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Maslinic acid in the treatment of heart damage in obesity hypertension through activating Nrf2 pathway

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Abstract

The present study is to explore the interventional effects and potential mechanisms of Maslinic acid on heart damage in obesity hypertension. The rats were given SHR high-fat diet for 16 weeks. WGA staining and Masson staining were applied to detect heart structure and heart fibrosis degree. WB method was used to detect the expressions of ANP, BNP, P-MHC, TGF-β1, CTGF, Collagen I, Collagen III, Fibronectin and other proteins in SHR heart tissue. ELISA method was used to detect serum TNF-α, IL-6, and PAI-1 content. IF method was employed to detect the expression of Nrf2 in heart tissue. Maslinic acid can significantly improve the morphology of the heart tissue, reduce the hypertrophy and swelling of cardiomyocytes, inhibit the deposition of collagen fibers, and significantly inhibit the expressions of ANP, BNP, MYH7, CTGF, TGFpl, Fibronectin, CollagenI and CollagenIII proteins in the heart tissue. Maslinic acid can effectively inhibit TNF-a, IL-6 and PAI-1 expression, reduce heart inflammation, and significantly elevate Nrf2 level in heart tissue. Maslinic acid can significantly improve the heart structure of obese SHR, inhibit myocardial hypertrophy, cardiac fibrosis, and cardiac inflammation. The underlying mechanism may be related to elevating the expression of Nrf2 protein in the cytoplasm of heart tissue, promoting Nrf2 to enter the nucleus, thereby promoting the expression and activity of mitochondria-function-related proteins. The capabilities to inhibit the expression of hypertrophy-related proteins are also related.

Keywords: maslinic acid; obesity; hypertension; heart damage.

Practical Application: Maslinic acid on heart damage in obesity hypertension.

1 Introduction

The heart damage of obesity hypertension mainly characterizes myocardial hypertrophy and cardiac fibrosis, cardiac redox imbalance, cardiac tissue inflammation, myocardial mitochondrial dysfunction and energy metabolism imbalance, abnormal myocardial autophagy, myocardial cell necrosis and apoptosis. These pathological changes are independent and can influence each other to jointly promote the occurrence and development of heart damage (Termklinchan et al., 2019; Chen et al., 2019; Wang et al., 2019a). The cardioprotective effect of Nrf2 is a hot topic in recent research. Nrf2 is a transcription factor belonging to the leucine zipper family.

It is found that the expression and transcriptional activity of Nrf2 in heart tissue were significantly inhibited in the obese hypertension animal model. A series of studies have shown that heart tissue Nrf2 is an important regulatory molecule that can play a protective role in heart damage caused by obesity, diabetes and hypertension. First of all, in obesity, diabetes and hypertension disease models induced by high-fat, high-sugar and high-salt diets for a long time, the expression and/or transcriptional activity of Nrf2 is often significantly inhibited, and the degree of inhibition of the expression and or transcriptional activity of Nrf2 is often positively correlated with the degree of heart damage. Secondly, when Nrf2 knockout animals are fed with high fat, high sugar and high salt diet alone, their myocardial hypertrophy, fibrosis, peroxidation and inflammation will be more severe than wild-type animals (Liu et al., 2017; Wang et al., 2019b; Ding et al., 2015).

Maslinic acid has multiple pharmacological effects such as anti-inflammatory, anti-oxidative stress, anti-myocardial cell apoptosis, protecting vascular endothelial function, improving blood rheology, regulating lipid metabolism disorders, anti-thrombosis and platelet aggregation, increasing coronary blood flow, and improving myocardial blood deficiency, anti-ventricular remodeling after myocardial infarction, promoting angiogenesis, reducing infarct size and others. Our research is to explore whether Maslinic acid can treat obese hypertensive heart damage by activating Nrf2 pathway.

