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Dual PPARα/γ ligand TZD18 improves myocardial metabolic remodeling after myocardial infarction in rats

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Abstract. – OBJECTIVE: To investigate the changes in myocardial energy metabolism and the effect of peroxisome proliferator-activated receptor alpha/gamma (PPARα/γ) dual agonist TZD18 on myocardial energy metabolism in rats with heart failure after myocardial infarction.

MATERIALS AND METHODS: The myocardial infarction model was established by ligating the left anterior descending coronary artery. The rats were randomly divided into the myocardial infarction group (MI group), the TZD18 intervention group (TZD18 group), and the shame surgery group (sham group). 8 weeks later, the blood flow parameters were measured by carotid arterial cannulas, and ventricular remodeling indexes were calculated. Hearts were extracted from rats after the execution. The expressions of PPARα/γ mRNA and α/β-MHC mRNA were detected by reverse transcription-polymerase chain reaction (RT-PCR). The mitochondrial oxidative respiration activity was measured by a bio-tissue oxygen consumption meter, the content of adenosine in mitochondria was measured by high-performance liquid chromatography, and tritium-labeled adenosine diphosphate incorporation assay was used to detect the transport activity of adenosine nucleotide translocases (ANT).

RESULTS: The expression of PPARα/γ mRNA and the ratio of α/β-MHC mRNA in the MI group were significantly decreased, the content of high energy phosphates, respiration activity, ANT transport activity in mitochondria were significantly decreased, the hemodynamic indexes were disturbed and left ventricular weight/body weight ratio (LVW/BW) significantly became higher. TZD18 intervention could increase the expression level of PPARα/γ mRNA and up-regulate the ratio of α/β-MHC mRNA, thus improving mitochondrial respiratory activity and ANT transport activity in rats with heart failure after myocardial infarction, increasing the content of high energy phosphates in mitochondria and improving the remodeling indexes in the ventricle.

CONCLUSIONS: TZD18 increases both the expression of enzymes related to myocardial energy metabolism and the content of high-energy phosphates in mitochondria. Also, it improves the respiratory activity and ANT transport activity by activating PPARα/γ genes, thus improving the generation and delivery of myocardial energy and protecting the myocardial cells.

Key Words:
PPARα/γ, TZD18, Myocardial metabolic remodeling, Myocardial infarction.

Introduction

Myocardial metabolic remodeling refers to the phenomenon that there are changes in the action path of myocardial cell substrates, mitochondrial dysfunction and abnormalities of high energy phosphates caused by a variety of pathological factors acting on the heart, which can lead to changes in cardiac structures and functions, eventually causing heart failure[1,2]. Ischemic cardiomyopathy caused by myocardial infarction and other symptoms is a metabolism-related disease and also an important reason for the occurrence of heart failure[3,4]. Therefore, revealing the possible mechanism of myocardial energy metabolism in the development of chronic heart failure (CHF) by studying the characteristics of the energy metabolism in ischemic myocardium is of great significance in improving the cardiac function of ischemic cardiomyopathy, preventing the progress of heart failure and other aspects. The heart is one of the important energy-consuming organs in the body, and previous studies have confirmed that the metabolic imbalance of myocardial metabolic substrates, glucose and fatty acid, may be

