Original Research Article

Danshensu exerts cardioprotective effects in rats with acute myocardial infarction via reduction of infiltration of inflammatory cells and mitigation of myocardial fibrosis

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Abstract

Purpose: To investigate the mitigative impact of danshensu on heart tissues in acute myocardial infarction (AMI) rats, and the mechanism of action involved.
Methods: Seventy-five male Sprague-Dawley (SD) rats were used in this study. After successful induction of myocardial infarction, the rats were divided into model group (MG), low-dose danshensu group (LDG; 15 mg/kg), middle-dose danshensu group (MDG; 30 mg/kg), and high-dose danshensu group (HDG; 60 mg/kg), with 15 rats per group. Rats in sham group (SG; n = 15) served as control. Serum levels of biochemical indicators and expressions of various proteins in myocardial tissue were determined using Western blotting, and compared amongst the rat groups.
Results: Serum cTnI concentrations in MDG and HDG were significantly decreased, relative to the corresponding concentrations in MDG. There were significantly lower serum concentrations of IL-1 in MDG and HDG than in model rats. Rats in HDG had lower serum IL-6 concentration than MG rats, while TNF-α levels were down-regulated in MDG and HDG, relative to MG ($p < 0.05$).
Conclusion: Danshensu protects the heart function of rats with AMI by decreasing inflammation-derived cells and mitigating myocardial fibrosis. Thus, it may be useful in the management of AMI in humans but clinical trials are necessary to ascertain this.

Keywords: Danshensu, Acute myocardial infarction, Myocardial ischemia-reperfusion, Myocardial fibrosis

INTRODUCTION

Acute myocardial infarction (AMI) refers to myocardial ischemia-reperfusion (MI/R) due to cardiac muscle lesions as a result of severe and prolonged ischemia and hypoxia in heart arteries [1]. It is a serious disease often seen in cardiology clinics. When AMI occurs in heart failure, the main pathophysiological event is cardiac remodeling which leads to apoptosis and hypertrophy of cardiac cells, as well as myocardial fibrosis and inflammatory response [2]. The use of anti-inflammatory drugs results in significant inhibition of AMI, thereby protecting myocardial function, and preventing extracellular matrix accumulation. Fibroblasts in heart muscle hyperplasia are prone to cardiomyocyte fibrosis

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which, if not timely treated, will lead to more serious cardiac damage and sudden death [3].

Salvianic acid A, a bioactive compound in *Salvia miltiorrhiza* Bge, has been reported to exert protective effect on myocardial cells and improve microcirculation and anti-thrombosis in the treatment of cardiovascular diseases [4]. However, there are limited studies on its therapeutic effect on AMI. The purpose of this study was to investigate the protective effect of *danshensu* on cardiac function in acute myocardial infarction rats, and the associated mechanism.

EXPERIMENTAL

Animals

Seventy-five mature SPF male Sprague Dawley (SD) rats were selected as experimental animals. The rats were provided by Beijing Weitong Lihua Experimental Animal Co. Ltd.

Ethical approval

This study received approval from the Ethical Authority of Shenyang Medical College (approval 2021032) and conducted according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [5].

Drugs, reagents and equipment

The drugs, reagents and instruments used, and their sources (in brackets) were: *danshensu* (Xi 'an Baichuan Biotechnology Co. Ltd, purity: 99.99 %.); TaqPathTM 1-STEP fluorescence quantification kit (Thermo Fisher Scientific, USA); kits for creatine kinase isoenzyme (CK-MB), troponin (cTnI), interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor (TNF-α) (Shanghai Xitang Biotechnology Co. Ltd); RNAprep Pure Animal Tissue Total RNA Extraction Kit (Beijing Tiagen Biochemical Technology Co. Ltd); 7500 Fast Dx Real-time quantitative PCR (ABI); protein vertical and horizontal electrophoresis tank (Bio-rad Corporation, USA); Multiskan Sky Full wavelength Enzyme marker (Thermo Fisher Company, USA), and small animal ventilator (Chengdu Taimeng Technology Co. Ltd).

