Effect and Mechanism of Sacubitril/valsartan on Ventricular Remodeling in STZ induced Diabetic Cardiomyopathy Rats

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Abstract

Background: Diabetic cardiomyopathy (DCM) has a high incidence of heart failure and poor prognosis, so its prevention and treatment should be more active. In this study, we investigate the effect and mechanism of sacubitril/valsartan (Sac/Val) on ventricular remodeling in DCM rats, so as to provide a new idea for clinical prevention of DCM.

Methods: Twenty 8-week-old male Wistar rats were randomly divided into four groups: normal rat group (NOR), DCM group, DCM+ Sac/Val group and DCM+ Perindopril (PER) group , with 5 rats in each group. The DCM rat model was established by intraperitoneal injection of 1% streptozotocin (STZ). NOR and DCM groups were gavaged with normal saline (1ml/100g/d) for 8 weeks, while Sac/Val and PER groups were gavaged with Sac/Val (60mg/kg/d) and PER (2mg/kg/d) for 8 weeks. HE and Masson staining were used to evaluate the pathological changes of myocardial tissue, the left ventricular/ body mass index was calculated to evaluate ventricular remodeling, the levels of IL-6 and TNF- α in myocardial tissue were detected by ELISA, and the protein expression of p-38 and phos-p38 was detected.

Results: Compared with the NOR group, the arrangement of cardio myocytes in the DCM group was disordered and the content of collagen fibers increased. In the DCM group, the left ventricular/body mass index increased, the levels of IL-6 and TNF- α increased, and the phos-p38 protein expression increased (P 0.01). Compared with DCM group, fewer collagen fibers were found and the degree of fibrosis was reduced in Sac/Val group and PER group. The levels of left ventricular/body mass index, IL-6, TNF- α and phos-p38 protein expression in the Sac/Val group were all lower than those in the DCM group, and the differences were statistically significant (P 0.01).

Conclusion: Sac/Val can improve ventricular remodeling in DCM, and its mechanism may be related to its anti-inflammatory effect and inhibition of p38 signaling pathway in myocardial tissue.

Background

Diabetic cardiomyopathy (DCM) is a kind of cardiomyopathy, which is caused by diabetes and leads to a series of malignant heart events such as heart failure and arrhythmia. Studies have shown that the changes of myocardial structure and function in DCM patients are not related to coronary heart disease, hypertensive heart disease and other cardiovascular diseases [1]. The pathophysiological process of DCM is complex. It has been found that glucose toxicity, lipid toxicity, insulin resistance, hyperinsulinemia, cardiac autonomic neuropathy, renin-angiotensin-aldosterone system activation, p38 signaling pathway activation, inflammatory response and other factors are related to the incidence of DCM [2]. According to the pathophysiological characteristics and imaging changes of DCM, the cardiac damage was divided into four stages. Firstly, myocardial fibrosis increased, left ventricular mass (LVW) increased, diastolic dysfunction and normal ejection fraction. Secondly, LVW was increased. Diastolic function and systolic function were decreased. And then the heart was slightly enlarged. Thirdly, microvascular lesions and diastolic dysfunction cause more severe systolic dysfunction. Fourthly, there is

more severe contractile dysfunction, myocardial fibrosis, cardiac dilatation, microvascular and macrovascular lesions [3]. The incidence of heart failure in type 2 diabetic patients is 2.5 times higher than that in non-diabetic patients, and the prognosis of patients with heart failure due to diabetic cardiomyopathy is poor, with a 5-year survival rate of only about 50%, which seriously threatens people's health [4]. Therefore, further study on the pathogenesis of DCM and search for appropriate treatment can help to reduce the morbidity and mortality of DCM.

Sacubitril/valsartan (Sac/Val) is an angiotensin- receptor enkephalin inhibitor consisting of an enkephalin inhibitor (Sacubitril) and an angiotensin II receptor antagonist (Valsartan), which was approved to be marketed in China in 2017. According to PARADIGM study，compared with enalapril, Sac/Val reduced the risk of major composite endpoint events by 20%, all-cause mortality by 16%, sudden cardiac death by 20%, and heart failure death by 21%[5]. A total of 4822 heart failure patients with ejection fraction retention were included in the 57-month PARAGON-HF study. The results showed that compared with valsartan alone, Sac/Val reduced NT-proBNP levels at week 12 and improved NYHA cardiac grade at week 36[6]. In the PIONEER- HF study, which included 884 patients with reduced ejection fraction hospitalized for acute decompensated heart failure, Sac/Val was found to have a greater reduction in NT-proBNP one week after initiation of treatment than enalapril in patients with acute decompensated heart failure[7].At causal analysis of the PARADIGM HF test, Sac/Val reduces the risk of cardiovascular death and hospitalization for heart failure, regardless of blood sugar status, compared with enalapril[8]. However, there is a lack of basic research on the effect of Sac/Val on ventricular remodeling in DCM patients. In this study, we observed the effects of Sac/Val on ventricular structural changes, pathological changes and inflammatory changes in the hearts of DCM rats, to explore the primary mechanism of Sac/Val's improvement on ventricular remodeling in DCM rats, and to provide a new idea for clinical prevention and treatment of DCM.

