Experimental research on the protective effect of PPAR γ agonist rosiglitazone on myocardial reperfusion injury

Bo Yuan1*, Dong-Qi Wang2, Sheng-Qiang Liu3, Hui Ren1, Jie Gong1, Rui Wang1, Tian-Lin Gao1

1First ward of Cardiovascular Medicine, Ankang City Central Hospital, Ankang City, Shaanxi Province, 725000, China
2The First Affiliated Hospital of Xian Jiaotong University, Xi’an City, Shaanxi Province, 710061, China
3Ankang Hospital of Traditional Chinese Medicine, Ankang City, Shaanxi Province, 725000, China

ARTICLE INFO

Objective: To study the protective effect of PPAR γ agonist rosiglitazone on myocardial reperfusion injury. Methods: SD rats were chosen for study and randomly divided into sham group, ischemia-reperfusion injury group and rosiglitazone treatment group. Then myocardial infarct size, apoptotic index, myocardial performance index and mRNA contents of apoptosis-related molecules were detected. Results: Infarct size and apoptotic index of I/R group were greater than those of Sham group, and infarct size and apoptotic index of ROC group were less than those of I/R group; CK-MB and cTnT contents in plasma of I/R group were higher than those of Sham group, and CK-MB and cTnT contents in plasma of ROC group were lower than those of I/R group; β-MHC/α-MHC proportion as well as bax and caspase-3 contents in myocardial tissue of I/R group were higher than those of Sham group, and bcl-2 and miR-208a contents were lower than those of Sham group; β-MHC/α-MHC proportion as well as bax and caspase-3 contents in myocardial tissue of ROC group were lower than those of I/R group, and bcl-2 and miR-208a contents were higher than those of I/R group. Conclusion: PPAR γ agonist rosiglitazone has protective effect on myocardial reperfusion injury; it can reduce the infarct size, decrease the number of infarction cells and regulate the expression of pro-apoptotic and anti-apoptotic genes.

ABSTRACT

1. Introduction

Ischemia-reperfusion injury (I/R) refers to the pathological phenomena of aggravated structural damage and functional impairment after the blood perfusion restoration of ischemic myocardium. After receiving interventional therapy, myocardial infarction patients will go through the process of ischemia reperfusion and ischemia reperfusion injury will affect myocardial structure and function, which is not conducive to the recovery of heart function. In clinical practice, I/R injury will adversely affect the prognosis of myocardial infarction patients with interventional therapy, and reduction and prevention of I/R injury can improve the prognosis of patients with myocardial infarction[1]. In recent years, studies have shown that decreased expression of peroxisome proliferator-activated receptor γ (PPAR γ) is associated with the occurrence of myocardial I/R injury[2]. In the following research, the protective effect of PPAR γ agonist rosiglitazone on myocardial reperfusion injury was analyzed.

2. Experimental materials, animals and methods

2.1. Experimental materials

Rosiglitazone was purchased from Sigma Company, TUNEL kit was purchased from Roche Company, and RNA extraction kit and PCR amplification kit were purchased from Beijing ComWin Biotech Company; small animal ventilator was purchased from Chengdu Taimeng Company.
2.2. Experimental animals and groups

Male SPF level of adult SD rats were purchased in the university animal center, received conventional light, diet and water supply feeding, and were randomly divided into sham group (Sham group), ischemia-reperfusion injury group (I/R group) and rosiglitazone treatment group (ROS group).

2.3. Experimental methods

2.3.1. I/R model establishing methods

For I/R group and ROS group, the following methods were referred to establish myocardial ischemia-reperfusion injury model. Details were as follows: they were given intraperitoneal injection of 3 mL/kg of 10% chloral hydrate anesthesia, laid flat on the operating table and connected to small animal ventilator after tracheal intubation; chest was opened to reveal heart, anterior descending branch of coronary artery was identified and separated, No.6 thread went through the deep part of anterior descending branch of coronary artery and was ligated along with the water infusion balloon, making the balloon oppress the anterior descending branch of coronary artery to cause myocardial ischemia; after 30 min, fluid in the balloon was sucked out to relieve the oppression on coronary artery, making myocardium gain blood reperfusion, and after 2 h, materials were collected for the next test. Sham group revealed coronary artery only without ligation and chest exposure time was the same as that of I/R group and ROS group.

2.3.2. Treatment methods

ROS group received intraperitoneal injection of 6 mg/kg of rosiglitazone; Sham group and I/R group received intraperitoneal injection of the same volume of normal saline.

2.4. Index detecting methods

2.4.1. Infarct size detecting methods

After reperfusion ended, 2 mL of 2% Evans blue dye was injected through caudal vein; after fully myocardial dye, heart tissue was cut out, washed with normal saline, rapidly frozen, cut into slices with 2 mm thickness along the cross section and fixed with 4% paraformaldehyde; then photos were taken. In myocardium, blue was for normal tissue, red for ischemic tissue and white for infarction tissue. Image-J software was used to calculate the percentage of infarct size in total size.

