Astragaloside IV alleviates heart failure by modulating Nrf-2

Wenxiao Feng[1], Jie Yang[2], Yan Li[2], Haiyan Sun[3], Juan Zhang[2], Yitao Xue[2]

[1]Department of Cardiovascular, First Clinical Medical College, Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250014, China;
[2]Department of Cardiovascular, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250014, China;
[3]Department of Emergency Intensive Medicine, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250014, China.

To the Editor: Heart failure (HF) is currently recognized as a disease that affects approximately 1%–2% of adults worldwide.[1] The most frequent reasons for HF are valvular heart diseases, chronic or acute cardiac ischemia, and prevalent cardiomyopathies that are caused by alcohol, doxorubicin (Dox), hypertension, diabetes, and metabolic syndrome.[2] The diverse therapeutic approaches that have been developed in recent years have usually only been successful in preclinical subjects, and efficient treatment for HF still needs to be executed.[3] Therefore, a more practical therapeutic strategy and therapeutic candidates are urgently needed. Astragaloside IV (AS-IV) is a principle active constituent of *Astragalus membranaceus Bunge,* which is a Chinese protective medicine that is widely used in HF patients.[4] Increasing evidence confirms the efficiency and safety of AS-IV for the treatment of HF *in vivo* and *in vitro*. However, to date, the investigations in this field have been limited to the broad roles of AS-IV.

The HF model was established in Wistar rats by treatment with Dox. Wistar rats were purchased from Shandong Provincial Laboratory Animal Center (China). Echocardiography was performed, and rats with left ventricular ejection fraction (EF) ≤45% were diagnosed as having HF.[5] Briefly, three-month-old male Wistar rats (180–200 g) were randomly assigned to three groups ($n = 6$ each). The HF was induced by intraperitoneal injection of Dox (Sigma-Aldrich, St. Louis, MO, USA, 3 mg/kg, 2 times/week for 6 weeks). The sham group was treated with an equal volume of saline. After 1 week of injections, 1.0 mg/kg AS-IV (Sigma-Aldrich, USA) was intraperitoneally injected into the rats each day, and an equal volume of saline was used as the control. At 9 weeks, rats were sedated with 3% isoflurane inhalation and studied on an echocardiography measurement with an ultrasound system (Panoview, Beijing, China) equipped with a 30-MHz phased array. Echocardiography analysis demonstrated that the percentage of EF was decreased in the rats with HF compared with the sham-operated rats. Significantly, AS-IV treatment increased the levels of EF [Figure 1A]. Wheat germ agglutinin (WGA) staining showed that Dox treatment resulted in enlarged cardiomyocytes. This increased size was reduced by AS-IV treatment [Figure 1B]. The number of terminal deoxynu-cleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells was increased in the hearts from the rats with HF compared with that from the sham rats, and AS-IV treatment inhibited this increase [Figure 1C]. The expression levels of dynamin-related protein 1 (DRP1) were increased and the expression levels of mitofusin and mitofusin were decreased in the rats with HF compared with the sham rats, and AS-IV treatment significantly reversed these phenotypes [Figure 1D]. Remarkably, we showed that the HF-impaired expression of nuclear factor erythroid 2-related factor 2 (Nrf-2) and its downstream gene heme oxygenase-1 (HO-1) were rescued by treatment with AS-IV [Figure 1E]. Together, these results were consistent with the previous reports,[6,7] and AS-IV can modulate DRP1/mitofusin1/2 expression and activate Nrf-2/HO-1 signaling.

The H9C2 cell line was purchased from American Type Tissue Culture Collection. The cells were cultured in DMEM (Gibco, Amarillo, TX, USA) containing 10% fetal bovine serum (Gibco), 0.1 mg/mL streptomycin (Gibco), and 100 units/mL penicillin (Gibco) at 37°C in 5% CO_2. To assess the role of AS-IV *in vitro*, the H9C2 cells were treated with Dox (1 μmol/L), cotreated with Dox (1 μmol/L) and AS-IV (low dose: 50 μmol/L or high dose: 100 μmol/L) for 24 hour before further analysis. Treatment with Dox significantly promoted the apoptosis and necrosis of the H9C2 cells, and AS-IV reversed this effect in a dosedependent manner [Figure 1F]. Dox treatment increased the percentage of cells with low membrane potential, and AS-IV could attenuate this phenotype in the cells in a dosedependent manner [Figure 1G]. Moreover, Dox treatment markedly increased the expression of DRP1 but decreased

