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Original Article

Effects of fucoidan on NF-κB-mediated inflammatory response in rats with acute myocardial infarction

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Abstract: Incidence rates of cardiovascular disease in China have increased year by year. Previous studies have suggested that fucoidan provides anti-coagulation, anti-tumor, and anti-thrombosis effects. However, the effects of fucoidan on acute myocardial infarction rats have not been reported. In the current study, a rat model of acute myocardial infarction (AMI) was established by coronary artery ligation. NF-κB inhibitor PDTC or fucoidan intervention was administered. This was followed by analysis of the degree of pathological damage of myocardial infarction via hematoxylin-eosin (HE) staining. Expression of related proteins was examined by Western blotting and levels of myocardial enzymes and inflammation were examined by ELISA. Severe myocardial damage was observed in rats in the myocardial infarction model group. However, the damage was improved in the PDTC and fucoidan groups. At the same time, NF-κB signaling pathways were significantly activated in the myocardial infarction model group. Both PDTC and fucoidan were shown to inhibit activation of NF-κB signaling pathways and reduce secretion of inflammatory factor tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Administration of PDTC and fucoidan also improved myocardial enzymes and decreased expression of aspartate aminotransferase (AST), creatine kinase (CK), and creatine kinase isoenzyme (CKMB). Fucoidan can inhibit NF-κB-mediated inflammation and improve myocardial injuries in rats with acute myocardial infarction, providing a theoretical basis for the development of anti-infarction drugs for treatment of AMI.

Keywords: Fucoidan, NF-κB, acute myocardial infarction

Introduction

Incidence rates of cardiovascular disease in China have increased year by year. Mortality rates of cardiovascular disease rank first, much higher than tumors and other diseases, accounting for more than 40% of deaths of residents. This includes the highest incidence of myocardial infarction [1, 2]. Therefore, it is necessary to find new drugs for prevention or treatment of myocardial infarction, improving prognosis. Fucoidan is a kind of polysaccharide mainly derived from brown algae. It is also known as fucus polysaccharide, fucoidan sulfate, and fucoidan sulfate [3, 4]. Previous studies have suggested that fucoidan provides anti-coagulation, anti-tumor, anti-thrombotic, anti-viral, and anti-oxidation effects. However, the experimental basis remains relatively limited [5, 6]. Therefore, it has been widely used in medical and health and food processing industries. Whether fucoidan can be used in the field of medicine requires more foundation and further clinical research. However, current studies on polysaccharides of salt algae have mainly focused on extraction and purification, as well as their effects on water quality, bacteriostatic, and anti-inflammation. Studies have suggested that fucoidan has prominent effects on regulation of blood pressure, lowering of blood lipids, and lowering of blood sugar. However, reports are relatively limited.

The NF-κB signaling pathway is a classical signaling pathway that regulates inflammation-related responses. Its central role in regulating the body's immune response is unquestionable [7, 8]. Studies have shown that NF-κB sig-

naling can promote the development and progression of myocardial infarction through transcriptional regulation of multiple genes. Early studies have also detected a significant increase in expression of NF-κB in the myocardial infarction model, caused by surgery. Decreasing NF-κB levels can reduce myocardial infarct size and improve prognosis of myocardial infarction [9]. However, it is not clear whether fucoidan can reduce the area of myocardial infarction and improve prognosis of myocardial infarction. In this study, a rat acute myocardial infarction (AMI) model was established. Changes of NF-κB signaling pathways in a rat model of myocardial infarction, as well as the effects of salt algae polysaccharides, were observed.

Materials and methods

Reagents

PDTC was purchased from Sigma (Shanghai, China). RT-qPCR one-step kit was purchased from Quanjin Biotech (Beijing, China). Anti-rabbit NF-κB p65, IκB, and hitine polyclonal antibody was purchased from Abcam Co., Ltd. (Hong Kong, China). Goat Anti-Rabbit IgG (H + L) and β-actin antibody was purchased from Proteintech Co., Ltd. (Wuhan, China). The whole protein extraction kit and the nuclear protein extraction kit was purchased from Biyuntian (Shanghai, China). Experimental rats (male, 6-8 weeks) were provided by the Experimental Animal Center. Western Blot Lysis Buffer and BCA Protein Assay Kit was provided by Biyuntian Biotechnology Research Institute (Suzhou, China). All other reagents were purchased from Sigma.

