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Original Research Article

Ling gui shen fu decoction ameliorates cardiac injury in ischemic heart disease rats by regulating ANP32A/HIF-2α/HMGB1 signal route

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Abstract

Purpose: *To investigate the effect of ling gui shen fu decoction (LGSFD) on cardiac injury and cardiomyocyte apoptosis in rats with ischemic heart disease (IHD), and to elucidate the underlying molecular mechanism.*
Methods: *An IHD rat model was established by performing coronary artery occlusion surgery on the left anterior descending (LAD) of rats. The rats were assigned equally to 5 groups: sham-operated group (sham group), IHD group, IHD + perindopril group, IHD + low-dose LGSFD group (IHD + LGSFD-L group), and IHD + high-dose LGSFD group (IHD+LGSFD-H group). The LGSFD was given via gavage. A hypoxia-induced cardiomyocyte model was established by subjecting H9C2 cells to hypoxia. Then, LGSFD-containing serum or blank serum was used to treat the hypoxic rat cardiomyocytes (H9C2). The severity of cardiac injury and extent of apoptotic changes in cardiomyocytes was determined using hematoxylin-eosin (H&E) staining and TUNEL staining, while immunoblotting was used to measure the protein expressions of ANP32A, p-AKT, AKT, HIF-2α, p-mTOR, mTOR and HMGB1.*
Results: *The cardiac injury of rats in the LGSFD groups was significantly reversed, relative to the IHD group ($p < 0.05$). Moreover, LGSFD reduced the level of apoptosis in hypoxia-induced H9C2 cells and upregulated the concentrations of ANP32A, p-AKT, HIF-2α and p-mTOR. However, the expression levels of HMGB1 were decreased in the heart tissues of IHD rats and hypoxic H9C2 cells. Silencing of ANP32A with ANP32A siRNA (siANP32A) down-regulated ANP32A, p-AKT, HIF-2α and p-mTOR, but up-regulated apoptosis and expression levels of HMGB1 in hypoxic H9C2 cells.*
Conclusion: *LGSFD ameliorates cardiac injury in IHD rats by inhibiting cardiomyocyte apoptosis via the ANP32A/HIF-2α/HMGB1 axis. Further investigations are recommended into the specific mechanism of LGSFD effect using clinical samples, in order to facilitate its clinical development.*

Keywords: *Ischemic heart disease, Cardiac injury, Hypoxia, LGSFD, Apoptosis*

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INTRODUCTION

An imbalance between coronary artery blood supply and myocardial demand causes ischemic heart disease (IHD), and it results in myocardial ischemia and hypoxia which eventually lead to myocardial cell necrosis and apoptosis [1]. IHD is also the most common cause of cardiovascular diseases, usually with acute myocardial infarction (AMI) as the main clinical manifestation. This

causes serious damage to normal heart function, and it results in high mortality rate which poses a serious threat to public health [2]. Hence, elucidating the mechanism of IHD and finding new therapeutic drugs with few or low side effects are important for facilitating the treatment of IHD. Traditional Chinese medicine (TCM) has gained widespread attention as a therapy for diseases on account of its prominent efficacy and limited toxic side effects. Currently, TCM is widely prescribed for the relief of IHD. Many extant studies show that Chinese herbal medicines have therapeutic effects on IHD, in addition to improving the survival rate of patients with IHD when used as adjunctive therapy [3]. Therefore, it is worthwhile to investigate the efficacy and mechanism of action of TCM in the treatment of IHD. According to TCM theory, TCM prescriptions with the effects of benefiting *qi* and *yang* should be recommended for the treatment of IHD. Therefore, ling gui shen fu decoction (LGSFD) was used in the current study as a TCM prescription with that level of efficacy, i.e., benefiting *qi* and *yang*.

Ling gui shen fu decoction (LGSFD) is composed of Radix Codonopsis, Radix Astragali, processed aconite (previously decocted), *Salviae miltiorrhiza*, *Cinnamomum cassiae*, Radix Paeoniae Alba, *Atractylodes macrocephala*, Poria, *Leonurus japonicus* Houtt, and Licorice. Most of these components are effective in the treatment of cardiovascular diseases. Astragaloside IV (AS-IV) is an active component of Radix Astragali which protects animals against ischemic and hypoxic cardiomyocyte injury, inhibits myocardial hypertrophy and myocardial fibrosis, enhances myocardial contractility, and improves diastolic dysfunction [4]. It has been found that *Salviae miltiorrhiza* is efficacious in the treatment of cardiovascular and cerebrovascular diseases. For instance, it has multi-component and multi-target effects in the treatment of myocardial ischemia-reperfusion injury (MIRI) [5]. 2-Methoxycinnamaldehyde (2-MCA), one of the active components of *Cinnamomum cassia*, has been found to reduce myocardial ischemia and reperfusion injury in rats [6]. *Atractylodes Macrocephala* may reverse ventricular remodeling via its antioxidative effect and inhibition of the activation of rennin-angiotensin-aldosterone system (RAAS) [7]. Poria improved cardiac function in rats with chronic heart failure via the AVP-V2R-AQP2 axis [8]. *Leonurus japonicus* Houtt has been proven to be effective in treating cardiovascular diseases (CVD), particularly IHD, in various ways. Leonurine, an alkaloid present in *Leonurus japonicus* Houtt, has been found to exert biological effects by preventing IHD [9]. Licorice has been shown to restore cardiac function and protect the myocardium in rat models of myocardial injury [10]. Therefore, the efficacy and mechanism of action of LGSFD in the treatment of IHD were investigated in this study. An IHD rat model and a cell model of hypoxia were established and used to investigate the effect of LGSFD on cardiac and cardiomyocyte injury in rats with IHD, as well as the associated potential molecular mechanisms.

