

Pioglitazone Ameliorates Hypertension Induced Cardiac Hypertrophy and Down Regulates Cardiac Hypoxia Inducible Factor-1 α in Rats

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ABSTRACT

It is known that cardiac hypertrophy induced by hypertension always goes with abnormal myocardial glucolipid metabolism. However, whether or not pioglitazone may alleviate the cardiac hypertrophy and reverse abnormal myocardial glucolipid metabolism is still unknown. We conducted this experiment to explore the influence of pioglitazone on cardiac hypertrophy in rats. Rats with cardiac hypertrophy induced by renovascular hypertension were given a gavage of pioglitazone 5–10 mg/kg for 4 weeks. Our crew determined systolic blood pressure (SBP) and diastolic blood pressure (DBP). Heart mass index (HMI), left ventricular mass index (LVMI), myocardial cell diameter (MCD) and surface area (SA) were further estimated. We members determined FFA and Ang II levels. HE and Masson staining were estimated histopathological changes. The protein and mRNA expression of PPAR- α , CPT-1 as well as PDK-4 were measured by western blot and qRT-PCR. The results showed that pioglitazone could reduced SBP, DBP, MCD, SA, HMI, LVMI as well as myocardial fibrosis and FFA and Ang II levels in serum. Moreover, pioglitazone inhibited the expression of HIF-1 α protein and simultaneously enhanced the expressions of PPAR- α , CPT-1 as well as PDK-4 mRNA and proteins. So we infer that pioglitazone could improve hypertension-induced cardiac hypertrophy and redress abnormal myocardial glucolipid metabolism in rats by down-regulating the protein expression of myocardial HIF-1 α and increasing the protein expressions of myocardial PPAR α , CPT-1 and PDK-4.

INTRODUCTION

Cardiac hypertrophy is a common pathological outcome of hypertension, myocardial infarction, coronary heart disease, and other cardiovascular diseases. Sustained cardiac hypertrophy eventually leads to heart failure. Several therapeutic approaches have been used extensively in the treatment of cardiac hypertrophy and general cardiac failure (Peterzan *et al.*, 2017). Despite recent advances in drug therapy for cardiac hypertrophy and heart failure, these conditions have an extremely poor survival rate with an annual mortality rate of 29.6% (Chen *et al.*, 2011). With the progression of this disease,

the enlarged myocardium can counteract the increase in wall pressure, and in the case of myocardial hypoxia, the utilization of myocardial energy also changes from fatty acids to glucose to produce more adenosine triphosphate (ATP) (Ritchie and Delbridge, 2006). This alteration of glucolipid metabolism could eventually contribute to the excessive accumulation of fatty acids in the heart and more serious deterioration of cardiac hypertrophy considering the impaired oxidation of fatty acids (Brandt *et al.*, 1998; Purushothaman *et al.*, 2011). Hence, the reversion of dysfunctional myocardial glucolipid metabolism plays a crucial part in ameliorating cardiac hypertrophy.

Increasing evidence has shown that hypoxia inducible factor (HIF)-1 α , highly expressed in hypertrophied hearts, is closely related to the glucolipid metabolism, as well as the alteration of hypoxic myocardial energy utilization (Abe *et al.*, 2017; Roncaro *et al.*, 2018). Stimulation of HIF-1 α could restrain the expression of peroxisome proliferator activated receptor (PPAR) α in anoxic myocardial cells (Czibik, 2010). The alterations in the expression of myocardial PPAR α could further lead to enhanced glucose utilization and decreased fatty acid oxidation under the influence of their respective downstream genes- PPAR α

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Authors' Contribution

Y-MT and W-ZL designed the research. LY performed the research. Y-MT provided help and advice on the experiments. W-ZL analyzed the data. Y-MT wrote the manuscript. All authors contributed to editorial changes in the manuscript and approved the final manuscript.

Key words

Hypertension, Cardiac hypertrophy, Pioglitazone

target genes carnitine palmitoyl transferase (CPT)-1 and pyruvate dehydrogenase kinase (PDK)-4 (Barger and Kelly, 2000; Finck and Kelly, 2002).

