Review Article
Hypericin protects against doxorubicin-induced dilated cardiomyopathy in rats via down-regulating expression of Cx43 and inhibiting of TNF-α, ET-1, BNP

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Abstract: Hypericin (Hyp) is a naphthodianthrone monomer extracted from Hypericum. In the present study, we investigated the effects and underlying mechanisms of Hyp on doxorubicin-induced rat dilated cardiomyopathy (DCM). 50 SD rats were randomly divided into normal control group (10 rats), Hyp group (10 rats), DCM group (15 rats) and DCM+Hyp treated group (15 rats). After 8 weeks, serum levels of TNF-α, BNP, plasma ET-1 levels, hemodynamics of rats were measured. HE and Masson staining were performed in left ventricular muscle biopsy, and myocardial connexin 43 were detected by RT-PCR and immunohistochemical techniques. The enhancement of heart weight index and mortality was found in the DCM group, which was significantly attenuated by DCM+ treatment with Hyp. Meanwhile, elevated TNF-α, ET-1, and BNP levels as well as LVEDP reduced and LVSP, $\pm dP/dt_{max}$ increased in haemodynamics were significantly remitted by DCM+Hyp. The cardiac pathological changes in DCM+Hyp treated group were ameliorated. Furthermore, RT-PCR and immunohistochemical data showed that After DCM+Hyp treatment, Cx43 mRNA and protein expression were higher than that of the DCM group ($P<0.01$). These results suggest that Hyp can evidently relieve cardiac damage in rats with DCM, which mechanism may be related to lowered blood TNF-α, ET-1 and BNP levels and enhanced Cx43 expression.

Keywords: Dilated cardiomyopathy, hypericin, hemodynamics, myocardial connexin 43, doxorubicin

Introduction

Dilated cardiomyopathy (DCM) is a kind of left ventricle and (or) right ventricular chamber enlargement with myocardial systolic dysfunction, and it is also one of the major causes of heart failure in addition to coronary heart disease and hypertension. The main clinical manifestations were progressive congestive heart failure (CHF), arrhythmia, thrombosis and sudden death. It has been shown to be associated with profound morphological and histological changes including fibrosis formation and dilated cardiomyopathy, its etiology and pathogenesis is still not very clear, but some factors have been recognized that play an important role in the pathogenesis of DCM, such as virus infection (coxsackie virus infection), infection immune mechanism and genetic factors [1]. Over the past ten years, progress has been made in

treating DCM, but improving outcome remains a difficult goal to achieve. At present, there is still a lack of effective and specific treatment for DCM, which is still mainly aimed at heart failure and the drugs were used in the Department of Iinternal medicine [2]. Traditional Chinese medicine application is increasingly applied for this disease [3].

A number of basic and clinical studies have showed that ET-1 deteriorated heart function by promoting blood vessel contraction, cardiac hypertrophy and myocardial cell damage and so on. BNP concentration of plasma is a very important biochemical marker for clinical diagnosis of CHF. Plasma BNP levels for diagnosis and prognosis of CHF has an important value, Serum TNF-level is closely related to the degree of CHF and clinical characteristics, and can be used as an important indicator of the severity of the disease [2, 4].

Gap junctions of cells are the sole electrical connection among the myocardial cells. The exchange of information and energy is performed via a gap junction protein connexin 43 (Cx43), which plays an important role in cell metabolism, homeostasis, proliferation, differentiation and other physiological processes [5].