EXPERIMENTAL

Animals

A total of forty (40) mature male SD rats (mean weight = 220 ± 20 g) were purchased from the Laboratory Animal Center of Hangzhou Medical College, Hangzhou, China. All the rats were housed in an environment with an average room temperature of 22 ± 2 ℃ and a 12 h daylight/12 h dark photoperiod. After 7 days of adaptive feeding, the rats were assigned equally to 5 groups: sham-operated group (sham group), IHD group, IHD + perindopril group, IHD + low-dose LGSFD group (IHD + LGSFD-L group), and IHD + high-dose LGSFD group (IHD+LGSFD-H group). All experiments were evaluated and approved by the Institutional Animal Care and Use Committee, Zhejiang Center of Laboratory Animals, with approval no. ZJCLA-IACUC-20030095. The animal studies followed international guidelines for animal studies.

Establishment of IHD rat model

In the establishment of the IHD rat model, all the experimental rats were anesthetized and the thoracic cavity of each rat was opened to expose the heart for ligation of the left anterior descending (LAD) coronary artery with 5-0 polypropylene thread. After the operation, the thoracic cavity of each rat was closed and the wound was sutured. For comparison, rats in the sham group were not subjected to ligation.

Preparation of LGSFD

The LGSFD was prepared by first mixing the following Chinese herbal medicines: 10 g of Radix Codonopsis, 30 g of Radix Astragali, 10 g of processed aconite which was decocted earlier, 15 g of *Salviae miltiorrhiza*, 10 g of Cinnamomum Cassiae, 10 g of Radix Paeoniae Alba, 10 g of *Atractylodes macrocephala*, 15 g of Poria, 20 g of *Leonurus japonicus* Houtt, and 15 g of Radix Glycyrrhiza. The mixture was subsequently soaked for 2 h in an appropriate amount of water. Thereafter, the mixture was decocted 2 times, first with gentle heat and then with high heat.

Then, filtration and debris removal were performed, and the liquid obtained was concentrated via heating in a water bath. The concentration of LGSFD used in this study was 4 mg/mL.

Administration of LGSFD

After the surgery, rats in the sham group were administered physiological saline, while rats in the IHD + LGSFD-L and IHD + LGSFD-H groups were administered LGSFD at the doses of 10 mg/kg and 40 mg/kg, respectively. Rats in the IHD + perindopril group were administered perindopril solution at a dose of 4.6 mg/kg. All groups were given their respective treatments *via* gavage. The dose of solution used for gavage was 20 mL/kg/day per rat, and all the rats were intragastrically administered treatments once a day for 28 days.

Preparation of LGSFD-containing serum (LGSFD serum)

All the experimental rats were fasted for 12 h before the last administration of LGSFD. Blood samples collected from the heart of each rat 60 min after the last LGSFD administration, were allowed to stand for 30 min at 4 °C, followed by centrifugation at 1000 rpm for 15 min using a cryogenic centrifuge. The separated serum samples were filtered using a microporous membrane to remove bacteria, followed by inactivation in constant-temperature water bath at 56 °C for 30 min. The sera were kept at -20 °C refrigerator prior to subsequent use.

Cell culture

Rat cardiomyocyte (H9C2) cells purchased from Procell (Wuhan, China) were maintained in DMEM having 10 % FBS in a cell incubator containing 5 % CO_2 at 37 °C. To establish the hypoxic cell model, the cells were incubated with serum-free DMEM for 6 h and then transferred to a hypoxic incubator containing 1 % O_2 for 12 h. The cells were evenly divided into 6 groups: control group, hypoxia group, hypoxia + blank serum group, hypoxia + LGSFD serum group, hypoxia + LGSFD serum + negative control siRNA group (hypoxia + LGSFD serum + siNC group), and hypoxia + LGSFD serum + ANP32A siRNA group (hypoxia + LGSFD serum + siANP32A group).

Hematoxylin-eosin (H&E) staining

Heart tissues were taken from the cardiac apex of the left ventricle of rats in each group, fixed in 10 % phosphate-buffered formalin, and then embedded. Ventricular tissue sections of the rats were prepared and subjected to H&E staining.

