Protective Effect of Naringenin on Acute Myocardial Ischemia-reperfusion Injury in Rats

Xia ZHANG[1], Ping ZHOU[1], Juan LI[2], Zhaojun XIANG[1], Qianqian LUO[1], Qing DENG[1]*

1. Department of Neurosurgery, Taihe Hospital of Shiyan City, Hubei University of Medicine, Shiyan 442000, China; 2. Gynecology and Obstetrics, Taihe Hospital, Hubei University of Medicine, Shiyan 442000, China

Abstract [**Objectives**] To investigate the protective mechanism of naringenin on acute myocardial ischemia-reperfusion injury (AMI-RI) in Sprague-Dawley (SD) rats. [**Methods**] A total of 32 SD rats with AMI-RI model construction were randomly divided into AMI-RI model control group and citrus pigment A/B/C groups ($n=8$). The naringenin A, B, and C groups were administrated 20, 40 and 80 mg/(kg · d) for 10 d. The AMI group served as the negative control and was not treated. At the conclusion of the treatment regimen, a sample of intraventricular blood was collected for the purpose of measuring lactate dehydrogenase (LDH), glutathione peroxidase (GLH-PX), nitric oxide (NO), and superoxide dismutase (SOD) levels. Additionally, myocardial tissue was identified within the ischemic region. The content of malondialdehyde (MDA) was determined by inducing nitric oxide synthase (iNOS) and endodermal nitric oxide synthase (eNOS) positive cells in the left anterior descending coronary artery. [**Results**] Following citrus treatment, the contents of GLH-PX and SOD in ventricular blood of the citrus B group were found to be significantly elevated, while the contents of NO and LDH in myocardial MDA and ventricle were observed to be significantly reduced. The number of eNOS-positive cells was significantly increased, while the number of iNOS-positive cells was significantly decreased. The difference was statistically significant when compared with the AMI-RI group ($P<0.05$). The changes observed in the above indicators in the citrus C group were more pronounced than those observed in the citrus B group. The difference between the citrus C and the B group was statistically significant ($P<0.05$), indicating that this effect is concentration dependent. [**Conclusions**] In addition to its ability to inhibit myocardial lipid peroxidation during AMI-RI by increasing SOD activity, naringenin may also affect the synthesis and release of NO by regulating eNOS and iNOS, thereby achieving protection against AMI-RI. One effect is enhanced as the dose of the drug increases.

Key words Rat, Naringenin, Acute myocardial ischemia-reperfusion, Lipid peroxidation, Inducible/endothelial nitric oxide synthase

1 Introduction

Acute myocardial infarction (AMI) is the leading cause of fatal heart disease. The pathological factors causing AMI are mainly insufficient blood supply caused by coronary artery stenosis. Consequently, the prompt restoration of coronary blood flow represents a fundamental measure for the preservation of ischemic myocardium and the maintenance of cardiac pumping function. Nevertheless, while ischemic myocardial tissue rapidly restores blood flow, acute myocardial ischemia-reperfusion injury (AMI-RI) is a significant concern[1]. Studies have demonstrated that the primary cause of tissue injury in AMI patients is not myocardial ischemia itself, but rather the tissue redestruction caused by lipid peroxidation of oxygen free radicals after reperfusion, or reperfusion injury[2]. SOD is a free radical antioxidant enzyme that primarily functions by removing oxygen free radicals and inhibiting lipid peroxidation reactions. Naringenin has been demonstrated to possess free radical scavenging, anti-atherosclerotic, myocardial protective and other pharmaceutical effects[3]. It has been reported that naringenin has pharmaceutical effects, including scavenging free radicals, inhibiting tumor cell proliferation, anti-atherosclerosis, hypoglycemic effects, protection heart muscle, and inhibition of inflammatory responses caused by inflammatory factors[4]. A paucity of systematic studies on cardiovascular research has been identified. Consequently, an investigation was conducted to assess the impact of naringenin on AMI-RI and to elucidate its potential mechanism of action.