Hyp is a naphthodianthrone monomer extracted from Hypericum (Hypericum perforatum L. HP), and has anti-depression, anti-bacterial, anti-viral anti-tumor effects. In Germany, it has been used for antidepressant treatment in AIDS clinically [6]. Recent studies have shown that Hyp is against many viruses (DNA or RNA viruses), including HIV (HIV), herpes virus, stomatitis virus, parainfluenza virus, vaccinia virus, hepatitis virus, and avian influenza viruses, particularly effective for retroviral RNA viruses such as the AIDS virus, parainfluenza virus, avian influenza virus and etc., and the role of the mechanism may be related to inhibition of reverse transcriptase. We found that Hyp has significant anti-CVB3 replication effects and protects myocardial cells, which are mediated by affecting the production of interferon and tumor necrosis factor in VMC mice, inhibiting the myocyte apoptosis in chronic phase of VMC, and decreasing Fas/FasL protein expression [7]. Our previous study has used pig cardiac myosin to immunize BALB/C mice to establish autoimmune myocarditis model and observed the effect of Hyp in acute autoimmune myocarditis. The results showed that Hyp had a therapeutic effect, not only could reduce the extent of acute autoimmune myocarditis in mice, but also could relieve myocardial immune injury, whose mechanisms were related to suppression of perforin/granzyme B pathway, and improvement of the body antioxidant capacity (to be published data). Based on this knowledge, we set up a Wistar DCM rat model induced by intraperitoneal injection of doxorubicin hydrochloride to investigate the therapeutic effect and mechanisms of Hyp.

Experimental

Medicines, reagents and instruments

Doxorubicin hydrochloride for injection was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd.; batch number was 20120101. Hyp was purchased from Sigma. It was prepared as 1.5% solution in DMSO, autoclaved, and stored at 4°C refrigerator. Serum TNF-α, ET-1 radioimmunoassay kits were purchased from Beijing North Institute of Biotechnology. Plasma BNP (ELISA) kit was purchased from Shanghai Jin chemical Co. Trizol was purchased from Ambion Company. Reverse transcription kit and SYBR Premix PCR kit were purchased from TaKaRa Company. Rabbit anti-Cx43 antibody was purchased from Cell Signaling Technology Company. PCR primers were synthesized by Shanghai Ying Jie Wei biotechnology Co., Ltd.. Vertical electrophoresis was purchased from USA Biorad Company. Pathology slicer was Germany Leica RM2135. Photomicrography and image analysis system were German Leica QWIN3. SN-697 automatic dual probe 7 was used as the radioimmunoassay counter and purchased from Shanghai Institute of Nuclear Research on the Environment and Instrument Plant.

Preparation and treatment of animals

50 healthy, weighting 200 g~215 g wistar male rats were purchased on the West Coast Poole-Rubicam animal company of Shanghai in China (qualified No. 2008001617932). The rearing conditions were: temperature: (25±2)°C, humidity: 55% to 60%, day and night illumination time: 8:00 opening fluorescent, 17:00 off fluorescent; sub cages. The rats were randomly divided into normal group (n=10), Hyp group (n=10), DCM model group (n=15) and DCM+Hyp treated group (n=15). DCM group and DCM+Hyp group received ip doxorubicin 2.0 mg/kg, once a week for eight weeks. Hyp group and DCM+Hyp treated group also received Hyp ip 40 mg/kg once a day, while the Con group and the DCM group were given saline. During the experiment, rats were observed regularly. After 8 weeks, animals were sacrificed, body weight and heart weight were measured, and heart weight index was calculated.

Serum TNF-α, BNP and plasma ET-1 determination

3 ml blood from rat femoral artery was collected without anticoagulant and shaking. After it stayed at 4°C for 10 minutes, it was centrifuged at 300 rpm for 5 minutes. The supernatant was collected and stored at -20°C for future serum TNF-α and BNP level detection. Another 3 ml blood was obtained from femoral artery and

collected in collection tube containing heparin anticoagulant. The plasma level of ET-1 was determined by kits.

Hemodynamic examination

The rats were fasted for 12 h before the end of the experiment. 20% urethane was used to anesthetize rats (0.6 ml/kg). Then animals were fixed on the mounting plate. Neck hair was removed after disinfection with iodine surgical site. An incision was made vertically to neck-right ventral axis on the rat's skin, and the right carotid artery at the inner side of sternocleidomastoid muscle was separated for about 2 cm. The distal end of the artery was ligated and the proximal end was inserted with a ventricular cannula filled with heparin saline [21]. Blood pressure waveform changes were observed. When the lower edge of the blood pressure waveform reached 0 mmHg with obvious diastolic period and the top edge of the diagram had a clear flat waveform, it indicated that the catheter has entered the left ventricular cavity via aorta. The catheter was further inserted for about 0.2 to 0.3 cm, and the pressure-volume curve was recorded. Hemodynamic indicators for left ventricular systolic and diastolic function were recorded: maximum speed of left ventricular pressure rise ($+dp/dt_{max}$), maximum speed of left ventricular pressure drop ($-dp/dt_{max}$), left ventricular end diastolic pressure (LVEDP) and left ventricular stress peak (LVSP).

