

Original Article

The protective effect of trimetazidine on cardiac myocardial cells in rats with heart failure

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Abstract: Trimetazidine is a drug that promotes myocardial energy metabolism. This study established a heart failure rat model and analyzed the impact of trimetazidine on myocardial energy metabolism and structure in a heart failure rat model. Abdominal aortic contraction was applied to establish the pressure overload heart failure rat model, followed by an analysis of the general conditions, including eating, drinking, breathing, activity, and hair. In addition, myocardial tissue structure changes were observed under lighted and electron microscopes. Cardiac hemodynamic changes were tested using cardiac ultrasonography. Serum ATP, ADP, AMP, and lactic acid content were detected using the perchlorate extraction enzyme reaction method. Myocardial cell apoptosis was determined using a TUNEL assay. The rats in the experimental group presented slight cardiac failure symptoms and a degree of mild myocardial tissue damage. The thickness of the interventricular septum (IVS), the left ventricular posterior wall thickness (LVPW), the left ventricular end-diastolic diameter (LVD), and the left ventricular end systolic diameter (LVS) in the experimental group were significantly lower than they were in the model group but were higher than they were in the control group. The left ventricular ejection fraction (LVEF) and the left ventricular short axis fractional shortening (FS) were significantly elevated in the model group but lower compared with the control group ($P < 0.05$). The ATP, ADP, AMP, and lactic acid levels in the experimental group were significantly higher than the levels in the model group but lower than the levels in the control group ($P < 0.05$). Myocardial AI in the experimental group was significantly lower than it was in the model group, but it was higher than it was in the control group ($P < 0.05$). The application of trimetazidine can treat heart failure, possibly by reducing myocardial apoptosis.

Keywords: Trimetazidine, heart failure, myocardial cell, protection

Introduction

In recent years, positive innovations have been made by researchers in the diagnosis and treatment of heart failure, and the mortality rate is significantly reduced. However, the incidence of heart failure keeps rising in China [1, 2]. Drug treatment is mainly applied in the clinic, mainly including nitrate esters, β -blockers, and calcium antagonists [3-5]. Van Bilsen reported that the glucose and fat metabolic disorder in myocardial cells can lead to changes in the energy metabolic pathways, resulting in cardiac structure variation and dysfunction [6]. Several researchers determined that myocardial ischemia is a type of metabolism-related disease, and myocardial energy metabolism can effectively treat heart failure. Trimetazidine is mainly used in angina pectoris as it can improve the myocardial cell energy metabolism [7]. Previous

studies pointed out that trimetazidine plays a role in the myocardial cell energy metabolism by inhibiting fatty acid β oxidation and reducing myocardial cell apoptosis [8-11]. However, the exact effect of trimetazidine on myocardial cells under heart failure conditions remains poorly understood. In this study, we established a rat heart failure model and adopted trimetazidine as an intervention to analyze the protective effect of trimetazidine on myocardial cells in rat heart failure.

Materials and methods

Experimental animals

A total of 30 SD rats in SPF grade were enrolled. There were 15 males and 15 females aged 12-14 weeks old and weighing 150 to 200 g.

Trimetazidine's effect on HD

The rats were provided by the Shandong University laboratory animal center.

The rats were used for all experiments, and all the procedures were approved by the Animal Ethics Committee of Dongjing Clinical College of Henan University (Kaifeng, Henan, China).

Instruments and reagents

Trimetazidine was provided by the Servier company (France). A TUNEL assay kit was bought from Roche (USA). An ultrasound cardiograph (VIVID7) was purchased from GE (USA). A MHz high-frequency drive-by-wire probe was obtained from GE (USA).

Grouping

Experimental group: Abdominal aortic contraction was applied to establish the pressure overload heart failure rat model. Each rat was anesthetized through an intraperitoneal injection of 2% pentobarbital sodium followed by opening the abdominal cavity and separating the abdominal aorta. The abdominal aorta was ligated and sutured using a 0.6 mm needle. Four weeks after modeling, trimetazidine was intragastrically administrated at a dose of 10 mg/kg once a day for four weeks.

Modeling group: The heart failure rat model was established. Four weeks after modeling, double distilled water was intragastrically administrated once a day for four weeks.

Normal control group: The abdominal aorta was separated without contraction treatment. Four weeks after modeling, double distilled water was intragastrically administrated once a day for four weeks.

Cardiac ultrastructure observation

The rats were sacrificed and their hearts were isolated and washed using ice and a normal saline solution. The heart was incised along the left ventricle and fixed with paraformaldehyde. After HE staining, a tissue at 1 × 1 × 1 mm size was collected at 2 mm from the edge of infarction and fixed with glutaraldehyde. Finally, the tissue was observed under an electron microscope.

