

Original Article

Effects of metoprolol on serum inflammatory factors and myocardial ischemia in rats modeled with coronary heart disease

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Abstract: Objective: This study was designed to observe the effects of metoprolol on serum inflammatory factors, cardiac function and oxidative stress response in rats modeled with coronary heart disease (CHD). Methods: Thirty clean SD rats aged 6-8 weeks were randomized into a control group (CG), treatment group (TG) and model group (MG), with 10 in each group. Rats in the CG were fed regular chow, while those in the MG and TG were fed a high-fat diet. After successful CHD modeling, those in the TG were given metoprolol every day, 10 mg/kg once a day. The effects of cardiac function indexes, myocardial injury indexes, blood lipids, inflammatory factors and oxidative stress indexes, myocardial apoptosis-related factors and apoptosis rate were observed and recorded before and after treatment. Results: Compared with the CG, the cardiac function indexes of the MG decreased significantly, while the myocardial injury indexes increased markedly. After metoprolol treatment, the cardiac function and myocardial injury of the TG were significantly improved. Also, the expression of serum lipid indexes in the MG increased obviously, and the hyperlipidemia in the TG was improved after metoprolol treatment. Besides, the expression of inflammatory factors in serum of the MG increased remarkably, and metoprolol could reduce the inflammatory state in rats. Furthermore, MDA in serum of the MG increased, SOD, CAT, GSH-Px decreased; revealing that metoprolol can improve oxidative stress in rats. Finally, the apoptosis rate of cardiomyocytes in the MG increased dramatically. Metoprolol treatment can reduce the apoptosis rate and improve the expression of apoptosis related proteins. Conclusion: Metoprolol reduces the degree of myocardial injury, inhibits inflammatory reaction and oxidative stress *in vivo*, reduces myocardial apoptosis and improves myocardial ischemia in CHD modeled rats.

Keywords: Metoprolol, coronary heart disease, inflammatory factors, myocardial ischemia

Introduction

Coronary heart disease (CHD) is a vascular disease that threatens human health, and it is also the main disease that causes disability and death of elderly people [1]. Clinically, it is mainly caused by coronary artery stenosis and blockage, resulting in hypoxia of myocardial tissue, ischemia and necrosis [2, 3]. Patients will also have palpitations, angina pectoris, fatigue and other symptoms. If the disease is not treated in time, it will also cause myocardial infarction, heart failure and even affect the life and safety of patients in severe cases [4, 5]. Therefore, it is particularly important to find new treatments for CHD.

Metoprolol is a cardiac selective competitive β -1 adrenergic receptor antagonist, which has

antihypertensive effects and no endogenous sympathomimetic activity [6]. It can also weaken the catecholamine effect related to physiological and psychological load, and reduce heart rate, blood pressure and cardiac output [7]. A recent study has shown that metoprolol can slow down heart rate, inhibit cardiac contractility, reduce the resistance of peripheral circulation, and decrease myocardial oxygen consumption and blood viscosity [8]. Thus, this study gave metoprolol intervention for CHD and observed its myocardial improvement effect. Wu W et al. [9] mentioned that metoprolol intervention could improve the structure and function of the left ventricle in acute myocardial infarction patients, effectively inhibit inflammatory cytokine expression in the myocardium and enhance cardiac function. Another study has shown that [10] giving metoprolol treat-

ment intervention to patients with chronic heart failure can improve their heart, motor function and quality of life.

In this study, the effects of metoprolol on inflammatory cells, stress response, myocardial injury and apoptosis in rats, as well as the protective mechanism of metoprolol on the myocardium were observed.

Materials and methods

Thirty SD rats (Junke Biotech Co., Ltd., Nanjing, China, J006) aged 6-8 weeks were purchased and kept in a clean environment with good ventilation. The environmental adaptation conditions: They were housed in both light and darkness for 12 h each, at an indoor temperature (22 ± 2)°C, humidity 50-65%, and water and food were provided according to standard animal care procedures; the adaptation time was one week. This test was approved by the Hospital Ethics Committee, and it followed the Guide for the Protection and Use of Experimental Animals [11].

Establishment of CHD rat models

Thirty rats were randomized into a control group (CG), model group (MG) and treatment group (TG), with 10 animals in each group. Those in the MG and the TG were fed for 6 weeks with a high-fat diet (propylthiouracil 0.2%, sodium taurocholate 0.5%, cholesterol 2%, lard 10%, basal diet 87.3%). Afterwards, rats in both groups were injected with 5 mg/kg isoprenaline hydrochloride once a day, for 3 consecutive days. Three days after injection, 0.3 ml/100 mg 10% chloral hydrate was employed for intraperitoneal anesthesia, and the changes of ST segment were observed by electrocardiograph. The elevation of the ST segment in electrocardiogram ≥ 0.1 mv indicated that a CHD rat model was successful [12]. The rats in the TG were given metoprolol once a day, 10 mg/kg [13], while those in the CG were fed with regular chow for 6 weeks.

