Fufang Danshen Pian (Danshen Tablets) Ameliorates Myocardial Infarction Injury via Endothelial Protection in Rats

ABSTRACT

Objective Compound Danshen Tablets (CDT), also known as Fufang Danshen Pian in China, has been commonly used to prevent and treat cardiovascular diseases in the clinic in Asia-Pacific region, especially in China. This study investigated the effects and possible mechanism of CDT against isoproterenol-induced myocardial infarction (MI) in rats.

Methods Male rats were randomly assigned to six groups: control, model, isosorbide dinitrate (ISDN, 2.72 mg/kg, i.g.), low-dose CDT (0.315 g/kg, i.g.), medium-dose CDT (0.63 g/kg, i.g.), and high-dose CDT (1.26 g/kg, i.g.). After 10 consecutive days of administration, except for the control group, others were subcutaneously injected with 5 mg/kg isoproterenol for three consecutive days to establish MI models. Variables including electrocardiogram (ECG), cardiac function, infarct size, myocardium structure, and the activities of serum creatine kinase (CK), lactate dehydrogenase (LDH), cardiac troponin I (cTnI), nitric oxide (NO), endothelial nitric oxide synthase (eNOS), and brain natriuretic peptide (BNP) were determined.

Results CDT significantly decreased infarct size, BNP level in myocardial tissues, and the concentrations of serum CK, LDH and cTnI. In addition, CDT could remarkably increase the levels of serum NO, eNOS and ameliorate myocardial injury.

Conclusion CDT has endothelial protective effect against MI injury in rats by protecting cardiomyocytes, reducing myocardial infarct size, oxidative stress, the levels of serum myocardial enzymes (LDH and CK), cTnI, and BNP.

KEYWORDS compound danshen tablet (CDT), fufang danshen pian, radix salvia miltiorrhiza, isoproterenol, myocardial infarction

INTRODUCTION

Cardiovascular disease (CVD) is the most alarming cause of death worldwide to date. The incidence of CVD is expected to continue to rise, and the expected worldwide CVD-related deaths are projected to climb to 23.3 million by 2030.1,2 As one of the most dominant forms of CVD, myocardial infarction (MI) was caused by partial or complete coronary artery occlusion from plaques.3

At present, MI is mainly treated with chemical medicines or surgery. The commonly used medicines include anticoagulants, antiplatelet agents, β-blockers, angiotensin-converting enzyme inhibitors (ACEI), lipid-lowering drugs, and so on. While they yield desired outcomes and reduce the mortality rate of MI, they may also lead to serious adverse effects, such as bleeding, orthostatic hypotension, bradycardia, or even congestive heart failure. Reperfusion therapies including percutaneous coronary intervention (PCI) and coronary artery bypass graft surgery (CABG) can directly rescue the necrosed myocardium more timely and effectively, but the surgical conditions and cost are demanding, and the patients are likely to suffer from bleeding, allergy, infection or other adverse cardiac events.4,5 Based on these statistics, it is apparent that new therapies are needed to combat the enormous burden CVDs have put on the quality and lifespan of the global population.

Fufang Danshen Pian (Danshen Tablets, Compound Danshen Tablet, CDT), an herbal preparation consisting of Salvia miltiorrhiza Radix et Rhizoma, Radix et Rhizoma,
Panax notoginseng Radix et Rhizoma, and Borneolum Syntheticum, is widely used for treating CVDs in China and other Asia-Pacific countries, and it has been listed in the Chinese pharmacopoeia\(^6\). CDT possesses various pharmacological effects, including dilation of coronary arteries, improvement of coronary circulation, reduction of myocardial oxygen consumption, memory improvement in Alzheimer’s disease, and so on\(^7\). Recent studies have found that the bioactive constituents of Salvia miltiorrhiza can be divided into lipophilic compounds and hydrophilic compounds. Lipophilic compounds include tanshinones, and hydrophilic compounds include salvianolic acids, both of which are capable of alleviating CVDs\(^8\)-\(^10\). The major bioactive constituents in Panax notoginseng are notoginseng triterpenes, which can antagonize thrombosis, dilate blood vessels, and protect cardiac capillaries\(^11\). While CDT has been widely used in the clinic for treating CVDs, its detailed protective effects and underlying mechanisms against each disease still lack complete elucidation. In our previous study, we had found that CDT could protect cardiomyocytes against MI/R injury by regulating myocardial enzyme, decreasing the infarct size, enhancing the ability of eliminating oxidant radical et al. At the same time, CDT can inhibit apoptosis in rats via activating Akt-eNOS signaling pathway. This study applies isoproterenol to establish MI models in rats, and investigates the protective effects and their possible mechanisms of CDT against drug-induced MI.

