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Influence of naringin on the myocardial ultrastructure and NF-κB expression in rats with diabetic cardiomyopathy

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We investigated the effects of naringin on myocardial nuclear factor-kappa B (NF-κB) expression in rats with diabetic cardiomyopathy (DC), and its protective effect on DC. Wistar rats were randomly assigned into a normal control group, DC group and naringin intervention group. The DC rat model was established using high-sugar, high-fat feeding and streptozotocin (STZ) injection. The ultrastructural changes in the myocardial tissues were observed under electron microscopy; NF-κB expression in the myocardia was detected using immunohistochemistry. The electron microscopy results showed that the myocardial arrangement in the control group and the naringin intervention group were better than in the diabetic group, with little fibrosis. Compared with the control group, the myocardial NF-κB expression was significantly increased in the diabetes group and the intervention group (p < 0.05), and the NF-κB expression in the intervention group was significantly lower than in the diabetic group (p < 0.05). Naringin reduces the NF-κB expression in myocardial cells, which prevents structural changes, and protect them from DC.

Key words: Diabetes, cardiomyopathy, naringin, nuclear factor–kappa B (NF-kB), inflammation.

INTRODUCTION

Clinical and experimental studies have shown that inflammatory reactions possibly play an important role in the development of diabetic cardiomyopathy (DC) (Dasu et al., 2008; Jiao et al., 2009; Van Linthout et al., 2007; Westermann et al., 2009; Lorenzo O et al., 2011). Nuclear factor-kappa B (NF-kB) is the key factor in the inflammation signal pathway. The NF-κB family includes five subsets: NF-κB1 (p50/p105), NF-κB2 (p52/p100), P65 Rel A, Rel B and c-Rel 5. Among these subsets, the P50/P65 heterodimer is the most common (Neurath et al., 1998). NF-kB occurs nearly in all types of tissues and cells, and it participates in the signal transduction of many inflammatory reactions. Numerous studies have investigated the effectiveness of anti-inflammatory drugs as myocardial protectants for preventing. However, drugs that effectively prevent DC and inhibit inflammation have not been developed (Adeghate et al., 2008; Aneja et al., 2008; Boudina and Abel, 2007, 2010; Opie et al., 2011). Therefore, finding potential anti-inflammatory drugs, especially those derived from anti-inflammatory traditional Chinese medicine, and under-standing their mechanisms of action would be academically and clinically significant.

Naringin is the main effective component of the Herbal drugs such as Rhizoma Drynariae, Fructus Aurantii Immaturii, Fructus Aurantii and Exocarpium Citri Rubrum. Its glycoside ligand is 4′,5,7-hydroxy flavone, and its saccharide constituent is rhamnose β-1,2-glucose. In recent years, many animal studies showed that naringin helps regulate glycolipid metabolism, has anti-inflammatory anti-oxidative stress, myocardial preservation effects (Rajadurai and Prince, 2007a, b, 2009).

In this study, we established a DC model in rats to observe changes in the expression of the key inflammatory factor NF-kB in myocardial tissues and the ultrastructure of myocardial cells. We used naringin from medicinal plants as an inflammation inhibitor to determine its effect on the pathogenesis of DC. The results provide an experimental basis for DC prevention and treatment.

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Animal grouping and establishing DC model

Specific pathogen-free (SPF) grade male Wistar rats, weighing 150 ± 180 g, were provided by the Experimental Animal Centre of Southern Medical University. The blood was collected from tail veins to ascertain whether the blood glucose level is normal or not. Thirty Wistar rats were fed adaptively for two weeks and divided into three groups: the normal control group (NC group, n = 10); the DC group (DC group, n = 10); and the naringin intervention group (NC+ naringin group, n = 10 and DC+ naringin group, n = 10). The rats in the NC group and NC+ naringin group were fed basic feed, whereas the rats in the DC group and DC+ naringin group were fed high-fat, high-sugar feed (20% sugar, 10% lard, 2.5% cholesterol and 67.5% basic feed). During the 6th week, 1% streptozotocin (STZ, U.S. Sigma Company) at 30 mg/kg dose (pH = 4.5, freshly prepared at 4°C) was injected once intraperitoneally. After 72 h, the fasting blood glucose levels of the rats were determined. The rats with fasting blood glucose levels exceeding 11.1 mmol/L were considered successful type 2 DM rat models. They were continuously fed high-fat, high-sugar feed until the 12th week. Transmission electron microscopy revealed that myocardial cells developed apparent pathologic changes. Therefore, the rat DC model was successfully established.