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bases for the occurrence and development of heart diseases[5-7]. Peroxisome proliferator-activated receptors (PPARs) regulate the energy metabolism at the level of gene transcription and are closely related to the energy metabolism disorder of myocardial cells[8,9]. PPARs are receptors of ligand-activated nuclear transcription factors, belonging to one of the steroid hormone receptor super-families. In mammals, when PPARs combined with ligands are activated, there will be conformational changes, and they will regulate the transcription level of enzymes related to myocardial energy metabolism, thus regulating the myocardial energy metabolism. A large number of studies have confirmed that PPARs are ideal targets for drug therapy as their hydrophobic ligand binding sites can be activated by a variety of compounds. PPAR alpha/gamma (PPARα/γ) are the most widely studied subtypes, both of which are fully expressed in myocardial tissues. PPARα acts as a pivot in the myocardial energy metabolism, and the abnormal expression of PPARγ also leads to disorders of the myocardial energy metabolism, thus leading to cardiac insufficiency. TZD18 is a dual PPARα/γ agonist that stimulates PPARα and PPARγ receptors in a dose-dependent manner. Animal experiments showed that dual PPARα/γ agonists can significantly reduce liver triglyceride (TG) aggregation caused by the high fat diet, inhibit the formation of visceral fat, increase the sensitivity of the liver, muscle, fat and other tissues to insulin, reduce blood lipids and improve the insulin resistance[10,11]. TZD18 not only reduces the blood glucose levels in diabetes (db/db) mice and insulin-resistant obese Zucker rats to normal levels, but also reduces blood cholesterol and TG levels in both rats and dogs. TZD18 can inhibit the biosynthesis of cholesterol and reduce the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase step by step before the synthesis of mevalonate. It can also significantly inhibit the expression of genes regulating the synthesis of fatty acids and those inducing the degradation of fatty acids and TG elimination[12,13]. Lipids will not be reduced only by the activation of PPARγ affecting the expression of liver genes, but the PPARα/γ agonist TZD18 can affect lipid metabolism through this way and maintain serum lipids within a safe range. Therefore, it has been speculated that dual PPARα/γ agonists may be more beneficial for the treatment of type 2 diabetes and the improvement of lipid metabolism, and be able to protect the cardiovascular system. However, the effect of TZD18 on the energy metabolism of heart failure caused by ischemia induced by myocardial infarction and other causes is rarely reported. This study aimed to investigate the effect of TZD18 on the energy metabolism in the process of heart failure in ischemic myocardium after its intervention in rats with myocardial infarction caused by left anterior descending coronary arteries.

Materials and Methods

Experimental Animal and Modeling

Male Wistar rats weighing 150-250 g were provided by Wenzhou Medical University Experimental Animal Center. Intraperitoneal injection of 1% pentobarbital sodium (30 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA) was conducted for anesthesia, and after the tracheal intubation, an artificial ventilator was used for assisting breathing. The chest was opened at the sternal left edge of the intercostal space between the 4th and 5th rib, and the left anterior descending coronary artery was ligated using non-invasive sutures at 1-2 mm below the junction of the pulmonary artery and left auricle. If the myocardium under the ligation site turned white, pulsation became weakened and electrocardiogram showed there were arch elevation in the ST segment, the modeling was considered successful[14]. After surgery, each rat received intramuscular injection of 10^5U penicillin to prevent infection for 3 days. This study was approved by the Animal Ethics Committee of Wenzhou Medical University Animal Center.

Determination of Hemodynamic Parameters and Ventricular Remodeling Indexes

8 weeks after surgery, rats in each group were anaesthetized with intraperitoneal injection of 1% pentobarbital sodium (30 mg/kg), respectively. The right carotid artery was separated and intubated. The mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP) and the maximum speed of left ventricular pressure increase or decrease ($\pm dp/dt_{max}$) were measured with a physiological recorder of PowerLab/8sp 8 channel model (AD Instruments, Shanghai, China). The heart was taken out immediately after pressure curves were recorded and it was flushed with pre-cooling saline until the flushing fluid was not red. Wet weights of the left ventricle (including interventricular septum) and right ventricle were weighed, and the remodeling indexes of left

and right ventricular myocardium, left ventricular weight/body weight ratio (LVW/BW) and right ventricular weight/body weight ratio (RVW/BW) were calculated. The ventricular myocardium in 4 mm range from the myocardial infarction was preserved in liquid nitrogen for the detection of content by reverse transcription-polymerase chain reaction (RT-PCR). The other myocardium was used for isolation of mitochondria.