Received: January 18, 2024 Accepted: April 10, 2024

* Corresponding author. E-mail: 524078242@qq.com

2 Materials and methods

2.1 Experimental animals The study involved 32 male SD rats (SPF grade), aged 6 - 7 weeks old, with a body mass of 160 - 170 g. The animals were maintained in accordance with the principles of animal welfare, and all experimental procedures were conducted in accordance with the 3R principle of experimental animals.

2.2 Instruments and reagents The citrus pigment was procured from Shanghai Keshun Biotechnology Co., Ltd. (batch No.:180724); Chloral hydrate was purchased from Shanghai Haling Biotechnology Co., Ltd. (batch No.:01802719); Lactate dehydrogenase (LDH), glutathione peroxidase (GLH-PX), nitric oxide (NO), superoxide dismutase (SOD), malondialdehyde (malondialdehyde, MDA), the induced nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) ELASA test kits were provided by Nanjing Jiancheng Bioengineering Institute (batch No.:H18050, H18013, A01807, A018094, A7025, A8021, A18097).

2.3 Methods

2.3.1 AMI-RI Model building. Rats of similar weight were selected and injected with 10% chloral hydrate intraperitoneally at a dose of 0.3 mL/kg. Following the administration of the anesthetic, the abdomen was fixed on the mouse table and connected to the BW-BM1103 small animal ventilator. In the case of iodine disinfection of the chest and abdomen, a sterile towel should be spread, and carefully opened to reveal the pericardium. The heart

should then be held in place while a small, round needle should be prepared and used to puncture the anterior descending branch. However, care should be taken to avoid puncturing the ventricular wall, as this could result in significant bleeding. A thin silicone tube should be placed parallel to the anterior descending branch, with the wire and ligation tightened. This would result in acute ischemia of the left anterior coronary artery for 30 min. Subsequently, the silicone tube should be removed, allowing reperfusion of the descending branch of the left anterior coronary artery. Finally, the chest and skin should be sutured 45 min after the initial procedure[5]. The following criteria must be met for enrollment in the AMI-RI model: typical AMI electrocardiographic changes, including presystole, T-wave elevation or inversion, and ST-segment elevation on the electrocardiogram, must be observed after 45 minutes of reperfusion[6].

2.3.2 Group allocation and treatment. All animals were randomly divided into AMI-RI group and naringenin A/B/C groups. Each group consisted of 8 animals. After the animals were awake, the citrus A/B/C groups were treated for 10 d at 20, 40 and 80 mg/(kg · d), administrated 8 h per day. The AMI-RI group was not treated and served as a negative control. By the conclusion of the treatment period, 6, 7, 8, and 8 animals survived in the AMI-RI and naringenin A/B/C groups, respectively.

2.3.3 Detection of myocardial tissue in blood and ischemic region. Following the conclusion of the treatment regimen, a 1-mL of blood sample was collected directly into the chamber with a blood sampling needle. The levels of LDH, NO GLH-PX and SOD were then quantified via enzyme-linked immunosorbent assay (ELISA). The animals were subsequently euthanized, and myocardial tissue from the ischemic region of the left anterior descending coronary artery was harvested. The content of MDA was then measured according to the ELASA test box.

2.3.4 Detection of the number of vascular endothelial iNOS/eNOS positive cells. The heart was excised and the ventricles and atria were perfused with normal saline injection from the aorta, The myocardial tissue in the ischemic area of the left anterior descending coronary artery was fixed with formaldehyde, dehydrated with ethanol 24 h later, embedded in paraffin, continuously sectioned into approximately 5 μm thick tissue sections, and then stained with hematoxylin and eosin. Four fields were randomly selected for the examination of iNOS/eNOS positive expression at 400x magnification, and the number of iNOS/Enos-positive cells in 100 cells in each field was counted.

2.4 Statistical analysis A statistical analysis was conducted using SPSS 21.0 statistical analysis software. The experimental data were expressed as mean ± standard deviation ($\bar{x} \pm s$). A one-way homogeneity of variance analysis was initially performed, and pairwise comparisons between groups were conducted using the Dunnet t test for two-sample means, with a significant level of $P < 0.05$.