Outcome measures

(1) Cardiac function indexes: Left ventricular diastolic pressure (LVDP), left ventricular end diastolic pressure (LVEDP), and maximum ascending and descending rate of left ventricular internal pressure ($\pm dp/dt$ max) were measured by pressure transducer.

(2) Detection of physiological and biochemical indexes: 5 mL femoral artery blood from rats in all three groups was collected, and then stored 10 min in a freezer at -70°C, and centrifuged at 1500×g. The myocardial injury and blood lipid indexes consisted of creatine kinase MB isoenzyme (CK-MB), lactate dehydrogenase (LDH), total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL), which were detected via automatic biochemical analyzer. The expression levels of inflammatory factors and oxidative stress factors were tested via enzyme-linked immunosorbent assay (ELISA) [14], including tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px).

(3) Detection of myocardial apoptosis: After the rats in the three groups were anesthetized, 2-3 ml 10% potassium chloride was injected to arrest and relax their heart, and after death the hearts were cut out after thoracotomy, and the cultured myocardial cells were isolated. According to the instructions of Annexin V-FITC/PI double staining apoptosis kit, the apoptosis rate of myocardial cells in each group was tested via flow cytometry. Cells were digested with trypsin and washed twice with PBS. Next, they were collected into centrifuge tubes. First, 20 μ l Annexin-V-FITC labeling solution was added into 1 ml buffer solution. Then, 20 μ l PI reagent was added. After that, they were incubated for 5 min at room temperature under dark conditions. Finally, levels were detected through flow cytometry. To get the average value, the experiment was conducted 3 times.

(4) WB detection: 50 mg myocardial tissue was isolated and 500 μ L lysis solution was added, and then, was homogenized in an ice bath and centrifuged for 20 min at 12,000×g, 4°C. The supernatant was separated, and the protein concentration was determined by BCA kit. Afterwards, it was separated with 12% SDS-PAGE electrophoresis, ionized, and transferred to a PVDF membrane. The membrane was sealed for 5 min in 5% nonfat milk. Subsequently, it produced an immune reaction. At last, it was incubated with 1:1000 primary antibody at 4°C all night, washed to remove the primary antibody, added with 1:1000 horseradish peroxidase labeled goat anti-rabbit secondary antibody, cultivated 1 h at 37°C,

