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RESEARCH ARTICLE

Effect of Sini decoction on angiotensin Ⅱ, transforming growth factor β_1 and connective tissue growth factor in rats with myocardial fibrosis-induced banding of the abdominal aorta

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Abstract

OBJECTIVE: To investigate the effect of Sini decoction on rats with myocardial fibrosis induced by banding the abdominal aorta, and explore the mechanism underlying its actions on angiotensin Ⅱ (Ang Ⅱ), transforming growth factor-β_1 (TGF-β_1) and connective tissue growth factor (CTGF).

METHODS: Forty-eight male Sprague-Dawley rats were randomly divided into sham operation, model, Captopril, and Sini decoction groups. The models were established by the partial banding of the abdominal aorta according to Doering's method. Eight weeks later, heart weight indexes were calculated; hemodynamic changes of the hearts were tested; changes in myocardial tissue morphology were observed by Masson staining; and myocardial collagen volume fraction was calculated. Enzyme-linked immunosorbent assay was used to measure the concentration of Ang Ⅱ in serum. The expression of TGF-β_1 and CTGF were determined by immunohistochemistry and Western blotting.

RESULTS: Compared with the sham operation group, the heart weight index, collagen volume fraction of the myocardium, serum levels of Ang Ⅱ, and the expression of myocardial TGF-β_1 and CTGF in the model group were significantly increased ($P < 0.05$). Compared with the model group, the heart weight index, collagen volume fraction of the myocardium, serum levels of Ang Ⅱ, and the expression of myocardial TGF-β_1 and CTGF in all treatment groups were significantly reduced ($P < 0.05$).

CONCLUSION: Sini decoction reduced Ang Ⅱ level and inhibited the expression of myocardial TGF-β_1 and CTGF, which may explain the mechanism of its protective effect on myocardium with fibrosis.

Keywords: Myocardial fibrosis; Angiotensin Ⅱ; Transforming growth factor beta1; Connective tissue growth factor; Sini decoction

INTRODUCTION

Myocardial fibrosis is characterized by interstitial fibroblast proliferation and excessive extracellular matrix deposition, and leads to heart failure, arrhythmia, sudden cardiac death and other serious complications.[1] Long-term pressure overload induces ventricular remodeling, including myocyte hypertrophy and interstitial fibrosis. Myocardial fibrosis is one of the most important factors contributing to the transition from compensatory ventricular hypertrophy to heart failure.[2]

Therefore, inhibiting the process of myocardial fibrosis is a crucial issue in clinical treatment. Sini decoction is described in the "Treatise on Febrile Diseases" and is thought to revive the Yang based on the theory of Traditional Chinese Medicine (TCM). A study suggested the Sini decoction prevents myocardial ischemia, protects myocardial cells, antagonizes atherosclerosis, and reduces neointimal proliferation after vascular injury.[3] Our study investigated the Sini decoction efficacy on rats with myocardial fibrosis induced by banded abdominal aorta according to Doering's method,[4] and the possible mechanism involved.

MATERIALS AND METHODS

Drugs and reagents

The Sini decoction was prepared with a Chinese medicinal formula consisting of Fuzi (*Radix Aconiti Carmichaeli*) [130801], Ganjiang (*Rhizoma Zingiberis*) [121201], and Gancao (*Radix Glycyrrhizae*) [130501], using granules free of frying (Beijing Kang Ren Tang Pharmaceutical Co., Ltd., Beijing, China). Other reagents were Captopril Tablets (20130910, Shanghai Xudong Haipu Pharmaceutical Co., Ltd., Shanghai, China); Ang Ⅱ Kit (Biotechnology Research Institute of Beijing North, Beijing, China); rabbit anti-TGF-β1 polyclonal antibody (Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China); diaminobenzidine (DAB) horseradish peroxidase color development kit (Beyotime Biotechnology Research Institute, Beijing, China); rabbit anti-CTGF polyclonal antibody (Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China). Goat anti rabbit IgG antibody (Santa Cruz Biotechnology Inc., Dallas, TX, USA); and modified Masson's trichrome staining kit (Beijing Leagene Biotechnology Co., Ltd., Beijing, China). Urethane was obtained from Shanghai Yika Biological Technology Co., Ltd., Shanghai, China. A silver clip with a diameter of 0.7 mm was made by Shanghai Alcott Biotechnology Co., Ltd., Shanghai , China.

Animals

Forty-eight male Sprague-Dawley rats [8 weeks old; (200 ± 20) g] of Specific Pathogen Free grade were purchased from Liaoning Changsheng Biotechnology Limited Company [Certificate of quality: SCXK (liao) 2010-0001]. Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006), and were approved by the animal ethics committee of Jinzhou Medical University. All rats were maintained at 22-24 ℃ with a relative humidity of 50% and normal photoperiods (12 h light/12 h dark). The rats were fed a normal diet and allowed access to water ad libitum in the Laboratory of the First Affiliated Hospital of Jinzhou Medical University.

Animal modeling and grouping

Overall, 48 rats were randomly divided into four groups using a random number table as follows: sham operation group, model group, Captopril group, and Sini decoction group with 12 rats per group. The models were established by the partial banding of the abdominal aorta according to Doering's method. The rats were anaesthetized by intraperitoneal injection with 10% chloral hydrate (3 mL/kg body weight), fixed in an operating frame, shaved and sterilized. Then, the abdominal cavity was opened at 1 cm below the left costal margin, the retroperitoneal soft tissues were separated at the upper edge of the left kidney to expose the abdominal aorta, which was separated from both kidneys. Next, the abdominal aortas were banded with silver clip (0.7 mm diameter), and finally the abdominal cavities were closed in layers. In the sham operation group, the abdominal cavities were only opened and separated from abdominal aortas without constriction. Three days later, all rats were given an intramuscular injection of gentamicin (0.09 mg/kg) to prevent infection, once a day.

Treatments

The fourth day after the operation, the sham operation group and model group were orally administered with distilled water (1.5 mL/100 g), the Captopril group was perfused with captopril (100 mg/kg) dissolved in distilled water, and the Sini Decoction group was administered with 3.8 g/kg[5] Sini Decoction [Fuzi (*Radix Aconiti Carmichaeli*)∶ Ganjiang (*Rhizoma Zingiberis*)∶ Gancao (*Radix Glycyrrhizae*) = 5∶3∶2, containing granules free of frying (based on a human and animal body surface area ratio, this dose was 5 times that of a clinical dose, equivalent to the pharmacological medium dose). All rats were perfused once a day for 8 weeks.

Hemodynamic measurements

After 8 weeks, all rats were anesthetized by intraperitoneal injection of 20% urethane (5 mL/kg). The right common carotid artery was separated, and the distal end was ligated. A v-type cut was made at the proximal part and a polyethylene tube of 1 mm diameter connected to the pressure transducer was dipped in liquid paraffin and sent to the left ventricle. Then, the left ventricular pressure curve was determined. The signal of the left ventricular pressure curve was input into a BL-420S biological signal collecting and processing system. The left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP) and maximum rates of rise and decline in left ventricular pressure (± dp/dtmax) were recorded.

Determination of weight index

After measuring the cardiac function, the chest was opened and the heart was removed. Then, the residual root of the large vessel was cut, the heart was placed in saline to remove the blood, and then put on filter pa-