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Effect of peroxisome proliferator−activated receptor gamma agonist on heart of rabbits with acute myocardial ischemia/reperfusion injury

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ABSTRACT

Objective: To explore protective effect of rosiglitazone on myocardial ischemia reperfusion injury. **Methods:** A total of 48 male SD rats were randomly divided into control group (A), I/R group(B),high dose of rosiglitazone (C), low dose of rosiglitazone (D). Plasm concentration of creatine kinase (CK), CK−MB, hsCRP, Superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH−Px), nitric oxide (NO) and endothelin (ET) were measured 1 h later after I/R. 24 h after I/R hearts were harvested to observe pathological and ultrastructural changes. Immunohistochemistry and western blotting was used to test CD40 expression in myocardial tissue. Area of myocardial infarction were tested, arrhythmia rate during I/R was recorded. **Results:** Plasm concentration of creatine kinase (CK), CK−MB, hsCRP, NO, MDA and ET were decreased in group C, D compared with group B. Plasm concentration of T−SOD and GSH−Px was increased significantly in group C, D compared with group B. Compared with group B, pathological and ultrastructural changes in group C, D were slightly. Myocardial infarction area and arrhythmia rate were lower in group C, D compare with group B. **Conclusions:** Rosiglitazone can protect myocardium from I/R injury by enhancing T−SOD and GSH−Px concentration, inhibit inflammatory reaction, improve endothelial function, reduce oxidative stress and calcium overload.

1. Introduction

Myocardial ischemia/reperfusion injury often occurs after thrombolysis, percutaneous transluminal coronary angioplasty, and coronary artery bypass surgery for acute myocardial infarction. The underlying pathophysiological mechanism is that the expression of cytokines, in particular cell surface adhesion molecules induces the aggregation and adherence of inflammatory cells, resulting in immune−mediated injury to histiocytes[1,2], which greatly influences the therapeutic effects and prognosis in patients with myocardial infarction. Peroxisome proliferator−activated receptor gamma (PPAR−γ) is mainly expressed in adipose tissue and immune system and closely related to adipocyte differentiation, insulin resistance and organism immunity[3−5]. Rosiglitazone can regulate insulin gene transcription, which is involved in glucose production, transport and utilization, by activating PPAR−γ and it can also alleviate myocardial ischemia/reperfusion injury by inhibiting inflammatory reaction, improving endothelial function and reducing oxidative stress[6−8]. Whether rosiglitazone exhibits protective effects on myocardial ischemia/reperfusion injury remains poorly understood. This study investigated this problem, hoping to provide new thoughts for clinical treatment of myocardial ischemia/reperfusion injury and achieve favorable economic and social benefits.

2. Materials and methods

2.1. Animals and reagents

Male Japanese big−ear rabbits, weighing (2.50±0.25) kg, were provided by Laboratory Animal Center, Zhengzhou University, China.

Rosiglitazone was produced by Shanghai No.1 Biochemical

and Pharmaceutical Co., Ltd., China (batch No. 06050211). It was used together with physiological saline in the clinic and should be sterilized before use. Glucose injection was produced by Ji′nan Limin Pharmaceutical Co., Ltd., China (batch No. 0512547) and sodium chloride injection by Shenyang Zhiying Pharmaceutical Factory, China (batch No. 06080204). Superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO), glutathione peroxidase (GSH–Px), and endothelin (ET) kits were purchased from Nanjing Jiancheng Bioengineering Institute, China.

2.2. Instruments

The following instruments were used in this study: 7170A full–automatic biochemical analyzer (Hitachi, Japan), 721 visible spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd., China), UV300 ultraviolet–visible spectrophotometer, K15–C low–temperature ultracentrifuge (Beijing Medical Centrifuge Factor, China), MDFU40865 low temperature and ultra low temperature freezer, HX–200 animal respirator, SHH.7121.600 electrothermal constant–temperature water tank (Beijing Ever Light Medical Equipment Co., Ltd., China), MC–ASCENT enzyme reader, electronic balance, DYY–6C electrophoresis apparatus, CH20 optical microscope (Olympus, Shinjuku–ku, Tokyo, Japan), CIAS–1000 image analyzer, RF–535 fluorescence detector, LC–9A high performance liquid chromatograph (Shimadzu, Japan), C–35A microscope (Olympus), and IX–70 inverted fluorescence microscope.

