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Original Article

Effects of PPARα/PGC-1α on the myocardial energy metabolism during heart failure in the doxorubicin induced dilated cardiomyopathy in mice

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Abstract: Objective: This study aims to investigate the effects and their mechanisms of PPARα and PGC-1α pathways in doxorubicin induced dilated cardiomyopathy in mice. Methods: The model of dilated cardiomyopathy (DCM) was established by injecting doxorubicin in mice. The 40 surviving mice were divided randomly into control group, doxorubicin model group, PPARα inhibitor and PPARα agonist group. The PPARα/PGC-1α proteins were detected. The size of adenine acid pool (ATP, ADP, AMP) and phosphocreatine (Pcr) in mitochondria were measured by HPLC. The ANT activity was detected by the atractyloside-inhibitor stop technique. The echocardiography and hemodynamic changes were detected in each group after PPARα inhibitor and PPARα agonist treatment for 2 weeks. Results: The DOX induced DCM model were successfully established. The expression of PPARα and PGC-1α protein level in normal group were significantly higher than that in DOX model group ($P<0.05$). Both the high-energy phosphate content and the transport activity of ANT were decreased in DOX group ($P<0.05$), and the hemodynamic parameters were disorder ($P<0.01$). Compared with Dox group, PPARα inhibitor intervention significantly reduce the expression of PPARα/PGC-1α, high-energy phosphate content in the mitochondria had no significant change ($P>0.05$), but the ANT transport activity of mitochondria decreased significantly ($P<0.05$), the left ventricular function decreased. On the other side, PPARα agonist intervention significantly increased the expression of PPARα and PGC-1α, improved transport activity of ANT, the hemodynamic parameters was ameliorated ($P<0.05$), but the high-energy phosphate content of mitochondria did not change significantly ($P>0.05$). Conclusion: There was lower expression of PPARα and PGC-1α in DOC induced DCM in mice. Promotion of PPARα can improve myocardia energy metabolism and delay the occurrence of heart failure.

Keywords: PPARα, PGC-1α, doxorubicin, dilated cardiomyopathy, energy metabolism

Introduction

Doxorubicin (DOX, trade name Adriamycin; liposome-encapsulated trade name Doxil), also known as hydroxydaunorubicin, derived by chemical semisynthesis from a bacterial species, is a drug used in cancer chemotherapy. In its unaltered form, doxorubicin has shown great treatment potential, being regarded as one of the most potent of the Food and Drug Administration-approved chemotherapeutic drugs. The ability to combat rapidly dividing cells and slow disease progression has been widely acknowledged for several decades [1]. It is commonly used in the treatment of a wide range of cancers, including hematological malignancies (blood cancers, like leukaemia and lymphoma), many types of carcinoma (solid tumors) and soft tissue sarcomas [2]. It is known to bind to DNA-associated enzymes, intercalate with DNA base pairs, and target multiple molecular targets to produce a range of cytotoxic effects. However, it is limited by its toxicity on noncancerous cells in the human body, with the most serious adverse effect being life-threatening heart damage. It can induce chronic cardiac toxicity with the increase of its dosage and lead to irreversible myocardial damage, dilated cardiomyopathy (DCM) and congestive heart failure can occur in patients finally [2, 3]. DCM is the recognized difficult problem in the international medical circles and its pathogenesis and treatment still need to do further research [4].