2 Methods

2.1. Animals and drug administration

60 male 6-week-old SHRs were randomly divided into 4 groups: the standard diet group (STD), the high-fat diet group (HFD) (high-fat diet for 16 consecutive weeks), the 20 mg/kg Maslinic acid administration group (16 consecutive weeks of high-fat diet, plus 20 mg/kg Maslinic acid administration for 16 weeks), and the 40 mg/kg Maslinic acid administration group

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(16 consecutive weeks of high-fat diet, plus 40 mg/kg Maslinic acid administration for 16 weeks) .

2.2 WGA fluorescent staining.

Heart tissues were obtained from each group of rats. WGA fluorescence staining was performed according to the instructions. Images were taken under a fluorescence microscope.

2.3 Masson dyeing.

Heart tissues were obtained from each group of rats. Masson staining was performed according to the instructions. Following this, the tissues were examined and photographed under a microscope.

2.4 ELISA method to detect the contents of TNF-α, IL-6 and PAI-1 in serum.

The serum of each group of rats was collected, and the contents of TNF-α, IL-6, and PAI-1 in the serum were detected according to the instructions of the ELISA kit.

2.5 IF method to detect the expression of Nrf2 in heart tissue.

Heart tissues were obtained from each group of rats. The expression of Nrf2 in the heart tissues was detected according to the instructions, and images were taken under a fluorescence microscope.

2.6 WB method to detect the expression of each protein.

The heart tissues of each group were minced into small pieces, and homogenized in the cell lysate. The tissues were centrifuged at 12000g. Following this, the supernatant was aspirated, and the protein concentration was detected by the BCA method. After adding the SDS-PAGE loading buffer, the same amount of protein samples was drawn from each sample. The samples were added into 8%, 10%, and 12% precast gels for gel electrophoresis. After this, the membrane was transferred and blocked with Quickblock. The tissues were incubated with primary antibody, then incubated with HRP-labeled goat anti-rabbit. HRP fluorescence intensity was detected with ECL luminescent solution.

2.7 Statistical analysis

The results are expressed as mean ± standard deviation (Mean ± SD). The comparison between two groups was conducted using t-test, and the comparison between multiple groups was performed using one-way analysis of variance (one-way ANOVA) and two-way analysis of variance (two-way ANOVA). GraphpadPrism7 software was employed.

3 Results

3.1 The effect of Maslinic acid on the cross-sectional area of cardiomyocytes from SHR fed with high fat diet.

The results showed that, after sixteen weeks of high-fat feeding, the cross-sectional area of cardiomyocytes in the SHR of the HFD group was significantly larger than that of the STD group. After 16 weeks of continuous drug intervention, compared with the SHR of the HFD group, the cross-sectional area of the cardiomyocytes of the SHR was significantly reduced, as shown in Figure 1.

3.2 The effect of Maslinic acid on heart tissue fibrosis induced by high-fat feeding in SHR

Compared with the SHR of the HFD group after the drug intervention, the degree of fibrosis in the administration group was relieved to a certain extent, while the degree of fibrosis of SHR was significantly reduced, as shown in Figure 2.

3.3 The effect of Maslinic acid on the contents of TNF-α, IL-6 and PAI-1 in serum of SHR induced by high-fat feeding.

The results showed that, compared with the SHR of the HFD group, the contents of TNF-a, IL-6, and PAI-1 in the serum of the SHR administration group were all reduced to some extent, as shown in Figure 3.

3.4 The effect of Maslinic acid on various proteins.

The effect of Maslinic acid on the expression of myocardial hypertrophy marker protein in the heart tissue of high-fat fed SHR was significantly lower than the expressions of ANP, BNP and MYH7 proteins in the administered SHR in the HFD group. As to the cardiac fibrosis marker protein in the heart tissue of SHR, the expressions of TGF-βl, CTGF, Collagen I, Collagen III and Fibronectin proteins in the heart tissue of SHR of the HFD group were significantly higher than that of the SHR in the STD group. However, all the proteins were significantly reduced after drug administration. See Figure 4.

