Effect of *Atractylodes macrocephala* rhizoma on isoproterenol-induced ventricular remodeling in rats

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Abstract. Myocardial infarction (MI) is the primary cause of ventricular remodeling (VR). The aim of the present study was to determine the effect of *Atractylodis macrocephalae* rhizoma (AMR) on VR induced by isoproterenol (ISO) in rats. Male Sprague Dawley rats were randomly divided into the normal control, ISO-induced and AMR groups. Rats in the ISO-induced and AMR groups were subcutaneously injected with 85 mg/kg/day ISO for two consecutive days. Compared with the ISO-induced group, AMR normalized the levels of hemodynamic parameters, markedly attenuated myocardial pathological damage, decreased the level of N-terminal prohormone of brain natriuretic peptide, and inhibited cardiac hypertrophy and myocardial fibrosis. In addition, AMR inhibited oxidative stress and activation of the rennin-angiotensin-aldosterone system (RAAS) when compared with the ISO-induced group. The results of the present study suggest that AMR may reverse VR via its anti-oxidative effect and inhibition of RAAS activation.

Introduction

Isoproterenol (ISO) is a β-adrenoceptor agonist and synthetic catecholamine. Treatment with a high dose of ISO can result in myocardial infarction (MI) and the development of necrotic lesions in the myocardium of experimental animals (1,2). The majority of these undesirable consequences in patients with MI are associated with ventricular remodeling (VR), which occurs post-infarction (3). The mechanism underlying VR following MI remains to be elucidated, despite the advances in medical treatment over previous decades. VR is associated with an increased risk of cardiovascular death and heart failure (HF) (4,5). VR occurs in a similar terminal sequence of molecular, biochemical and mechanical events that lead to HF. Therefore, inhibition of VR in post-MI patients is beneficial. VR, a myocardium-associated response to noxious, hemodynamic, metabolic and inflammatory stimuli, is associated with mortality in patients with acute coronary syndromes (6). Furthermore, myocyte hypertrophy and loss following necrosis or apoptosis, interstitial cell growth and fibroblast proliferation, which in turn leads to myocardial fibrosis, are associated with VR (7). VR is also affected by preload and afterload activation of the neurohumoral system, and other factors that further adversely influence the remodeling process (8). The net result of these events is the development of left ventricular hypertrophy with or without fibrosis, which ultimately progresses to left ventricular dilation and systolic failure (9).

Atractylodis macrocephalae rhizoma (AMR), the dry rhizome of *Atractylodes macrocephala* koidz, is an edible Chinese medicinal herb. In traditional Chinese medicine, herbal medicinal compounds containing AMR are frequently administered in oral treatments for a number of diseases, including congestive HF. For example, Ling Gui Zhu Gan Tang, composed of four herbal components, Hoelen, Cinnamomi cortex, AMR and *Glycyrrhizae radix*, is frequently used for the treatment of diseases associated with edema, including chronic bronchitis, congestive HF and chronic nephritis (10). However, the protective effect of AMR, as a single drug therapy in the cardiovascular system, has not been extensively studied. The present study investigated the effect of AMR treatment on ISO-induced VR in order to provide experimental evidence for potential future clinical treatments.

Materials and methods

Animals. Healthy male Sprague Dawley rats (n=37, 180-200 g, aged 6-7 weeks) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The animals were individually housed at the ambient temperature of 22-24˚C and humidity of 30-50% with a 12-h light/dark cycle, and free access to standard food and water. All rats received humane care and animal experiments were performed in accordance with the guidelines of the Animal Care and Use

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Committee of Shanghai University of Traditional Chinese Medicine and conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (11) (NIH publication number: 85-23, revised in 1996). Ethical approval for all animal experiments performed in the present study was obtained from the Medical Ethics Committee of Shanghai University of Traditional Chinese Medicine (Shanghai, China; reference no. SZY201504022).

