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Hypercholesterolemia abrogates the protective effect of ischemic postconditioning by induction of apoptosis and impairment of activation of reperfusion injury salvage kinase pathway

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ABSTRACT

Ischemic postcontioning (IPoC) is an effective method to prevent myocardial ischemia reperfusion injury (MIRI), but its cardioprotection is usually blocked in the presence of hypercholesterolemia (HC) and the potential mechanism is still unknown. In this study, we investigated the roles of reperfusion injury salvage kinase (RISK) and apoptosis-related pathways in the attenuation of cardioprotection of IPoC in the presence of HC. The results showed that IPoC significantly decreased the infarct size and apoptosis, improved the recovery of ischemic myocardium, but these beneficial effects were reversed by high cholesterol diet-induced HC. Moreover, we also found that HC inhibited the phosphorylation of Akt and ERK1/2 which usually activated by IPoC in normal heart, induced excessive apoptosis by down-regulating Bcl-2 and up-regulating Bax, cytochrome c, caspase 9 and caspase 3 when compared with that in normal heart. Taken together, our results demonstrated that the cardioprotection of IPoC was abolished by HC, which was associated with inactivation of RISK signal pathway and dysregulation of downstream apoptosis-related pathway.

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1. Introduction

Ischemic heart disease is one of major causes of death worldwide [1]. Reperfusion therapy, such as coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI), is believed to be the most effective method to salvage ischemic myocardium, especially on the treatment of acute myocardial infarction (AMI) [2]. However, the abrupt reperfusion may lead to further damage of ischemic myocardium, known as myocardial ischemia reperfusion injury (MIRI) [3]. At present, there is still lack of effective therapy for preventing MIRI [4]. Therefore, it is extremely urgent to develop an effective approach to cope with MIRI during the implement of reperfusion therapy.

Recently, much attention has been focused on the ischemic postconditioning (IPoC), repetitive cycles of brief reperfusion and ischemia performed after sustained ischemia, which is considered as an effective method to attenuate MIRI as evidence by reduction in infarct size and accelerating recovery of cardiac function [5,6]. However, the cardioprotection of IPoC is most demonstrated in healthy animals, and several studies indicate that many risk factors of cardiovascular diseases including hyperlipidemia, diabetes, hypertension and aging may impair the protective effect of IPoC [7]. So far, the mechanism of attenuation of the cardioprotective effect of IPoC in the presence of these risk factors is not well understood.

Our previous research also confirms that hypercholesterolemia abrogates the cardioprotection of IPoC in isolated rat hearts and the abrogation of IPoC in hypercholesterolemic rat heart is involved in the impairment of anti-apoptosis effect and the excessive opening of mitochondrial permeability transition pore (mPTP) [8]. Although the cardioprotection of IPoC is involved in several signaling pathways, activation of reperfusion injury salvage kinase (RISK) signaling pathway, which subsequently acts to prevent mPTP opening and further regulate the apoptosis-related pathway, is still considered as a major mechanism of IPoC [9,10]. Therefore, we infer that the abrogation of IPoC in the presence of hypercholesterolemia is involved in inactivation of RISK signaling pathway and dysregulation of apoptosis-related pathways. In the present study,

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we investigated the roles of RISK and apoptosis-related pathways in the attenuation of IPoC in the presence of hypercholesterolemia.

2. Materials and methods

2.1. Animals and induction of experimental hypercholesterolemia

All the experimental procedures concerning animals were performed strictly in adherence to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH). The study procedure was approved by the institutional Ethics Committee of China Medical University (Shenyang, China).

Sixty male Wistar rats weighing 200 ± 10 g were randomly divided into hypercholesterolemia (HC) group and normocholesterolemia (NC) group. Animals in HC group were fed with a diet including 1.5% cholesterol, 5% egg yolk powder, 10% lard, 0.5% sodium cholate, 3% sugar, and 80% normal feedstuff for 8 weeks, and this formula was based on our previous report [8], whereas animals in NC group received a nomal diet (Qian Ming Experimental animal feed factory, Shenyang, China) for the same period. At the end of 8-week feeding period, blood samples were collected from the rats' vena caudalis for determination of serum levels of total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.2. Heart preparation

