Biomedicine & Pharmacotherapy 88 (2017) 617–624

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Dang Gui Bu Xue Tang ameliorates coronary artery ligation-induced myocardial ischemia in rats

Ma Chunhua[a], Long Hongyan[a,*], Zhu Weina[a], He Xiaoli[b], Zhang Yajie[a], Ruan Jie[a]

[a] Central Laboratory, Nanjing Municipal Hospital of T.C.M, The Third Affiliated Hospital of Nanjing University of T.C.M, Nanjing 210001, China
[b] Department of Cardiology, Nanjing Municipal Hospital of T.C.M, The Third Affiliated Hospital of Nanjing University of T.C.M, Nanjing 210001, China

ARTICLE INFO

Article history:
Received 25 December 2016
Received in revised form 12 January 2017
Accepted 13 January 2017

Keywords:
Dang Gui Bu Xue Tang
Myocardial ischemia
Inflammation

ABSTRACT

Dang The present study was designed to investigate cardioprotective effects of Dang Gui Bu Xue Tang (DGBUT) on coronary artery ligation-induced myocardial ischemia. Myocardial ischemia (MI) model was induced in SD rats by surgical ligation of the left anterior descending coronary artery. ST segment elevation of Electrocardiograph (ECG) infarct size, levels of lactate dehydrogenase (LDH), creatine kinase (CK), glutathione (GSH) and catalase (CAT), catalase (SOD), malondialdehyde (MDA), and inflammatory cytokines and phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, p38, c-Jun NH2 terminal kinases (JNK), nuclear factor (NF)-κBp65, inhibitory kappa B (IκB) α, IκB kinase (IKK) α and IKKβ were evaluated in rats treated with or without DGBUT. DGBUT treatment significantly reduced the elevation of the ST segment of ECG, the myocardial infarct size of MI. The level of LDH, CK and MDA were suppressed, the contents of SOD, GSH and CAT were enhanced with DGBUT. The elevated concentration of inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and IL-6 in MI rats were effectively reversed by the DGBUT administration. Also, highly expressed p-JNK, p-ERK, p-p38, p-NF-κBp65, p-IκBα, p-IKKα and p-IKKβ in MI rats were restored respectively by DGBUT treatment.

The protective effect of DGBUT against MI injury might be associated with MAPK/NF-κB pathway.

1. Introduction

DGBUT, a Chinese medicinal decoction consisted of Astragali Radix (AR; roots of *Astragalus memebranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge) Hsiao) and Angelicae Sinensis Radix (ASR; roots of *Angelica sinensis* Oliv.) at a ratio of 1:5, has been extensively employed in traditional Chinese medicine (TCM) because of its remarkable hematopoietic, anti-inflammatory, anti-oxidative stress, and anti-lipid peroxidation activities [1,2]. DGBUT is also widely explored to treat ischemia associated conditions, including cardiovascular and cerebrovascular diseases [3,4]. The therapeutical effects of DGBUT on myocardial injury have been investigated recently. For example, experimental researches showed that DGBUT was capable of protecting myocardial issues from isoproterenol-induced myocardial fibrosis lesion, hydrochloric doxorubicin-induced heart failure, and defensing cardiac aging by regulating mitochondria functions [5–7]. Studies suggested that benefit effects of DGBUT might involved with promoting hematopoietic functions, inhibiting platelet aggregation and stimulating cardiovascular circulations [8,9]. Furthermore, AR and ASR alone, or in combination with each other, have been demonstrated to have potent anti-inflammatory, anti-oxidative stress, and anti-lipid peroxidation activities on various animal models [10,11]. However, little information is available about the myocardial protective effects of DGBUT on coronary artery ligation-induced myocardial ischemia. Meanwhile, the underlying mechanisms of its preventative effects of DGBUT remain largely unknown.

Cardiovascular disease is a major health problem and is recognized as one of the most burdensome diseases in modern society [12]. Although the pathogenesis of myocardial ischemia is far from clear, the anatomic changes and the characterized biochemical markers have been well illustrated. The overproduction of reactive oxygen species (ROS) and the activation of inflammatory cascades are major causative factors of cardiomyocyte abnormalities [13]. MAPKs family, mainly consists of ERK, JNK and p38 MAPK pathway, are important mediators of cellular inflammation responses to various signals. ERK1/2 activation

Abbreviations: Dang Gui Bu Xue Tang, DGBUT; electrocardiograph, ECG; lactate dehydrogenase, LDH; creatine kinase, CK; glutathione, GSH; catalase, CAT; catalase, SOD; Amalondialdehyde, MD; extracellular signal-regulated kinase (ERK), ERK; c-Jun NH2 terminal kinases, JNK; tumor necrosis factor-alpha, TNF-α; interleukin, IL; traditional Chinese medicine, TCM.

* Corresponding author.
E-mail address: longhongyantcm@163.com (L. Hongyan).

http://dx.doi.org/10.1016/j.biopha.2017.01.079
0753-3322/

participates in cell survival and the recovery of damaged myocardium ischemia, while JNK and p38 are involved in myocardial apoptosis [14,15]. In addition, reactive oxygen species induced by excessive oxidative stress could activate MAPKs signaling and promote the phosphorylation of classical inflammation-relevant NF-κB pathway [16]. The activation of NF-κB pathway further triggers the overproduction of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, which are detrimental to the myocardial functions [17].