2.3. Methods

2.3.1. Establishment of acute myocardial ischemia/reperfusion injury models in rabbits

Following anesthesia by 20% urethane (1 g/kg), chest hair was sheared, and an incision was made along the midsternal line and started over the clavicle and till the xiphoid process of the sternum. The second, third and fourth ribs were interrupted at the left sternal border and the pericardium was cut open to expose the heart. A 2/0 silk suture was introduced into the root region of the circumflex branch of left coronary artery for later ligation

2.3.2. Grouping and drug administration

Forty–eight rabbits were randomly divided into four groups. In the sham operation group, the suture was introduced into the circumflex branch of the left coronary artery and twisted, and ligation was not performed; before surgery, rabbits were raised with common pellet feeds for 3 d. In the ischemia/reperfusion group, 30–minute ischemia followed by 60 min reperfusion was performed, and before surgery, rabbits were also raised with common pellet feeds for 3 d. Based on the procedure in the ischemia/reperfusion group, 3 mg and 0.5 mg rosiglitazone was orally administered in the rosiglitazone high–dose and low–dose groups, respectively. On the day of surgery, the circumflex branch of the left coronary artery

was ligated in the latter three groups. When an obvious ST–segment elevation, a sign of successful ligation, was observed in the electrocardiogram, 1–hour reperfusion was performed. Then blood was taken from the common carotid artery through the use of heparin anticoagulation tube for detection of biochemical indices.

2.3.3. Index detection

Plasma plasma cardiac troponin Ⅰ (CTnⅠ), creatinine kinase–MB (CK–MB) and C–reactive protein (hsCRP) levels were detected in the blood immediately taken from the common carotid artery.

After 10–minute centrifugation at 2 500 r/min, the supernatant of the common carotid artery blood was collected for detection of plasma total superoxide dismutase (T–SOD), GSH–Px, MDA, NO and ET levels.

2.3.4. Sample preparation

Following washes with fixing solution, myocardial tissue on the left ventricular anterior wall was dissected into several strips, fixed in the fixing solution, rinsed with 0.09 mmol/L KH_2PO_4 for 15 min, and preserved in KH_2PO_4. Strip–shaped tissue was hydrated in a graded series of ethanol, cleared by xylene, and embedded by paraffin. Then part of tissue was used for immunohistochemical detection and the remainder was sliced, stained with hematoxylin–eosin, and observed under the optical microscope.

2.3.5. Immunohistochemistry

Paraffin sections were dehydrated, dewaxed with xylene and dehydrated alcohol, retrieved by citric acid antigen, enveloped, hybridized, developed and mounted by gum.

2.4. Statistical analysis

All data were statistically processed using SPSS 17.0 software. Measurement data were expressed as mean±SD. Q–test was used for difference comparison between two groups and one–way analysis of variance for difference comparison among multiple groups. A level of $P<0.05$ indicates significant difference and $P<0.05$ highly significant difference. Pathomorphological data were analyzed by contrast description.

3. Results

3.1. Effects of rosiglitazone on plasma CTnⅠ, CK–MB and hsCRP levels in rabbits with myocardial ischemia/reperfusion injury

Compared with sham operation group, plasma CTnⅠ, CK–MB and hsCRP levels in the ischemia/reperfusion group were significantly increased. Plasma CTnⅠ, CK–MB and hsCRP levels in the rosiglitazone high–dose group and rosiglitazone low–dose group were significantly lower than

in the ischemia/reperfusion group (Table 1).

Table 1
Plasma CTn Ⅰ, CK–MB and hsCRP levels in each group.

Group	n	CTn Ⅰ (μg/mL)	CK–MB (U/L)	hsCRP (U/L)
A	12	110.35±25.96	134.75±35.12	47.10±2.82
B	12	182.37±30.46△	214.52±43.78△	77.35±1.32△
C	12	120.53±31.03★	142.36±40.13★	50.24±1.47★
D	12	136.42±28.37★	157.21±45.91★	53.38±1.56★

Note: ★$P<0.01$, *vs*. ischemia/reperfusion group; △$P<0.01$, *vs*. sham operation group.

3.2. Effects of rosiglitazone on plasma NO, ET and GSH–Px levels in rabbits with myocardial ischemia/reperfusion injury

Compared with sham operation group, plasma NO and GSH–Px levels were significantly decreased, while plasma ET level was significantly increased in the ischemia/reperfusion group. Compared with ischemia/reperfusion group, plasma NO and GSH–Px levels were significantly increased and plasma ET level was significantly decreased in the rosiglitazone high and low dose groups. There were no significant differences in plasma NO, ET and GSH–Px levels between rosiglitazone high and low dose groups (Table 2).

Table 2
Plasma NO, ET and (GSH–Px levels in each group.

