Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The protective effect of nicorandil on cardiomyocyte apoptosis after coronary microembolization by activating Nrf2/HO-1 signaling pathway in rats

Wenkai He, Qiang Su, Jiabao Liang, Yuhan Sun, Xiantao Wang, Lang Li*

Department of Cardiology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

ARTICLE INFO

Article history:
Received 29 January 2018
Accepted 1 February 2018
Available online xxx

Keywords:
Nicorandil
Coronary microembolization
Apoptosis
Cardiac function
Nuclear factor erythroid 2-like
Heme oxygenase-1

ABSTRACT

Background: Myocardial apoptosis is considered to be the chief cause of progressive cardiac dysfunction induced by coronary microembolization (CME), and the Nrf2/HO-1 signaling pathway is involved in CME-induced myocardial apoptosis. Nicorandil (NIC) has multiple beneficial cardiovascular effects on myocardial injury. Therefore, this study was undertaken to analyze the role of NIC pretreatment in the inhibiting myocardial apoptosis after CME in rats.

Methods: Forty rats were divided into Sham group, CME group, CME plus NIC (NIC) group, and CME plus AAV9-Nrf2 (AAV9-Nrf2) group (n = 10 per group). CME-induced myocardial apoptosis model was established through injecting plastic microspheres (42 μM) into the left ventricle except the Sham group. NIC group received nicorandil 3 mg/(kg.d) for 7 days before the operation. Cardiac function was assessed by echocardiography. The mRNA expression level of Nrf2 was detected by RT-PCR. The protein expression levels of Nrf2, HO-1, Bcl-2, Bax and cleaved caspase-3 were detected by Western blot. The size of the microinfarction area was measured by HBFP staining; myocardial apoptosis was analyzed by TUNEL staining.

Results: Compared with the sham group, the cardiac function and the expression level of Nrf2, HO-1 and Bcl-2were decreased, while myocardial apoptosis and the expression of Bax and cleaved caspase-3 were increased in the CME group. Compared with the CME group, cardiac function was significantly improved, the expression levels of Nrf2, HO-1, and Bcl-2 were increased, the expression of Bax and cleaved caspase-3 were decreased, and the myocardial apoptosis was attenuated in the NIC group and AAV9-Nrf2 group.

Conclusion: NIC pretreatment effectively inhibit CME-induced myocardial apoptosis and improve cardiac function. The protective effects are mediated through the activation of the Nrf2/HO-1 signaling in cardiomyocytes.

1. Introduction

Coronary microembolization (CME) is a serious complication that is caused by atherosclerotic plaque rupture in patients with acute coronary syndromes and a potential iatrogenic complication in patients undergoing thrombolytic therapy or therapeutic percutaneous coronary interventions [1,2]. Once CME occurs, it could induce malignant arrhythmias, contractile dysfunction, coronary reserve reduction, irreversible myocardial damage and necrosis [3–5]. Moreover, CME causes transient 'slow flow' or 'no blood flow', which severely affect patient's cardiac function and long-term prognosis and could even lead to death [1,6]. However, the mechanism of CME induced myocardial damage is complicated. Numerous studies have demonstrated that myocardial apoptosis is involved in the pathogenesis of CME and plays a crucial role in declining cardiac function [7–9]. Therefore, the suppression of myocardial apoptosis can be a promising therapeutic target for CME related cardiac dysfunction. In our previous studies, we found that CME-induced myocardial apoptosis mediated through Nrf2/HO-1 signaling was the chief cause of progressive cardiac dysfunction [10]. Thus, the activation of this signaling pathway would effectively inhibit myocardial apoptosis and improve the cardiac function after CME.

Nicorandil (NIC) is an adenosine triphosphate (ATP)-sensitive

* Corresponding author.
E-mail addresses: 972975622@qq.com (W. He), drlilang@163.com (L. Li).

https://doi.org/10.1016/j.bbrc.2018.02.003
0006-291X/

potassium channel opener and nitrate agonist, which has been shown to possess multiple beneficial cardiovascular effects besides its anti-angina effect, including anti-oxidative effect, anti-inflammatory and anti-apoptotic properties [11–13]. Recently, many studies have indicated the anti-apoptotic effect of nicorandil, such as in cultured cardiac myocytes [13], animal models of myocardial ischemia-reperfusion injury [12], isoproterenol-induced heart failure [14], and doxorubicin-induced cardiotoxicity [15]. However, the underlying anti-apoptotic effect of nicorandil on coronary microembolization has not been examined. Therefore, in this study we investigated the effects of nicorandil pretreatment on cardiomyocyte apoptosis and on Nrf2/HO-1 signaling to elucidate the mechanisms of nicorandil in enhancing CME-induced cardiac dysfunction after coronary microembolization in rats.