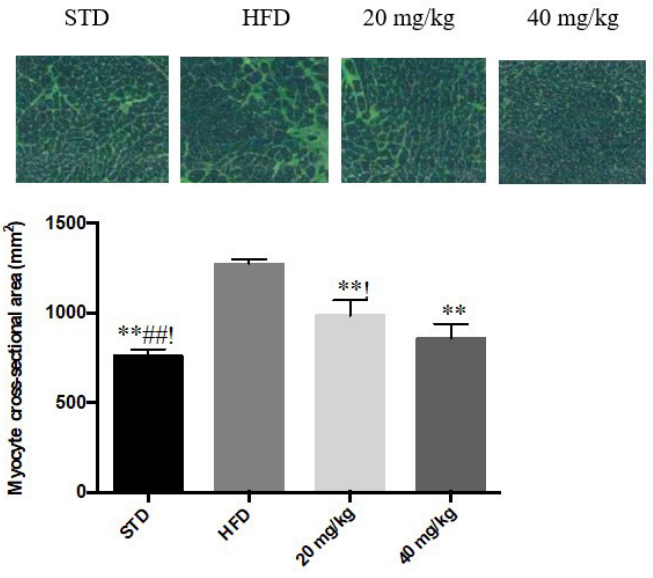

Figure 1. The effect of Maslinic acid on the cross-sectional area of cardiomyocytes from SHR fed with high fat diet. ** $p < 0.01$ vs HFD ## $p < 0.01$ vs 20 mg/kg ! $p < 0.05$ vs 40 mg/kg

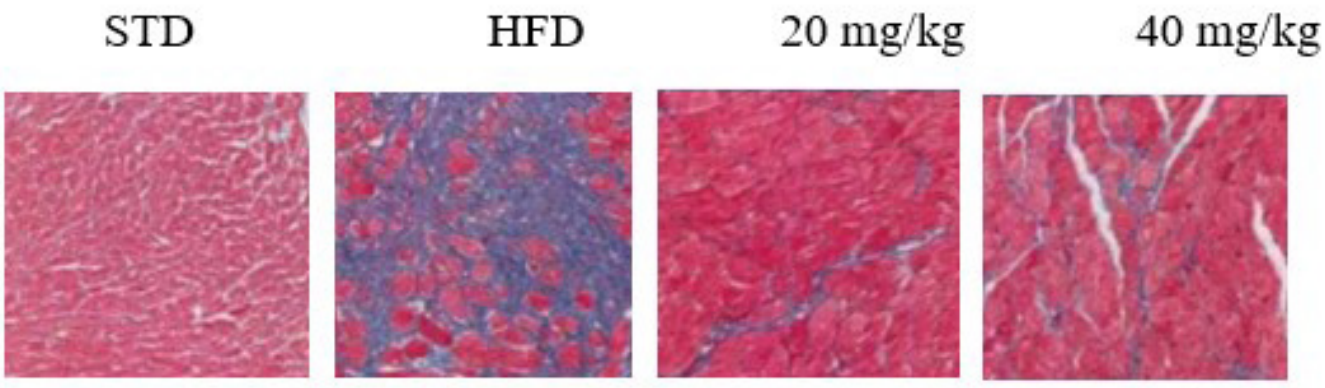

Figure 2. The intervention results of Maslinic acid on SHR cardiac tissue fibrosis induced by high-fat feeding. ** $p < 0.01$ vs HFD ## $p < 0.01$ vs 20 mg/kg ! $p < 0.05$ vs 40 mg/kg !! $p < 0.01$ vs 40 mg/kg

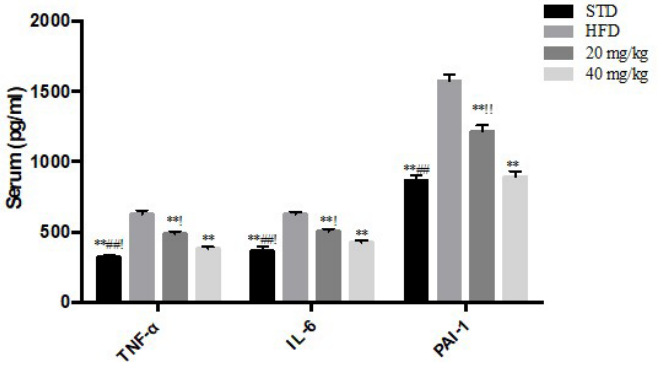

Figure 3. The effect of Maslinic acid on the inflammatory factor content in the serum of SHR induced by high-fat feeding.

