Wang et al., Afr J Tradit Complement Altern Med. (2017) 14(2):65-74
doi:10.21010/ajtcam.v14i2.8

CARDIOPROTECTIVE ROLES OF THE CHINESE MEDICINAL FORMULA BAO-XIN-TANG ON ACUTE MYOCARDIAL INFARCTION IN RATS

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Abstract

Background: Bao-Xin-Tang (BXT) is a traditional Chinese medicinal formula used for the treatment of coronary heart disease and known to have favorable therapeutic benefits. The current study was designed to determine whether BXT has a cardioprotective role for acute myocardial infarction. The underlying mechanisms were also explored.

Materials and Methods: The Sprague-Dawley rat model of acute myocardial infarction was established by occluding the left anterior descending branch of the coronary artery. After a 3-h ischemic period, we determined the myocardial infarction size, inflammatory components, and antioxidant activities.

Results: The data showed that BXT could reduce the infarction size and lower the levels of C-reactive protein, interleukin-6, and myeloperoxidase, and increase the activities of superoxide dismutase and the anti-inflammatory cytokine, interleukin-10. These results indicate that administration of BXT, following acute myocardial infarction, could reduce infarct size.

Conclusion: The effects of BXT may be related to its anti-inflammatory and anti-oxidative properties.

Key words: Bao-Xin-Tang, cardioprotective, infarction size, anti-inflammatory, anti-oxidative

Introduction

In the United States (US), over 800,000 people suffer from acute myocardial infarction (AMI) every year, with 27% mortality (Boateng and Sanborn, 2013). Although the incidence of AMI has decreased in the US, according to the American Heart Association, heart disease remains the leading cause of death worldwide (O'Gara et al., 2013). Acute intravascular coronary thrombus formation within a visceral pericardium coronary artery is the underlying pathology of AMI (Kloner, 2006). Pathological and clinical studies have suggested that arterial inflammation is the most common cellular and molecular mechanism, after the rupture of an atherosclerotic plaque (O'Gara et al., 2013; Kloner, 2006). Oxidative stress also plays a crucial role in the process of AMI (Przybyt et al., 2013). The main concern is to find an effective way to minimize myocardial damage and rapidly rescue the affected myocardium after AMI. Although primary percutaneous coronary intervention (PCI) and thrombolytic therapy are important revascularization treatments (Yip et al., 2002), aborted reperfusion, such as during miscarry after the infarct artery reopens through thrombolytic therapy,

or during a lack of coronary reflow when undergoing primary PCI, have always been areas of concern to the clinician (Reffelmann and Kloner, 2002).

Chinese herbal medicines, widely utilized for thousands of years in China and other Asian countries (Ou et al., 2003), have recently received more attention because they target multiple pathways and regulatory mechanisms. Modern pharmacological studies have shown that a number of traditional Chinese herbs and herbal extracts prevent or slow the progression of cardiovascular disease through their anti-inflammatory and antioxidant functions (Shang et al., 2013; Wu et al., 2007; Li et al., 2011). For example, to treat AMI, Suxiao Jiuxin Pill and Compound Danshen dropping is effective in clinical practices and is used by the majority of AMI patients (Li et al., 2011; Luo et al., 2013).

Bao-Xin-Tang (BXT), a traditional Chinese medicinal formula, is mainly used in the field of coronary heart disease (CHD) and has good curative effects. BXT contains numerous plant extracts (see Materials and methods) of Codonopsis pilosula (dangshen), Astragalus (huangqi), Atractylodes macrocephala (baizhu), Wolfiporia extensa (fulin), Fructus crataegi (shanzha), Radix puerariae (gegen), Salvia miltiorrhizae (danshen), peach seed (taoren), Ligusticum wallichii (chuanxiong), and Carthamus tinctorius (honghua). In the traditional Chinese medicine theory, it improves the movement of Qi in body, and promotes blood circulation to protect cardiac muscle in patients with myocardial infarction (Zhang et al., 2006). Moreover, our previous studies have confirmed that BXT is exact effective to cure coronary heart disease with stable anginapectoris by elevate the Nitric Oxide (NO) level in the patients. Some studies suggest it also can promote the collateralization in ischemic zone, and enhances the recovery of heart functions through the improvement of endothelial functions, by elevating plasma vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) levels (Zhang et al., 2006; Xing et al., 2003; Xing et al., 2006; Peng et al., 2011). In BXT, Codonopsis pilosula and Astragalus are the principal drugs of this decoction.

