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Metformin improves cardiac function in mice with heart failure after myocardial infarction by regulating mitochondrial energy metabolism

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ABSTRACT

To investigate whether metformin can improve the cardiac function through improving the mitochondrial function in model of heart failure after myocardial infarction. Male C57/BL6 mice aged about 8 weeks were selected and the anterior descending branch was ligatured to establish the heart failure model after myocardial infarction. The cardiac function was evaluated via ultrasound after 3 days to determine the modeling was successful, and the mice were randomly divided into two groups. Saline group (Saline) received the intragastric administration of normal saline for 4 weeks, and metformin group (Met) received the intragastric administration of metformin for 4 weeks. At the same time, Shame group (Sham) was set up. Changes in cardiac function in mice were detected at 4 weeks after operation. Hearts were taken from mice after 4 weeks, and cell apoptosis in myocardial tissue was detected using TUNEL method; fresh mitochondria were taken and changes in oxygen consumption rate (OCR) and respiratory control rate (RCR) of mitochondria in each group were detected using bio-energy metabolism tester, and change in mitochondrial membrane potential (MMP) of myocardial tissue was detected via JC-1 staining; the expressions and changes in Bcl-2, Bax, Sirt3, PGC-1α and acetylated PGC-1α in myocardial tissue were detected by Western blot. RT-PCR was used to detect mRNA levels in Sirt3 in myocardial tissues. Metformin improved the systolic function of heart failure model rats after myocardial infarction and reduced the apoptosis of myocardial cells after myocardial infarction. Myocardial mitochondrial respiratory function and membrane potential were decreased after myocardial infarction, and metformin treatment significantly improved the mitochondrial respiratory function and mitochondrial membrane potential; Metformin up-regulated the expression of Sirt3 and the activity of PGC-1α in myocardial tissue of heart failure after myocardial infarction. Metformin decreases the acetylation level of PGC-1α through up-regulating Sirt3, mitigates the damage to mitochondrial membrane potential of model of heart failure after myocardial infarction and improves the respiratory function of mitochondria, thus improving the cardiac function of mice.

1. Introduction

Congestive heart failure (CHF) is the end-stage manifestation of various cardiovascular diseases, which is a major cause of death of cardiovascular diseases and has become huge medical and social burden [1,2]. Population aging increases the incidence of various cardiovascular diseases, including coronary heart disease and hypertension. Although the development and maturity of coronary artery intervention, coronary artery bypass graft surgery and other treatment techniques greatly improve the diagnosis and treatment rates of cardiovascular diseases, and significantly decrease the death rate of patients with acute myocardial infarction, new problems have emerged: the incidence of heart failure, a main complication of myocardial infarction, has been increasing year by year [3,4]. Heart failure is the final battlefield of cardiovascular disease treatment, so preventing the occurrence and development of heart failure is essential.

Myocardial remodeling after MI is the main pathological basis for congestive heart failure (CHF) [5–7]. Neurohormone theory in the 80–90s argued that neurohormonal dysfunction was a key reason for the occurrence and development of CHF, so the proposed treatment improved the prognosis of CHF to a large extent by inhibiting the sympathetic nervous system and RASS system [8]. Although the current treatment strategy can stabilize the course of CHF patients, reduce mortality and even partially reverse myocardial remodeling and improve the prognosis to a certain extent, the disease of most patients is still in progress, eventually developing

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into the end-stage heart failure. It is obvious that the clinical treatment guided by neurohormone theory cannot solve all the problems in the progression of CHF, and more and more evidence proves that myocardial energy metabolic disorder is the main reason for the occurrence and development of heart failure [9,10]. Therefore, the mechanism of energy metabolic disorder of heart failure is widely concerned. In CHF, mitochondrial structure is damaged, mitochondrial dysfunction leads to the decreased production of ATP, and insufficient energy supply promotes the myocardial cell apoptosis [11]; the increased myocardial cell apoptosis is the main mechanism of myocardial remodeling in CHF. Therefore, the study on the molecular mechanism of mitochondrial energy metabolism in heart failure can provide new therapeutic targets and strategies for delaying the development of heart failure.

