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Huang-Lian-Jie-Du-Decotion induced protective autophagy against the injury of cerebral ischemia/reperfusion via MAPK-mTOR signaling pathway

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ABSTRACT

Ethnopharmacological relevance: Huang-Lian-Jie-Du-Decotion (HLJDD, Hwangryun-Hae-Dok-Decotion in Japan), an ancient antipyretic and detoxifying traditional Chinese medicine formula, was reported to have protective effect on ischemic stroke.

Aim of the research: To investigate the therapeutic effect of HLJDD on ischemic stroke and explore its mode of action.

Material and methods: A model of ischemic stroke in the rat was established after transient middle cerebral artery occlusion (MCAO) followed by reperfusion. Rats were assigned randomly to groups of control, sham, transient ischemia/reperfusion (I/R), and three treatment groups by HLJDD at 2.5, 5.0, 10.0 mg/kg. The neurological deficit, the cerebral infarct size, morphology abnormality, biochemical parameters were examined, and the levels of relevant proteins were determined by immunoblotting analysis to evaluate the protective effects of HLJDD on ischemic stroke and explore the underlying mechanism.

Results: Compared with I/R group, HLJDD significantly ameliorated neurological deficit and histopathology changes, decreased infarct area, and restored the levels of biochemical indicators including nitric oxide (NO), malondialdehyde (MDA), glutathione (GSH), glutathione disulfide (GSSG), total superoxide dismutase (T-SOD), Cu/Zn-SOD, Mn-SOD and glutathione peroxidase (GSH-PX). HLJDD also notably elevated the levels of microtubule-associated protein1 light chain 3 (LC3), Beclin-1, and other autophagy related genes (Atgs), promoted the activation of extracellular signal-regulated kinases (ERK), protein kinase B (Akt), 3-phosphoinositide-dependent kinase (PDK1), and inhibited the activation of mammalian target of rapamycin (mTOR), c-Jun N-terminal protein kinases (JNK), p38, phosphatase and tensin homolog (PTEN).

Conclusion: HLJDD showed neuroprotective effects on ischemic stroke, at least in part to the induced protective autophagy via the regulation of mitogen-activated protein kinase (MAPK) signals. This Akt-independent protective autophagy is favorable in the treatment of stroke, avoiding unfavorable side-effects

Abbreviations: HLJDD, Huang-Lian-Jie-Du-Decotion; MCAO, middle cerebral artery occlusion; TTC, 2,3,5-triphenyltetrazolium chloride; HE, hematoxylin–eosin; NO, nitric oxide; MDA, malondialdehyde; GSH, glutathione; GSSG, glutathione disulfide; T-SOD, total superoxide dismutase; GSH-PX, glutathione peroxidase; LC3, microtubule-associated protein1 light chain 3; Atg, Autophagy related genes; ERK, extracellular signal-regulated kinases; Akt, protein kinase B; PDK1, 3-phosphoinositide-dependent kinase; mTOR, mammalian target of rapamycin; JNK, c-Jun N-terminal protein kinases; PTEN, tensin homolog; MAPK, mitogen-activated protein kinase; t-PA, tissue-plasminogen activator; IPC, ischemic preconditioning; w/v, weigh/volume; i.g., intragastric administration; CMC-Na, carboxymethyl cellulose sodium salt; I/R, ischemia/reperfusion; Fig., Figure; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; MCA, middle cerebral artery; LCBF, local cortical blood flow; LDF, laser Doppler flow meter; NADH, (β-Nicotinamide adenine dinucleotide, reduced disodium salt, trihydrate); DTNB, 5,5′-Dithiobis-(2-nitrobenzoic acid); WST-1, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium; v: v, volume: volume; BCA, Bicinchoninic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA, Fetal Bovine Serum Albumin; TBS-T, tris-buffered saline containing 0.1% Tween-20; ECL, enhanced chemiluminescence; ANOVA, one-way analysis of variance; S.D., standard deviation; ROS, reactive oxygen species; mTORC1, mTOR complex1; PAPTOR, scaffolding protein regulatory-associated protein of mTOR; mLST8, mammalian lethal with Sec13 protein 8; PI3K, phosphatidylinositol 3-kinase; RICTOR, scaffolding protein rapamycin-insensitive companion of mTOR; mSIN1, mLST8 and mammalian stress-activated protein kinase interacting protein; S6K, p70 ribosomal S6 kinase; PIP_3, phosphatidylinositol (3,4,5) trisphosphate; PIP_2, phosphatidylinositol (4,5) bisphosphate.

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associated with the inactivation of Akt. The efficacy of HLJDD on ischemic stroke and its safety warranted by its long-term clinical use in traditional Chinese medicine favored further study to develop HLJDD as an effective therapeutic agent to treat ischemic stroke.

1. Introduction

Following cardiovascular diseases and cancer, ischemic stroke is the third worldwide leading cause of mortality and chief cause of handicap with documented high incidence and relapse rate (Hong and Saver, 2009; Doyle et al., 2008). Due to a short therapeutic time window of currently 3 h after stroke onset (Lees et al., 2010) and further progression of neuronal damage, most victims without effective treatment suffer serious physical and cognitive disabilities (Goldstein and Rothwell, 2008). Therefore, great efforts have been made to develop neuroprotective agents (Ginsberg, 2008). However, most of them failed due to poor efficacy or severe toxicity/side effects (Sahota and Savitz, 2011; Yu et al., 2012). It is extremely urgent to develop new therapeutic agents with favorable no or low side-effects for the treatment of ischemic stroke.