However, although BXT shows strong cardioprotection from coronary heart disease and angina pectoris, in this case, it is the first time to evaluate whether acute oral BXT could improve the prognosis of AMI rats. If so, we would then investigate the anti-inflammatory and anti-oxidant mechanisms involved.

Materials and Methods

Preparation of BXT

BXT consists of 10 herbal components, including 15g of Codonopsis pilosula, 15g of Astragalus, 10g of Atractylodes macrocephala, 10g of Wolfiporia extensa, 15g of Fructus crataegi, 15g of Radix puerariae, 15g of Salviae miltiorrhizae, 12g of peach seed, 10g of Ligusticum wallichii, and 5g of Carthamus tinctorius, purchased from Xiangya Hospital of TCM Pharmacy (Changsha, China). All herbal ingredients were obtained from optimal sources to ensure the consistency of the chemical components of traditional Chinese medicine, with GAP grade, and the extracted drugs were in agreement with the requirements of the Chinese Pharmacopeoia (2000 edition). Sample specimens were preserved (No. 140927). The BXT mixture was dissolved in distilled water for 30 min. After soaking, the mixture was boiled for 30 min, twice. The mixture was then filtered through gauze, and vacuum lyophilized to collect the dry powder. Distilled water was used to dissolve the final product to a concentration of 2.5 g/mL (dry weighting).

Experimental protocol

This research project was reviewed and approved by the Ethics Committee of the Center for Scientific Research with Animal Models at the Central South University [Certificate of Conformity:2014-09-1].

The Experimental Animal Center of Central South University (Hunan, Changsha, China) provided 24 male Sprague-Dawley

rats (180–220 g body weight). Rats were housed in polyethylene cages, three per cage, and food and water were provided *ad libitum*. Rats were housed under standard conditions at 22°C, with relative humidity at 40%–60% and a 12 h light/dark cycle. Before any experiment, rats were allowed a 1-week acclimatization period.

All rats were randomly divided into four groups as follows: i) sham operation group (sham MI; rats in this group had sutures passing around the left main coronary artery without ligations); ii) vehicle control group [MI + vehicle; rats were treated with vehicle (0.9% NaCl, 20 mL/kg)]; iii) lower dosage BXT group (MI + BXT; at 30 min before myocardial ischemia, rats were orally administered BXT extract at 12 g/kg); and, iv) higher dose BXT group (MI + BXT; at 30 min before myocardial ischemia, rats were orally administered BXT extract at 36 g/kg). The drug dose given to the rats was based on the following principles. The lower dose of BXT given to rat model was 12 g/kg, which is approximately 6.7 times that used by humans. This dose has been previously reported (PINKEL et al., 2011). BXT in the high dose group was 36 g/kg, which is 3 times of lower dose, without obvious side effects.

Acute myocardial infarction rat model preparation

We performed the surgical operations as previously described (Qin et al., 2009). The recommended dose of pentobarbital for intraperitoneal injection was 36 mg/kg. Intubation was through a tracheotomy, and ventilation used a DHX-500B small animal ventilator [RR = 60 beats per minute (bpm), V_T = 2–3 mL/100 g, I:E = 1:2]. Electrodes were inserted subcutaneously to record the electrocardiogram (ECG) of the rat throughout the entire experiment (RM6240BD; Extension Equipment Co., Shanghai, China). The chest was opened by means of a left thoracotomy, and the heart was exposed through the use of a retractor and forceps. A 3-0 suture was used to ligate the left anterior descending coronary artery. Typical arrhythmia from AMI was successfully established using this rat model. The heart was immediately returned to its anatomical location, and a sternum incision was done with final skin suturing. After 3 h of ischemia, the heart was quickly removed, and the myocardial tissue was processed as follows.