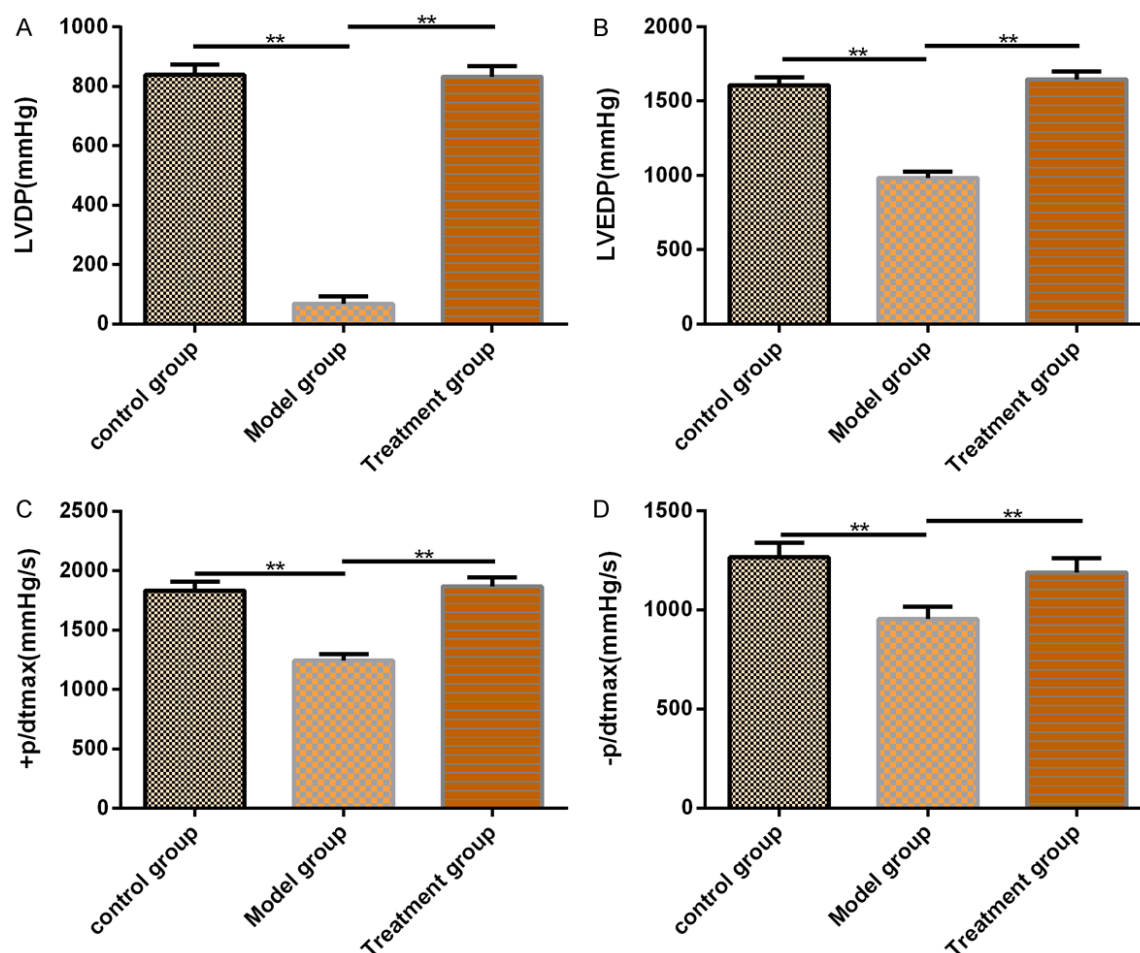


Figure 1. Comparison of cardiac function indexes of rats among three groups. A. Effect of metoprolol on LVDP in CHD modeled rats; B. Effect of metoprolol on LVEDP in CHD modeled rats; C. Effect of metoprolol on +dp/dtmax in CHD modeled rats; D. Effect of metoprolol on -dp/dtmax in CHD modeled rats. Note: compared with CG or between both groups, *P < 0.05, **P < 0.01.

rinsed 3 times with PBS, for 5 min each time. Soon afterwards, it was developed using ECL luminescent reagent and fixed, and pictures were taken by Quantity One infrared imaging system. The relative expression of the protein to be detected was equal to the gray value of the band detected/gray value of internal reference protein band.

Statistical methods

SPSS 25.0 (Easy Biotechnology Co., Ltd., Beijing, China) was used for statistical analysis. GraphPad 6 was employed for data analysis and figure illustration. All data were expressed in mean \pm SD. The comparison between the two groups was analyzed through independent-samples t test and that among multiple groups was assessed through one-way analysis of variance (ANOVA), and post hoc pairwise comparison was measured through LSD-t test. Expression at multiple time points was analyzed through repeated measures ANOVA, and back testing adopted Bonferroni. There were statistical differences when P < 0.05.

sis of variance (ANOVA), and post hoc pairwise comparison was measured through LSD-t test. Expression at multiple time points was analyzed through repeated measures ANOVA, and back testing adopted Bonferroni. There were statistical differences when P < 0.05.

Results

Comparison of cardiac function indexes of rats among three groups

We detected the cardiac function indexes of rats in the three groups, and found that compared with the CG, the LVDP, LVEDP, +dp/dtmax and -dp/dtmax indexes of the MG decreased obviously (P < 0.05), while those in the TG increased markedly after metoprolol intervention (P < 0.05), with statistically remarkable differences (Figure 1).