Huanglian-Jie-Du-Decotion (HLJDD, Hwangryun-Hae-Dok-Decotion in Japan), is a representative antipyretic and detoxifying prescription, composed of Coptidis rhizoma, Scutellariae radix, Phellodendri cortex and Gardeniae fructus in a weight ratio of 3:2:2:3. Mentioned firstly in Wang Tao's "Wai Tai Mi Yao" two thousand years ago, it has been widely used to treat various ailments, such as liver injury (Hsu et al., 2008; Ohta et al., 2004), gastrointestinal disorders (Ohta et al., 1999; Wang and Mineshita, 1996), inflammation (Lu et al., 2011), cardiovascular diseases (Sekiya et al., 2005) and multiple myeloma (Ma et al., 2005). HLJDD and its components (Cui et al., 2010; Kondo et al., 2000; Wu et al., 2004; Xu et al., 2000; Zhang et al., 2009) have been so far widely acknowledged to ameliorate acute or chronic cerebral ischemia-induced neuron losses, learning and memory deficits, or other neurodegeneration. Such abilities could be ascribed to their anti-oxidant, anti-inflammatory, anti-apoptotic properties (Hwang et al., 2002; Kabuto et al., 1997; Mu et al., 2009; Song et al., 2012; Ye et al., 2012a; Zhang et al., 2006; Zhou et al., 2008).

In this study, a model of ischemic stroke in the rat was established after 2 h of transient middle cerebral artery occlusion (MCAO) followed by 24 h reperfusion. The effect of HLJDD on ischemic stroke and its neuroprotective mechanism was investigated.

2. Experiment procedure

2.1. Materials

Component herbs of HLJDD (Coptidis Rhizoma, Scutellariae Radix, Phellodendri Cortex and Gardeniae Fructus) were purchased from Jiangsu Medicine Company (Nanjing, China) and authenticated by Professor Mian Zhang, Department of Medicinal Plants, China Pharmaceutical University, Nanjing, China (Table 1). Voucher specimens (2011066-CR, 2011067-SR, 2011068-PC and 2011069-GF for Coptidis Rhizoma, Scutellariae Radix, Phellodendri Cortex and Gardeniae Fructus, respectively) have been deposited at the herbarium of the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Standards of baicalin and baicalein were purchased from the National Institute for the Control of Pharmaceutical and Biology Products (Beijing, China). Columbamine, Jatrorrhizine, Epiberberine, Berberine, Coptisine, and Palmatine were isolated and purified from Coptidis Rhizoma; and Geniposide from Gardeniae Fructus in our laboratory with purities of more than 98%. Structures of the standard reference compounds were shown in Fig. 1. All of the chemical reagents evolved in this research were of analysis grade.

2.2. Preparation of HLJDD extraction

Coptidis Rhizoma, Scutellariae Radix, Phellodendri Cortex and Gardeniae Fructus, in a ratio of 3:2:2:3, were mixed to reach a final weight of 2 kg, which was refluxed thrice with 70% ethanol (1:10, 1:10 and 1:5, w/v), 3 h each. The decoction was concentrated to dryness on a rotary vacuum evaporator, affording 586.21 g HLJDD (yield: 29.31%), which was stored in refrigerator at 4 °C and suspended in 0.5% CMC-Na (carboxymethyl cellulose sodium salt) before intragastric administration.

2.3. HPLC analysis

HPLC analysis was made using Shimadzu LC2010 series coupled with Shimadzu SPD-M10A photodiode array detector (Shimazdu, Kyoto, Japan). Chromatographic separation was performed on Ultimate XB-C18 column (250 × 4.6 mm^2, ID 5 μm, Welch Materials, Inc., USA) with flow rate of 1 mL/min and injection volume of 5 μL (Lu et al., 2011). The mobile phase used was a mixture of water containing 10 mmol/L ammonium acetate (A), titrated with acetic acid to pH 3.0, and, acetonitrile (B) in gradient: 0–4 min, 10% B; 4–15 min, 10–26% B; 15–27 min, 26–28% B; 27–35 min, 28–70% B; 35–55 min, 70–90% B; 55–60 min, 90% B.

2.4. Experiment animal

Male Sprague-Dawley rats, clear grade, aged 10 weeks (280 ± 20 g) were purchased from Comparative Medicine Centre of Yangzhou University (Yangzhou, China). All rats were reared on a 12-h light/12-h dark regime at 25 ± 2 °C and provided with water and standard chow ad lib for the whole experiment procedure. The care and use of laboratory animals were strictly under obligations of the Animal Ethics Committee of China Pharmaceutical University and the experiment was conducted under the standard guidelines of laboratory animal care (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Table 1
Component herbs of HLJDD.

	Botanical plant name	Family	Part used	English name	Chinese name	Place of production
Coptidis Rhizoma	*Coptis chinensis* Franch	Ranunculaceae	Rhizome	Chinese Goldthread Rhizoma	Wei Lian	Si Chuan Province, China
Scutellariae Radix	*Scutellaria baicalensis* Georgi	Labiatae	Radix	Baikal Skullcap Root	Huang Qin	He Bei Province, China
Phellodendri Chinensis Cortex	*Phellodendron chinensis* Schneid	Rutaceae	Cortex	Amur Corktree Bark	Chuan Huang Bai	Hei Longjiang, China
Gardeniae Fructus	*Gardenia jasminoides* Ellis	Rubiaceae	Fructus	Common Gardenia Fruit	Zhi Zi	Hu Nan Province, China

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