Western blotting

Total protein was extracted from H9C2 cells and rat heart tissues by lysing with RIPA. The protein in each lysate was quantified with BCA, and equivalent amounts of proteins were resolved using polyacrylamide gel electrophoresis. The proteins were then transferred to PVDF membranes which were blocked with 5 % solution of skim milk powder for 2 h. This was followed by membrane incubation with primary antibodies (ANP32A, p-AKT, AKT, HIF-2α, p-mTOR, mTOR, HMGB1 and GAPDH) in a 4 ^oC refrigerator for 12 h. All antibodies were purchased from Abcam (USA) and diluted 1000 times. Thereafter, the membranes were incubated with secondary antibody (5000 times dilution, Abcam, USA) at room temperature for 2 h. Finally, freshly configured enhanced chemiluminescence (ECL) reagent was added dropwise to the membranes, for band visualization.

Transferase UTP nick end labeling (TUNEL)

The paraffin sections of the heart tissue were incubated with protease K for 0.5 h. Then, TUNEL solution (TdT enzyme: fluorescent labeling solution ratio = 1:9) was added, incubated at 37 °C for 1 h, nucleated with 4 ', 6-diamino-2-phenylindole (DAPI) for 5 min, and then examined under fluorescence microscope (Olympus, Japan) after sealing. The H9C2 cells were subjected to fixation with paraformaldehyde (4 %) at room temperature for 15 min and permeated with 0.5 %Triton X-100 at laboratory temperature for 0.5 h. Thereafter, TUNEL reaction solution (Beyotime, Nanjing) was added and incubated at 37 °C for 1.5 h. After washing with PBS, aqueous anti-quenching agent containing DAPI was added, and apoptosis of cells in each group was checked under a light microscope (Olympus, Tokyo, Japan).

Statistical analysis

Results were processed using GraphPad Prism 8.0. Statistical analyses were done with one-way ANOVA and Student's *t*-test. Statistical significance of differences amongst groups was assumed at $p < 0.05$.

RESULTS

LGSFD attenuated severity of cardiac injury and enhanced cardiomyocyte apoptosis

The histomorphological changes and apoptosis levels of IHD rats were compared via H&E staining and TUNEL assay, respectively, in order to study the effect of LGSFD on IHD. The results showed that rats in the sham group had clear tissue structures, with no disorganized interstitial myocardial cells, and neatly arranged myocardial fibers. In contrast, rats in the IHD group had disorganized tissue arrangement, extensive myocardial fiber degeneration, interstitial edema, myocardial fiber deformation and inflammatory cell infiltration. However, the extent of myocardial fiber arrangement, interstitial edema and inflammatory cell infiltration were decreased in the IHD + LGSFD-H group (Figure 1 A). The levels of myocardial apoptosis were higher in the IHD group than in the sham group, whereas LGSFD significantly reduced the myocardial apoptosis levels, when compared with the IHD group. The inhibitory effect of high doses of LGSFD on myocardial apoptosis was more remarkable in Figure 1 B. There were no significant differences in cardiac injury and apoptosis between the IHD + LGSFD-H group and IHD + perindopril group ($p < 0.05$; Figure 1 A and B). These results suggest that LGSFD reduced cardiac injury and myocardial apoptosis in IHD rats.

LGSFD affected the expression of ANP32A, HIF-2α and HMGB1 in heart tissues

Studies have found that ANP32A, HIF-2α and HMGB1 are closely associated with cardiac injury and AKT/mTOR pathway. To clarify whether LGSFD regulates the expressions of ANP32A, HIF-2α, HMGB1 and the AKT/mTOR pathway in the heart tissues of IHD rats, the expression levels of ANP32A, HIF-2α and HMGB1 and phosphorylation of AKT/mTOR in the sham, IHD, IHD + LGSFD-L and IHD + LGSFD-H groups were measured with immunoblot assay and compared.

