

Research Article

Acute Lead Exposure Weakens Myocardial Contractile Function and the Therapeutic Effect of Alpha Lipoic Acid

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Abstract

This study comprehensively utilized toad hearts and myocardial cell models to explore the effects of acute lead exposure on myocardial contractile function and the therapeutic effect of alpha lipoic acid (ALA). The results showed that lead acetate (PbAc) dose dependently inhibited the contractile function of toad hearts. Acute treatment with 50, 150, and 450 mM PbAc reduced the amplitude of heart contraction to 92.8%, 78.0%, and 69.7% of the control before medication ($P < 0.01$), respectively, and reduced heart rate to 92.4%, 92.5%, and 54.9% of the control before medication ($P < 0.05$). After treatment with a middle concentration of PbAc (150 mM) for 24 hours, the cell viability of H9c2 decreased to 71.9% of the control group ($P < 0.05$). Compared with the lead exposure group, ALA at a concentration of 10 μ M significantly improved cardiac contractile function, increasing the amplitude of cardiac contractions and heart rate to 109.4% and 134.2% of the control, respectively ($P < 0.05$). Correspondingly, ALA at a concentration of 20 μ M increased the cell viability of H9c2 to 112.6% of the control group ($P < 0.05$). This study indicates that PbAc can significantly inhibit myocardial contractility and reduce cell viability, while ALA can antagonize lead induced myocardial injury and has significant cardioprotective potential.

Keywords

Lead Exposure, Myocardial Cells, Cardiac Contractility, Heart Rate, Alpha Lipoic Acid

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1. Introduction

Lead, as a persistent pollutant in the environment, can enter the human body through the respiratory tract, skin, and gastrointestinal tract, accumulate in organs such as the heart, and cause toxic effects [1]. Epidemiological studies have shown that individuals exposed to lead have a significantly increased risk of cardiovascular disease, but the molecular mechanisms underlying lead induced myocardial injury have not been fully elucidated [1]. Lead can induce myocardial cells apoptosis and oxidative stress, among which inhibition of cardiac contractile function is an important manifestation of lead poisoning [2, 3]. As a natural antioxidant, α -lipoic acid (ALA) has been proved to reduce diabetes cardiomyopathy and ischemia-reperfusion injury [4]. However, the research on its therapeutic effect on lead induced myocardial injury is still lacking. Given this research gap, the present study aims to systematically investigate the acute cardiotoxic effects of lead acetate (PbAc) and the potential cardioprotective role of α -lipoic acid. The significance of this research problem lies in addressing the urgent need for effective therapeutic interventions against cardiovascular complications resulting from environmental lead exposure. This study combines in-vivo cardiac perfusion and cell models to explore the acute effects of lead acetate on myocardial contractile function and the protective efficacy of ALA, providing experimental evidence for the prevention and treatment of cardiac toxicity caused by acute lead exposure. Specifically, this research seeks to address the following key questions: (1) How does acute lead exposure impair myocardial contractile function at the physiological level? (2) To what extent can α -lipoic acid mitigate lead-induced cardiac dysfunction? (3) What are the underlying mechanisms through which ALA confers protection against lead-induced cardiotoxicity?

2. Materials and Methods

2.1. Cardiac Perfusion

Adult toads (*Xenopus laevis*, weighing 50-70 g, purchased from the Experimental Animal Center of Zhejiang University) were perfused with Ringer's solution. Under normal perfusion conditions, the RM6240 multi-channel physiological signal acquisition system (Chengdu Instrument Factory, China) was used to record the original heart contraction curve for 15 minutes as a control. Then, PbAc solution was added in sequence to achieve final concentrations of 50, 150, and 450 mM, respectively. The raw data of the heart were recorded for 10 minutes under each concentration condition. Finally, ALA was added to achieve a concentration of 10 and 20 μ M, and the data were record for 10 minutes (Figure 1).

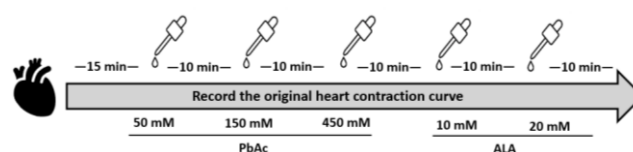


Figure 1. Schematic diagram of drug application process for toad heart perfusion experiment.

2.2. Cell Culture and Grouping

H9c2 cells (purchased from the Shanghai Cell Resource Center of the Chinese Academy of Sciences, China) were cultured in DMEM medium containing 10% fetal bovine serum (FBS, HyClone, USA). The experiment was divided into a control group (normal culture medium), a low concentration PbAc group (50 mM), a medium concentration PbAc group (150 mM), and an ALA treatment group (PbAc-150 mM +ALA-20 μ M). After 24 hours of drug treatment, each group was tested for corresponding biological indicators.

