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Exploring the therapeutic effect of Qijia Wuling soupon cardiomyocyte mast cells

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Abstract: The formation of cardiac hypertrophy is an important pathological change leading to chronic heart failure, which determines the development and prognosis of CHF. When the heart is damaged and stressed, the cardiomyocytes become hypertrophic and lead to changes in cellular metabolic patterns, which ultimately affect the structure and function of the heart. Qijia Wuling soup is the experience prescription of treating heart failure by the chief physician of Staff Liqin. In this study, we found that it could improve the echocardiographic parameters and relieve the symptoms of CHF in rats. In this study, the effect and mechanism of Qijia Wuling soup on myocardial hypertrophy in rats with CHF were investigated on the basis of the previous studies which proved that Qijia Wuling soup was effective in the treatment of CHF, to provide evidence for improving cardiac hypertrophy. H9C2 cells were inoculated into 6-well plate and randomly divided into control group and model group. Study it. This study demonstrated that qjwlt-containing serum had a significant protective effect on H9C2 cardiomyocytes injury induced by (-)-noradrenaline. This provides an important experimental and theoretical basis for the clinical application of qjwlt in the treatment of heart failure and improvement of cardiac hypertrophy. With the in-depth study of its mechanism of action, Qijia Wuling soupis expected to become one of the effective drugs in the treatment of CHF, bringing better therapeutic effect and quality of life for patients.

According to the 2019 Fuwai Hospital statistical survey, HF data from 31 provinces showed an incidence of approximately 1.3% (13.7 million) in patients aged ≥35 years. It was found that the prevalence rate in the north was significantly higher than that in the south, the prevalence rate in the city was higher than that in the countryside, and 60% of the patients were over 60 years old. Therefore, cardiovascular disease has become a serious threat to the Chinese People's physical and mental health and life expectancy of an important disease [1]. And as China gradually enters the aging stage, as well as the impact of modern society on People's work, diet and lifestyle. The development of cardiovascular diseases will cause irreversible changes in the structure and morphology of the heart, leading to the occurrence and deterioration of heart failure. Therefore, it is of great significance to prevent and delay the occurrence of heart failure by treating cardiovascular

diseases [2].

1. The pathogenesis of CHF

1.1 Hemodynamics reaction

When cardiomyocyte contractility decreases and/or haemodynamic load is exacerbated, the heart produces a range of adaptive changes to maintain normal cardiac output [3]. Activation of endocrine system (especially Raas) and sympathetic nervous system can also enhance cardiac contractility and increase cardiac preload by regulating volume balance and increasing ventricular perfusion [4] . These mechanisms may initially improve myocardial function, but as the disease progresses and cardiomyocytes are destroyed, there will eventually be a series of serious consequences due to maladaptation.

1.2 Cardiomyocyte apoptosis

CHF usually involves the injury and loss of myocardial cells, which leads to the loss of normal contractile function of myocardial tissue. This damage can be caused by a variety of causes, including coronary artery disease, high blood pressure, myocarditis and cardiomyopathy. Once the cardiac muscle cells are damaged, various mechanisms such as nerve and endocrine will further accelerate the death of cardiac muscle cells. In addition, the loss of cardiomyocytes may lead to thinning of ventricular wall, heart enlargement, further impaired cardiac function. Therefore, the damage and loss of cardiomyocytes is one of the fundamental reasons for the continuous progress of heart failure. Protection and repair of cardiac myocytes may be an important strategy for the prevention and treatment of heart failure[5].

1.3 Cytokines

During cardiac remodeling, compensatory hypertrophy of cardiomyocytes is regulated by multiple factors, including directly acting hypertrophic mediators as well as upstream stimuli of other cell types. The injury and death of cardiomyocytes may release bioactive substances, such as Transforming growth factor beta (TGF-β)[6]. Moreover, in myocardial infarction, the death of cardiomyocytes and other cardiomyocytes leads to the release of damage-associated molecular patterns, thereby activating inflammation and ultimately leading to hypertrophy [7]. The relative role of various cell types in the hypertrophic response depends on the type of myocardial injury.

