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Original Article

A traditional Chinese medicine, *Lujiao* prescription, as a potential therapy for hypertrophic cardiomyocytes by acting on histone acetylation

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Abstract

Background: Chronic heart failure (CHF) is a complex clinical syndrome, and a serious stage of various heart diseases. Dysfunction of histone acetylation is involved in pathogenesis of CHF. *Lujiao* is a clinical and traditional prescription that has been previously used in the treatment of heart failure. The objective of our study was to explore the effects of traditional Chinese Medicine intervention with *Lujiao* prescription on hypertrophic cardiomyocytes with histone acetylation abnormality.

Methods: Myocardial cells from neonatal rats were stimulated via phenylephrine (PE) and then randomly divided into seven groups: normal group (without any treatment), model group (treated with saline), TSA group (treated with trichostatin A), perindopril group (treated with perindopril), and the high, medium, and low dose of *Lujiao* groups (treated with 2.4 g/mL, 1.2 g/mL, and 0.6 g/mL of *Lujiao*, respectively). The test drug of perindopril group or *Lujiao* group was derived from serum after drug treatment in rats. Real-time polymerase chain reaction and Western blot were performed to analyze expression of myocyte enhancer factor 2 (MEF-2), α-major histocompatibility complex (MHC), and β-MHC and acetylation level of histone H3.

Results: Expressions of MEF-2 and β-MHC were significantly increased after PE treatment and decreased after drug treatment. Expression of α-MHC mRNA was significantly reduced after PE treatment and increased after being treated with *Lujiao* prescription, perindopril, and TSA. The acetylation level of histone H3 decreased in rat myocardial cells stimulated by PE 48 for hours and this decrease was reversed after treatment with high and medium doses of *Lujiao* prescription, perindopril and TSA.

Conclusion: Histone acetylation-MEF-2-α-MHC/β-MHC axis was discovered in myocardial hypertrophy, and intervention of *Lujiao* prescription exhibited good effects.

Keywords: histone acetylation; intervention; *Lujiao* prescription; myocardial hypertrophy; myocyte enhancer factor 2

1. Introduction

The phenomenon of chronic heart failure (CHF) is a complex clinical syndrome, and further represents a serious stage of various heart diseases.[1] According to epidemiological data released in 2003, the average prevalence rate of heart failure in China was 0.9% (age 35–74 years).[2] In addition, the exorbitant costs as a result of long-term treatment and repeated exacerbations and hospital costs have become a burden of families and society, and increasingly represent a serious global public health problem.[1,2]

CHF treatment decisions have changed from an emphasis on short-term, hemodynamic/pharmacological measures to a long-term maintenance and repair strategy.[3] Efforts to repair failed myocardial biology, blocking of the vicious spiral

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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between factor neuroendocrine, cell activation, and myocardial remodeling have become the key for treatment of CHF. American Heart Association/American College of Cardiology heart failure guidelines classify the high-risk population of CHF and the refractory heart failure patients into four stages (A–D) according to the process of the development of CHF, and provides comprehensive prevention treatment in different stages.[4] Nevertheless, in the face of a rising prevalence rate, the continued decline of high-risk-of-death and hard-to-reverse malignant diseases, CHF treatment is still an ongoing challenge. Not only do the symptoms and prognosis of the diseases need to be improved, but also new drugs with fewer side effects or treatment are urgently needed.[3,4]

It is well known that myocardial remodeling is the basic mechanism of the occurrence and development of heart failure, including hypertrophy and apoptosis of cardiomyocytes, myocardial extracellular matrix quality, and composition change.[5] In the development of CHF, the sympathetic activation and long-term pressure overload is an important cause of myocardial remodeling, and cardiac hypertrophy is one of the most important myocardial remodeling elements in the pathological process.[6] Focusing on myocardial cell hypertrophy, an important pathological process, there are clinical and social significances for the further study of the pathogenesis and intervention of myocardial hypertrophy.[2,5,6]

The present study will innovatively introduce histone acetylation modification to explore the role of traditional Chinese medicine intervention during myocardial hypertrophy. Many investigations revealed that altered expression of β-major histocompatibility complex (MHC) was observed in CHF hypertrophy myocardial cells in compensatory stage with the force of contraction, with decreased α-MHC/β-MHC ratio.[7–9]

Class II histone deacetylase (HDAC2) regulates histone deacetylase myocyte enhancer factor 2 (MEF-2) to prevent myocardial cell hypertrophy[10]; acetyl transferase P300 and histone acetyltransferases (HATs-p300) promote cardiac hypertrophy combined with MEF-2 after histone acetylation.[11] The transcription factor MEF-2 shows activity only in the adult human heart, but it is reported that MEF-2 activity increases rapidly in the hypertrophy of cardiomyocytes induced by calcium dependent stimulation signal under stress.[5] MEF-2 provides a correlation between HDACs, HATs, and downstream response genes (such as myocardial hypertrophy of embryonic gene), and it also plays positive or negative roles in gene expression of pathological myocardial cells.[12]