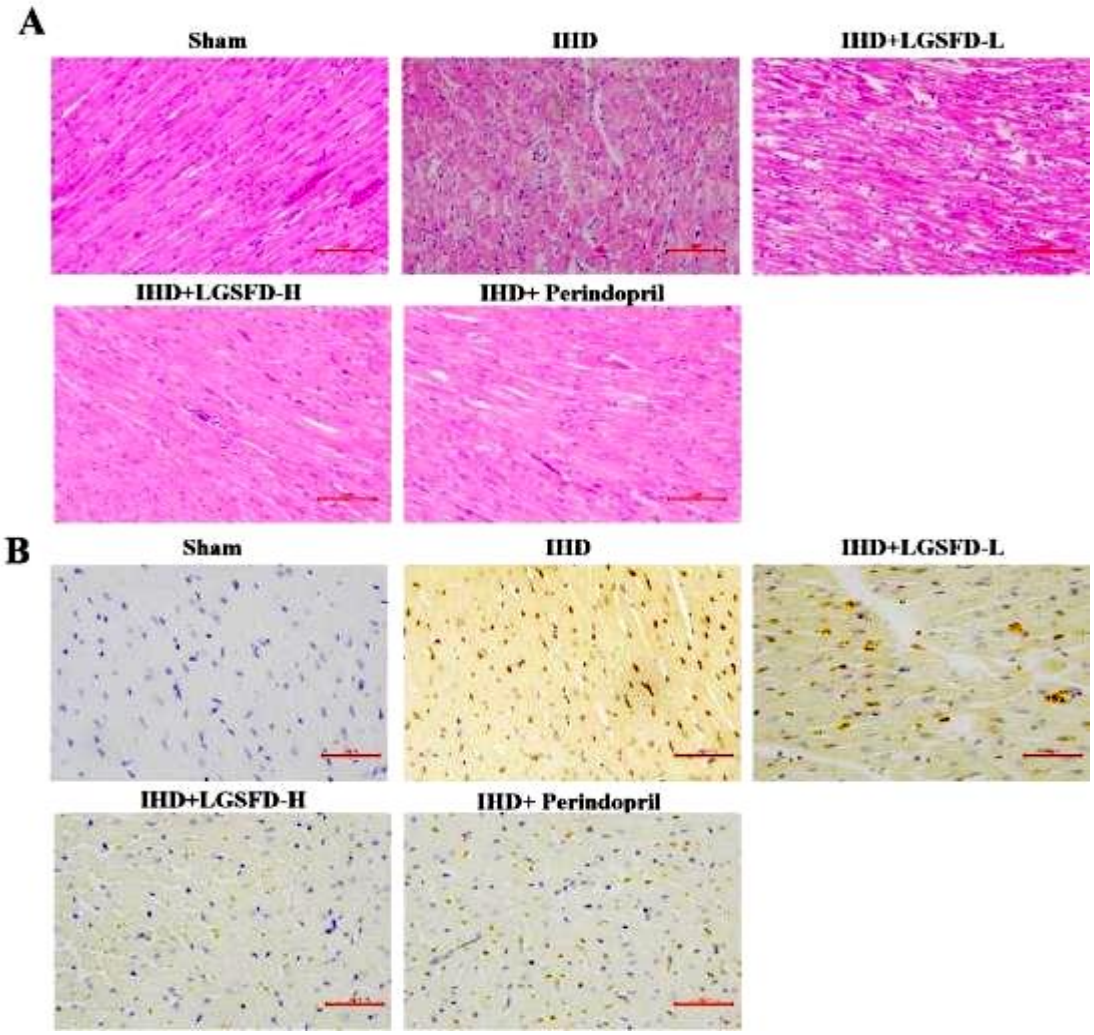

Figure 1: LGSFD reduced the severity of cardiac injury and enhanced myocardial apoptosis in IHD rats. (A) Histomorphological changes in the heart tissues of IHD rats (H&E staining). (B) Cell apoptosis, as determined using TUNEL staining

Figure 2 shows that the concentrations of ANP32A, p-AKT and p-mTOR were reduced, while those of HIF-2α and HMGB1 were upregulated in the IHD group when compared with the sham group. However, the low and high doses of LGSFD reversed the trends in protein levels of ANP32A, p-AKT, p-mTOR and HMGB1 in IHD rats, and the reversal was shown to be most obvious in the LGSFD-H group.

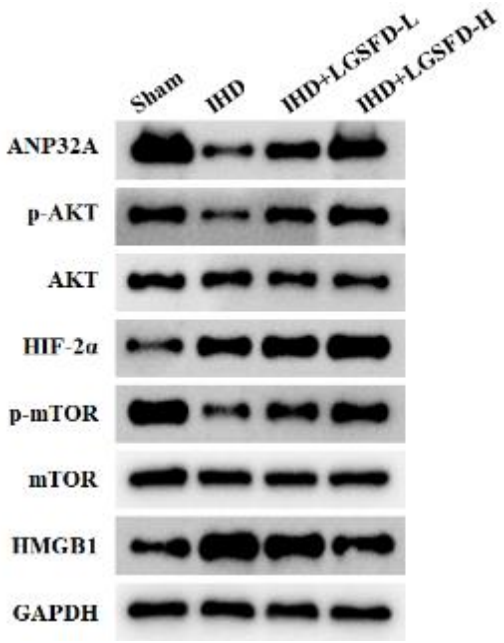

Figure 2: Protein expression levels of ANP32A, HIF-2α and HMGB1 in the heart tissues of IHD rats. Protein concentrations of ANP32A, p-AKT, AKT, HIF-2α, p-mTOR, mTOR and HMGB1 in the heart tissues of rats were determined with Western blot assay

LGSFD reduced cardiomyocyte apoptosis

A hypoxic H9C2 cell model was established by exposing H9C2 cells to a hypoxic environment. This model was used to investigate whether LGSFD inhibited apoptosis in cardiomyocytes. TUNEL assay was used to determine the levels of cardiomyocyte apoptosis in each group. A significant increase was observed in the apoptosis level of hypoxic H9C2 cells when compared with the control group. Moreover, the LGSFD serum reduced the apoptosis levels of hypoxic H9C2 cells (Figure 3). Collectively, these results suggest that LGSFD inhibited apoptosis of cardiomyocytes.

