

Cardiac contractility modulation regulates the structural and electrical remodeling in chronic heart failure rabbit model

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Abstract

Background Chronic heart failure (CHF) is accompanied by complex changes in cardiac electrophysiology and functional properties of cardiomyocytes which cause the structural and electrical remodeling process. Cardiac contractility modulation (CCM) is a novel therapeutic method and has proven to be effective in improving cardiac function. This study evaluated the effects of CCM on structural and electrical remodeling in a rabbit model of CHF. **Methods** Thirty rabbits were randomly divided into sham group, heart failure group and CCM group. CHF model was induced 12 weeks after trans aortic constriction by pressure unloading. Then cardiac contractility modulation was delivered to the myocardium lasting six hours per day for 4 weeks. Structural changes were assessed by hydroxyproline assay and picosirius red staining. The QTc intervals, ventricular effective refractory period and the inducibility of ventricular tachycardia were measured by electrophysiological examination. Protein levels of CTGF, Gal-3, Kv4.3, KCNQ1, KCNH2 and CX43 were measured by western blot analysis. **Results** Our study revealed that CHF rabbits developed significant prolonged QTc, ventricular effective refractory period and increased inducibility of ventricular tachycardia. Prominent myocardial fibrosis and increased levels of hydroxyproline content were observed in the heart failure group. Changes mentioned above can be suppressed with CCM therapy in CHF rabbits. The protein levels of CTGF, Gal-3, Kv4.3, KCNQ1, KCNH2 and CX43 significantly increased in the heart failure group, but these changes were prevented in the CCM group. **Conclusions** The present study demonstrated that CCM treatment prevented myocardial structural and electrical remodeling in a rabbit model of CHF. The beneficial effect of CCM may be related to prevention of downregulation of the CTGF, Gal-3, Kv4.3, KCNQ1, KCNH2 and CX43. These findings provide experimental evidence for the clinical use of CCM in the treatment of HF.

Background

Chronic heart failure (CHF) is a common cardiovascular disease which have a continuously increasing prevalence. Although there has been remarkable progress in the treatment of CHF, morbidity and mortality are still substantial[1]. There is strong support that much of the relationship between a poor prognosis and high mortality in CHF is related to the increased susceptibility to lethal ventricular arrhythmias. The development of heart failure is accompanied by complex changes in cardiac electrophysiology and functional properties of cardiomyocytes which cause the structural and electrical remodeling process of ventricular[2].

Cardiac contractility modulation (CCM) is a device-based therapy for heart failure (HF) that involves applying relatively high-voltage, long-duration, biphasic electric signals to the myocardial during the absolute myocardial refractory period. Evidence from animal models and patients with CHF demonstrate that CCM therapy has the potential to have beneficial effects in HF via processes involved in Ca²⁺ handling, the cytoskeleton, the extracellular matrix, and potentially the autonomous nervous system[3, 4]. Although experimental animal studies and human clinical trials have reported CCM could improve cardiac function and remodeling by echocardiography and histological assessment, the link between

CCM and arrhythmic complications and the effect of CCM on electrical remodeling coincide with structural remodeling are rarely investigated[5-8].

It has been demonstrated that connective tissue growth factor (CTGF) and galectin-3 (Gal-3) are novel pro-fibrotic markers. Those bio-makers are involved in physiological conditions, such as promoting fibroblast proliferation and collagen deposition, thus lead to myocardial structural remodeling[9, 10]. In addition to alterations in Connexin43 (Cx43) expression, changes in potassium currents are important contributors to myocardial electrical remodeling[11, 12]. These pathologies are associated with the increasing propensity for arrhythmias.

We therefore identify the effect of CCM on structural and electrical remodeling in heart failure rabbit models by histological and electrophysiological study. Furthermore, the protein level of CTGF, Gal-3, Kv4.3 (the subunit of the Ito channel), KCNH2 (the subunit of the Ikr channel), KCNQ1 (the subunit of the Iks channel) and Cx43 were measured to investigate the underlie mechanism.