Animals

The 6-8 week old male SPF SD rats were provided by the Animal Center. The experimental animals were divided into four groups, including the sham operation group, myocardial infarction model group, myocardial infarction + PDTC group, and myocardial infarction + fucoidan Polysaccharide group, with n=10 in each group. All experimental procedures involving animals complied with ARRIVE guidelines. The current study was approved by the Ethics Committee.

Establishment of rat AMI model

The rat AMI model was established using coronary artery ligation [10]. Briefly, after anesthetizing the rats, the small animal ventilator was connected. The skin was cut through the fourth intercostal space on the left side, bluntly separating the muscle tissue. The chest cavity was opened and the happy capsule was cut. The heart was squeezed, threaded between the left atrial appendage and the pulmonary artery cone. It was ligated to make the anterior descending coronary artery. Electrocardiogram information was recorded and ST segment elevation was observed, evaluating whether the AMI model was successfully established. The heart was then placed back into the chest and sutured. The sham operation group was operated on with the surgery group, except that the artery was not ligated. In the myocardial infarction + PDTC group and the myocardial infarction + fucoidan group, the rats received respective administration of PDTC (5 mg/kg) or fucoidan (20 mg/kg) one day before surgery. This continued until 7 days after surgery. On the 8th day, after analyzing cardiac function, the specimens were collected. The tissues were weighed and stored in a -80°C refrigerator for Western blot analysis.

Western blot

Western blotting was were performed with reference to the literature [11]. Lysis buffer lysate was first thawed and benzyltrifluoride fluoride (PMSF) was added to a final concentration of 1 mM. Lysis buffer homogenate was added into 100 mg of fresh rat myocardial tissue and lysed at 4°C for 15 minutes. This was followed by centrifugation at 140,00 rpm for 15 minutes. Aspiration of the supernatant into a new pre-cooled EP tube was conducted. After quantification by BCA assay and denaturation in boiling water for 5 minutes, the protein was separated on 8-12% SDS-PAGE. It was then transferred to PVDF membranes and incubated with the corresponding primary antibody (1:1000) at 4°C overnight. After the membranes were washed three times with TTBS, they were incubated with corresponding secondary antibodies (1: 1000) at 37°C for 2 hours. This was followed by development via chemiluminescence.

H&E staining

Rat myocardial tissues were fixed in paraformaldehyde. After routine paraffin sectioning, they were immersed in 3% hydrogen peroxide methanol for 10 minutes, washed twice with PBS, and stained with H&E staining solution,

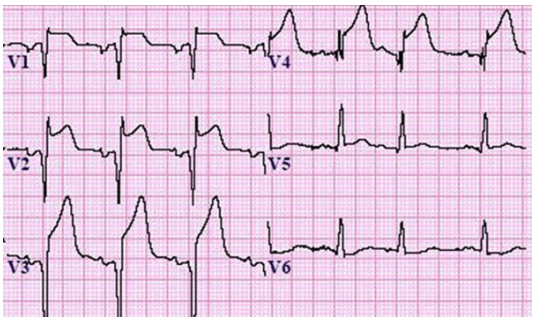

Figure 1. Electrocardiogram of acute myocardial infarction rats.

according to manufacturer instructions. After washing with PBS three times, pathological changes were observed under the microscope.

Detection of myocardial enzyme levels

Rat myocardial tissues were collected and homogenized by adding physiological saline (1:10). According to kit instructions, AST, alkaline phosphatase (AKP), lactate dehydrogenase (LDH), LDH-1, α-hydroxybutyrate dehydrogenase (α-HBDH), CK, and CKMB were determined.

Determination of inflammatory cytokines

Peripheral blood was collected from each group of rats and centrifuged, obtaining serum. This was used to measure levels of TNF-α and IL-6 using an ELISA kit, according to kit instructions.

Statistical analysis

Experimental data were obtained from at least three independent experiments. They are expressed as mean ± standard deviation (SD). Comparisons between the two groups were performed using Student's t-tests. Comparisons between groups of the samples were performed via one-way ANOVA. Pairwise comparisons were performed using the SNK method. $P<0.05$ indicates statistical differences.

Results

Electrocardiogram changes in rats with acute myocardial infarction

In the electrocardiogram of the acute myocardial infarction model group, the ST segment was significantly elevated (**Figure 1**).