Cardiac ultrasonography

The rats were anesthetized and cardiac ultrasonography was applied to test LVEF, FS, IVS, LVPW, LVD, and LVS.

Serum ATP, ADP, AMP, and lactic acid content detection

The perchlorate extraction enzyme reaction method was adopted. A total of 200 mg tissue was mixed with 5% perchloric acid and centrifuged to obtain the supernatant. After being neutralized with KOH/K₂HPO₄ mixed liquid, the supernatant was used for analysis.

TUNEL assay

Myocardial cells were developed using DAB. Sepia staining of the nucleus indicated positive. The apoptosis index (AI) = apoptotic cell nuclear number/total cell nuclear number × 100%.

Data analysis

SPSS 17.0 software was applied for the statistical analysis. The measurement data was depicted as the mean ± standard deviation and compared using ANOVA. P < 0.05 was considered statistically significant.

Results

General conditions

The rats' general conditions were observed after the rat heart failure model was established. Rats in the control group exhibited normal eating, breathing, and activity. Rats in the model group showed shortness of breath, pink foam secretions at the mouth and nose, they were tired, they had reduced activity, they did not eat or drink, and their hair became dark. However, the rats in the experimental group presented with improved general conditions but with slightly accelerated breathing and decreased activity.

Cardiac tissue structure observation

Under the lighted microscope, the myocardial cells in the control group ranged regularly and tightly with normal form and no inflammatory cells. However, they exhibited a disordered

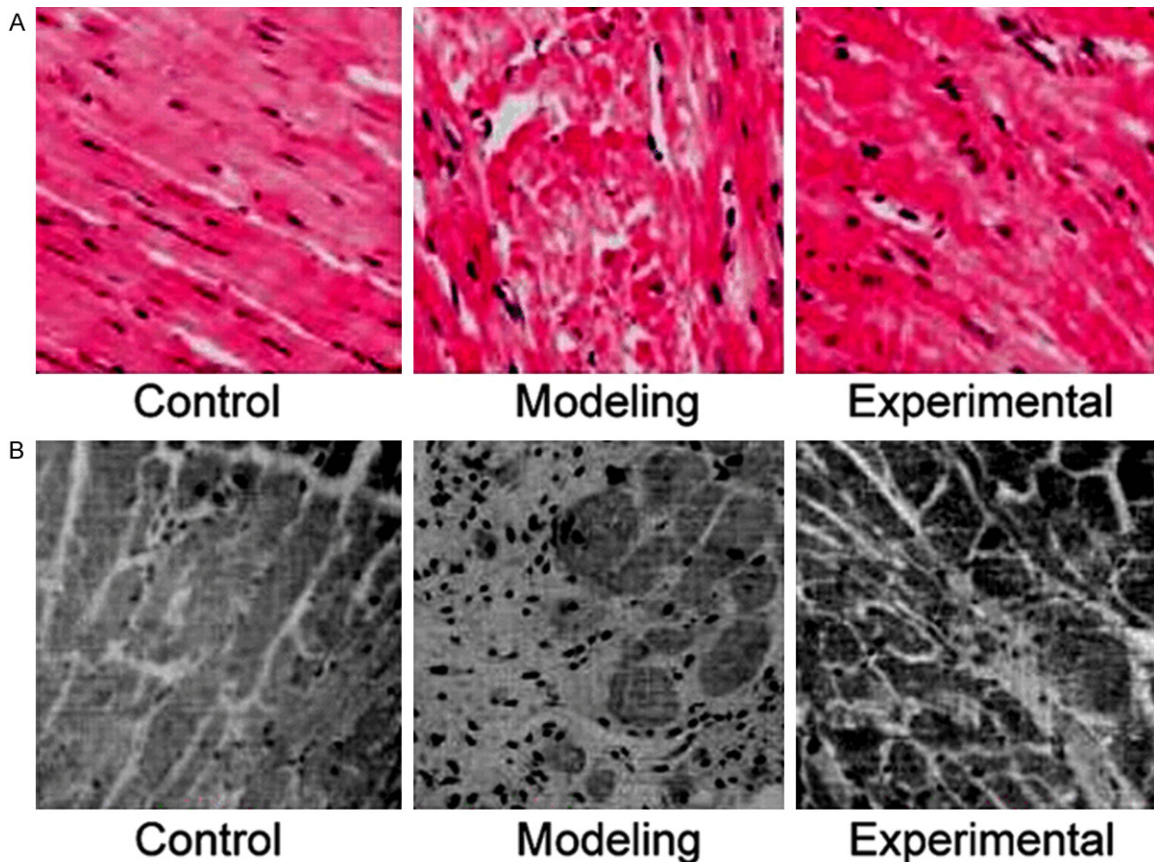


Figure 1. Cardiac tissue structure changes. A. Under the light microscope ($\times 400$). B. Under the electron microscope ($\times 3000$).