Histochemistry staining

At the end of the experiment, the rats were sacrificed and the apical portion of the left ventricle was fixed in 10% formalin (pH=10) for HE and Masson staining.

Immunohistochemistry of Cx43

Left ventricular muscle biopsy was dewaxed, incubated with primary antibody and hematoxylin, serial dehydrated by ethanol, incubated in xylene, mounted by mounting medium, air dried and observed. Using 800 × optical microscope, 5 areas were randomly selected for each slice and 6 slices were chosen in each group (n=6). Image Pro Plus (IPP) 6.0 software was used to determine Cx43 protein expression levels according to the dye darkness and the positive area. AOI tool was used to selecte the desired area, "count/size" tool was used to analyze the selected area.

RT-PCR assay of Cx43

Total RNA was extracted from left ventricle using Trizol one step method. OD 260/OD280 value was detected by the minim spectrophotometer. The primers for Cx43 and internal reference β-actin were designed and synthesized. Cx43 primers: F, 5'-GAAGCACCATCTCCAACTC-GC-3'; R, 5'-CGTTGGTCCACGATGGCTAAT-3'; total length is 115 bp. β-actin primers: F, 5'-GA-GGGAAATCGTGCGTGAC-3'; R, 5'-CATTGCCGAT-AGTGATGACCT-3'; total length is 143 bp. Amplification conditions: (1) denaturation at 95°C, 4 min (2) TS at 95°C, 10 s (3) annealing extension at 56°C, 20 s, a total of 40 cycles.

Statistical analysis

All data were expressed as mean ± SD., using SPSS 17.0 statistical software for data analysis and processing. Single factor analysis of variance was used among group comparison. LSD method was used between two groups, $P<0.05$ was considered statistically significant.

Results

General physiological conditions, weight and heart weight index

Throughout the experiment, there was no death in the control (Con) group and Hyp group. DCM group had symptoms such as lethargy, appetite decreasing, less activity, diarrhea, ascites, and etc. At eight weeks, six rats were dead in DCM group and the mortality rate was 40%. In contrast, Hyp-treated group had two deaths, a mortality rate of 13.3%. Also, Hyp-treated rats had less symptoms of poisoning, and had peritoneal irritation, such as ascites.

Compared with Con group, Hyp group had no obviously changed, DCM rats had decreased body weight and heart weight and increased heart weight index ($P<0.01$). After Hyp treatment, heart weight and heart weight index reduced ($P<0.01$) as shown in **Table 1**.

Hemodynamics

At 8 weeks, compared with the Con group, Hyp group had no obviously changed; LVEDP of DCM group increased ($P<0.05$); LVSP, $+dp/dt_{max}$

Table 1. The changes of body weights, heart weighs and heart weigh indexes of rats (mean ± SD)

Group	n	Body weight/g	heart weigh/mg	heart weight index/(mg/g)
Con	10	283.9±15.6	546.4±44.5	1.9±0.2
Hyp	10	273.4±16.2	541.9±48.2	1.9±0.1
DCM	9	217.0±13.5*	601.6±39.7*	2.8±0.3*
Hyp+DCM	13	257.1±26.4#	519.2±56.0#	2.3±0.2#

Heart weight index = heart weight/body weight; compared with normal control group, *$P<0.01$; compared with the DCM group, #$P<0.01$.

and -dp/dt$_{max}$ were lower ($P<0.05$). LVEDP of Hyp treatment group was lower ($P<0.05$) compared with DCM group LVSP increased as well as +dp/dt$_{max}$ and -dp/dt$_{max}$ ($P<0.05$). The results showed that Hyp effectively improved blood flow kinetics in DCM rats as shown in **Table 2**.