Measurement of infarct size

The infarct size was measured as previously described (Yu et al., 2013). The heart was quickly removed at 3 h after occlusion of the left anterior descending coronary artery (LAD). Samples were frozen at −20°C, then the left ventricles were sectioned into 2-mm thick sections from the apex to the coronary sulcus. Infarct size was determined using 1% triphenyl tetrazolium chloride (TTC) staining (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) at 37°C for 30 min. The normal cardiac tissue was bright red while the ischemic myocardium appeared pale. Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA) was to measure the size of the infarct area.

Inflammatory factor assays

An enzyme-linked immunosorbent assay (ELISA) was used to measure the serum levels of C-reactive protein (CRP), interleukin-6 (IL-6), and interleukin-10 (IL-10). Each kit was provided by Weier Research (Hunan Province, China). After 3 h of acute myocardial ischemia, a serum separator tube was used for 30 min on all clotted blood samples. Whole blood was separated from serum at 1,000 rpm, according to the manufacturer's instructions, and 100 µL of standard blank or serum sample were incubated for 2 h at 37°C, with detection reagent A (100 µL) added to each well, then incubated for 1 h at 37°C. Samples were washed with buffer, then 100 µL of detection reagent B was added to each well, and further incubated for 1 h at 37°C, with rinsing, once. After the addition of 90 µL of substrate solution, followed by incubation for 20 min, 50 µL of stop solution was added at room temperature. Microplate spectrophotometry was used to detect the optical density of each well (BioTek, Winooski, VT, USA). The final results

doi:10.21010/ajtcam.v14i2.8

were determined from a standard curve.

Antioxidant assays

Blood samples were collected from the abdominal aorta to measure the activities of superoxide dismutase (SOD) and myeloperoxidase (MPO) activities. Whole blood was separated for 10 min at 3,000 rpm, and blood samples were then used to measure levels of SOD and MPO using diagnostic kits, following the manufacturer's instructions (Huamei, Wuhan, China).

Statistical analysis

All data were expressed as mean ± standard deviation (SD) for 6 rats in each group. Experimental results were analyzed using one-way ANOVA followed by least significant difference (LSD) tests for individual comparisons between group means, while the Tamhane *t*-test method was used when the variance was arrhythmic. All data were analyzed using Social Science (SPSS) 17.0 software (Armonk, New York, USA). A value of $P < 0.05$ was considered statistically significant.

Results

Effects of BXT on myocardial infarct size

As shown in **Figure. 1**, we measured the myocardial infarct size to determine the protective effects of BXT on the acute myocardial infarction range in experimental rats. Interestingly, in the vehicle-treated group the myocardial infarct was 53.37 ± 2.30%. After oral treatment with BXT at doses of 12 and 36 g/kg, the myocardial infarct area was significantly smaller compared with the vehicle-treated group, respectively (MI+12 g/kg, 31.96 ± 1.70%; MI+36 g/kg, 23.24 ± 1.33%; $P < 0.01$).

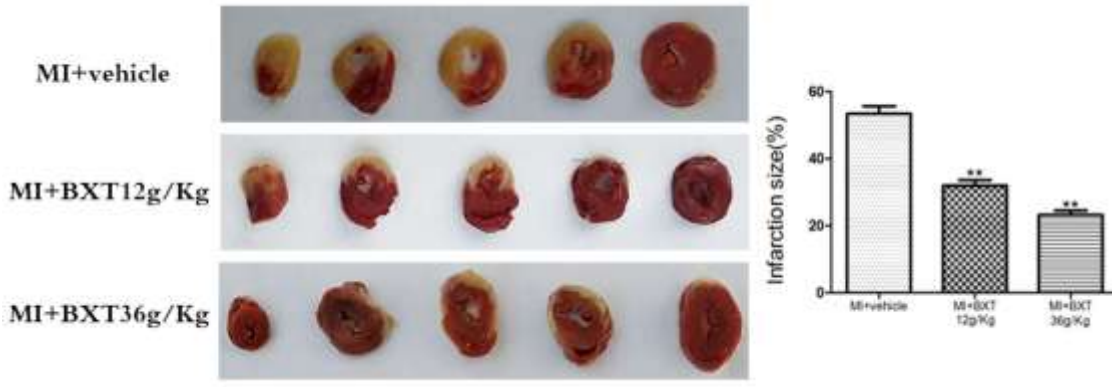

Figure 1: Effects of BXT on myocardial infarct size (Mean ± SD, n = 6). ** $P < 0.01$ vs. vehicle-treated group.