Rats were anesthetized through an intraperitoneal injection of 10% chloral hydrate (4 ml/kg). Heparin (1500 IU/kg) was administered intravenously priors to the surgery to prevent intracoronary clot formation. After opening the chest, the heart was rapidly excised and immediately immersed in ice-cold heparinized Krebs–Henseleit solution (KH solution: 127 mmol/l NaCl, 17.7 mmol/l $NaHCO_3$, 5.1 mmol/l KCl, 1.5 mmol/l $CaCl_2$, 1.26 mmol/l $MgCl_2$, 11 mmol/l D-glucose, pH = 7.4) for trimming. Then the heart was mounted on a Langendorff-perfusion apparatus and retrogradely perfused through the aorta with KH solution saturated with 95% O_2–5% CO_2 under a constant pressure of 75 mmHg at 37 °C. The fluid-filled latex balloon was inserted in the left ventricle via the left atrium for pressure measurement. The balloon was connected to a pressure transducer and the homodynamic parameters including heart rate (HR), left ventricular developed pressure (LVDP), positive first order derivative of ventricular pressure (+dp/dt) and negative first order derivative of ventricular pressure (−dp/dt) were continuously recorded and digitally processed via a homodynamic system (BIOPAC MP150, USA).

2.3. Experimental protocol

Rats in NC and HC groups were further assigned to two subgroups: (i) ischemia reperfusion (IR) group: the isolated rat hearts were allowed for 20 min of stabilization, and then subjected to 30 min of global ischemia and 120 min of reperfusion; (ii) ischemic postconditioning (IPoC) group: the isolated rat hearts were also allowed for 20 min of stabilization and subjected to 30 min of global ischemia. At the onset of long time of reperfusion, six cycles of IPoC (6 × 10 s) were conducted, and then followed by 120 min of reperfusion.

2.4. Measurement of infarct size

Measurement of infarct size was previously described [11]. Briefly, at the end of reperfusion, the heart was harvested and stored at − 20 °C for 1 h. The whole heart was sectioned from apex to base into 1–2 mm sections, and incubated in 1% triphenyltetrazolium chloride (TTC) solution for 20 min at 37 °C, fixed by 4% paraformaldehyde for 24 h, and then photographed by a digital camera. The infarct myocardium tissues were unstained and turned into white, whereas viable myocardium tissues were stained and presented red.

2.5. Determination of myocardial apoptosis

The specific procedure for determination of myocardial apoptosis was previous described [12]. Briefly, myocardial apoptosis was detected using In Situ Cell Death Detection Kit (Roche, USA) according to the manufacturer's instructions. The apoptotic nuclei were stained dark brown. In contrast, the normal nuclei were presented blue. Three sections from each myocardial sample were randomly selected and 10 microscopic fields (Olympus BX51 microscope) per section were evaluated by two independent blind observers. For each field, the number of nuclei was counted and the percentage of TUNEL-positive nuclei was calculated.

2.6. Western blotting

Proteins were extracted from the myocardial tissues using a protein extraction reagent (Beyotime, Shanghai, China) and protein concentration was measured using the BCA Protein Assay kit (Beyotime, Shanghai, China). 50 μg protein of each sample was separated by electrophoresis on SDS-PAGE and transferred onto polyvinylidenedifluoride-plus membrane. The membranes were blocked with 5% skim milk followed by incubated overnight at 4 °C with the antibodies: Akt (1:500; Santa Cruz, California, USA); phospho-Akt (at Ser473, 1:500; Santa Cruz, California, USA); ERK1/2 (1:500; Santa Cruz, California, USA); phospho-ERK1/2 (at Thr 202 and Tyr 204, 1:500; Santa Cruz, California, USA); Bcl-2 (1:1000; Abcam, Hongkong); Bax (1:1000; Abcam, Hongkong); cytochrome c (1:1000; Abcam, Hongkong), caspase 9 (1:1000; Abcam, Hongkong) and caspase 3 (1:1000; Abcam, Hongkong). After incubation, the membranes were washed three times with 0.1% Tween-20 for 15 min and incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (1:2000; Santa Cruz, California, USA) at 37 °C for 2 h. Detection was performed by enhanced chemi-luminescence (ECL) using a Western blotting luminological reagent (Santa Cruz, California, USA) according to the manufacturer's instructions. The levels of phosphorylated proteins were normalized to their total protein levels. Relative densitometry was performed using a computerized software package (NIH Image 1.63 software).

2.7. Statistical analysis

The data were expressed as mean ± SD values. Statistical analysis was performed by using Sigma Stat software version 3.5 (Systat software). Differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by Student–Newman–Keuls post hoc test. All P values less than 0.05 were considered statistically significant.

3. Results

3.1. The levels of serum lipids

The values for serum lipids of rats in NC and HC groups were listed in Table 1. The levels of TC and LDL were significantly increased in HC group compared with NC group ($P < 0.05$).

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