Lujiao prescription is a clinical and traditional prescription in the treatment of heart failure, and is mainly composed of antler, epimedium, psoralea fruit, dogwood, glossy privet fruit, and dried tangerine or orange peel.[13] The function of *Lujiao* prescription is to nourish Yin and Yang, warm the kidney, and strengthen the heart. The benefit effects of *Lujiao* prescription are apparent primarily through positive constriction, dilation of blood vessels, and a creation of a diuretic result.[13] The medicine can promote the influx of extracellular calcium, reduce plasma atrial natriuretic peptide (ANP), angiotensin II (AngII), and aldosterone concentrations, and reduce left ventricular regional AngII levels and angiotensin receptor I mRNA expression. Furthermore, the medicine can block and reverse ventricular remodeling process to a certain extent, and significantly improve the symptoms of heart failure without obvious side effects.[2,10,12–16]

In the present study, neonatal rat cardiomyocytes were cultured *in vitro*, and hypertrophied myocardial cell culture models were constructed. *Lujiao* prescription was used to intervene in the hypertrophy of cardiac muscle cells to explore the mechanism of *Lujiao* prescription from the histone acetylation avenue.

2. Methods

2.1. Ethical approval of studies

All studies have been approved by the Ethics Committee of Shanghai Shuguang Hospital Affiliated with Shanghai University of Traditional Chinese Medicine, Shanghai, China and performed in accordance with proper ethical standards.

2.2. Lujiao prescription

Lujiao prescription was bought from the Traditional Chinese Medicine Pharmacy in Shuguang Hospital. Raw materials of *Lujiao* prescription by weight percent were as follows: 1–5% deerhorn glue or 8–25% antlers, 15–40% Epimedium, 15–40% Fructus Ligustri Lucidi, 10–30% Fructus Psoraleae, 8–25% Fructus Corni, and 8–25% orange. According to the above proportion, antler glue or crushed antlers were prepared via 80 mesh sieving. About 10–30 times the amount of water was added to the remaining components. The solution was boiled at 90 ± 10°C two or three times (1.5 h for each time). After filtering, the decoction was merged to a concentration with the relative density of 1.04–1.05 at room temperature. After high-speed centrifugation 289,249*g*, the supernatant was concentrated to a relative density of 1.10–1.14 at 90°C. Spray drying or the addition of a small amount of dextrin spray was used to dry and extract the powder. The above antler powder and the extracted powder mixture were added to the medicinal materials according to the conventional methods of granular preparation. The particles were dissolved in water before use, with a crude drug concentration of 1.2 g/mL.

2.3. Perindopril (tablets)

Perindopril tablets (4 mg/tablet) were used in the present study. The perindopril (tablets) were produced by French pharmaceutical company Servier Pharmaceutical (Suresnes, France). The tablets were crushed and subjected to a pharmacopoeia 100 mesh sieve, and then added into twice distilled water with an 0.5% suspension agent (sodium carboxymethyl cellulose). Finally, a 0.036 mg/mL concentration of perindopril suspension was produced.

2.4. Drug administration and serum samples collection

Male Sprague–Dawley (SD) rats (body weight 220–300 g, Shanghai SLRC Laboratory Animals Co., Shanghai, China) were fasted for 12 hours, and then randomly divided into the following groups: normal control group treated with vehicle; perindopril group treated with perindopril; the high, medium, and low dose of *Lujiao* groups treated with high, medium, and high dose of *Lujiao*, with 12 rats in each group. For experiments involving animals, approval was obtained from the Institutional Review Board of the Shanghai Shuguang Hospital Affiliated with Shanghai University of Traditional Chinese Medicine. According to the human and animal surface area of the equivalent dose conversion ratio table conversion, the concentrations of *Lujiao* in high, medium, and low dose of *Lujiao* groups were 2.4 g/mL, 1.2 g/mL, and 0.6 g/mL, respectively. The *Lujiao* prescription or perindopril was dissolved in water solvent and administrated by gavage. In the normal control group, double distilled water was administered. Drugs were used with a volume of 100 mL/g of body weight two times/d for 7 continuous days. At 2 hours after the last drug administration, ether anesthesia was conducted. Abdominal aortic blood was collected under aseptic conditions. After resting in a sterile tube for 4 hours, the samples were centrifuged at 13,282*g* for 15 minutes, and then serum was collected. The protein concentration of the serum was measured for the purpose of quality control by absorbance value measurement using spectrophotometry via an enzyme-linked immunosorbent assay reader. The serum of the same group was mixed and inactivated at 56°C for 30 minutes to eliminate interference of virus and other factors. After the samples were packed, they were frozen at −80°C until analysis.

