

Original Article

Reversal of lipopolysaccharide-induced cardiomyocyte apoptosis via $\alpha 7$ nAChR by dexmedetomidine

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Abstract

This study aims to analyze the reversal of lipopolysaccharide (LPS)-induced cardiomyocyte apoptosis via $\alpha 7$ nAChR by dexmedetomidine (Dex), so as to provide references for clinical treatment of myocardial disorders in the future. First, the research team divided cardiomyocytes (H9C2) were divided into a control group (normal culture), an LPS group (LPS-induced injury model), and an experimental group (pretreated with Dex before LPS induction). Subsequently, lactate dehydrogenase (LDH) and cell activity were detected, and the research team found that the LDH content of the control, experimental and LPS groups were in ascending order ($P < 0.05$). The cell viability decreased and apoptosis increased in the LPS group, with cells mainly concentrating in the G2-M phase; the viability increased and apoptosis decreased in the experimental group, with blocked G1-G0 phase ($P < 0.05$). This demonstrates that Dex can reverse LPS-induced apoptosis in cardiomyocytes. Subsequently, the research group also detected the expression of $\alpha 7$ nAChR and NF- κ B/AKT pathway, and it was seen that the expression of $\alpha 7$ nAChR in the LPS group was higher than that in the control group, with activated NF- κ B/AKT pathway; the $\alpha 7$ nAChR expression in the experimental group was further elevated, but the NF- κ B/AKT pathway was inhibited ($P < 0.05$). The effects of Dex on cardiomyocytes were seen to be related to the $\alpha 7$ nAChR and NF- κ B/AKT pathways.

Keywords: Dexmedetomidine, $\alpha 7$ nAChR, Cardiomyocytes, Lipopolysaccharide, Apoptosis

1. Introduction

Myocardial disorder remains the primary disease that threatens the health and life of the middle-aged and elderly. According to the statistics from the World Health Organization (WHO), more than 40% of individuals aged 60 and above have a potential risk of myocardial disorder worldwide [1, 2]. Among various cardiovascular diseases, diabetes mellitus (DM) is one of the most common contributing factors. High blood sugar levels in the body may trigger serious conditions such as diabetic cardiomyopathy and coronary heart disease [3]. Prevention of cardiovascular diseases has always been a hot and difficult topic in clinic. Research has confirmed that the basis of myocardial disorder lies in apoptosis of cardiomyocytes and mitochondrial dysfunction [4]. Hence, exploring effective drugs that effectively inhibit high blood sugar-induced mitochondrial damage and cell apoptosis is the key to preventing and treating the disease.

Dexmedetomidine (Dex) is a highly selective $\alpha 2$ -adrenoceptor agonist with sedative and analgesic effects; pretreatment with Dex can protect the body from myocardial ischemia-reperfusion injury [5]. In addition, it has been demonstrated that Dex pretreatment relieves ischemia-reperfusion-induced myocardial mitochondrial impairment and improves myocardial function [6]. These studies fully confirmed the excellent cardioprotective effect of Dex.

Nicotinic acetylcholine receptors (nAChRs) have a significant impact on the regulation of the cholinergic anti-inflammatory system, with $\alpha 7$ nAChR being one of the most extensively studied subtypes [7]. Previous studies have suggested that $\alpha 7$ nAChR agonists may protect against apoptosis of neurons in the rat brain induced by extracorporeal circulation by blocking the TLR4/MyD88/NF- κ B signaling pathway [8]. In a sepsis model, stimulation of $\alpha 7$ nAChRs suppresses TLR4/NF- κ B expression, thereby reducing septic injury [9]. However, it is still

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unclear whether $\alpha 7$ nAChRs is involved in the effect of Dex on myocardial disorder. To further investigate the effects of Dex on cardiomyocytes, lipopolysaccharide (LPS) was used to induce a myocardial cell injury model in this study to observe whether Dex mediates injury changes via $\alpha 7$ nAChR, thus providing more reliable references and guidance for myocardial disorder prevention and treatment in the future.