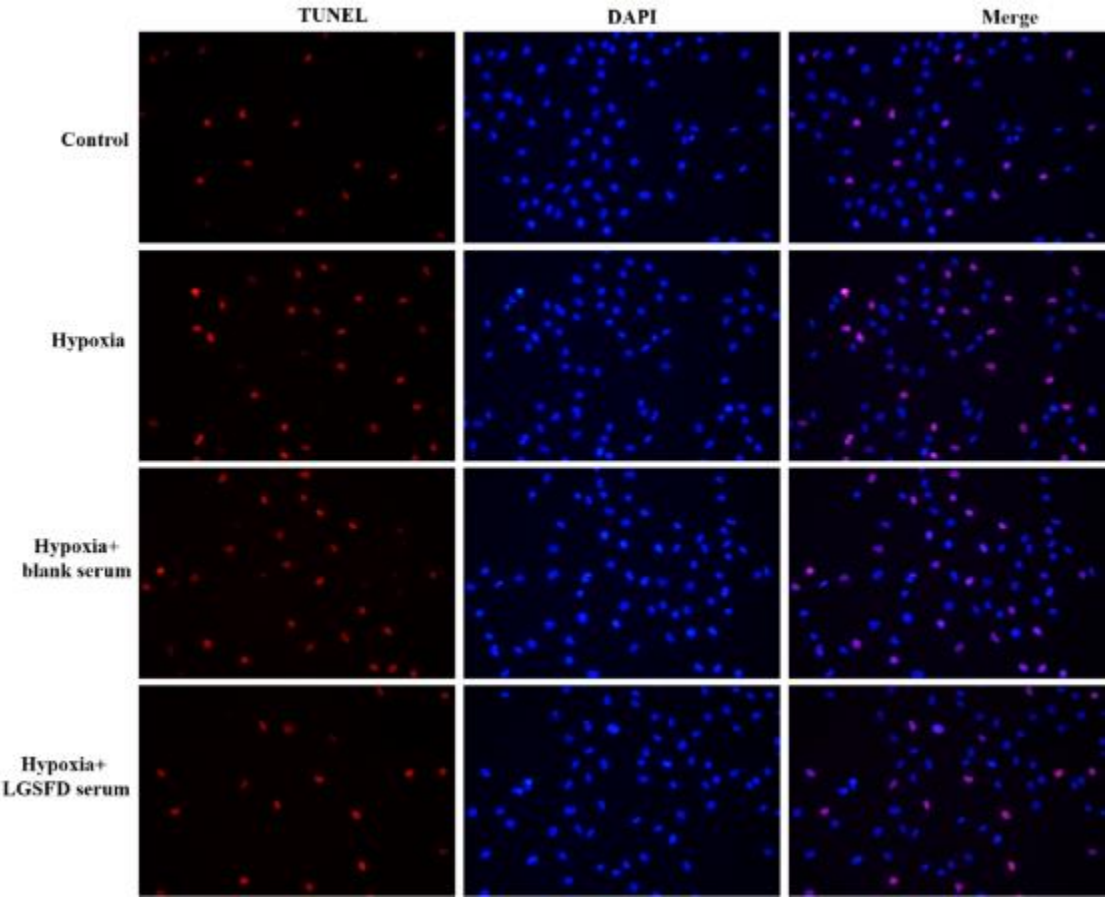

Figure 3: LGSFD serum inhibited apoptosis of hypoxic H9C2 cells. Cell apoptosis was determined with TUNEL assay

LGSFD upregulated the expression of ANP32A in cardiomyocytes

To further investigate whether LGSFD upregulated ANP32A expression in cardiomyocytes, Western blot was used to determine ANP32A expression in cells treated with blank serum or serum containing LGSFD.

Reduced expression of ANP32A was found in hypoxia and hypoxia + blank serum group. LGSFD increased the expression of ANP32A in hypoxia-induced H9C2 cells (Figure 4). These results suggest that LGSFD up-regulated ANP32A expression in cardiomyocytes.

LGSFD inhibited cardiomyocyte apoptosis via ANP32A

To silence ANP32A, ANP32A siRNA was transfected into hypoxic H9C2 cells (Figure 5A). Then, TUNEL was used to assess whether LGSFD inhibited cardiomyocyte apoptosis via ANP32A. It was revealed that LGSFD inhibited apoptosis of hypoxic H9C2 cells. However, siANP32A reversed the inhibitory effect of LGSFD on cardiomyocyte apoptosis (Figure 5B). These results suggest that LGSFD inhibited apoptosis in cardiomyocytes via ANP32A.

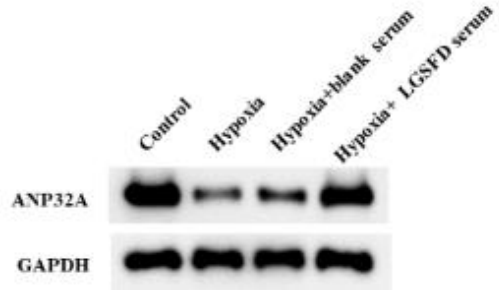

Figure 4: The expression levels of ANP32A in hypoxic H9C2 cells. Immunoblot assay was used to measure ANP32A protein expression in H9C2 cells.