2.5. Myocardial cell culture and packet processing

Methods of myocardial cell culture and packet processing were used according to the literature.[17] Ten neonate SD rats (P2-3, provided by Shanghai SLRC Laboratory Animals Co., Shanghai, China) were soaked in 0.1% bromogeramine. The hearts were quickly collected, and placed into petri dishes containing 5 mL Dulbecco's modified Eagle medium (DMEM). The atria and great vessels were trimmed, and the residual blood washed away. The apical portion was put in another petri dish. Then, the apical part was quickly cut into 1 mm^3 pieces and transferred into an Erlenmeyer flask with 5 mL trypsin and incubated in a 34°C water bath for 10 minutes. The supernatant was transferred into a centrifuge tube, and centrifuged at 944*g* for 10 minutes. The residue was then added to 5 mL of trypsin and incubated at 34°C in a water bath for 10 minutes. After that, the supernatant was transferred to another tube and centrifuged at 944*g* for 10 minutes. If there were residual tissues, the above procedures were repeated two to three additional times. The collected digestive supernatant was centrifuged at 4°C at 1475*g*, after which the supernatant was removed. Cell pellets were washed with liquid suspension culture of DMEM containing 10% fetal bovine serum (Gibco, Grand island, N.Y., USA). The cells were cultured in cell culture flasks for 90–100 minutes in 5% CO_2, 37°C, and in high humidity. Then nonadherent myocardial cell suspension was carefully aspirated and divided into 7 groups (Fig. 1).

Phenylephrine (PE, final concentration 20μM, Sigma–Aldrich Co., St Louis, MO, USA) was added in the cultured myocardial cells to establish myocardial hypertrophy cell model. The expression of ANP was detected by real time polymerase chain reaction (PCR) to test whether the model was successfully established. Cell diameters were also detected using the cell imagine analysis system (CAIS-1000; China Daheng Group, Beijing, China) to record volume changes. Trichostatin A (TSA, final concentration of 100nM; Wako, Osaka, Japan) served as a positive control. Different treatments were administrated to the separated groups, as shown in Fig. 1. In the normal control group and model group, an equal amount of DMEM was added. All cells were co-cultured for 48 hours for further real-time PCR or Western blot analysis.

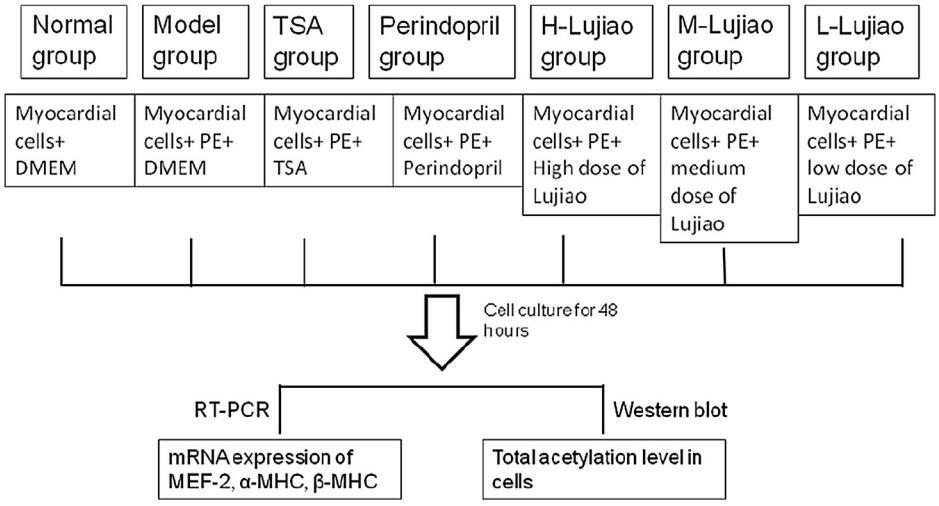

Fig. 1. Different treatments for different groups.

Table 1
Real-time polymerase chain reaction primers.

Gene	Primer sequence	Temperature
MEF-2	Forward 5′-TACAACGAGCCGCATGAGAG-3′ Reverse 5′-CCTGTGTTACCTGCACTTGG-3′	61°C
α-MHC	Forward 5′-ACAGAGTGCTTCGTGCCTGAT-3′ Reverse 5′-CGAATTTCGGAGGGTTCTGC-3′	63°C
β-MHC	Forward 5′-GCTACCCAACCCTAAGGATGC-3′ Reverse 5′-TCTGCCTAAGGTGCTGTTTCA-3′	61°C
β-actin	Forward 5′-CATCGTACTCCTGCTTGCTG-3′ Reverse 5′-CCTCTATGCCAACACAGTGC-3′	60°C

MEF-2 = myocyte enhancer factor-2; MHC = major histocompatibility complex.

2.6. Total RNA extraction and real-time fluorescence quantitative PCR

The conventional TRIzol method (Invitrogen) was used to extract the total RNA of myocardial cells and real-time quantitative PCR was employed for analysis. Expressions of MEF-2, α-MHC, and β-MHC mRNA were determined with β-actin as internal reference. The primers used in this analysis are listed in Table 1.

2.7. Immunoblotting detection of histone H3 acetylation

Western blot was conducted to detect the expression of histone H3 acetylation according to earlier studies.[18] Primary antibodies against MEF-2, α-MHC, and β-MHC, as well as secondary antibodies against goat-anti mouse-HRP (Sigma) were used.

2.8. Statistical analysis

Experimental data were expressed as mean ± standard deviation. Mean comparisons were compared with one-way analysis of variance (ANOVA), followed by use of the Bonferroni *t* test for multiple comparisons between groups. A *p* value <0.05 indicated that the difference was statistically significant. In order to facilitate the display and analysis, the data of the control group were set to 1, and the data of other groups were calculated by comparison with the control group.

