Pharmacogn. Mag.
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ORIGINAL ARTICLE

Effects of *Atractylodes macrocephala* Rhizoma on Isoproterenol-Induced Myocardial Hypertrophy in Mice

Ke-Zhao Wei[1], Xiao-Hua Cui[1,2]*, Jia-Hua Feng[1], Ping-An Yao[1], Jian-Ping Gao[1]

[1]Department of Pharmacology, School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, [2]Department of Pharmacy, Zhejiang Hospital, Hangzhou, Zhejiang Province, China
*This author has contributed equally

Submitted: 02-12-2018 Revised: 02-01-2019 Published: 19-09-2019

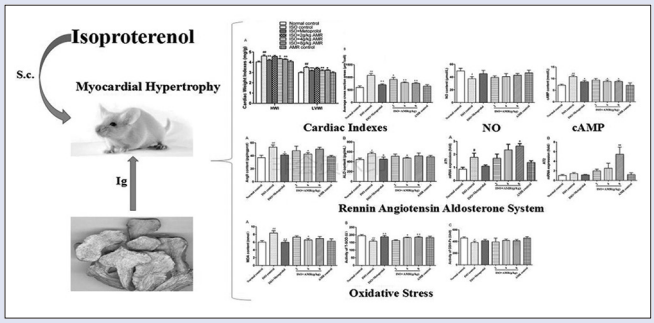
ABSTRACT

Background: The studies about the protective effect on the heart of single *Atractylodes macrocephala* rhizoma (AMR) herb and mechanisms have not been reported. **Objective:** The purpose of this study was to assess the effects of AMR on attenuating myocardial hypertrophy induced by isoproterenol (ISO) in mice. **Materials and Methods:** Mice were randomly divided into normal control group, ISO control group, ISO plus metoprolol (60 mg/kg) group, ISO plus AMR (2, 4, and 8 g/kg) groups, and AMR (4 g/kg) control group. The mice with myocardial hypertrophy were established by subcutaneous (s.c.) injection with ISO (2 mg/kg/d) and administered intragastrically with the corresponding drugs in the volume of 0.2 mL/10 g/d for 7 days. In the normal and AMR control groups, mice were injected (s.c.) with physiological saline (the solvent for ISO) and administered intragastrically with drinking water for 7 days. **Results:** Compared with the ISO-induced group, AMR significantly decreased heart weight index, left ventricular weight index, and average transverse area of cardiomyocytes, significantly increased the activity of total superoxide dismutase in serum and the level of the angiotensin II receptor type (AT) gene expression in myocardium and significantly decreased the contents of malondialdehyde, cyclic adenosine monophosphate , and aldosterone in serum and angiotensin II (Ang II) in myocardium. **Conclusion:** The ability of AMR to mitigate myocardial hypertrophy is partly associated with its anti-oxidative effect, restraining excessive secretion or activation of neuroendocrine factors, and the stronger upward effect on AT_2 gene expression than AT_1.

Key words: Angiotensin II receptor type, *Atractylodes macrocephala* rhizoma, isoproterenol, myocardial hypertrophy, rennin angiotensin aldosterone system

SUMMARY

- The aim of this study was to explore the potential mechanism of *Atractylodes macrocephala* rhizoma (AMR) mitigate myocardial hypertrophy. Results: AMR mitigates myocardial hypertrophy caused by isoproterenol by regulating rennin angiotensin aldosterone system.



Abbreviations used: AMR: *Atractylodes macrocephala* rhizoma; ISO: Isoproterenol; BW: Body weight; HW: Heart weight; LVW: Left ventricular weight; HWI: Heart weight index; LVWI: Left ventricular weight index; MDA: Malondialdehyde; T-SOD: Total superoxide dismutase; GSH-Px: Glutathione peroxidase; NO: Nitric oxide; cAMP: Cyclic adenosine monophosphate; Ang II: Angiotensin II; ALD: Aldosterone; AT_1: Angiotensin II receptor type 1; AT_2: Angiotensin II receptor type 2; RAAS: Rennin angiotensin aldosterone system; VR: Ventricular remodeling; MI: Myocardial ischemia.

Correspondence:

Prof. Jian-Ping Gao,
Department of Pharmacology, School of Pharmacy, Shanghai University of Traditional Chinese Medicine, No. 1200 Cailun Road, Shanghai 201203, China.
E-mail: zydgjp@aliyun.com
DOI: 10.4103/pm.pm_617_18

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INTRODUCTION

Myocardial hypertrophy is usually caused by stress stimuli or diseases such as hypertension, valvular heart disease, myocardial infarction, and excessive activation of cardiac neurohormones.[1] Myocardial hypertrophy is a compensatory response to the heart after increased postload, which increases the risk of heart failure for a long time.[2] It is a key part in the occurrence and development of heart failure.[3] Myocardial hypertrophy is an independent risk of many cardiovascular diseases. Patients with myocardial hypertrophy and heart failure often have fatal ventricular arrhythmias, leading to cardiac rhythm disorder, and sudden cardiac death.[4,5] Furthermore, myocardial hypertrophy, myocardial loss from necrosis or apoptosis, interstitial cell growth, and especially fibroblast proliferation, which leads to myocardial fibrosis, are related to ventricular remodeling (VR).[6] VR is also affected by preload and afterload activation of the neurohumoral system and other factors that further adversely influence the remodeling process.[7] The net result of these events is the development of left ventricular (LV) hypertrophy with or without fibrosis, which ultimately progresses to LV dilation and systolic failure.[2]

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Cite this article as: Wei KZ, Cui XH, Feng JH, Yao PA, Gao JP. Effects of *Atractylodes macrocephala* rhizoma on isoproterenol-induced myocardial hypertrophy in mice. Phcog Mag 2019;15:579-85.