1. Materials And Methods

1.1 material

A total of twenty 8-week-old male Wistar rats were selected and provided by the Animal Model Center of Nanjing University, with the animal qualification number SCXK (Su) 2015-0001. They were raised in an environment of 22 ± 2°C, freely feeding and drinking water, and were adaptive fed for 1 week before the experiment. Streptozotocin (STZ) (Sigma, St. Louis, MO, USA), Sacubitril valsartan tablets (Novartis, Switzerland), HE kit, and Masson kit were provided by Jiangsu KeyGEN Biotech, NanJing, China. Rat IL-6 ELISA Kit (ab100772), Rat TNF- ELISA Kit (ab100785), Anti-p38 antibody (ab31828), and Anti-phos-p38 antibody (Thr180/Tyr182) (ab4822) were purchased from Abcam, Cambridge, UK.

1.2 Model Establishment

At a dose of 70mg/kg, 1% STZ (pH = 4.5, compound sodium citrate buffer 4 ℃, instantly make immediate use) was injected into the abdominal cavity of rats to destroy the function of islet cells, tail venous blood of rats was extracted 3 days and 1 week after injection of STZ to detect fasting blood

glucose (fasting for 8h), When blood glucose ≥16.7mmol/L and polydipsia, polyphagia and polyuria occurred in the rats, the diabetic cardiomyopathy model of rats was considered to be established.

1.3 Group setting

Rats were divided into the following four groups. Firstly, normal rats (NOR) were given normal saline (1ml/100g/d) by gavage for 8 weeks without modeling. Secondly, DCM model group, after modeling, normal saline (1ml/100g/d) was given by gavage for 8 weeks. Thirdly, DCM+ Sac/Val group (Sac/Val) after modeling, Sac/Val (60mg/kg/d) was given orally for 8 weeks. Fourthly, DCM+ Perindopril group (PER), PER (2mg/kg/d) was given orally for 8 weeks after modeling.

1.4 Left ventricular/body mass index (LVW/BW)

After measured BW, the rats were killed immediately, and then the heart was taken out immediately and washed repeatedly in 0.9% sodium chloride injection. After removing the residual blood, the atrial, great vessels and epicardial adipose tissue were removed, and the left ventricle was dissociated and dried by filter paper. Finally, we use an electronic balance to weigh the lvw. LVW / BW (mg/g) = left ventricular weight (mg) / body weight (g).

1.5 Myocardial histology

We prepared paraffin sections of heart tissue for routine xylene dewaxing, followed by gradient ethanol hydration, distilled water washing, and then stained with HE to evaluate the myocardial arrangement. The paraffin sections of heart tissue were placed in xylene and xylene for 10 min. After dewaxing, they were washed with water, stained with hematoxylin for 10 min, and then washed with water until they turned blue. Then they were stained with Ponceau and fuchsin for 5 min, 1% molybdic acid solution for 10 min, aniline blue solution for 15 min, 0.2% glacial acetic acid solution for 5 min, and 0.2% glacial acetic acid solution for 5 min. Finally, the myocardial histological changes were evaluated by Masson's trichrome staining.

1.6 ELISA

We quickly extracted the hearts of the rats immediately after they were killed and removed about 100mg of myocardial tissue, then rinsed them with PBS buffer. The tissue homogenate was centrifuged at 12,000 g for 10 minutes, and the contents of IL-6 and TNF-α in the supernatant were determined with ELISA kit.

1.7 Western Blot

We isolated cardiac tissue and cut about 100 mg tissue into a precooled EP tube. Then we added 400 μl RIPA lysate into a homogenizer for homogenized the tissue, crushed the tissue as much as possible, put it on ice for 30 min, then transferred the lysate to 1.5 ml Eppendorf tube with a pipette, centrifuged for 5 min, and part of the supernatant was put into 200 μl Eppendorf tube, and the total protein concentration

was determined according to the instructions of BCA kit. The SDS-PAGE gel was prepared. The size of the gel was sheared PVDF film and activated in methanol for 1 min, and then immersed in the membrane buffer. The filter paper was placed in the transfer film buffer to soak for 15 min. According to the principle of PVDF membrane≥ gel≥ filter paper, we make transfer sandwich to ensure constant pressure transfer after bubble removal. Add the TBST diluted to the appropriate concentration of a single antibody, close the bag mouth, stay overnight at 4 ℃, add appropriate amount of two resistance, close the bag mouth, and incubate 1 h at room temperature. Tanon 6600 luminous imaging workstation was used for image acquisition. Finally, we detected the expression levels of p38 and phos-p38 in myocardium tissue of rats in each group by Western blot.

1.8 Statistical analysis

We used Single factor analysis of variance (ANOVA) to compare the mean of multiple independent samples. Kruskal-wallis test is used for analysis if there are other conditions such as uneven variance or non-normal distribution. The significance level was bilateral α = 0.05. All data were analyzed using SPSS 20.0.