Determination of serum and plasma cytokines

DCM group had higher TNF-α, ET-1 and BNP: 22.61±3.31 fmol/ml, 74.22±4.16 pg/ml and 47.26±2.82 pg/ml respectively, compared with the Con group: 0, (40.13±3.48) pg/ml and (16.22±2.14) pg/ml (all $P<0.05$) respectively. DCM+Hyp treated group had lower TNF-α, ET-1 and BNP: 10.14±1.23 fmol/ml, 51.45±2.33 pg/ml and 29.43±2.35 pg/ml respectively compared with the DCM group ($P<0.05$ mean ± S.D., n=9-10). Hyp group had no obviously changed for comparison with the Con group.

Pathology

DCM rats had partial myocardial fiber rupture, widened myocardial cell gap with vacuolar degeneration, mild inflammatory cell infiltration, and hypertrophy in myocardium. Hyp treated rats had orderly arranged myocardial fibers, normal and uniform cell gap, and only few vacuoles. The action of Hyp rats was similar to that of the Con group as shown in **Figures 1** and **2**.

DCM had disordered myocardial fiber and higher interstitial collagen deposition compared with control group and the Hyp treated group, indicating myocardial fibrosis in rats with DCM. The action of Hyp rats was similar to that of the Con group.

Immunohistochemical staining of Cx43 protein

Immunohistochemistry showed that lots of Cx43 (brown) expression in Con group, and most of it located in the myocardial fibers per-

pendicular to the long axis of the end-end connections. Cx43 in DCM group was lower than that in the Con group and there was a side-side connection ($P<0.01$), and sparse distribution. Cx43 expression was significantly increased in Hyp treated group had darker staining of Cx43 compared with DCM group ($P<0.05$), and more orderly distribution, as shown in **Figure 3**: Nine-ten semi-quantitative results are shown in **Table 3**.

Analysis of Cx43 gene expression using semiquantitative RT-PCR

Figure 4 shows that DCM group had lower Cx43 mRNA expression compared with Con ($P<0.01$); in the Hyp treated group Cx43 mRNA expression increased compared with the DCM group ($P<0.01$).

Discussion

Doxorubicin may reduce endogenous myocardial intracellular antioxidants (including SOD, catalase [CAT] and etc.) thereby causing extensive lipid peroxidation, mitochondrial damage, and mitochondrial DNA mutation, ultimately leading to myocardial cell degeneration, necrosis, interstitial changes, and severe myocardial systolic and diastolic dysfunction. In the current study, these effects were utilized to establish DCM model [8].

Hemodynamics is the study of blood flow dynamics in the cardiovascular system. Its basic object is to study the relationship between flow resistance and pressure and commonly used to study drug effects and mechanisms on cardiovascular system. Currently used hemodynamic parameters are: blood pressure (BP), heart rate (HR), left ventricular pressure (LVSP), left ventricular isovolumic maximum rate of pressure change (±dp/dt$_{max}$), left ventricular begins to shrink to left ventricular pressure rising peak rate time, left ventricular end-diastolic pressure (LVEDP), T values (time constant of left ventricular isovolumic relaxation), and etc [9].

LVSP and +dp/dt$_{max}$ are indicators of systolic function. LVSP displays the left ventricular systolic pressure change; elevated preload or afterload or strengthened myocardial contractility leads to increased LVSP. +dp/dt$_{max}$ is the maximum rate of left ventricular isovolumic pressure rise, and it also represents the increasing tension rate in muscle wall. +dp/dt$_{max}$ issensitive to various inotropic effects,

Table 2. Changes of hemodynamics indexes (mean ± SD)

Group	n	LVSP/mmHg	LVEDP/mmHg	+dp/dt$_{max}$/(mmHg/s)	-dp/dt$_{max}$ (mmHg/s)
Con	10	126.5±5.2	5.7±0.3	5 989.8±214.5	5 410.9±103.8
Hyp	10	129.2±3.7	6.1±0.4	6098.2±201.3	5 489.3±112.4
DCM	9	108.8±3.9[*]	18.2±0.5[*]	3 306.3±191.6[*]	2 576.4±142.5[*]
DCM+Hyp	10	137.3±3.5[*,#]	3.6±0.3[*,#]	4 874.3±146.1[*,#]	3 780.4±305.6[*,#]