Group	n	NO(μmol/L)	ET (pg/mL)	GSH–Px(U)
A	12	71.28±4.32	40.23±6.35	218.23±21.75
B	12	32.51±5.25△	297.57±7.58△	112.42±31.58△
C	12	65.84±5.05★	121.28±8.82★▲	187.34±27.29★★▲
D	12	57.32±6.34★★	143.56±6.54★	176.56±33.81★★

Note: ★★$P < 0.05$, ★$P < 0.01$, *vs*. ischemia/reperfusion group; △$P < 0.01$, *vs*. sham operation group; ▲$P > 0.05$, *vs*. rosiglitazone low dose group.

3.3. Effects of T–SOD and MDA levels in rabbits with myocardial ischemia/reperfusion injury

Compared with sham operation group, plasma MDA level was significantly increased and plasma T–SOD level was significantly decreased in the ischemia/reperfusion group. Compared with ischemia/reperfusion group, plasma MDA level was significantly decreased and plasma T–SOD level was significantly increased in the rosiglitazone high and low dose groups (Table 3).

Table 3
Plasma MDA and ET levels in each group.

Group	n	T–SOD(U/L)	MDA(nmol/mL)
A	12	521.38±34.42	7.14±0.42
B	12	390.54±33.86△△	12.84±0.79△
C	12	489.27±15.62★★	7.46±0.37★
D	12	454.58±25.43★★	8.10±0.54★

Note: ★$P<0.05$, ★★$P<0.01$, *vs*. ischemia/reperfusion group; △$P<0.05$, △△$P<0.01$, *vs*. sham operation group.

3.4. Optical microscope observation of pathological structure

Through the optical microscopy, in the sham operation group, myocardial fibers were orderly arranged, and no invasion of inflammatory cells in the myocardial interstitium was observed; in the rosiglitazone high and low dose groups, nuclei of myocardial cells were uniform in size and distribution, but myocardial fibers exhibited slight swelling, and fibrocytes and inflammatory cells invaded occasionally; in the ischemia/reperfusion group, myocardial fibers were swollen and disordered, a large number of inflammatory cells invaded the myocardial interstitium, and obvious local necrosis was even observed.
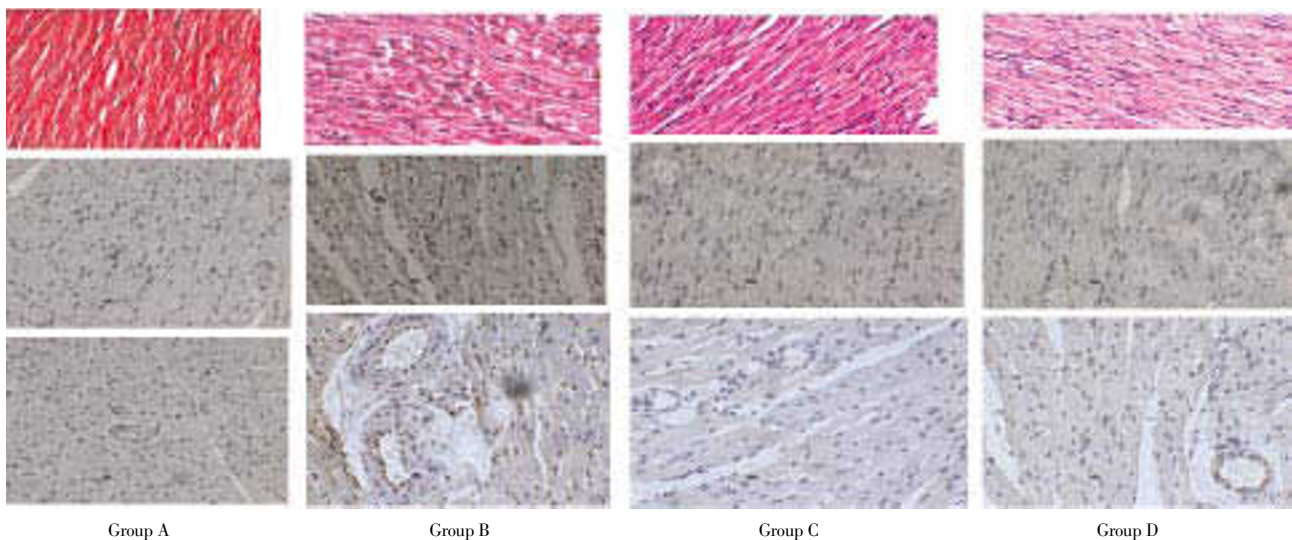

Figure 1. Optical microscope observation of pathological structure.
Group A: full of muscle fiber, the nuclear shape is normal.
Group B: extensive necrosis, muscle fiber fracture, severe muscle cell nucleus dissolved, disappear, neutrophils large seepage.
Group C: myocardial cell nuclei are uniform and mild myocardial fibers swelling, occasional fibroblast cells and inflammatory cells infiltration.
Group D: myocardial cell nuclei are uniform and mild myocardial fibers swelling, occasional fibroblast cells and inflammatory cells infiltration.