Pioglitazone (PIO) has been proved to alleviate cardiac hypertrophy induced by pressure overload in mice (Shiomi *et al.*, 2002). PIO could inhibit atherosclerosis and improved left ventricular remodeling in mice with post myocardial infarction. In this study, we used an animal model of cardiac hypertrophy induced by renovascular hypertension to determine whether PIO could protect against cardiac hypertrophy, and we also uncovered the molecular mechanisms underlying the protective effects.

Chemicals and reagents

Pioglitazone hydrochloride (99% purity) was purchased from Sigma-Aldrich Co. Ltd (St. Louis, USA). Free fatty acids (FFA) kit was provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and Rat angiotensin II ELISA kit was purchased from Shanghai Xitang Biotechnology Co. Ltd (Shanghai, China). Anti-HIF-1 α , anti-CPT-1, anti-PDK-4 and anti-PPAR α antibodies were the products of Abcam (Cambridge, UK). Anti-GAPDH antibody was obtained from Cell Signaling Technology (Boston, USA). All other reagents used in this study were of analytical grade.

Animals

Male Sprague-Dawley rats, weighted 200±20g, were provided by Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). These rats were kept in a temperature-controlled (20±4°C) and humidity-controlled (55%±5%) environment and fed freely. The animal study was approved by the University Ethics Committee and performed according to the guiding principles for the use and care of experimental animals at Soochow University.

Establishment of rat renovascular hypertensive model and treatment

Two-kidney, one-clip method was used to make rat model of hypertrophy induced by renal hypertension (Ménard *et al.*, 2018). The experimental rats were randomly divided into 4 groups with 6 rats respectively, including the control group (left renal artery isolation without ligation), the model group (left renal artery ligation), 5 mg per kg (in 5% DMSO, i.p.) pioglitazone group and 10 mg per kg (in 5% DMSO, i.p.) pioglitazone group. After treatment with pioglitazone for 4 weeks (the control and model groups were orally administered with an equivalent volume of 0.5% sodium carboxymethyl cellulose solution according to the same schedule). Finally, all these rats were fasted for 12 h and then sacrificed to collect blood and heart after chloral anaesthesia. The samples of these blood and heart

were used for related parameter measurements.

Measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP)

The SBP and DBP of rat tail artery was measured every 2 weeks with the CODA 4 tail-cuff non-invasive blood pressure system (Kent Scientific Corporation, Torrington, USA) before and after renovascular ligation. After fixing the rats on the blood pressure meter, their blood pressure was measured when they calmed down, during which the temperature was maintained at 30°C, also the measurement was conducted at fixed time of day (8:30–14:30) to avoid the influence of the circadian cycle. The blood pressure values were finally obtained by computing the average reading of 5 measurements. The blood pressure values were finally obtained by computing the average reading of 5 measurements.

Measurement of heart mass index (HMI), left ventricular mass index (LVMI), myocardial cell diameter and surface area

After the rats were anesthetized, their HMI and LVMI were measured. The HMI was calculated as heart weight (mg)/weight (g). The LVMI was calculated as left ventricular weight (mg)/weight (g).

Measurements of FFA and Ang II levels in serum

After removing the rat's blood for half an hour, whole blood was centrifuged at 3500 g and 4 °C for 5 min. Serum FFA and Ang II were measured in accordance with the methods provided in the instructions.

Morphological observation

The left ventricle of the rat was removed, fixed in 4% formaldehyde solution and embedded in paraffin, then routinely sectioned and stained with HE and Masson to visualize architecture of heart under light microscope.

qRT-PCR detection

Our crew used Trizol reagents to take total RNAs from cardiac tissue. Reverse transcription was applied to compose cDNA. The circulation system is as follows: 95°C for 30 min, 40 cycles, 50 °C for 5 min. GAPDH were used as internal control. 2 $^{-\Delta\Delta CT}$ approaches were used to proceed data.