Determination of the expressions of PPARα/γ mRNA and α/β-MHC mRNA by RT-PCR

100 mg myocardial tissue was added to 1 ml Tripure (Roche, Basel, Switzerland), thoroughly homogenized and transferred to Eppendorf (EP) tube. They were mixed for 15 s, and stood for 2-5 min at room temperature. After centrifugation for 15 min, the colorless liquid phase (about 0.5 ml) of the upper layer was transferred to the new EP tube, and 0.5 ml isopropanol was added. It was mixed completely, and then incubated at room temperature for 10 min. The supernatant was centrifuged at 4°C, 12000 g for 10 min. 1 ml 75% ethanol was added to the tube, the vortex oscillation for 15 s. After centrifugation for 5 min, the supernatant was discarded. The solution was dissolved in DEPC water and incubated at 55°C for 10 min. Solution of RNA was diluted and OD260/OD280 was measured by UV spectrophotometer. The purity and the concentration were estimated. The polymerase chain reaction (PCR) was carried out after reverse transcription. Primer design software Primer premier 5.0 (Palo Alto, CA, USA) was used for primer design. PPARγ 5'-GGTTGATTTC/TCCAGCATTTC-3', 5'-TCAATCGGATGGTTCTTCG-3'; PPARα 5'-AAGCCATCTTCACGATGCTG-3', 5'-TCAGAGGTCCCTGAACAGTG-3'; α-MHC 5'-ACCAA/GCAGC/CACGC/CAGTA-3', 5'-TCCAG/CCAGC/CCAAG/ATGTT-3'; β-MHC 5'-AGGAA/GAACC/TACTG/CGACT/G-3', 5'-CATCC/TTAGG/GTTGG/GTAGC/AC-3'; β-actin 5'-TTGTC/ACCAA/CTGGG/ACGAT/ATGG-3', 5'-GATCT/TGATC/TTCAT/GGTGC/TAGG-3'. After the agarose gel electrophoresis, photos were taken and scanned, and the statistical analysis was conducted.

Determination of Mitochondria Separation and Respiratory Functions

1.3 mL reaction medium was added to the pool for incubation for 2 min, saturated with air, and then the pool was added with 0.1 mL mitochondrial suspension. After pre-warm for 1 to 2 min, 5 μL reaction substrate were added successively. Then, changes in mitochondrial respiration states were observed, and the oxygen consumption curve was recorded. The oxygen consumption after adenosine diphosphate (ADP) was added (State 3 respiration, ST3) and ADP was depleted (State 4 respiration, ST4) was calculated. The control rate of mitochondrial respiration (RCR), ST3/ST4, was expressed as the number of moles of oxygen atoms consumed by the unit mitochondrial protein per unit time. The oxidative phosphorylation rate (OPR) was expressed as the amount of adenosine triphosphates (ATPs) synthesized by mitochondrial proteins per unit time (OPR=ST3×P/O), in which P/O was the ratio of ADP added to the reaction system to the corresponding consumed atomic oxygen.

Determination of Adenosine Nucleotides in Myocardial Tissues

100 μL fresh mitochondria suspension was taken and added with 200 ul 0.2 mol/L perchloric acid ($HClO_4$) (Sigma-Aldrich, St. Louis, MO, USA). The membranes were collected by centrifugation at 20000 g for 10 min at 4°C. 200 ul of the supernatant were taken and added with 1 mol/L potassium carbonate (K_2CO_3) (Sigma-Aldrich, St. Louis, MO, USA) for neutralization, and then they were fully mixed. After standing for 5 min, 20000 g centrifugation was conducted again for 10 min at 4°C. The supernatant was taken for the separation by high-performance liquid chromatography, and the contents of ATP, ADP and adenosine monophosphate (AMP) were quantified[15].

Determination of the Transport Activity of Adenosine Nucleotide Translocases (ANT) in Myocardial Mitochondrial Membranes

50 μL prepared mitochondrial suspension was taken to separate media, and after the dilution, ^{3}H-ADP solution was added. Then, the ANT inhibitor atractyloside (ATR) was added and mixed immediately to terminate ANT transport function. After centrifugation, the supernatant was discarded, and hydrogen peroxide (H_2O_2) was added into the precipitation after being dissolved and cleaned with the separation medium. Then, it was digested for 40 min in 70°C water bath. The radioactivity of 200 μL of the mitochondrial suspension was measured by liquid scintillation counting method. ATR was added into the control tube before the addition of ^{3}H-ADP. ANT transport activity was

calculated by the experimental tube radioactivity minus the control tube radioactivity.