3 Results and analysis

3.1 Rat blood and myocardial tissue As illustrated in Table 1, SOD and GLH-PX exhibited a notable increase, while NO and MDA demonstrated a significant decline when compared to the AMI-RI group ($P < 0.05$). Furthermore, the observed change in naringenin group C was more pronounced, and the disparity between naringenin group C and group B was statistically significant ($P < 0.05$).

Table 1 Comparison of lipid metabolites and active enzymes in 4 groups of rats ($\bar{x} \pm s$)

Group (number)	MDA//nmol/mL	SOD//U/mL	LDH//U/mL	GLH-PX//U/mL	NO//U/mL
AMI-RI (6)	21.47 ± 1.64	218.39 ± 12.01	34.24 ± 2.20	207.18 ± 13.54	67.39 ± 4.02
Naringenin A (7)	19.25 ± 1.95	227.17 ± 12.93	32.09 ± 1.32	215.33 ± 14.72	64.28 ± 5.87
Naringenin B (8)	15.34 ± 1.27^{ab}	268.21 ± 17.80^{ab}	22.53 ± 1.94^{ab}	309.27 ± 19.89^{ab}	51.97 ± 4.15^{ab}
Naringenin C (8)	9.01 ± 1.02^{abc}	291.50 ± 22.42^{abc}	17.91 ± 1.66^{abc}	341.22 ± 13.97^{abc}	44.91 ± 3.05^{abc}

NOTE The letters "a," "b," and "c" indicate statistical significance at the 0.05 level versus the AMI-RI, naringenin A, and naringenin B groups, respectively. The same below.

3.2 Number of iNOS/eNOS positive cells As illustrated in Table 2, the number of eNOS-positive cells in the naringenin B/C groups was found to be significantly higher than that in the AMI-RI group ($P < 0.05$). Furthermore, the change in naringenin concentration in the naringenin group C was more pronounced, and the difference between the naringenin group C and group B was statistically significant ($P < 0.05$).

Table 2 Comparison of test results of iNOS/eNOS positive cells

cells/100

Group (number)	iNOS	eNOS
AMI-RI (6)	41.09 ± 2.57	9.25 ± 1.04
Naringenin A (7)	39.51 ± 4.60	10.53 ± 1.43
Naringenin B (8)	31.97 ± 1.20^{ab}	15.30 ± 1.91^{ab}
Naringenin C (8)	25.04 ± 1.35^{abc}	19.24 ± 2.10^{abc}

4 Discussion

Naringenin, also known as naringenin, is a flavonoid that is widely distributed in lemon, Rosaceae, Rosaceae and citrus plant. Among these, the content of tangerine peel is the highest[7]. It was observed that SOD and GLH-PX exhibited a notable increase, while NO, MDA and LDH demonstrated a significant decline when compared to the AMI-RI group. The number of eNOS-positive cells in the naringenin B/C groups was found to be significantly higher than that in the AMI-RI group, and the change in naringenin concentration in the naringenin group C was more pronounced. The results indicate that the effect increases with an increase in drug dose, suggesting a concentration-dependent phenomenon. Our findings demonstrate that the lipid peroxidation product MDA is involved in the entire evolution of AMI-RI pathology, and that SOD

can exert a protective effect on AMI-RI by scavenging free radicals[8]. GLH-PX is a crucial peroxidase-like enzyme that is widely distributed throughout the body. The active center of GSH-Px is selenocysteine, and its vitality size can serve as an indicator of the body's selenium levels. Selenium is a crucial component of the GLH-PX enzyme system. It can catalyze the conversion of GSH into GSSG, facilitating the reduction of toxic peroxides into non-toxic hydroxyl compounds, This process protects protect the structure and function of cell membranes from peroxide-induced damage. Additionally, GLH-PX levels influence the body's ability to remove toxic metabolites. LDH plays a pivotal role in glycolysis and gluconeogenesis, acting as an essential enzyme between pyruvate and lactate. The body exhibits a clear increase in ischemia reperfusion, with a correspondingly specific distribution. This makes it an invaluable clinical reference standard and diagnostic tool for myocardial disease[9]. In this study, the content of SOD and GLH-PX increased in rats compared to the AMI-RI group, while myocardial MDA and ventricular LDH decreased. These results are consistent with those of the aforementioned study.