2. Treatment of CHF

The basis for treating chronic heart failure includes drugs that modulate the Renin–angiotensin system system (Raas), sympathetic nervous system system (SNS) or natriuretic peptide system. These include ACE inhibitor (ACEI), Angiotensin receptor neuroendocrine enzyme inhibitors (Arni), Beta-blockers and the mineral corticosteroid receptor antagonist (MRA)[8]. ACE inhibitor (e.g., captopril, enalapril, lisinopril, Ramipril, tradopril) not only block the conversion of AI to AII, it can also promote the conversion of AI to A1-7, thus further promoting cardiovascular benefi TS [9]. MRA (eplerenone, potassium canrelate, and spironolactone) is an aldosterone antagonist and is also considered to have cardiovascular benefits when used in combination with ACE inhibitors (beta blockers)[10-11]. Arbs (such as Valsartan, Irbesartan, and kandisartan) not only block the binding of ATII to ATI receptors produced by ACE and non-ACE pathways, it also attenuated the myocardial damage and HF progression induced by overactivation of Raas by AT1R [12-13]. In

clinical practice, the objective of dose increase should be the maximum tolerated dose [14~15].4. Various types of diuretics play an important role in reducing water and sodium retention in heart failure and reducing cardiac load [16~17]. 5. Positive inotropic drugs: it can increase the work of cardiac myocytes by blocking the Na, K, atpase pathway on the cell membrane. Representative drug: digoxin.6. Angiotensin receptor Enkephalinase inhibitor are a class of drugs that act on both Angiotensin receptor and enkephalinase. The mechanism of action of this drug makes it have some advantages in the treatment of heart failure. Angiotensinogen II. The receptor blocker (ARB) Valerian Chatain is used in combination with the NEP inhibitor shakubaqu. Compared with ACE inhibitor Enalapril Puli[18], shakubatrovalsartan has a lower rate of cardiac death and heart failure hospitalizations. Other additional benefits of shakubatrovalsartan include: improved symptoms and quality of life [19]; reduced incidence of diabetes requiring insulin therapy [20]; and reduced need for loop diuretics [21]. 7. Antithrombotic drugs, there is obvious evidence that antithrombotic therapy can reduce the probability of embolic events, and heart failure often combined with coronary heart disease, atrial fibrillation. This case should be given long-term antithrombotic treatment, commonly used drugs such as aspirin, clopidogrel bisulfate. Other drugs, such as vasodilators and cardiomyocyte nutrition, are also widely used in the clinic[22].

3. Experimental methods

3.1 Materials

3.1.1 Experimental animals

Healthy male SD rats with body weight of 200 ± 20 g and 6 weeks were selected as experimental animals. Purchased from Xi'an Enoch Biotechnology Co., Ltd. Licence No. SCXK (Shaanxi) 2018-001. The animals are kept in the laboratory of Xi'an Medical College, which belongs to the laboratory of constant temperature regulation. All animals are raised and used in full compliance with the provisions of the animal ethics law, and the animals were fed adaptively for 1 week before entering the experiment.

3.1.2 Cell line

H9C2 myocardial cell line

3.1.3 Experimental drug formulation

The formula of Qijia Wuling soupfor adults is astragalus, fangji, Poria, Poria, Alisma, plantain seed, atractylodes macrocephala, Cassia Twig. According to the method of pharmacological test, the dosage of Chinese herbal medicine was 10 mg/kg, the concentration ofQijia Wuling soupwas 5 mg/ml, and the daily dose was 2 ml. Keep at 2 °C for later use.

3.1.4 MAJOR reagents and instruments

(1) Reagent

The (-)-noradrenaline, Chloral hydrate, 20% formaldehyde solution, 75% medical alcohol, 100% ethanol, 75% ethanol, 1% ethanol hydrochloride and IODOPHOR disinfectant were purchased from Xi'an Jinrui Chemical Co. , Ltd. . Neutral resin glue, paraffin, hematoxylin and eosin dyes were purchased from Beijing Solebo Technology Co., Ltd. ANGII kit and TNF-α kit were purchased from Shanghai enzyme linked immunosorbent assay (M1002859).

(2) Equipment

Small Animal Heart Imaging Ultrasound system: Beijing Yiren Hengye Technology Co. , Ltd. . 2-8 degree medical refrigerator, medical cryopreservation, oven, precision electronic balance, vortex apparatus, thermostatic water bath, xi'an Friendship Medical Laboratory; embedding machine, automatic tissue dehydrator: Wuhan Junjie company; enzyme marker: ThermoFisher company.

3.2 Method

3.2.1 Reagent preparation

The (-)-noradrenaline and chloral hydrate for anesthesia were mixed with the solvent according to the method in the reagent manual and prepared at the relevant working concentration of 2 °C for storage and reserve.