Compared with normal control group, [*]$P<0.01$; compared with DCMgroup, [#]$P<0.01$.
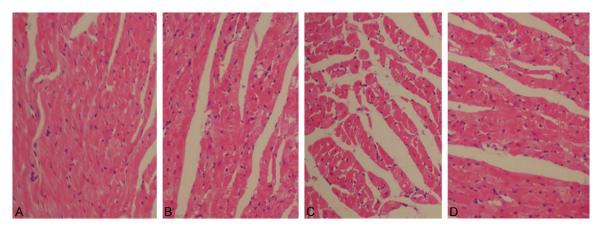

Figure 1. The histology of cardiac muscle of rat. A. Control group; B. Hyp 40 mg/k group; C. DCM model group; D. DCM+Hyp 40 mg/k treated group; Hyp: hypericin. (mean ± SD, n=9-10, HE staining ×200).
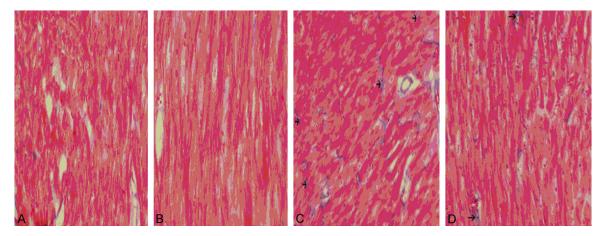

Figure 2. The histology of cardiac muscle of rat. A. Control group; B. Hyp 40 mg/k group; C. DCM model group; D. DCM+Hyp 40 mg/k treated group; Arrow means fibrosis; Hyp: hypericin (mean ± SD. n=9-10, Masson staining ×200).

and to a certain extent is affected by and positively correlated withthe heart rate, preload and afterload. When +dp/dt$_{max}$ is increased or unchanged with unchanged or lowered heart rate, preload and afterload, it indicates enhanced cardiac contractility [9]. When +dp/dt$_{max}$ is increased with increased LVSP, it also indicates enhanced myocardial contractility.

The maximum rate of left ventricular pressure drop -dp/dt$_{max}$ is a sensitive indicator of early diastolic myocardial function changes. It represents the degree of left ventricular filling, diastolic function and compliance. It is affected by LVSP, afterload and heart rate. LVEDP represents left ventricular preload. It changes with the ventricle volume, diastolic function and
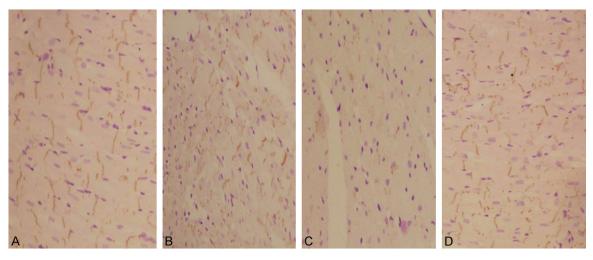
Figure 3. Immunohistochemistry in the myocardium of left ventricle. A. Control group; B. Hyp 40 mg/k group; C. DCM model group; D. DCM+Hyp 40 mg/k treated group. Brown indicate Cx43 protein, Hyp: hypericin (mean ± SD, n=9-10, Masson staining ×200).
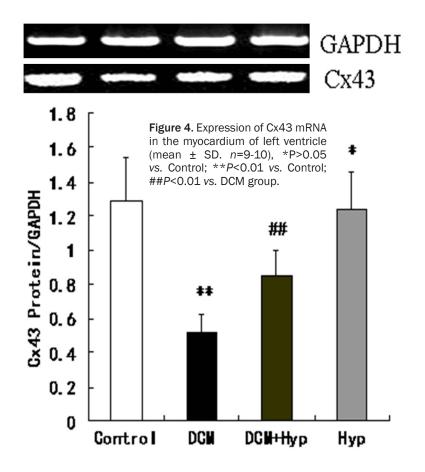
Table 3. Cx43 protein expression in the myocardium of left ventricle (mean ± SD, n=9-10)

Groups	OD	Area
Con	0.5871±0.034	2.64±0.25
Hyp	0.5921±0.043	2.57±0.30
DCM	0.112±0.023**	0.97±0.13**
DCM+Hyp+	0.4103±0.212#	1.86±0.16#