doi:10.21010/ajtcam.v14i2.8

Effects of BXT on the serum levels of CRP, IL-6, and IL-10

We determined the serum levels of CRP, IL-6, and IL-10 to confirm if BXT reduced the inflammatory response. As shown in **Figure. 2A–C**, the serum levels of CRP and IL-6 in the BXT-treated group were reduced compared to the vehicle-treated group ($P < 0.05$). The serum levels of IL-10 in the BXT (36 g/kg)-treated group were significantly increased compared to the vehicle-treated group ($P < 0.05$). However, there was no significant difference between the 12 g/kg BXT dose and the vehicle-treated groups ($P > 0.05$).

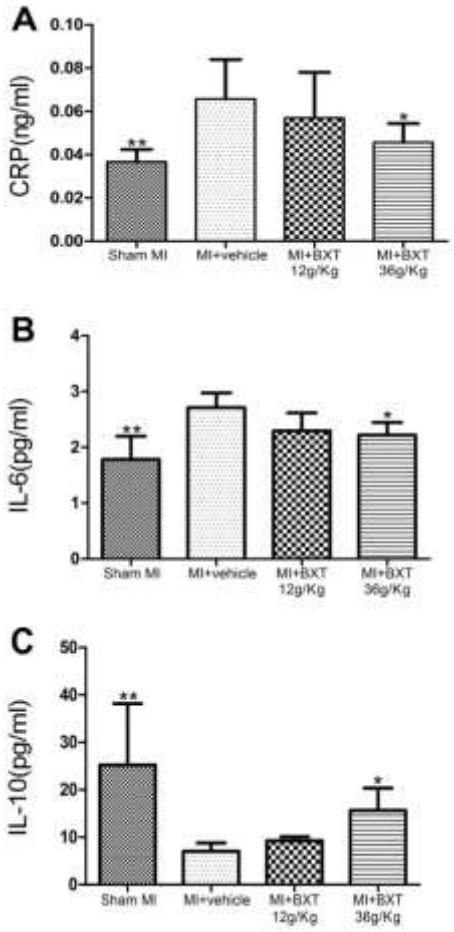

Figure 2: Effects of BXT on the concentrations of circulating inflammatory markers, C-reactive protein (CRP), serum interleukin-6 (IL-6), and interleukin-10 (IL-10) in the sham MI group (n = 6), the MI + vehicle group (orally administered vehicle, n = 6), the MI + BXT (orally administered BXT extract at 12 g/kg; n = 6), and the MI + BXT group (orally administered BXT extract at 36 g/kg; n = 6). (A) Serum CRP levels; (B) serum IL-6 levels; (C) serum IL-10 levels. *P < 0.05 and **P < 0.01 vs. the vehicle-treated group. MI, myocardial infarction; BXT, Bao-Xin-Tang.

Antioxidant assays

In the BXT-treated groups, the serum SOD levels were significantly increased ($P < 0.01$ or 0.05; **Figure. 3A**), while the levels of serum MPO were decreased compared to the vehicle-treated groups ($P < 0.01$; **Figure. 3B**). In addition, there were no

doi:10.21010/ajtcam.v14i2.8

significant differences between the 12 g/kg BXT-treated group and the 36 g/kg BXT-treated group in the serum levels of SOD and MPO (all P > 0.05).