Statistical Analysis

All quantitative data were expressed as mean ± standard deviation. Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) package was used for one-way analysis of variance. Comparisons among groups were conducted using the least significant difference (LSD) test. $p<0.05$ represented that the difference was statistically significant.

Results

Changes in Hemodynamic Parameters and Ventricular Remodeling Indexes in Rats

There were low appetite, depression, polypnea in the resting state and other symptoms of heart failure in rats in each group receiving surgery. Compared with those in the Sham surgery group (Sham group), LVEDP and MAP were significantly increased, $\pm dp/dt_{max}$ and LVSP were significantly decreased and the heart rate was increased in the myocardial infarction group (MI group). Compared with those in the MI group, LVEDP was significantly decreased, $+dp/dt_{max}$ was increased and there was no significant difference in hemodynamic parameters in the TZD18 intervention group (TZD18 group). LVW/BW in the MI group and the TZD18 group was significantly higher than that in the Sham group, but RVW/BW was slightly increased in the MI group. LVW/BW was enhanced in the TZD18 group compared with that in the MI group (Figure 1).

Changes in the Expression Levels of PPARα/γ and α/β-MHC mRNA in the Myocardium of Rats

Results of PCR amplification showed that, in the myocardium of rats with heart failure after myocardial infarction, the expression of α-MHC mRNA was decreased while that of β-MHC mRNA was increased, and α/β-MHC mRNA ratio was significantly decreased. The expression of PPARα/γ mRNA was up-regulated after the treatment with TZD18 for 8 weeks, which reversed the expression of α-MHC/β-MHC mRNA (Figure 2).

Changes in Mitochondrial Respiratory Activity in the Myocardium of rats

Compared with those in the sham group, ST3 and ST4 mitochondrial respiratory activity, OPR and respiratory control ratios (RCR) were decrea-