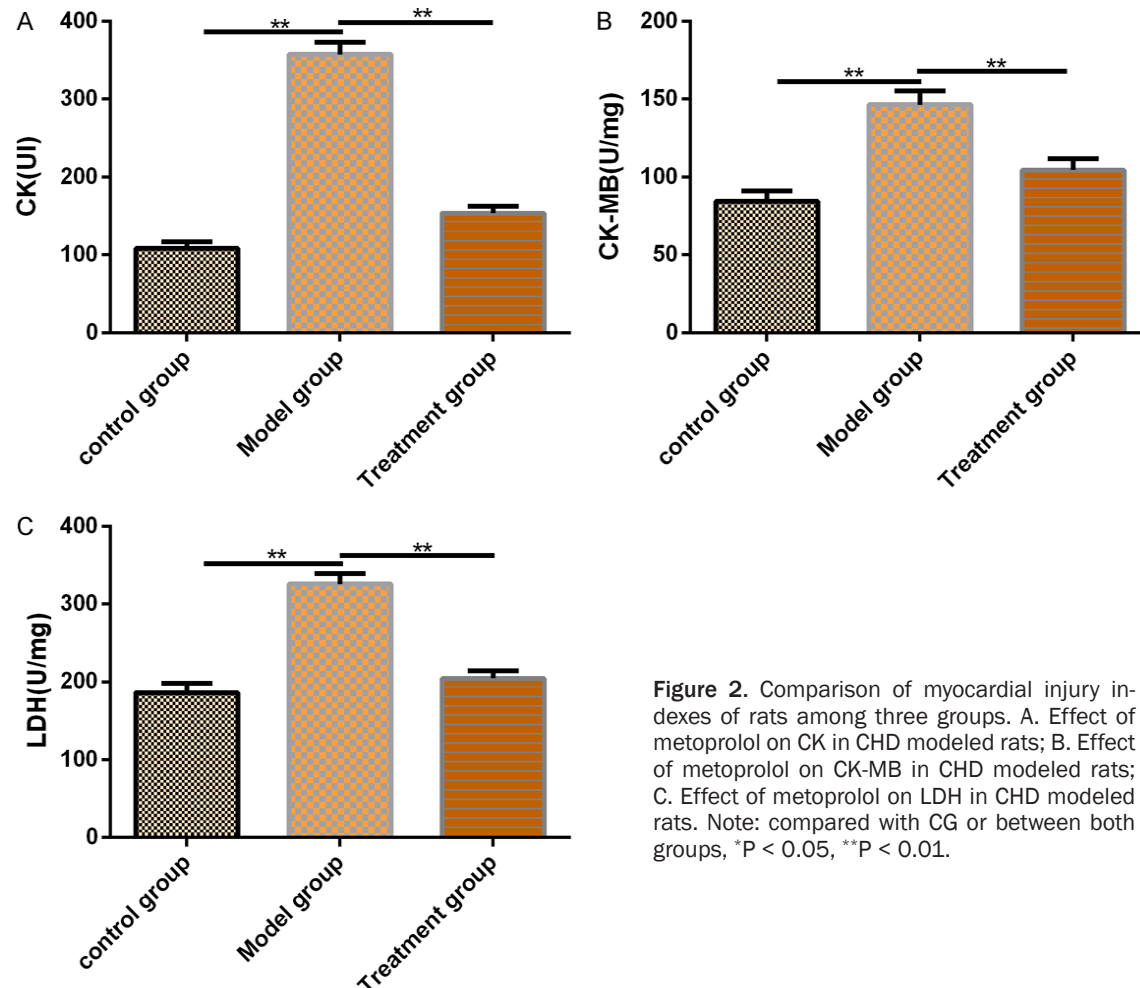


Figure 2. Comparison of myocardial injury indexes of rats among three groups. A. Effect of metoprolol on CK in CHD modeled rats; B. Effect of metoprolol on CK-MB in CHD modeled rats; C. Effect of metoprolol on LDH in CHD modeled rats. Note: compared with CG or between both groups, *P < 0.05, **P < 0.01.

Comparison of myocardial injury indexes of rats among the three groups

Compared with the CG, CK, CK-MB and LDH concentrations in the MG increased remarkably ($P < 0.05$), while those in the TG decreased dramatically after metoprolol intervention, and the differences were statistically remarkable ($P < 0.05$) (**Figure 2**).

Metoprolol's effect on blood lipids in CHD modeled rats

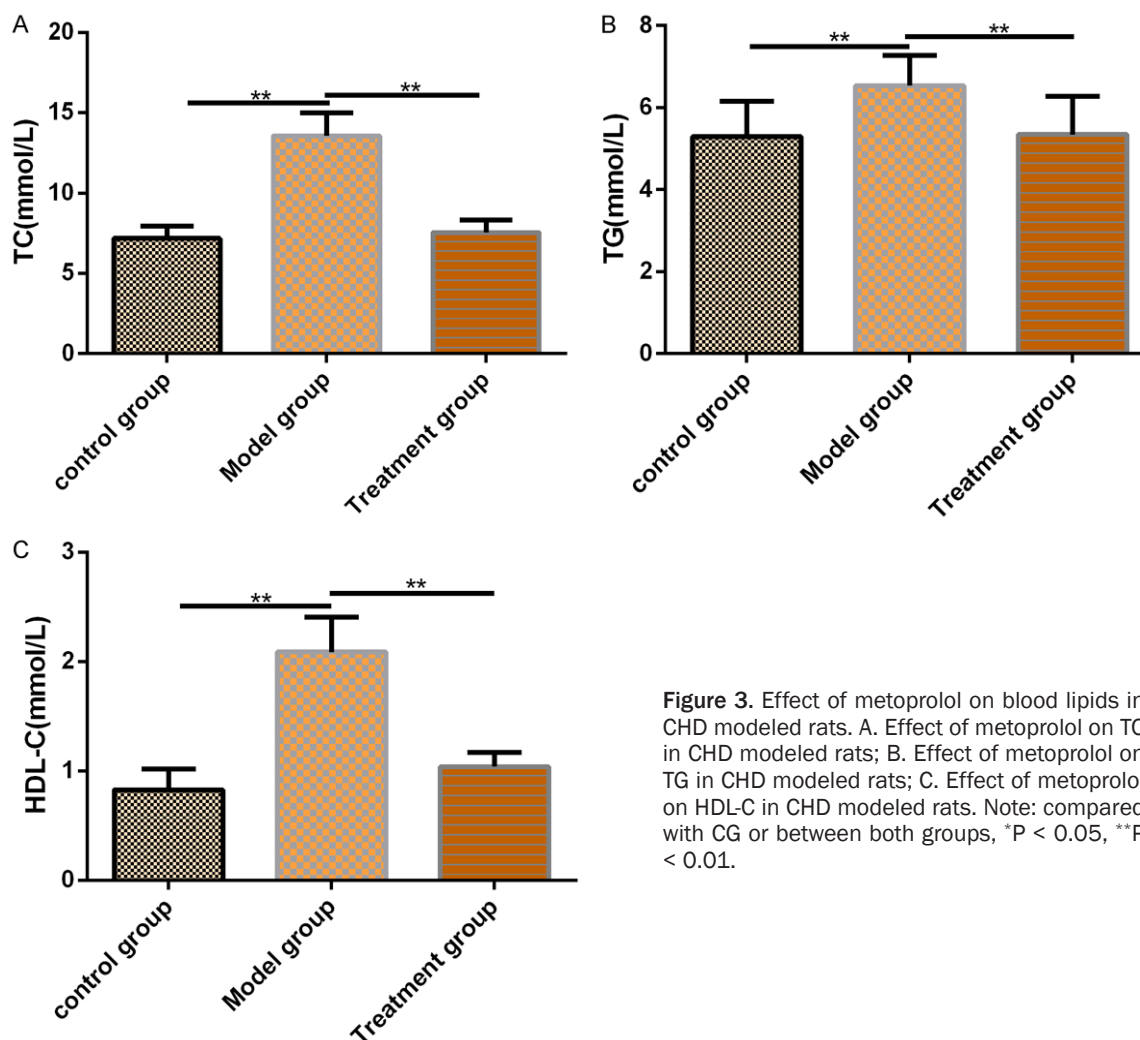
We examined the effect of metoprolol on blood lipids in CHD modeled rats, and found that compared with the CG, the TC, TG and HDL-C expression levels in the MG increased dramatically ($P < 0.05$), while the levels in the TG decreased dramatically after metoprolol intervention ($P < 0.05$) (**Figure 3**).