2.4.2. TUNEL staining

Paraformaldehyde-fixed infarction tissue was taken. TUNEL kit was used for apoptotic cell staining. DAPI staining solution was used for nucleus staining, and after sealing, the number of apoptotic cells was observed under fluorescence microscope. Four to five independent visions were randomly selected, and the number of TUNEL staining positive cells in 1 000 cells was observed to calculate TUNEL staining positive rate.

2.4.3. Plasma myocardial enzyme levels

The rats were killed by decapitation. Plasma was collected and ECLIA was used to detect creatine kinase isoenzyme (CK-MB) and troponin T (cTnT) contents.

2.4.4. Contents of related molecules in myocardial tissue

Myocardial tissue was collected. RNA extraction kit manual was referred to extract total RNA in the tissue; after its reverse transcription into cDNA, PCR kit manual was referred to amplify Bax, Caspase-3, Bcl-2 and miR208a; β-actin was used as internal reference to standardize mRNA content and finally mRNA contents were calculated.

2.5. Statistical methods

Detected data was input by SPSS 18.0 software, comparison of measurement data among three groups by variance analysis and pairwise comparison by LSD- t test. Differences were considered to be statistically significant at a level of P<0.05.

3. Results

3.1. Infarct size

After 2% Evans blue staining, normal tissue was blue, ischemic tissue was red and infarction tissue was white, diagram shown in the above figure of Figure 1; infarct sizes were calculated and statistically analyzed, results shown in the below figure of Figure 1. Details were as follows: infarct size of I/R group was greater than that of Sham group; infarct size of ROC group was less than that of I/R group.

![Figure 1. Comparison of infarct size among three groups. The figure above: diagram of the infarct parts; the figure below: chart of infarct sizes. *: compared with Sham group, there were differences; &: compared with I/R group, there were differences.](image-url)
3.2. Cardiomyocytes' apoptosis index

Apoptotic cells in infarction tissue were marked by TUNEL staining and they showed green fluorescence under microscope; nuclei were marked by DAPI staining and they showed blue fluorescence under microscope, diagram shown in the left figure of Figure 2; apoptosis index was calculated and statistically analyzed, results shown in the right figure of Figure 2. Details were as follows: cardiomyocytes' apoptosis index of I/R group was greater than that of Sham group; cardiomyocytes' apoptosis index of ROC group was less than that of I/R group.

![Figure 2. Comparison of cardiomyocytes' apoptosis index among three groups.](image)

Left figure: diagram of TUNEL staining; right figure: chart of cardiomyocytes' apoptosis index. *: compared with Sham group, there were differences; &: compared with I/R group, there were differences.

3.3. Myocardial performance index

After cardiomyocyte injury, enzymes CK-MB and cTnT in the cytoplasm will be released into blood circulation, and expression proportion of contraction related proteins α-MHC and β-MHC will change. After rosiglitazone treatment, myocardial performance index was detected, and statistical analysis showed that CK-MB and cTnT contents in plasma of I/R group were higher than those of Sham group, and β-MHC/α-MHC proportion in myocardial tissue was higher than that of Sham group; that CK-MB and cTnT contents in plasma of ROC group were lower than that of I/R group, and β-MHC/α-MHC proportion in myocardial tissue was lower than that of I/R group.

3.4. mRNA contents of apoptosis related molecules in myocardial tissue

Bcl-2 is an anti-apoptotic molecule, and bax and caspase-3 are pro-apoptotic molecules. The three are all involved in the apoptotic process of cardiomyocytes; miR-208a can regulate the expression of bax and caspase-3. In the research, mRNA contents of related molecules in myocardial tissue were detected, and statistical analysis showed that bcl-2 and miR-208a contents in myocardial tissue of I/R group were lower than those of Sham group, and bax and caspase-3 contents were higher than those of Sham group; that bcl-2 and miR-208a contents in myocardial tissue of ROC group were higher than those of I/R group, and bax and caspase-3 contents were lower than those of I/R group.

4. Discussions

Ischemia-reperfusion injury is an important pathological link that affects heart function recovery after interventional therapy of myocardial infarction. How to prevent and reduce myocardial injury caused by ischemia reperfusion has been a hotspot of clinical research. Now the mechanism of ischemia-reperfusion injury is not fully elucidated, and pertinent treatment is also lacking. Peroxisome proliferator-activated receptors (PPAR) are a type of dependent ligand-activated transcription factor family, including three subtypes, β and γ. Among them, PPAR-γ belongs to hormone receptor gene transcription factor in the cell nucleus. Once inside the cell nucleus,
it can form heterodimer with RXR, combine specific reaction element PPRE in target gene promoter region, and then regulate the expression of corresponding genes\[3,4\]. Studies have shown that abnormal PPAR-γ content or function is closely related to ischemia-reperfusion injury of myocardial tissue and brain tissue; excessive PPAR-γ expression in local myocardial tissue can reduce the injury caused by ischemia reperfusion\[5\].