Myocardial enzyme levels in each group

In the myocardial infarction model group, AST, CK, and CKMB levels were significantly elevated, compared with the sham operation group. Administration of fucoidan or PDTC reduced levels of AST, CK, and CKMB, to a certain extent. There were no significant differences in levels of LDH, LDH-1, and α-HBDH between the groups (**Table 1**).

Heart/body ratios of each group

The heart is one of the most important organs of the animals. Heart/body ratio measurements are important parameters for the size of the heart. The structure and relative size of the heart can represent the ability of blood circulation [12, 13]. According to results, the heart/body ratio of the myocardial infarction model group was significantly higher than that of the sham-operated group, which was reduced after administration of PDTC or fucoidan, compared with the myocardial infarction model group (**Figure 2**).

Changes of myocardial structure in rats by H&E staining

H&E staining was used to evaluate changes in the myocardial structure after myocardial infarction in rats. As shown in **Figure 3**, the sham-operated group showed clear myocardial cells, regular arrangement, intact myofilament, abundant cytoplasm, and a uniform and complete gap. The myocardial infarction group showed obviously pathological changes in the myocardium. Some myocardial fibrinolysis was broken with a disappeared nucleus, fragmentation, and enhanced cytoplasmic acid staining. In addition, the myocardial cells were obviously hypertrophied. Myocardial tissue necrosis was severe. Staining results suggest that the acute myocardial death model was established successfully. In the group treated with fucoidan or PDTC, the myocardial alignment and degrees of myofibrillar rupture were between the sham operation group and model group. Significantly improvement was observed, compared with the model group. The overall shape was clear, indicating that fucoidan had a significant inhibitory effect on ventricular reconstruction and myocardial infarction.

Table 1. Changes of myocardial enzymes in each group (mean ± SD, n=10)

Parameters (U/L)	Sham	AMI	AMI + PDTC	AMI + fucoidan
AST	117.26 ± 26.48	176.70 ± 61.48**	129.21 ± 20.59##	124.54 ± 32.11##
AKP	148.50 ± 65.32	123.56 ± 45.65	135.88 ± 54.24	125.50 ± 46.67
LDH	247.20 ± 77.69	408.50 ± 56.23	321.98 ± 34.15	298.87 ± 34.98
LDH-1	254.90 ± 131.89	465.80 ± 189.65	407.00 ± 186.70	456.65 ± 135.29
α-HBDH	122.10 ± 69.79	159.90 ± 76.30	122.68 ± 45.65	156.65 ± 65.32
CK	388.40 ± 130.90	1200.60 ± 392.15**	950.62 ± 487.12##	934.80 ± 336.52##
CKMB	268.90 ± 91.76	910.95 ± 331.00**	674.56 ± 332.37##	640.00 ± 277.05##

**P<0.05 compared with sham group, ##P<0.05 compared with AMI group.

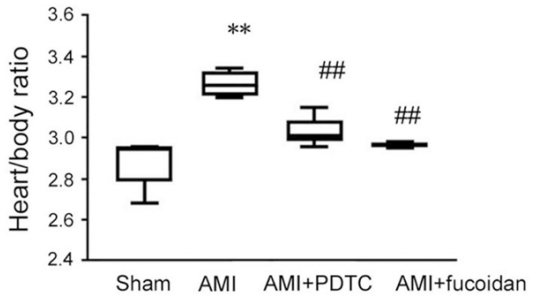

Figure 2. The heart/body ratio of each group was changed. **p<0.05 compared with the sham-operated group, ##p<0.05 compared with the acute myocardial infarction model group.

Changes of NF-κB in tissues of rats with myocardial infarction

Previous studies have suggested that high expression of NF-κB is closely related to occurrence of myocardial infarction. Inhibition of NF-κB signaling pathway activation can improve prognosis of myocardial infarction. Therefore, the current study examined the effects of fucoidan on NF-κB signaling pathways. As shown in **Figure 4**, compared with the sham-operated group, the degradation of IκB in the model group was significantly increased, accompanied by a significant increase in NF-κB p65 nuclear import and subsequent phosphorylation of p65. Activation of NF-κB signaling pathways was significantly inhibited after administration of PDTC. This was significantly different from that of the acute myocardial infarction group. For the fucoidan group, NF-κB signaling pathways were also inhibited, to a certain extent. This was accompanied by an improvement in myocardial structure. Thus, it can be speculated that fucoidan may improve prognosis of acute myocardial infarction by inhibiting NF-κB signaling pathways.