Metoprolol's effect on inflammatory factors in CHD modeled rats

We examined the influence of metoprolol on serum inflammatory factors in CHD modeled rats, and found that compared with the CG, the concentrations of TNF- α , IL-6 and IL-1 β in the MG increased obviously ($P < 0.05$), while the concentration in the TG decreased markedly after metoprolol intervention (**Figure 4**).

Metoprolol's effect on oxidative stress in CHD modeled rats

We examined metoprolol intervention's effect on oxidative stress in CHD rats, and found that compared with the CG, the MDA expression in serum of the MG increased markedly, while SOD, CAT and GSH-Px decreased obviously ($P < 0.05$). After metoprolol intervention, it was



found that the MDA expression in serum of the TG decreased markedly, while all three increased markedly, with statistically marked differences (Figure 5).

Metoprolol's effect on myocardial apoptosis and related factors in CHD modeled rats

The apoptosis of myocardial cells of rats in all three groups was observed. We analyzed via flow cytometry, and confirmed that compared with the CG, the apoptosis rate of myocardial cells in the MG increased markedly ($P < 0.05$), but after metoprolol intervention, apoptosis was alleviated and the rate in the TG decreased ($P < 0.05$). Compared with the CG, expression of apoptosis-related proteins caspase-9, caspase-3 and Bax and apoptosis rate in the MG increased dramatically, while apoptosis-

related protein Bcl-2 expression and cell viability reduced markedly. However, the TG treated with metoprolol had significantly reversed conditions to the above situation (Figure 6).

Discussion

CHD is a major disease that threatens human life and health, and it is also a main cause of death [15]. A clinical study has observed a variety of pathological and physiological abnormalities in CHD patients, including lipid content, oxidative stress, dysfunction of the autonomic nervous system, inflammation, and endothelial dysfunction [16]. This also shows that although great progress has been made in treating CHD, the morbidity and mortality have not decreased markedly and have caused great social and economic burdens [17].