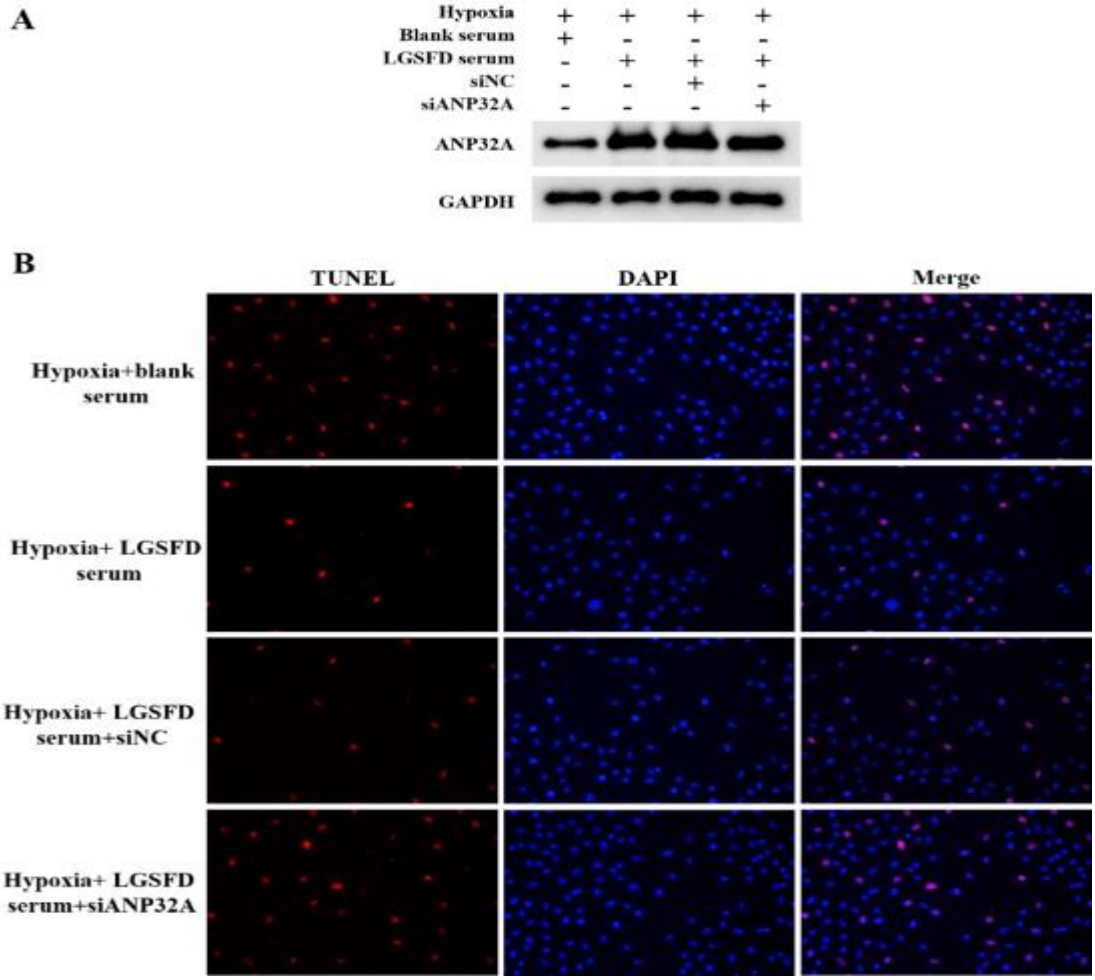

Figure 5: LGSFD serum inhibited the apoptosis of cardiomyocytes through increased expression of ANP32A. (A) Expression of ANP32A in hypoxic H9C2 cells, as determined using Western blot assay. (B) Cell apoptosis, as measured using TUNEL assay

LGSFD inhibited cardiomyocyte apoptosis via the ANP32A/HIF-2α/HMGB axis

In this study, LGSFD upregulated p-AKT, HIF-2α and p-mTOR, but down-regulated the expression of HMGB1 in hypoxic H9C2 cells, when compared to the hypoxia + blank serum group. However, the levels of p-AKT, HIF-2α and p-mTOR were reduced, while the expression level of HMGB1 was increased in the hypoxia + LGSFD serum + siANP32A group, when compared with the hypoxia + LGSFD serum + siNC group (Figure 6).