2 Results

2.1 Sac/Val reduced LVW/BW ratio in DCM rats

Compared with healthy rats in NOR group, the left ventricular weight of rats in DCM group increased (842.34 ± 53.18 mg vs. 536.27 ± 22.97 mg, P < 0.01), LVW/BW ratio increased (1.59 ± 0.12 mg/g vs. 3.03 ± 0.24 mg/g, P < 0.01), after treatment with Sac/Val or PER, LVW/BW ratio of Sac/Val group (3.03 ± 0.24 mg/g vs. 1.98 ± 0.15 mg/g, P < 0.01) and PER group（3.03±0.24 mg/g vs. 1.95±0.37 mg/g, P<0.01） was lower than that of DCM group (Fig. 1).

Figure. 1 Effect of Sac/Val on LVW/BW ratio in DCM rats

*P<0.05; **P<0.01

2.2 Sac/Val alleviated the degree of myocardial injury and fibrosis in DCM rats

As shown in Figure 2, compared with the NOR group, the arrangement of cardiomyocytes in the DCM group was disordered and the content of collagen fibers increased. Compared with the DCM group, after Sac/Val and PER were treated, the collagen fiber and fibrosis degree were reduced in the Sac/Val and PER groups.

Figure. 2 Comparison of HR staining (X100) and Masson staining (X200) in myocardial tissues of rats in each group

HE staining: CON group (A1), DCM group (B1), Sac/Val group (C1) and PER group (D1)

Masson staining: CON group (A2), DCM group (B2), Sac/Val group (C2) and PER group (D2)

2.3 Sac/Val reduced the levels of inflammatory cytokines IL-6 and TNF- in DCM rats

Compared with the NOR group, the levels of inflammatory factors IL-6 (125.28 + 10.44 pg/mg vs. 295.49 + 25.31 pg/mg, P < 0.01) and TNF-α (80.03 + 9.46 pg/mg vs. 240.61 + 40.53 pg/mg, P < 0.01) in the myocardial tissue of the DCM group were significantly increased, and the difference was statistically significant. Compared with the DCM group, the inflammatory cytokines IL-6295.49±25.31 pg/mg vs. 167.25±18.74 pg/mgP<0.01. 295.49±25.31 pg/mg vs. 171.50±13.12 pg/mgP<0.01 and TNF-α 240.61±40.53 pg/mg vs. 138.10±3.58 pg/mgP<0.01. 240.61±40.53 pg/mg vs. 137.35±7.36 pg/mg P<0.01decreased in the Sac/Val and PER groups after treatment with Sac/Val and PER, and the differences were statistically significant Fig. 3).

Figure 3 Sac/Val attenuates IL-6 and TNF-α levels in myocardial tissue of DCM rats

*P<0.05; **P<0.01

2.4 Sac/Val inhibits p38 signaling pathway in myocardial tissue of DCM rats

Compared with the NOR group, the expression of phos-p38 protein in myocardial tissue of rats in the DCM group was significantly increased (1.00 + 0.12 vs. 2.27 + 0.53, P = 0.001); after treatment with Sac/Val and P ER, the expression of phos-p38 protein in the Sac/Val group (2.27 + 0.53 vs. 1.25 + 0.23, P = 0.004) and the P ER group (2.27 + 0.53 vs. 1.29 + 0.16, P = 0.004) was lower than that in the DCM group, and the difference was statistically significant (Fig. 4).

3 Discussion

DCM is a cardiomyopathy caused by diabetes mellitus, which can not be explained by other cardiovascular diseases. Diabetic cardiomyopathy patients with heart failure have worse quality of life, higher hospitalization rate and mortality rate of heart failure than patients with other causes of heart failure [9], so its prevention and treatment should be more active. In this study, the rat model of DCM was established by STZ to investigate the effect of Sac/Val on ventricular remodeling in DCM rats and its preliminary mechanism. This study found that Sac/Val could reduce LVW/BW, myocardial inflammatory factors IL-6 and TNF-a levels, phos-p38 protein expression in myocardial tissue of DCM rats, and improve the degree of myocardial injury and fibrosis in DCM rats. Diabetes causes ventricular remodeling, which is independent of factors such as obesity and hypertension [10]. Patients with DCM often do not have heart failure symptoms even when LVW increases in the early stage, but as the disease progresses, cardiac enlargement decreases diastolic left ventricular filling, thereby causing left ventricular diastolic dysfunction. DCM can be seen under cardiac MRI mainly manifested as left ventricular hypertrophy, interstitial and peripheral vascular fibrosis and microvascular abnormalities [11]. This study found that compared with healthy rats, DCM rats showed a significant increase in LVW and a significant increase in the ratio of LVW to body weight, which also proved that the increase of LVW in DCM rats was not related to obesity, which was consistent with previous studies[10]. Sac/Val could improve ventricular remodeling and decrease LVW/BW in rats. This study also found that the arrangement of cardiomyocytes was