Materials. AMR was purchased from Kangqiao Traditional Chinese Medicine Co., Ltd. (Shanghai, China) and decocted in water to the concentration of 0.24 g crude drug/ml. Isoproterenol hydrochloride (99.0% purity; lot number: MI5VB-DI) was purchased from TCI (Shanghai) Development Co., Ltd. (Shanghai, China).

Experimental protocols. All rats were randomly allocated to the normal control group (n=7) or ISO-induced group (n=30). Rats in the normal control and ISO-induced groups were subcutaneously injected with physiological saline (4 ml/kg, the solvent for ISO) and ISO 85 mg/kg/day, respectively, for two consecutive days. Following ISO treatment, 18 rats survived in the ISO-induced group and were randomly allocated to the AMR and ISO-induced groups (n=9). Rats in the normal control and ISO-induced groups were administered intragastrically with drinking water (10 ml/kg/day), and rats in the AMR group were administered a AMR decoction at a volume of 10 ml/kg/day for 4 weeks from the second day following the administration of ISO.

Following 4 weeks of experimentation, all rats were anesthetized with an intraperitoneal injection of urethane (1.0 g/kg; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Once body weight (BW) and hemodynamic parameters were measured, blood samples were collected and centrifuged at 4˚C and 1,780 x g for 10 min to recover the serum. After blood sample collection, rats were sacrificed and hearts were removed immediately and washed in chilled physiological saline. The left ventricles (LVs) were separated from the atria, aorta and adipose tissue. The upper part of the LV was fixed in 10% formalin at 22-24˚C for one week and embedded in paraffin wax, and the lower part of the LV and serum were stored at -80˚C until further analysis.

Measurement of hemodynamic parameters. The right carotid artery was separated and cannulated with a polyethylene 90 catheter filled with 80 U/ml heparin saline, connected to a pressure transducer for the measurement of systolic blood pressure (SBP), diastolic BP (DBP), mean arterial BP (MABP), pulse pressure (PP), LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and the maximum rate of LV pressure increase and decline ($+dP/dt_{max}$ and $-dP/dt_{max}$, respectively). All parameters were continuously recorded using a multichannel biological signal analysis system (RM6240C; Chengdu Technology & Market Co., Ltd., Chengdu, China).

Determination of heart weight index (HWI), LV weight index (LVWI) and histopathological observations. Following the excision of hearts, excluding connective tissue and large blood vessels, heart weight (HW) and LV weight (LVW) were measured, and the HWI and LVWI were estimated by calculating the ratios of HW to BW and LVW to BW.

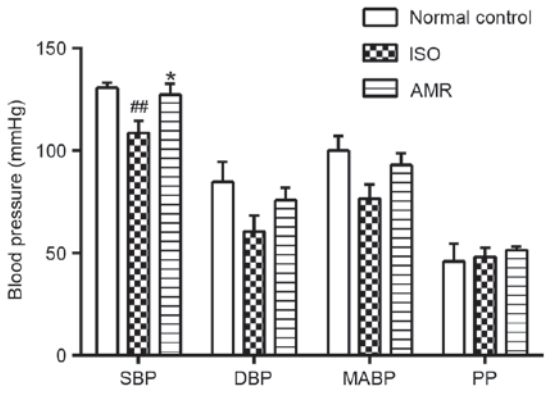

Figure 1. Effect of AMR on blood pressure parameters. Data are presented as the mean ± standard error of the mean (n=5). $^{\#\#}$P<0.01 vs. the normal control group; *P<0.05 vs. the ISO-induced group. SBP, systolic blood pressure; DBP, diastolic blood pressure; MABP, mean arterial blood pressure; PP, pulse pressure; ISO, isoproterenol; AMR, *Atractylodis macrocephalae* rhizoma.