Isoproterenol (ISO), a β-adrenoceptor agonist and synthetic catecholamine, exposed to the heart can result in myocardial hypertrophy with the development of necrotic lesions in the myocardium of previous myocardium research.[8] Most of these undesirable consequences in patients with myocardial ischemia (MI) are related to the VR after occurring postinfarction.[9] VR after MI remains a pivotal clinical question, despite of the advance of medical treatment over the past few decades. VR is associated with an increased risk of cardiovascular death and heart failure.[10,11] The model of cardiac hypertrophy prepared by mice is stable and commonly used.[12] In this study, the selection of the positive reference was based on the model mouse being prepared by β receptor stimulant, so metoprolol was chosen for control.

Atractylodes macrocephala rhizoma (AMR) is a dry rhizome of composite plant *A. macrocephala* Koidz (*A. macrocephalae* Koidz), which is distributed in China, Japan, Korea, Vietnam, and India.[13] As a herbal medicine, it is widely used in China and Korea.[14] Other countries have also studied AMR to discover its value.[15,16] There are many active ingredients of AMR such as polysaccharides, volatile oils, lactones, glycosides, and amino acids. Moreover, there are many prescriptions-containing AMR for treating heart failure and other diseases, for example, Zhang Zhongjing's Linggui Zhugan decoction-containing Tuckahoe, *Cinnamon*, AMR, and licorice can be used for the treatment of congestive heart failure and other related diseases,[17] but the studies about protective effect on the heart of single AMR herb and mechanisms have not been reported. Combining with its clinical application in the treatment of heart failure and other related diseases, the study of AMR on cardioprotective effect will expand its new application.

MATERIALS AND METHODS

Animals

Male Kunming mice (20 ± 2 g) were supplied by Shanghai Slac Laboratory Animal Co., Ltd. The mice were maintained at an ambient temperature of 23°C ± 1°C and the humidity at 50% ± 10% on alternatively 12 h light/dark cycles constant condition. All animals received humane care and had free access to standard food and water. The Principles of Laboratory Animal Care were followed according to the Animal Care and Use 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 (NIH publication No. 85-23, revised in 1996).

Drugs

AMR was purchased from Kangqiao Traditional Chinese Medicine Co. Ltd., (Shanghai, China) and decocted in boiling water to obtain water decoctions with the crude drug content of 0.1 g/mL, 0.2 g/mL and 0.4 g/mL, respectively. Metoprolol was from AstraZeneca Pharmaceutical Co., Ltd. (Wuxi, China). Isoprenaline hydrochloride injection was from Shanghai Harvest Pharmaceutical Co., Ltd., (Shanghai, China).

Experimental protocols

All mice were randomly divided into seven groups. Mice received a subcutaneous (s.c.) injection of ISO (2 mg/kg/d) for 7 consecutive days in ISO control group, ISO plus metoprolol (60 mg/kg) group, ISO plus AMR (2, 4, and 8 g/kg) groups, and AMR (4 g/kg) control group. In the normal control and AMR (4 g/kg) control groups, mice were injected (s.c.) with physiological saline (the solvent for ISO). The dosage of AMR is 6–12 g in the Pharmacopoeia of the People's Republic of China (2015 Edition, 103–104) and large doses of 30–150 g range are also used for clinical treatment of various diseases.[18] The dosage range for clinical use was converted to the equivalent dose of mice according to the volume surface area conversion algorithm, ranging from 1.2 to 30.8 g/kg. Moreover, according to the preliminary experimental results, we selected the 2–8 g/kg drug doses in the research, who are in the range of safe doses. Mice in the treatment groups and AMR control group were administrated with AMR or metoprolol, as positive control drug intragastrically for 7 days, while mice in the normal and ISO control groups were treated with the same volume drinking water.

Assessment of myocardial hypertrophy

At the end of the experiment, the body weight was recorded after fasting for 16 h. After mice were sacrificed, the heart was excised which excluding connective tissue and large blood vessels and the left ventricle was separated. Then, the heart weight (HW) and LV weight (LVW) were measured after blotting with filter paper so that HW index (HWI) and LVW index (LVWI) were calculated.

Moreover, the LV tissue was divided into two parts. The superior part of LV tissue was fixed in 10% formalin and embedded in paraffin wax. The lower half part was rapidly frozen in liquid nitrogen and then stored at −80°C freezer until assay.

Sections were cut into 4-μm thickness and stained with hematoxylin and eosin. Each sample slice was selected three fields randomly to photograph under the microscope (Nikon Eclipse LV100 POL, Nikon, Tokyo, Japan) and 30 myocardial cells of each field were chosen to calculate the average cross-section area of cardiomyocytes. All photos were analyzed with the image-pro plus 6.0 analyzing software (Media Cybernetics, Bethesda, MD, USA).