disordered. The content of collagen fibers were increased in the DCM group. Previous animal experiments have demonstrated that the metabolic disorder caused by diabetes mellitus can directly affect cardiomyocytes and fibroblasts, change the function of cardiomyocytes, make collagen fibers deposit in the myocardial interstitium, leading to reduced myocardial compliance and diastolic function [12]. Cardiac fibrosis in DCM patients assessed by cardiac MRI was associated with mortality and hospitalization for heart failure [13]. It was found that after treatment with Sac/Val, myocardial collagen fibers were significantly reduced and the degree of fibrosis was significantly reduced in rats, which indicated that Sac/Val could delay the myocardial fibrosis caused by DCM and possibly reduce the mortality and hospitalization rate of heart failure in patients with DCM, but this still needs to be confirmed by large-scale multicenter clinical studies. Previous studies have confirmed that the pathophysiological role of inflammatory signals is associated with diabetic complications, and infiltrating immune cell influx has become an important factor in the progression of cardiomyopathy and left ventricular dysfunction [14]. Inflammatory factors and related proteins such as TNF-α, IL-1β, IL-6, TGF-β, IFNγand NFκB are highly expressed in the myocardial tissue of diabetic rodent model. Increased cytokines, chemokines and various white blood cell counts in human DCM also prove that inflammation is one of the pathogenesis of DCM [15]. This study found that compared with the NOR group, the levels of inflammatory factor IL-6 (125.28 + 10.44 pg/mg vs. 295.49 + 25.31 pg/mg, $P < 0.01$) and TNF-α (80.03 + 9.46 pg/mg vs. 240.61 + 40.53 pg/mg, $P < 0.01$) in myocardial tissue of DCM group were significantly increased, and the difference was statistically significant. This is consistent with the previous results, and found that Sac/Val can reduce the levels of IL-6 and TNF-α in the myocardial tissue of DCM rats. The results showed that Sac/Val could inhibit the myocardial inflammatory response induced by diabetes in rats. In addition, p38 signaling pathway is a classic inflammatory signaling pathway, which mediates a variety of pathophysiological processes. P38 mitogen activated protein kinase (p38 MAPK) is a serine / threonine protein kinase, which can respond to various cellular processes and external stress signals, such as cell differentiation, cell proliferation, and inflammation and cell death. Therefore, the increase of p38 MAPK may lead to cardiac damage in DCM [16]. It has been proved that inhibition of p38 MAPK activity can reduce cardiomyocyte apoptosis and myocardial hypertrophy in DCM rats [17]. The expression of phos-p38 protein was significantly increased in DCM group, but decreased after treatment with Sac/val. the results suggest that Sac/val can reduce cardiomyocyte apoptosis and myocardial hypertrophy by inhibiting p38 signaling pathway in DCM rats.

There are also some shortcomings in this study. Firstly, the establishment of early diabetic animal model by STZ can not completely simulate the human diabetic patients. Secondly, LVW/BW index can not fully reflect the changes of ventricular remodeling in rats, and there is a lack of cardiac MRI data. Moreover, LVW/BW index can not fully reflect the changes of ventricular remodeling in rats, and there is a lack of cardiac MRI data. These deficiencies need to be further improved in the follow-up study.

4 Conclusion

Sac/Val can improve the ventricular remodeling of DCM, and its mechanism may be related to its anti-inflammatory effect and inhibition of p38 signaling pathway.

Abbreviations

Sac/Val: sacubitril/valsartan; DCM: diabetic cardiomyopathy; NOR: normal rat group; PER: Perindopril ; STZ: streptozotocin; LVW: left ventricular mass; p38 MAPK: P38 mitogen activated protein kinase .

Declarations

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Availability of data and materials

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

Ethics approval and consent to participate

The study was approved by the Ethics review Committee of the Affiliated Hospital of North Sichuan Medical College

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Authors' contributions

Conceptualization: ML; ZL; KT.

Statistical analysis: AJ; SZ; LZY; ZLW.

Writing – original draft: KT.

Writing – review and editing: ML and ZL.

All authors read and approved the final manuscript.

