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Pterostilbene protects against myocardial ischemia/reperfusion injury via suppressing oxidative/nitrative stress and inflammatory response

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ABSTRACT

Recent studies have shown that pterostilbene (Pte) confers protection against myocardial ischemia/reperfusion injury. The oxidative/nitrative stress and inflammation induce injury after myocardial ischemia/reperfusion. The present study was designed to evaluate whether treatment with Pte attenuates oxidative/nitrative stress and inflammation in myocardial ischemia/reperfusion (MI/R). Rats were subjected to 30 min of myocardial ischemia and 3 h of reperfusion, and the rats were administered with vehicle or Pte. The results showed that Pte (10 mg/kg) dramatically improved cardiac function and reduced myocardial infarction and myocardial apoptosis following MI/R. As an indicator of oxidative/nitrative stress, myocardial $ONOO^-$ content was markedly reduced after Pte treatment. And, Pte led to a dramatic decrease in superoxide generation and malondialdehyde (MDA) content and a dramatic increase in superoxide dismutase (SOD) activity. In addition, Pte treatment significantly reduced p38 MAPK activation and the expression of iNOS and $gp91^{phox}$ and increased phosphorylated eNOS expression. Pte treatment dramatically decreased myocardial TNF-α, and IL-1β levels and myeloperoxidase (MPO) activity. Furthermore, $ONOO^-$ suppression by either Pte or uric acid (UA), an $ONOO^-$ scavenger, reduced myocardial injury. In conclusion, Pte exerts a protective effect against MI/R injury by suppressing oxidative/nitrative stress. These results provide evidence that Pte might be a therapeutic approach for the treatment of MI/R injury.

1. Introduction

Myocardial infarction is one of the most common cardiovascular diseases with a high mortality and morbidity [1]. The reperfusion of the myocardium within the time window is the most important step for the treatment of myocardial infarction. However, some complications, such as arrhythmia, will occur during the reperfusion, leading to an exacerbated cardiac function [2]. In fact, it has been suggested that not only ischemia itself but also reperfusion contributes to the injury following myocardial ischemia, known as myocardial ischemia/reperfusion (MI/R) injury [3]. MI/R injury is a complicated process and various mechanisms are involved. Calcium overload is induced by MI/R, which can trigger myocardial apoptosis [4]. Inflammation after MI/R also contributes to cell death. In recent years, studies have shown that nitric oxide (NO) derived reactive nitrogen species (RNS) may induce myocardial injury by triggering nitrative stress [5]. Peroxynitrite ($ONOO^-$), results from the reaction of NO with superoxide (O_2^-), is indicated to be one of the most toxic RNS [6]. Additionally, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in myocardium acts as a major source of oxidative stress [7,8]. Therefore, reducing oxidative/nitrative stress after MI/R injury serves as a therapeutic target for the treatment of MI/R injury.

Pterostilbene (Pte), a natural dimethylated analog of resveratrol, is known to confer various pharmacological actions, including, anti-inflammatory, antioxidant, and anti-apoptotic effects [9–11]. It has been suggested that Pte and resveratrol significantly decreased inflammation without becoming cytotoxic and the highest non-cytotoxic concentrations for each of the compounds were the following: (A) pterostilbene 80 μM, and (B) resveratrol 160 μM [12]. A comparative study indicates that the cytotoxic potency of Pte is higher than resveratrol [13]. Additionally, Pte and resveratrol gain a significant amount of attention for their potent antioxidant property in neurodegenerative diseases [14]. A high dietary intake of Pte does not lead to toxicity in mice [15]. Wang et al. have suggested that Pte exerts its protective effects in murine hippocampal neuronal HT22 cells against glutamate-induced oxidative stress [9]. Guo et al. demonstrate that Pte attenuates hypoxia-reoxygenation injury in cardiomyocytes in vitro via restoration of sirt1 function [11]. However, it remains unclear whether Pte can decrease the oxidative/nitrative stress induced by MI/R injury. Therefore, the current study was designed (1) to determine whether Pte reduces the

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oxidative/nitrative stress induced by MI/R injury and, if so, (2) to investigate the potential mechanisms.

2. Materials and methods

2.1. Animals

All experiments were performed on adult male Sprague-Dawley rats (250–300 g). The rats were obtained from the Laboratory Animal Center of the Xinjiang Medical University. Rats were housed in a temperature-controlled room on a 12 h day/night cycle with free access to food and water. All experiments were performed in adherence with the National Institutes of Health (NIH) Guidelines on the Use of Laboratory Animals, and were approved by the Ethics Committee of the Xinjiang Medical University.