The effect of Maslinic acid on the expressions of NRF-1, HO-1, SOD2 and TFAM in the heart tissue of SHR induced by high-fat feeding were detected. Compared with the SHR of the HFD group, the expressions of NRF-1, HO-1, SOD2 and TFAM in the administration group has been improved, as shown in Figure 5.

3.5 The effect of Maslinic acid on the expression of Nrf2 in the heart tissue of SHR induced by high-fat feeding.

The result showed that the Nrf2 expression of the 40 mg/kg group is the most obvious, as shown in Figure 6.

4 Discussion

For myocardial injury factors such as neurohumoral activation, hypertension, and obesity, the heart is initially hypertrophic with myocardial adaptive hypertrophy, which has since compensated for it. However, continuous myocardial hypertrophy is harmful and can exacerbate heart failure and lead to sudden death (Xiao et al., 2016). In terms of cardiac morphology, WGA fluorescent staining results showed that the cross-sectional area of cardiomyocytes of SHR in the HFD group was significantly increased, and the intervention of Maslinic acid

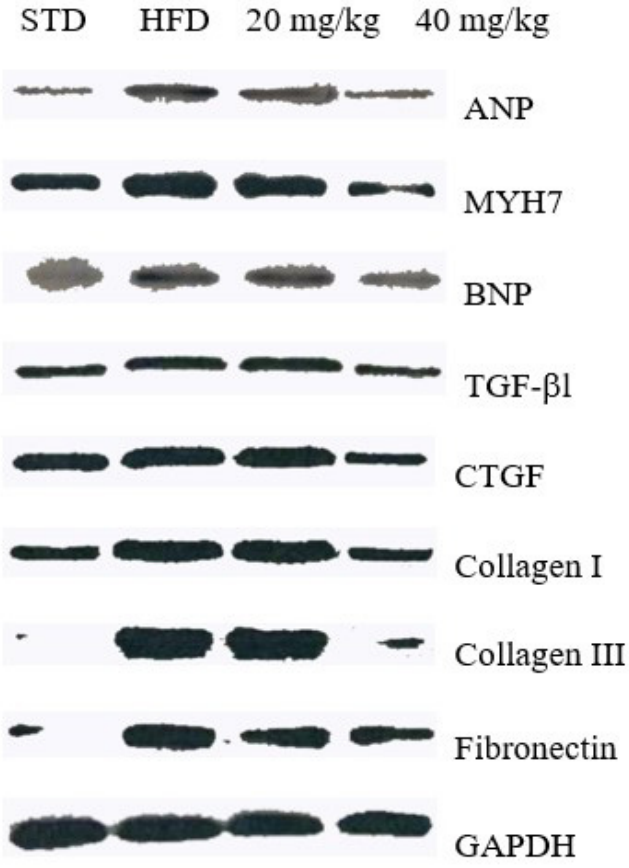

Figure 4. The effect of Maslinic acid on various proteins.

could significantly reduce the surface area of cardiomyocytes. In terms of mechanism, the results of WB showed that high-fat feeding can significantly induce the expressions of ANP, BNP and MYH7 proteins, while the intervention with Maslinic acid can significantly inhibit the expressions of ANP, BNP and MYH7.

