemicals

Nimodipine (NMP) tablets (Lot no. 101207) manufactured by Shanxi Yabao Pharmaceutical Group Co., Ltd. (Shanxi, China) and were diluted in saline to 2 mg/mL. 2,3,5-triphenyltetrazolium chloride (TTC) was purchased from Sigma-Aldrich Co. (St. Louis, USA). The kits for biochemical analysis, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), inducible nitric oxide synthase (iNOS) and total nitric oxide synthase (TNOS), were purchased from Nanjing Jian Cheng Bioengineering Institute (Nanjing, China). Caspase-3 antibody was purchased from Santa Cruz Biotechnology, Inc. (USA).

2.3. Preparation of SNK

The composition of SNK is listed in Table 1. Crude materials of SNK were supplied by the 323rd hospital of People's Liberation Army (Xi'an, China) and carefully identified by Professor Wenji Sun of Northwest University (Xi'an, China). Standards of tanshinol (110855-200506), protocatechuic aldehyde (110810-200205), paeoniflorin (0736-9608), puerarin (1111-081021), ferulic acid (110773-200611) and tanshinone IIA (0766-200213) were purchased from the National Institute for the Control of Pharmaceutical and Biology Products (Beijing, China).

Astragalus membranaceus (Fisch.) Bge. and *Leonurus japonicus* Houtt. were dried, mixed in proportion and decocted twice by refluxing with water (1:10 and then 1:8, w/v) for 2 h. The filtrates were combined and condensed. Then the other drugs were pulverized and added. Then it was freeze-dried under vacuum and made into drug powder eventually (yield: 72.99%). This procedure followed the TCM canon and remained consistent with the clinical preparation method. The common human daily dose of SNK is 7.86 g/75 kg in body weight. According to the formula: $d_{rat} = d_{human} \times 0.71/0.11$, the common dose of SNK for rat should be 0.68 g/kg/day. In general, rat's drug tolerance is higher than that of human (Huang et al., 2004). In the present study we selected 0.7, 1.4 and 2.8 g/kg/day as low, middle and high dosages for rat, respectively. Therefore, the drug powder was dissolved in saline and diluted to the concentrations of 70 mg/mL, 140 mg/mL or 280 mg/mL before use, respectively. To ensure the quality and stability of the SNK solution, we used high performance liquid chromatography (HPLC) to identify the active compounds.

2.4. HPLC analysis

The analysis of the extract was performed by HPLC using Waters 2695 HPLC system and Welch Materials C18 column (250 mm × 4.6 mm, ID 5 μm) with the column temperature at 30 °C. The mobile