Measurement of oxidative stress

Blood samples were collected and centrifuged (4°C, 2325 ×*g*, 10 min) to recover serum. Serum of malondialdehyde (MDA) content, the activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) were analyzed using thiobarbituric acid method, xanthine oxidase method, and rate assay with the corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Measurement of serum nitric oxide concentration

The concentration of nitric oxide (NO) in serum was tested with the corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Measurement of the content of cyclic adenosine monophosphate in serum

The concentration of cyclic adenosine monophosphate (cAMP) in serum was assayed using ELISA according to the manufacturer's instruction (Shanghai Haling Biological Technology Co., Ltd., Shanghai, China).

Measurement of the rennin angiotensin aldosterone system

The concentrations of angiotensin II (Ang II) in myocardium and aldosterone (ALD) in serum were measured using ELISA. Diagnostic kits were provided by Shanghai Haling Biological Technology Co., Ltd., (Shanghai, China). All measurements were performed according to the manufacturers' approaches.

The gene expression levels of angiotensin II receptor type 1 (AT_1), angiotensin II receptor type 2 (AT_2) and reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured by real-time polymerase chain reaction in the presence of GAPDH left primer (GAGAAACCTGCCAAGTATGATGAC), right primer (AGAGTGGGAGTTGCTGTTGAAG); AT_1 left primer (TGGGCGTCA TCCATGACTGTA), right primer (TGAGTGCGACTTGGCCTTTG); AT_2 left primer (AACTGGCACCAATGAGTCCG), right primer (CCAAAAGGAGTAAGTCAGCCAAG). We standardized the results by the formula: microRNA/gene ΔC_T = target average C_T-internal reference average C_T; $\Delta\Delta C_T$. X = sample (x) ΔC_T-calibration specimen ΔC_T. The relative calibrated quantity was calculated by calculating the $2^{-\Delta\Delta CT}$ value, which was considered the relative mRNA expression level of AT_1 or AT_2.

Statistical analysis

All values were presented as mean ± standard error of the mean and one-way analysis of variance was performed for multiple comparisons, followed by Dunnett's test for the heterogeneity of variance. A Mann–Whitney test was utilized to analyze non-normal distribution data. Statistical analysis was performed using SPSS (IBM Corp, Ltd., New York, USA) 21.0 software. Value of $P < 0.05$ was considered statistically significant.

RESULTS

Cardiac weight indexes and histopathologic analysis

As shown in Figure 1, HWI and LVWI of mice and the average cross-section areas of the myocardial cells were significantly increased in the ISO control group ($P < 0.01$). Compared with the ISO control group, HWI and LVWI in ISO plus metoprolol group and ISO plus AMR (4 and 8 g/kg) groups were significantly decreased, and the metoprolol and AMR significantly decreased the average cross-section areas of the myocardial cells ($P < 0.05$, $P < 0.01$). There was no significant change in HWI, LVWI, and the average cross-section areas of myocardial cells between the normal control group and the AMR control group [Figure 1].

Levels of malondialdehyde, total superoxide dismutase, and glutathione peroxidase

Figure 2 shows the level of MDA was significantly increased ($P < 0.01$) and the activities of T-SOD and GSH-Px were significantly decreased in the ISO control group ($P < 0.01$, $P < 0.05$). Compared with the ISO control group, the content of MDA was significantly decreased in ISO plus metoprolol and ISO plus AMR (4 g/kg) groups ($P < 0.01$, $P < 0.05$). The activity of T-SOD in ISO plus metoprolol group and ISO plus AMR (4 and 8 g/kg) groups were significantly higher than that in ISO control group ($P < 0.01$, $P < 0.05$), but there was no significant difference in GSH-Px after treating. In the AMR control group, there were no significant changes in the levels of MDA, T-SOD, and GSH-Px compared with the normal control group [Figure 2].

Nitric oxide content

In Figure 3, the content of NO in ISO control group was significantly decreased compared with the normal control group ($P < 0.05$). However, AMR-administered mice did not show any marked change. There were no significant changes in the NO content between the normal control group and the AMR control group [Figure 3].

Cyclic adenosine monophosphate content

As shown in Figure 4, a significant rise in the content of cAMP was observed in serum in ISO-induced mice compared with the normal control group ($P < 0.01$). Treatment with metoprolol and AMR (4 and 8 g/kg) significantly decreased the content of cAMP ($P < 0.05$). In the AMR control group, there were no significant changes in the cAMP content compared with the normal control group [Figure 4].

Contents of angiotensin II and aldosterone

As shown in Figure 5, ISO-induced mice showed a significant increase in the content of Ang II and ALD ($P < 0.01$, $P < 0.05$). After administering with metoprolol and AMR (4 g/kg), mice showed significant decrease in the contents of Ang II and ALD ($P < 0.05$). In the AMR control group, there were no significant changes in the Ang II and ALD contents compared with the normal control group [Figure 5].