3. Results

3.1. Establishment of the myocardial hypertrophy cell model

PE was added into the cells to establish the myocardial hypertrophy model (after the stimulation) and cell volume manifestly increased compared with the control cells (Fig. 2A). Real time PCR showed that there was increased expression of ANP compared with that in control cells ($p < 0.05$; Fig. 2B).

3.2. Expression of MEF-2 in neonatal rat hypertrophic cardiomyocytes and effects of drug intervention

The mRNA expression level of MEF-2 was detected by real-time PCR (Fig. 3). As shown in Fig. 3, expression of MEF-2 was significantly increased after the stimulation of PE for 48 hours ($p = 0.027$ vs. control). After being treated with high and medium doses of *Lujiao*, perindopril, and TSA, the expression of MEF-2 significantly decreased compared with that in the PE group, suggesting the role of high and medium doses of *Lujiao* in regulating MEF-2. However, there was no significant change of MEF-2 expression in the low concentration *Lujiao* group ($p > 0.05$ vs. PE group, and $p < 0.05$ vs. control group).

3.3. Expression of α-MHC mRNA in neonatal rat hypertrophic cardiomyocyte and effects of drug intervention

Compared with that in the normal control group, the expression of α-MHC mRNA in the PE group was significantly reduced, indicating that the expression of α-MHC mRNA decreased in rat myocardial cells with myocardial hypertrophy stimulated by PE. In respect to drug intervention, expression of α-MHC mRNA was significantly increased in the groups of medium and high concentration of *Lujiao* prescription, perindopril, and TSA groups, which was especially significant in the perindopril group (compared with the PE group, where $p < 0.05$). Although the expression of α-MHC

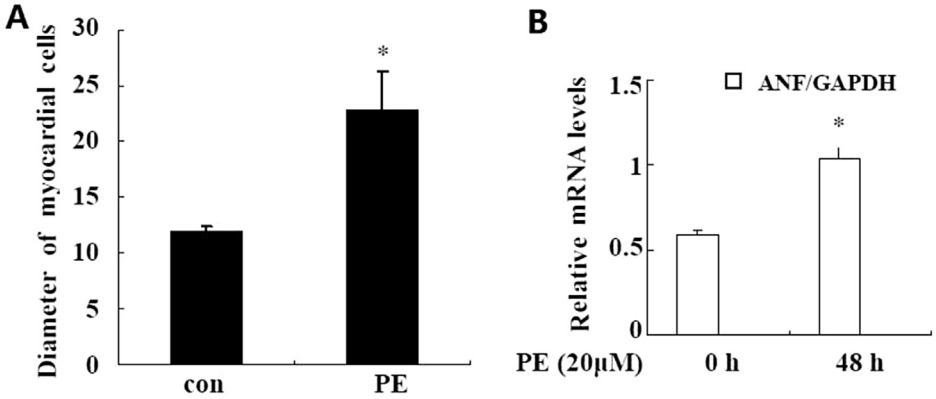

Fig. 2. Establishment of myocardial hypertrophy model via PE. (A) Cell diameters were determined using CAIS-1000 cell imagine analysis system; (B) Real-time polymerase chain reaction showed that there was increased expression of ANP before and after treatment of cells with PE for 48 hours ($p < 0.05$), which is used as a marker for myocardial hypertrophy. ANP = atrial natriuretic peptide; con = control group; PE = phenylephrine.

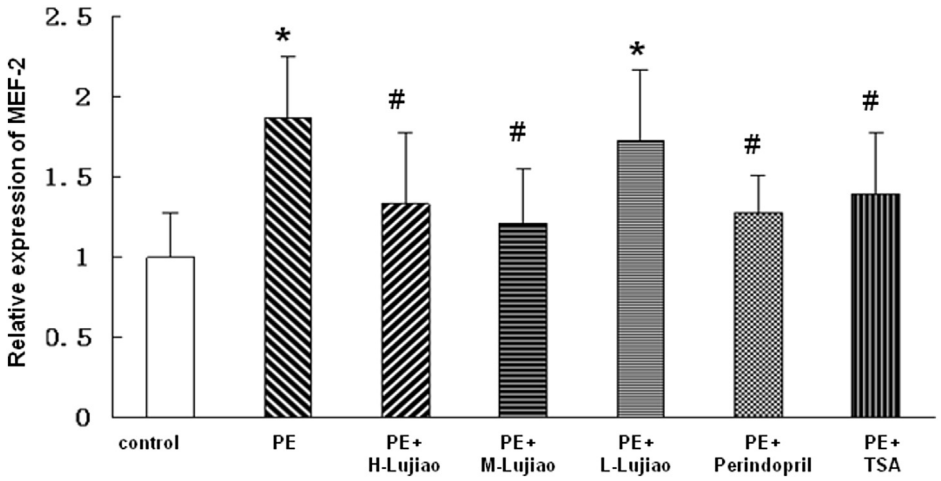

Fig. 3. Expression of myocyte enhancer factor 2 (MEF-2) in neonatal rat hypertrophic cardiomyocytes and effects of drug intervention. Methods of myocardial cell culture and packet processing were conducted. Ten neonate Sprague–Dawley rats were soaked in 0.1% bromogeramine. Hearts were collected quickly, and put in petri dishes containing 5 mL Dulbecco's modified Eagle medium. Western blot was conducted. Primary antibodies against MEF-2 and secondary antibody against goat-anti mouse-HRP were used. MEF-2 = myocyte enhancer factor 2, PE = phenylephrine. *$p < 0.05$ vs. control; #$p < 0.05$ vs. PE.

mRNA demonstrated an increased trend in the low concentration group, the difference was not statistically significant (Fig. 4).