Methods

Animals

Our study chose rabbit as experimental subject because the rabbit heart shares some physiological commonalities with the human heart. Thirty healthy New Zealand white rabbits (weight 2.5-3.5Kg), were provided by the Experimental Animal Center of the Hebei Medical University. Those animals were randomly divided into three groups; sham operation group (sham group), heart failure group (HF group), and CCM group (CCM group), with n=10 for each group. Rabbits only receive thoracotomy in the sham operation group. Thoracotomy and ascending aortic cerclage were performed in the heart failure group. In the CCM group, rabbits underwent thoracotomy, ascending aortic cerclage and receive 4 weeks CCM after the formation of chronic heart failure.

Induction of heart failure

The procedure of trans aortic constriction for the creation of the HF model has been established and described in our previous study. Briefly, all animals were anesthetized with 3% sodium pentobarbital (30 mg/kg) via the ear venous. The thoracic cavity was opened. After the ascending aorta was dissected, it was occluded to make a constriction to 60% of the original circumference. A pediatric temporary pacing lead (Medtronic 6491) was used to deliver CCM was sutured to left ventricular anterior wall and the other end of the electrode was fixed to the neck for later use. After 12 weeks, left ventricular ejection fraction \leq 40% meant that the heart failure model had been successfully established.

CCM protocols

EPS320 Cardiac Stimulator (BARD Micro Pace, Inc, USA) was used to deliver CCM stimulation to the absolute refractory period of heart by sensed R-wave. As detailed previously, the signals consisted of a biphasic square-wave pulses with phase duration=2 ms, stimulus amplitude=7 V, and 30 ms delay after

R-wave sensing[13, 14]. The CCM signals was conducted 6 hours per day for consecutive 4 weeks (Fig. 1A).

Electrocardiography

Needle electrodes were placed subcutaneously in the four limbs for surface ECG recordings as described previously[15]. The lead II of ECG was selected of further analysis. QT interval mainly focus on the repolarization of the action potential of the heart. Currently, the beat-to-beat instability of the QT interval is viewed as ECG biomarker that detect cardiac arrhythmias, mainly focus on the repolarization of the action potential of the heart. The QT interval was defined as the duration from the beginning of the Q wave to the end of T wave. In addition, heart rate-corrected QTc calculation was obtained by using the Carlson's formula ($QTc = QT - 0.175(RR - 300)$)[16].

Electrophysiology study

Programmed electrical stimulation protocols was performed by EPS320 Cardiac Stimulator (BARD Micro Pace, Inc, USA) to assess the electrophysiological characteristic. The effective refractory prior (ERP) was measured (2 times the diastolic threshold, 2-milliseconds pulse width) at basic cycle lengths of 250 milliseconds with a train of 8 basic (S1) stimuli followed by a premature (S2) stimulus with 2-milliseconds decrements(Fig. 1B). The longest S1S2 interval that failed to produce a propagated ventricular response was taken as the ERP[17]. ventricular tachycardia (VT) inducibility was assessed with 8 beat drive trains(S1) at 200 milliseconds, followed by 1-3 ventricular extra-stimuli. Single (S2), or double (S2-S3) premature stimuli were applied with a coupling interval of 160 milliseconds, progressively shortened in 2-milliseconds steps until VT induction or until ventricular effective refractory period was reached (Fig. 1C). VT was defined as 8 consecutive ventricular beats at a cycle length ≤ 150 ms[18].

Histological observation

At the end of the study, the rabbits were sacrificed by exsanguination under anesthesia. The heart from anesthetized rabbits was rapidly excised, and fixed in 4% paraformaldehyde solution. The heart was sectioned transversely across myocardial papillary muscle, and then embedded with paraffin. Myocardial tissue sections (5 μ m) were cut from the paraffin blocks and stained with picrosirius red liquid dye for 60 min. Hematoxylin was used as a nuclear counterstain for 10 min. The stained sections were observed under the polarized light which could distinguish type I from type III collagen by different colors. Yellow and red color indicated type I collagen, and green color indicated collagen III. Digital photographs were taken at 400 \times magnification for 10 random fields from each section, and percentages of CVF (collagen volume fraction = collagen area/total area $\times 100\%$) was detected by Image-ProPlus5.1 (Media Cybernetics). Similarly, [type I and III collagen](#) was also observed under a [polarization](#) microscope.