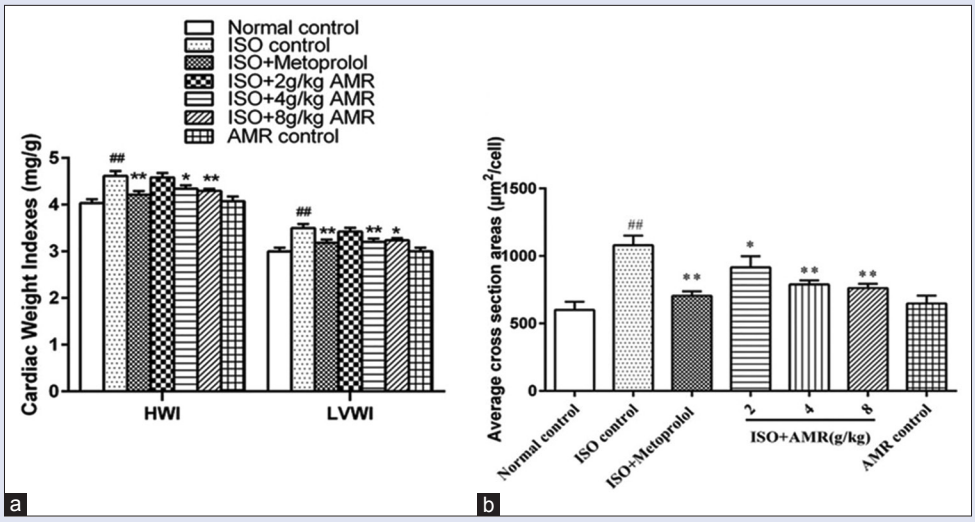

Figure 1: Effects of AMR on cardiac weight indexes (a) and average cross section areas (b) in ISO-induced mice. Values are means ± standard error of mean; AMR: *Atractylodes macrocephala* rhizoma; ISO: Isoproterenol; HWI: Heart weight index; LVWI: Left ventricular weight index; $^{\#\#}P < 0.01$ versus normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus ISO control group

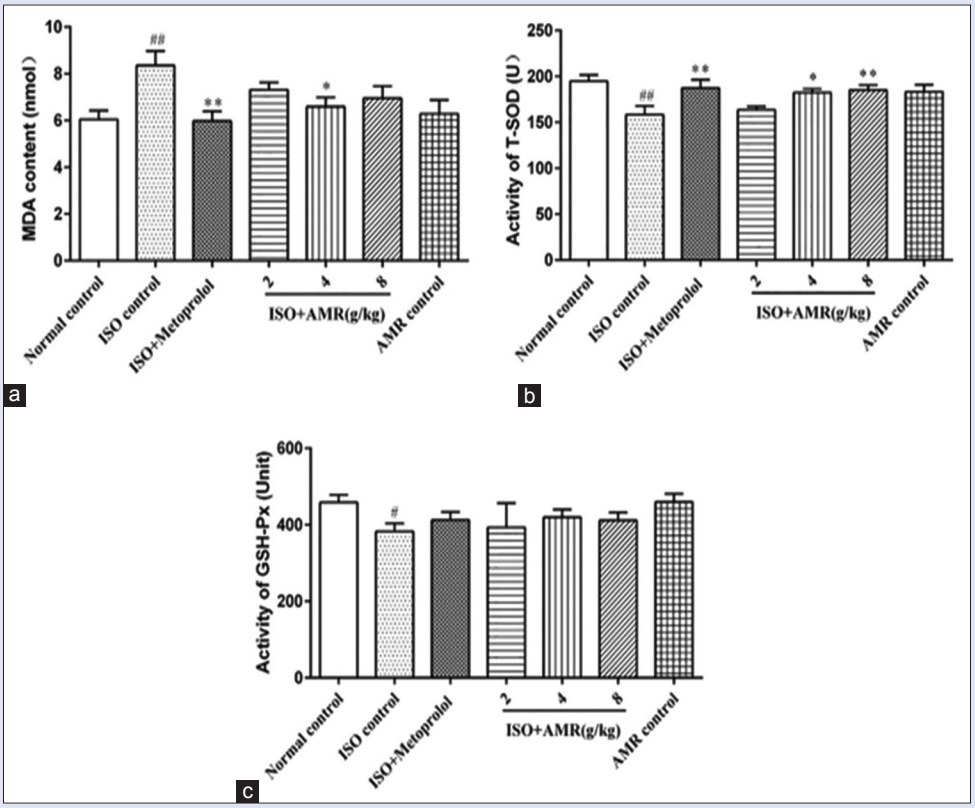

Figure 2: Effects of AMR on MDA (a), T-SOD (b) and GSH-Px (c) levels in ISO-induced mice. Values are means ± standard error of the mean; AMR: *Atractylodes macrocephala* rhizoma; ISO: Isoproterenol; MDA: Malondialdehyde; T-SOD: Total superoxide dismutase; GSH-Px: Glutathione peroxidase; $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ versus normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus ISO control group