The aforementioned fixed parts of the LVs were dehydrated in ethanol (70-100%), cleared in xylene, and embedded in paraffin. Each specimen was cut into 5 μm thick sections and heated overnight in a 60˚C incubator. The sections were stained with hematoxylin and eosin (H&E) and Masson stain at room temperature for one day. Images of each sample were captured (magnification, x400) under a light microscope (UB202i; Chongqing COIC Industrial Co., Ltd., Chongqing, China). A total of three random fields in each H&E stained sample were examined and 30 myocardial cells from each field were selected to calculate the mean cross section area of cardiomyocytes. The interstitial collagen volume fraction (ICVF), in the selected myocardium sections stained with Masson stain, was calculated as a percentage of the collagen area/field. Three sections of each sample were randomly selected to calculate the above parameters using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA).

Measurement of the level of N-terminal prohormone of brain natriuretic peptide (NT-proBNP) in serum. The levels of NT-proBNP in sera were determined using a rat NT-proBNP ELISA kit according to the manufacturer's protocol (cat no. JL15585; Shanghai Jianglai Biotechnology Co., Ltd., Shanghai, China).

Measurement of malondialdehyde (MDA) content and the activities of antioxidant enzymes in the myocardium. LV tissue (100 mg) was homogenized with 1 ml chilled physiological saline and centrifuged at 4˚C, 1,780 x g for 15 min to collect the supernatant. Myocardium protein and MDA levels, and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were measured using the Coomassie Brilliant Blue method, thiobarbituric acid method, xanthine oxidase method and a rate assay, respectively, using kits manufactured by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The MDA level and activities of antioxidant enzymes in the myocardium were expressed as their contents/mg protein of ventricular tissue.

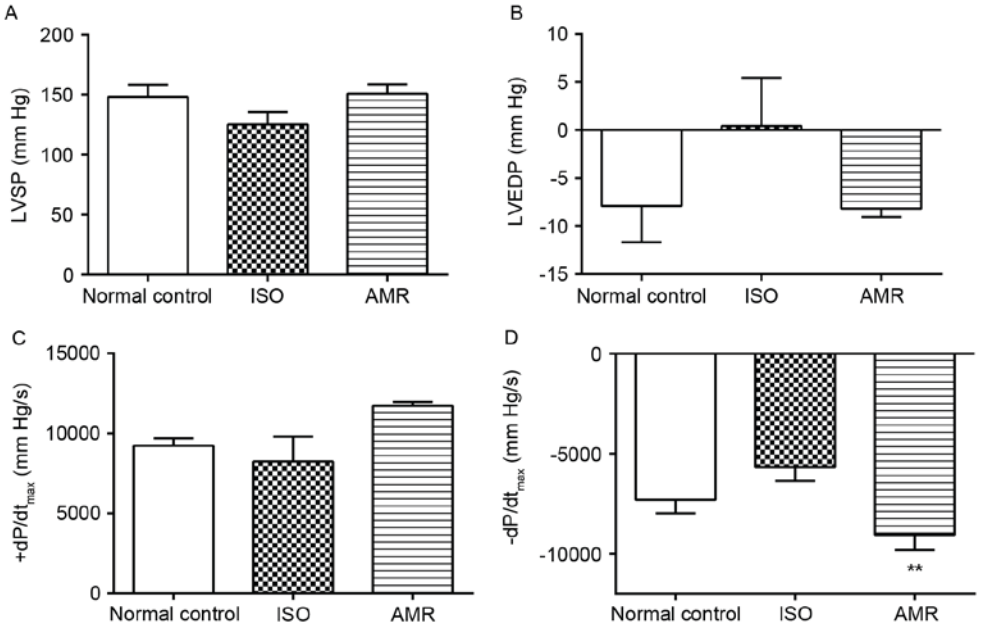

Figure 2. Effect of AMR on hemodynamic parameters. Levels of (A) LVSP, (B) LVEDP, (C) $+dP/dt_{max}$ (D) $-dP/dt_{max}$. Data are presented as the mean ± standard error of the mean (n=5). **P<0.01 vs. the ISO-induced group. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; $+dP/dt_{max}$, the maximum rate of LV pressure increase; $-dP/dt_{max}$, the maximum rate of LV pressure decline; ISO, isoproterenol; AMR, *Atractylodis macrocephalae* rhizoma.