At present, the changes and regulations of myocardial energy metabolism in DCM are poorly understand, especially the transcriptional regu-

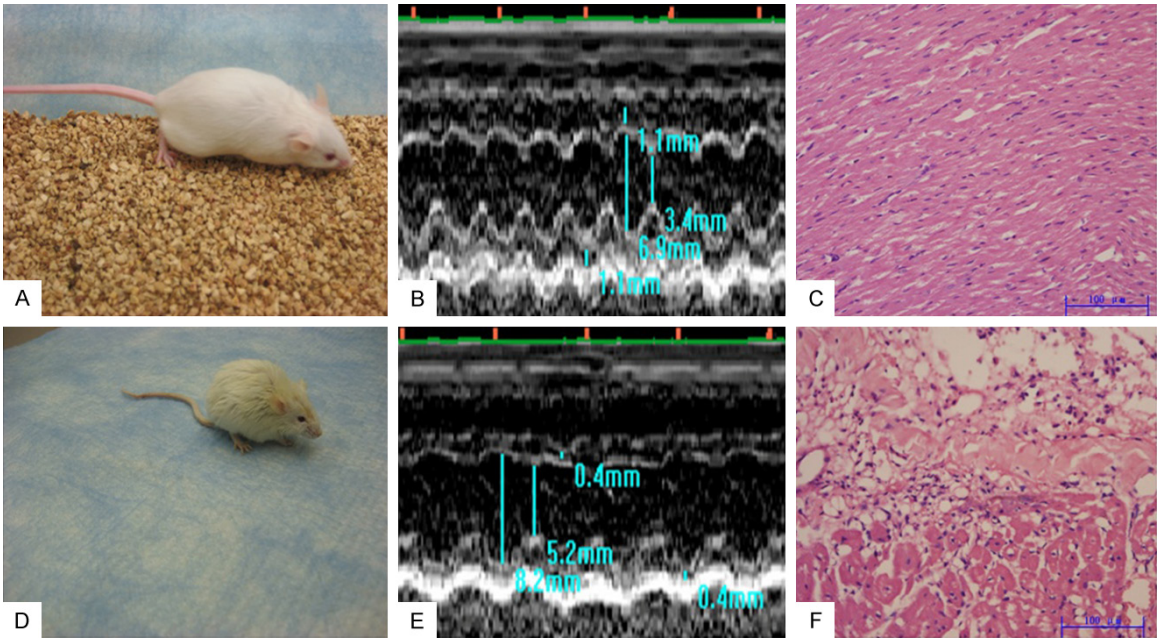

Figure 1. Echocardiography and myocardial HE staining results in DCM and control group. A: Mouse in control group, good activity and no edema; B: Echocardiography of control group, normal IVS, PWT, FS and EF; C: Myocardial HE staining in control group, myocardial fibers arranged neatly; D: Mouse in DCM group, weight loss, facial edema and sluggish; E: Echocardiography of DCM group, heart cavity significantly expanded, myocardial thinning, reduced FS and EF; F: Myocardial HE staining in DCM group, disordered arrangement of myocardial fibers and partial vacuolar degeneration.

lation of PPARα and PGC-1α on energy metabolism of drug-induced cardiomyopathy is rarely reported. This study intends to establish a mouse model of DCM induced by adriamycin, and detect the expression of PPARα and PGC-1α in mice to investigate the effects and their mechanisms of PPARα and PGC-1α pathways in doxorubicin induced DCM in mice.

Materials and methods

Experimental animals

40 adult FVB/NJ mice were obtained from the animal experimental center of Third Military Medical University. These FVB/NJ mice were kept under clean and quiet environment with room temperature of 22±1°C and relative humidity as 40-50%, provided with 12D:12N photoperiod cycle (6:00 AM-6:00 PM). The mice had free access to food and drinking water and were pre feeding for 3 days to adapt to the environment. They were randomly divided into 4 groups: control group, DOX group, PPARα inhibition (PPARα-) group and PPARα activation (PPARα+) group. Each group has 10 mice. PPARα- group used PPARα inhibitors GW 6471 and PPARα+ group used PPARα agonists Wy14643.

Housing and procedures involving experimental animals were in accordance with the Guide for the Care and Use of Laboratory Animals (eight edition, published by the National academies press). All experimental procedures were approved by the Care of Experimental Animals Committee of Third Military Medical University, Chongqing, China.