The heart shows strong plasticity in the damage of various pathological factors. This process is considered as pathological

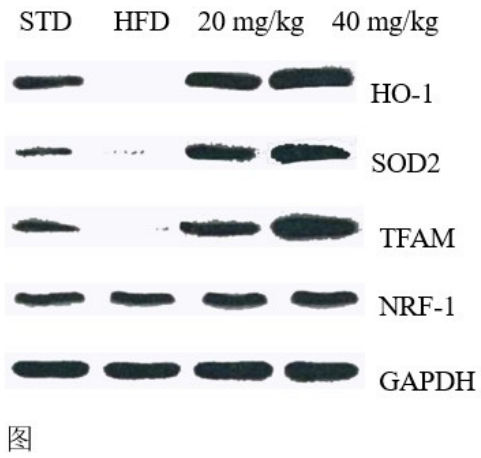

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Figure 5. The effect of Maslinic acid on each protein.

remodeling (Yang et al., 2017). Pathological myocardial remodeling is characterized by a process called myocardial fibrosis, which can lead to excessive accumulation of extracellular matrix. Physiologically, the extracellular matrix provides a structural scaffold for cardiomyocytes, shares mechanical forces in the myocardial tissue, and mediates the conduction of electrical pulses. Cardiac fibrosis is the ultimate common pathway for many heart damages. Extensive myocardial fibrosis will aggravate myocardial stiffness, worsen diastolic function, and ultimately lead to heart failure (Jing et al., 2019). Cardiac fibrosis reaction often suggests the aggregation of collagen fibers in phenotype, characterizing in mechanism the increased expressions of fibrotic mediators such as CTGF, TGF-β, Fibronectin, CollagenI and Collagen III proteins.

Inflammation is part of the non-specific immune response, occurring in response to any type of physical injury (Benjamin et al.,