Western blot analysis

Myocardial protein was extracted using a commercial kit (Keygen Biotech, Nanjing, China) according to the manufacturer's instructions and the protein concentration was determined using a bicinchoninic acid kit (Beyotime Institute of Biotechnology, Jiangsu, China). In brief,

an aliquot of 60–80 µg of protein from each sample was separated on 9–12% SDS-polyacrylamide gel by electrophoresis and then transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% skimmed milk at room temperature for 2 h. Subsequently, the membranes were incubated with specific primary antibodies for HIF-1 α (1:300 dilution), PPAR α (1: 500 dilution), CPT-1 (1:250 dilution), PDK-4 (1:1000 dilution) and GAPDH (1:1500 dilution) at 4 °C overnight with gentle shaking. Next, the membranes were washed and incubated with fluorescent secondary antibody at room temperature for 1 h. Finally, the reaction products were densitometrically quantified using an Odyssey infrared imaging system and Image J software. The ratio of the protein of interest was subjected to GAPDH, which acted as the internal control in the experiments.

Statistical analysis

All data were expressed as the mean \pm SD. Multiple comparisons between groups were analyzed by one-way ANOVA followed by a post hoc LSD test. Statistical analyses were conducted using SPSS 18.0 software, and p-values \leq 0.05 were considered statistically significant.

RESULTS

Effect on SBP and DBP in rats

As shown in Table I, the SBP and DBP in the model group was significantly higher at 2 weeks in the case of left renal artery ligation and kept at high levels compared with the control group ($p < 0.01$), indicating that a model of rat hypertension was successfully established. The SBP and DBP was gradually ameliorated in hypertensive rats with the treatment of 5–10 mg/kg pioglitazone ($p < 0.05$, $p < 0.01$).

Effects on heart mass index (HMI), left ventricular mass index (LVMI), myocardial cell diameter (MCD) and surface area (SA)

As shown in Table II, the HMI in the model group were increased than that in the control group, as well as the

LVMI ($p < 0.05$). After 4 weeks of pioglitazone treatment, both HMI and LVMI were decreased, especially in the 10 mg/kg group ($p < 0.01$). In addition, in the model group, MCD and SA were significantly increased ($p < 0.01$), which indicated that myocardial hypertrophy occurred after modeling. Nevertheless, after pioglitazone intervention, MCD and SA decreased significantly in a dose-dependent manner ($p < 0.05$, $p < 0.01$).

Table I. Rats SBP and DBP determination.

Groups	SBP (mm/Hg)	DBP (mm/Hg)
Control group	120.51 \pm 5.28	70.12 \pm 4.58
Model group	213.24 \pm 4.23**	148.76 \pm 8.29**
Pioglitazone (5 mg/kg) group	180.65 \pm 3.29#	100.92 \pm 7.14#
Pioglitazone (10 mg/kg) group	141.24 \pm 5.16##	81.32 \pm 5.63##

** $p < 0.01$ contrast to Control group. # $p < 0.05$, ## $p < 0.01$ contrast to Model group.

Effect on myocardial structure in rats

As presented in Figure 1, the arrangement of cardiomyocytes was disordered in model group, with severe inflammatory cells, compared with that in the control group. Even more impressively, the arrangement of cardiac myocytes was orderly, especially in the 10 mg/kg pioglitazone group.

Effects on myocardial fibrosis in rats

In the Figure 2, compared with control group, myocardial cells were stained unevenly, with fewer nuclei, and myocardial fibers were disordered in model group. Nevertheless, pioglitazone could relieve myocardial fiber condition. The best effect was pioglitazone 10 mg/kg.

Effects on Ang II as well as FFA levels in serum

As presented in Figure 3, the Ang II level in the model group was upgraded than that in the control group, as well as FFA level ($p < 0.01$). After 4 weeks of pioglitazone administration, these indices were remarkably decreased ($p < 0.05$ or $p < 0.01$), especially in the 10 mg/kg group.

Table II. Rats HMI, LVMI, MCD as well as SA determination.