DCM model was established using intraperitoneal injection of DOX in physiological saline solution (2.0 $mg \cdot kg^{-1}$) for 2 weeks, then the mice were continued to be treated with DOX after intermittent 2 weeks. The control group was treated with intraperitoneal injection of the same volume (0.1 mL) of physiological saline, PPARα inhibitor group was treated with intraperitoneal injection of PPARα inhibitor GW 6471 (20 $mg \cdot kg^{-1} \cdot d^{-1}$), PPARα agonist group was treated with intraperitoneal injection of PPARα specific activator Wy 14643 (20 $mg \cdot kg^{-1} \cdot d^{-1}$).

Echocardiography

The measurement of echocardiography was performed according to the USA ultrasonic Heartbeat figure Association recommended method and 3 continuous Heartbeat cycles

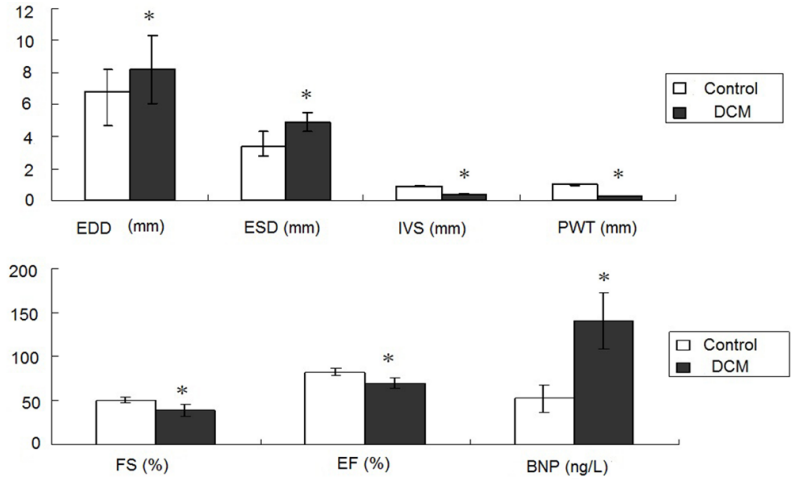

Figure 2. Comparison of echocardiography and heart function in control and DCM group. A: Comparison of echocardiography in control and DCM group, EDD and ESD increased and IVS and PWT decreased significantly in DCM group; B: BNP increased and FS and EF decreased significantly in DCM group.

were selected for measuring. The measurements data included end diastolic diameter of left ventricular (EDD), end systolic diameter of left ventricular (ESD), Heart rate (HR), end diastolic volume of left ventricular (EDV), end systolic volume of left ventricular (ESV). The systolic function of left ventricular was evaluated by fractional shortening (FS) and ejection fraction (EF) of left ventricular. HR was measured with aortic blood flow Doppler signals. FS=(LVEDD-LVESD)/LVEDD; $EDV=7\times EDD^3/(2.4+EDD)$; $ESV=7\times ESD^3/(2.4+ESD)$; EF=(EDV-ESV)/EDV×100%.

Detection of hemodynamic parameters and keep the myocardium specimens

The mice of each group were anaesthetized with intraperitoneal injection of 1% pentobarbital sodium (30 mg/kg) respectively. The right carotid artery was separated and intubated. Heart rate (HR), mean artery pressure (MAP), systolic aortic pressure (SAP), diastolic aortic pressure (DAP), left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP) and the maximum speed of left ventricular pressure increase or decrease (±dp/dtmax) were measured with physiological recorder of Powerlab/8sp 8 channel model.

The heart was taken out immediately after pressure curves were recorded and was flushed with pre-cooling saline. The ventricular myocardium in 4 mm range from the myocardial infarction was preserved in liquid nitrogen for RT-PCR and ATP detection. The other myocardium was used for isolation of mitochondria.

Detection of PPARα and PGC-1α

The expressions of PPARα and PGC-1α were detected using Western blotting and RT-PCR methods.