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Figures

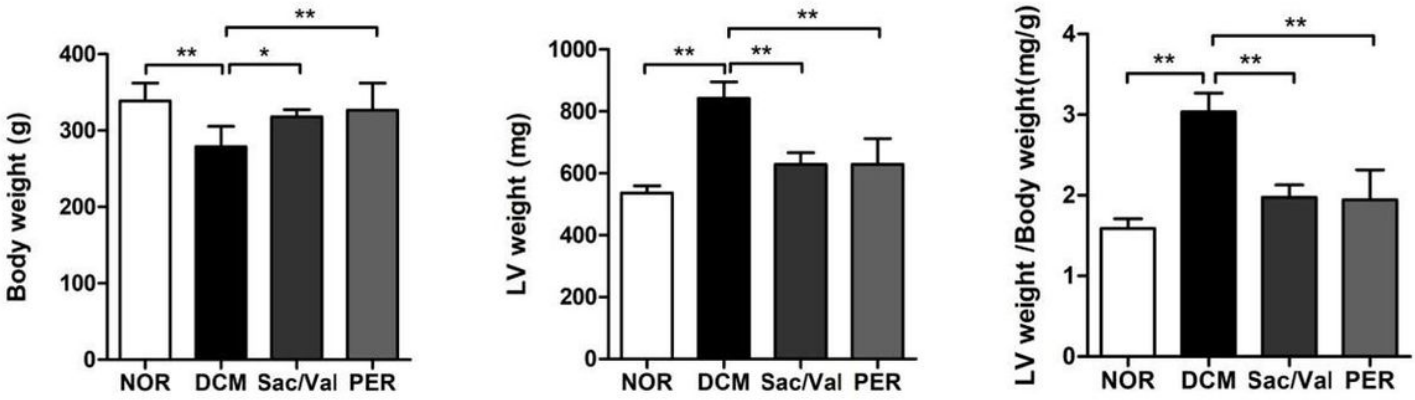
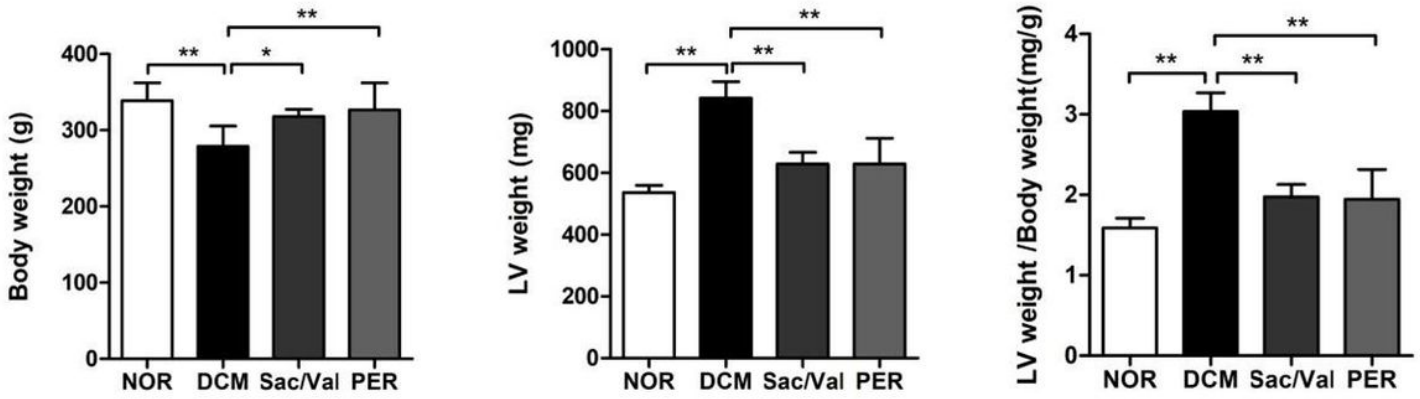