Groups	HMI(mg/g)	LVMI(mg/g)	MCD (μm)	DBP (μm^2)
Control group	2.35 \pm 0.23	2.42 \pm 0.13	18.25 \pm 2.15	300.46 \pm 60.34
Model group	5.13 \pm 0.18**	3.65 \pm 0.17**	30.26 \pm 3.41**	534.97 \pm 87.93**
Pioglitazone (5 mg/kg) group	3.91 \pm 0.31#	2.87 \pm 0.24#	26.87 \pm 4.68#	408.91 \pm 92.36#
Pioglitazone (10 mg/kg) group	2.94 \pm 0.16##	2.50 \pm 0.19##	20.56 \pm 3.97##	310.26 \pm 78.09##

** $p < 0.01$ compared to Control group. # $p < 0.05$, ## $p < 0.01$ compared to Model group.

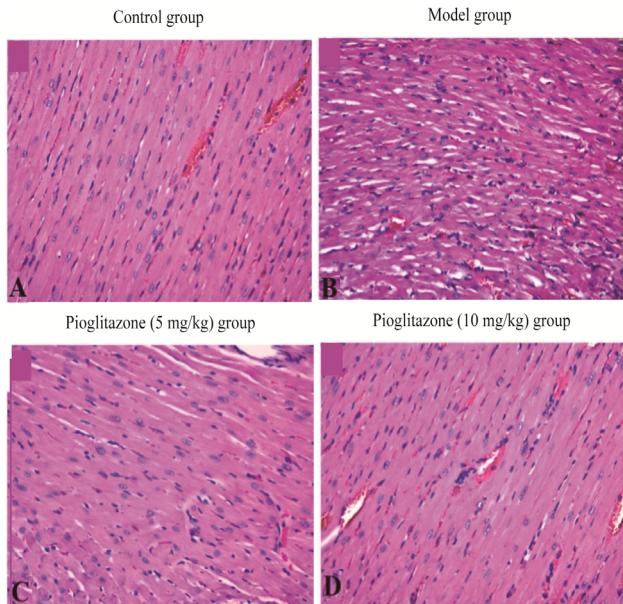


Fig. 1. Effects of pioglitazone on myocardial structure in rats. (A) Control group, (B) Model group, (C) Pioglitazone (5 mg/kg) group, (D) Pioglitazone (10 mg/kg) group.

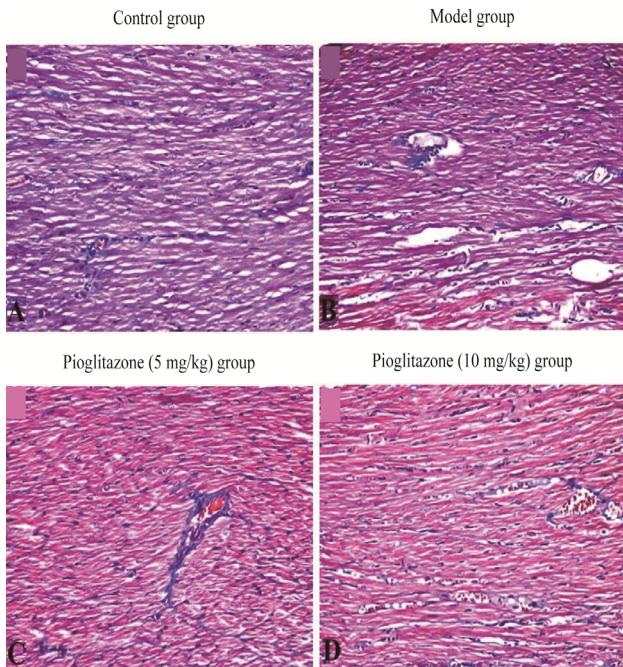


Fig. 2. Effects of pioglitazone on myocardial fibrosis in rats. (A) Control group, (B) Model group, (C) Pioglitazone (5 mg/kg) group, (D) Pioglitazone (10 mg/kg) group.

qRT-PCR assay

qRT-PCR outcomes displayed that the mRNA of HIF-1 α level in model group were evidently enhanced,

while the mRNA of PPAR α , CPT-1 as well as PDK-4 were substantially reduced (Fig. 4). Most surprising, this trend was completely reversed by pioglitazone (10 mg/kg).