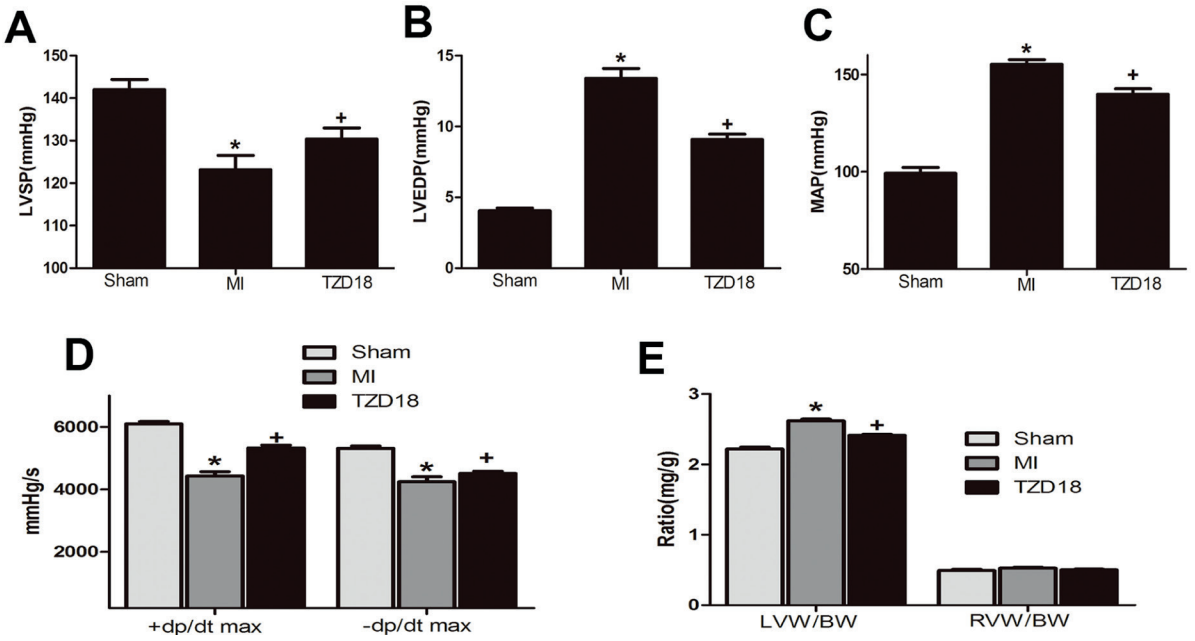

Figure 1. The result of the hemodynamic parameters and ventricular remodeling indexes 8 weeks after myocardial Infarction. ***(A)*** Analysis of left ventricular systolic pressure (LVSP). ***(B)*** Analysis of left ventricular end diastolic pressure (LVEDP). ***(C)*** Analysis of mean artery pressure (MAP). ***(D)*** Analysis of $\pm dp/dt_{max}$. ***(E)*** Analysis of ratio of LVW/BW and RVW/BW. n=10 per group. $^{*}p<0.05$ *vs.* Sham group, $+p<0.05$ *vs.* MI group.

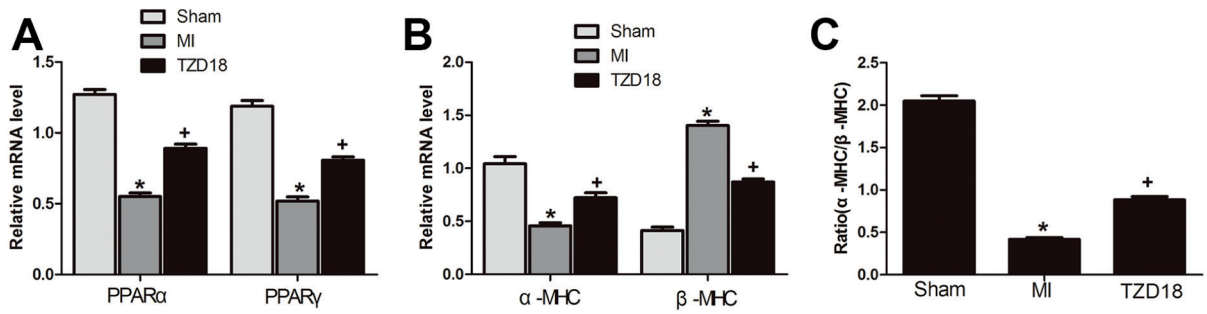

Figure 2. The expression of PPARα/γ and α/β-MHC mRNA 8 weeks after myocardial infarction. ***(A)*** Relative mRNA level of PPARα/γ. ***(B)*** Relative mRNA level of α/β-MHC. ***(C)*** Analysis of ratio of α/β-MHC mRNA. **p*<0.05 *vs.* Sham group, +*p*<0.05 *vs.* MI group.

sed at the 8th week in the MI group. 8 weeks after TZD18 intervention, ST3, OPR and RCR were significantly increased compared with those in the MI group (Figure 3). The results indicated that TZD18 intervention can significantly increase the mitochondrial oxidative capacity as a whole, improve the respiratory function, and enhance the reserve of rats with heart failure after myocardial infarction.

Changes in Adenosine Content and Mitochondrial ANT Transport Activity in Myocardial Mitochondria of rats

Compared with those in the Sham group, the contents of myocardial mitochondrial ATP, ADP, AMP and total adenosine pool were significantly decreased in the MI group at the 8th week. The intervention of TZD18 for 8 weeks increased the adenosine content in ischemic myocardial mito-