3.4. Expression of β-MHC mRNA in neonatal rat hypertrophic cardiomyocyte and effects of drug intervention

Compared with that of the normal control group, the expression of β-MHC mRNA involved a multifold increase in myocardial cells of neonatal rats in the PE group, suggesting that the expression of β-MHC mRNA in the hypertrophic cardiomyocytes was upregulated. The expression of β-MHC mRNA in the groups of medium and high concentrations of *Lujiao* prescription, perindopril group and TSA group was dramatically decreased, but it was still higher than that in the control group (Fig. 5).

3.5. Expression of acetylated histone H3 in rat myocardial cells with drug treatment

Western blot assay was used in the analysis of acetylated histone H3 expression in rat myocardial cells with drug treatment. The acetylation level of histone H3 significantly decreased in rat myocardial cells stimulated by PE for 48 hours. After treating with different doses of *Lujiao* prescription, perindopril, and TSA, the level of acetylated histone H3 was significantly increased, especially in the high and medium dose groups (Fig. 6).

4. Discussion

In the process of occurrence and development in relation to heart failure, myocardial remodeling is one of the most important basic pathogeneses, and cardiac hypertrophy is one of the most important pathological processes during the remodeling. Cardiac hypertrophy is of phenomenon whereby myocardial cell volume increases without cell division, which is found in many cardiovascular diseases. The biochemical basis of myocardial cells hypertrophy is the increase of myocardial protein synthesis, leading to an increase in cell volume.[12] An *in vitro* myocardial hypertrophy model was constructed by treating and stimulating cardiac myocytes with adrenaline or AngII. In the present study, PE stimulation method was used to establish a myocardial hypertrophy model.[11] There are differences among adrenaline, AngII, and PE-induced cell models of myocardial hypertrophy. The reason might be that there could be other mechanisms in the induced myocardial cell hypertrophy. Another reason might be that there were conflicting data by different methods in exploring and detecting myocardial hypertrophy.

In the present study, SD rats were randomly divided into normal control group, perindopril group, high dose of *Lujiao* group, medium dose of *Lujiao* group and low dose of *Lujiao* group. A myocardial hypertrophy model was established by PE stimulation. HDACs and HATs participate in the myocardial hypertrophy process,[10] and regulate downstream hypertrophic genes such as cardiac fetal gene by MEF-2. Thus, real-time PCR and western blotting were used to analyze the expression of MEF-2, α-MHC, and β-MHC mRNA and the acetylation level of histone H3. Expression of the MEF-2 gene was detected in rat myocardial cells with normal growth in the control group. Furthermore, MEF-2 expression was significantly increased and expression of α-MHC mRNA in the PE group was significantly reduced. Expression of α-MHC and β-MHC mRNA were significantly increased in the medium and high concentrations of *Lujiao* prescription, perindopril, and TSA groups.

Expression of HDACs was not detected in the present study, but the TSA intervention after PE stimulation in rat myocardial cells was used in the analysis. The results showed that TSA intervention significantly increased the acetylation

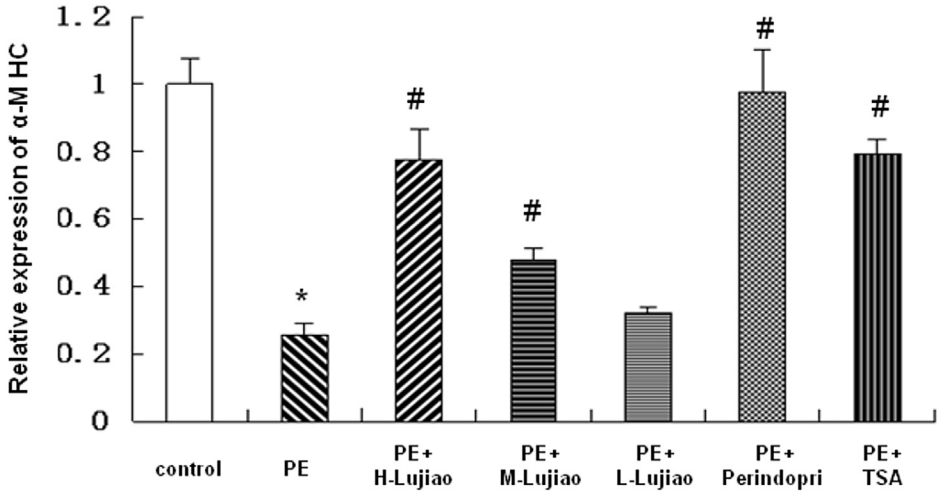

Fig. 4. Expression of α-major histocompatibility complex (MHC) mRNA in neonatal rat hypertrophic cardiomyocytes and effects of drug intervention. Methods of myocardial cell culture and packet processing were conducted. Ten neonate Sprague–Dawley rats were soaked in 0.1% bromogeramine. Hearts were collected quickly, and put in petri dishes containing 5 mL Dulbecco's modified Eagle medium. mRNA was extracted and real-time polymerase chain reaction was conducted using α-MHC primers. PE = phenylephrine. *$p < 0.05$ vs. control, #$p < 0.05$ vs. PE.