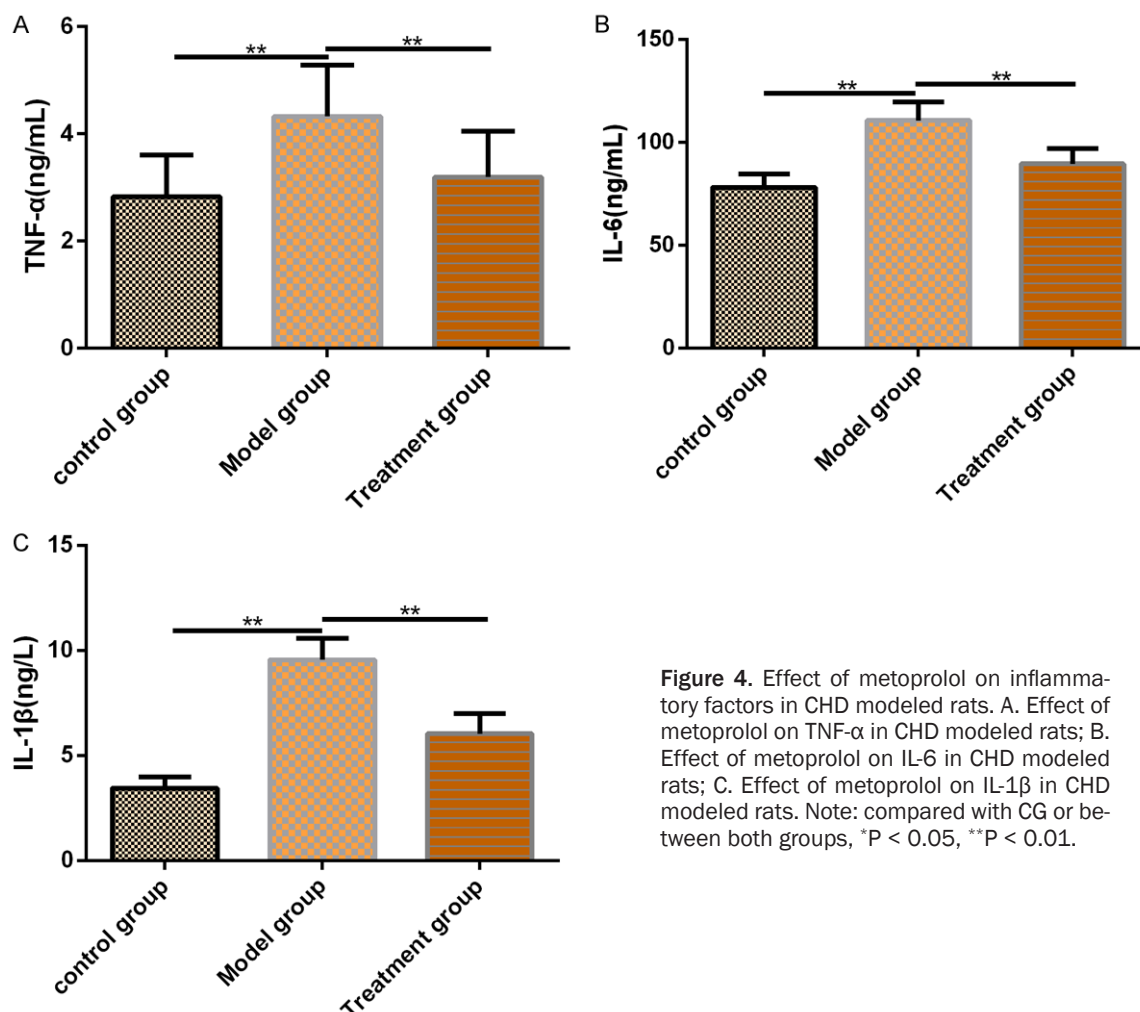


Figure 4. Effect of metoprolol on inflammatory factors in CHD modeled rats. A. Effect of metoprolol on TNF-α in CHD modeled rats; B. Effect of metoprolol on IL-6 in CHD modeled rats; C. Effect of metoprolol on IL-1β in CHD modeled rats. Note: compared with CG or between both groups, *P < 0.05, **P < 0.01.

A previous study has shown that metoprolol is a β -adrenergic blocker, which selectively blocks β_1 receptors, and it reduces adrenaline and norepinephrine, thus decreasing the stimulation of the sympathetic nervous system [18]. However, there are few studies on the myocardial protection of metoprolol on CHD and its influence on myocardial apoptosis. Thus, this experiment was designed to explore the myocardial protection of metoprolol on CHD rats. The results revealed that metoprolol can effectively reduce inflammatory cells, improve lipid content and oxidative stress levels, and promote cardiomyocyte apoptosis. For instance, studies have shown that metoprolol can improve the heart and motor function of patients with chronic mental failure [19]. This study revealed that LVDP, LVEDP, +dp/dtmax and -dp/dtmax in the MG decreased dramatically, while those in the TG increased markedly after meto-

prolol intervention, which indicated that metoprolol can significantly improve the cardiac function and cardiac exercise ability of CHD rats. Studies have shown that [20] the expression of serum LDH, CK and CK-MB in CHD patients increased obviously, similar to the results of this study. It was found that CK, CK-MB and LDH concentrations in serum of CHD modeled rats increased markedly, while the concentrations in the TG decreased markedly after metoprolol intervention, indicating that metoprolol could effectively improve the cardiac function of CHD modeled rats, stabilize their hemodynamics and reduce myocardial injury. A recent study has shown that dyslipidemia is a high risk factor for CHD [21]. It turned out that the TC, TG and HDL-C expression levels in serum of the MG increased markedly, while the levels in the TG decreased markedly after metoprolol intervention, indicat-