Establishment of AMI model, animal grouping and treatments

All rats were routinely maintained for 1 week, followed by induction of *i.p.* anesthesia with pentobarbital sodium. Then, the animals were placed supinely on a fixed table. After tracheal intubation, the rats were placed on a small animal ventilator (frequency: 90 times/min, tidal volume: 10 mg/kg). Thoracotomy was performed, the pericardium was removed, and the anterior descending coronary artery was accurately located and ligated. Fifteen SPF male SD rats were randomly selected as the sham group.

Rats in the sham operation group were threaded only, without ligation. The rats were examined to ensure successful induction of AMI, based on ECG showing ST dorsal elevation lasting more than 15 min and ≥ 0.1mV. The excess gas was discharged and the thoracic cavity was sutured. When the rats recovered from anesthesia and resumed spontaneous breathing, the small animal ventilator was turned off, and the organs were excised. Penicillin was intraperitoneally injected daily for 3 days, to prevent infection.

After electrocardiogram examination and continuous use of penicillin for prevention of infection, AMI was successfully established in 60 rats. The AMI model rats were randomly divided into MG, LDG (15 mg/kg), MDG (30 mg/kg) and HDDG (60 mg/kg), with 15 rats/group. *Danshensu* was administered via tail vein injection, continuously for 4 weeks. Sham and MG rats received equivalent amounts of normal saline in place of *danshensu*.

Assay of serum levels of biochemical indices

At the end of the treatments, the aorta blood of each rat was taken and allowed to stand at room temperature for 30 min, followed by centrifugation. The resultant serum was assayed for levels of CK-MB, cTnI, IL-1, IL-6 and TNF-α using their appropriate kits, in accordance with the manufacturers' protocols.

Assay of cardiac function indicators

After 4 consecutive weeks of treatment, all animals were given *i.p* pentobarbital Na anesthesia (0.030 g/kg). Then, with the rats supinely positioned on a fixed platform, M-mode echocardiography was measured with a small animal ultrasonic detector. The main parameters measured were ejection fraction (LVESD), left ventricular posterior wall end-diastolic diameter (LVEDD), left ventricular end-diastolic diameter (LVFS), and short axis shortening rate (LVFS).

Assay of myocardial protein expression levels of α-SMA, TGF-β and CTGF

Myocardial tissue (100 g) from each rat was thoroughly ground with cell lysis buffer (500 µL) at low temperature. The protein concentration of

the lysate was measured with BSA method. Then, the protein extract was resolved using SDS-polyacrylamide gel electrophoresis, and electro-transferred to PVDF membrane which was sealed using skim milk (5 %) at laboratory temperature for 60 min. Membrane incubation with 1° immunoglobulins for α-SMA, TGF-β and CTGF was done for 12 h at 4 °C. Next, the membrane was incubated with horse radish peroxidase-linked 2° immunoglobulin at laboratory temperature for 60 min, and protein bands were subjected to ECL. Grayscale analysis was used to determine relative protein levels.

Statistical analysis

This was done with SPSS 20.0 software and the data are depicted as mean ± SD. One-way ANOVA was used for multiple group comparison, while 2-groups were compared with LSD *t*-test. All count data are presented as n (%), and paired comparison were done using χ^2 test. Statistically significant difference was assumed at *p < 0.05*.

RESULTS

Influence of *danshensu* on cardiac function indices

Values of LVEDD and LVESD were significantly higher in MG than in sham rats, while LVFE and LEVF values were significantly lower in MG. Relative to MG, LVEDD and LVESD in each *danshensu* dose group were appreciably decreased ($p < 0.05$). However, LVFE and LEVF values were significantly higher in each *danshensu* dose group than in MG ($p < 0.05$; Table 1).