level of histone H3 in myocardial cells than those in the model group, while MEF-2 mRNA expression was downregulated. The expressions of α-MHC and β-MHC decreased. However, there was no significant change in the acetylation level of histone H3 by TSA treatments compared with that of the normal control group. The acetylation level of histone H3 decreased in rat myocardial cells stimulated by PE for 48 hours. The level of acetylated histone H3 in high concentrations did not increase significantly, while histone H3 acetylation level in the low concentrations of traditional Chinese medicine group and perindopril group, and TSA group increased.

HDACs and HATs were involved in the myocardial hypertrophy process by MEF-2 responses to hypertrophic gene downstream (such as cardiac fetal gene) regulation. It is reported that HDACs, as histone deacetylases, inhibited MEF-2 expression, thereby inhibiting cardiac hypertrophy[19]; HATs and the histone acetylation and MEF-2 interaction thereby promoted myocardial hypertrophy. HDACs inhibit MEF-2 expression via histone deacetylase, thus inhibiting cardiac hypertrophy[6]; additionally, the HATs promote myocardial hypertrophy through the histone acetylation and MEF-2 binding.[5] It has been established that the histone acetylation level is increased in the myocardial cell hypertrophy group.

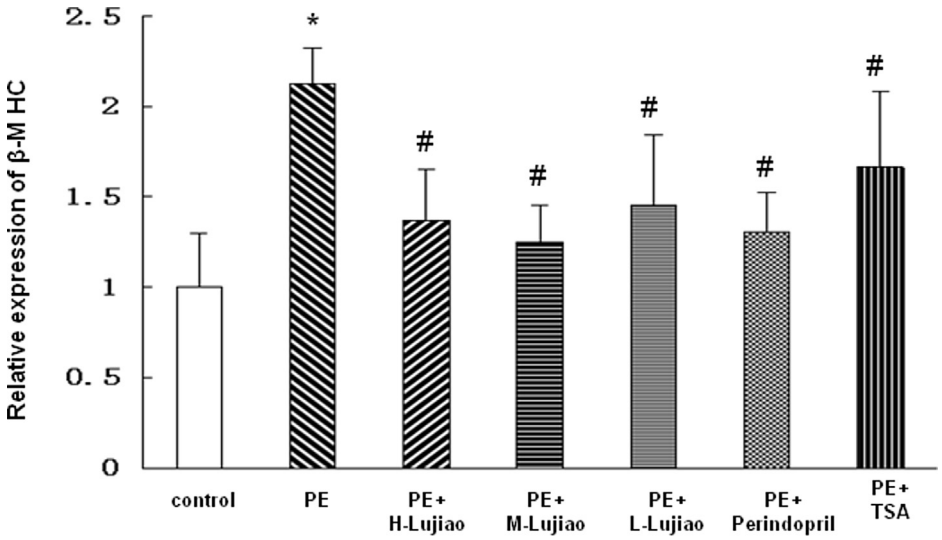

Fig. 5. Expression of β-major histocompatibility complex (MHC) mRNA in neonatal rat hypertrophic cardiomyocytes and effects of drug intervention. Methods of myocardial cell culture and packet processing were conducted. Ten neonate Sprague–Dawley rats were soaked in 0.1% bromogeramine. Hearts were collected quickly, and put in petri dishes containing 5 mL Dulbecco's modified Eagle medium. mRNA was extracted and real-time polymerase chain reaction was conducted using β-MHC primers. PE = phenylephrine. *$p < 0.05$ vs. control; #$p < 0.05$ vs. PE.

