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Roles of adiponectin in alleviating myocardial ischemia-reperfusion injury in rats with remote ischemic postconditioning.

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Abstract

The study was to evaluate the roles of Adiponectin (ADP) in alleviating myocardial ischemia-reperfusion injury in rats with Remote Ischemic Postconditioning (RIP). A total of 57 healthy male SD rats were randomly divided into 3 groups (the sham operation group (S), the myocardial ischemia-reperfusion group (I/R), and the RIP group (R). The myocardial ischemia-reperfusion injury model was established by ligating the left coronary artery for 30 min followed by 180-min reperfusion. Group R was performed RIP after 20 min ligation of the left coronary artery, and the ST segment amplitudes were recorded at the 30, 60, 120, and 180 min after reperfusion. 3 ml of jugular venous blood was sampled at the 180 min after reperfusion for the detection of serum ADP, creatine kinase-MB (CK-MB) activity, and cTnI concentration. At the end of the experiment, the rats were sacrificed to measure the myocardial Infarct Size (IS) and sample the myocardial tissue to detect the content of ADP. Compared with group S, the ADP contents in the serum and myocardial tissue in group I/R and R were deceased, but IS, serum CK-MB activity, and cTnI concentration were increased, and the ST segment was elevated (P<0.05). Compared with group I/R, the ADP contents in the serum and myocardial tissue in group R were increased, but IS, serum CK-MB activity, cTnI concentration, and the ST segment were decreased. ADP is involved in RIP that can alleviate the myocardial ischemia-reperfusion injury in rats.

Keywords: Adiponectin, Myocardial ischemia-reperfusion injury, Myocardial protection, Remote ischemic postconditioning.

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Introduction

Remote Ischemic Postconditioning (RIP) refers to repeated short-term intermittent remote organ reperfusion and re-ischemia before the reperfusion toward one long-term myocardial ischemic period, and it can reduce myocardial ischemia/reperfusion (M-I/R) injury and enhance myocardial tolerance against relatively long-time ischemia and hypoxia [1-3]. However, as one important mechanism in inducing autoprotection of cells, its specific mechanism is still unclear. At present, it is considered that the ability of RIP in mobilizing endogenous anti-injury may be related to multiple factors. Adiponectin (ADP) is one adipose cell-derived hormone protein secreted by the adipose tissue, abundant in human plasma, and can be expressed in the bone marrow, osteoblasts, cardiomyocytes, etc.; it has such activities as anti-inflammation, antioxidation, reducing blood glucose, etc., thus exhibiting protective effects on cells, tissues, or organs [4-9]. Our previous studies have found that the postoperative serum ADP level in elderly patients with general anesthesia is negatively correlated with the incidence of postoperative cognitive dysfunction [10]. However, whether RIP can activate this protective factor has not been studied. In this study, we established the rat RIP model and the M-I/R model, aiming to observe the myocardial protective roles of RIP and the serum and myocardial ADP changes in rats.

Materials and Methods

Animal grouping and processing

A total of 57 healthy clean male SD rats were provided by the Animal Center, Dongguan branch, Guangdong Medical College (Certificate No: SCXK (Yue) 2008-0001, 8 weeks of age, weighing 250~350 g), and divided into three groups according to the random number table method: the sham operation group (S, n=19): only performed threading toward the left anterior descending artery; the I/R group (R/I, n=19), and the RIP group (R, n=19). The rats in group R were performed RIP 20 min after ligating the left coronary artery, which used tourniquet to ligate the hind limbs for 10 min. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The

animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Dongguan People's Hospital.

Preparation of animal model

The rats were performed 12 h fasting while free to drinking water, and then prepared the R/I model according to the literature [11]: after intraperitoneally injected 20% urethane (5 ml/kg) for anesthesia, each rat was performed tracheal intubation, artificial mechanical ventilation, Electrocardiogram (ECG) monitoring, left internal jugular vein intubation for fluid infusion or medication, and right carotid artery intubation to measure the carotid Artery Blood Pressure (ABP) or sample blood. Needle electrodes were inserted subcutaneously into the four limbs to monitor reciprocal II ECG. Left Axis Deviation (LAD) was then ligated to form local myocardial ischemia with the epicardium at the distal end of the ligation appearing cyanosis or gray and synchronous ECG exhibiting ST segment elevation as the sign of successful LAD ligation. The LAD ligation was then loosened to restore the myocardial blood flow, which resulted in the elevated ST segment relatively reduced and the epicardium in the ischemic region restoring rosy. All the rats except for those in group S were performed the LAD ligation for 30 min and restored the blood flow for 180 min reperfusion. Operations of limb RIP: at the midupper 1/3 site of the both lower extremities of each rat, one elastic tourniquet was used for the ligation, with the skin surface at the distal end of the ligation appearing cyanosis and lower limb skin temperature decreasing as the sign of successful lower extremity ischemia. After re-opened the lower limb ligation and restored the blood flow for reperfusion, the skin surface at the distal end of the ligation should restore rosy gradually.