3.2.2 Preparation of drug-containing serum

20 rats were randomly divided into normal group (n = 10) and traditional Chinese medicine group (n = 10). The Rats in theQijia Wuling soup group were given Qijia Wuling soup granule 1 mL/100g, which was equivalent to 12.1 g crude drug/kg body weight. The normal group was given the same amount of normal saline, once a day for 6 days. The rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital 1 hour after administration in the morning of the seventh day. Blood was taken from the abdominal aorta and left for 2 hours, then centrifuged at a speed of 3000 rpm, the serum was obtained after centrifugation for 10 minutes. The serum was then inactivated in a 56 ° C water bath for 30 minutes. After gastric perfusion, the serum was collected to prepare the drug-containing serum without exosome.

3.2.3 Cell grouping and model preparation

The cells were divided into three groups: control group (normal saline H9C2 cells), model group ((-)-noradrenaline H9C2 cells) and model + traditional Chinese medicine group (H9C2 cells treated with (-)-noradrenaline and drug-containing serum). The rats were cultured in DMEM medium containing 10% drug-containing serum (blank serum) 24 h and 1 h before modeling, respectively, 1 hour before modeling, 3-MA (100 nmol/l Rapa) with final concentration of 5 mmol/L was pretreated, and the constant temperature, constant humidity and hypoxia (95% n 2,5% co 2,1% O 2) were prepared by EVOS desktop cell culture chamber.

3.2.4 The growth and morphological changes of H9C2 cardiomyocytes were observed

(1) Take the cells of logarithmic growth period.

(2) After trypsin digestion, cells were counted and the cell density was adjusted to 5 × 10 ^ 5 cells per well.

(3) The cells were seeded in 6cm2 culture dish.

(4) When the density of the cells reached 70% ~ 80%, the cells were grouped and treated according to the above methods.

(5) After the treatment, the morphological changes of cells were observed under inverted microscope and photographed.

3.2.5 Statistical treatment

After data statistics were completed, GraphPad Prism 8.0.2 software was used for mapping

analysis. In the chart, the data are presented as mean ± standard deviation. For comparisons between groups, one-way analysis of variance (ANOVA-RRB- was used for statistical analysis. If the P value is less than 0.05, the difference is statistically significant.

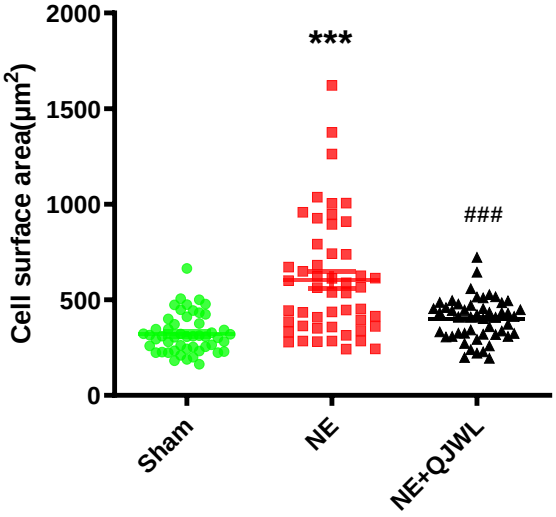
3.3 Results

3.3.1 Determination of serum concentration

The activity of normal H9C2 cardiomyocytes was obviously affected by different amount of serum, and the serum could promote the activity of H9C2 cardiomyocytes. Specifically, in the range of 10% to 30% serum addition, cell activity gradually increased, while in the range of 30% to 50%, cell activity gradually decreased. The cell activity reached the highest level at 30% serum level, but decreased significantly at 40% and 50% serum levels. This suggests that high serum concentrations may have a negative effect on cells. Taking the results into account, 20% serum was determined to be the most suitable condition, because at this concentration the cell activity was highest. This conclusion is of great significance for the design of subsequent experiments and the interpretation of data, and provides important guidance for further research.

3.3.2 Cell surface area was measured

Further analysis showed that the surface area of H9C2 cardiomyocytes increased significantly after NE treatment for 24 hours ($p < 0.05$). However, in the context of Qijia Wuling souppretreatment, the NE-induced increase in cardiomyocyte surface area was observed to be significantly inhibited ($p < 0.05$), a result further supported by figures 1. This finding reinforces the potential role of QiJia Wu Ling Tang in inhibiting NE-induced cardiomyocyte hypertrophy, suggesting that it may have potential cardioprotective effects. This inhibition may be achieved by multiple mechanisms, including inhibition of troponin synthesis or regulation of intracellular calcium levels, but the specific mechanisms still need to be elucidated by further studies.