**$P<0.01$ vs. Control; #$P<0.05$ vs. DCM group.

ventricular compliance. It also indirectly reflects left ventricular diastolic function. LVEDP may be elevated when there is a left ventricular diastolic dysfunction or increased venous blood return. Similarly, it is decreased when there is increased left ventricle shrinks or reduced venous blood return. Reduced -dp/dt$_{max}$ and LVEDP reflect worsened ventricular diastolic performance [10]. Our experimental results showed that compared with normal Con group, DCM group had reduced LVSP and ±dp/dt$_{max}$ and elevated LVEDP ($P<0.01$), in line with their hemodynamics. Also, the heart weight, heart weight index, and mortality increased in DCM group compared with Con group ($P<0.05$), which were also consistent with the hemodynamics. After Hyp treatment, heart weight and heart weight index reduced, LVSP increased, ±dp/dt$_{max}$ increased, LVEDP decreased compared with DCM group ($P<0.01$), indicating Hyp treatment has improved rat cardiac function in DCM.

Many studies have shown that in heart failure, large number of biologically active substances change in the plasma, among which endothelin-1 and B-type natriuretic peptide (BNP) receive a lot of attention [11, 12]. BNP is a peptide hormone composed of 32 amino acids, mainly secreted by the ventricles. Its functions include natriuresis, diuresis, dilation of blood vessels, and inhibiting renin-aldosterone system-angiotensin. The change in pressure and cardiac ventricular wall tension caused by increased load during cardiac dysfunction is the most effective factor stimulating BNP release. Thus, plasma BNP concentration is positively correlated with and often used as the indicator for the degree of heart failure [4, 12]. ET-1 is one of the major bioactive substances of endothelin. It is mainly secreted by vascular endothelium, endocardium, myocardium cells, smooth muscle cells and fibroblasts. ET-1 mainly functions on the heart and blood vessels, as well as activates the renin-angiotensin-aldosterone system and the sympathetic nervous system. It exerts its functions through the cell membrane ET-1 receptors. Currently the role of ET-1 on CHF is not clear because the results of in vivo and in vivo are not completely consistent. Kakita [13] and other in vitro studies have shown that ET-1 can inhibit the oxidation- and isoproterenol-induced myocardial apoptosis, suggesting that exogenous ET-1 has a protective role in CHF; however, Ishikawa [14] and other in vivo studies concluded that in-

Figure 4. Expression of Cx43 mRNA in the myocardium of left ventricle (mean ± SD. *n*=9-10), *P>0.05 *vs.* Control; **P<0.01 *vs.* Control; ##P<0.01 *vs.* DCM group.

and deterioration of heart function. Hyp decreased TNF-α level, BNP and improved heart function, suggesting TNF-α may be associated with cardiac function. We speculate that TNF-α induced iNOS mRNA expression and myocardial NO levels, leading to imbalance of ET-1 and NO and accelerated heart failure process.

We observed that in DCM, $+dp/dt_{max}$ and $-dp/dt_{max}$ were lower compared with the control group, while BNP expression in rat plasma was higher compared with the Con group. This result is consistent with previous report demonstrating that BNP synthesis is associated with ventricular volume overload, expansion and pressure. Hyp improved cardiac function, hemodynamics, and reduced plasma BNP levels which indicates the role of Hyp in heart function and ventricular remodeling improvement. In addition, ET-1 increased in blood from DCM group and it decreased in Hyp group. Cx43 is the most important ventricular gap junction proteins, mainly located in the intercalated disc. It participates and influences cardiac electrophysiology, including excited electricity conduction velocity, myocardial electrical coupling, and etc. It plays an important role in maintaining a stable environment, cell proliferation, differentiation, and metabolism. Changes of Cx43 in the structure or the number can cause increased channel resistance, vertical conduction delay, severe declined conduction velocity, and significant changes conduction in all directions. These changes will result in conduction block and reentry, inducing arrhythmias [16]. When DCM rats developed heart failure, reduced expression and abnormal distribution of Cx43 caused its remodeling, resulting in uncoupling potential, prolonged ventricular myocyte repolarization, and arrhythmia [17].

creased ET-1 levels are associated with increased extent of hemodynamic abnormalities and myocardial apoptosis in CHF.