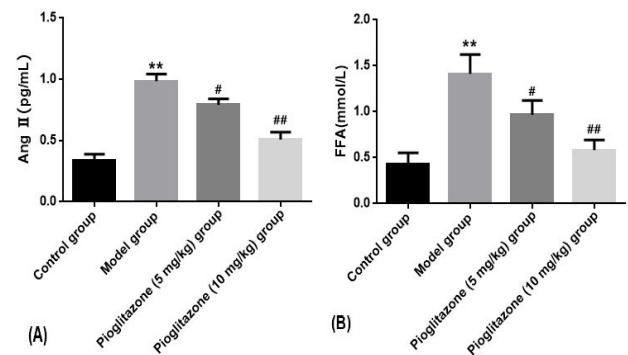


Fig. 3. Effects of pioglitazone on the levels of serum cytokine. (A) Ang II, (B) FFA. ** $p < 0.01$ compared to Control group. # $p < 0.05$, ## $p < 0.01$ compared to Model group.

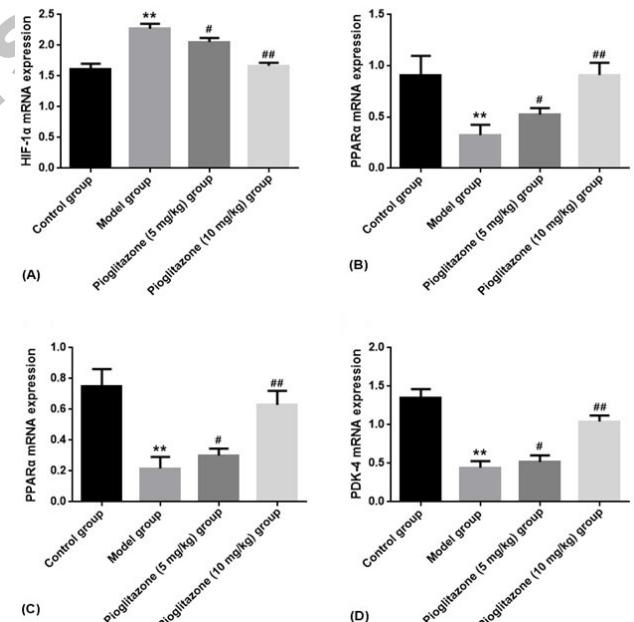


Fig. 4. Effects of pioglitazone on expressions of HIF-1 α , PPAR α , CPT-1 and PDK-4 mRNA expression. ** $p < 0.01$ compared to Control group. # $p < 0.05$, ## $p < 0.01$ compared to Model group.

Effects on myocardial HIF-1 α , PPAR α , CPT-1 and PDK-4 protein expression

As illustrated in Figure 5, the expression of HIF-1 α were upgraded in the model group, while PPAR α , CPT-1

and PDK-4 were prominently reduced, compared with the control group ($p < 0.001$ or $p < 0.0001$). After 4 weeks of treatment with pioglitazone, the HIF-1 α protein expression level was significantly downgraded. On the contrary, the PPAR α , CPT-1, and PDK-4 protein expression levels were upgraded, as we speculated, especially in the 10 mg/kg group ($p < 0.01$ or $p < 0.001$).

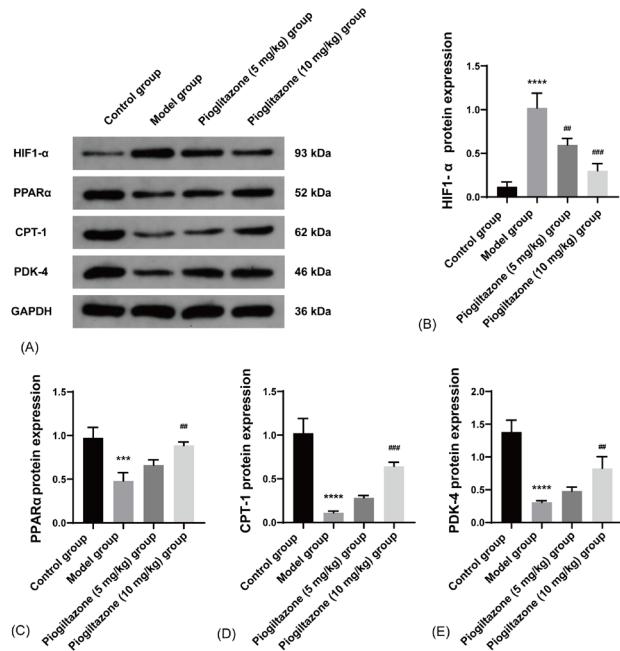


Fig. 5. Effects of pioglitazone on myocardial HIF-1 α , PPAR α , CPT-1 and PDK-4 protein expression. **** $p < 0.0001$, *** $p < 0.001$ compared to Control group. # $p < 0.01$, ## $p < 0.001$ compared to Model group.