Table 1. Hemodynamics changes

Item	Experimental group	Modeling group	Control
IVS (mm)	$0.65 \pm 0.04^{*,\#}$	$1.08 \pm 0.07^*$	0.52 ± 0.04
LVPW (mm)	$0.73 \pm 0.05^{*,\#}$	$1.24 \pm 0.05^*$	0.56 ± 0.05
LVS (mm)	$0.74 \pm 0.06^{*,\#}$	$0.98 \pm 0.08^*$	0.56 ± 0.05
LVD (mm)	$0.86 \pm 0.05^{*,\#}$	$1.43 \pm 0.07^*$	0.72 ± 0.03
LVEF (%)	$27.75 \pm 1.72^{*,\#}$	$11.65 \pm 0.45^*$	42.54 ± 2.72
FS (%)	$11.34 \pm 0.67^{*,\#}$	$4.06 \pm 0.21^*$	17.68 ± 2.06

* $P < 0.05$, compared with control. # $P < 0.05$, compared with the model group.

arrangement and an obscure structure with cell edema and inflammatory cell infiltration in the modeling group. The myocardial cells in the experimental group presented an improved performance.

Under the electron microscope, the myocardial cells in the control group showed uniform mitochondria, an integrate structure, and regularly arranged muscle fiber. However, in the myocardial cells of the modeling group, variable mito-

chondria in size, vacuoloid changes, incomplete structure, and muscle fiber edema and fracture were found. The myocardial cells in the experimental group showed an improved performance compared with the modeling group (**Figure 1**).

Cardiac ultrasonography detection of hemodynamics

The hemodynamics index, including IVS, LVPW, LVS, LVD, LVEF, and FS were tested and we found that IVS, LVPW, LVS, and LVD were significantly lower in the experimental group than they were in the model group but higher than they were in the control group. LVEF and FS were elevated in model group but were lower than in the control group ($P < 0.05$) (**Table 1**).

Serum ATP, ADP, AMP, and lactic acid content detection

Serum ATP, ADP, AMP, and lactic acid levels in experimental group were significantly higher

Table 2. Serum ATP, ADP, AMP, and lactic acid content

Item	Experimental group	Modeling group	Control
ATP	1.55 ± 0.24*.#	1.28 ± 0.47*	2.32 ± 0.44
ADP	0.61 ± 0.19*.#	0.64 ± 0.15*	0.86 ± 0.35
AMP	1.24 ± 0.16*.#	0.92 ± 0.18*	1.66 ± 0.55
Lactic acid	26.81 ± 5.05*.#	23.13 ± 5.01*	31.72 ± 6.03

*P < 0.05, compared with control. #P < 0.05, compared with modeling group.

Table 3. Myocardial cell apoptosis changes

Group	Cases	AI (%)
Experimental group	10	23.67 ± 1.55*.#
Modeling group	10	40.12 ± 1.35*
Control	10	1.67 ± 0.46

*P < 0.05, compared with control. #P < 0.05, compared with modeling group.

than the levels in the modeling group, but lower than the levels in the control group (P < 0.05) (Table 2).

Myocardial cell apoptosis

The myocardial cell AI in the experimental group was lower than it was in the model group but higher than in the control group (P < 0.05) (Table 3, Figure 2).

Discussion

In recent years, although the treatment for heart failure has been updated, the 5-year survival rate has not significantly improved. It is understood that the myocardial cell metabolism imbalance is involved in the occurrence and development of heart failure [10]. The normal metabolism of myocardial cells involves 65% fatty acid and 30% glucose [11]. The myocardial energy metabolism using fatty acids turns into glucose during heart failure, leading to increased energy consumption. Not only that, but free fatty acids affect the normal energy metabolism of myocardial cells by restraining the activity of mitochondria, resulting in cardiac function deterioration [12-14]. Normal heart energy metabolism can maintain the heart in a stable environment and constantly provide new material sources for the heart tissues. As one of a new generation of drugs that work against myocardial ischemia, trimetazidine can directly act on ischemic myocardial

cells to affect the oxygen balance between the supply and demand [15-17]. In this study, we established a heart failure rat model to analyze the impact of trimetazidine on myocardial cells during heart failure.