Total proteins were extracted from the excised myocardial tissue samples of each group. They were quantified (Bradford method) and analyzed with SDS-PAGE electrophoresis. After electrophoresis, they were electro-transferred to the PVDF membrane. The membrane containing the proteins was used for immunoblotting with required antibodies. The protein bands were scanned and quantified as a ratio to β-actin.

Total RNA was extracted from the excised myocardial tissue samples of each group. The primers for PPARα are as follows. F: 5'-GGGTGGTTGAATCGTGAGG-3', R: 5'-TGCCTTTTGCCAACAGTAGTAC-3'. The primers for PGC-1α as follows. F: 5'-ATGGCACGCAGCCCTATTC-3', R: 5'-GCATCCTTTGGGGTCTTTG -3'.

Determination of adenylate in myocardial tissue

The adenine nucleotide in myocardial tissue was determined according to the method of Bøtker et al [5]. The detection wavelength of HPLC was 254 nm, the sensitivity was 0.01 AUFS. Chromatographic column was 4.6 nm×250 nm, YWG-ODS C_{18} 10 μm. The mobile phase was 2 mmol/L PBS (pH: 5.5). It was eluted for 20 min with constant speed of 1 mL/min.

Determination of myocardial mitochondrial ANT transport activity

ANT transport activity was determined by inhibitors termination method. 20 μl of ^{3}H-ADP solution (0.3 μmol/L) was added into 50 μl of prepared mitochondrial suspension for 10 s, then 50 μl of ANT inhibitor ATR (3.2 nmol/L) was

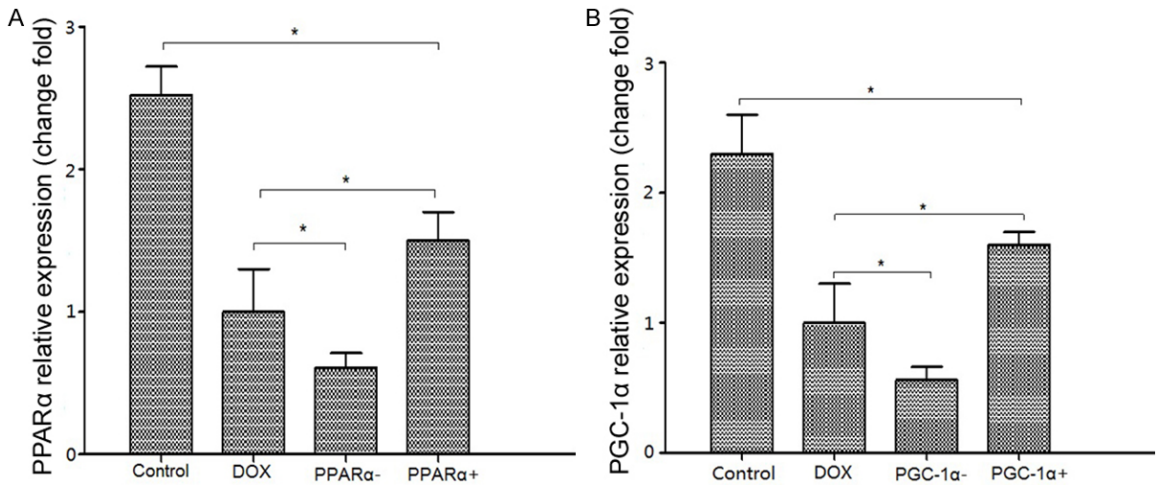

Figure 3. RT-PCR results of PPARα and PGC-1α relative expression in different groups (A: PPARα; B: PGC-1α), *P<0.05.