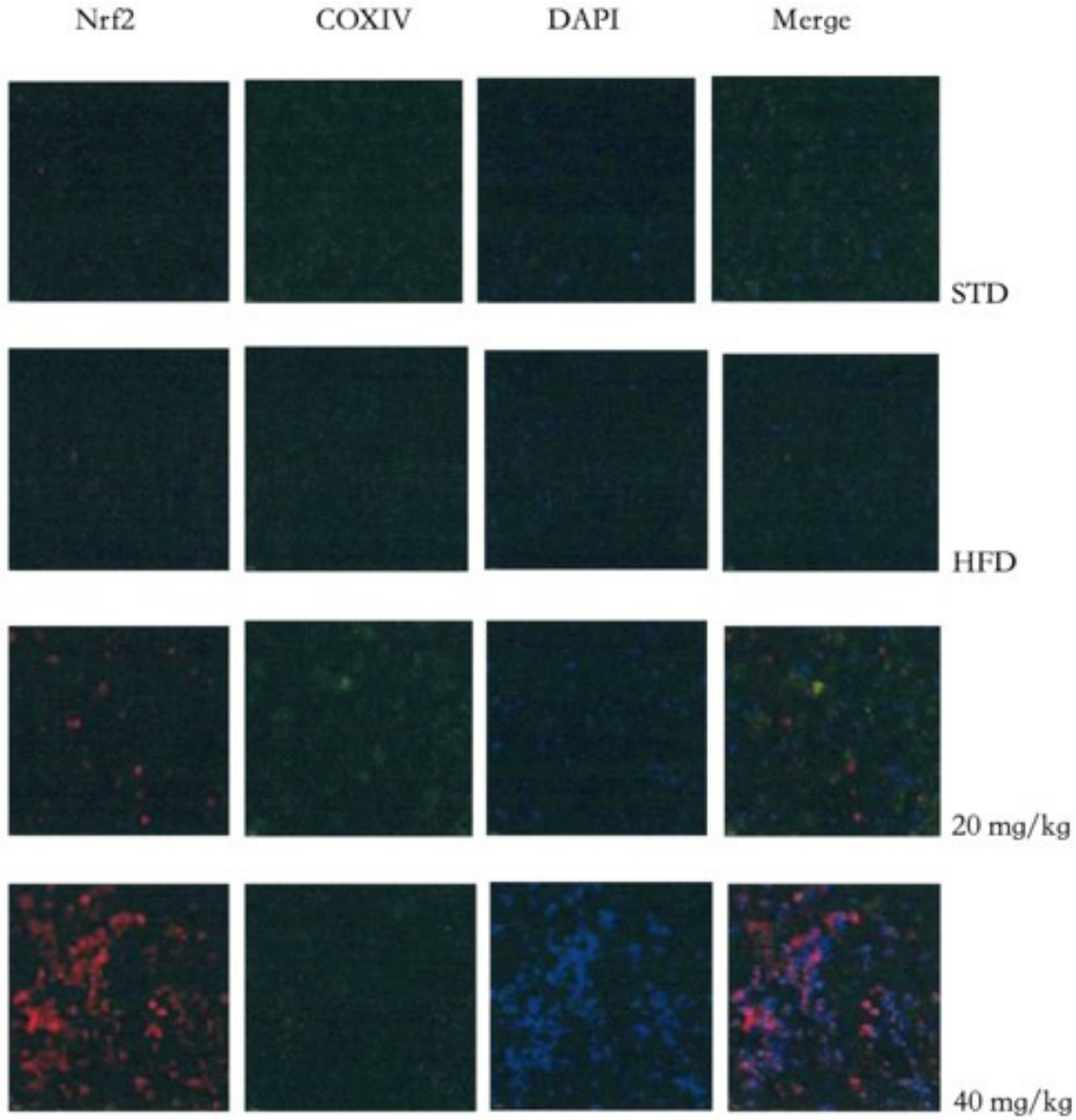

Figure 6. Maslinic acid on the expression of Nrf2 in the heart tissue of SHR induced by high-fat feeding.

2018). More and more evidences show that inflammation plays an important role in the occurrence and development of heart failure and myocardial infarction. Obesity can cause adipose tissue to present a chronic and persistent low-inflammatory state. This low-inflammatory state is also called adipose tissue microenvironment disorder, which is characterized by increased secretion of pro-inflammatory factors such as TNF-α, IL-6, and PAl-1, affecting other tissues and organs, and inducing insulin resistance, diabetes, and cardiovascular disease, etc. (Stojanoska et al., 2017). Our research showed that high-fat diet feeding can significantly increase the expressions of TNF-α, IL-6 and PAI-1 proteins in the serum and heart. However, the use of Maslinic acid, especially high doses, can effectively inhibit these three inflammatory factors, suggesting that Maslinic acid can effectively reduce heart inflammation.

In long-term obesity, diabetes and high blood pressure disease models induced by high-fat, high-sugar and high-salt diets, the expression and/or transcriptional activity of Nrf2 are often significantly inhibited. Firstly, increasing the expression of Nrf2, promoting its nucleus and binding with ARE, can activate its downstream antioxidant proteins, promote the production and metabolism of GSH, reduce the content of ROS, and inhibit the degree of peroxidation of DNA, protein, and lipids in cardiomyocytes, thus protecting cardiomyocytes. Secondly, activating Nrf2 can inhibit myocardial hypertrophy by inhibiting the expression of ANP, BNP, and P-MHC, etc. Third, activating Nrf2 can inhibit the expression of TGF-β, CTGF, Collagen I/III, and Fibronectin, thereby inhibiting the deposition of cardiac collagen fibers and combating cardiac fibrosis. Fourth, up-regulation of Nrf2 can inhibit the content of pro-inflammatory factors such as TNF-cu, IL-6, and PAI-1), thereby inhibiting myocardial inflammation. Fifth, Nrf2 can increase the regulation of myocardial mitochondrial biosynthesis by up-regulating HO-1, NRF-1, TFAM, and SOD2, and improve the structure and function of mitochondria (Reddy et al., 2018). Our research showed that high-fat diet can significantly reduce the level of Nrf2 protein in the heart tissue, and the intervention of Maslinic acid can significantly increase the expression of Nrf2 in the heart tissue. Therefore, the protective effect may be related to the activation of Nrf2.

5 Conclusions

In summary, Maslinic acid can significantly improve the heart structure of obese SHR, inhibit myocardial hypertrophy, cardiac fibrosis, and cardiac inflammation. The underlying mechanism may be related to increasing the expression of Nrf2 protein in the cytoplasm of the heart tissue and promoting Nrf2 to enter the nucleus, thereby promoting the expression and activity of mitochondrial function-related proteins, and inhibiting the expression of hypertrophy-related proteins.

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