Measurement of ECG ST segment

After being anesthetized, each rat was fixed and inserted the needle electrodes into the skin of the four limbs to record the standard limb reciprocal II ECG. Each rat was continuously monitored ECG, and recorded the ST segment changes at the 30, 60, 120, and 180 min after reperfusion.

Biochemical indexes

The ST segment amplitudes were recorded at the 30, 60, 120, and 180 min after reperfusion; meanwhile, 3 ml of jugular venous blood of 13 rats in each group was sampled at the 180 min after reperfusion for the detection of serum ADP, creatine kinase-MB (CK-MB) activity, and cTnI concentration. The plasma CK-MB activity was measured with a 7060 automatic biochemical analyzer (Hitachi, Japan). The cTnI concentration was determined using an XD711 multifunctional microplate reader (Shanghai Xunda Medical Co. Ltd., China), and the serum ADP level was measured by Enzyme Linked Immunosorbent Assay (ELISA).

Measurement of IS

After 120 min reperfusion, the left coronary artery was re-ligated, and the left internal jugular vein was injected with 1 ml of 3% Evan blue and 100 g/L potassium chloride to terminate the heartbeat. After the heart was fully stained, the heart was cut and washed with normal saline. The non-ischemic myocardium was stained blue, but the ischemic myocardium was not stained; after isolated the above two parts, the left ventricular muscle of the ischemic region was sliced to form 2 mm slices along the longitudinal axis, which were then placed in 1% TTC phosphoric acid buffer for 15 min water bath at 37°C. The non-infarcted area was stained red, but the infarcted area was not. The two parts were then isolated and weighed, respectively. The sum of these two parts was the weight of the ischemic myocardium, and the weight percentage of infarcted area to the ischemic myocardium was the IS.

Detection of ADP

Six rats of each group were quickly killed at the 180 min after reperfusion to sample the apical tissue for the homogenization. The homogenate was centrifuged (3000 rpm, centrifugation radius 12 cm) for 10min, and the supernatant was then stored at -80°C for further detecting the ADP concentration by ELISA (Rapidbio, USA); the concentration was then conversed into the ADP content in the myocardial tissue.

Statistical analysis

SPSS14.0 was used for the statistical processing; the data were expressed as mean ± standard deviation ($\bar{x} \pm s$); the intragroup comparison used the paired t test, the multi-group comparison used the analysis of variance, and the comparison between two groups used the SNK-q Test, with $P<0.05$ considered as statistically significant difference.

Results

General information

A total of 54 rats, out of 57 rats, were successfully performed the modeling, and the rest 3 were excluded due to the occurrence of intractable arrhythmias when ligating or re-opening LAD (2 rats in group R/I, and 1 rat in group R).

ST segment changes

Compared with group S, the ST segments in group I/R and R at different time points were significantly increased. Compared with group I/R, the increasing of the ST segment in group R was alleviated ($P<0.05$, Table 1).

Comparison of ADP, cTnI, and CK-MB

The serum and myocardial ADP levels in group I/R and R were reduced ($P<0.05$), but IS, serum CK-MB activity, and cTnI concentration were increased ($P<0.05$); Compared with group I/R, the ADP contents in the serum and myocardial tissue in group R were increased ($P<0.05$), but IS, serum CK-

MB activity, and cTnI concentration were decreased (P<0.05; Tables 2 and 3).

Table 1. *Comparison of ST-segment elevation after reperfusion among the three groups (mv, $\bar{x} \pm s$).*

Group	n	30 min	60 min	120 min	180 min
S	19	0.06 ± 0.03	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.01
I/R	17	0.23 ± 0.03[a]	0.22 ± 0.06[a]	0.18 ± 0.02[a]	0.15 ± 0.03[a]
R	18	0.15 ± 0.01[ab]	0.13 ± 0.02[ab]	0.14 ± 0.03[ab]	0.12 ± 0.02[ab]

Compared with group S, [a]P<0.05; Compared with group I/R, [b]P<0.05.

Table 2. *Comparison of ADP, cTnI, CK-MB, and MI rate among the three groups after reperfusion ($\bar{x} \pm s$).*

Group	n	ADP (ug/l)	cTnI (ug/l)	CK-MB (U/l)	MI rate (%)
S	13	1.13 ± 0.23	0.3 ± 0.2	383.2 ± 93.1	0.21 ± 0.03
I/R	11	0.38 ± 0.18[a]	4.3 ± 1.3[a]	1323.1 ± 112.3[a]	53.63 ± 8.36[a]
R	12	0.78 ± 0.33[ab]	1.8 ± 1.1[ab]	693.8 ± 103.5[ab]	38.68 ± 7.13[ab]

Compared with group S, [a]P<0.05; Compared with group I/R, [b]P<0.05.