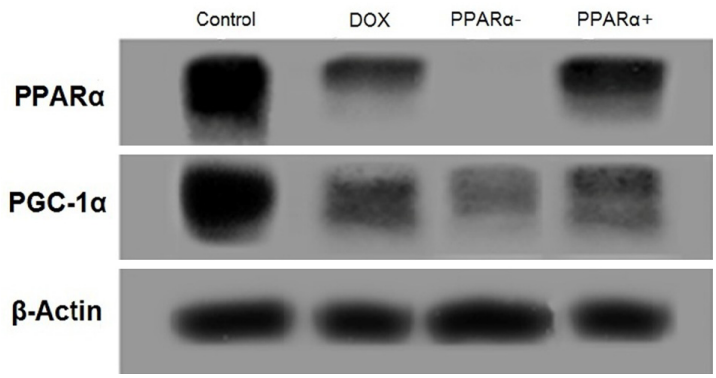

Figure 4. Western blotting results of PPARα and PGC-1α expression in different groups.

added and mixed immediately to terminate ANT transporter function. The mixture was centrifuged for 20 min with 12000 r/min speed at 4°C and the supernatant was discarded. 400 μl of H_2O_2 (8.8 mol/L) was added into the precipitation after being dissolved and cleaned with-1ml separation medium, then it was digested for 40 min in 70°C water bath. The radioactivity was measured by liquid scintillation counting method.

Statistical analysis

All the experimental data were analyzed by SPSS17.0. Comparison among the groups was carried out by One-way ANOVA. The comparison between two groups was carried out by LSD. P<0.05 was considered to be statistically significant differences.

Results

Establishment of DCM model induced by DOX

We successfully established the FVB/NJ mouse model of DCM congestive heart failure. The observed indexes including echocardiography examination, left ventricular diameter measurement, myocardial pathological findings were consistent with the changes in DCM (**Figures 1** and **2**).

The expression of PPARα and PGC-1α in different groups

The results of RT-PCR showed that the expression of PPARα and PGC-1α in DCM groups was lower than that of control group, while the expression of PPARα and PGC-1α in PPARα+ group was higher than that of DOX and PPARα- group (*P*<0.05) (**Figure 3**). The Western blotting results showed in **Figures 4** and **5**.

Echocardiographic results in different groups

Compared with control group, EDD and ESD increased and FS and EF decreased significantly in DOX group and PPARα- group (*P*<0.05). There was no significant difference between PPARα+ group and control group, however they are higher than that of DOX group (*P*<0.05) (**Table 1**; **Figure 6**).

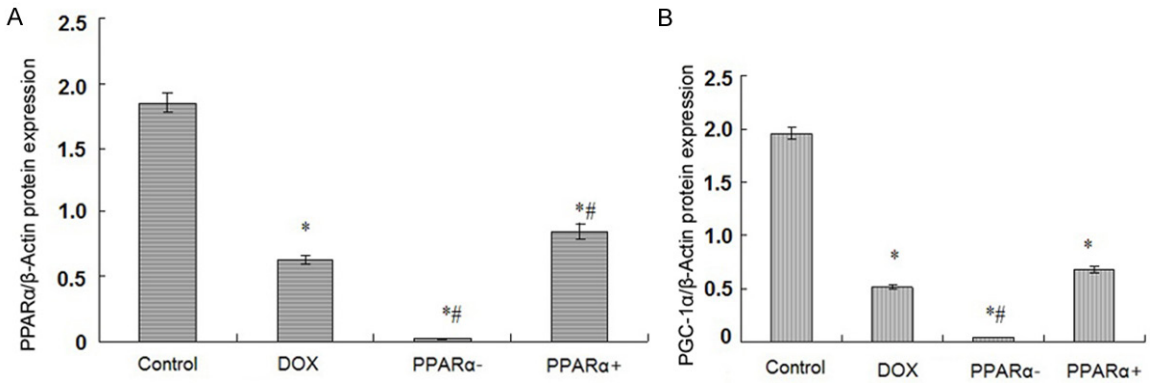

Figure 5. Western blotting results of PPARα and PGC-1α/β-actin relative expression in different groups (A: PPARα; B: PGC-1α). *Compared with control group, $P<0.05$; #Compared with DOX group, $P<0.05$.

