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Protective effects of naringenin in cardiorenal syndrome

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ABSTRACT

Background: Cardiorenal syndrome is a complicated and bidirectional interrelationship between the heart and kidneys. Naringenin (NG) is a naturally occurring flavonoid possessing various biological and pharmacological properties.

Materials and methods: We tested whether NG could improve cardiac and renal function in a rat model of cardiorenal syndrome.

Results: The results showed that NG-attenuated cardiac remodeling and cardiac dysfunction in rats with cardiorenal syndrome, as evidenced by decrease of left ventricle weight (LVW), increase of body weight (BW), decrease of LVW/BW, decrease of concentrations of serum creatinine, blood urea nitrogen, type-B natriuretic peptide, aldosterone, angiotensin (Ang) II, C-reactive protein, and urine protein, increase of left ventricular systolic pressure and falling rates of left ventricular pressure (dp/dtmax), and decrease of left ventricular diastolic pressure, left ventricular end-diastolic pressure, and −dp/dtmax. NG significantly inhibited the increase of lipid profiles including low-density lipoprotein, TC, and TG in rats. In addition, NG significantly inhibited the increase of cardiac expression of IL-1β, IL-6, and interferon γ. Moreover, NG decreased malonaldehyde level, increased superoxide dismutase activity and glutathione content in rats, and increased the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and catalytic subunit of γ-glutamylcysteine ligase (GCLc) in rats and Ang II-treated cardiac fibroblasts. Inhibition of Nrf2 and glutathione synthesis significantly suppressed NG-induced decrease of ROS level. Inhibition of Nrf2 markedly suppressed NG-induced increase of GCLc expression in Ang II-treated cardiac fibroblasts.

Conclusions: The data provide novel options for therapy of patients and new insights into the cardioprotective effects of NG in cardiorenal syndrome.

Introduction

The heart has a complicated and bidirectional interrelationship with the kidneys.[1] Impairment of one organ could influence the other one, which in turn results in damage of both organs. This phenomenon is defined as cardiorenal syndrome.[1] Cardiovascular morbidity is considered to be the major cause of death among those patients with impaired

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renal function.[2,3] In turn, myocardial infarction has been reported to result in injury of renal function.[4] Therefore, impairment of renal and cardiovascular function forms a vicious cycle through cardiorenal interaction, leading to increase of morbidity and mortality in patients with cardiovascular and renal diseases. Numerous literature has suggested that the renin-angiotensin-aldosterone system, sympathetic nervous system, endothelial dysfunction, oxidative stress, and inflammation are involved in cardiorenal syndrome.[5-10]

Naringenin (NG) is a naturally occurring flavonoid which is widely distributed in grapefruit, lemon, and tomato.[11] NG possesses various biological and pharmacologic properties, including antioxidant, anti-inflammatory, antithrombotic, anticarcinogenic, antidiarrheal, and antiulcer activities. In the last decade, several studies have shown that NG exhibits cardiovascular and renal protective effects. Subburaman *et al.* showed that NG protected against doxorubicin-induced cardiotoxicity in a rat model.[12] Karuppagounder *et al.* found that NG-ameliorated daunorubicin-induced nephrotoxicity through inhibition of inflammation.[13] However, whether NG could improve the outcomes of cardiorenal syndrome is unclear.

In the present study, we sought to assess whether NG could improve cardiac and renal function in a rat model of cardiorenal syndrome. We found that NG attenuated cardiac remodeling and cardiac dysfunction in rats with cardiorenal syndrome. NG decreased lipid profiles, inhibited cardiac inflammation, and reduced oxidative stress. Nuclear factor erythroid 2-related factor 2 (Nrf2) and/or catalytic subunit of γ-glutamylcysteine ligase (GCLc)-regulated glutathione (GSH) synthesis contributed to NG-induced antioxidant effects and thus the cardioprotective effects against cardiorenal syndrome.

Materials and methods

Animal treatment

All the experimental procedures were approved by the Animal Ethnics Committee of the Yan'an University. All experiments were carried out in accordance with the approved guidelines. 80 Male Sprague Dawley rats (180-220 g) were purchased from Laboratory Animal Centre of Yan'an University. The animals were housed under conditions of controlled temperature (23 ± 2°C) and humidity (60%) with 12-h light and/or dark cycles.

Rat model of cardiorenal syndrome was established as previously reported.[14,15] After 1 wk of preparative administration, rats were intraperitoneally injected with 10% chloral hydrate (0.3 mL/100 g). Then, the lower pole (1/2) of the left kidney was excised. One week later, the right kidney was totally removed. After another week, rats were subcutaneously injected with 100-mg/kg isoproterenol (ISO, Sigma−Aldrich) twice at an interval of 24 h. The diet, performance and condition of rats were observed. Four weeks later, the surviving rats (15 rats died) were moved into separate metabolic cages, and 24-h urine was collected. Blood samples were collected from ocular venous, and serum was separated. Urinary protein content, serum creatinine (Scr), blood urea nitrogen (BUN), type-B natriuretic peptide (BNP), aldosterone (ALD), angiotensin (Ang) II, and C-reactive protein (CRP) level were determined. Rats with the values of these parameters beyond normal values were identified with cardiorenal syndrome. According to body weights, the rats were randomly divided into control, low dose of NG and high dose of NG groups, with 16 rats in each group. Rats in NG groups were orally given 25 mg/kg/day or 50 mg/kg/day NG for 4 weeks. The dose of NG was selected according to previous studies.[13,16] Rats in the control group were given vehicle. After the experiments, 24-h urine and blood samples were collected as mentioned above.

Determination of heart function

Left ventricular function was detected as previously reported.[15,17] Briefly, the right carotid artery of rats was exposed. The left ventricular catheter was prefilled with saline containing 10% heparin, and then retrograde catheterized into the left ventricle through the right common carotid artery. The other end of the catheter was connected to the pressure transducer. Measurements were taken using a computerized CODA blood pressure monitor (Kent Scientific, CT, USA), equipped with an occlusion cuff and a volume pressure recording sensor. Hemodynamic tests were conducted, including left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and falling rates of left ventricular pressure (±dp/dtmax).

Weighing of heart weight

After the determination of cardiac functions, the heart was removed and rinsed with saline. The left ventricle was removed and weighed. The weight of left ventricle (LVW) was divided into body weight (BW) to calculate the heart weight index (LVW/BW).

Biochemical determination

Scr, BUN, BNP, ALD, Ang II, CRP level, and blood lipid profiles were determined by ELISA kits (Wuhan Jiyinmei Biotechnology, China).

Evaluation of cardiac oxidative stress

Homogenates of left ventricular tissue were centrifuged at 3000 g at 4°C for 10 minutes. The activity of superoxide dismutase (SOD) and levels of glutathione (GSH) and malonaldehyde (MDA) in the supernatants were measured using commercial kits (Nanjing Jiancheng, China).

Cell culture and treatment

Cardiac fibroblasts were isolated and treated as previously reported.[18] Cells were transfected with siNrf2 or scramble siRNAs using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol. Forty eight hours after the transfection, cells were exposed to 100-nmol/L Ang II