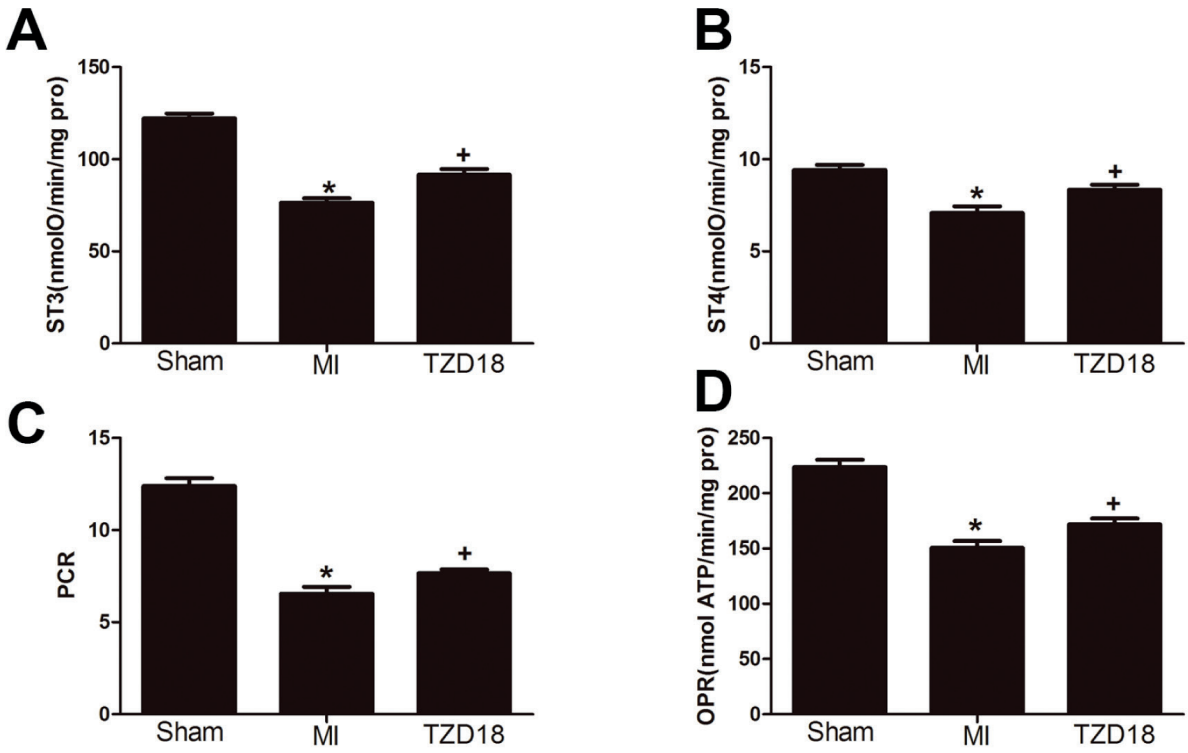

Figure 3. The level of mitochondrial respiratory function 8 weeks after myocardial infarction. ***(A)*** Relative ST3 level in each group. ***(B)*** Relative ST4 level in each group. ***(C)*** The mitochondria respiratory control rate (RCR) in each group. ***(D)*** The mitochondrial oxygen consumption rate (OCR) in each group. **p*<0.05 *vs.* Sham group, +*p*<0.05 *vs.* MI group.

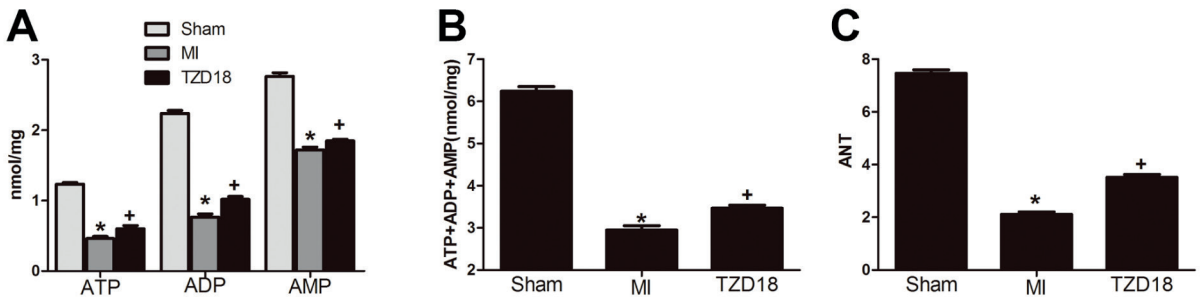

Figure 4. The high-energy phosphate content and the ANT activity 8 weeks after myocardial infarction. ***(A)*** Relative level of ATP/ADP/AMP in each group. ***(B)*** Analysis of ATP+ADP+AMP. ***(C)*** Analysis of the ANT activity. **p*<0.05 *vs.* Sham group, +*p*<0.05 *vs.* MI group.

chondria of rat, improved the energy metabolism, and delayed the progress of heart failure to a certain extent. Compared with that in the Sham group, ANT transport activity of myocardial tissues at the 8th week in the MI group was significantly reduced, indicating that mitochondrial ANT transport activity is inhibited after myocardial infarction caused by myocardial ischemia, thus reducing the turnover rate of energy generation and utilization (Figure 4).