Table 1. Echocardiographic results in different groups

group	EDD (mm)	ESD (mm)	FS (%)	EF (%)
Control	5.62±0.41	2.51±0.20	55.80±2.14	90.37±1.49
DOX	6.94±0.52*	4.06±0.25*	41.51±3.15*	78.08±2.21*
PPARα-	7.94±0.34*,#	5.61±0.72*,#	39.37±2.26*,#	76.91±9.82*,#
PPARα+	6.41±0.56	4.91±1.04	53.80±2.18	86.34±5.94

*Compared with control group, $P<0.05$; #Compared with DOX group, $P<0.05$.

Hemodynamics results in different groups

Compared with control group, LVSP, $+dp/dt_{max}$ and $-dp/dt_{max}$ decreased and LVEDP increased significantly in DOX group and PPARα- group ($P<0.05$). There was no significant difference between PPARα+ group and control group (**Table 2**).

Changes of adenylate content in myocardial tissue in different groups

Compared with control group, ATP, ADP and AMP decreased significantly in DOX, PPARα+ and PPARα- groups ($P<0.05$) (**Figure 7**).

Changes of myocardial mitochondrial ANT transport activity in different groups

Compared with control group, the myocardial mitochondrial ANT transport activity decreased significantly in DOX, PPARα+ and PPARα- groups, the results were showed in **Figure 8**.

Discussion

DOX was widely used in a variety of cancer chemotherapy. However, its obvious irreversible dose-dependent cardiac toxicity limited its clinical application seriously. It is often used to induce the model of non ischemic cardiomyopathy these years. At present, the rabbits and rats were often used as objects in many studies, but they had high cost and high mortality disadvantage [6, 7]. Therefore, we used FVB/NJ mice in this study to establish the DCM model, it had the economic and reliable advantages and was consistent with the clinical course of treatment of DOX, which providing a guarantee for the research of DOX induced DCM.

The pathogenesis of DOX induced DCM is not clarified until now. Generally, mitochondria are the main target organs in the pathogenesis of DOX induced cardiac toxicity. DOX redox to semiquinone after its affinity with myocardium and produced a large amount of oxyradical which attacking mitochondria directly, mitochondrial dysfunction occurred, such as mitochondria swelling, cracking, cristae broken until dissolved. The oxidative phosphorylation was inhibited, ATP generation was blocked, which influencing myocardial systolic and diastolic function seriously [8-10].

Peroxisome proliferator-activated receptors (PPARs) is an important regulator of myocardial lipid and energy metabolism, which regulating most of the nuclear gene expression of fatty acid oxidation (FAO) encoded by myocardial mitochondria [11].

PGC-1α was found firstly as the member of the PGC-1 family, then its homologous proteins

