JTCM 中医杂志 Journal of Traditional Chinese Medicine

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J Tradit Chin Med 2017 June 15; 37(3): 333-340
ISSN 0255-2922

RESEARCH ARTICLE

Neuroprotective effect of Naomaitong extract following focal cerebral ischemia induced by middle cerebral artery occlusion in rats

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Supported by National Natural Science Foundation of China (Study on the Material Basis and the Ratio of the Effective Components of Naodesheng Based on the Combination of Fingerprint and Metabolic Network, No. 81274059; Study on the Material Basis of Naomaitong in the Treatment of Ischemic Stroke Based on the *in vivo* Dynamic Effect and Bioinformatics, No. 81274060; Study on the *in vivo* Process and Compatibility Rule of Naomaitong Based on the PK-PD of Effective Components and the Multiobjective Optimization, No. 81473413)
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Accepted: June 17, 2016

Abstract

OBJECTIVE: To examine the neuroprotective effect of extract from Naomaitong following focal cerebral ischemia reperfusion induced by occlusion of middle cerebral artery (MCA), and to determine the biochemical alterations in urine using proton nuclear magnetic resonance spectroscopy and principal component analysis.

METHODS: Wistar rats were randomly assigned to three groups: sham-operated group, MCA focal cerebral ischemia reperfusion model group, and active extract of Naomaitong treatment group. The model was established by an improved MCA occlusion (MCAO) method. Sham-operated rats received the same surgical procedure, but without occlusion. The Naomaitong treatment group were treated with active extract from Naomaitong at a dose of 3.0 $g \cdot kg^{-1} \cdot d^{-1}$. Brain tissues and urine samples were collected from all groups for histopathological assessment and proton nuclear magnetic resonance spectroscopy-based metabonomics, respectively.

RESULTS: Hematoxylin-eosin and triphenyl tetrazolium chloride staining of brain tissues showed a significant decrease in cerebral infarction area in the Naomaitong group. In model rats, metabonomic analyses showed increased urinary levels of glutamate, taurine, trimetlylamine oxide, betaine, and glycine, and reduced levels of creatinine and creatine. Naomaitong regulated the metabolic changes by acting on multiple metabolic pathways, including glycine metabolism, glutaminolysis, transmethylation metabolism and creatinine metabolism.

CONCLUSION: These data demonstrate that extract from Naomaitong is neuroprotective against focal cerebral ischemia induced by MCAO, and can alleviate biochemical changes in urinary metabolism. Metabonomics may be a useful approach for assessing the biochemical mechanisms underlying the neuroprotective actions of extract from Naomaitong.

Keywords: Cerebrovascular circulation; Reperfu-

sion; Middle cerebral artery; Principal component analysis; Metabonomics; Magnetic resonance spectroscopy; Naomaitong

INTRODUCTION

Cerebral apoplexy, the acute occurrence of neurological symptoms induced by a vascular lesion in brain, accounts for approximately 60%-80% of all cerebrovascular accidents.[1] Occlusion of the middle cerebral artery (MCAO) is a general cause of stroke in humans,[2] and is commonly used in animal models for studying cerebral apoplexy.[3] There are numerous mechanisms considered to be involved in ischemic brain damage, including lipid peroxidation, overproduction of free oxygen radicals, increased intracellular calcium, and neuroinflammation.[4] Nevertheless, there are limited therapeutic options, largely due to the complications resulting from ischemic/reperfusion injury.[5]

Western Medicines used for clinical prevention of stroke primarily include aspirin, clopidogrel, and rosuvastatin.[6] Aspirin is an essential drug used to prevent and control stroke, and it may also be useful for preventing stroke relapse. However, 47% of stroke patients exhibit a resistance to aspirin. Further, even enteric-coated aspirin can have detrimental effects on the gastrointestinal system.[7] Clopidogrel also has a potentially serious side effects associated with hepatotoxicity,[8] while rosuvastatin can cause ischemic colitis.[9] Traditional Chinese Medicine (TCM) can provide therapeutic benefits for numerous diseases, including Complex Salvia Miltiorrhiza Dripping pills for cardiovascular diseases[10] and Liuwei Dihuang pills for kidney lesions.[11] In addition, the effects of TCM are usually more prolonged than Western Medicine, with less adverse reactions or rebound phenomena after withdrawal of medication.