2.3. Cell Viability Assay

Cell viability was measured using the reagents of the cell counting kit-8 (CCK-8, Beyotime, China). Inoculate each group of cells into a 96 well plate, with 5000 cells/100 μ L per well cultured at 37 $^{\circ}$ C. After 24 hours of drug treatment, add 10 μ L of CCK-8 reagent to each well and continue to cultivate at 37 $^{\circ}$ C for 4 hours. Afterwards, shake the 96 well plate with a shaker for about 1 minute to ensure uniform color inside the wells, and then measure the absorbance at a wavelength of 450nm using a microplate reader.

2.4. Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean (Mean $\pm$ SEM). Statistical analyses were performed using one-way ANOVA and t-tests with GraphPad Prism (version 10.5.0). A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of PbAc and ALA on the Contraction Amplitude of Isolated Toad Hearts

Lead acetate solution was added in three portions to the cardiac perfusion fluid to achieve final concentrations of 50, 150, and 450 mM, respectively. It was observed that PbAc could dose dependently inhibit cardiac contractile function. The contraction amplitude of the isolated heart decreased to 92.8%, 78.0%, and 69.7% of the control group before medi-

cation ($P < 0.01$). Subsequently, ALA was added to the perfusion solution twice, with final concentrations of 10 and 20 μM . It was found that 10 μM of ALA could significantly reverse the contractile function of isolated toad hearts, restoring the contraction amplitude to 109.4% of the normal control ($P < 0.05$, Figure 2).

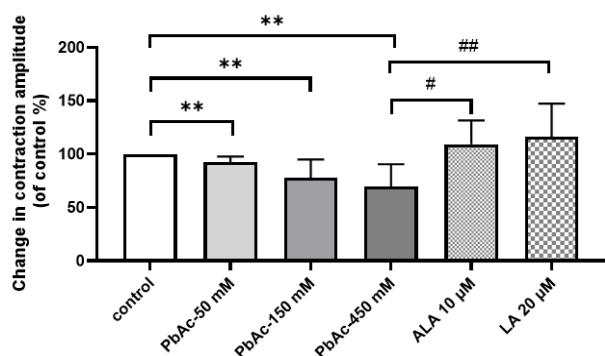


Figure 2. Effects of PbAc and ALA on the contraction amplitude of isolated hearts.

Acute exposure to PbAc at concentrations of 50, 150, and 450 mM significantly reduced the amplitude of cardiac contractions, while ALA at concentrations of 10 and 20 μM effectively increased the amplitude of cardiac contractions. $^{**}P < 0.01$ vs control group, $^{\#}P < 0.05$, $^{###}P < 0.01$ vs PbAc-450 mM group.

3.3. Effects of PbAc and ALA on the Heart Rate of Isolated Toad Hearts

Lead acetate solutions with concentrations of 50, 150, and 450 mM could also dose dependently inhibit heart rate, reducing the heart rate of isolated hearts to 92.4%, 92.5%, and 54.9% of the control, respectively ($P < 0.05$). Adding ALA to the perfusion solution could also reverse the heart rate of isolated toad hearts. A concentration of 10 μM ALA restored the heart rate to 109.4% of the normal control ($P < 0.05$, Figure 3).

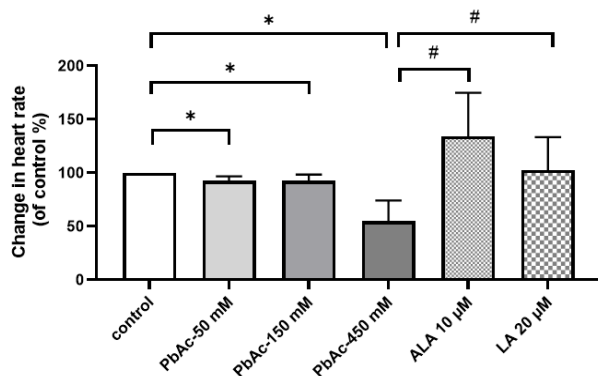


Figure 3. Effects of PbAc and ALA on the heart rate of isolated hearts.

Acute exposure to PbAc at concentrations of 50, 150, and 450 mM also significantly reduced the heart rate of isolated hearts, while ALA at concentrations of 10 and 20 μM also significantly increased heart rate. $^{*}P < 0.05$ vs control group, $^{\#}P < 0.05$ vs PbAc-450 mM group.

3.3. Effects of PbAc and ALA on Cell Viability

To determine the effect of PbAc on cell viability, we calculated the following using the optical density (OD) values measured in each well: Cell viability % = (OD of drug-induced cells/OD of control cells) $\times$ 100%. The results showed that after treatment with different concentrations of PbAc for 24 hours, compared with the control group cells, there was no significant difference in cell viability after treatment with low concentrations of PbAc (50 mM), while the higher concentrations of PbAc (150 mM) significantly reduced cell viability to 71.9% ($P < 0.05$, Figure 3). However, if incubated with 20 μM ALA at the same time, H9c2 cells were effectively protected, and their viability was significantly increased to 112.6% in the PbAc-150 mM treatment group ($P < 0.05$, Figure 4).