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Correspondence to: Dr. Yitao Xue, Department of Cardiovascular, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, No. 16369, Jingshi Road, Jinan, Shandong 250014, China
E-Mail: xueyitao_szy@163.com

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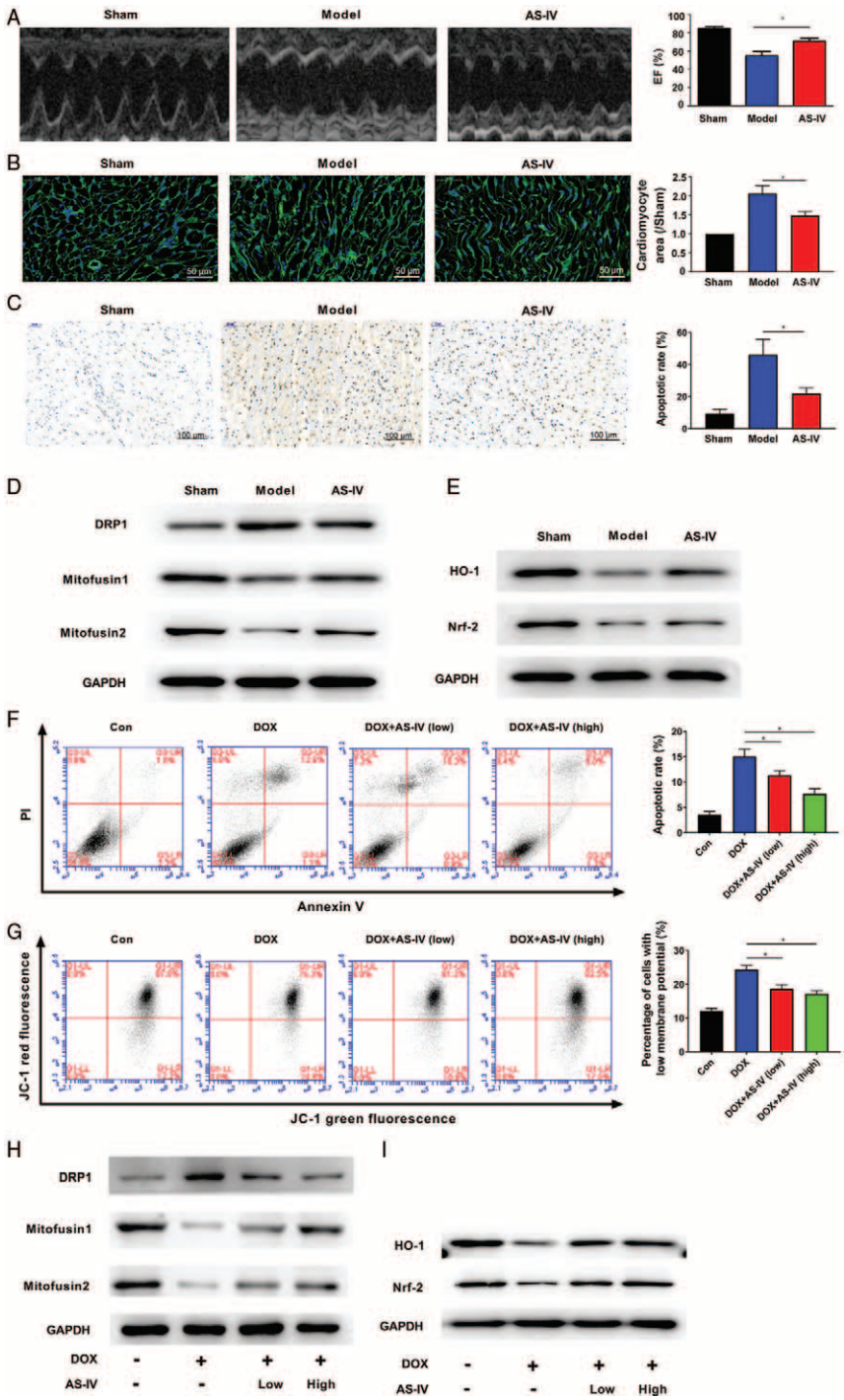