Discussion

In this study, coronary artery ligation was used to establish a rat myocardial infarction model. There were low appetite, depression, polypnea in the resting state and other symptoms of heart failure in rats after myocardial infarction. The heart rate, LVEDP, and MAP were significantly increased, while $\pm dp/dt_{max}$ and LVSP were significantly decreased, indicating that there were significant hemodynamic disorders. Remodeling indexes in the left ventricle were significantly changed, suggesting there was heart failure and the model was successfully established. It was also proved that the myocardial energy dysbolism is one of the important factors of ischemic myocardium developing into heart failure. After the intervention of TZD18 for 8 weeks, LVEDP and $\pm dp/dt_{max}$ were improved, indicating that TZD18 can improve cardiac functions of rats after myocardial infarction. Peroxisome proliferator-activated receptor is a ligand-activated nuclear transcription factor. When the ligands bind to the ligand-binding domain of PPARs and are activated, the conformational changes occur, and enzymes related to myocardial energy metabolism are regulated at the transcriptional level, thus regulating the energy metabolism of the myocardium. PPARα/γ plays an important role in energy metabolism in glucose uptake[16,17], oxidation[18], lipid metabolism[19] and so on. The appearance of myocardial ischemia and myocardial energy metabolism disorders, including changes in the main energy substrate, mitochondrial oxidative respiratory dysfunction, high-energy phosphate abnormalities, etc. under the pathological conditions, can lead to myocardial remodeling and cardiac function decline, thus ultimately progressing to heart failure[20]. Therefore, to improve myocardial mitochondrial function and optimize energy metabolism may be an effective way to protect the myocardium after infarction. This study showed that the expression of PPARα/γ mRNA in the left ventricular myocardium of the MI group was lower than that of the Sham group, indicating that PPARα/γ plays an important role in ventricular remodeling in rats with myocardial infarction. PPARα/γ genes were activated by the treatment with the intervention of PPARα/γ dual agonist TZD18 for 8 weeks, and the α/β-MHC ratio was decreased. Therefore, we considered that TZD18 enhances ATP-ase activity, improves myocardial energy metabolism and delays cardiac hypofunction. Normal energy metabolism is the basis of all physiological functions of myocardial cells. ATP is the most important high-energy phosphate carrier in the cell, which has three phosphate groups. When the phosphate group is divided and transferred, ADP and AMP are generated, and ATP is an energy substance that can be directly used by cells and can directly supply energy during the myocardial fiber contraction. Previous studies have shown that long-term hypoxia weakens the phosphorylation function of myocardial mitochondria, gradually decreases the mitochondrial adenosine content and significantly reduces ATP content[21,22], so the