**METHODS**

**Preparation of compound danshen tablets**

The CDT was produced by Hutchison Whampoa Baiyunshan Chinese Medicine Co., Ltd., Guangzhou, China as follows: *Radix Salvia Miltiorrhiza* (450 g), *Radix Notoginseng* (141 g) and *Borneolum Syntheticum* (8 g) were extracted with ethanol and heated under reflux for 1.5 hours, filtered, then ethanol was recycled, and the filtrate was concentrated to moderate volume. Drugs were extracted with 50% ethanol and heated under reflux for 1.5 hours, filtered, then ethanol was recycled and the filtrate was concentrated to moderate volume. Drugs were extracted again with water for another 2 hours, and the filtrate was concentrated to moderate volume. *Radix Notoginseng* was crushed into fine powder and combined with the above concentrated solution and excipients to make granules, then granules were dried afterwards. *Borneolum Syntheticum* was pulverized, blended with granules, compressed into 1000 tablets, and then packed with film coating. Each tablet weighs 0.32 g\(^12\).

**Animals**

Male Sprague-Dawley rats, weight 180–220 g (Certificate No.: SCXX (Guangdong) 2013-0002) were provided by Guangdong Medical Laboratory Animal Center. The experiments were performed in accordance with the guidelines of the National Institute of Health for the use of laboratory animals and were approved by the Jinan University Committee on Animal Care.

**Reagents**

CDT (Lot no.: D3A008, Hutchison Whampoa Baiyunshan Chinese Medicine Co., Ltd., Guangzhou, China); Isosorbide Dinitrate (ISDN, Lot no.: 20130303, Shimao Tianjie Pharmaceutical Co., Ltd., Jiangsu, China); Triphenyltetrazolium chloride (TTC, Lot no.: 130311, Shanghai Ruji Biology Technology Co., Ltd., China); Isoproterenol hydrochloride (Lot no.: CBC7466, Sigma-Aldrich Co., Ltd., St. Louis, USA); ELISA kits for cTnI, BNP, and eNOS (Lot no.: Z10015846, Z02015848, Z10015847,CUSABIO Biotech Co., Ltd., Wuhan, China).

**Group arrangement and administration**

The rats were randomly assigned to the following six groups with 12 rats in each group: Control, Model, ISDN (2.72 mg/kg)\(^13\), Low-dose CDT (0.315 g/kg), Medium-dose CDT (0.63 g/kg), and High-dose CDT (1.26 g/kg). All drugs were dissolved in normal saline and produced suspension with different concentration before administration. Rats were administered with respective drugs intragastrically once daily, while rats in control and model groups received the same amount of normal saline, for 10 consecutive days.

**Model establishment**

Isoproterenol-induced MI models were produced via the method introduced by Rona et al\(^14\),\(^15\). On the 8th, 9th, and 10th days, except control group, other groups received subcutaneous injection with isoproterenol 5 mg/kg 30 minutes after gavage once daily, for 3 consecutive days.

**Cardiac function examination**

Cardiac function was evaluated by using the noninvasive *in vivo* micro-ultrasound imaging system (VisualSonics Inc. Toronto, Canada). heart rate (HR), ejection fraction (EF), and cardiac output (CO) were measured.