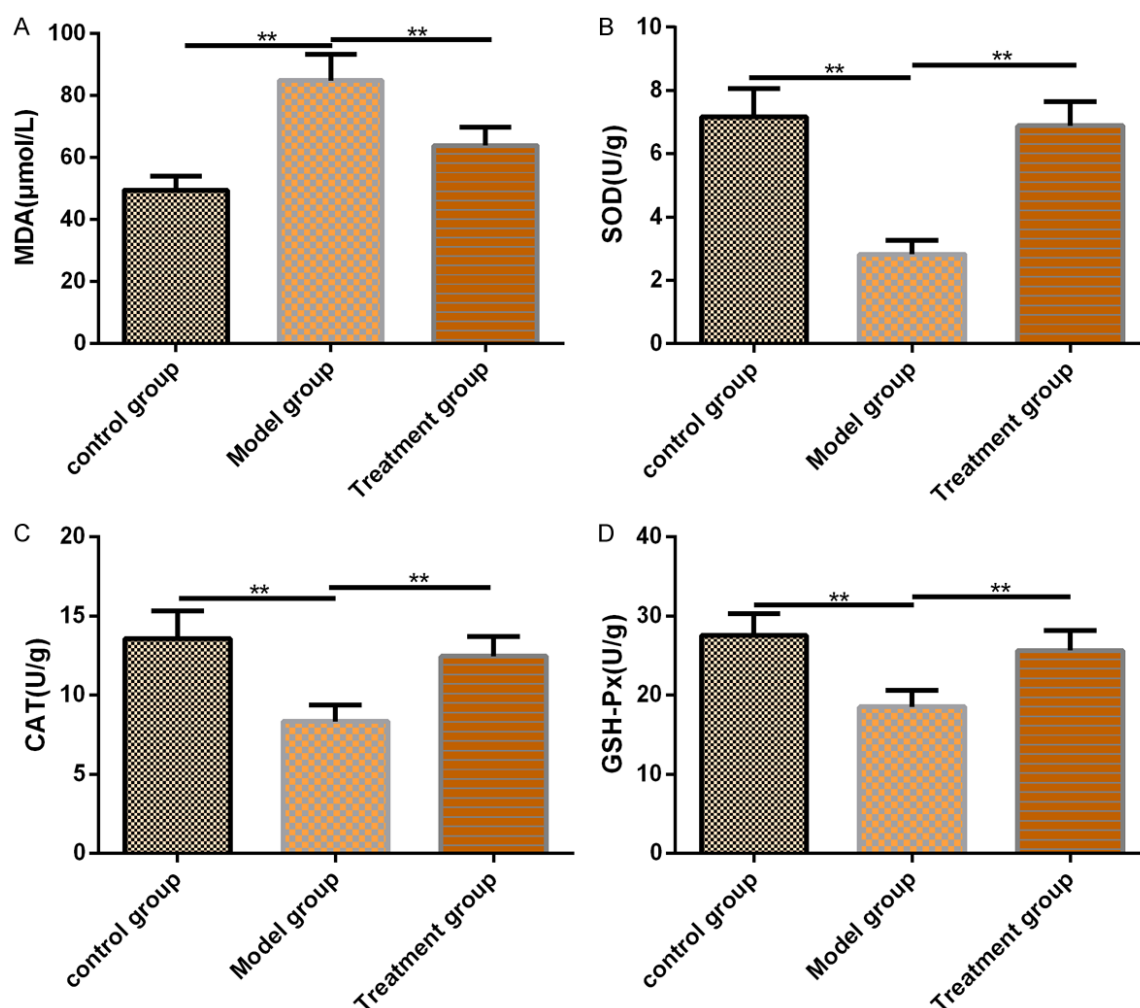


Figure 5. Effect of metoprolol on oxidative stress in CHD modeled rats. A. Effect of metoprolol on MDA in CHD modeled rats; B. Effect of metoprolol on SOD in CHD modeled rats; C. Effect of metoprolol on CAT in CHD modeled rats; D. Effect of metoprolol on GSH-Px in CHD modeled rats. Note: compared with CG or between both groups, * $P < 0.05$, ** $P < 0.01$.

ing that metoprolol intervention could further improve blood lipid levels and thus has a good lipid-lowering effect.

Clinically, inflammation plays a key part in atherosclerotic disease development and progression. For example, IL-6 is regarded as an upstream inflammatory cytokine, which acts as a mediator of inflammatory reactions [22]. For example, Lu Y et al. [23] pointed out that metoprolol intervention could reduce the TNF- α , IL-10 and IL-1 β expression levels in inflammatory cells and improve left ventricular function. It showed that TNF- α , IL-6 and IL-1 β concentrations in the serum of rats in the MG increased obviously, while the concentrations in the TG decreased markedly after metoprolol treat-

ment. It indicated that metoprolol could reduce the release of inflammatory factors in rat heart, so as to protect the heart. This study also observed the expression of oxidative stress indicators in three groups of rats, which showed that oxidative stress plays a vital role in CHD pathogenesis [24]. Metoprolol intervention in patients with myocardial infarction can reduce their expression of inflammatory factors and oxidative stress [25]. Also, in this study, the MDA expression in serum of rats in the MG increased markedly, while SOD, CAT, GSH-Px decreased dramatically. After metoprolol intervention, it was found that the expression in serum of rats in the TG decreased obviously, while those three increased markedly, indicating that metoprolol regulated oxidative stress