There are numerous TCM for treatment of stroke, including Qingkailing injection[12] and Mailuoning injection.[13] Naomaitong, which comprises Renshen (*Radix Ginseng*), Dahuang (*Radix Et Rhizoma Rhei*), Gegen (*Radix Puerariae Lobatae*) and Chuanxiong (*Rhizoma Chuanxiong*), was reported to be neuroprotective against cerebral ischemia injury, by reducing the degree of cerebral ischemia, water content, and brain infarction area.[14,15] Nevertheless, the metabolic mechanisms underlying these actions of Naomaitong remain unclear.

Metabolomics/metabonomics is a relatively new approach for monitoring biochemical changes caused by endogenous and exogenous factors.[16] Analysis techniques for metabolomics include proton nuclear magnetic resonance spectroscopy (^{1}H NMR), mass spectrometry (MS), and gas/liquid chromatograph-MS (GC/LC-MS).[17] Nicholson *et al* [18] reported that ^{1}H NMR spectroscopy of biofluids allowed the simultaneous measurement of endogenous metabolites, providing a biochemical fingerprint of the organism, as well as potential biomarkers. Based on the combination of ^{1}H NMR with multivariate data analysis, metabonomics is now a well-established technique for metabonome analysis, including widespread applications in pharmacological studies.[19] For example, Huo *et al* [20] adopted NMR-based metabonomics to successfully determine the essence of blood deficiency syndrome and the active mechanism of Siwutang. ^{1}H NMR-based metabonomics was also applied to study the restorative effect and potential mechanisms of Buzhongyiqi Tang in a spleen-*Qi* deficiency rat model, providing a useful tool for assessment of the restorative effects of Buzhongyiqi Tang both dynamically and holistically.[21]

In the present study, we analyzed the neuroprotective action of Naomaitong treatment following cerebral ischemia in MCAO model rats, and assessed the urinary metabolites changes using a ^{1}H NMR-based metabonomics approach.

MATERIALS AND METHODS

Materials and reagents

Renshen, Dahuang, Gegen and Chuanxiong were purchased from Guangzhou Zhixin Pieces of Chinese Medicine Co., Ltd. (Guangzhou, China), and were identified by Prof. Li Shuyuan (Department of Chinese Medicine of Guangdong Pharmaceutical University). The extract of Naomaitong was prepared in our laboratory. The four herbs were weighed according to the prescription of Naomaitong, and placed into a round bottomed flask and dissolved in 60% ethanol with ten times the amount of the herbs. The mixture was then extracted using the water bath reflux method for 1 h, and the procedure was repeated twice more. A 60% ethanol elution solvent and recycled ethanol were collected by rotating evaporation, and then concentrated to 400 mL. Finally, the extract (0.3 g/mL dose) was prepared for purification of the active extract of Naomaitong.

The optimal parameters for purification of the active extract of Naomaitong using D101 macroporous absorption resin included a 1∶6 diameter height ratio, a 0.3 g/mL concentration of the extract fluid, and 1 bed volume (BV)/h flow rate. After 2 h of sample loading, we rinsed the macroporous absorption resin using 2 BV water until the solution became colorless, and discarded the eluent. The macroporous absorption resin was then rinsed with 8 BV 50% alcohol. A 50% alcohol elution solvent and recycled alcohol were collected by rotating evaporation. Finally, the concentrated alcohol elution solvent was diluted with distilled water to a concentration of 0.3 g/mL as the active extract of Naomaitong formula.

Sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), and heavy water (D_2O) containing sodium 3-trimethylsilyl-(2, 2, 3,