Measurement of angiotensin II (Ang II) and aldosterone (ALD) levels in the myocardium. The levels of Ang II and ALD in the myocardium were measured by radioimmunoassays with an Iodine [^{125}I] Angiotensin II Radioimmunoassay kit (cat no. D02PZB) and Iodine [^{125}I] Aldosterone Radioimmunoassay kit (cat no. D03PZB). All measurements were performed according to the manufacturer's protocol (Northern Biotechnology Research Institute, Beijing, China). The contents of Ang II and ALD were expressed as their mass/mg total protein in ventricular tissue and mass/ml serum, respectively.

Statistical analysis. Data were analyzed using one-way analysis of variance and presented as the mean ± standard error of the mean. A Student-Newman-Keuls post hoc test was performed for multiple comparisons. A rank-sum test was used as an alternative test for variance heterogeneity. Statistical analysis was performed using SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). Experiments were repeated ≥5 times. P<0.05 was considered to indicate a statistically significant difference.

Results

Hemodynamic parameters. SBP levels significantly decreased in the ISO-induced group compared with the normal control group (Fig. 1; P<0.01). Administration of AMR significantly increased SBP levels when compared with the ISO-induced group (P<0.05), presenting levels similar to those of the normal control group (Fig. 1). Compared with the ISO-induced group, DBP increased in the AMR group, however, the difference was not statistically significant. There were no differences in MABP and PP values between all groups.

The levels of $-dP/dt_{max}$ were not significantly different in the ISO-induced group compared with the normal control group; however, they significantly increased in the AMR group when compared with the ISO-induced group (Fig. 2; P<0.01). LVSP, LVEDP and $+dP/dt_{max}$ levels did not significantly differ between the groups.

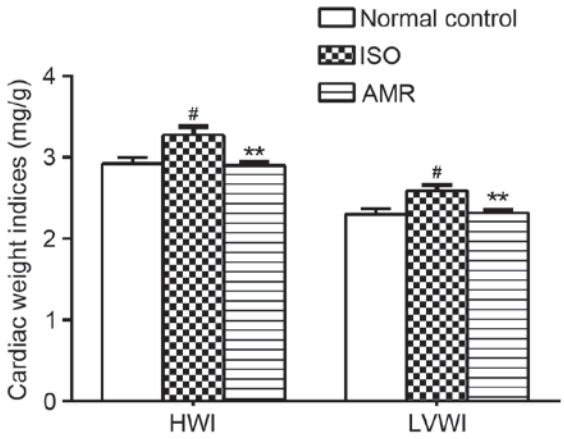

Figure 3. Effect of AMR on cardiac weight indices. Data are presented as the mean ± standard error of the mean (n=7). $^{\#}$P<0.05 vs. the normal control group; **P<0.01 vs. the ISO-induced group. HWI, heart weight index; LVWI, left ventricular weight index; ISO, isoproterenol; AMR, *Atractylodis macrocephalae* rhizoma.

Cardiac weight indices and histopathology. In the present study, LVWI and HWI significantly increased in the ISO-induced group when compared with the normal control group (both P<0.05; Fig. 3). Following 4 weeks of AMR treatment, LVWI and HWI levels in the AMR group significantly decreased, compared with the ISO-induced group (P<0.01).