Levels of TNF-α and IL-6 in each group

Levels of TNF-α and IL-6 in each group were significantly increased in the myocardial infarction model group. Results were significantly different from the sham operation group (**Figure 5**). After administration of PDTC and fucoidan, levels of TNF-α and IL-6 were significantly decreased.

Discussion

AMI refers to acute necrosis caused by persistent and severe myocardial ischemia, plaque ruptures, and thromboembolisms, which are the pathological basis of acute coronary occlusion. The dynamic evolution of electrocardiograms and abnormal myocardial enzymes are the basic criteria for clinical diagnosis of AMI [14, 15].

Studies have shown that fucoidan has a wide range of pharmacological effects, including anti-oxidation, anti-inflammatory, anti-thrombotic, and anti-atherosclerosis effects [16]. However, basic research is still scarce. In this study, a rat model of AMI was established by coronary artery ligation, aiming to investigate the pharmacological effects of fucoidan. In this study, it was found that administration of fucoidan could improve myocardial enzyme levels after myocardial infarction, reduce heart/body mass ratios, and reduce levels of myocardial damage. Present results suggest that fucoidan can improve myocardial damage caused by myocardial infarction.

NF-κB is a nuclear transcription factor that functions and is widely distributed. It initiates transcription of genes through binding to specific gene promoters or enhancers [17]. Under normal circumstances, NF-κB binds to IκB to

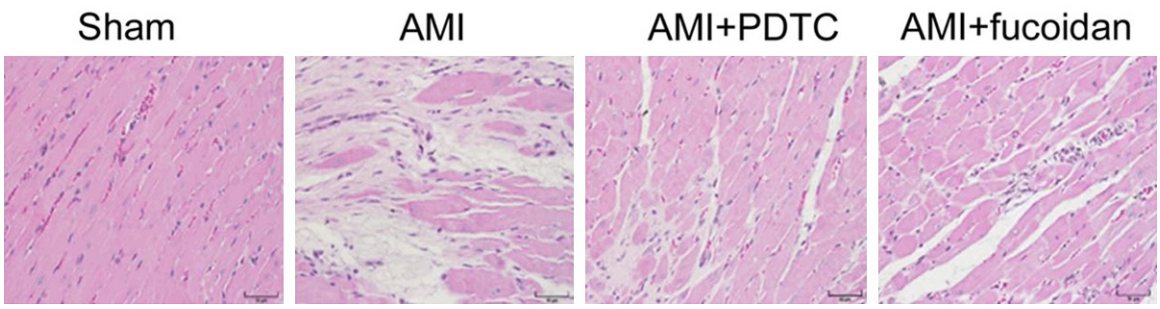

Figure 3. HE staining of myocardial tissues of each group of rats (magnification × 40).

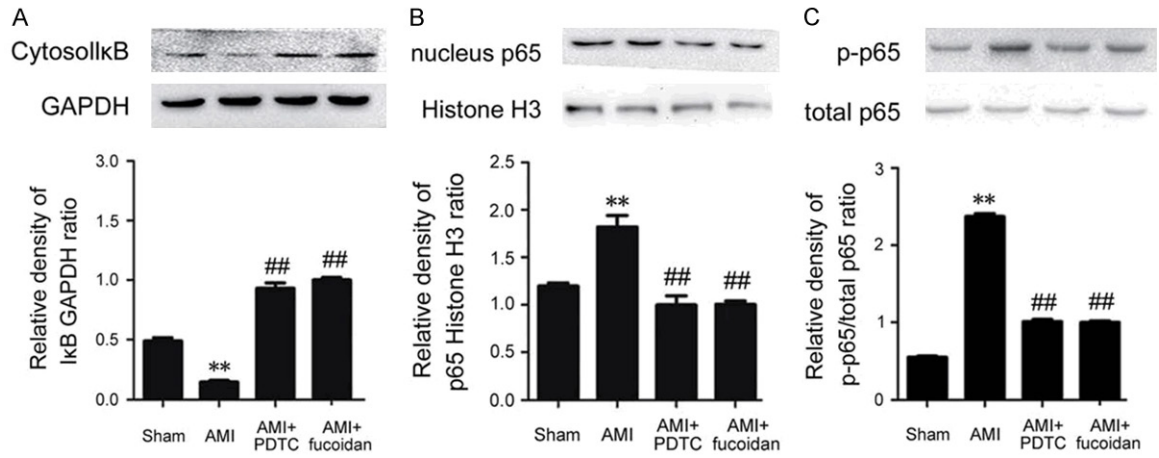

Figure 4. Activation of NF-κB signaling pathways in each group of rats. A. IκB degradation; B. NF-κB p65 into the nucleus; C. p65 phosphorylation. **p<0.05 compared with the sham-operated group, ##p<0.05 compared with the acute myocardial infarction model group.