**Determination of infarct size**

Rats were injected with 10 mL of 1% TTC in phosphate buffer (pH = 7.4) pre-incubated at 37°C via the abdominal aorta after blood collection, then the aorta was clamped for 15 minutes. Next, the heart was cross-sectioned into five slices in 4°C phosphate buffer saline (PBS), and then incubated at 37°C in 1% TTC
in phosphate buffer (pH = 7.4) for 15 minutes. The infarcted myocardium was cut off and weighed by LAC-110.4 analytical balance (Sartorius Group, Germany). The infarct size could be obtained by

\[
\frac{W_{\text{infarcted myocardium}}}{W_{\text{total myocardium}}} \times 100% ^{16}
\]

### Histological observation of myocardium

After blood collection, the apex of the ischemic heart was separated, and it was washed in normal saline and dried with filter paper. Next, it was fixed in 10% formalin and sent to the Pathology Department of the First Affiliated Hospital of Jinan University (Guangzhou, China) for pathological section and hematoxylin and eosin (H&E) staining. At the end, the structure of myocardium was observed with Nikon ECLIPSE TS100 fluorescence inverted microscope (Nikon Co., Ltd., Japan).

### Enzyme assays

After blood collection, the whole blood was left standing for 2 hours. Then, it was centrifuged at 3000 rpm at 4°C for 10 minutes by refrigerated centrifuge (Sigma Laborzentrifugen GmbH, Germany). The supernatant was collected and stored at -20°C for tests of CK, LDH, cTnI, NO, and eNOS. A myocardium homogenate was prepared with 100 mg of refrigerated heart tissue and ice-cold normal saline. The homogenate was centrifuged at 4°C, and the supernatant was collected for BNP assay.

### Statistical analysis

SPSS Statistics 17.0 was used for statistical analysis. All data were expressed as the mean ± standard error of the mean (SEM). Comparisons between groups were calculated by using one-way ANOVA and independent-samples t test, while comparisons between percentages were calculated by using rank-sum test. Probability (\(P\)) values less than 0.05 were considered statistically significant.

### RESULTS

#### Effect of CDT on electrocardiogram (ST segment)

Compared with the control group, the ST segments of the model group elevated significantly, which demonstrated that the model of MI was successfully established. In contrast, the ST segments of CDT and ISDN (2.72 mg/kg) groups declined (Fig. 1).

#### Effect of CDT on myocardial infarct size

TTC staining of myocardium revealed that MI injury was strongly related to the development and progression of irreversible ischemic injury of myocardium (Fig. 2a). The proportion of infarct size in the model group was remarkably greater than that in the control group (\(P < 0.01\)), and the infarct size decreased significantly with treatments of low-dose CDT (0.315 g/kg), medium-dose CDT (0.63 g/kg), high-dose CDT (1.26 g/kg), and ISDN (2.72 mg/kg) (\(P < 0.01\) or 0.05, Fig. 2b).

#### Effect of CDT on myocardial histology

The H&E staining of myocardial tissue showed that the myocardial fibers of rats in the control group were in an orderly arrangement, and there was no atrophy, hypertrophy, necrotic foci, inflammatory cell infiltration or any other pathological changes. However, the myocardial damages were evident in the model group. The myocardial fibers were in a disorderly arrangement. Myocardial interstitial edema was found, and the tissue space was significantly widened. Neutrophil infiltration was also present. Many myocardial fibers were swollen, lysed, or ruptured, and the cross striation was vague or even disappeared. The myocardial cells underwent vacuolar degeneration. The myocardial damages in CDT and ISDN groups were milder than those in the model group. Local mild swelling was found, mild interstitial edema was present, and the tissue space was slightly widened. A few inflammatory...
cells were infiltrated, and vacuolar degeneration could be observed sporadically (Fig. 3).

**Effect of CDT on cardiac function**

Compared with the control group, only the heart rate of model group displayed a remarkable increase ($P < 0.05$), while there were no differences on the EF, CO and HR after treated with CDT or ISDN, compared with the model group ($P > 0.05$, Table 1).

**Effect of CDT on serum LDH, CK, and cTn-I**

To examine how CDT influences myocardial injury, the activities of CK, LDH, cTn-I were measured. As shown in Table 2, activities of serum CK, LDH, and cTn-I of rats in the model group were higher than those of the control group (all $P < 0.01$). However, after treated with CDT or ISDN, levels of serum CK, LDH, and cTn-I decreased significantly, compared with the model group ($P < 0.01$ or 0.05).