It is believed that TNF-α may affect the heart in the following aspects. First, it increases inducible NO synthase (iNOS) mRNA expression in macrophages, endothelial cells and cardiac myocytes, promotes the production of NO within the myocardium, inhibits cardiac contraction. Second, it promotes troponin uncoupling and-degradation, reduces myocardial contractility; affects myocardial energy metabolism and membrane ion channel function. Third, it induces cardiac hypertrophy, promotes myocardial necrosis, and the mechanisms may be associated with TNF-α-induced free oxygen radicals. Forth, it changes myocardial extracellular matrix by activating matrix metalloproteinases and inhibiting of matrix metalloproteinase inhibitors, resulting in ventricular dilatation, heart failure, and fibroblast proliferation. Fifth, it stimulates the secretion of ET-1 and deteriorates the CHF [15]. The results of our experiment showed that, compared with the Con group, DCM group had increased serum TNF-α

Studies have shown that the renin-angiotensin-aldosterone system (RACC) is involved in myocardial remodeling due to that angiotensin and

aldosterone can stimulate collagen synthesis in cardiac muscle cells, and this induced myocardial fibrosis is independent of the hemodynamic changes [18].

In pathological processes, Ang II increases PKC activity, induces phosphorylation of Cx43, accelerates Cx43 degradation, leading to increased susceptibility to arrhythmias. PKA activation can induce phosphorylation of Cx43, and its expression [19], however, the exact mechanism is not yet clear. Our study found a significant reduction in mRNA and protein expression of Cx43 in DCM rats Cx43. In early heart failure, elevated concentrations of norepinephrine stimulate β receptor activation. However, long-term sympathetic nerve stimulation causes noradrenaline (NA) accumulation including catecholamines accumulation. With the rise in NA concentration, β receptor is downregulated, receptor uncoupling appears, and β receptor desensitization causes reduced Cx43 expression [20].

DCM causes increased cardiac preload and afterload. If not it is promptly treated it can lead to difficulties in myocardial energy generation and usage, myocardial fibrosis and future ventricular remodeling. Decreased expression and abnormal distribution of Cx43 can be considered as cardiac remodeling on the molecular level, and it ultimately leads to death due to irreversible heart failure. Our experimental results show that the Hyp treatment reduced mortality in DCM rats, decreased plasma AngII, induced the expression of Cx43 mRNA and protein in myocardial tissues, which may help alleviate DCM.

In summary, prophylactic treatment of Hyp alleviated myocardial histological changes, improved heart function and reduced mortality in rats. The mechanisms may be associated with lowered blood TNF-α, ET-1 and BNP levels, increased Cx43 expression and reversed connection disorders.

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Disclosure of conflict of interest

None.

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References

[1] Sanbe A. Dilated cardiomyopathy: a disease of the myocardium. Biol Pharm Bull 2013; 36: 18-22.

[2] Towbin JA, Lowe AM, Colan SD, Sleeper LA, Orav EJ, Clunie S, Messere J, Cox GF, Lurie PR, Hsu D, Canter C, Wilkinson JD, Lipshultz SE. Incidence, causes, and outcomes of dilated cardiomyopathy in children. JAMA 2006; 296: 1867-1876.

[3] Li L, Zhen Y, Li SY. Clinical experience in treating dilated cardiomyopathy. JETCM 2012; 21: 1879-1881.

[4] Bielecka-Dabrowa A, von Haehling S, Aronow WS, Ahmed MI, Rysz J, Banach M. Heart failure biomarkers in patients with dilated cardiomyopathy. Internati J Cardiol 2013; 168: 2404-2410.

[5] Jeyaraman MM, Srisakuldee W, Nickel BE, Kardami E. Connexin43 phosphorylation and cytoprotection in the heart. Biochimica Biophysica Acta 2012; 1818: 2009-2013.

[6] Gulick RM, McAuliffe V, Holden-Wiltse J, Crumpacker C, Liebes L, Stein DS, Meehan P, Hussey S, Forcht J, Valentine FT. Phase I studies of hypericin, the active compound in St. John's wort, as an antiretroviral agent in HIV infected adults: AIDS clinical trials group protocols 150 and 258. Ann Intern Med 1999; 130: 510-514.