Figure 1

Effect of Sac/Val on LVW/BW ratio in DCM rats *P<0.05; **P<0.01



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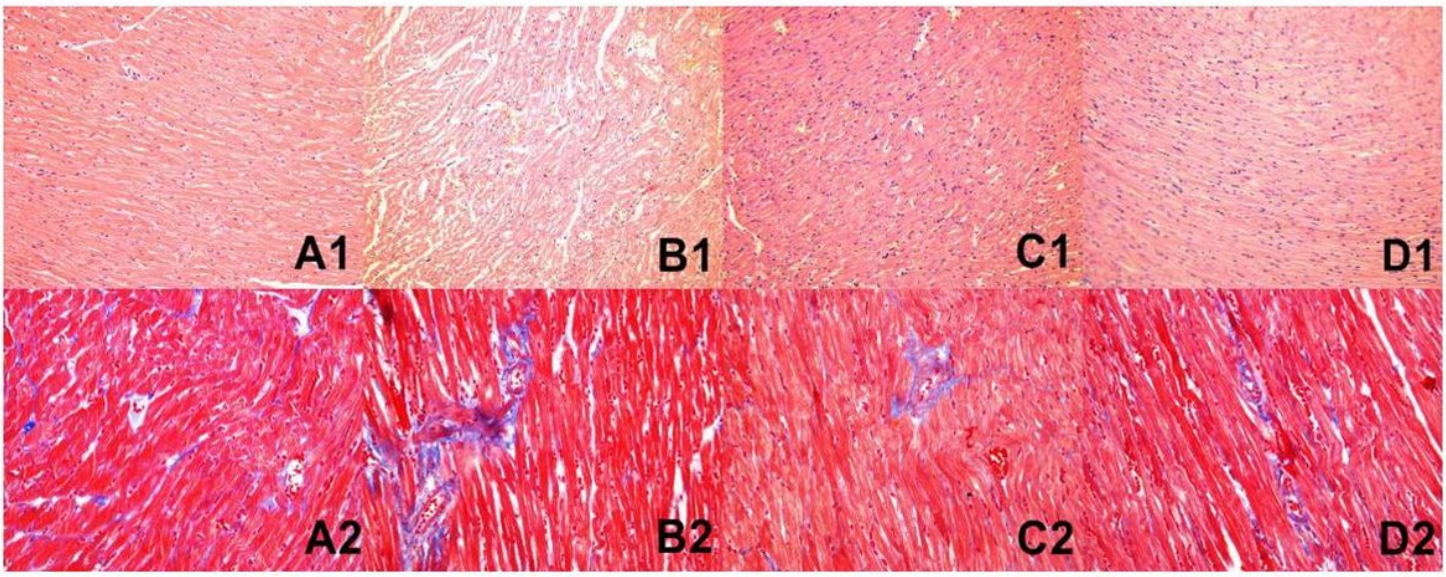
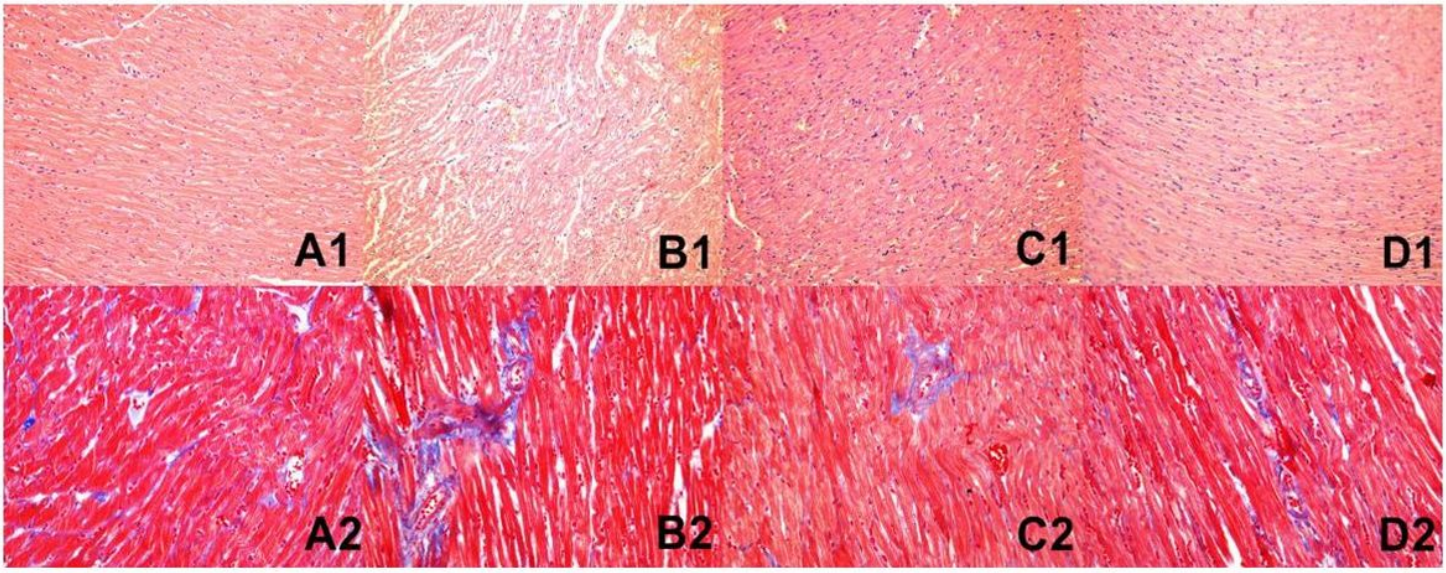


Figure 2

Comparison of HR staining (X100) and Masson staining (X200) in myocardial tissues of rats in each group