**Effect of CDT on serum NO, eNOS and myocardial tissue BNP**

As shown in Table 3, compared with the control group, the model group rats showed significantly more BNP but lower activities of NO and eNOS (all $P < 0.01$). The administration of CDT or ISDN caused significantly less BNP content but higher NO and eNOS enzyme activities, compared with the model group ($P < 0.01$ or 0.05).

**DISCUSSION**

The elevation of the ST segment of ECG is the characteristic sign of MI and generally measured in...
Effect of CDT on serum LDH, CK and cTnI in myocardial infarction rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH (U/mL)</th>
<th>CK (U/mL)</th>
<th>cTnI (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.43 ± 0.16</td>
<td>0.43 ± 0.05</td>
<td>1.90 ± 0.22</td>
</tr>
<tr>
<td>Model</td>
<td>7.80 ± 0.61</td>
<td>0.69 ± 0.06</td>
<td>2.56 ± 0.42</td>
</tr>
<tr>
<td>ISDN (2.72 mg/kg)</td>
<td>5.41 ± 0.36</td>
<td>0.53 ± 0.03</td>
<td>2.36 ± 0.24</td>
</tr>
<tr>
<td>CDT (1.26 g/kg)</td>
<td>5.07 ± 0.52</td>
<td>0.50 ± 0.05</td>
<td>2.99 ± 0.28</td>
</tr>
<tr>
<td>CDT (0.63 g/kg)</td>
<td>5.77 ± 0.53</td>
<td>0.58 ± 0.01</td>
<td>3.66 ± 0.24</td>
</tr>
<tr>
<td>CDT (0.315 g/kg)</td>
<td>6.07 ± 0.63</td>
<td>0.62 ± 0.01</td>
<td>4.16 ± 0.26</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, Compared with model group.

Effect of CDT on serum BNP, NO and eNOS in myocardial infarction rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>BNP (ng/mL)</th>
<th>NO (μmole/L)</th>
<th>eNOS (mLU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.65 ± 0.27</td>
<td>22.06 ± 2.04</td>
<td>27.48 ± 3.70</td>
</tr>
<tr>
<td>Model</td>
<td>4.96 ± 0.62</td>
<td>8.14 ± 1.87</td>
<td>10.03 ± 2.35</td>
</tr>
<tr>
<td>ISDN (2.72 mg/kg)</td>
<td>2.87 ± 0.36</td>
<td>18.56 ± 2.00</td>
<td>24.69 ± 3.60</td>
</tr>
<tr>
<td>CDT (1.26 g/kg)</td>
<td>3.00 ± 0.35</td>
<td>17.67 ± 2.33</td>
<td>24.18 ± 3.74</td>
</tr>
<tr>
<td>CDT (0.63 g/kg)</td>
<td>3.36 ± 0.30</td>
<td>16.02 ± 2.86</td>
<td>22.27 ± 3.57</td>
</tr>
<tr>
<td>CDT (0.315 g/kg)</td>
<td>3.54 ± 0.29</td>
<td>14.76 ± 2.34</td>
<td>21.24 ± 3.15</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, Compared with model group.

Myocardial cell death can be recognized by the presence of different proteins released into the circulation from the damaged myocytes, such as myoglobin, cardiac troponin T and I, CK, LDH, BNP as well as many others, and their leakage to blood is an indicator of MI.

CONCLUSIONS

The above-mentioned results proved that CDT has endothelial protective effects against isoproterenol-induced MI injury in rats. CDT could protect rats from MI by improving ECG of ischemia, reducing myocardial infarct size and oxidative stress, and modulating the amount of CK, LDH, cTnI, eNOS, and BNP. NO protects myocardium by affecting oxygen consumption, platelet aggregation, leukocyte adhesion, and free radical scavenging. A large volume of research has determined that after the occurrence of acute MI, the level of NO declines remarkably, which was also demonstrated in the results of this study. The amount of eNOS and NO in the model group was significantly lower than that of the control group. All CDT groups showed remarkably higher eNOS phosphorylation level. The increased synthesis and release of NO could reduce damages to myocardial tissue so as to protect the heart.

However, in this experiment, the main cardiac function indicators of MI rats were not significantly improved. This might have been caused by a small sample size, or isoproterenol might not have induced serious enough myocardial lesions that could have changed all the cardiac function indicators.

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REFERENCES