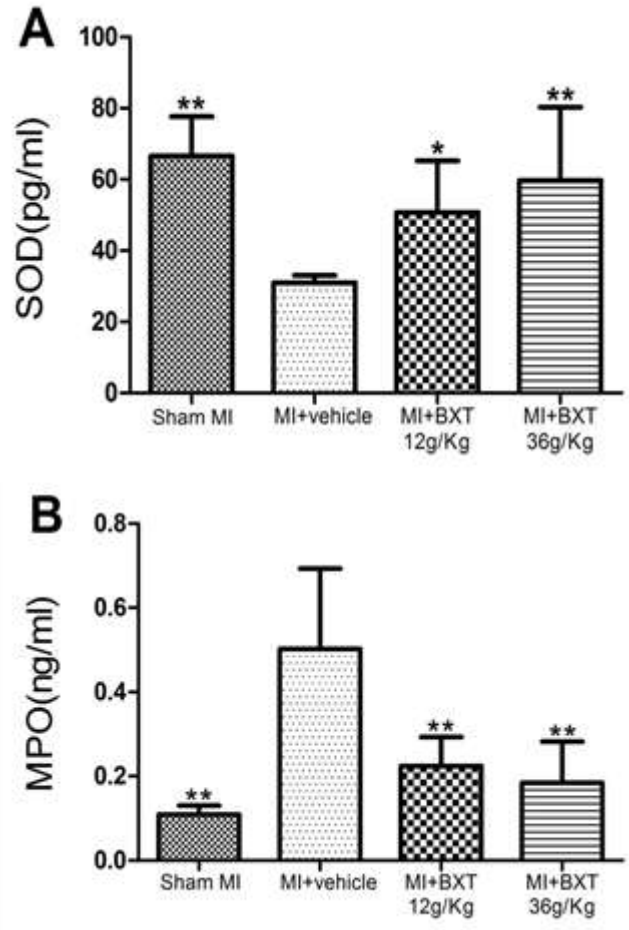

Figure 3: Antioxidant assay and BXT effects. (A) Superoxide dismutase (SOD); (B) myeloperoxidase (MPO). Sham MI group (n = 6), MI + vehicle group (orally administered vehicle, n = 6), MI + BXT (orally administered BXT extract at 12 g/kg; n = 6), and MI + BXT (orally administered BXT extract at 36 g/kg; n = 6). Serum levels of SOD and MPO were determined by ELISA. *P < 0.05 and **P < 0.01 vs. the vehicle-treated group.

Discussion and Conclusion

The results of this study confirm that: (Boateng and Sanborn, 2013) BXT significantly reduced infarct size compared with the vehicle-treated group, (O'Gara et al., 2013) myocardial infarction in rats pre-treated with BXT led to a sharp decrease of CRP as well as IL-6, and enhanced anti-inflammatory cytokine IL-10 activities, and (Kloner 2006) BXT markedly ameliorated oxidative stress (MPO), and enhanced the activity of SOD in acute myocardial infarct rat models. These findings support the hypothesis that BXT protects rat hearts from acute cardiac injury and may mediate the cardioprotection via inhibiting inflammatory and oxidative damage.

BXT effectively reduced myocardial infarct size in AMI. Infarct size is an important indicator of the effectiveness of cardiovascular drugs in the treatment of CVD (Maulik et al., 1999). For this reason, reduction of infarction size should be considered a

principal therapeutic goal (Ferreira, 2010).