In the ISO-induced group, the average cross section area markedly increased compared with the normal control group (P<0.01); however, it markedly decreased in the AMR group

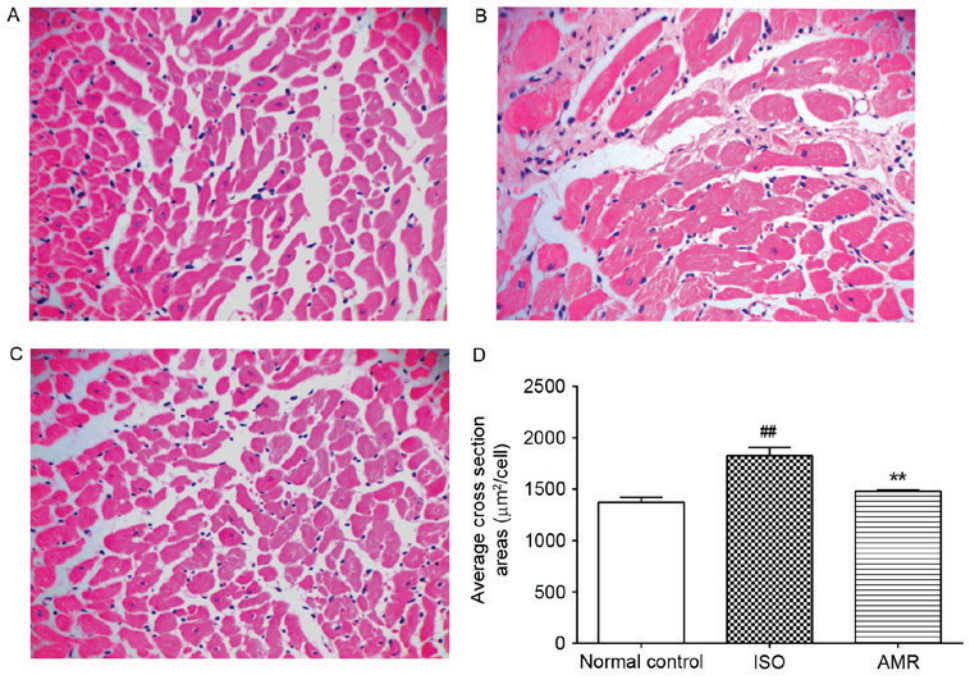

Figure 4. Effect of AMR on the average cross section area of cardiomyocytes determined by histopathological observations of cardiac tissue stained using hematoxylin and eosin (magnification, x400). (A) The normal control group. (B) The ISO-induced group. (C) The AMR group. (D) Effect of AMR on average cross section area of cardiomyocytes. Data are presented as the mean ± standard error of the mean (n=5). ##P<0.01 vs. the normal control group; **P<0.01 vs. the ISO-induced group. ISO, isoproterenol; AMR, *Atractylodis macrocephalae* rhizoma.

