Phytomedicine 19 (2012) 193–205

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Effects of ethanolic extract from Radix Scrophulariae on ventricular remodeling in rats

Xiao Yan Huang[a], Chang Xun Chen[a,*], Xue Mei Zhang[b], Ying Liu[a], Xi Min Wu[b], Yi Ming Li[b]

[a] Department of Pharmacology, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China
[b] Department of Natural Product Chemistry, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

ARTICLE INFO

Keywords:
Radix Scrophulariae
Ethanolic extract
Rat
Coronary artery ligation
Ventricular remodeling

ABSTRACT

Purpose: To explore the effects of ethanolic extract of Radix Scrophulariae (EERS) on ventricular remodeling in rats.
Methods: Rats with coronary artery ligation (CAL) were randomly assigned to 5 groups: CAL model; CAL plus 40 mg/kg captopril; CAL plus 60 mg/kg, 120 mg/kg, 240 mg/kg EERS. Sham operation rats were randomly assigned to 2 groups, sham-operated control and sham-operated plus 120 mg/kg EERS. The rats were orally administered with the corresponding drugs or drinking water for 14 weeks. The left ventricular weight index (LVWI) and heart weight index (HWI) were determined. Myocardium tissue was stained with hematoxylin and eosin or picric acid/Sirius red for cardiomyocyte cross-section area or collagen content measurements respectively. The concentrations of hydroxyproline (Hyp), matrix metalloproteinase 2 (MMP-2), angiotensin II (Ang II), aldosterone (ALD), endothelin 1 (ET-1), atrial natriuretic peptide (ANP), tumor necrosis factor α (TNF-α) and renin activity (RA) in myocardium or serum were determined. Real-time RT-PCR was used to detect the mRNA expressions of angiotensin converting enzyme (ACE), ET-1 and ANP.
Results: EERS could significantly reduce the LVWI and HWI, decrease heart tissue concentrations of Hyp and collagen deposition, diminish cardiomyocyte cross-section area, reduce the tissue level of Ang II, ET-1, ANP and TNF-α. EERS could also down regulate the mRNA expression of ACE, ET-1 and ANP in myocardium.
Conclusion: EERS attenuates ventricular remodeling. The mechanisms may be related to restraining the excessive activation of RAAS, TNF-α and modulating some gene expressions associated with cardiac hypertrophy.

Introduction

Chronic heart failure (CHF) is the ultimate consequence of a vast number of cardiovascular diseases and constitutes one of the worldwide leading causes of morbidity and mortality. It is considered to be an irreversible and progressive process characterized by ventricular remodeling (VR), diminished pump performance and a number of neurohormonal perturbations (Rong et al. 2009). Most of all, ventricular remodeling is considered as the basic mechanism in the process of CHF (Nagase and Woessner 1999). Therefore, inhibiting ventricular remodeling early may be an effective way to postpone heart failure induced by myocardial infarction, hypertension and other cardiovascular diseases.

Radix Scrophulariae, a traditional Chinese herb medicine derives from the *Scrophularia ningpoensis* (Xuanshen), has long been used in clinic to treat febrile diseases with impairment of *Yin* manifested by deep red tongue and dire thirst or with eruptions, constipation due to impairment of body fluid; phtisis with cough, conjunctivitis, sore throat, scrofula, diphteria, boils and sores, internal bleeding (Wagner et al. 2011). The previous study reveals that total rough extracts of Radix Scrophulariae has beneficial effect against ventricular remodeling induced by ligating the left coronary artery of the rats (Gu et al. 2010). The present study was aimed at investigating the midterm effects of ethanolic extract of Radix Scrophulariae on ventricular remodeling in rats, and the underlying mechanisms.

Materials and methods

Drugs and reagents

Preparation of ethanolic extract of Radix Scrophulariae (EERS): 60 kg of dried rough powdered roots of *Scrophularia ningpoensis* was refluxed with 600 l 70% ethanol for 2 h, after taking the filtered solution, the residue was refluxed with the same solvent in the same

* Corresponding author. Tel.: +86 21 51322200; fax: +86 21 51322200.
E-mail address: cxchen6@126.com (C.X. Chen).

0944-7113/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2011.09.079

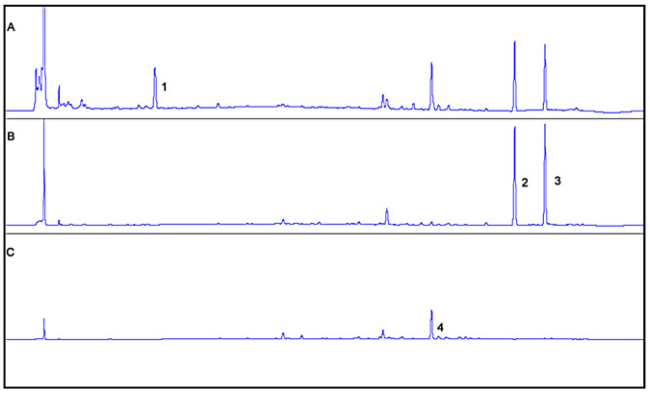

Fig. 1. HPLC chromatograms of ethanolic extract of Radix Scrophulariae (A: 210 nm, B: 280 nm, C: 330 nm). 1, Harpagide; (10.1 min) 2, Harpagoside (33.3 min); 3, Cinnamic acid (35.4 min); 4, Angoroside C (27.9 min).

condition for two more times. The combined extract solution was concentrated under reduced pressure to remove the ethanol. Then, the concentrated solution was subjected to D-1400 macro resin chromatograph column eluted with water, 10% ethanol water solution and 50% ethanol water solution respectively. The eluent parts (the 50% EtOH eluent part was removal of solvent under vacuum at 50 °C first) were subjected to spray dryer with inlet air temperature of 100 °C and outlet air temperature of 60 °C. The extract yield after spray drying was 360 g.

