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Corydalis hendersonii Hemsl. protects against myocardial injury by attenuating inflammation and fibrosis via NF-κB and JAK2-STAT3 signaling pathways

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ABSTRACT

Ethnopharmacological relevance: Corydalis hendersonii Hemsl. (CH) with heat clearing and detoxifying effects are well described in Tibetan folk medicine. It has been used for centuries in China largely for the treatment of high altitude polycythemia, a pathophysiological condition referred to “plethora” in Tibetan medicine, hypertension, hepatitis, edema, gastritis, and other infectious diseases.

Aim of the study: To investigate the cardioprotective effects of *Corydalis hendersonii* extract in an ICR mouse model of myocardial ischemic injury.

Materials and methods: Ethanol [85% (v/v)] extract of CH whole plant was prepared, and their chemical profile was analyzed with use of HPLC-DAD and IT-TOF-ESI-MS. A mouse model of AMI was established by ligation of the left ventricular dysfunction (LAD) coronary artery. Mice were randomly divided into six groups (n = 12 per group): sham group, model group, CH groups treated with three doses of CH (100, 200, and 400 mg/kg, intragastric), and a positive control group (captopril, 16.67 mg/kg, intragastric). Heart function was evaluated by measurement of ejection fraction (EF) and fractional shortening (FS) by echocardiography. Serum levels of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH), plasma levels of angiotensin II (AngII), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1ß (IL-1ß) and expressions of matrix metalloproteinase-2 (MMP-2) and MMP-9 in the cardiac tissue homogenate, protein expressions of signal-transduction proteins, p65, IκBα, JAK2, and STAT3 in heart tissues were measured by ELISA and Western blot analyses. Inflammatory cell infiltration and changes in collagen deposition in the myocardial ischemic heart tissues were observed by histopathological examination. Platelet aggregation in vitro was also assessed.

Results: CH treatment showed a dose-dependent cardioprotective effect. It significantly reduced left ventricular end-diastolic diameter (LVEDd) and left ventricular end-diastolic diameter (LVEDs), improved EF and FS as compared to those in the model group; attenuated the increase levels of CK-MB and LDH in serum; reduced expressions of AngII, TNF-α, IL-6 and IL-1ß in plasma, MMP-2 and MMP-9 expressions in the cardiac tissue homogenate; and down-regulated myocardial expressions of p-p65, p-IκBα, p-JAK2, p-STAT3, MMP-2, and MMP-9 in AMI mice. Also, an obvious reduction in inflammatory cell infiltration in the myocardial infarct was

Abbreviations: AA, arachidonic acid; ACEI, angiotensin converting enzyme inhibitor; ADP, adenosine 5-diphosphate; AMI, acute myocardial infarction; AngII, angiotensin II; CH, *Corydalis hendersonii Hemsl.*; CK-MB, creatine kinase-MB; EF, ejection fraction; FS, fractional shortening; HAPC, high altitude polycythemia; HE, hematoxylin-eosin; HF, heart failure; HRMS, high resolution mass spectrometer; IHD, ischemic heart disease; IL-6, interleukin-6; JAK2-STAT3, janus kinase 2-signal transducers and activators of transcription 3; LAD, left anterior descending; LDH, lactate dehydrogenase; LVD, left ventricular dysfunction; LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; PRP, platelet-rich plasma; RAAS, renin-angiotensin aldosterone system; TCM, Traditional Chinese Medicine; THR, thrombase; TNF-α, tumor necrosis factor-α

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[4] These authors are involved in the analysis of related biochemical indexes, Western blotting experiments, echocardiography, and immunofluorescence assays.

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found in all CH treated groups. Besides, CH also inhibited platelet aggregation induced by THR, ADP, and AA. *Conclusion:* CH extract exerted a protective effect against myocardial ischemic injury via inhibition of inflammation, myocardial fibrosis, and platelet aggregation. This study demonstrates such protection for the first time and provides a basis for development of CH-based drugs for treatment of ischemic heart disease in clinical settings.

1. Introduction

Acute myocardial infarction (AMI) is the leading cause of congestive heart failure and death in the Western world (Shen et al., 2011). In the United States, there are about 1.5 million individuals suffering from AMI each year (Ma et al., 2013). Pathogenesis of myocardial ischemic injury involves complex interplay between several molecular pathways. Inflammation and fibrosis play a fundamental roles in the development of AMI (Seropian et al., 2014; Wang et al., 2015). The most common complications of AMI are recognized as left ventricular dysfunction (LVD) and heart failure (HF), along with platelet hyperactivity in the early hours following the event (Guha et al., 2011). Therefore, inhibition of inflammation, fibrosis, and platelet aggregation may attenuate inflammatory injury, decrease myocardial fibrosis and alleviate microvascular resistance, which are key factors in the prevention of myocardial ischemic injury. Owing to the complex pathogenic mechanism, a single molecule approach to treatment of AMI therapy is inherently challenging. In this context, traditional medicines have evoked much interest for treatment of AMI. In particular, TCM which has been used to treat cardiovascular diseases for thousands of years is a potential avenue for discovery of potential remedies for IHD (Ferreira et al., 2011).