Hydroxyproline Assay

Myocardial hydroxyproline content was analyzed using a commercially available kit (Jiancheng Bioengineering Institute, Nanjing, China) according to manufacturer's instructions. Briefly, the myocardial

tissue (30–100 mg) was digested at 95 °C for 20 min in an acidic buffer solution. After the hydrolysis, the samples were centrifuged at 3500 rpm for 10 min. Absorbance of the final solution was determined using a microplate spectrophotometer at 550 nm, and hydroxyproline content was calculated as µg per mg of tissue according to the protocol.

Western blot analysis

The total protein samples were extracted from myocardia tissues and quantified using the bicinchoninic acid (BCA) protein assay (Beyotime, Shanghai, China). Samples were separated with 10% SDS–PAGE electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. After blocked with 5% fat-free milk for 2 h, the membrane was incubated with primary antibodies overnight at 4°C.

The following primary antibodies were independently used to detect specific proteins: Gal-3 (1:1000 dilution, Abcam, USA), CTGF (1:1000 dilution, Santa Cruz, USA), Kv4.3 (1:400 dilution, Santa Cruz, USA), KCNH2 (1:500 dilution, Santa Cruz, USA), KCNQ1 (1:500 dilution, Santa Cruz, USA), CX43 (1:500 dilution, Abcam, USA). An antibody against β-actin (1:1000 dilution, Santa Cruz, USA) was used as an internal control. Following washing with PBS, membranes were treated with horseradish peroxidase-conjugated secondary antibodies (dilution, 1:1000 dilution, Santa Cruz, USA). The immunoreactive bands were visualized using the enhanced chemiluminescence kit (ECL Millipore Corp., Bedford, MA, USA). Developed films were scanned and Image-ProPlus 5.1 was used for quantitative analysis.

Statistical analysis

Continuous data were reported as mean value ± SE. Categorical data were presented as absolute values and percentages. Differences among multiple groups were compared with single factor analysis of variance (ANOVA) while the comparison between two groups was detected with LSD method. The Fisher exact test was used to check for the significance of frequency data. Analyses were performed using the Software Package for Statistical Science (SPSS for Windows; Version 22, SPSS Inc.; Chicago, IL). $P < 0.05$ is considered statistically significant.

Results

As described in our previous study^[14], rabbits in HF group and CCM group with 12 weeks trans aortic constriction showed significant decreasement of ejection fraction and met the criteria of heart failure. Only one rabbit dies from surgery complication in each group mentioned above. All animals survived after CCM protocols in all groups.

Effect of CCM on Electrophysiological characteristics

Rabbits in HF group (152.00 ± 4.80) and CCM group (151.44 ± 3.97) developed significant prolonged QTc compared to sham group (140.70 ± 4.42 , $P \leq 0.05$) before CCM protocols (Fig. 1D). After 4 weeks of CCM

stimulation, the QTc of CCM group (144.67 ± 3.84) showed significant shorting compared to HF group (152.11 ± 5.49 , $P < 0.05$) (Fig. 1E).

Consistent with QTc changing, the ERP was significantly longer in HF group (140.56 ± 5.10) compared to sham group (121.50 ± 9.54 , $P < 0.05$). CCM therapy significantly reduced ERP in rabbits with heart failure (133.33 ± 6.12 , $P < 0.05$) (Fig. 1F).

Inducibility of VT was significantly increased in HF group rabbits (8 out of 9, 89%) compared with those in sham group (1 out of 10, 10%). Whereas decreased VT inducibility was observed in CCM group rabbits (4 out of 9, 44%) (Fig. 1G).

Effect of CCM treatment on myocardial fibrosis

The collagen fiber performs a critical role in cardiac structural remodeling. Excessive deposition of collagen known as myocardial fibrosis. Hydroxyproline is unique to collagen and is a well-recognized quantitative marker for fibrosis. Picrosirius red staining was performed to differentiate depositions of collagen I (red and yellow staining) and collagen III (green staining) (Fig. 2A, B, C). The results showed that type I, III in the HF group increased significantly comparing with the sham group. These changes in the levels of these two types of collagen were suppressed by CCM treatment (Fig. 2D, E). The hydroxyproline content ($0.98 \pm 0.04 \mu\text{g}/\text{mg}$) of the HF group was significantly higher than that of the sham group ($0.41 \pm 0.03 \mu\text{g}/\text{mg}$, $P < 0.01$). With the treatment of CCM, the hydroxyproline content was remarkably reduced ($0.69 \pm 0.05 \mu\text{g}/\text{mg}$, $P < 0.01$) compared with HF group (Fig. 2F).