2. Materials and methods

2.1. Animal and modeling

Forty Sprague Dawley (SD) rats weighing 250–300 g, 14–16 wk old, were purchased from the Experimental Animal Center of Guangxi Medical University. All rats were randomly divided into four group, including Sham group, CME group, CME plus NIC(NIC) group and CME plus AAV9-Nrf2 (AAV9-Nrf2) group. The experiment was approved by the Ethic Committee for Animal Use of Guangxi Medical University, and performed in accordance with the Guidelines for Care and Use of Laboratory Animals. The model of CME was successfully established as described previously [8]. In brief, anesthesia was induced in rats with 10% chloral hydrate and a respirator was connected. The left lateral thoracotomy was performed through the third and fifth intercostal space. Next, the pericardium and the ascending aorta were fully exposed. Then, with a vascular clamp, 0.1 mL of a suspension containing 3000 polyethylene microspheres with 42 μm diameter (Biosphere Medical Inc. Rockland, USA) in physiological saline was injected into the left ventricle. After the heart rate and breathing returned to normal, the chest was sutured. Using the same procedure, the sham group was only given physiological saline without microspheres. The NIC groups received NIC powder dissolved in physiological saline (3 mg/kg/d) orally (Nipro Pharma Corporation, Japan) for 7 d before the CME modeling operation [14]. The AAV9-Nrf2 group was transfected with AAV9-Nrf2 (AAV9, Biotechnology, shanghai China) via the tail vein 2 wk before CME modeling operation [10]. The rats were sacrificed 6 h after the operation.

2.2. Transfection of AAV-Nrf2

The AAV-Nrf2 was transfected successfully as described previously [10,13]. In brief, the adeno-associated virus subtype 9 (AAV9, Biotechnology, shanghai China) carrying the Nrf2 sequence mixed with saline was injected into rats via the tail vein 2 wk before CME modeling operation—1×10^{11} TU/rat. The Nrf2 gene sequence was found in GenBank (Gene ID: 83619).

2.3. Echocardiography

In this study, the cardiac function reached the lowest level at 6 h after CME, so we selected that time point to detect the cardiac function [8]. Transthoracic echocardiographic study was performed by an experienced physician who was blinded to the study. The rats were lightly anesthetized with an intraperitoneal injection of 10% chloral hydrate, and were laid in a supine position on the experimental platform. Hewlett Packard Sonos 7500 ultrasound instrument equipped with a 12.0 MHz transducer (Philips Technologies) was used to research the LVEF (left ventricle ejection fraction), LVEDd (left ventricle end-diastolic dimension), CO (cardiac output) and FS (fractional shortening) on the left anterior chest wall [8,16].

2.4. Tissue sampling and sample treatment

Each heart was arrested in diastole by injecting 10% KCl into the tail vein of the rats, and the heart was retrieved immediately. Then, the cold normal saline was used to inculcate the ventricle until the rinse solution was achromatous. Next, the left ventricle was segmented into apex and base portions from the middle point of its long axis in a plane parallel to the atrioventricular groove. Finally, the apex portion was frozen and stored at −80 °C, and prepared for western blot and RT-PCR analysis. In addition, the base portions were fixed by 4% paraformaldehyde for 24 h, embedded in paraffin, and sectioned into 4 μm sections for HE staining, HBFP (ematoxylin-basic fuchsin picric acid) staining, TUNEL staining.

2.5. Myocardial microinfarction area

HBFP staining was used to detect early myocardial ischemia and infarct area. It dyes red for the ischemic myocardium and yellow or brown for the normal myocardium. In addition, a pathological image pattern analysis instrument (DMR-Q550, Leica, Wetzlar, Germany) was used to analyzed HBFP-stained slices. The planar area method was used to measure the infarction zone, which was indicated as the average percentage area of bulk analysis slices [17]. In addition, the relative ischemic areas were analyzed through the following formula: ischemic area/total area × 100%.

2.6. Apoptosis assay

Myocardial apoptosis was determined by the TUNEL apoptosis detection kit (Roche, USA), according to the manufacturer's instructions. The apoptotic nuclei are displayed yellow-brown (TUNEL-positive), while the normal nuclei are stained light blue. In each specimen, a total of 20 non-overlapping zones (× 400) belonging to the microinfarction zone [18]. Overall cell number and the number of apoptotic cardiomyocytes were determined, and the myocardial apoptotic rate was analyzed through the following formula: apoptotic cells/total number of cells × 100%.

2.7. RNA extraction and real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from apex portion by the Trizol reagent kit (Gibco, USA) according to the manufacturers' protocol. The concentration of RNA was quantified by a NanoDrop (Thermo Fisher Scientific Inc, USA), and then reverse transcription was done by Reverse Transcriptase Kit (TaKaRa, Japan). RT-PCR for gene expression level was analyzed on a StepOnePlusTM Real-Time PCR System (Applied Biosystems, USA). The primers for RT-PCR amplification were as follows:

Nrf2: Forward primer:5′-GCTGCCATTAGTCAGTCGCTCTC-3′
Reverse primer 5′-ACCGTGCCTTCAGTGTGCTTC-3’;
β-actin: Forward primers 5′-GAGATTACTGCCCTGGCTCCTA-3′
Reverse prime 5′-CATCGTACTCCTGCTTGCTGAT-3′

(all primers were designed by TaKaRa, Japan). The data were collected at the end of each cycle.

2.8. Western blot analysis

Total proteins were extracted by lysis buffer, and then the protein concentration was determined by the Lowry method. Equal

Please cite this article in press as: W. He, et al., The protective effect of nicorandil on cardiomyocyte apoptosis after coronary microembolization by activating Nrf2/HO-1 signaling pathway in rats, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.003