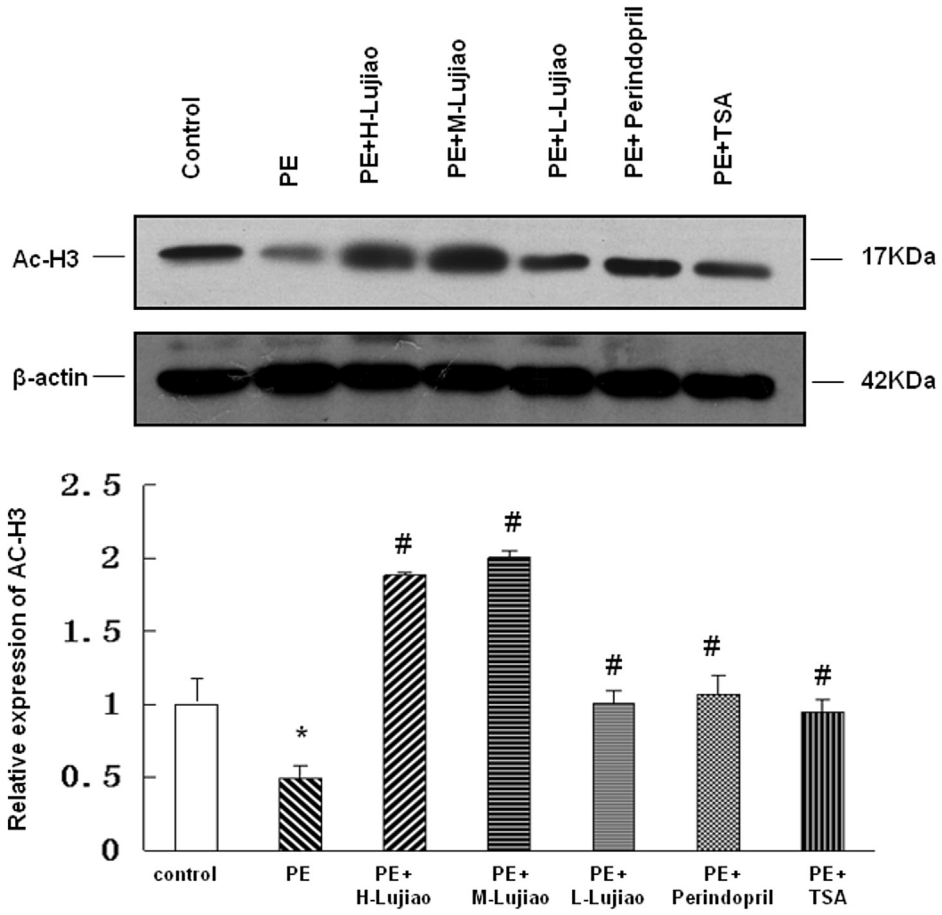

Fig. 6. Expression of acetyl histone H3 in rat myocardial cells with drug treatment. Methods of myocardial cell culture and packet processing were conducted. Ten neonate Sprague–Dawley rats were soaked in 0.1% bromogeramine. Hearts were collected quickly, and put in petri dishes containing 5 mL Dulbecco's modified Eagle medium. Western blot was conducted to detect expression of histone H3 acetylation. PE = phenylephrine. *$p < 0.05$ vs. control; #$p < 0.05$ vs. PE.

However, other studies have shown that HDACs inhibitors can prevent cardiac hypertrophy, inhibit cardiac fetal gene (such as β-MHC) and ANP activity, increase expression of adult α visoforms of MHC, and the effect was dose dependent. At the same time, the total H4 histone acetylation in myocardial cells increased.[20,21] An HDACs inhibitor, TSA, also can significantly reduce the myocardial hypertrophy heart coefficient of homeodomain-only protein-1 transgenic mice and rats induced by isoproterenol. In addition, the research of Kook[22] also showed HDAC2 could form complex mechanisms with homeodomain-only protein and inhibited serum response factor (serum response factor), thereby inhibiting anti-hypertrophic gene expression. In hypertrophy myocardial cells, HDACs have double functions of promotion and antagonist. Our study did not detect the expression of HDACs, but used the TSA intervention after PE stimulation in rat myocardial cells. The results showed that TSA intervention increased significantly the acetylation level of histone H3 in myocardial cells over those in the model group, while MEF-2mRNA expression was downregulated. The expressions of α-MHC and β-MHC decreased. However, there was no significant change in acetylation level of histone H3 by TSA treatments compared with that of the normal control group.

Chinese traditional medicine of *Lujiao* prescription and perindopril are effective drugs in the treatment of heart failure, and they have been shown to inhibit left ventricular remodeling.[23] We established rat cardiac hypertrophy models to explore the possible mechanism of two kinds of drugs from the angle of histone acetylation using the method of serum pharmacology. The experimental results show that compared with that of the model group, the expression of MEF-2 mRNA and β-MHC mRNA expression in the high, medium concentration group and perindopril group all significantly decreased, and the expression of α-MHC mRNA was upregulated. At the same time, the acetylation level of histone H3 increased. The myocardial cell histone H3 acetylation level was high with *Lujiao* prescription intervention compared with that of the normal control group.

This study examined the histone acetylation-MEF-2-α-MHC/β-MHC axis in myocardial hypertrophy, and intervention results of *Lujiao* prescription. In fact, many factors can lead to myocardial cell hypertrophy, the above axis and the other known signaling pathways such as the relationship between CaN, MAPK, PKD/HDAC5/MEF2 path to mediate cardiac hypertrophy still need to be further studied.

The present investigation demonstrates that the combination of SalB with Rg1, but not with Rb1 alone, demonstrated

significant improvement on cardiac contractility in rats with myocardial infarction. Unfortunately, Rb1 alone did not improve the infarct size. The current data indicate that the histone acetylation-MEF-2−α-MHC/β-MHC axis is found in myocardial hypertrophy, and the intervention effects of *Lujiao* prescription are positive. Because various protein kinases or signaling proteins such as CaN, MAPK, PKD/HDAC5/MEF2 also contribute to myocardial cell hypertrophy, there is the possibility that CaN, MAPK, PKD/HDAC5/MEF2 also play important roles in *Lujiao* prescription-induced potential therapy for hypertrophic cardiomyocytes. There might be a novel strategy in therapy for hypertrophic cardiomyocytes by targeting MAPK, PKD/HDAC5/MEF2.