Sham operation group: intact muscle fiber and normal nuclear morphology.

Ischemia/reperfusion group: wide necrosis, muscle fiber was greatly broken, a large number of myocyte nuclei dissolved or disappeared, and many neutrophil granulocytes effused.

Rosiglitazone high and low dose groups: myocyte nuclei were uniform in size, myocardial fiber was slightly swollen, and fibroblasts and inflammatory cells invaded occasionally (Figure 1).

4. Discussion

Acute myocardial infarction is recently a leading health– or life–threatening disease, and myocardial ischemia/ reperfusion can cause irreversible damage to, even death or apoptosis of, injured myocardial cells, which further aggravates myocardial damage and increases mortality[9,10]. There is evidence that various inflammatory factors, including neutrophil, monocyte, mastocyte, macrophage, tumor necrosis factor– α, interleukin–1, –6, and –8 participate in myocardial ischemia/reperfusion[11–13]. Protein–protein interaction between PPAR– γ and nuclear factor κ B (NF– κ B) effectively inhibits homogenous cis–element binding in the promoter region of NF– κ B and inflammatory factor genes[14–16].

PPAR– γ is expressed in many immunocytes and PPAR– γ ligand 15d–PGJ2 regulates various immunological responses. PPAR– γ even in a very low concentration of 15d–PGJ2 can inhibit lipopolysaccharide–induced or NF– κ B–, STAT1– and activator protein 1 (AP–1)–mediated transcription[17,18]. Both 15d–PGJ2 and rosiglitazone can inhibit the production of PPAR– γ, thereby inhibiting interleukin–2 gene expression and PHA–induced human T cell proliferation, and finally hampering homogenous cis–acting element binding in the promoter region between activated T cells and interleukin–2[19–21]. In addition, PPAR– γ also regulates the differentiation of some immunocytes, including T cells, mononuclear macrophages and nature killer cells. PPAR– γ ligand inhibits the production of interferon– γ in natural killer cells and T cells by PPAR γ –dependent and non–dependent approach. Gene chip technique showed that PPAR γ expression was 5–8 times greater in type 2 T cells than in type 1 T cells[22–24]. After addition of interleukin–4 and interferon– γ antibodies, type 2 immunocytes can induce PPAR– γ expression in natural killer cells. The activated PPAR– γ can successfully inhibit the expression of inflammatory factors, including tumor necrosis factor– α, and interleukin–1, –2 and –6 in monocytes, exhibiting anti–inflammatory effects. An effective control of interleulin–2 gene expression in the early stage of lymphocyte differentiation is very important for activation of lymphocytes. The activated PPAR– γ can effectively inhibit interleukin–2 gene expression and prevent the early activation of T lymphocytes, thereby exerting anti–inflammatory effects[25,26].

Results from this study showed that pretreatment with PPAR– γ agonist rosiglitazone can significantly increase plasma SOD, GSH–Px and NO levels and decrease plasma cTNI, CK–MB, hsCRP, MDA and ET levels, significantly decrease the incidence of arrhythmia, reduce myocardial infarct volume, protect cardiocyte mitochondrial function, and maintain intact mitochondrial ultrastructure in a rat model of myocardial ischemia/reperfusion injury.

Based on the present results, we can infer that rosiglitazone pretreatment can protect myocardium and alleviate ischemia/ reperfusion injury. However, the precise mechanism remains unclear. Rosiglitazone, as a PPAR– γ agonist, influences the formation and activation of inflammatory cells or inflammatory factors and simultaneously inhibits the expression of monocyte chemoattractant protein–1, intercellular adhesion molecule–1 and inducible nitric oxide synthase by regulating PPAR– γ expression[27,28]. In addition, Khandoudi et al[30] found that thiazolidinediones can inhibit c–Jun amino–terminal kinase phosphorylation and activator protein–1 DNA binding activity, and thereby alleviate myocardial ischemia/ reperfusion injury and improve cardiac function.

Taken together, rosiglitazone can be used to treat myocardial ischemia/reperfusion injury by inhibiting inflammatory reaction, improving endothelial function, and reducing reactive oxygen species. Nevertheless, the precise mechanism will be further studied.

Conflict of interest statement

We declare that we have no conflict of interest.

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