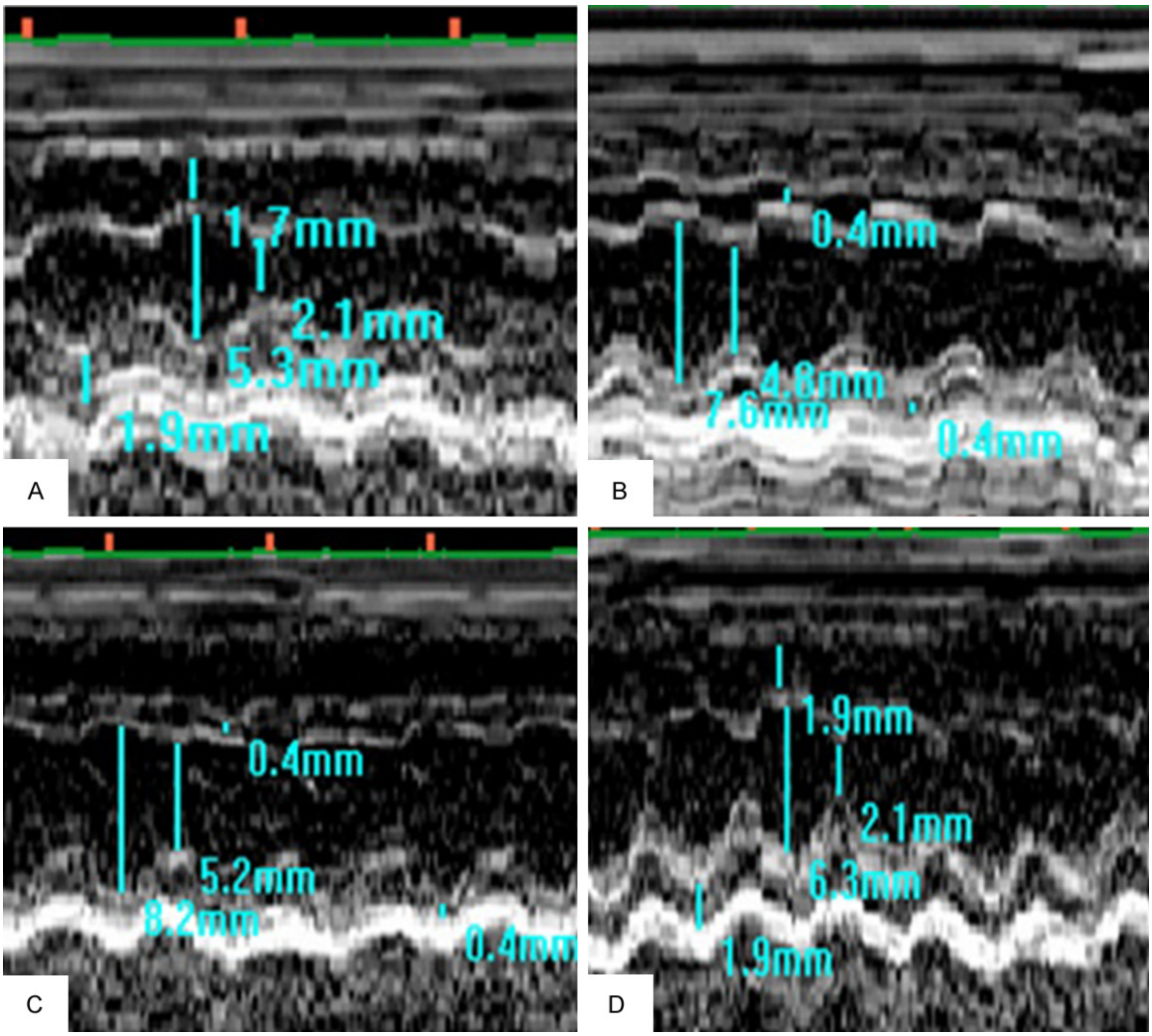

Figure 6. Echocardiography in different groups. A: Control group; B: DOX group; C: PPARα- group; D: PPARα+ group.

Table 2. Hemodynamics results in different groups

Group	LVSP (mmHg)	+dp/dt$_{max}$ (mmHg/s)	-dp/dt$_{max}$ (mmHg/s)	LVEDP (mmHg)
Control	127.76±3.15	6045.42±205.04	5137.26±183.84	4.68±0.60
DOX	109.21±4.11*	3488.17±470.62*	2722.81±201.42*	22.36±2.91*
PPARα-	118.64±3.57*,#	3551.64±386.52*,#	2999.61±235.27*,#	24.07±2.91*,#
PPARα+	130.58±9.28	6024.51±309.24	5109.64±203.54	5.05±1.68

*Compared with control group, $P<0.05$; #Compared with DOX group, $P<0.05$.

such as PGC-1β and PRC (PGC-1-related coactivator) were found in succession. They constitute a small coactivator family and PGC-1 is a key regulator of mitochondrial biogenesis and breathing [12, 13]. Studies have found the strategy that mitochondrial biogenesis could be regulated through up-regulation of the PGC-1 pathway for the prevention and reversal of various diseases, including obesity and diabetes [14]. PPARα/PGC-1α pathway had a beneficial effect on the heart function in myocardial infarction model, but its effect in the DOX induced cardiomyopathy is not clear.