In AMI, the inflammatory response plays a critical role (Frangogiannis et al., 2002), through the secretion of inflammatory cytokines, and inflammatory cells that aggregate and infiltrate during the inflammatory process (Speyer and Ward, 2011). Atherosclerosis is a serious inflammatory disease of the blood vessels, and inflammatory processes participate during the entire period of atherosclerosis (Li, 2005), causing the delay of coronary reflow, coronary microvascular endothelial dysfunction, and vascular restenosis (Li, 2004; Li et al., 2007). Oxidative stress and inflammatory pathways correlate with the development of atherosclerotic cardiovascular diseases (Nabel and Shurin, 2007). The levels of IL-6 and CRP are biomarkers for systemic inflammation and are positively associated with CHD risk (Ridker et al.,2000). CRP is an acute-phase protein generated by liver cells and synthesized in the course of tissue damage or infection (Agrawal et al., 2010; Du Clos, 2013; Di Napoli et al.,2011). Studies have shown that both CRP and components of the activated complement system are deposited and colocalized in myocardial infarcts, and further shows that complement activation is due to the presence of CRP (Pegues et al., 2013). CRP has been shown to exacerbate left ventricular dysfunction and promote adverse left ventricular remodeling after myocardial infarction. It may promote inflammation and thrombosis via directly interacting with atherosclerotic vessels or ischemic myocardium, thus stimulating the complement system (Ridker et al.,2000; Ridker, 2003). IL-6 is a pro-inflammatory cytokine that stimulates hepatic production of CRP as an acute-phase protein, modulates cell adhesion, and promotes coagulation of platelets. It also is a mediator of host responses to infection and inflammation and is an early marker of the acute-phase response (ARP) to infection or organ damage (Schieffer et al., 2000). IL-10 is an essential anti-inflammatory cytokine secreted by immune cells. It is a powerful Th1 cytokine inhibitor, for both interferon-gamma (IFN-γ) and IL-2 (Lalani et al., 1997; Howard and O'Garra, 1992; Opal et al., 1998). In the present study, BXT was linked with the augmentation of the anti-inflammatory cytokine IL-10 in rats with AMI. However, in the BXT-treated groups, the serum level of pro-inflammatory markers IL-6 and CRP were reduced compared to the vehicle-treated groups. Hence, BXT possesses anti-inflammatory properties, to protect against the cardiomyocyte damage of AMI.

There are numerous studies describing the effects of myocardial infarction. Data show that heart failure follows myocardial infarction, by altering the oxidative condition and decreasing myocardial antioxidant ability (Hill and Singal 1996). Oxidative stress is often caused by an imbalance between reactive oxygen species (ROS) and the defense system against antioxidants (Steinberg, 1997; Stocker and Keaney, 2004; Quinn et al., 1987). Free radicals and ROS are the basis for many diseases and play a harmful role in cardiac function. Experimental and clinical trials have demonstrated that ROS, such as superoxide, hydrogen peroxide, and hydrogen radicals, are detected in the myocardium of failing hearts of rats (Rajadurai and Stanely, 2006). Lipid peroxidation is a biomarker of oxidative stress, and it participates in the injury mechanism of cells. Increased lipid peroxidation, derived from oxidative stress, alters the cellular balance between pro-oxidants and antioxidants (Kumari and Menon, 1987). Superoxide can remove lipid peroxides by regulating the balance between oxidation and anti-oxidation reactions, thus preventing the formation of atherosclerosis (Halliwell, 1994; Kiruthiga et al., 2007). Myeloperoxidase (MPO) is a heme protein, which is secreted by activated human leukocytes. It promotes lipid peroxidation *in vitro* through the production of reactive intermediates, and MPO is a major source of oxidation during stress-induced inflammation (Zhang et al., 2002). The activities of SOD and MPO show a close relationship with cardiac muscle injury. Our results suggest that SOD activity in the treatment group was increased, and MPO levels were significantly decreased when compared with the vehicle-treated group. Because BXT increases the oxygen free radical scavenging system in cells, reduces free radical production, and decreases lipid peroxidation product formation, the damage from myocardial oxygen free radicals and myocardial ischemia injury is reduced. In summary, the BXT cardioprotective mechanism is still not fully understand because BXT is a complex mixture of herbs, and the role of each individual component has not been determined. Consequently, additional studies are necessary to identify and characterize the actions of the individual components of BXT. The current study demonstrates that Bao-Xin-Tang (BXT) ameliorates AMI and alleviates myocardial cell injury. The cardioprotective profile of BXT shows potent anti-inflammatory and anti-oxidative properties. Oral BXT may be a possible therapeutic agent for acute myocardial infarction.

doi:10.21010/ajtcam.v14i2.8

Acknowledgments

The research was supported by the Science and Technology Project of Changsha City of China (Grant No. K1205018-31)

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