reduction of myocardial high-energy phosphate compounds is considered to be the direct cause of cardiac dysfunction. This study also showed that compared with those in the Sham group, the contents of myocardial mitochondrial ATP, ADP, AMP and total adenosine pool were significantly decreased in the MI group at the 8th week. As the total adenosine pool of the infarcted myocardium and ATP content were decreased, the production of energy was blocked. With the mitochondrial respiratory hypofunction, the mitochondrial oxidative phosphorylation function was further impaired, indicating that the insufficient mitochondrial energy plays important roles in the occurrence and development of heart failure in rats after myocardial infarction. It was found in this study that the intervention of TZD18 for 8 weeks increased the adenosine content in ischemic myocardial mitochondria of rat, improved the energy metabolism and delayed the progress of heart failure of a certain extent. Mitochondria are important organelles in myocardial cells, whose main function is to synthesize ATP by oxidative phosphorylation, meeting the need for energies in various chemical reactions and functions in cells. ST3, ST4, RCR and OPR are important indicators reflecting oxidative phosphorylation of mitochondria. The addition of a certain amount of ADP in the suspension of mitochondria *in vitro* can stimulate the oxygen consumption flow so that the mitochondria reached the ST3 respiration with the maximum phosphorylation rate and oxygen consumption flow, indicating that ADP stimulates the oxidative phosphorylation. When ADP is all converted to ATP, the mitochondria reach the so-called ST4 respiration, which is an ineffective oxygen consumption process with no ATP synthesis, reflecting the "proton leakage" situation. RCR is the ratio of ST3/ST4, which is an important index to evaluate the functional status of mitochondria. Its size reflects the structural integrity of mitochondria and the degree of coupling of oxidative phosphorylation. OPR represents the efficiency of oxidative phosphorylation of mitochondria, that is, the efficiency of the conversion from the released energy to the energy representing mitochondrial oxidative phosphorylation, or the efficiency of the conversion from the released energy into ATP. Previous studies have shown that in the acute phase of myocardial infarction in rats, myocardial mitochondrial ST4 was increased, while ST3, RCR and OPR were decreased significantly, indicating that mitochondrial respiratory function and oxidative phosphorylation were significantly impaired, which may be an important reason for the progression to CHF after myocardial ischemia[23,24]. The results of this research showed that compared with those in the Sham group, myocardial mitochondrial ST3, ST4, OPR and RCR were significantly reduced at the 8th week in the MI group, indicating that after myocardial infarction, oxidative respiratory function was severely impaired. When the demand for myocardial ATP was increased, oxygen consumption and ATP synthesis were not increased by ADP stimulating the electron transport chain; this might be related to the inhibition of the electron transport on the respiratory chain by calcium overload, O^{2-}, OH^{-}, etc. when there were heart failure and myocardial remodeling induced by myocardial ischemia and other reasons. When the mitochondrial function was gradually decreased, myocardial mitochondrial function and myocardial ATP were gradually decreased. When ATP was decreased to a certain degree, the heart function was affected, which reduced the myocardial contractility, thus leading to heart failure. 8 weeks after TZD18 intervention, ST3, OPR and RCR were significantly increased compared with those in the MI group. The results indicated that TZD18 intervention can significantly increase the mitochondrial oxidative capacity as a whole, improve the respiratory function and enhance the reserve of rats with heart failure after myocardial infarction. TZD18 improved the excitation-contraction decoupling and the efficiency of mitochondrial oxidative phosphorylation to a certain extent. ANT on the inner mitochondrial membrane plays an important role in the process of mitochondrial energy generation and utilization, which is the main carrier to realize the energy supply of cells[25,26]. Studies have shown that the activity of ANT on the inner mitochondrial membrane changes accordingly when the energy demand and/or supply imbalance occur(s) in the body[27,28]. This study indicated that compared with that in the Sham group, ANT transport activity of myocardial tissues at the 8th week in the MI group was significantly reduced, indicating that mitochondrial ANT transport activity is inhibited after myocardial infarction caused by myocardial ischemia, thus reducing the turnover rate of energy generation and utilization. This may be an important mechanism of the energy dysbolism of cells during myocardial infarction. After the intervention of TZD18 for 8 weeks, ANT transport activity was improved compared with that in the MI group, which increased the turnover rate of the energy generation and utilization of cells, indica-

ting that TZD18 can delay cardiac hypofunction by improving myocardial energy utilization and turnover.

Conclusions

Heart failure occurred 8 weeks after myocardial infarction in rats, accompanied by metabolic remodeling. The myocardial adenosine content was decreased and the mitochondrial respiratory function and ANT activity were changed. TZD18 increases the expression of enzymes related to myocardial energy metabolism, increases the content of high energy phosphate in mitochondria, and improves the respiratory activity and ANT transport activity, thus enhancing the production and delivery of myocardial energy by activating PPARα/γ genes.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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