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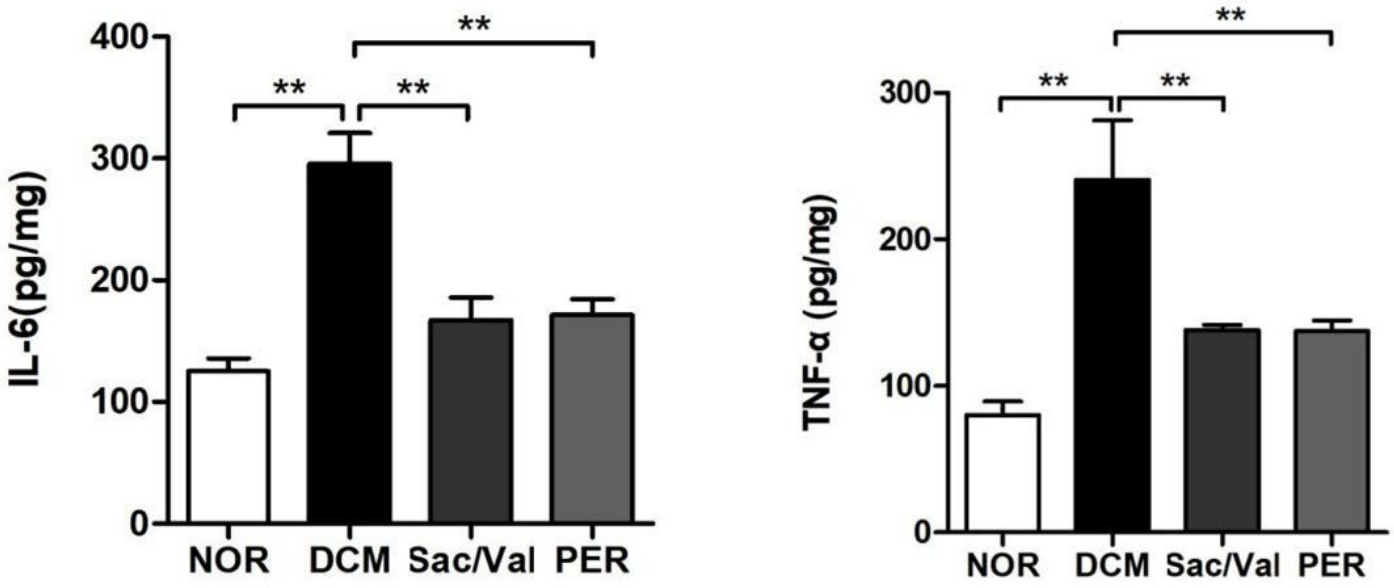
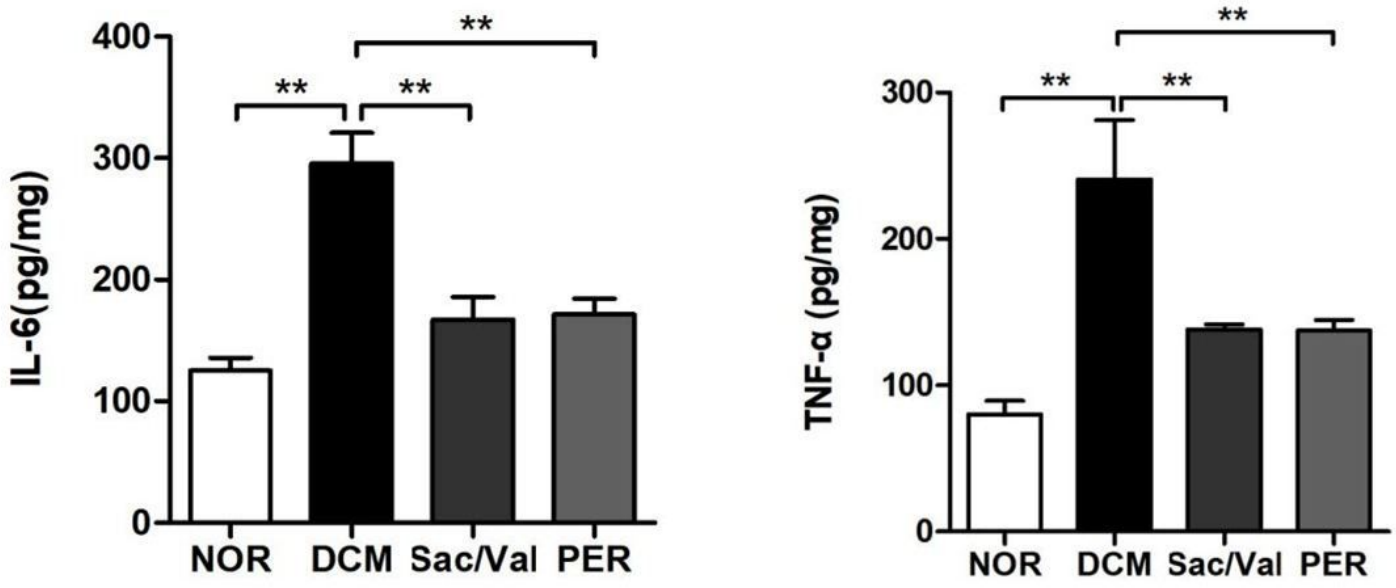


Figure 3

Sac/Val attenuates IL-6 and TNF-α levels in myocardial tissue of DCM rats *P<0.05; **P<0.01