Effect of CCM treatment on expression of CTGF and GAL-3 detected by western blot

CTGF and Gal-3 is characterized by increased production of ECM components, strongly contributes to the pathogenesis of myocardial fibrosis. The protein level of CTGF and Gal-3 were significantly increased in the HF group compared with the sham group. This upregulation rectified by CCM therapy. CTGF and Gal-3 expression was reduced by in CCM group compared with HF group (Fig. 3).

Effect of CCM treatment on expression of Kv4.3, KCNQ1, KCNH2 and CX43 detected by western blot

To further investigate the underlying mechanisms by which CCM change electrophysiological characteristics of heart failure, we studied Kv4.3, KCNQ1, KCNH2 and CX43 expression on the protein level. The protein level of Kv4.3, KCNQ1, KCNH2 and CX43 in HF group were significantly decreased in the HF group compared with the sham group. Treatment with CCM restore the expression level of Kv4.3, KCNQ1, KCNH2 and CX43 (Fig. 4).

Discussion

Chronic heart failure is a kind of myocardial overload disease caused by multiple diseases with many manifestations and may cause high mortality and disability in clinics. The remodeling process of myocardial structure and electricity deteriorate the function of heart and increase the susceptibility of

arrhythmia. CCM is a novel therapeutic method and has proven to be effective in improving cardiac function. It has been suggested by the European Society of Cardiology guidelines that patients with symptomatic HF on OMT and with normal or mildly prolonged QRS duration and reduced EF may consider CCM therapy[1]. Due to the rabbit heart shares some physiological commonalities with the human heart, e.g. a similar alteration of myocardial function in the failing stage and similarities in ion channel, gap junction's expression pattern, we chose rabbits CHF model induced by TAC to investigate the effects of CCM on structural and electrical remodeling. The main findings of this study include: (1) CCM therapy attenuate the deposition of collagen, prolonged QTc and ERP and the increasing Inducibility of VT in CHF rabbits thus reverse myocardial structural and electrical remodeling. (2) Expression of CTGF, Gal-3, CX43, KCNH2 and KCNQ1 restored by CCM might contribute to the underlying mechanism.

Effect of CCM treatment on structural remodeling in heart failure

Myocardial fibrosis is a hallmark of heart failure and plays a vital role in myocardial structural remodeling, which is characterized by an increase in collagen synthesis and decrease in collagen degradation in the myocardium, resulting in disproportionate collagen deposition including collagen I and collagen III[19]. Type I collagen has a strong anti-tensile strength and small extension resilience. Whereas, type III Collagen belongs to embryo-type collagen and has largely extended resilience. The ratio of type I and type III increased in the process of remodeling, As the ratio of type I and type III collagen changed, myocardial stiffness increased, and then myocardial compliance was decreased manifesting systolic dysfunction, diastolic dysfunction and eventually led to the failure function. Previous studies have reported CCM could improve cardiac remodeling by echocardiography and histological assessment. In the current study, picrosirius red staining and a hydroxyproline biochemical assay revealed that CCM reduced the deposition of collagen and prevent the development of cardiac fibrosis[7, 20]. The result is consistent with our previous study result that CCM therapy could down regulate the expression of collagen I and collagen III by inhibiting TGF- β 1/Smad3 signaling pathway[14]. As mediators of myocardial fibrosis, CTGF and Gal-3 involved in decreasing collagen deposition in myocardial interstitium and adverse structural remodeling of CHF. It has been demonstrated that overexpressing CTGF induce cardiac hypertrophy and collagen production and CTGF is a well-characterized downstream mediator of TGF- β 1/Smad3 signaling pathway during the fibrotic response[21, 22]. Another study shown the galectin-3 inhibitor alleviated collagen deposition and significantly decreased fibrosis[10]. In our study, the protein level of CTGF, Gal-3 were suppressed with CCM therapy in CHF rabbits. These may be the potential mechanism by which CCM prevent structural remodeling.