DISCUSSION

The two-kidney one-clip method, a common renovascular hypertension animal model, could stimulate the renin-angiotensin system, increase the angiotensin II release and blood pressure, which in the end would cause cardiac hypertrophy and abnormal myocardial glucolipid metabolism (Polizio *et al.*, 2008). The myocardial energy utilization, mainly 30% glucose and 65% fatty acids, indicated normal levels of circulating substrate (Shao and Tian, 2015; Robinson and Grieve, 2009). Under normal physiological condition, the main substrates of myocardial energy metabolism were from fatty acids, but recent studies have shown that the myocardium of cardiac hypertrophy might switch from fatty acids to glucose utilization (Umbarawan *et al.*, 2018). In this study, the findings verified that the serum angiotensin II, blood pressure, cardiac weight index, cardiomyocyte cross-sectional area,

and serum FFA levels were decreased after the treatment of pioglitazone for 4 weeks in hypertensive rats, indicating that pioglitazone might serve beneficial ameliorative functions on hypertension-induced cardiac hypertrophy and abnormal myocardial glucolipid metabolism.

Hypoxia was known to be one of the common features in cardiovascular including heart failure which could induce the HIF-1 α expression and subsequent abnormal glucolipid metabolism (Narravula and Colgan, 2001). In our study, we found that HIF-1 α protein expression was prominently enhanced compared with control group, while the HIF-1 α protein expression was decreased after treatment with pioglitazone. It might show that pioglitazone could inhibit HIF-1 α to serve its anticardiac hypertrophy effect.

Growing studies reported that HIF-1 α in cardiomyocytes played an important role in modulating its intracellular metabolism by regulating PPAR α (Krishnan *et al.*, 2009). PPAR α showed a high expression level and played a key part in heart. Thereby, other studies confirmed that PPAR α served a critical role in substrate utilization alterations of cardiac energy metabolism during pathologic cardiac hypertrophy and played an important part in FFA metabolism and transport (Korman *et al.*, 2005). In our study, we found that PPAR α was significantly decreased in the model group, indicating that abnormal glucolipid metabolism was formed. After treatment with pioglitazone, pioglitazone could up-regulate PPAR α protein expression (Soñanez-Organis *et al.*, 2016).

PPAR α is involved in maintaining the balance of myocardial energy metabolism, and its activation may increase the protein expressions of CPT-1 and PDK-4 (Osman and Segar, 2016). The former is a key enzyme - mediated fatty acid into the mitochondria for fatty acid oxidation and the latter is a negative regulator of glucose oxidation through its inhibition of pyruvate dehydrogenase complexe. Current research results show that pioglitazone can increase the expression of CPT-1 and PDK-4 proteins in myocardium (Ichihara *et al.*, 2006). The results suggested that myocardial fatty acid oxidation was increased but glucose oxidation was decreased, indicating that pioglitazone could reverse the disorder of myocardial glucolipid metabolism.

The current results show that pioglitazone can effectively improve hypertensive cardiac hypertrophy and abnormal myocardial glucose and lipid metabolism in rats (Wei *et al.*, 2016). The mechanism may be related to the down-regulation of HIF-1 α expression in myocardium, thereby increasing the expression of PPAR α and its target genes CPT-1 and PDK-4. These findings are in line with our hypothesis, which may be a new pharmacological effect of pioglitazone in treating cardiovascular diseases.

Ethics approval and consent to participate

The present study was approved by the Guangxi Medical University Animal Experimental Ethics Committee (Guangxi, China).

Statement of conflict of interest

The authors have declared no conflict of interest.

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