We selected rats in the SPF grade and established the heart failure rat model upon abdominal aortic contraction. The rats were divided into the control, modeling, and experimental groups according to the different interventions. The rats in the control group exhibited normal eating, breathing, and activity. The rats in the model group showed shortness of breath, a pink foam secretion at the mouth and nose, they were tired, showed reduced activity, did not eat or drink, and their hair darkened. The rats in the experimental group presented improved general conditions but with slightly accelerated breathing and decreased activity. The rats were sacrificed 4 weeks after modeling, and their cardiac muscle tissue was taken. Under the lighted microscope, the myocardial cells in control group appeared regularly and tightly arranged with a normal form and no inflammatory cells. The myocardial cells in the modeling group exhibited a disordered arrangement and an obscure structure with cell edema and inflammatory cell infiltration. The myocardial cells in the experimental group showed an improved performance. Under the electron microscope, the myocardial cells in the control showed uniform mitochondria, an integrated structure, and regularly arranged muscle fiber. The myocardial cells in the modeling group exhibited variable mitochondria in size, vacuoloid changes, an incomplete structure, and muscle fiber edema and fracture. The myocardial cells in the experimental group presented improved performance compared with the modeling group. It suggested the successful establishment of a heart failure rat model. Trimetazidine significantly alleviated the clinical symptoms of heart failure and reduced myocardium structure injuries.

This study further analyzed the impact of trimetazidine on hemodynamics in rats. IVS, LVPW, LVS, and LVD in experimental group were significantly lower than they were in the model group but higher than they were in the control group. LVEF and FS were elevated in the model group but lower than in the control group, sug-

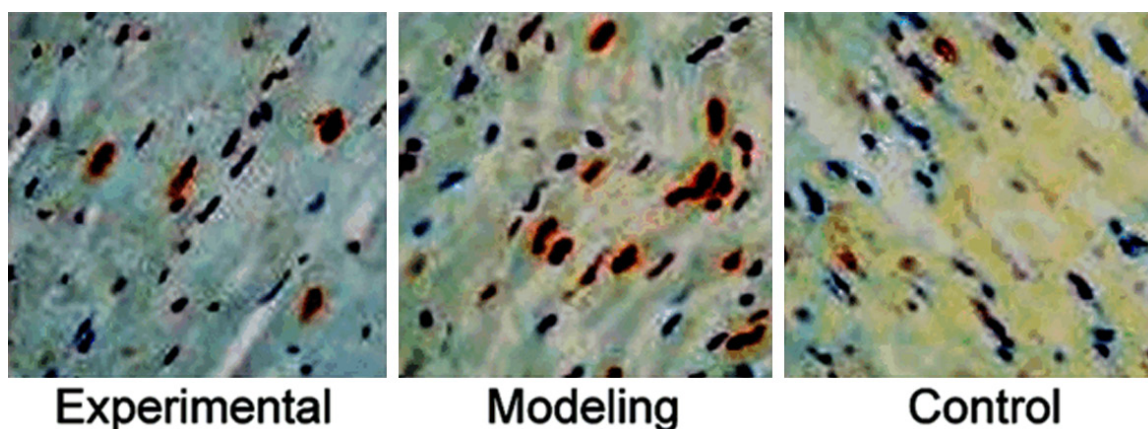


Figure 2. Myocardial cell apoptosis changes ($\times 400$).

gesting that IVS, LVPW, LVS, and LVD increased, but LVEF and FS declined in the heart failure rats. However, trimetazidine treatment improved left ventricular remodeling and cardiac function which possibly occurred by reducing IVS, LVPW, LVS, LVD and increasing LVEF and FS [17].

Trimetazidine has certain inhibitory effects on mitochondrial enzyme-long chain 3-ketone acyl coenzyme A thiolase, so it can suppress the fatty acid β oxidation process, increase glucose oxidation, and promote production capacity. In addition, trimetazidine plays an inhibitory role in the balance between free fatty acids and glucose oxidation to maintain cell function [18, 19]. Serum ATP, ADP, AMP, and lactic acid levels in the experimental group were significantly higher than they were in the modeling group but lower than in the control group. The levels revealed that trimetazidine promoted the cardiac energy metabolism process from fat metabolism to glucose oxidation metabolism, increasing the production of ATP and promoting phosphate ester synthesis to protect myocardial cells and improve the physiological function of the heart.

The myocardial cell AI in the experimental group was lower than it was in the model group, but it was higher than in the control, suggesting that trimetazidine restrained myocardial cell apoptosis in the heart failure rat model. It was reported that trimetazidine can reduce myocardial cell apoptosis and suppress ventricular remodeling [20, 21], which is in agreement with our results.

In conclusion, the application of trimetazidine can improve clinical symptoms and alleviate myocardial tissue structure injuries in heart failure model rats. It decreases IVS, LVPW, LVS, and LVD, but it upregulates LVEF and FS, leading to improved ventricular remodeling. It can also promote the cardiac energy metabolism process from the fat metabolism to glucose oxidation metabolism and increase phosphate ester synthesis to protect myocardial cells and improve the physiological function of the heart. It is worthy of generalized use in clinical applications.

Disclosure of conflict of interest

None.

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