Effect of CCM treatment on electrical remodeling in heart failure

Electrophysiological remodeling includes the changes of a variety of ion channels, sarcoplasm reticulum calcium cycle and the gap junctions between cells. These changes lead to reduced cardiac electrical stability, conduction slowing, prolonged the action potential duration and make cardiac prone to arrhythmia[23]. The down regulation of potassium currents with CHF are important contributors to electrical remodeling, which noticeable prolongation of the ERP and the QT-intervals as a consequence of a

prolonged repolarization process. The excessive prolongation of action potential duration leading to early afterdepolarization (EAD) and an increase of spatial dispersion of repolarization can act as a substrate for the occurrence of proarrhythmia. Cx43 is the main component of gap junction channel and is the main conductor of the current conduction between ventricular myocytes. Evidence has shown that the expression of the CX43 is downregulated with heart failure[11, 24]. Decreased CX43 expression contribute to conduction slowing thus increasing the inducibility of reentrant arrhythmias[25]. Here, we show that rabbits with CHF shown the prolongation of QT-intervals, ERP and increasing inducibility of VT. The underlying mechanism may be a down-regulation of the Ito subunit, Kv4.3, the Ikr subunit, KCNH2, the Iks subunit, KCNQ1, and CX43. This is partially consistent with previous research[26-28]. It can be explained that the methods used to induce CHF in previous study are different. Clinical studies demonstrate that CCM improves long-term survival and without proarrhythmia[5]. Former experimental indicates CCM can shorten the ADP duration in isolated rabbit heart without HF partially dependent on the activation of Iks[29]. In the present study we found CCM restore the prolonged QTc and ERP, and reduce the susceptibility of ventricular arrhythmia. At the molecular level, CCM could reverse the Kv4.3, KCNQ1, KCNH2 and CX43 protein expression which contribute to the prevention of electrical remodeling in heart failure. In addition, progressive cardiac fibrosis fosters the development of malignant arrhythmias based on local reentry. Consequently, the prevention of cardiac fibrosis of CCM might therefore additionally protect the heart from the occurrence of arrhythmias.

Conclusions

The present study demonstrated that CCM treatment prevented myocardial structural and electrical remodeling in a rabbit model of TAC-induced HF. The beneficial effect of CCM may be related to prevention of downregulation of the CTGF, Gal-3, Cx43, Kv4.3, KCNQ1, KCNH2 and CX43. These findings provide experimental evidence for the clinical use of CCM in the treatment of HF.

Limitations

Several limitations of this study should be noted. Firstly, the sample size was relatively small. Secondly, heart failure is a complex process, it remains unclear whether heart failure from another model as volume overload or tachycardia leads to similar results. Last but importantly, we did not perform patch clamp studies to explore the effect of CCM on ion channel's function.

Abbreviations

CHF: Chronic heart failure; CCM: Cardiac contractility modulation; ERP: Effective refractory period; VT: Ventricular tachycardia; CTGF: Connective tissue growth factor; Gal-3: Galectin-3; Cx43: Connexin43; CVF: Collagen volume fraction; OMT: Optimal medical treatment; TAC: trans aorta constriction; TGF- β 1: Transforming growth factor beta 1;

Declarations

Acknowledgments

None

Availability of data and materials

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

Ethics approval and consent to participate

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85–23, revised 1996), and The protocol was approved by the Ethics Committee on Scientific Research of Hebei General Hospital.

Authors' contributions

FFZ participated in the design, performed the experiment, collected the data, performed statistical analyses and drafted the manuscript. XYQ and BN participated in the design, performed statistical analyses and helped to draft the manuscript. YD and YXL performed the experiment, collected the data and performed statistical analyses. QQH, RL and SXZ performed the experiment and collected the data. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figure Legends

Fig. 1. Effect of CCM myocardial electrophysiological characteristics. (A) Representative ECG recordings of CCM signals. (B) Representative the measurement of ventricular ERP. (C) Representative the inducibility of ventricular tachycardia (VT). (D) Comparison of QTc intervals before CCM among the 3 groups. (E) Comparison of QTc intervals after CCM among the 3 groups. (F) Comparison of ventricular ERP among the 3 groups. (G) VT inducibility in the Sham group, HF group and CCM group. * $P < 0.05$ vs. Sham group; # $P < 0.05$ vs. HF group.