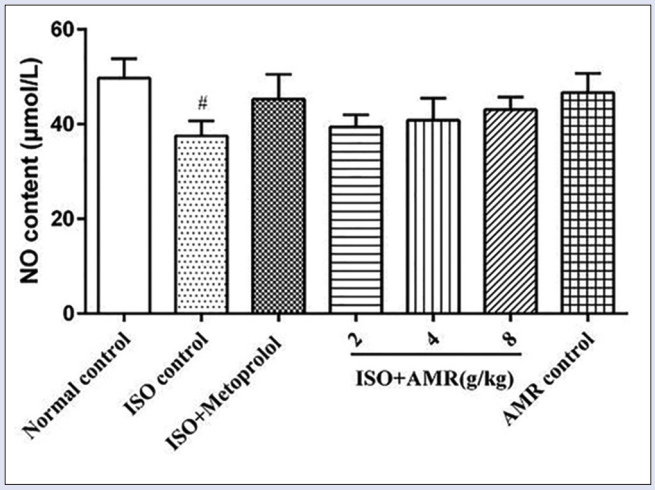

Figure 3: Effects of AMR on the content of NO in ISO-induced mice. Values are means ± standard error of the mean; AMR: *Atractylodes macrocephala* rhizoma; ISO: Isoproterenol; NO: Nitric oxide; $^{\#}P < 0.05$ versus normal control group

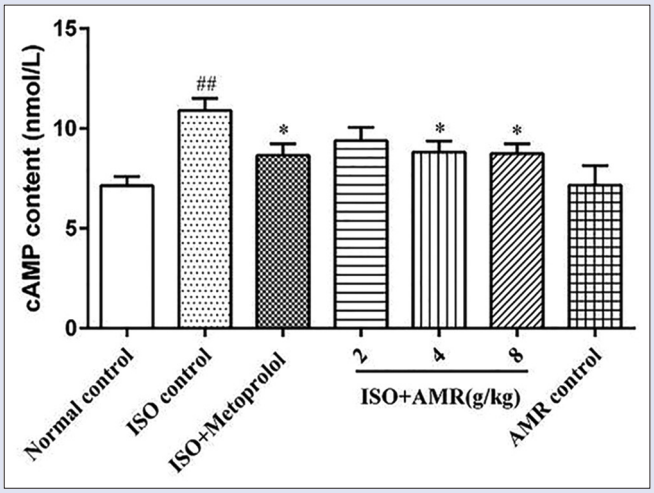

Figure 4: Effects of AMR on the content of cAMP in ISO-induced mice. Values are means ± standard error of mean; AMR: *Atractylodes macrocephala* rhizoma; ISO: Isoproterenol; cAMP, Cyclic adenosine monophosphate; $^{\#\#}P < 0.01$ versus normal control group; $^{*}P < 0.05$ versus ISO control group

Angiotensin II receptor type 1 and angiotensin II receptor type 2 mRNA expressions

As shown in Figure 6, in ISO control group, the expression of AT_1 in myocardium showed an increase compared with the normal control group. The AT_1 and AT_2 expressions in ISO plus AMR (8 g/kg) group were significantly higher than those in ISO control group ($P < 0.01$) and the up-regulation of AT_2 gene expression presented more obviously than AT_1 gene expression [Figure 6].

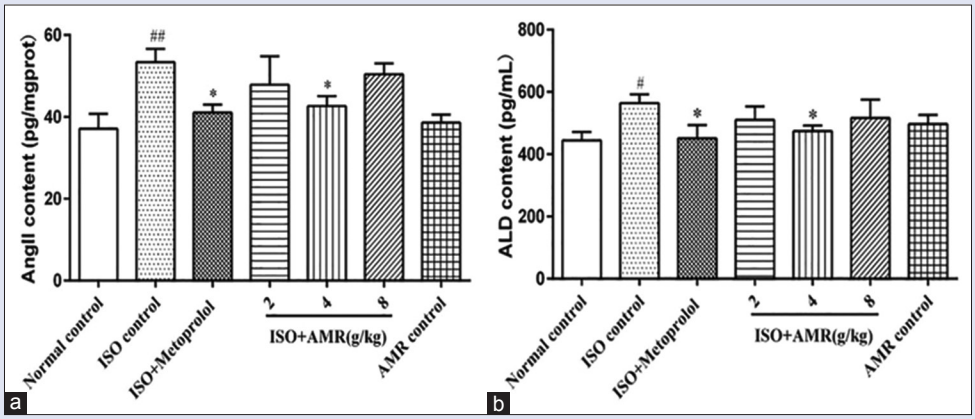

Figure 5: Effects of AMR on contents of Ang II and ALD in ISO-induced mice. Values are means ± standard error of mean; AMR: *Atractylodes macrocephala* rhizoma; ISO: Isoproterenol; Ang II, Angiotensin II; ALD, Aldosterone; $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ versus normal control group; $^{*}P < 0.05$ versus ISO control group