In addition, naringin powder (98% purity; Guangdong Meiyang Cyanobacteria Company) (40 mg/kg) was administered intragastrically to the NC+ naringin group and DC+ naringin group. Furthermore, distilled water was similarly administered to the NC and DC groups according to bodyweight. The rats were sacrificed at the end of 12th week.

Ultrastructure of the myocardial cells

The hearts of the sacrificed rats were rapidly taken out. A 1 mm3 sample of the left ventricular myocardium was taken, fixed with 3% glutaraldehyde, and examined under a transmission electron microscope. The myocardium was washed with phosphate-buffered saline (PBS), fixed with 1% osmic acid, progressively dehydrated with ethanol and acetone, replaced, embedded, polymerized, sliced and stained. Finally, Japan Hitachi H-600 transmission electron microscope was used for observation.

Immunohistochemistry

The Streptavidin-Peroxidase (SP) method (SP kit was purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) was used. The specimen was embedded with paraffin wax and sliced into 4 μm sections. The sections were conventionally dehydrated and washed with water. Then, 3% hydrogen peroxide (H2O2) was used to clear endogenous peroxidase. Subsequently, the sections were soaked in 0.01 M citrate buffer solution with a pH of 6.0 and heated under high pressure for 3 min. After they were sealed with 10% normal goat serum, an NF-kB polyclonal antibody kit (Beijing Biosynthesis Biotechnology Co., Ltd.), secondary antibodies, and tertiary antibodies were added in proper order. Benzidine (DAB) staining was regulated under a microscope. Brown granules represented positive markers, and the samples were subjected to hematoxylin restaining, dehydration and vitrification. At the same time, PBS was used to replace the first antibody as a negative control.

Optical microscopy was performed at 400× magnification. For each section, 10 visual fields were randomly selected to acquire images for saving and analysing. In addition, Motic 6.0 Digital medical image analysis system was used to determine the gray value and positive optical density.

Statistical analysis

All data were processed using the SPSS 17.0 statistical software, and the measurement data were expressed as X±s. For comparison between groups and among groups, t test or variance analysis of completely random grouping was carried out. Differences with p < 0.05 were considered significant.

RESULTS

Success rate of the rat DC model and death situations

After the rats were fed high-fat, high-sugar feed for 12 weeks and intraperitoneally injected once with 1% STZ (30 mg/kg), the rat DC model was successfully established, with a 100% success rate. Only two rats died during the experiment, one in the DC group and one in the DC+naringin group. The deaths were possibly caused by excessively high blood glucose that caused diabetic ketoacidosis, infection, or other diabetic complications.

General data

Body weight, heart/body index, haemoglobin Alc (HbAlc), free fatty acids (FFA), and the homeostasis model assessment–insulin resistance index (HOMA-IR) of the DC group were significantly higher than those of the NC group (p < 0.05). These indicators for the DC+ naringin group were significantly lower than those of the DC group (p < 0.05). No significant difference was observed between the NC group and the NC+ naringin group (p > 0.05) (Table 1).

Ultrastructure of myocardial cells

The rat myocardial cells in the NC and NC+ naringin groups had numerous regularly arranged myofilaments. Light and dark bands were clear and visible; mitochondria were normally round or oval, with closely arranged developed ridges. Regularly arranged intercalated discs connected the myocardial cells. In the DC group, the myocardial intracellular myofibril content of the rat clearly decreased, while the myofilaments were arranged irregularly and sparsely. The myofilaments were partially fractured, distorted, and presented local dissolution and loss. Furthermore, the sarcomeric lengths were inconsistent, and the periodic sarcomeric structures disappeared. Light and dark bands were unclear, and the mitochondria were irregularly arranged. Some of the mitochondria were apparently swollen, with cristae that were broadened, fractured, and some even disappeared. The mitochondrial matrix density decreased, and vacuoles formed in the matrix. The regional gaps in the myocardial intercalated disc were broadened, and some were irregularly connected or fractured. Meanwhile, the sarcoplasmic matrix was also destroyed.
reticula were clearly loose and vacuoles formed within them.

In the DC+ naringin group, the myofibril content of the myocardial cells clearly increased more than in the DC control rats, and it was close to the normal level. In addition, the myofilaments were almost regularly arranged, the sarcomeres were apparent, the light and dark bands were clear, and the intercalated disc structure was nearly close to normal. Moreover, cell gap widening and mitochondrial swelling were not obvious. The cristae gaps were not broaden, and the cristae were orderly arranged. No fusion and fractures were observed and no vacuoles formed (Figure 1).