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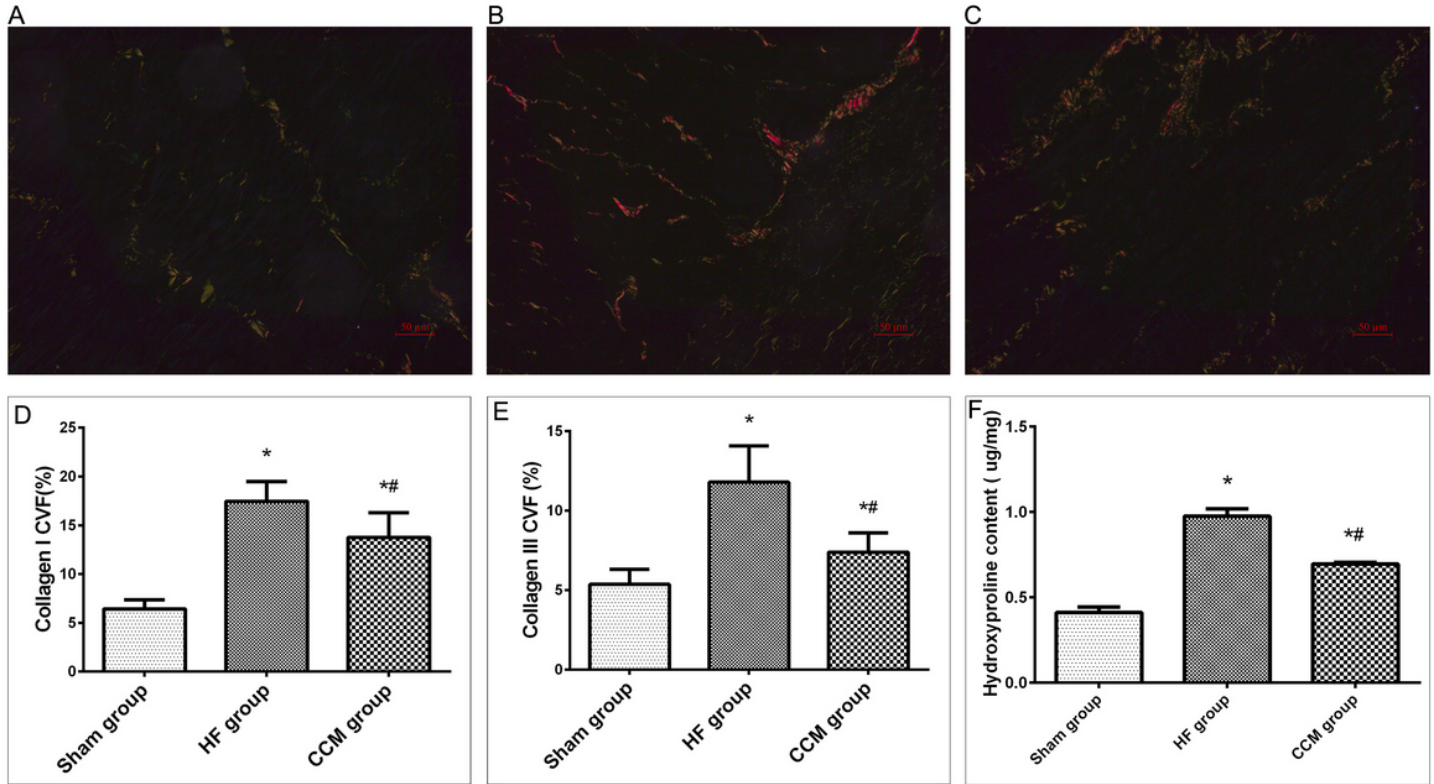


Figure 2

Effect of CCM treatment on myocardial fibrosis. (A), (B), (C) Representative picrosirius red staining of myocardium in Sham group, HF group and CCM group. (The magnification is $\times 400$, scale bar: 50 μ m). (D) Percentage of areas of collagen I among the 3 groups. (E) Percentage of areas of collagen III among the 3 groups. (F) Comparison of hydroxyproline content among the 3 groups. Each bar represents the means \pm standard deviation. * $P \leq 0.05$ vs. Sham group; # $P \leq 0.05$ vs. HF group.