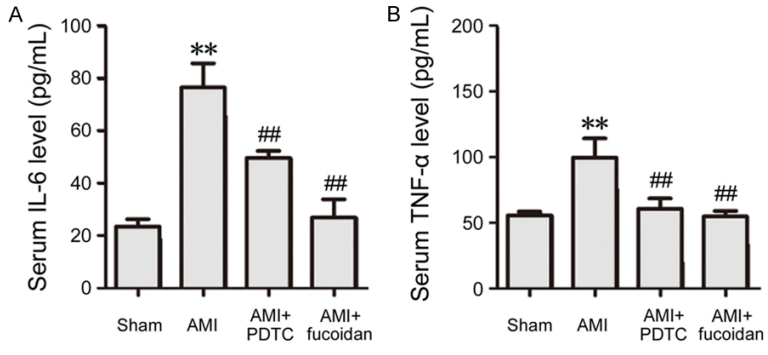

Figure 5. Levels of TNF-α and IL-6 in each group were changed. A. IL-6 levels in rats of each group; B. Levels of TNF-α in each group of rats. **p<0.05 compared with the sham-operated group, ##p<0.05 compared with the acute myocardial infarction model group.

form an inactive complex. When receiving external stimulation, IκB degrades after phosphorylation, releasing NF-κB p50/p65 into the nucleus and binding to the κB site on the target gene, thereby initiating or enhancing the transcription of the target gene [18]. Previous studies have suggested that expression of NF-κB is positively correlated with myocardial damage caused by myocardial infarction. NF-κB signaling pathways can affect cardiac function in many ways. For example, NF-κB activation promotes macrophage polarization and improves cardiac dysfunction after myocardial infarction [19]. Beetroot juice can also alleviate isoproterenol-induced myocardial damage by reducing oxidative stress, inflammation, and apoptosis through NF-κB pathways [20]. Thus, it was speculated that NF-κB pathways may serve as targets for improving the prognosis of myocardial infarction. The current study investigated the degradation

of IκB by NF-κB signaling pathways, as well as the transcription of NF-κB p65 into the nucleus and activation of p65. It was found that fucoidan inhibited NF-κB signaling pathways. Results suggest that fucoidan may prevent myocardial infarction and improve myocardial infarction through NF-κB-mediated pathways. PDTC is a potent inhibitor of NF-κB. It inhibits the phosphorylation and subsequent degradation of IκB by inhibiting inactivation of IκBα through chelation of metal ions. Thus, it prevents the transfer of NF-κB into the nucleus [21]. Fucoidan has similar effects on PDTC. Inhibition of NF-κB signaling pathways by fucoidan was verified from the side.

Inflammatory response after myocardial infarction is positively correlated with the severity of the disease. NF-κB entry into the nucleus is an important step in activating inflammatory response [22]. It has been reported that circulating inflammatory factors, including TNF-α and IL-6, are increased in patients with chronic heart failure. In this study, levels of TNF-α and IL-6 in the blood were also increased in rats after myocardial infarction, in accord with previous studies [23]. In the current study, administration of fucoidan and PDTC significantly inhibited NF-κB nuclear activation and secretion of TNF-α and IL-6, indicating that fucoidan inhibits NF-κB in rats with acute myocardial infarction and reduces inflammatory response.

Although the current study confirms that fucoidan can inhibit NF-κB pathways and reduce inflammatory response in acute myocardial infarction rats, the exact mechanisms by which fucoidan acts on NF-κB pathways remain unclear. Further investigation is necessary.

Conclusion

In conclusion, the current study shows that fucoidan can inhibit NF-κB-mediated inflammatory response and improve myocardial damage in rats with acute myocardial infarction. Thus, present results may provide a theoretical basis for the development of anti-infarction drugs for treatment of AMI.

Disclosure of conflict of interest

None.

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