2.2. Myocardial ischemia-reperfusion model

Male Sprague-Dawley rats were anesthetized i.p. with sodium pentobarbital (Sigma, St. Louis, USA, 40 mg/kg). The right femoral vein was cannulated for the drug administration. A left thoracic incision was made and myocardial ischemia was produced by exteriorizing the heart with a slipknot (5-0 silk) around the left anterior descending coronary artery (LAD). After 30 min of ischemia, the slipknot was released and the animal received 3 h of reperfusion. The rats in the Sham-operated group rats underwent the same surgical procedures without LAD artery occlusion.

2.3. Drug injection and animal groups

Pte (Sigma-Aldrich, St. Louis, MO, USA; purity was ≥97%) was first dissolved in DMSO and then diluted in 0.9% NaCl solution and was administered intravenously 10 min before reperfusion at a dose of 10 mg/(kg body weight). Uric acid (UA), an $ONOO^-$ scavenger, was administered intravenously 10 min before reperfusion at a dose of 5 mg/kg. The rats in the MI/R group received equal amount of vehicle. In the first part of this study, rats were randomly assigned to Sham, MI/R, MI/R + Pte groups. In the second part, the animals were randomly assigned to MI/R, MI/R + UA, MI/R + UA + Pte groups.

2.4. Assessment of cardiac function

A microcatheter was inserted into the left ventricle through the right carotid artery to measure the left ventricular pressure (LVP). The artery pressure was measured by right femoral artery intubation. Electrocardiogram (ECG) and LVP were simultaneously recorded on a polygraph (RM-6200C). Left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), and the instantaneous first derivation of LVP ($\pm dp/dt_{max}$) were recorded by computer algorithms.

2.5. Assay of myocardial infarct size

The slipknot of the LAD artery was retied at the end of the reperfusion, and 1 mL of 1% Evans blue dye was injected into the aorta. The heart was quickly excised and frozen at −80 °C. Then, the frozen heart was cut transversally into 1-mm thick sections and then incubated in 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution at 37 °C for 30 min. The digital pictures were captured. TTC-unstained pale area (infarct zone), TTC-stained red area (ischemic but viable myocardium) and Evans blue-unstained regions (area-at-risk, AAR) were analyzed by using an image analysis system (Image Pro Plus 6.0). Myocardial infarct size was determined as INF/AAR × 100%.

2.6. Evaluation of serum creatine kinase-MB and lactate dehydrogenase levels

After the 3 h reperfusion period, blood samples were collected from the carotid artery. Serum creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) levels were determined using the commercial kits (Nanjing Jiancheng Bioengineering, China). The activities of these two enzymes were expressed as U/L.

2.7. Terminal deoxynucleotidyl nick-end labeling staining and myocardial caspase-3 activity

Terminal deoxynucleotidyl nick-end labeling (TUNEL) staining was performed by using an in situ cell death detection kit. In brief, the slides were incubated with TUNEL reaction mixture and then counterstained with DAPI to detect the nuclei. The apoptotic index was calculated as a percentage of the number of TUNEL-positive apoptotic cells over the total number of nucleated cells. Myocardial caspase-3 activity was determined by using a caspase colorimetric assay kit (Chemicon, Temecula, CA, USA) according to the manufacturer's instructions.

2.8. Quantification of tissue 3-nitrotyrosine content

After 3 h of reperfusion, the myocardial tissues were collected and 3-nitrotyrosine content was measured using a by a nitrotyrosine ELISA kit (Chemicon, CA, USA).

2.9. Quantification of superoxide production

Lucigenin-enhanced luminescence was used to determine myocardial superoxide content. Cardiac tissues were weighed, cut into uniform cubes (0.5 mm^3), and transferred into a polypropylene tube containing 1 mL PBS and lucigenin (Sigma, 0.25 mmol/L). The tube was placed in an FB12-Berthold luminometer. The relative luminescence units emitted were recorded and integrated over 30 s intervals for 5 min. Activity was normalized with dry tissue weights. For in situ superoxide detection, 20-μm-thick optimal cutting temperature-embedded cardiac tissues were thaw-mounted on Fisher-Plus slides and stained with 5 μM dihydroethidium at 37 °Cfor 30 min. Ethidium staining (red) was confirmed with an Olympus BX51 fluorescence microscope. Superoxide production was presented as relative light units (RLU) per second per milligram heart weight (RLU/mg/s).

2.10. Assessment of malondialdehyde content and superoxide dismutase activity

The MDA content and activity of SOD in heart homogenates were assessed spectrophotometrically using commercial available assay kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.11. Assay of p38 MAPK activity, inflammatory cytokines levels, and MPO activity

The assay of p38 MAPK activity was performed with a p38 MAPK assay kit (Cell Signaling Technology) as described in previous study [16]. Inflammatory cytokines in the myocardial tissue were assessed by using commercially available TNF-α and IL-1β ELISA kits, under the guidance of the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China). MPO test kit was used to detect level of MPO in the myocardial tissue according to manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China).