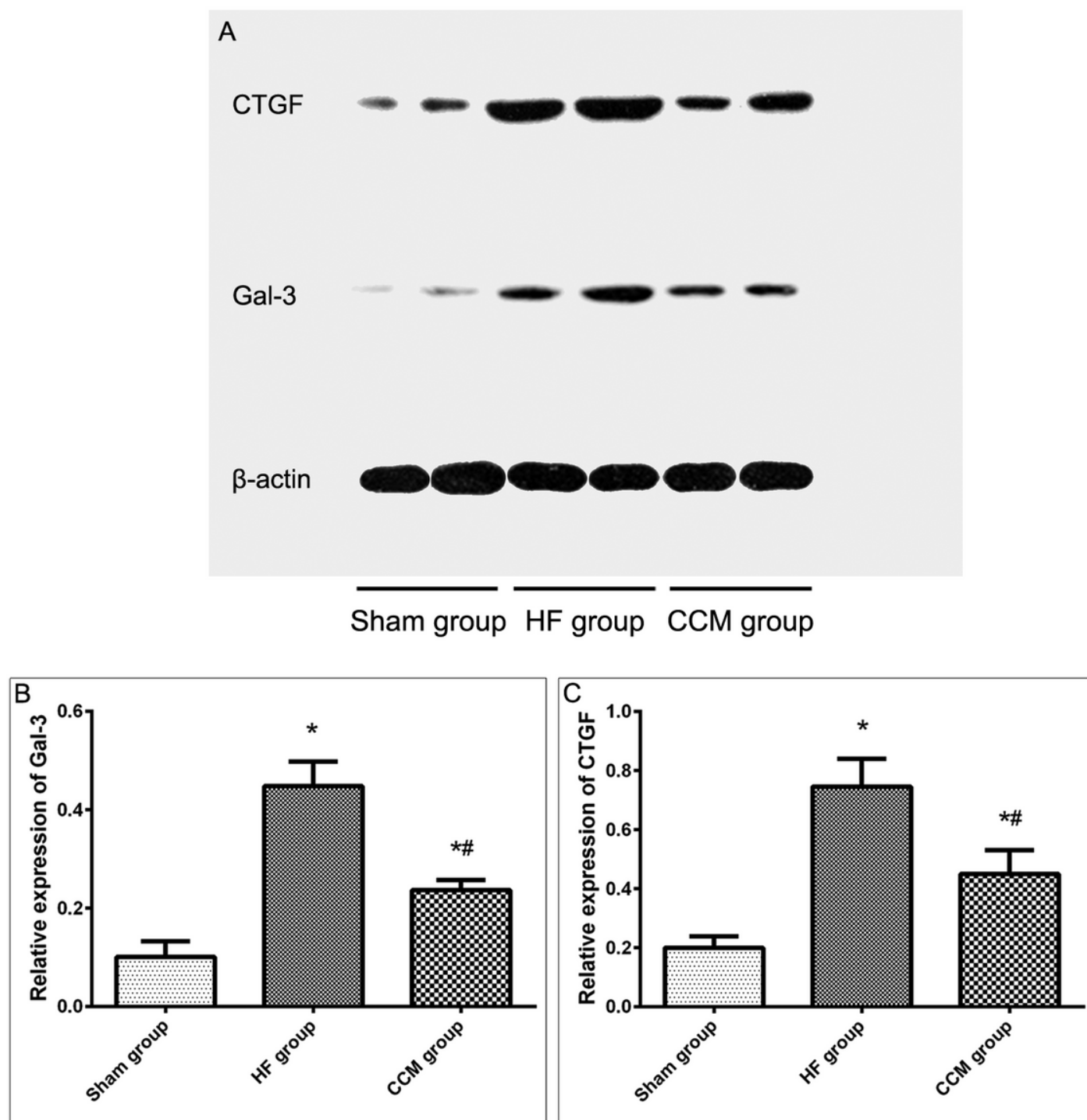


Figure 3

The protein levels of CTGF and Gal-3 in myocardium. (A) Representative western blot gels depict the protein expression levels of CTGF and Gal-3. (B) Comparison of the protein relatively expression levels of Gal-3 in the 3 groups. (C) Comparison of the protein relatively expression levels of CTGF in the 3 groups. Each bar represents the means±standard deviation. * $P \leq 0.05$ vs. Sham group; # $P \leq 0.05$ vs. HF group.