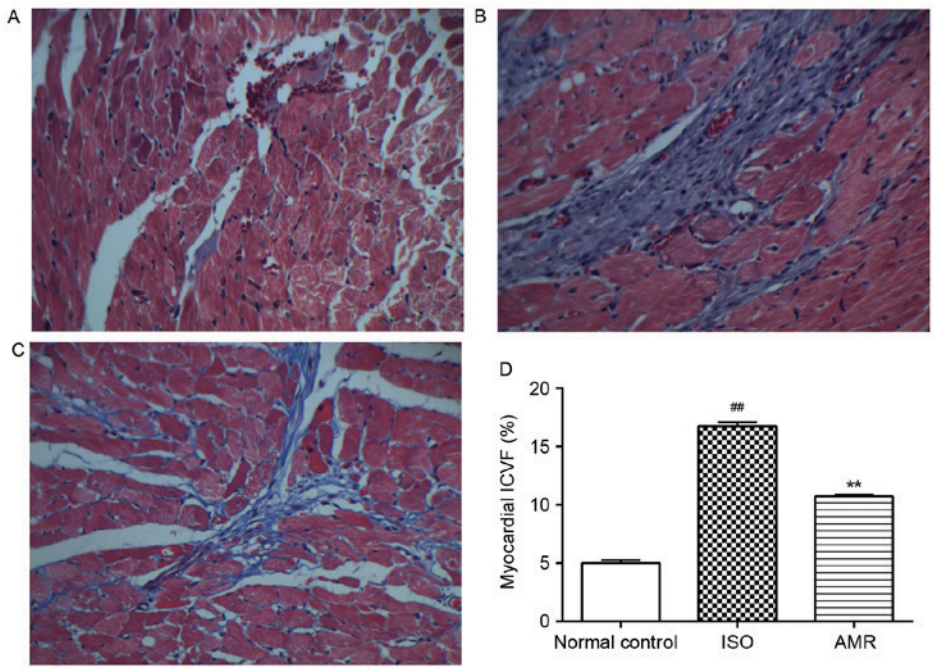

Figure 5. Effect of AMR on the ICVF and histopathological observations in cardiac tissue Masson staining of tissues from the (A) normal control, (B) ISO-induced and (C) AMR groups (magnification, x400). (D) Effect of AMR on ICVF. Data are presented as the mean ± standard error of the mean (n=5). ##P<0.01 vs. the normal control group; **P<0.01 vs. the ISO-induced group. ICVF, myocardial interstitial collagen volume fraction; ISO, isoproterenol; AMR, *Atractylodis Macrocephalae* rhizoma.

(P<0.01; Fig. 4). In the myocardium of the normal control group, a low level of collagen was identified in the interstitial space. There was a significant increase in the accumulation of collagen in the ventricle of the ISO-induced group (P<0.01; Fig. 5). Decreased levels of collagen deposition were identified in the AMR group compared with the ISO-induced group (P<0.01). The above results were confirmed by quantification of the ICVF (Fig. 5).

Levels of NT-proBNP in the myocardium. Compared with the normal control group, treatment with ISO resulted in a significant increase in the levels of NT-proBNP in the myocardium

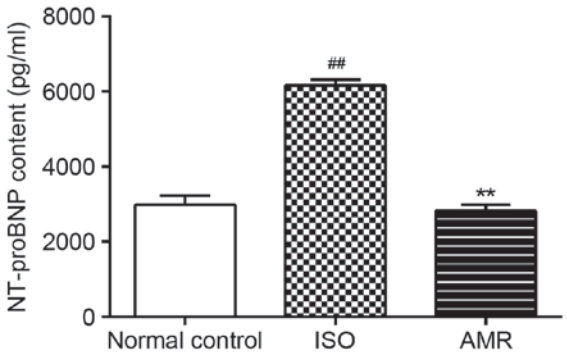

Figure 6. Effect of AMR on NT-proBNP levels in the myocardium. Data are presented as the mean ± standard error of the mean (n=7). ##P<0.01 vs. the normal control group; **P<0.01 vs. the ISO-induced group. NT-proBNP, N-terminal B-type natriuretic peptide; ISO, isoproterenol; AMR, *Atractylodis macrocephalae* rhizoma.

(P<0.01; Fig. 6). Administration of AMR decreased the levels of NT-proBNP to similar levels to those observed in the normal control group (Fig. 6).

Levels of MDA, SOD and GSH-Px in the myocardium. The levels of MDA significantly increased and the activity of GSH-Px significantly decreased in the myocardium of the ISO-induced group when compared with the normal control group. In the AMR group, the level of MDA significantly decreased compared with the ISO-induced group (P<0.01; Fig. 7). All other comparisons were not significantly different.

Levels of Ang II and ALD in the myocardium. In the present study, the levels of Ang II in the myocardium significantly increased in ISO-induced rats (P<0.05). Administration of AMR significantly decreased the levels of Ang II and ALD, when compared with the respective ISO-induced groups (both P<0.01; Fig. 8).