HPLC analyses of the principal constituents of EERS: A HPLC method was developed to check the principal constituents of the extract. 10 mg EERS sample powder was put into a 10 ml of volumetric flask, 8 ml of distilled water was added, sonicated for 3 min, cooled to room temperature and diluted to 10 ml. The solution was filtered for HPLC injection.

Chromatographic analyses were performed on an Agilent1200 series HPLC instrument, quaternary HPLC pump, column heater, diode array detector, and Agilent Chem-Station for data collection and manipulation. Reverse phase separations of the procyanidin oligomers were performed on a 5 μm silica column (250 × 4.6 mm) (Phenomenex, Torrance, CA). Samples were analyzed with a linear gradient from 97% solvent A (water with 0.03% phosphoric acid) and 3% solvent B (100% acetonitrile) to 50% solvent A and 50% solvent B in 42 min at a flow rate of 1.0 ml/min. UV data were collected by using a diode array detector set at 210 nm, 280 nm and 330 nm.

The HPLC chromatograms of EERS were shown in Fig. 1. Four main peaks were determined by comparing with the standard compounds. They were Harpagide with the remain time at 10.1 min under 210 nm, Harpagoside with the remain time at 33.3 min under 280 nm, Cinnamic acid with the remain time at 35.4 min under 280 nm, Angoroside C with the remain time at 27.9 min under 330 nm. After setting up the regression equations of these four standards respectively, the contents of them in the EERS were assayed as Harpagide 18.7%, Harpagoside 13.4%, Cinnamic acid 5.7%, Angoroside C 14.6%. Their chemical formulas were shown in Fig. 2 (Wagner et al. 2011). The other main constituents in EERS were saccharides which had no peaks under UV detector.

The standard compounds of Harpagide, Harpagoside, Angoroside C and Cinnamic acid were purchased from Shanghai Hotmed Sciences Co., Ltd. (Shanghai, China).

Captopril tablets (Lot number: 090727) were from Jiangsu Huanghe River Pharmaceutical Co., Ltd. (Jiangsu, China). They were dissolved in distilled water before use.

Animals and experimental protocols

Male SD rats (180–200 g, grade of specific pathogen free) were supplied by Shanghai Slac laboratory animal Co., Ltd. All animals were maintained in a 12 h light/dark cycle room with the temperature at 22–24 °C and the humidity at 40 ± 5%. The rats received humane care and had free access to a standard diet and drinking water. The animal experiments were approved by the Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine and conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH publication no. 85-23, revised in 1996).

Left ventricular remodeling was created by left coronary artery ligation (CAL) in rats. In brief, rats were anesthetized by intraperitoneal administration of sodium pentobarbital (40 mg/kg), and the chest wall was shaved. Animals were then intubated, ventilated with a type HX-300s animal respirator (Chengdu Technology & Market Co., Ltd.). A left thoracotomy was performed, the heart was exposed and pericardiotomy was then performed, the left coronary artery was ligated approximately 2 mm from its origin with a 4–0 silk suture. Coronary artery occlusion with myocardial infarction (MI) was demonstrated by grossly visible scarring of the change in colour of the left ventricle and ischaemia was confirmed by the raising of ST (ECG-6511 Electrocardiograph, Shanghai Nihon Kohden). Then the thorax was closed immediately and the skin sutured.

Sham-operated rats underwent a similar procedure, but no coronary ligation was performed. After cardiac surgery, each rat was given benzyl-penicillin by intramuscular injection for three days to prevent infection. The operated rats were burdened by elevated mortality during the initial 24 h after CAL (Agnoletti et al. 2006). In our study, the survival rate from the surgery was about 55%.

On the second day after operation, the rats were randomly divided into seven groups: sham-operated control, sham-operated plus 120 mg/kg EERS, CAL plus drinking water (model), CAL plus 40 mg/kg captopril, CAL plus 60 mg/kg EERS, CAL plus 120 mg/kg EERS and CAL plus 240 mg/kg EERS.

The rats were orally administrated with EERS or captopril at above described doses once a day. And drinking water was administered in the same manner to the sham-operated control and model groups. Treatment started from 1 day after operation and continued for 14 weeks.

Hemodynamic parameters measurements

Cages were inspected daily in all groups, 14 weeks after treatment, rat body weight (BW) was recorded after fasting for 12 h and then anesthetized with intra-peritoneal injection of urethane (1.0 g/kg). A polypropylene catheter was inserted into the right carotid artery. The arterial catheter was filled with heparinized saline solution and connected to a pressure transducer. After an equilibrium period for about 5 min, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were recorded with a multi-channel biological signal analysis system. The catheter was advanced into the left ventricle to measure the left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP) and the maximal rate of rise and fall of the ventricular pressure (±dp/dtmax).

Cardiac weight indexes calculation and histological examination

After hemodynamic parameters being recorded, the blood sample was collected from carotid artery and centrifuged (4 °C, 2325 × *g*, 10 min) to recover serum which was stored immediately in a −70 °C freezer until being assayed. The heart was taken out, rinsed with cold saline solution, and the left ventricle was separated from the atria, aorta and adipose tissue. The left ventricle weight (LVW) and heart weight (HW) were measured, and then