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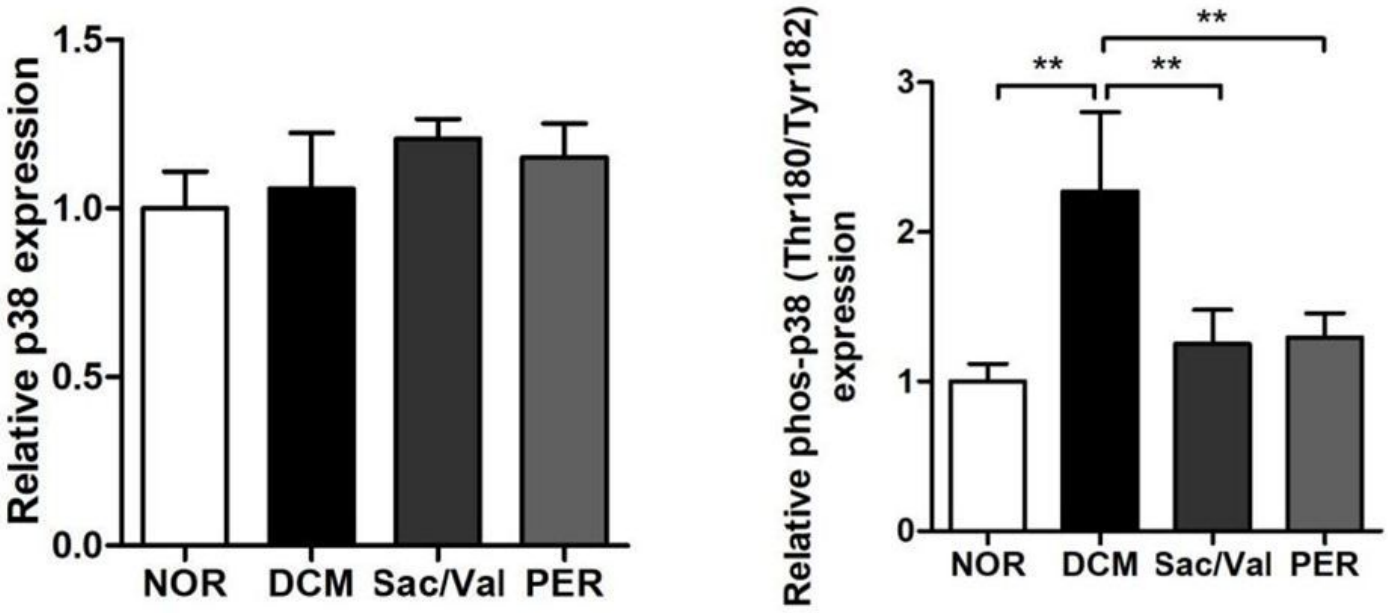

Figure 4

Sac/Val inhibits p38 signaling pathway in myocardial tissue of DCM rats *P<0.05; **P<0.01

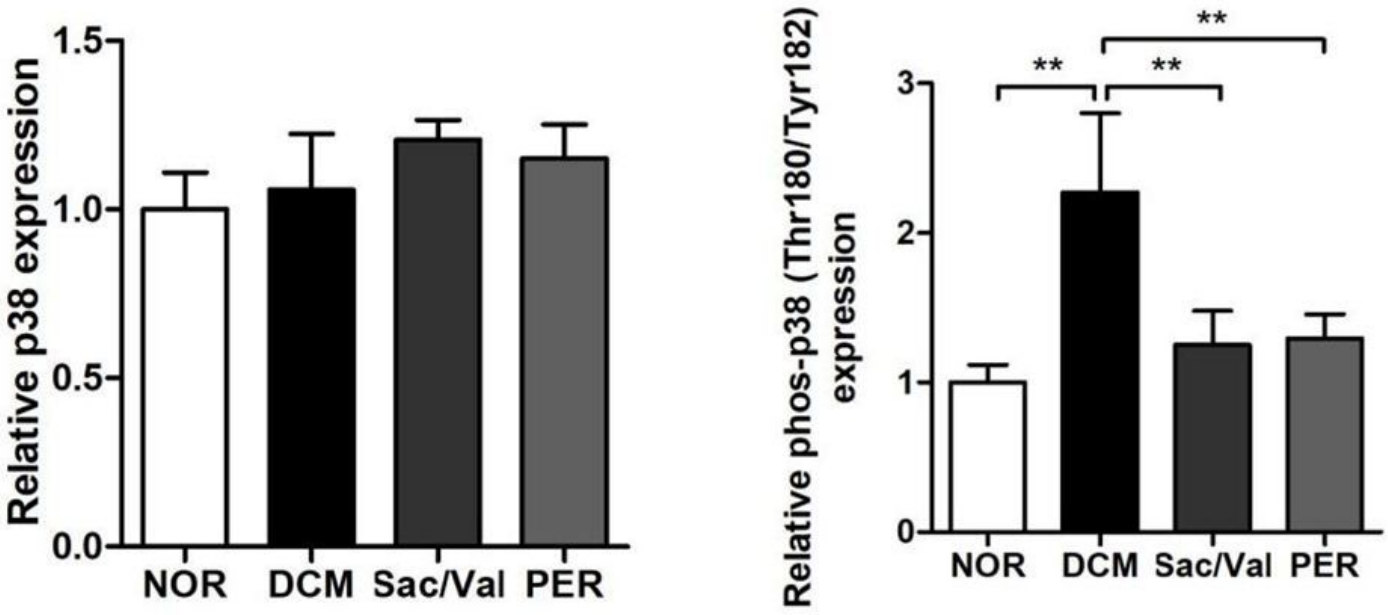

Figure 4

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