Serum concentrations of CK-MB and cTnI

There were significantly higher blood levels of CK-MB and cTnI in MG than in sham rats, while serum CK-MB was markedly lower in HDG than in MG. However, serum concentrations of CK-MB in LDG and MDG were comparable. There were no marked differences in serum cTnI concentration between LDG and MG, but serum cTnI concentrations in MDG and HDG were markedly reduced, relative to MD ($p < 0.05$; Table 2).

Effect of *danshensu* on levels of inflammatory factors

There were higher serum concentrations of IL-1, IL-6 and TNF-α in model group than in sham group, but serum level of IL-1 in LDG and MG were comparable. However, serum levels of IL-1 were markedly lower in MDG and HDG than in MG. The serum IL-6 concentrations in LDG, MDG and MG were comparable, but the serum IL-6 concentration was markedly lower in HDG than that in MG. Serum TNF-α concentration in LDG and MG were comparable. However, TNF-α concentration in MG and HDG were markedly lower, relative to MG ($p < 0.05$; Table 3).

Table 1: Influence of *danshensu* on cardiac function indexes (mean ± SD, n = 15)

Group	LVEDD (mm)	LVESD (mm)	LVFS (%)	LEVF (%)
Sham	5.56±0.43	3.27±0.31	48.62±5.31	85.12±6.43
Model	9.35±0.62 [a]	6.82±0.56 [a]	23.54±3.50 [a]	42.95±4.31 [a]
Low dose	8.53±0.60 [b]	6.12±0.49 [b]	27.93±3.84 [b]	49.53±4.38 [b]
Middle dose	7.63±0.52 [b]	5.53±0.42 [b]	30.14±4.32 [b]	54.93±5.37 [b]
High dose	6.82±0.53 [b]	4.99±0.39 [b]	36.31±3.58 [b]	60.37±5.92 [b]
F	110.12	138.80	67.78	133.31
P-value	＜0.001	＜0.001	＜0.001	＜0.001

[a]$P < 0.05$, vs sham; [b]$p < 0.05$, vs MG

Table 2: Serum CK-MB and cTnI concentrations (mean ± SD, n = 15)

Group	CK-MB (ng/kg)	cTnI (ng/kg)
Sham	36.92±14.83	3.01±0.94
Model	89.93±13.06[a]	12.30±2.42[a]
Low dose	84.31±12.64	11.42±1.15
Middle dose	79.68±9.82	10.05±1.39[b]
High dose	55.37±16.92[b]	7.68±1.17[b]
F	40.22	91.43
P-value	＜0.001	＜0.001

[a]$P < 0.05$; compared with sham group; [b]$p < 0.05$, vs model

Table 3: Levels of IL-1, IL-6 and TNF-α in rat serum (mean ± SD, n = 15)

Group	IL-1 (pg/kg)	IL-6 (pg/kg)	TNF-α (pg/kg)
Sham	66.21±24.87	120.93±36.68	156.82±34.80
Model	240.67±36.73 [a]	236.42±29.31 [a]	214.58±13.99 [a]
Low dose	231.31±38.24	232.81±23.27	201.80±25.72
Middle dose	186.54±24.28 [b]	226.17±25.37	169.27±34.26 [b]
High dose	155.37±36.24 [b]	100.54±28.34 [b]	159.82±23.81 [b]
F	69.73	79.82	13.44
P-value	＜0.001	＜0.001	＜0.001

[a]*P* < 0.05, vs sham; [b]*p* < 0.05, vs model

Table 4: Protein expression levels of α-SMA, TGF-β and CTGF in rat myocardial tissues (mean ± SD, n = 15)

Group	α-SMA	TGF-β	CTGF
Sham	0.29±0.05	0.62±0.04	0.31±0.05
Model	1.38±0.12 [a]	1.86±0.16 [a]	1.76±0.19 [a]
Low dose	1.12±0.13 [b]	1.41±0.14 [b]	1.32±0.20 [b]
Middle dose	0.79±0.12 [b]	1.21±0.11 [b]	1.01±0.16 [b]
High dose	0.61±0.10 [b]	0.78±0.20 [b]	0.62±0.12 [b]
F	234.5	187.64	205.6
P-value	＜0.001	＜0.001	＜0.001

[a]*P* < 0.05, compared with sham group; [b]*p* < 0.05, compared with model group

Myocardial expression levels of α-SMA, TGF-β and CTGF in the rat

Myocardial protein expression levels of α-SMA, TGF-β and CTGF were significantly higher in MG than in sham group, but the levels of these proteins were markedly lower in each *danshensu* dose group than in MG (*p* < 0.05; Table 4).