Table 3. *Comparison of ADP in the myocardium of the three groups after reperfusion (n=6, $\bar{x} \pm s$).*

Group	ADP (mg/mgpro)
S	0.33 ± 0.07
I/R	0.12 ± 0.06[a]
RLIP	0.25 ± 0.09[ab]

Compared with group S, [a]P<0.05; Compared with group I/R, [b]P<0.05.

Discussion

Targeting various factors being involved in I/R, various kinds of myocardial protective measures and drugs have been paid more and more attention, especially the mechanisms that can start the *in vivo* endogenous cardiovascular protection. It was firstly found in 2003 that postconditioning exhibited the same effects as ischemic preconditioning in limiting IS and protecting ischemic endothelial functions, and then the concept of Ischemic Postconditioning (IPOC) was proposed. Because of the invasive nature of the IPOC implementation toward the heart, unpredictable risks still exist, and in this condition, RIP was proposed. In recent years, its occurrence mechanism and prevention methods have obtained more and more in-depth studies, which reveal that RIP can significantly reduce the I/R injury. Certain study has confirmed its exact myocardial protective roles [12-15], but the specific mechanism is still not clear.

TTC staining is a well-established method for evaluating the desaturase activity in gross tissue, and IS is considered as the gold standard for studying myocardial protective effects [16,17]. The myocardial TTC staining results in this study showed that the MI rate in group R was decreased than group I/R, suggesting that RIP can reduce the M-I/R injury. Under normal circumstances, cTnI mainly exists in the myocardium and skeletal muscle, but it will enter the blood largely when myocardial degeneration and necrosis occur, so serum cTnI can reflect the extent of myocardial injury to some extent. M-I/R injury-caused by serum cTnI content and CK-MB activity increasing is parallel to the degree of myocardial injury. In this study, the plasma cTnI content and CK-MB activity in group I/R were higher than group S; meanwhile, the ST segment in group I/R was elevated at different time points after reperfusion, suggesting severe M-I/R injury; the plasma cTnI concentration and CK-MB activity in group R were decreased than group I/R. Although the ST segment in group R was elevated to varying degrees at different time points after reperfusion, the degrees were significantly reduced than group I/R, suggesting that RIP played its role in reducing M-I/R injury.

ADP is abundant in human plasma with the concentration as about 5-30 mg/L, which is 106-fold higher than Tumor Necrosis Factor (TNF-α) and Interleukin (IL-6) and accounts for about 0.01% of the total plasma proteins [18]. The expression and secretion of ADP is regulated by certain *in vivo* inflammatory factors, reactive oxygen species, transcription factors, or hormones, such as Reactive Oxygen Species (ROS), TNF-α, or IL-6 can inhibit the expression and secretion of ADP, among which TNF-a can strongly inhibit the activity of ADP promoter, thus playing its roles. In recent years, studies of ADP have shown that ADP has a wide range of biological effects in the cardiovascular field, including regulating pro-proliferation, promoting neovascularization, improving endothelial function, regulating cardiac energy metabolism, inhibiting cardiac hypertrophy and remodeling, confronting apoptosis and inflammation, confronting M-I/R injury, etc. The rats with the *ADP* gene knockout exhibited increased M-I/R injury and showed significantly expanded IS, but adenovirus-mediated ADP transfection can reduce IS and myocardial cell apoptosis [19]. These results suggest that ADP has protective effects on the cardiovascular system, but whether RIP can activate ADP is not clear. The results of this study showed that the ADP levels in the serum and myocardial tissue of group I/R were lower than group S, as well as negatively correlated with the MI rate, serum cTnI concentration, and CK-MB activity; however, the ADP levels in the serum and myocardial tissue of group R were increased than group I/R, suggesting that the reduction of the ADP contents in the serum and myocardial tissue are related to the M-I/R injury, and ADP and RIP both have protective effects on the M-I/R injury, so the increase of the ADP contents in the serum and myocardial tissue may be a beneficial positive feedback of RIP.

The possible mechanisms of RIP in staring ADP to alleviate the M-I/R injury may be the following: 1. RIP can downregulate the body's apoptosis: certain studies have found that RIP can activate the PI3K/Akt and JAK/STAT signal transduction pathways, thus mediating the apoptosis of myocardial cells and reducing the M-I/R injury in rats [20]; 2. RIP can confront inflammation and oxidation: certain studies

have found that RIP can downregulate the high expressions of TNF-α, High Mobility Group Box chromosomal protein 1 (HMGB-1), Intercellular Adhesion Molecule (ICAM-I), IL-1, and IL-6 in the myocardial tissue of the rats with M-I/R injury and decrease the activity of serum Malonaldehyde (MDA) and Myocardial Morphine (MOP) [21].

In summary, this study showed that the ADP levels in the serum and myocardial tissue were negatively correlated with the indexes of M-I/R injury, suggesting that RIP may play its role in reducing the M-I/R injury by changing the ADP levels in the serum and myocardial tissue.

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Conflicts of Interest

The authors declare no conflict of interest.

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