Discussion

VR is associated with alterations in the structure and function of the myocardium, which include cardiac dilatation, myocardial hypertrophy, interstitial fibrosis and a reduction in contractility and relaxation of the heart (12,13). VR frequently occurs as a result of MI and hypertension (14,15). Administration of a supramaximal dose of ISO can induce cardiomyocyte necrosis, and has been used to induce MI in animal experimental research through catecholamine toxicity (16,17) and the subsequent stimulated VR and myocardial hypertrophy as a result of infarct expansion. In VR animal models, cardiac WI and the average cross section area of cardiomyocytes are indicators used to measure the severity of cardiac hypertrophy (18,19). In the present study, ISO treatment resulted in myocardial hypertrophy, demonstrated by elevated levels of HWI and LVWI, and an increased average cross section area of cardiomyocytes in rats. These results suggested that AMR may limit the extent of myocardial hypertrophy induced by ISO.

VR is not only measured by the extent of myocardial hypertrophy, but also by the alterations in myocardial interstitial composition. In cardiac tissues, as well as in other organs,

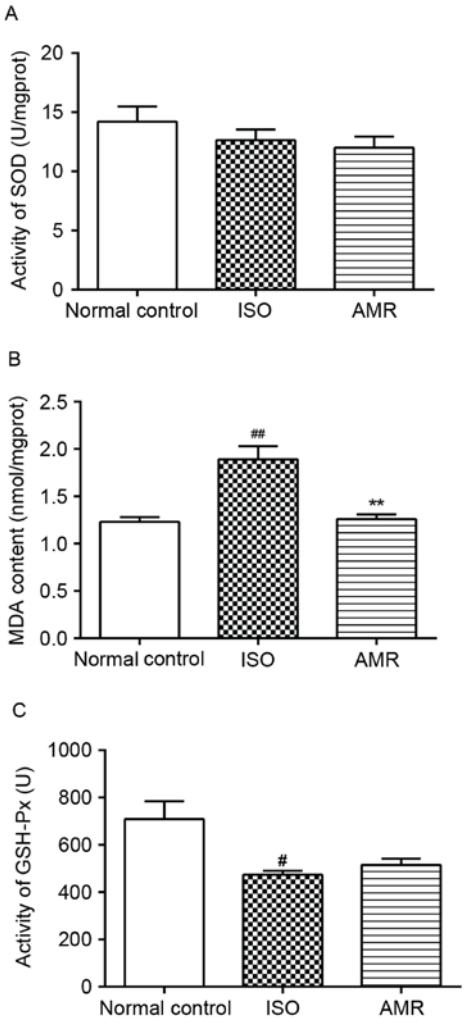

Figure 7. Effect of AMR on the levels of SOD, MDA and GSH-Px in the myocardium. Levels of (A) SOD activity, (B) MDA content and (C) GSH-Px activity. Data are presented as the mean ± standard error of the mean (n=7). #P<0.05 and ##P<0.01 vs. the normal control group; **P<0.01 vs. the ISO-induced group. SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; ISO, isoproterenol; AMR, *Atractylodis macrocephalae* rhizoma.

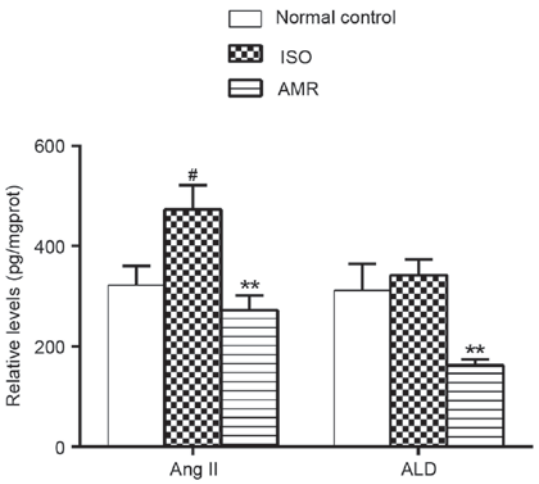

Figure 8. Effect of AMR on the levels of Ang II and ALD in the myocardium. Data are presented as the mean ± standard error of the mean (n=7). #P<0.05 vs. the normal control group; **P<0.01 vs. the ISO-induced group. Ang II, angiotensin II; ALD, aldosterone; ISO, isoproterenol; AMR, *Atractylodis macrocephalae* rhizoma.