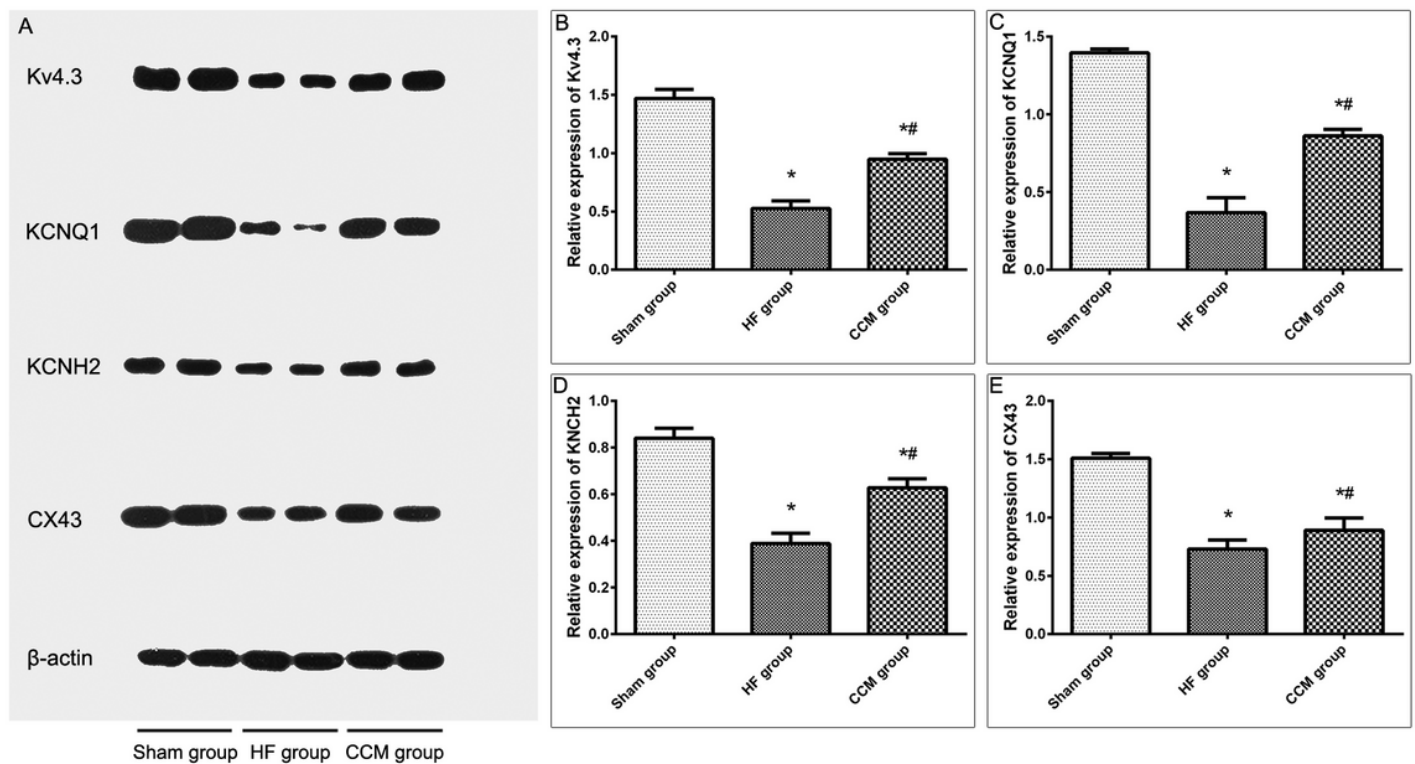


Figure 4

The protein levels of Kv4.3, KCNQ1, KCNH2 and CX43 in myocardium. (A) Representative western blot gels depict the protein expression levels of Kv4.3, KCNQ1, KCNH2 and CX43. (B), (C), (D), (E) Comparison of the protein relatively expression levels of Kv4.3, KCNQ1, KCNH2 and CX43 in the 3 groups. Each bar represents the means±standard deviation. * $P \leq 0.05$ vs. Sham group; # $P \leq 0.05$ vs. HF group.

Supplementary Files

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- [ARRIVEGuidelinesChecklist.pdf](#)