Figure 1: AS-IV attenuates Dox-induced heart failure *in vivo* and *in vitro*. (A–E) A model of HF was successfully established by Dox treatment. After 1 week, 1.0 mg/kg AS-IV was intraperitoneally injected into the rats each day, and the same volume of distilled water was used as the control ($n = 6$). (A) The EF and fractional shortening of the rats were assessed by echocardiographic analysis. (B) WGA staining revealed cardiomyocyte cross-sectional area. Bar = 50 −mm. The quantification of the cardiomyocyte size in the cardiomyocyte cross-sections is shown. (C) Apoptosis in the rats was measured by TUNEL staining. Bar = 100 μm. The quantification of TUNEL staining is shown. (D) The expression levels of DRP1, mitofusin1, mitofusin2, and GAPDH in the rats were measured by Western blotting analysis. (E) The expression levels of Nrf-2, HO-1, and GAPDH in the rats were examined by Western blotting analysis. (F–I) H9C2 cells were treated with Dox(1 μmol/L) or cotreated with Dox(1 μmol/L) and AS-IV (low dose: 50 μmol/L or high dose: 100 μmol/L) for 24 h before further analysis ($n = 3$). (F) Cell apoptosis in the indicated cell groups was analyzed by flow cytometry analysis. The quantification of the flow cytometry analysis is shown. (G) The mitochondrial membrane potential in the indicated cell groups was analyzed by JC-1 fluorescence using flow cytometry analysis. The quantification of the flow cytometry analysis is shown. (H) The expression levels of DRP1, mitofusin1, mitofusin2, and GAPDH in the indicated cell groups were measured by Western blotting analysis. (I) The expression levels of Nrf-2, HO-1, and GAPDH in the indicated cell groups were examined by Western blotting analysis. AS-IV: Astragaloside IV; Dox: Doxorubicin; DRP1: Dynamin related protein 1; EF: Ejection fractions; GAPDH: Glyceraldehyde phosphate dehydrogenase; HF: Heart failure; HO-1: Heme oxygenase-1; JC-1: 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide; Nrf-2: Nuclear factor erythroid 2-related factor 2; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling; WGA: Wheat germ agglutinin. $^{*}P < 0.05$.

the expression of mitofusin1 and mitofusin2, and AS-IV treatment reversed these effects [Figure 1H]. The Dox-impaired expression of Nrf-2 and HO-1 was partially rescued by AS-IV treatment [Figure 1I].

We discovered that AS-IV exerted a cardioprotective effect against HF partially by modulating Nrf-2, and AS-IV attenuated Dox-induced cardiomyocyte apoptosis and mitochondrial dysfunction. Our findings provide new insights into the mechanism by which AS-IV improves cardiac function during HF, presenting molecular evidence for the therapeutic value and clinical application of AS-IV during HF progression. AS-IV may contribute to the alleviation of Dox-induced cardiotoxicity during Dox-dependent antitumour therapy.

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Conflicts of interest

None.

References

1. Tanai E, Frantz S. Pathophysiology of heart failure. Compr Physiol 2015;6:187–214. doi: 10.1002/cphy.c140055.
2. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol 2016;13:368–378. doi: 10.1038/nrcardio.2016.25.
3. Rosik J, Szostak B, Machaj F, Pawlik A. Potential targets of gene therapy in the treatment of heart failure. Expert Opin Ther Targets 2018;22:811–816. doi: 10.1080/14728222.2018.1514012.
4. Zang Y, Wan J, Zhang Z, Huang S, Liu X, Zhang W. An updated role of astragaloside IV in heart failure. Biomed Pharmacother 2020;126:110012. doi: 10.1016/j.biopha.2020.110012.
5. Fu J, Chang L, Harms AC, Jia Z, Wang H, Wei C, *et al*. A metabolomics study of Qiliqiangxin in a rat model of heart failure: a reverse pharmacology approach. Sci Rep 2018;8:3688. doi: 10.1038/s41598-018-22074-6.
6. Liu X, Wang W, Song G, Wei X, Zeng Y, Han P, *et al*. Astragaloside IV ameliorates diabetic nephropathy by modulating the mitochondrial quality control network. PLoS One 2017;12:e0182558. doi: 10.1371/journal.pone.0182558.
7. Nie P, Meng F, Zhang J, Wei X, Shen C. Astragaloside IV exerts a myocardial protective effect against cardiac hypertrophy in rats, partially via activating the Nrf2/HO-1 signaling pathway. Oxid Med Cell Longev 2019;2019:4625912. doi: 10.1155/2019/4625912.

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