NO plays a pivotal role in maintaining homeostasis within the internal environment, particularly with regard to the cardiovascular system. Three distinct types of NOS are involved in the catalytic synthesis of NO, namely nNOS, eNOS and iNOS. However, it is iNOS that is primarily responsible for the induction of NO. The experimental observation of the citrus B/C group of rats revealed a significant increase in the number of vascular endothelial eNOS-positive cells, accompanied by a significant decrease in the number of iNOS-positive cells. This was accompanied by a significant decrease in ventricular blood NO levels. It was therefore postulated that citrus may improve eNOS activity, reduce iNOS expression, and indirectly reduce the synthesis of NO, thereby maintaining NO in a physiological state and expanding the effect of blood vessels, improving myocardial blood supply.

In conclusion, in addition to inhibiting cardiac lipid peroxidation during AMI-RI by improving SOD activity, citrus may also affect NO synthesis and release by regulating eNOS and iNOS, thus realizing the protective effect on AMI-RI, which is enhanced with increasing drug dose. This suggests that this effect is concentration-dependent. In view of the above observed phenomenon, it is imperative to conduct further research on the pharmacological mechanism of citin and to investigate its potential medicinal applications.

References

[1] ZHANG CY, CAO Z, FAN XQ, *et al.* The mechanism of myocardial ischemia-reperfusion injury in rabbits[J]. Chinese Medicine Emergency, 2016, 25(5): 804 - 807. (in Chinese).

[2] GUO J, LIU Y, LI YP, *et al.* Oxygen free radicals and myocardial ischemia and reperfusion injury[J]. Chinese Journal of Cardiovascular Medicine, 2008, 13(5): 384 - 387. (in Chinese).

[3] MA DZ, YU J, XI M, *et al.* Protective effect of free radical scavenging by superoxide dismutase on myocardial ischemia-reperfusion injury and its mechanism[J]. Chinese Journal of Experimental Surgery, 2014, 31(10): 2220 - 2222. (in Chinese).

[4] JI SOH, KIM H, VIJAYAKUMAR A, *et al.* Association of dietary flavonoid intake with prevalence of type 2 diabetes mellitus and cardiovascular disease risk factors in Korean women aged ≥30 years[J]. Journal of Nutritional Science and Vitaminology, 2017, 63(1): 51 - 58.

[5] CHEN YN, ZHOU YP, WANG KM, *et al.* Improvement and evaluation of the rat model preparation method for acute myocardial ischemia-reperfusion injury[J]. Chinese Medicine Emergency, 2018, 17(3): 359 - 360. (in Chinese).

[6] CAO M, XU LQ, LIU QZ, *et al.* Protective effect of phycocyanin against acute myocardial ischemia and reperfusion injury in rats[J]. China Medical Herald, 2017, 17(14): 25 - 28. (in Chinese).

[7] MENG LM. Effect of naringeninpre treatment on energy metabolism after ischemia / reperfusion heart injury in rats[J]. China Medical Herald, 2018, 15(35): 22 - 24. (in Chinese).

[8] KE JY, BANH T, HSIAO YH, *et al.* Citrus flavonoid naringenin reduces mammary tumor cell viability, adipose mass, and adipose inflammation in obese ovariectomized mice[J]. Molecular Nutrition & Food Research, 2017: 1600934.

[9] WANG CY, WANG LH, YI M, *et al.* Glutathione peroxidase 4 mediates the protective effect of probucol on hepatic ischemia / reperfusion injury in rats[J]. Chinese Journal of Clinical Pharmacology, 2018, 34(11): 1334 - 1337. (in Chinese).