We established the FVB/NJ mice model of DCM induced by DOX in this study and found that the expression of PPARα and PGC-1α in DCM groups decreased significantly than that of con-

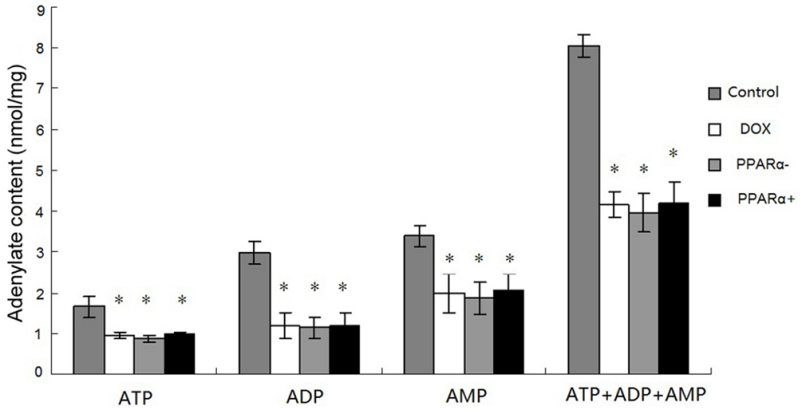

Figure 7. Changes of adenylate content in myocardial tissue in different groups. *Compared with control group, $P<0.05$.

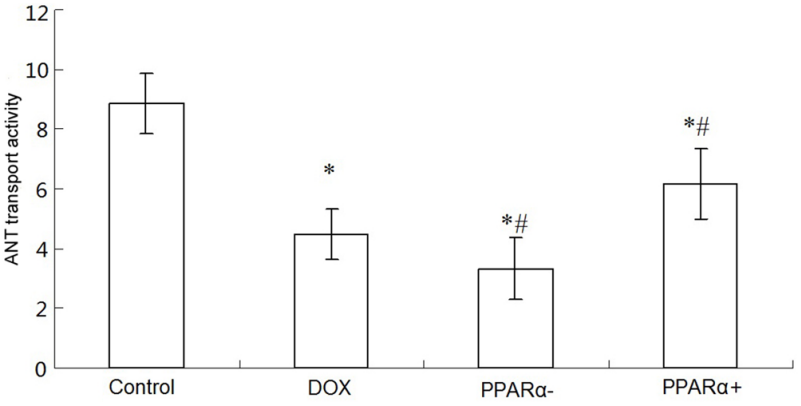

Figure 8. Changes of myocardial mitochondrial ANT transport activity in different groups. *Compared with control group, $P<0.05$; #Compared with DOX group, $P<0.05$.

trol group. The cardiac function decreased and hemodynamic disorder in DOX and PPARα- groups, while they were improved in PPARα+ group. We also found the expression of PPARα/PGC-1α increased through activating PPARα/PGC-1α, which improving myocardial mitochondrial ANT transport activity and delaying the development of heart failure. The inhibition of PPARα/PGC-1α has the opposite effect. Previous study thought that PPARα/PGC-1α pathway will become an effective means for treatment of mitochondrial myopathy [15]. In this study we also found that treatments for mitochondrial protection is effective against DOX induced cardiomyopathy.

In a word, this study revealed that the expression of PPARα and PGC-1α was low in DOX induced DCM mice model, promoting transcription and activation of PPARα/PGC-1α can improve the pathological myocardial energy metabolism and protection of mitochondrial function can reduce or even reverse myocardial lesions, which Plays a protective effects on DOX induced congestive heart failure. It provides a potential new therapeutic target and develops new ideas for the treatment of DCM.

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Disclosure of conflict of interest

None.

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