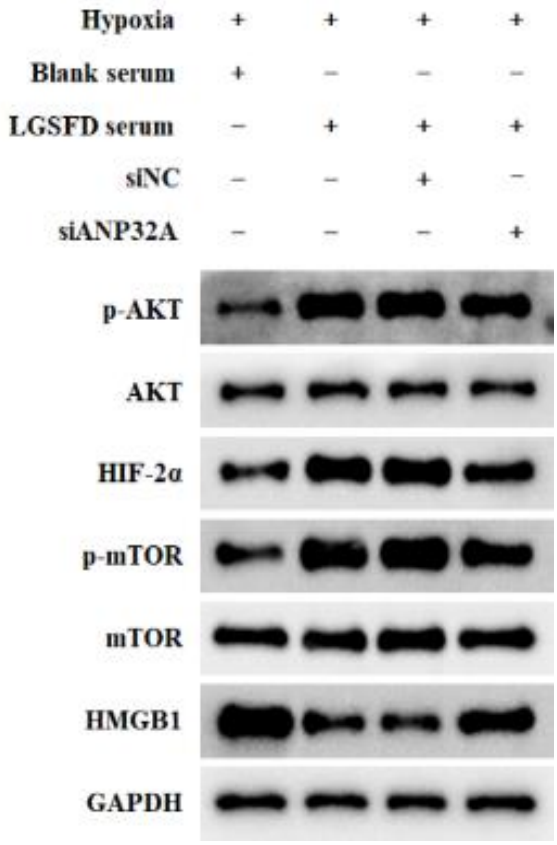

Figure 6: LGSFD regulated the expression of HIF-2α/HMGB via ANP32A. Immunoblot assay was used to determine protein expressions of ANP32A, p-AKT, AKT, HIF-2α, p-mTOR, mTOR, and HMGB1 in hypoxic H9C2 cells

DISCUSSION

Ischemic heart disease (IHD) generally causes heart damage that seriously threatens the lives of the affected patients. Currently, there are no effective and specific treatment methods for IHD, and this is a serious problem in the clinical management of IHD. In order to find effective therapies for IHD, a rat model of IHD was established in this study, and a hypoxic cell model was also established. It was found that the severity of cardiac injury and the levels of cardiomyocyte apoptosis were increased in IHD rats. In addition, it was found that LGSFD ameliorated cardiac injury and decreased the levels of apoptosis in the IHD rats.

Cardiomyocyte apoptosis is known to be a key pathological mechanism leading to IHD. It has been reported that ANP32A which belongs to the ANP32 group, participates mainly in cell adhesion, cell transport and cell apoptosis [11]. It has been demonstrated that ANP32A prevents the occurrence of heart diseases [12]. Besides, the expression of ANP32A is down-regulated in animal models of cardiac hypertrophy [13]. When there is oxygen deficiency caused by myocardial ischemia, the expression of hypoxia-inducible factor HIF-2α is increased, and it plays a protective role, while the downregulation of HIF-2α in hypoxic cardiomyocytes leads to increased infarct size [14].

The nucleus is the location of HMGB-1 which is released into the vesicles and exosomes of various sizes during apoptosis. Studies have found that HMGB1 induces apoptosis and autophagy during myocardial I/R injury, thereby exerting adverse effects on the heart [15]. Moreover, HMGB-1 mediates myocardial cell damage and accelerates cell apoptosis by releasing inflammatory factors [16]. The expression levels of ANP32A, HIF-2α and HMGB1 in the myocardium of IHD rats after LGSFD treatment were determined, in order to further study the specific mechanism involved in the treatment of IHD with LGSFD. Results from Western blot assay showed that LGSFD upregulated the protein expressions of ANP32A and HIF-2α, and inhibited the expression of HMGB1. These results confirm that LGSFD exerted therapeutic effects on IHD by regulating ANP32A, HIF-2α, and HMGB1.

In this study, LGSFD-containing serum significantly inhibited hypoxia-induced apoptosis and HMGB1 expression in H9C2 cells, and enhanced the expressions of ANP32A and HIF-2α. In order to clarify the possible relationship between ANP32A, HIF-2α and HMGB1, the expression of ANP32A in H9C2 cells was regulated using ANP32A-targeting siRNA. It was found that after ANP32A knockdown, HIF-2α expression decreased, while HMGB1 expression increased. Indeed, HIF-2α has been shown to activate the AKT/mTOR signaling pathway [17]. Moreover, the AKT/mTOR signaling inhibited the expression of HMGB-1 [18]. Therefore, the levels of these proteins were determined in H9C2 cells after regulating ANP32A expression. It was found

that the phosphorylation levels of AKT and mTOR decreased, which was consistent with the results of previous studies. Therefore, it is likely that LGSFD mitigated IHD via regulation of the ANP32A/HIF-2α/HMGB1 pathway.

Study limitations

First, this study is only a preliminary investigation in rats and cells, i.e., no clinical samples were used to verify data. Secondly, this study reached only an initial conclusion. Therefore, there is a need for more in-depth experiments on the mechanisms of action involved.

CONCLUSION

The present study has demonstrated the therapeutic effects of LGSFD on IHD through experiments at the animal and cellular levels; it preliminarily investigated the mechanism underlying the use of LGSFD in treating IHD. The major findings of this study provide important insights into the role and mechanism of LGSFD as an interventional therapy for IHD. Further investigation into the specific mechanism of action of LGSFD is required.

DECLARATIONS

Acknowledgement

None.