Corydalis hendersonii Hemsl. (CH, Papaveraceae) is mainly distributed in the northern temperate regions, growing at the overflow lands and alpine screes in a high altitude of 4200–5200 m area in Tibet, China. It is referred to as “ai-zi-jin” in Chinese, and “ri-gun-zi-ma” or “ri-gun” in Tibetan. As a prototype Tibetan medicinal herb, CH has been used for hundreds of years in China mainly to treat high altitude polycythemia (HAPC), a pathophysiological condition referred to “plethora” in Tibetan medicine, hypertension, and fever, hepatitis, edema, gastritis, and cholecystitis (Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 2002). Previous studies have shown that alkaloids especially isoquinolines are the main ingredients of CH. The pharmacological activities of CH extract include effects on the cardiovascular and central nervous system, anti-inflammatory, antibacterial, analgesic, hepatoprotective, and anti-oxidative effects (Shang et al., 2014).

Considering its traditional use in the treatment of HAPC, a hypoxia-induced disease characterized by hyperplasia of red blood cells, and the fact that myocardial injury caused by hypoxia is one of the major pathological manifestations of HAPC (Lu et al., 2016), CH was proposed to have cardioprotective effects. However, the effect of CH extract on cardiac function has not been determined. Therefore, we investigated the cardioprotective effect of CH against myocardial injury using a model of ligating the LAD coronary artery in ICR mice in this study.

2. Materials and methods

2.1. HPLC-DAD-IT-TOF-MS instrument and reagents

A Shimadzu LC system (Shimadzu, Kyoto, Japan) was employed for liquid chromatographic analysis. The LC system was connected by a hybrid ion trap/time-of-flight mass spectrometer quipped with an electrospray ionization (ESI) source to perform high-resolution tandem mass spectrometry. The HPLC analysis was conducted on an Eclipse XDB C_{18} column (250 × 4.6 mm, 5 μm, Agilent). The mobile phase (1.0 mL/min) consists of acetonitrile (A)–0.1% aqueous with formic acid (B) with a gradient program as follows: 0–20 min, 5–20% A; 20–30 min, 20–30% A; 30–40 min, 30–37.5% A; 40–50 min, 37.5–47.5% A; 50–60 min, 47.5–65% A; 60–71 min, 65–95% A. Roughly 25% portion of the effluent was introduced into the ESI source by splitting the effluent via two PEEK tubes with length ratio of 1:3. A detection wavelength of 254 nm and an injection volume of 10 μL were set.

Optimization of the operating conditions, and the equipment and reagents for HRMS analysis were identical to that reported previously (Cao et al., 2016).

2.2. Preparation of CH extracts

The whole plant of *Corydalis hendersonii* was collected in July 2013 from Shannan Autonomous Prefecture, Tibet, China, and authenticated by Dr. Zhang Y. (Beijing University of Chinese Medicine) (BUCM). A voucher specimen is deposited in the Modern Research Center for Traditional Chinese Medicine, BUCM (No. CH201308). CH (900 g) was refluxed with 85% EtOH (3 × 3.0 L, 2 h each time). The dried extract (150 g) was obtained after removal of the solvent under reduced pressure (Yin et al., 2016). Based on the recommended clinical dosage of 5–9 g/d (Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 2002), three doses of CH of 100, 200, and 400 mg/kg, respectively, suspended in purified water were prepared for the animal experiment.

2.3. Experimental animal

This research was approved by the Ethics Committee at the Beijing University of Chinese Medicine (BUCM-3–2015090701-3003) in accordance with the guidelines for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publications No. 85-23, revised 1996). Male ICR mice (weight: 25–28 g; purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd.) were bred in SPF animal room, under conditions of 22–26 °C temperature, with a 12 h light-dark cycle, and adlibitum access to drinking water. They were divided randomly into six groups with 12 mice per group: sham, model, low dose CH (CH-L, 100 mg/kg), medium dose CH (CH-M, 200 mg/kg), high dose CH (CH-H, 400 mg/kg) (National Pharmacopoeia Committee, 1998), and a positive control (captopril [Cap], 16.67 mg/kg) (Xu, 2002) per day. The doses of CH groups and Cap group are 10 times of that used in clinical settings. Intragastric doses were administered for seven consecutive days. All test components were diluted in MQ water. Mice in the sham and model groups were treated with MQ water.

2.4. Myocardial ischemia model

A model of myocardial ischemia was induced by direct ligation of the left anterior descending (LAD) coronary artery as previously described (Tao et al., 2015). Briefly, mice were anesthetized with 0.5% pentobarbital sodium (50 mg/kg intraperitoneal), then tracheal intubation with a ventilator (Alott Biotech Co., LTD, Shanghai, China). To expose the heart a thoracotomy was performed in the third and fourth intercostal spaces. The LAD coronary artery was ligated 2–3 mm from the left atrial appendage origin with a 7-0 polyester suture (Shanghai Yiling Medical Equipment Sales Co., LTD, Shanghai, China). A 5-0 polyester suture was used to close the thorax. Sham group received the same thoracotomy procedure except that their LAD