Thiazolidinediiones rosiglitazone and pioglitazone are synthetic PPARγ ligands that can activate the transcription activity of PPARγ\[6\]. In vitro studies have confirmed that PPARγ agonist rosiglitazone treatment can reduce cardiomyocyte apoptosis caused by hypoxia-reoxygenation. Its molecular mechanism is inhibiting the expression of apoptosis related genes Bax, Caspase-3, and so on\[7\]. Therefore, we speculated that PPARγ agonist had myocardium-protecting effect and could reduce myocardial injury caused by ischemia-reperfusion. In order to test the hypothesis, SD rats were used as research objects and surgical ligation of the anterior descending branch of coronary artery was adopted to establish myocardial ischemia- reperfusion injury animal model; research results showed that infarct size of I/R group was greater than that of Sham group, and apoptotic index of myocardial tissue was also higher than that of Sham group, which indicated that myocardial ischemia- reperfusion injury animal model was successfully established. Before surgery, PPARγ agonist pretreatment was given and it was observed that infarct size of ROC group was less than that of I/R group, and apoptotic index of myocardial tissue was also lower than that of I/R group, which indicated that PPARγ agonist rosiglitazone pretreatment could relieve myocardial ischemia-reperfusion injury and reduce the infarct size, with specific myocardium-protecting effect.

In the case of ischemia-reperfusion injury of myocardial cells, there will be corresponding function assessment change on the basis of massive myocardial infarction, increased number of necrotic cells and other pathological change. There are rich enzymes in cytoplasm of myocardial cells, including creatine kinase isoenzyme (CK-MB), troponin T (cTnT) and so on. They are involved in energy metabolism, skeleton structure, etc of myocardial cells; in the case of myocardial cell necrosis, CK-MB and cTnT in the cytoplasm will be released into blood circulation, characterized by significantly increased contents of myocardial enzymes in plasma. In clinical practice and animal experiments, myocardial enzyme levels in plasma can all accurately reflect the degree of myocardial injury. Myosin heavy chain (MHC) is a key protein to determine the contraction function of myocardial cells, including two subtypes α-MHC, β-MHC. Among them, α-MHC has extremely strong ATP enzyme activity, and can hydrolyze ATP and provide energy for myocardial contraction; catalytic ability of β-MHC is relatively weak\[8\]. When α-MHC/β-MHC proportion increases, myocardial contractility weakens and cardiac output decreases\[9\]. In the research, function assessment related index of ischemia-reperfusion injury rats was analyzed and results showed that CK-MB and cTnT contents in plasma of ROC group were lower than those of I/R group, and α-MHC/β-MHC proportion in myocardial tissue was lower than that of I/R group, which indicated that rosiglitazone treatment could improve function assessment index of ischemia-reperfusion injury rats.

Rosiglitazone-agitated PPARγ is an intranuclear transcription factor that can regulate the expression of a variety of target genes. In ischemia-reperfusion myocardial tissue, cardiomyocyte apoptosis related genes include anti-apoptotic gene bcl-2 as well as pro-apoptotic genes bax and caspase-3\[10,11\]. In the research, analysis of apoptosis related genes showed that mRNA content of bcl-2 in myocardial tissue of I/R group were lower than that of Sham group, and mRNA contents of bax and caspase-3 were higher than those of Sham group; that after rosiglitazone treatment, mRNA content of bcl-2 in myocardial tissue of ROC group were higher than that of I/R group, and mRNA contents of bax and caspase-3 were lower than those of I/R group, which indicated that rosiglitazone could increase the expression of anti-apoptotic gene bcl-2 and decrease the expression of pro-apoptotic genes bax and caspase-3. Studies have shown that there are binding sites of PPARγ in promoter region of bcl-2 gene and after agitating PPARγ, rosiglitazone can directly increase bcl-2 expression. However, there is no binding site of PPARγ found in promoter region of bax and caspase-3, which indicates that rosiglitazone may inhibit the expression of the two genes through indirect way. miRNAs are a type of micromolecule RNA that can regulate gene expression, and there are action sites of miR-208a in 3'UTR region of both bax and caspase-3\[12,13\]; in cardiomyocytes, rosiglitazone, after agitating PPARγ, can increase the generation of miR-208a to inhibit the expression of bax and caspase-3\[14,15\]. Detection of miR-208a content in myocardial tissue showed that miR-208a content in myocardial tissue of I/R group was lower than that of Sham group, which indicated that rosiglitazone could increase miRNA-208a content to inhibit the expression of bax and caspase-3.

Based on above discussions, it can be concluded that PPARγ agonist rosiglitazone has protective effect on myocardial reperfusion injury; that it can reduce the infarct size, decrease the number of infarction cells and regulate the expression of pro-apoptotic and anti-apoptotic genes.
References