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Ethical approval

Approval was granted by the Institutional Animal Care and Use Committee, Zhejiang Center of Laboratory Animals (ZJCLA-IACUC-20030095).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Gagno G, Ferro F, Fluca AL, Janjusevic M, Rossi M, Sinagra G, Beltrami AP, Moretti R, Aleksova A. From brain to heart: possible role of amyloid-beta in ischemic heart disease and ischemia-reperfusion injury. Int J Mol Sci 2020; 21(24).*
2. *Severino P, D'Amato A, Pucci M, Infusino F, Adamo F, Birtolo LI, Netti L, Montefusco G, Chimenti C, Lavalle C, et al. ischemic heart disease pathophysiology paradigms overview: from plaque activation to microvascular dysfunction. Int J Mol Sci 2020; 21(21).*
3. *Hung IL, Chung CJ, Hu WL, Liao YN, Hsu CY, Chiang JH, Hung YC. Chinese herbal medicine as an adjunctive therapy improves the survival rate of patients with ischemic heart disease: a nationwide population-based cohort study. Evid Based Complement Alternat Med 2022; 2022: 5596829.*
4. *Tan YQ, Chen HW, Li J. Astragaloside IV: An effective drug for the treatment of cardiovascular diseases. Drug Des Devel Ther 2020; 14: 3731-3746.*
5. *Jiang Z. Mechanism research of Salvia miltiorrhiza on treating myocardial ischemia-reperfusion injury according to network pharmacology combined with molecular docking technique. Medicine (Baltimore) 2021; 100(48): e28132.*
6. *Golshahi H, Araghi A, Baghban F, Farzad-Mohajeri S. Protective effects of 2-methoxycinnamaldehyde active ingredients of Cinnamomum cassia on warm hepatic ischemia-reperfusion injury in rat model. Iran J Basic Med Sci 2019; 22(12): 1400-1407.*
7. *Cui XH, Wang HL, Wu R, Yao PA, Wei KZ, Gao JP. Effect of Atractylodes macrocephala rhizoma on isoproterenol-induced ventricular remodeling in rats. Mol Med Rep 2018; 17(2): 2607-2613.*

8. Shi Y, Liu C, Xiong S, Yang L, Yang C, Qiao W, Liu Y, Liu S, Liu J, Dong G. Ling-Gui-Qi-Hua formula alleviates left ventricular myocardial fibrosis in rats with heart failure with preserved ejection fraction by blocking the transforming growth factor-beta1/Smads signaling pathway. J Ethnopharmacol 2023; 317: 116849.
9. Huang L, Xu DQ, Chen YY, Yue SJ, Tang YP. Leonurine, a potential drug for the treatment of cardiovascular system and central nervous system diseases. Brain Behav 2021; 11(2): e01995.
10. Li M, Wen Z, Xue Y, Han X, Ma D, Ma Z, Wu Z, Guan S, Sun S, Chu L. Cardioprotective effects of glycyrrhizic acid involve inhibition of calcium influx via L-type calcium channels and myocardial contraction in rats. Naunyn Schmiedebergs Arch Pharmacol 2020; 393(6): 979-989.
11. He Y, Zisan Z, Lu Z, Zheng L, Zhao J. Bergapten alleviates osteoarthritis by regulating the ANP32A/ATM signaling pathway. FEBS Open Bio 2019; 9(6): 1144-1152.
12. Monteagudo S, Cornelis FMF, Wang X, de Roover A, Peeters T, Quintiens J, Sermon A, de Almeida RC, Meulenbelt I, Lories RJ. ANP32A represses Wnt signaling across tissues thereby protecting against osteoarthritis and heart disease. Osteoarthritis Cartilage 2022; 30(5): 724-734.
13. Gao J, Li Y, Wang T, Shi Z, Zhang Y, Liu S, Wen P, Ma C. Analyzing gene expression profiles with preliminary validations in cardiac hypertrophy induced by pressure overload. Can J Physiol Pharmacol 2018; 96(8): 701-709.
14. Koeppen M, Lee JW, Seo SW, Brodsky KS, Kreth S, Yang IV, Buttrick PM, Eckle T, Eltzschig HK. Hypoxia-inducible factor 2-alpha-dependent induction of amphiregulin dampens myocardial ischemia-reperfusion injury. Nat Commun 2018; 9(1): 816.
15. Foglio E, Pellegrini L, Germani A, Russo MA, Limana F. HMGB1-mediated apoptosis and autophagy in ischemic heart diseases. Vasc Biol 2019; 1(1): H89-H96.
16. Xue J, Ge H, Lin Z, Wang H, Lin W, Liu Y, Wu G, Xia J, Zhao Q. The role of dendritic cells regulated by HMGB1/TLR4 signaling pathway in myocardial ischemia reperfusion injury. J Cell Mol Med 2019; 23(4): 2849-2862.
17. Chen J, Chen J, Huang J, Li Z, Gong Y, Zou B, Liu X, Ding L, Li P, Zhu Z, et al. HIF-2alpha upregulation mediated by hypoxia promotes NAFLD-HCC progression by activating lipid synthesis via the PI3K-AKT-mTOR pathway. Aging (Albany NY) 2019; 11(23): 10839-10860.
18. Niu Z, Lin J, Hao C, Xu X, Wang C, Dai K, Deng X, Deng M, Guo Y, Yao W. Glycyrrhizic acid attenuates pulmonary fibrosis of silicosis by inhibiting the interaction between HMGB1 and BRG1 through PI3K/Akt/mTOR pathway. Int J Environ Res Public Health 2022; 19(14).