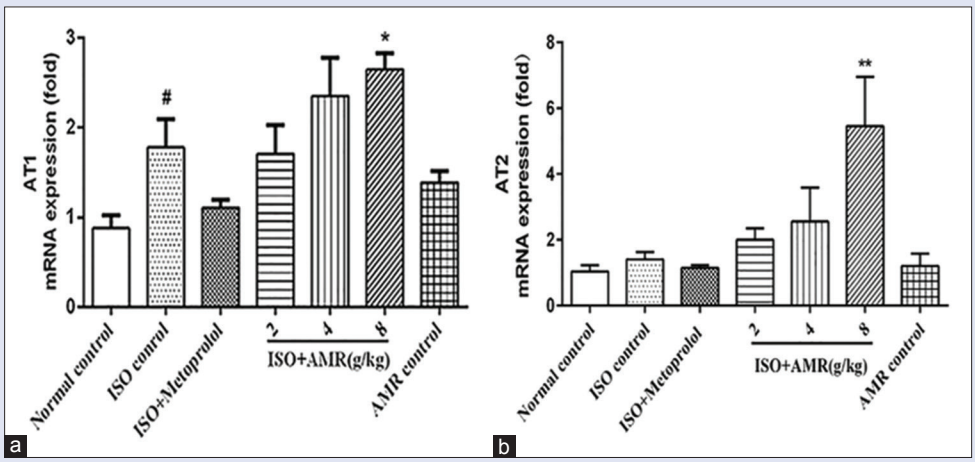

Figure 6: Effects of AMR on AT_1 and AT_2 mRNA expressions in myocardium of mice. Values are means ± standard error of mean; AMR: *Atractylodes macrocephala* rhizoma; ISO: Isoproterenol; AT_1, Angiotensin II receptor type 1; AT_2, Angiotensin II receptor type 2; $^{\#}P < 0.05$ versus normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus ISO control group

DISCUSSION

Myocardial hypertrophy follows the changes of the structure and function including ventricular wall thickening and ventricular dilatation, which will lead to VR. High dose of ISO can induce an extensive amount of cardiomyocyte necrosis which is generally applied to induce myocardial hypertrophy in experimental animal research through catecholamine toxicosis. In myocardial hypertrophy animal models, the cardiac weight indexes and the average cross section area of cardiomyocytes are the general indicators to assess the severity of myocardial hypertrophy.[12,19] In this research, ISO results in obvious myocardial hypertrophy in mice, which is manifested as elevations of HWI, LVWI, and average cross section area of cardiomyocytes in mice. The results show that AMR can significantly inhibit the occurrence of myocardial hypertrophy caused by ISO.

In systemic circulation, endothelial cells play a crucial role in the regulation of vascular tone. The endothelium-derived relaxing factor, such as NO, which can be synthesized and released by myocardium and vascular smooth vascular endothelial cells and have the functions of regulating heart function, protecting myocardium and inhibiting the division and proliferation of vascular smooth muscle cells.[20] However, reduction of NO synthesis will increase the myocardial oxygen consumption and further damage the body's oxygen supply and demand balance, which will lead to diastolic and systolic dysfunction in myocardium. Therefore, inhibition of NO synthesis will lead to myocardial hypertrophy.[21] The study showed that ISO caused significant reduction in NO and less obvious increase in AMR treated mice. These results indicate that AMR cause slight effect on myocardial relaxation and systolic dysfunction in mice.

Oxidative stress (OS) is also an important factor in heart injury. ISO-induced cardiac damage can increase the accumulation of reactive oxygen species (ROS),[22] which, in turn, leads to OS and lipid membrane peroxidation. MDA, as the final product of lipid peroxidation,[23] can cause tissue damage and apoptosis which reflects the degree of OS *in vivo*.[24] This research shows the level of MDA was significantly increased in ISO control group. Moreover, AMR can decrease the content of MDA. OS can react with oxygen by ISO quinone metabolism to produce superoxide anion (O^{2-}), hydroxyl (OH^{-}), and hydrogen peroxide (H_2O_2) and interfere with antioxidant enzymes.[25] SOD and GSH-Px, as important antioxidants, can remove ROS to inhibit OS damage and protect organisms from free radicals' attacks.[26] SOD through the disproportionation reaction of superoxide into oxygen (O_2) and H_2O_2 and its activity directly reflects the ability of the body to scavenge free radicals. GSH-Px is

widely found in the body which can promote the reaction of H_2O_2. It can reduce GSH to form H_2O and oxidized glutathione free radicals to remove cells from damage. When lots of ROS was produced *in vivo*, it can block the lipid peroxidation of the chain reaction by removing harmful peroxidic metabolites. Our results indicate that AMR can increase the activity of T-SOD and GSH-Px and the improvement of T-SOD level presented more obviously than GSH-Px. In conclusion, AMR can resist heart damage by inhibiting of lipid peroxidation and scavenging free radicals.

When cardiomyocyte β receptor was continually stimulated by ISO, it will cause sympathetic excitability increased. In this case, adenylate cyclase will be activated and ATP converts to cAMP, leading to activation of protein kinase A (PKA). β receptor excitability can also increase myocardial contraction and heart rate, result in increase of myocardial oxygen consumption and then cause myocardial hypertrophy.[27,28] In our research, AMR can significantly reduce cAMP level increased by ISO. The results show that AMR can inhibit cardiac damage and the activation of sympathetic nerve caused by ISO.