DISCUSSION

Reperfusion during myocardial ischemia in AMI patients aggravates myocardial ischemic injury [6]. Clinically, infarct size is a vital parameter for measuring MI/R lesion, and myocardial infarction size is closely related to prognosis of the disease [7]. Salvianic acid A, a bioactive compound in *Salvia miltiorrhiza* Bge, is a phenolic compound which has been reported to exert therapeutic effects on patients with cardiac hypertrophy and atherosclerosis. Moreover, it improves patients' blood flow dynamics and myocardial energy metabolism. Salvianic acid A has been widely applied for preventing and treating cardiovascular illnesses [8,9].

The clinical indicators for the diagnosis of MI/R injury generally involve specific enzymes associated with myocardial lesions. In this respect, intracellular energy metabolism involves CK-MB and cTnI, which presence in serum are important indicators of AMI-associated myocardial injury [10]. In this study, it was found that serum CK-MB and cTnI were appreciably higher in model group than in control rats, but they were reduced to different degrees by different doses of *danshensu*. Inflammatory response during MI/R injury may run through the whole process of myocardial cell injury, with early MI/R injury resulting in liberation of three inflammation-linked factors, i.e., IL-1, IL-6 and TNF-α [11]. Studies have shown that TNF-α regulates inflammatory response and stimulates other cells to secrete IL-1 and IL-6. In addition, it induces the expression of adhesion factors, improves the adsorption capacity and function of endothelial cells and neutrophils, and blocks micro-vessels, all of which ultimately lead to myocardial cell injury [12].

In this study, it was found that different concentrations of *danshensu* reduced the levels of pro-inflammatory factors, and also decreased myocardial injury. Myocardial fibrosis is a critical stage in the etiology of many cardiovascular events. Myocardial fibrosis is activated by myocardial fibroblasts and deposition of large quantities of collagen I and III in the cells and blood vessels, eventually resulting in increased myocardial stiffness, heart failure and death [13,14].

This research revealed markedly higher LVEDD and LVESD in MD than in sham rats, while LVFE and LEVF were markedly decreased in MG. Relative to MG, LVEDD and LVESD in each *danshensu* dose group were significantly decreased, but there were higher LVFE and LEVF in each *danshensu* dose group than in MG. As a multifunctional growth factor, TGF-β regulates cell multiplication and programmed cell death, while α-SMA is the marker protein in myofibroblasts [15].

Research has shown that TGF-β is an important fibrosis-promoting factor in clinical experiments

[16]. It affects the barrier function of endothelial cells: the proliferation of fibroblasts also slows down the degradation the extracellular matrix. The results of this study indicated significant myocardial protein up-regulations of α-SMA, TGF-β and CTGF in MG, relative to control rats, but the protein expressions of these factors in each *danshensu* group were significantly lower than those in model group. These results indicate that *Salvia miltiorrhizin* alleviated myocardial fibrosis in the rats.

CONCLUSION

This study has demonstrated that *danshensu* protects the cardiac function of acute myocardial infarction rats by reducing inflammatory cell infiltration and myocardial fibrosis. Therefore, it may be useful in the clinical treatment of the disease after successful clinical trials.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the contents of this article will be borne by the authors. Ying Wang designed the study, supervised the data collection, and analyzed the data. Zhong Zhang interpreted the data and prepared the manuscript for publication. Min Zhang supervised the data collection, analyzed the data and reviewed the draft of the manuscript. Ying Wang and Zhong Zhang contributed equally to this work and should considered as co-first authors.

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