Myocardial NF-κB expression

The rat myocardial tissues in the NC and NC+ naringin groups exhibited slight NF-κB expression, and no significant difference was observed between the NC group and the NC+ naringin group (p > 0.05). The NF-κB expression in the DC group was significantly higher than that in the NC group (p < 0.05). The NF-κB expression in the DC+ naringin group was significantly lower than in the DC group (p < 0.05) (Figure 2).

DISCUSSION

DC is a lesion specific for diabetic cardiomyopathy. Rubler et al. (1972) observed a specific cardiomyopathy in diabetic patients without apparent coronary atherosclerosis. Hambly et al. (1974) initially proposed the DC concept in a further study. Although, theoretical and clinical studies have been carried out for more than 30 years, the pathogenesis of DC is still unclear. Furthermore, glycolipid metabolism disorder, insulin resistance, oxidative stress, cell apoptosis, microvascular lesions and myocardial fibrosis have all been linked to DC. The main pathologic changes in DC include myocardial hypertrophy, myocardial fibrosis, microvascular lesions, and so on. Inflammatory reactions are involved in the pathogenesis and progression of DC. NF-κB is one of the key transcription factors in B lymphocytes discovered by Sen and Baltimore in 1986. NF-κB specifically binds the enhancer sequences of immunoglobulin κ light chain gene (Neurath et al., 1998) and participate in many pathologic and physiologic processes, including the immune response, cell apoptosis, carcinogenesis and inflammatory reactions. NF-κB is involved in the “gene switching” of inflammatory reaction chain, which participates in regulating the expression of many cytokines. High blood glucose induces AGE formation and protein kinase C (PKC) activation, thereby activating NF-κB to cause microcirculation lesions (Picchi et al., 2010).

Furthermore, Mohan and colleagues (Dasu et al., 2008) proved that high blood glucose rapidly activates PKC and NADPH oxidase to induce the expression of toll-like receptors, thereby promoting intracellular reactive oxygen species (ROS) generation and NF-κB activation to cause inflammation. In addition, several studies reported that NF-κB activation increases oxidative stress and induces mitochondrial dysfunction and cardiac insufficiency in rats with type 2 DM (Mariappan et al., 2010). In this experiment, NF-κB expression in the myocardial tissues of the DC model rats was significantly higher than in the NC group (p < 0.05), and changes in the myocardial ultra-structure were indicative of significant cardiomyopathy. Therefore, the activated NF-κB–mediated inflammatory reaction during diabetes is possibly involved in the pathological damage to myocardial cells in DC.

In China, Xiong et al. (2010) reported that naringin suppresses the ROS generation induced by high blood glucose to inhibit NF-κB activation. Naringin further inhibits the expression of intercellular adhesion molecules and vascular endothelial cell adhesion molecules, thereby inhibiting inflammatory reactions. This study indicated that both the regular arrangement and structural integrity of myocardial myofilaments and mitochondria in the DC+ naringin group were better than those in the DC group, and the extent of fibrosis was milder. In addition, NF-κB expression in the myocardium of the DC+ naringin rats was lower than that of the DC group. Hamid et al. (2009) confirmed the feasibility of reducing apoptosis by suppressing NF-κB. Sandeep and colleagues (Kumar et al., 2011) showed that the NF-κB inhibitor PDCT reduces the deposition of extracellular matrix and the expression of cell adhesion molecules by suppressing NF-κB pathways, thereby relieving myocardial fibrosis. Thus, naringin reduces the myocardial damage in DC by inhibiting the inflammatory reactions mediated by NF-κB.

Table 1. Comparison of NF-κB expression and positive vascular density of rat’s myocardium in different groups (t±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gray value</th>
<th>Mean positive vascular density</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10</td>
<td>145.60±1.85</td>
<td>0.23±0.07</td>
</tr>
<tr>
<td>MC</td>
<td>9</td>
<td>111.45±3.11</td>
<td>0.34±0.22</td>
</tr>
<tr>
<td>YC</td>
<td>9</td>
<td>135.46±1.25</td>
<td>0.26±0.14</td>
</tr>
</tbody>
</table>

Compared with NC group, *p<0.05; Compared with MC, *p<0.05.
Therefore, it can be suggested that naringin delays the occurrence and progression of DC and protects the myocardium.

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REFERENCES