Rennin angiotensin aldosterone system (RAAS) is a complex endocrine system, being largely considered as a main role in the development and progression of myocardial hypertrophy.[29,30] When ISO increases the sympathetic excitability, increased catecholamine content can stimulate RAAS activation. Ang II and ALD, as the primary influencing factors and mediators of RAAS, continue to increase adverse effects on the cardiovascular system, which can affect cardiovascular structure, growth, and fibrosis. In this study, AMR can reduce the levels of Ang II and ALD induced by ISO. ALD, as one of the important molecules in RAAS, can upregulate ERK1/2 pathway to promote the proliferation of cardiac fibroblasts, while fibroblast growth can promote the development of fibrosis and induce VR and heart failure. ALD can also cause water and sodium retention, induce apoptosis and enhance the biological effects of Ang II, thereby increasing cardiac damage.[31] In our results, after administering with AMR, it shows significant decrease in the contents of Ang II and ALD in mice.

Ang II plays an important physiological role in cardiovascular system through AT_1 and AT_2 receptors. Binding of Ang II to AT_1 receptor produces vasoconstriction. These negative consequences of Ang II are partly counteracted by AT_2 receptor stimulation, which has favorable effects on cardiac damage. Ang II binds to AT_2 and the bradykinin B2 receptor in the endothelium was phosphorylated by PKA-dependent signaling pathways, causing sustained production and release of NO, cAMP content elevation and vascular relaxation.[32] Studies have shown that anti-fibrosis effects are also observed in mice with cardiac-specific AT_2 receptor overexpression, in addition to improvements in cardiac function.[33] In addition to improving cardiac function and anti-fibrosis, activation of AT_2 receptor can also play a role in reducing myocardial hypertrophy.[34,35] In our study, a slight increase in AT_1 expression may be a negative feedback effect because of the decrease of Ang II synthesis in the treatment group and the increase in AT_2 expression is more obvious. It shows that AMR can significantly reduce cardiac damage by up-regulating the expression of AT_2 receptor gene in mice.

CONCLUSION

AMR produces a certain effect in preventing myocardial hypertrophy induced by ISO in mice. Moreover, the ability of AMR to mitigate myocardial hypertrophy is part associated with its anti-oxidative effect, inhibiting the activation of sympathetic nerves, the activation of the RAAS and up-regulating the expression of AT_2 receptor.

Financial support and sponsorship

This work was supported by Shanghai Municipal Education Commission and the Shanghai University of Traditional Chinese Medicine budget project (2014YSN07).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hunter JJ, Chien KR. Signaling pathways for cardiac hypertrophy and failure. N Engl J Med 1999;341:1276-83.
2. Linicus Y, Laufs U. Epidemiology, etiology and pathophysiology of heart failure. Med Monatsschr Pharm 2016;39:10-2.
3. Tham YK, Bernardo BC, Ooi JY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: Signaling pathways and novel therapeutic targets. Arch Toxicol 2015;89:1401-38.
4. Lorell BH, Carabello BA. Left ventricular hypertrophy: Pathogenesis, detection, and prognosis. Circulation 2000;102:470-9.
5. Okin PM, Hille DA, Kjeldsen SE, Dahlöf B, Devereux RB. Persistence of left ventricular hypertrophy is associated with increased cardiovascular morbidity and mortality in hypertensive patients with lower achieved systolic pressure during antihypertensive treatment. Blood Press 2014;23:71-80.
6. Heusch G, Libby P, Gersh B, Yellon D, Böhm M, Lopaschuk G, *et al.* Cardiovascular remodelling in coronary artery disease and heart failure. Lancet 2014;383:1933-43.
7. Hamilton DJ. Mechanisms of disease: Is mitochondrial function altered in heart failure? Methodist Debakey Cardiovasc J 2013;9:44-8.
8. Gao JP, Chen CX, Wang Y, Lü J, Gu WL. Effect of sodium houttuyfonate on myocardial hypertrophy in mice and rats. J Pharm Pharmacol 2009;61:677-83.
9. Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Mitochondrial bioenergetics and cardiolipin alterations in myocardial ischemia-reperfusion injury: implications for pharmacological cardioprotection. Am J Physiol Heart Circ Physiol 2018;315:H1341-52.
10. Kokorin VA, Spassky AA, Volov NA, Kokorin IA. Current aspects of correction of neurohumoral system activity in patients after myocardial infarction. Pol Arch Med Wewn 2012;122 Suppl 1:47-54.
11. Yoon HJ, Jeong MH, Jeong Y, Kim KH, Song JE, Cho JY, *et al.* Progressive dilation of the left atrium and ventricle after acute myocardial infarction is associated with high mortality. Korean Circ J 2013;43:731-8.
12. Hohimer AR, Davis LE, Hatton DC. Repeated daily injections and osmotic pump infusion of isoproterenol cause similar increases in cardiac mass but have different effects on blood pressure. Can J Physiol Pharmacol 2005;83:191-7.
13. Wikipedia Contributors. Atractylodes. The Free Encyclopedia; 2018. Available from: https://en.wikipedia.org/w/index.php?title=Atractylodes&oldid=823262998. [Last accessed on 2019 Mar 22].
14. Bose S, Kim H. Evaluation of *in vitro* anti-inflammatory activities and protective effect of fermented preparations of rhizoma atractylodis macrocephalae on intestinal barrier function against lipopolysaccharide insult. Evid Based Complement Alternat Med 2013;2013:363076.
15. Nikles S, Monschein M, Zou H, Liu Y, He X, Fan D, *et al.* Metabolic profiling of the traditional Chinese medicine formulation Yu Ping Feng San for the identification of constituents relevant for effects on expression of TNF-α, IFN-γ, IL-1β and IL-4 in U937 cells. J Pharm Biomed Anal 2017;145:219-29.
16. Lenon GB, Li CG, Xue CC, Thien FC, Story DF. Inhibition of inducible nitric oxide production and iNOS protein expression in lipopolysaccharide-stimulated rat aorta and raw 264.7 macrophages by ethanol extract of a Chinese herbal medicine formula (RCM-101) for allergic rhinitis. J Ethnopharmacol 2008;116:547-53.
17. Song ZH, Dai SJ, Li HQ, Bi KS, Feng D. Study on the compatibility of composite herbal medicines of the lingguizhugan decoction. Zhongguo Zhong Yao Za Zhi 2002;27:760-2.
18. Zhang JY, Li Y, Du LY, Wang LN. Clinical application of large dose of Rhizoma *Atractylodes macrocephala*. Henan Tradit Chin Med 2018;38:161-4.
19. Ji K, Minakawa M, Fukui K, Suzuki Y, Fukuda I. Increased superoxide radical with a decrease in vascular endothelial growth factor and inducible nitric oxide synthase level leads to the progression of left ventricular hypertrophy in a pressure-overload rat heart model. Ann Thorac Cardiovasc Surg 2008;14:210-7.