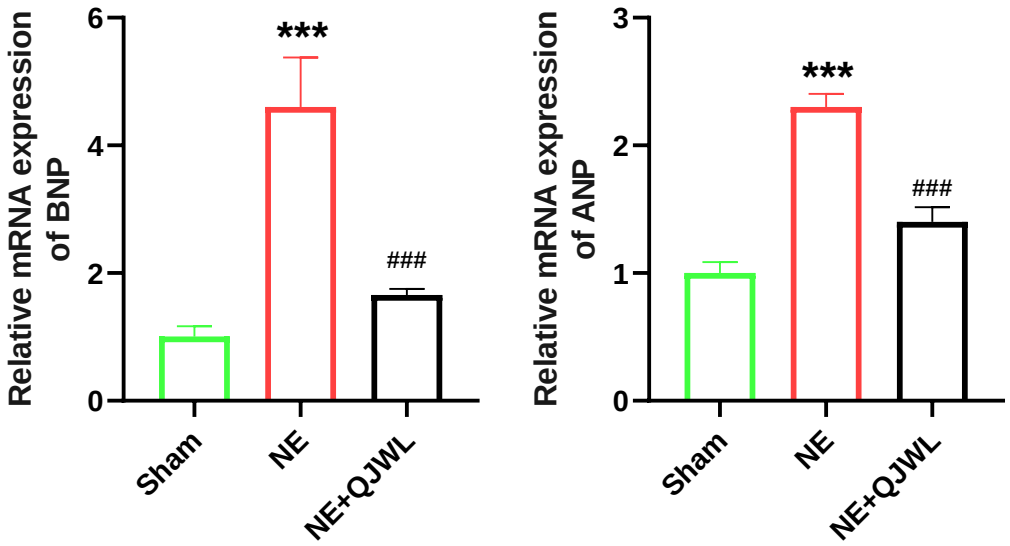


Note: compared with SHAM group, * * * P & lt; 0.001; compared with NE group, # # # P & Lt; 0.001. Note: * * * P & lt; 0.001 versus Sham; # # # P & Lt; 0.001 versus NE.

Figure 1: qjwld inhibited NE-induced increase in cardiomyocyte surface area

3.3.3 Gene expression detection

The expression levels of ANP and BNP mRNA were detected by RT-PCR. The results showed that the expression levels of ANP and BNP mRNA were significantly increased after (-)-noradrenaline intervention (P & Lt; 0.01). However, it should be noted that the Qijia Wuling soup-containing serum-treated group showed significant inhibition, significantly reducing the expression levels of ANP and BNP mRNA (P & Lt; 0.01), see Figure 2.This finding underscores the potential role of Qijia Wuling soup in regulating the expression of ANP and BNP genes, suggesting that it may exert cardioprotective effects by inhibiting the transcription of these genes.



Note: compared with SHAM group, * * * P & lt; 0.001; compared with NE group, # # # P & Lt; 0.001. Note: * * * P & lt; 0.001 versus Sham; # # # P & Lt; 0.001 versus NE.

Figure 2: anp and BNP mRNA levels

4. Summary

Heart failure is a heart disease characterized by an inability of the heart to pump blood efficiently, resulting in hypoxia and malnutrition in all parts of the body. With the increasing prevalence of population ageing and chronic diseases, heart failure has become a major public health problem worldwide. The pathogenesis of heart failure is complex, including cardiac hypertrophy, inflammatory reaction, cardiac remodeling and other factors. Myocardial hypertrophy plays a key role in the development of heart failure. Cardiac hypertrophy refers to the cardiac myocytes under a variety of conditions under the stimulation of cell volume increases, the ability to shrink weakened symptoms. This process is usually caused by damage to the heart and a stress response. Cardiac hypertrophy not only results in the impairment of cardiac systolic and diastolic function, but also increases cardiac load, aggravates cardiac workload and leads to clinical symptoms such as arrhythmia and heart failure. In addition, cardiac hypertrophy also affects the heart's blood supply and metabolism, further aggravating the heart damage and dysfunction. In order to prevent and treat heart failure, it is very important to control and delay the development of cardiac hypertrophy. Studies have shown that Chinese herbal medicine has a certain therapeutic effect in the treatment of heart failure, including Qingxin Jieyu granule. Qijia Wuling soup is a traditional Chinese medicine

compound, which has been shown to have the potential to inhibit cardiac hypertrophy and treat heart failure.

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