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EXPERIMENTAL STUDY

Neuroprotective effect of ethyl acetate extract from gastrodia elata against transient focal cerebral ischemia in rats induced by middle cerebral artery occlusion

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Abstract

OBJECTIVE: To investigate the protective effect of neuroprotection against transient focal cerebral ischemia of the extract from Tianma (*Rhizoma Gastrodiae*) and the possible mechanisms underlying the action.

METHODS: Cerebral ischemia-reperfusion injury was induced through middle cerebral artery occlusion (MCAO). Adult male Sprague-Dawley rats were randomly divided into four groups: sham-operated, ischemia-reperfusion model, 102.6 mg/kg extract treated and 11.4 mg/kg extract treated groups. The extract was prepared from gastrodia elata with ethyl acetate. The effect of the extract tested on rat neurological deficits and Cerebral index, cerebral infarct volume, brain injury, terminal dexynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and B-cell lymphoma-2 (Bcl-2) positive cells.

RESULTS: The extract was able to reduce neurological scores, cerebral index and cerebral infarction rate. The brain injury was also relieved by the extract. The results of immunofluorescence staining analysis indicated that the extract increased the expression of Bcl-2 and reduced TUNEL-positive cells significantly in the extract treated groups.

CONCLUSION: These results suggested that the extract relieved ischemic injury induced by transient focal cerebral ischemia in rats, and this neuroprotective effect might be partially due to the attenuated apoptosis pathway.

Key words: Ethyl acetate; Gastrodia; Neuroprotective agents; Reperfusion injury; Apoptosis

INTRODUCTION

In China, 1.5 million people die from stroke every year, and in developed countries, stroke is the third leading cause of death after cancer and heart diseases. Among all stroke cases, ischemic strokes account for approximately 87% , and it has produced negative im-

pacts on our society and economy.[1] A large number of studies found that apoptosis was the delayed neuronal death after the occurrence of chemic lesions.[2] The Bcl-2 family proteins play a crucial role in the control and the execution of the intrinsic, or mitochondrial, pathway of apoptosis.[3] Bcl-2, an inhibitor of apoptosis, preserves mitochondrial integrity and prevents the release of apoptogenic molecules such as cytochrome c.[4] The inhibition of cytochrome c release can prevent the DNA fragmentation, and eventually inhibit apoptotic cell death. The HSP-70 expression has been extensively demonstrated in the brain during brain ischemia, which suggested that the expression aided cell survival and recovery after ischemic injury.[5] Some studies have found that up-regulation of HSP 70 expression, at least in part, played a role in the induction of ischemic tolerance.

Gastrodia elata (GE), Blume (Orchidaceae), Tianma (*Rhizoma Gastrodiae*), is a traditional Chinese herbal medicine that has been used as an anti-convulsant in oriental countries for centuries. GE has also been used for the treatment of rheumatism, sedation, paralysis, hemiplegia, lumbago and headaches.[6] The herb was authorized for medical utilization by China Pharmacopeia. Data from study suggest that the protective effects of GE extract on 1-Methyl-4-phenylpyridinium (MPP^+)induced cytotoxicity in dopaminergic cells, which can be ascribed to its significant anti-oxidative and anti-apoptotic properties.[7] Vanillin, 4-hydroxybenzyl aldehyde and 4-hydroxybenzyl alcohol can prevent hippocampal CA1 cell death following global ischemia.[8,9] The ethyl acetate extract of Gastrodia elata (EEGE) demonstrated high anti-angiogenic activity in the Chorioallantoic membrane (CAM) assay.[10] Previous works had been reviewed and the cumulative evidence suggested that EEGE has protective effect on cerebral ischemia-reperfusion injury. This study was aimed to examine its neuroprotective effects against infarction, neurological deficits, and apoptosis in rat model of focal cerebral ischemia.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats of Specific Pathogen Free (SPF) grade (five-month-old, weighing 300 ± 50 g), were used in the experiments. The rats were purchased from the Central Animal Facility of sichuang Chinese [Laboratory animal certificate: scxk (chuang) 2008-24, Sichuan, China] and fed in the Laboratory Animal Center of Yunnan University of Traditional Chinese Medicine. The study was approved by the experimental animal ethics committee of Yunnan University of Traditional Chinese Medicine (TCM).

Preparation of EEGE

Dried and ground rhizomes of GE (Xiaocaoba, Zhaotong, China) 10.0 kg were extracted with 70% Ethanol (EtOH) (Kemiou Chemical Reagent Co., Ltd., Tianjin, China) (25L × 3) at room temperature. The EtOH extract, after removal of the solvent by evaporation, was suspended in H_2O and partitioned with ethyl acetate (EtOAc). The resulting fractions is a portion of the EtOAc-soluble fraction (EEGE, 114 g). The maximum trolerated dose (MTD) of EEGE is 634 mg/kg, equivalent to 205 times the clinical dose. EEGE-treated groups including 102.6 mg/kg EEGE and 11.4mg/kg EEGE,were safe.

EEGE administration

Thirty-two adult male Sprague-Dawley ratswere randomly divided into A, B, C and D groups (n = 8 per group) by random number table method: group A, sham-operated; group B, schemia-reperfusion models; group C, treated with 102.6 mg/kg of EEGE, and group D treated with 11.4 mg/kg of EEGE. The EEGE was administered for 5 days (q.d.) before right middle cerebral artery was occluded. Sham-operated and modeled animals were given equal volumes of same vehicle.

Establishment of transient cerebral ischemia models in rats

Transient cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) in rats as previously described.[11-13] Briefly, rats were anesthetized by 10% chloral hydrate (3 mL/kg, intraperitoneally). Body temperature was maintained at (37.0 ± 0.5) ℃ during and shortly after surgery by a heating lamp. After a midline neck incision, the right common carotid artery was carefully isolated from the vagal nerve, and external carotid artery was ligated. A nylon filament (diameter 0.26 mm) (Prodo Co., Ltd., Tokyo, Japan) was gently introduced into the right internal carotid artery through the common carotid artery, so as to occlude the origin of the middle cerebral artery. After 2 h ischemia, the nylon filament was carefully removed to establish reperfusion. Sham-operated animals underwent exposure of vessels without occlusion of common carotid arteries.

Measurement

Focal cerebral ischemia in the rats was induced by MCAO. After 6 h and 24 h reperfusion, the neurological deficits were evaluated by Bederson's improved method.[14,15] The neurological evaluation parameters are described in Table 1.

Cerebral index

The cerebral index was defined as: cerebral index = brain wet weight / body weight. The brains were removed and weighed 24 h after MCAO. The preoperative weight and postoperative weight (24 h after MCAO) were weighed.

Rate of decreased weight = (preoperative weight − postoperative weight) / preoperative weight × 100%