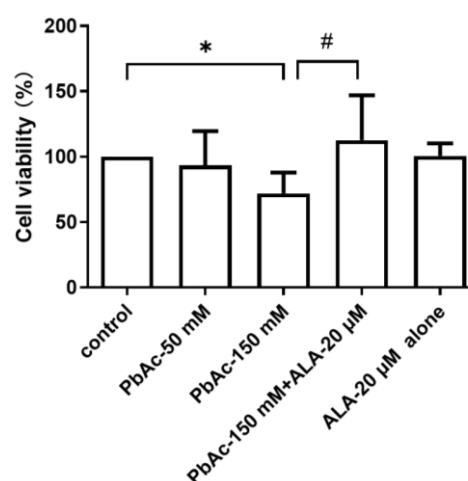


Figure 4. Effects of PbAc exposure and ALA on cell viability.

The CCK-8 experiment results showed that PbAc (150 mM) significantly reduced cell viability, while ALA had a significant reversal effect. $^{*}P < 0.05$ vs control group; $^{\#}P < 0.05$ vs PbAc-150 mM group.

4. Discussion

There have been reports of heart function damage caused by lead poisoning, such as a significant increase in abnormal electrocardiogram rates among lead exposed workers [5]. This study confirms for the first time in an isolated heart perfusion model of toads that acute lead exposure can rapidly lead to a decline in myocardial contractile function. Our results indicate that lead inhibits myocardial contractile function

before the decline in survival rate of myocardial cells. According to existing literature reports, a possible explanation is that lead can directly interfere with the calcium homeostasis of myocardial cells, inhibit the activity of sarcoplasmic reticulum Ca^{2+} -ATPase [5], leading to excitation contraction coupling dysfunction, resulting in a rapid decrease in contraction amplitude. This study did not detect intracellular calcium concentration and further exploration is needed in the future.

ALA is a potent antioxidant derived from nature, widely studied for its good protective effect against lead induced toxicity in various tissues, including the heart. Research has shown that ALA has cardioprotective effects through various mechanisms, such as directly clearing reactive oxygen species (ROS) to reduce oxidative stress [6], activating the extracellular signal regulated kinase 1/2 (ERK1/2) signaling pathway to prolong cell lifespan [7], and enhancing aldehyde dehydrogenase 2 (ALDH2) to alleviate reactive aldehydes and protect cardiac cells [8]. These pathways collectively contribute to maintaining myocardial function under toxic injury. Although this study did not directly explore the molecular mechanism, the short-term antagonistic effect of ALA on acute lead induced myocardial contraction inhibition and its partial reversal of myocardial cell toxicity were described in this article. These findings are consistent with the established role of ALA in improving cardiac dysfunction in other situations, such as diabetes cardiomyopathy. It has been proven to improve cardiac function by reducing oxidative stress and enhancing mitochondrial bioenergy [9, 10]. In addition, the metal chelation properties of ALA may reduce the accumulation of lead in cardiac tissue, further reducing toxicity [11, 12]. Combining its antioxidant and anti-apoptotic effects, these results indicate that ALA has significant potential as a therapeutic agent for preventing and treating lead induced cardiac toxicity, and its clinical applicability deserves further investigation.

Despite these promising findings, there are still some research gaps that need to be addressed to fully elucidate the therapeutic potential of ALA in lead induced cardiac toxicity. Future research should focus on three aspects. Firstly, it is important to describe and refine specific molecular pathways, such as ERK1/2 [13] signaling or ALDH2 [14] activation, through which ALA alleviates lead induced myocardial injury. In addition, exploring the best case of drug administration, such as appropriate dosage or potential synergistic effects with other antioxidants or chelating agents, can greatly enhance the clinical applicability of ALA [11, 15, 16]. Finally, studying the effect of ALA on lead induced epigenetic changes in cardiac tissue may also reveal new therapeutic targets. These research directions will provide a more solid foundation for developing lead related cardiac toxicity interventions based on ALA.

5. Conclusions

Lead acetate dose dependently inhibited myocardial con-

tractility and cell proliferation, while alpha lipoic acid effectively counteracted the myocardial toxicity of lead. The research results provided potential intervention strategies for the prevention and treatment of cardiovascular damage in lead exposed populations.

Abbreviations

ALA	Alpha Lipoic Acid
PbAc	Lead Acetate
FBS	Fetal Bovine Serum
CCK-8	Cell Counting kit-8
OD	Optical Density
ROS	Reactive Oxygen Species
ERK1/2	Extracellular Signal Regulated Kinase 1/2
ALDH2	Aldehyde Dehydrogenase 2

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Author Contributions

Zhengyi Zhou: Investigation, data curation, methodology
Zhuo Tang: Investigation, Data analysis, writing - original draft
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Yujie Ge: Investigation and data curation
Yuemin Ding: Conceptualization, supervision, writing - review & editing
Xiong Zhang: Conceptualization, supervision, writing - review & editing

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Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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