20. Shin DH, Yu H, Hsu WH. A paradoxical stimulatory effect of berberine on guinea-pig ileum contractility: Possible involvement of acetylcholine release from the postganglionic parasympathetic nerve and cholinesterase inhibition. Life Sci 1993;53:1495-500.
21. Sun Y, Carretero OA, Xu J, Rhaleb NE, Wang F, Lin C, *et al.* Lack of inducible NO synthase reduces oxidative stress and enhances cardiac response to isoproterenol in mice with deoxycorticosterone acetate-salt hypertension. Hypertension 2005;46:1355-61.
22. Parthasarathy A, Gopi V, Devi KM, Balaji N, Vellaichamy E. Aminoguanidine inhibits ventricular fibrosis and remodeling process in isoproterenol-induced hypertrophied rat hearts by suppressing ROS and MMPs. Life Sci 2014;118:15-26.
23. Pryor WA, Stanley JP. Letter: A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation. J Org Chem 1975;40:3615-7.
24. Pirincciogluo AG, Gökalp D, Pirincciogluo M, Kizil G, Kizil M. Malondialdehyde (MDA) and protein carbonyl (PCO) levels as biomarkers of oxidative stress in subjects with familial hypercholesterolemia. Clin Biochem 2010;43:1220-4.
25. Punithavathi VR, Shanmugapriya K, Prince PS. Protective effects of rutin on mitochondrial damage in isoproterenol-induced cardiotoxic rats: An *in vivo* and *in vitro* study. Cardiovasc Toxicol 2010;10:181-9.
26. Rathore N, John S, Kale M, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. Pharmacol Res 1998;38:297-303.
27. Meinkoth JL, Alberts AS, Went W, Fantozzi D, Taylor SS, Hagiwara M, *et al.* Signal transduction through the cAMP-dependent protein kinase. Mol Cell Biochem 1993;127-128:179-86.
28. Yang JH, Polanowska-Grabowska RK, Smith JS, Shields CW 4th, Saucerman JJ. PKA catalytic subunit compartmentation regulates contractile and hypertrophic responses to β-adrenergic signaling. J Mol Cell Cardiol 2014;66:83-93.
29. Sayer G, Bhat G. The renin-angiotensin-aldosterone system and heart failure. Cardiol Clin 2014;32:21-32, vii.
30. Gregori M, Tocci G, Marra A, Pignatelli G, Santolamazza C, Befani A, *et al.* Inadequate RAAS suppression is associated with excessive left ventricular mass and systo-diastolic dysfunction. Clin Res Cardiol 2013;102:725-33.
31. Wang Q, Cui W, Zhang HL, Hu HJ, Zhang YN, Liu DM, *et al.* Atorvastatin suppresses aldosterone-induced neonatal rat cardiac fibroblast proliferation by inhibiting ERK1/2 in the genomic pathway. J Cardiovasc Pharmacol 2013;61:520-7.
32. Unger T. The role of the renin-angiotensin system in the development of cardiovascular disease. Am J Cardiol 2002;89:3A-9A.
33. Qi Y, Li H, Shenoy V, Li Q, Wong F, Zhang L, *et al.* Moderate cardiac-selective overexpression of angiotensin II type 2 receptor protects cardiac functions from ischaemic injury. Exp Physiol 2012;97:89-101.
34. Oishi Y, Ozono R, Yoshizumi M, Akishita M, Horiuchi M, Oshima T. AT2 receptor mediates the cardioprotective effects of AT1 receptor antagonist in post-myocardial infarction remodeling. Life Sci 2006;80:82-8.
35. Xu J, Carretero OA, Liu YH, Shesely EG, Yang F, Kapke A, *et al.* Role of AT2 receptors in the cardioprotective effect of AT1 antagonists in mice. Hypertension 2002;40:244-50.