Clinically, myocardial ischemia (MI) is defined as an interrupted blood supply to the left ventricle (LV), which is caused by blockading the coronary artery [18]. Myocardial ischemia produced by surgical ligation of the left coronary artery in rats is a commonly used experimental model. A series of hemodynamic and morphological changes were observed in this model which were similar to human myocardial ischemia [19,20]. Thus, in our present study, cardioprotective effects of DGBUT were evaluated on coronary artery ligation-induced myocardial ischemia model in rats, and the underlying mechanisms were explored for this action.

2. Materials and methods

2.1. Preparation of Dang Gui Bu Xue Tang

DGBUT which was composed of stragali Radix (AR; roots of *Astragalus memebranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge) Hsiao) and Angelicae Sinensis Radix (ASR; roots of *Angelica sinensis Oliv.)* The authentication of plant materials was identified morphologically by Dr. Qin Mingjian at China Pharmaceutical University. AR and ASR at a ratio of 1:5 was immersed for 1 h with 8 times volume of distilled water. The medicinal materials were decocted twice at boiling temperature for half an hour, and then the decocted liquids were collected. Decocted liquids were centrifuged at 3000 rpm for 5 min, the supernatant was concentrated and dried in vacuum at 55 °C. The yield of dried powder was 19.9% according to the original dry materials. The final dried powder of DGBUT was dissoluted with some distilled water and the final concentration of DGBUT was 2 g/ml. The sample was stored at 4 °C. The doses of distilled water extract of DGBUT were expressed as gram of the original dry materials per kilogram body weight and the doses (3 g/kg, 6 g/kg) of distilled water extract of DGBUT were given to animals according to human dose.

2.2. Main reagents and kits

Enzyme-linked immunosorbent assay (ELISA) kits for the determination of IL-6, IL-1β and TNF-α were produced by Nanjing KeyGEN Biotech. CO., LTD. (Nanjing, China). CK, LDH, MDA, SOD, GSH and CAT kits were provided by Jiancheng Bioengineering Institute (Nanjing, China). All antibodies were purchased from Cell Signaling Technology Inc (Beverly, MA, USA).

2.3. HPLC analysis of DGBUT

HPLC was performed on a Waters Acquity HPLC system (Waters, Milford, MA, USA), including a binary solvent delivery system, an on-line degasser, an autosampler and a photo-diode array detector (PDA) system. All the analyses were conduct by an ACQUITY UPLC™ BEH C_{18} (2.1 × 100 mm I.D., 1.7 mm, Waters, Milford, USA) column. The mobile phase consists of A (0.1% formic acid, v/v) and B (acetonitrile) with a gradient elution: 0–6 min, 90–70% A; 6–12 min, 70–0% A; 12–16 min, 0% A; 16–30 min, 90% A. The flow rate of the mobile phase was 0.4 ml/min. The temperatures of the column and auto-sampler were maintained at 30 °C and 10 °C, respectively.

2.4. Animal and experimental protocol

50 Sprague-Dawley (SD) rats, weighting 250–300 g, were provided by Comparative Medicine Centre of Yangzhou University. Rats were housed in an animal facility under a 12 h light/12 h dark cycle environment at a constant temperature (22 ± 1 °C) and humidity (40–70%). Standard food and water were provided *ad libitum*. Rats were randomly divided into five groups (with 10 rats in each group) as follows: sham group, MI group, MI + DIL (10 mg/kg) group, MI + DGBUT (3 g/kg) group and MI + DGBUT (6 g/kg) group.

2.5. Ethics statement

All animal procedures were performed in strict accordance to the Institutional Animal Research Committee guidelines and approved by the Animal Ethics Committee of Jinan University.

2.6. Surgical ligation of the left coronary artery

The method of coronary artery ligation to establish MI model was based on previous report [16] with minor modifications. Rats were anesthetized and restrained. An incision was made on trachea and a purse string catgut suture was placed surrounding the wound for later use. After that, an incision was made on the skin at the position of the heart. The underlying ribs were exposed by blunt-dissection. Experimental rats were placed on a positive pressure respirator before the ribs were separated using curved hemostatic forceps. The ribs were separated and held to ensure convenient operation on the heart. The pericardium was stripped using a cotton applicator. Left anterior descending coronary artery was observed between the lower edge of the left atrial appendage and the pulmonary cone. The left anterior descending coronary was blocked with special suture. The thoracic cavity could not be tied until a strong and regular heart rhythm returned. The retractors were disconnected subsequently once strong and regular spontaneous respiration had been established. An ST-segment elevation was recognized as the sign of a successfully established model. Finally, the skin was carefully closed. Rats in the DIL and DGBUT groups were pretreated for 5 days, while sham-operated rats were treated using the same method except that the suture was not tied.

Rats were anesthetized 24 h after the surgery. Blood samples were obtained from carotid artery and centrifuged at 3500*g* for 15 min, and then the supernatant were collected and set aside at −80 °C for further analysis. Thereafter, the rats were sacrificed and hearts were harvested for TTC staining, western blot and pathology analysis.

2.7. Electrocardiographic (ECG)

Electrocardiograms (ECGs) recorded ST-segment changes along with the whole surgery using the BL-420S Biologic Function Experiment system (Chengdu, China).

2.8. Assay of myocardial infarct area

The frozen heart was sectioned into 1 mm thick short-axis slices from the apex towards the base of the heart. Sections were incubated in 1% TTC in PBS for 15–20 min at 37 °C, and then photographed with a digital camera. TTC stained area (red, normal area) and non TTC stained areas (white, infarct area) were analyzed with a digital imaging system by computer.