In conclusion, histone acetylation-MEF-2−α-MHC/β-MHC axis is found in myocardial hypertrophy, and the intervention effects of *Lujiao* prescription are good.

Acknowledgments

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References

1. McGovern BH. The epidemiology, natural history and prevention of hepatitis B: implications of HIV coinfection. *Antivir Ther* 2007;**12**:H3−13.
2. Gill S, Porter DL. CAR-modified anti-CD19 T cells for the treatment of B-cell malignancies: rules of the road. *Expert Opin Biol Ther* 2014;**14**:37−49.
3. Chen M, Li Y, Zhang D, Wang Z, Zeng W, Shi X, et al. Therapeutic effect of autologous dendritic cell vaccine on patients with chronic hepatitis B: a clinical study. *World J Gastroenterol* 2005;**11**:1806−8.
4. Bertoletti A, Gehring A. Immune response and tolerance during chronic hepatitis B virus infection. *Hepatology Research* 2007;**37**:S331−8.
5. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* 1976;**263**:663−5.
6. Pulendran B, Tang H, Manicassamy S. Programming dendritic cells to induce TH2 and tolerogenic responses. *Nat Immunol* 2010;**11**:647−55.
7. Landford E, Epsteun ND, Fananapazir L, Sweeney H. Abnormal contratile properties of muscle fibers expressing of β-myosin heavy chain gene mutation in patients with hypertrophic cardiomyopathy. *J Clin Invest* 1995;**95**:1409−14.
8. James J, Martin L, Krenz M, Quatman C, Jones F, Klevitsky R, et al. Forced expression of alpha-myosin heavy chain in the rabbit ventricle results in cardio-protection under cardiomyopathic conditions. *Circulation* 2005;**18**:2339−46.
9. Azahab I, Spicard N, Fortin N, Reudelhuber T, Deschepper C. Expression of constitutively active guanylate cyclase in cardiomyocytesin hibits the hypertrophic effects of isoproterenol and aortic constriction on mouse hearts. *J Biol Chem* 2003;**48**:47694−9.
10. Schiff ER, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, et al. Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre- and post-liver transplantation patients. *Hepatology* 2003;**38**:1419−27.
11. Shi M, Fu J, Shi F, Zhang B, Tang Z, Zhang Z, et al. Viral suppression correlates with dendritic cell restoration in chronic hepatitis B patients with autologous cytokine-induced killer cell transfusion. *Liver Int* 2009;**29**:466−74.
12. Simpson P, Savion S. Differentiation of rat myocytes in single cell cultures with and without proliferating nonmyocardial cells. Cross-striations, ultrastructure, and chronotropic response to isoproterenol. *Circ Res* 1982;**50**:101−16.
13. Cai H, Hu W, Dong Y. Changes of myocardial tissue and plasma angiotensin II in rats with pressure overload and effect of *lujiao* prescription. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2000;**20**:282−3 [Article in Chinese].
14. Jia J, Zhuang H. A winning war against hepatitis B virus infection in China. *China Med J* 2007;**120**:2157−8.
15. Lin N, Cai D, Jin D, Chen Y, Shi J. Ginseng panaxoside Rb1 reduces body weight in diet-induced obese mice. *Cell Biochem Biophys* 2013;**68**:1−6.
16. Zheng Z, Li G, Ping A, Shan S, O'Brien SJ, Winkler CA, et al. A population-based study to investigate host genetic factors associated with hepatitis B infection and pathogenesis in the Chinese population. *BMC Infect Dis* 2008;**8**:1.
17. Gui Y, An G, Ni J, Jia H. Involvement of MLP in Cardiac Hypertrophy is Related to Calcineurin/NFAT Signaling Pathway. *Chin J Biochem Mol Biol* 2007;**8**:660.
18. George JW. Activation of NK cell responses and immunotherapy of cancer. In: Rosenblatt JD, Podack ER, Barber GN, Ochoa A, editors. *Advances in tumor immunology and immunotherapy*. New York: Springer; 2014. p. 57−66.
19. Cao DJ, Wang ZV, Battiprolu PK, Jiang N, Morales CR, Kong Y, et al. Histone deacetylase (HDAC) inhibitors attenuate cardiac hypertrophy by suppressing autophagy. *Proc Natl Acad Sci U S A* 2011;**108**:4123−8.
20. Pedram A, Razandi M, Narayanan R, Dalton JT, McKinsey TA, Levin ER. Estrogen regulates histone deacetylases to prevent cardiac hypertrophy. *Mol Biol Cell* 2013;**24**:3805−18.
21. Liu Z, Chen J, Huang W, Zeng Z, Yang Y, Zhu B. Ginsenoside Rb1 protects rat retinal ganglion cells against hypoxia and oxidative stress. *Mol Med Report* 2013;**8**:1397−403.
22. Kook H, Yung W, Simpson R, Kee H, Shin S, Lowry J, et al. Analysis of the structure and function of the transcriptional coregulator HOP. *Biochemistry* 2006;**35**:10584−90.
23. Liu D, Zhang H, Gu W, Liu Y, Zhang M. Neuroprotective effects of ginsenoside Rb1 on high glucose-induced neurotoxicity in primary cultured rat hippocampal neurons. *PLoS One* 2013;**8**:e79399.