myofibroblasts are hypothesized to serve a role as predominant cellular mediators of fibrosis (20). Myofibroblasts, the primary source of cardiac fibrosis, can secrete extracellular matrix components including collagen, fibronectin and laminin to promote the development of fibrosis (21,22). An increase in collagen secretion and aggregation can lead to alterations in cardiac structure and the occurrence of VR or ventricular dysfunction (23,24). Therefore, inhibition of myocardial fibrosis can improve cardiac function. In the present study, treatment with AMR markedly reduced the accumulation of intercellular collagen. It can be hypothesized that AMR may delay and inhibit fibrosis by reducing the synthesis and secretion of collagen, and subsequently delay or inhibit the process of VR and myocardial failure initiated by myocardial hypertrophy or fibrosis.

In the present study, hemodynamic parameters were used to evaluate VR. Characteristics of ISO-induced cardiac dysfunction include diastolic and systolic dysfunction (25,26), which may result from cardiac apoptosis and disruption of myofibrils. Validation of effective screening tools for the identification of patients with early stage VR (diagnostic markers) is therefore required. In the present study, AMR inhibited the increase in LVEDP and the reduction of SBP, DBP, LVSP and $\pm dP/dt_{max}$ induced by ISO. These results indicated that following 4 weeks of treatment, AMR can alleviate LV dysfunction.

BNP is synthesized and stored as a full-length prohormone and cleaved to yield equimolar amounts of NT-proBNP. NT-proBNP is secreted into the blood from cardiac ventricles in response to excessive stretching of cardiomyocytes and volume overload (27,28). The level of NT-proBNP is a biomarker for the diagnosis and monitoring of VR (29). In the present study, rats treated with AMR demonstrated a decrease in the level of NT-proBNP, suggesting that AMR may protect the heart from ISO-induced VR and heart failure.

ROS accumulate to promote oxidative stress (OS) and lipid membrane peroxidation (30,31). Accumulation of quinone metabolites induced by ISO may result in OS, which reacts with oxygen to produce superoxide anions (O^{2-}), hydroxyl radicals (OH^{-}) and hydrogen peroxide (H_2O_2) species, and interfere with antioxidant enzymes (32). ROS degrade polyunsaturated lipids, leading to the formation of MDA as a final product of lipid peroxidation (31). MDA causes toxic stress in cells and it is used as a biomarker to measure the level of OS (33). Antioxidant enzymes, including SOD and GSH-Px, can remove ROS to inhibit OS-associated injury and protect organisms from the release of free radicals (32). In the present study, the activity of GSH-Px markedly decreased and MDA content increased in ISO-induced rats. AMR inhibited the increase in MDA levels induced by ISO. These results suggested that AMR may alleviate OS injury induced by ISO following 4 weeks.

The rennin-angiotensin-aldosterone system (RAAS) is an endocrine system that serves a role in the development and progression of cardiovascular diseases. Activation of RAAS is associated with VR (34,35). Ang II serves a role in promoting cardiac hypertrophy, fibrosis, the production of ALD, retention of water and sodium, and the activation of the sympathetic nervous system (36). Therefore, constant elevated levels of Ang II and ALD in the myocardium can induce deleterious effects on the cardiovascular system. Treatment with AMR can reduce the levels of Ang II and ALD. These results indicated that AMR can reduce the activation of RAAS in the myocardium.

In conclusion, AMR may prevent VR induced by ISO in rats, by inhibiting cardiac hypertrophy, myocardial fibrosis, NT-proBNP levels and by improving the levels of hemodynamic parameters. It can be hypothesized that AMR-induced mitigation of VR is associated with its anti-oxidative effect and prevention of activation of RAAS. The results may be helpful to fundamental pharmacology research and AMR may be an effective treatment for patients with VR and improve the clinic therapy strategies.

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