Metformin is a kind of first-line drug widely used in the treatment of type 2 diabetes. Recent studies have shown that metformin has a cardiovascular protective effect, which can significantly reduce the patient's cardiovascular events [12,13]. But its mechanism is still unclear. Studies have shown that metformin has the effect of improving energy metabolism [14,15], and whether it can improve the cardiac function via improving the mitochondrial energy metabolism of heart failure after myocardial infarction remains unclear.

This study established the model of heart failure induced by myocardial infarction to investigate the mechanism of energy metabolic disorder in heart failure after myocardial infarction, and further study the protection of metformin on myocardial mitochondrial energy metabolism in heart failure after myocardial infarction and its mechanism, so as to provide new treatment targets for energy metabolism of heart failure.

2. Materials and methods

2.1. Experimental animals and myocardial infarction model

A total of 100 male C57BL/6 mice aged about 8 weeks weighing about 20 g were fed in the animal center to adapt to the environment for one week; after fasting for 4–6 h, mice received the intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg), fixed in the supine position and the simulated ECG machine was connected. After successful tracheal intubation, small animal ventilator was quickly connected, followed by blunt separation layer by layer to expose the heart and explore the position of left auricle; and the anterior descending branch was ligatured with needle inserted from about 1 to 2 mm on the left side below the left auricle. After the successful ligation, local cardiac muscle became white, and ECG suggested the ST segment elevation. All steps were the same for sham group except no ligation. The chest was closed carefully layer by layer, and the mice were placed on electric blanket for recovery.

2.2. Experimental scheme

The experimental animals were randomly divided into three groups: Sham group, Saline group (myocardial infarction + saline gavage) and Met group (myocardial infarction + metformin gavage). Saline group received the intragastric administration of normal saline for 4 weeks, and Met group received the intragastric administration of metformin (200 mg/kg) for 4 weeks.

2.3. High-resolution ultrasound detection of cardiac function of small animal

Chest hair of mice was removed using depilatory paste at 1 d before the detection of heart function for preserved skin. The mice to be tested were inhaled with isoflurane for anesthesia; after successful anesthesia, mice were placed on the heating working table. M-mode ultrasound was performed using the high-resolution imaging system Vevo2100 (VisualSonics, Canada) and high-frequency probe specifically used for mouse ultrasound. The parameters were measured in the direction of long axis and short axis, and LVFS, LVEF, LVEDD and LVESD were calculated. The above values were continuously detected for 5–6 cardiac cycles, and the averages were taken.

2.4. Detection of myocardial cell apoptosis

After heart tissue section, myocardial apoptosis was detected using terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) and myocardial cell marker, cTnI. After sealing via 50% glycerol (prepared by PBS), results were observed under laser scanning confocal microscope.

2.5. Protein extraction, quantification and western blot analysis

The tissues in ischemic region of heart in mice were taken and the total protein was extracted according to the standard procedure. The protein was quantified by BCA protein concentration detection. Appropriate amount of protein was taken for Western blot detection, and the separation gel concentration and electrophoretic voltage were adjusted according to the molecular weight of target protein, followed by development via chemiluminescence kit; and the gray value received the semi-quantitative analysis.

2.6. RNA extraction and real-time quantitative PCR

RNA in ischemic myocardial tissue was extracted according to standard procedure. Primer design and synthesis were completed by Shanghai Sangon Company. PCR was performed after reverse transcription according to the instructions of Invitrogen kit.

2.7. Mitochondrial extraction and mitochondrial function detection in myocardial tissue

Tissue ATP was extracted and the ATP content was measured using Promega ATP detection kit according to the instructions. Ischemic myocardial mitochondria were extracted and the oxygen consumption rate of mitochondria was measured by hippocampus bio-energy tester, and the myocardial mitochondrial membrane potential was measured by mitochondrial membrane potential detection kit (Beyotime, China).

2.8. Statistical processing

SPSS 17.0 was used for statistical analysis. One-way ANOVA was used for comparison among groups, and *t*-test was used for pairwise comparison. $p < 0.05$ suggested that the difference was statistically significant.

3. Results

3.1. Metformin improved cardiac systolic function in mice with heart failure after myocardial infarction

M-mode ultrasound was used to evaluate changes in cardiac function of mice in each group at 4 weeks after MI. Typical M-mode ultrasound showed that compared with sham group, the amplitude of anterior wall movement of heart in MI group was decreased (Fig. 1A). Through measuring and analyzing the results of M-mode

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