Protective effect of metoprolol on the myocardium in rats modeled with CHD

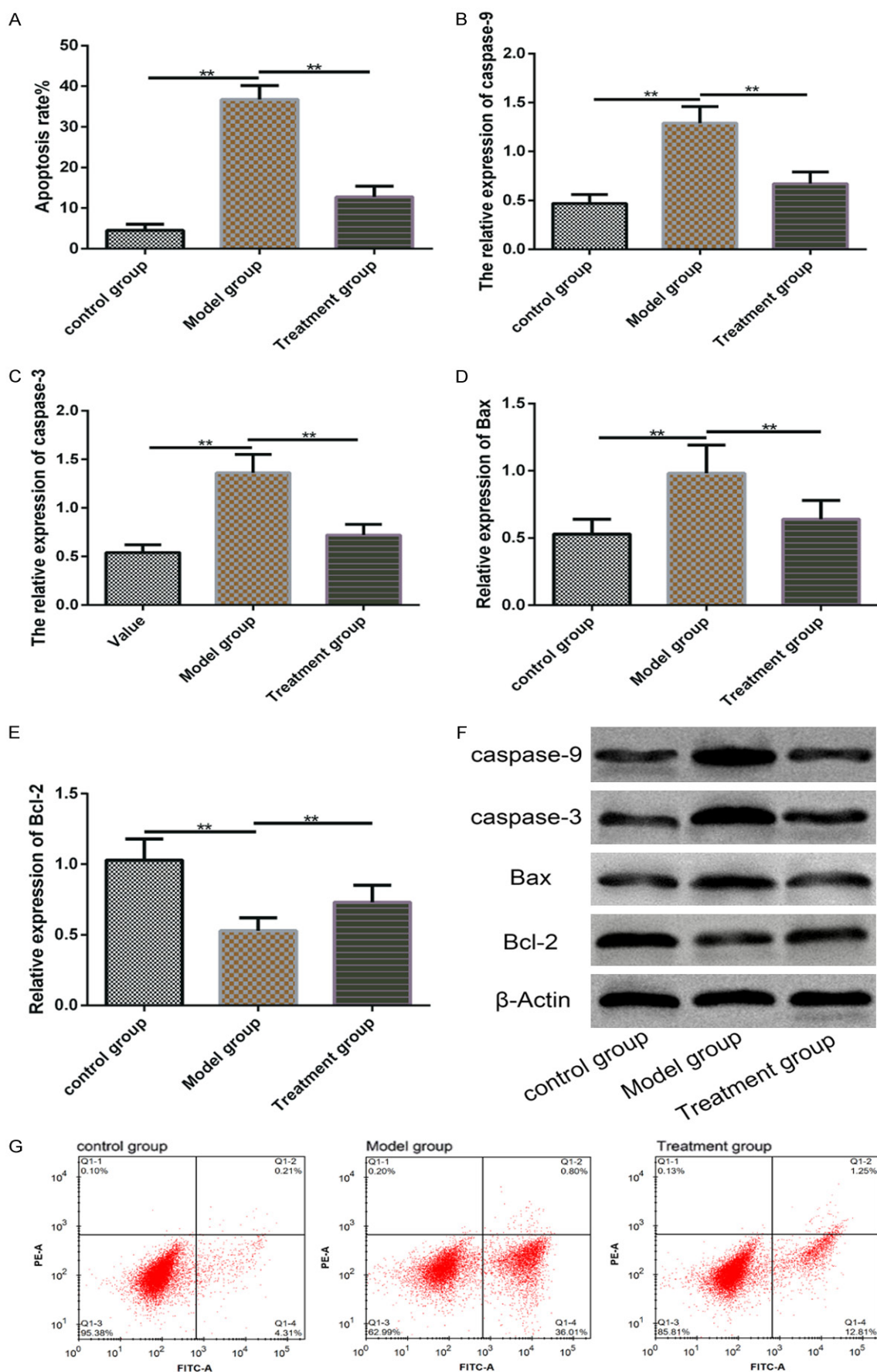


Figure 6. Effect of metoprolol on myocardial apoptosis and related factors in CHD modeled rats. A. Effect of metoprolol on myocardial apoptosis rate in CHD modeled rats; B-E. Effects of metoprolol on caspase-9, caspase-3, Bax and Bcl-2 in myocardial cells of CHD modeled rats; F. Protein map; G. Flow cytometry of apoptosis. Note: compared with CG or between both groups, *P < 0.05, **P < 0.01.

response in CHD rats, which could reduce the level of peroxide products and help control the disease. A recent study has shown that [26] the rate of myocardial cell apoptosis in CHD patients increased markedly, and that apoptosis is the main factor leading to myocardial dysfunction. We found that metoprolol could alleviate the apoptosis injury of myocardial cells in CHD rats, inhibit the transcription level of pro-apoptotic genes caspase-3, caspase-9 and Bax, and increase the anti-apoptotic gene Bcl-2 expression. However, there are still some limitations to our study. First of all, we also intend to carry out different doses of metoprolol in our research. Secondly, the human application of metoprolol needs to be verified in clinical practice.

Metoprolol can reduce the degree of myocardial injury, inhibit inflammatory reactions and oxidative stress *in vivo*, reduce myocardial apoptosis and improve myocardial ischemia in CHD modeled rats.

Disclosure of conflict of interest

None.

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