[7] Li L, Qi MY and Ding BX. Inhibitory Effect of Hypericin on Apoptosis and Fas/FasL Protein Expressions of Myocardium in Mice with Chronic Coxsackievirus B$_3$ Myocarditis. World Sci-Tech R & D 2009; 31: 366-369.

[8] Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. Cardiology 2010; 115: 155-162.

[9] Li L, Sun XD, Gao XL, Ding BX, Yuan BX. Protective effect of total flavonoids of Gynostemma pentaphyllum in dogs with acute myocardial ischemia. Chin J Pathophysiol 2008; 24: 388-391.

[10] Peng FL, Guo YJ, Mo WB, Xu SM, Liao HP. Cardioprotective effects mitochondrial ATP-sensitive potassium channel in exercise conditioning. Genet Mol Res 2014; 13: 7503-7512.

[11] Gombos T, Förhécz Z, Pozsonyi Z, Wallentin S, Papassotiriou J, Kunde J, Morgenthaler NG, Jánoskuti L, Prohászka Z. Adrenomedullin and endothelin-1 are related to inflammation in chronic heart failure. Inflamm Res 2009; 58: 298-305.

[12] Sherbuk JE, Okamoto EE, Marks MA, Fortuny E, Clark EH, Galdos-Cardenas G, Vasquez-Villar A, Fernandez AB, Crawford TC, Do RQ, Flores-Franco JL, Colanzi R, Gilman RH, Bern C. Biomarkers and Mortality in Severe Chagas Cardiomyopathy. Glob Heart 2015; 10: 173-180.

[13] Kakita T, Hasegawa K, Iwai-Kanai E, Adachi S, Morimoto T, Wada H, Kawamura T, Yanazume T, Sasayama S. Calcineurin pathway is required for endothelin-1-mediated protection against oxidant stress-induced apoptosisin cardiaoc-myocytes. Circ Res 2001; 88: 1239-1246.

[14] Engel D, Peshock R, Armstong RC, Sivasubramanian N, Mann DL. Cardiac myo-cyte apoptosis provokes adverse cardiac re-modeling in transgenicmice with targeted TNF overexpression. Am J Physiol 2004; 287: H1303-1311.

[15] Clark IA. "How TNF was recognized as a key mechanism of disease". Cytokine Growth Factor Rev 2007; 18: 335-343.

[16] Dou GH, He WL. Cx43 connexin and heart development. Heart J 2011; 23: 260-264.

[17] Van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, Cox MG, van Lochem LT, de Boer RA, Hofstra RM, Christiaans I, van Spaendonck-Zwarts KY, Lekanne dit Deprez RH, Judge DP, Calkins H, Suurmeijer AJ, Hauer RN, Saffitz JE, Wilde AA, van den Berg MP, van Tintelen JP. Phospholamban R14del mutation inpatients diagnosed with dilated cardiomyopathy or ar-rhythmogenic right ventricular cardiomyopa-thy. Evidence supporting the concept of ar-rhythmogenic cardiomyopathy. Eur J Heart Fail 2012; 14: 1199-1207.

[18] Van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, Cox MG, van Lochem LT, de Boer RA, Hofstra RM, Christiaans I, van Spaendonck-Zwarts KY, Lekanne dDe Maria R, Landolina M, Gasparini M, Schmitz B, Campolo J, Parolini M, Sanzo A, Galimberti P, Bianchi M, Brand SM, Parodi O, Lunati M. Genetic Variants of the Renin-Angiotensin-Aldosterone System and Reverse Remodeling After Cardiac Resynchronization. Therapy J Cardiac Fail 2012; 18: 762-768.

[19] Miura T, Yano T, Naitoh K, Nishihara M, Miki T, Tanno M, Shimamoto K. Delta-opioid receptor activation before ischemia reduces gap junc-tion permeability in ischemic myocardium by PKC-epsilon-mediated phosphorylation of con-nexin 43. Am J Physiol 2007; 293: 1425-1431.

[20] Boengler K. Stimulation of cardiac beta-adre-noceptors targets connexin 43. Br J Pharmacol 2009; 158: 195-197.

[21] Xu SY, Bian RL, Chen X. Pharmacological ex-periments methodology. Version 2 Beijing: People's Health Publishing House; 1992. pp. 854-856.