2. Materials and methods

2.1. Cell information

Cardiomyocyte H9C2 (provided by Bowers Type Culture Collection, Beijing) was cultured in DMEM containing 10% fetal bovine serum, 1% penicillin and streptomycin in an environment containing 5% CO₂ at 37°C. The medium was changed every 2 d. The cells were passaged three consecutive times, and those in the logarithmic growth phase were taken for experiments.

2.2. Grouping and treatment

For the experimental group, H9C2 cells in logarithmic growth phase were pretreated with 1 μ mol/L Dex hydrochloride for 1 h, and then incubated in high sugar medium containing 33 mmol/L glucose. For the LPS group, H9C2 cells were incubated in high sugar medium. For the control group, H9C2 cells were incubated in normal medium.

2.3. Lactate dehydrogenase (LDH) test

The supernatant of cell culture was absorbed from each group, subject to the operations specified in the instructions of the LDH kit, mixed, and allowed to stand for 5 min at room temperature. The OD values were measured at 440 nm.

2.4. Cell viability assay

When the cells in each group were cultured for 48 h, another 4-hour incubation was started after the addition of 20 μ L of MTT solution. After the medium was removed, 150 μ L of DMSO was added, followed by 10-minute shaking. The absorbance (OD) was measured at 490 nm with a microplate reader. Cells in each group were obtained and seeded into 6-well plates (500 cells/well). The medium was changed every 2 d, and the culture was terminated after visible cell clones appeared on the plate. The cells were fixed for 30 min with 4% paraformaldehyde, stained for 30 min with 1% crystal violet, dried, and photographed to count the number of cell clones formed.

2.5. Apoptosis assay

Cells were digested with trypsin, centrifuged, and washed with pre-cooled phosphate-buffered saline (PBS). As per the instructions of the Annexin V-FITC/PI cell apoptosis detection kit, Annexin V FITC (5 μ L) and PI (10 μ L) were added for incubation away from light for 15 min at room temperature. Cell apoptosis was determined using a flow cytometer. In addition, 20 μ L of RNase A solution and 400 μ L of PI were added following the instructions of a cell cycle analysis kit, and the cell cycle was analyzed by the flow cytometer.

2.6. Protein assay

Cells were subject to the extraction of total protein, and the concentration of protein samples was determined by a BCA assay kit. Then, electrophoresis was performed to

transfer the proteins onto a PVDF membrane, which was sealed with 5% skim milk powder for 1 h (room temperature) and added with $\alpha 7$ nAChR, Bax, Bcl-2, NF- κ B, AKT, and p-AKT primary antibody (1:1 000) for incubation overnight at 4°C. On the next day, the membrane was washed, and a secondary antibody (1:2000) was added for 2-hour incubation (room temperature). After that, the membrane was washed three times with TBS + Tween (TBST), and the proteins were visualized by ECL. The Quantity One gel analysis software was adopted to quantitatively analyze the results.

2.7. Mitochondrial damage detection

Cells were collected by centrifugation, added with 500 μ L of JC-1 in each well, and subjected to 20-minute incubation in an incubator containing 5% CO₂ at 37°C. Then, the cells were washed twice, added with 500 μ L of 1 \times staining binding solution, and resuspended. The proportion of mitochondrial membrane potential was analyzed by flow cytometry.

2.8. Oxidative stress damage detection

Cells were lysed with RIPA buffer, and the supernatant was collected by centrifugation. The levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were measured as per the instructions of the ELISA kit.

2.9. Immunofluorescence

Cells were sealed with 3% BSA, incubated with $\alpha 7$ nAChR primary antibody overnight, decolorized and washed, and added with secondary antibody for incubation. Then, another cycle of decolorization and washing was performed, and the nuclei of the cells were re-stained by DAPI, followed by washing. After sealing with anti-fluorescence quenching agent, a fluorescence microscope was utilized for slide observation and photographing.

2.10. Statistical analysis

All the experiments were performed in triplicates, and the results were expressed as ($\bar{x} \pm s$). Analysis of variance and LSD test (post-hoc) was adopted for statistical analysis. A *P* value of less than 0.05 was considered statistically significant.

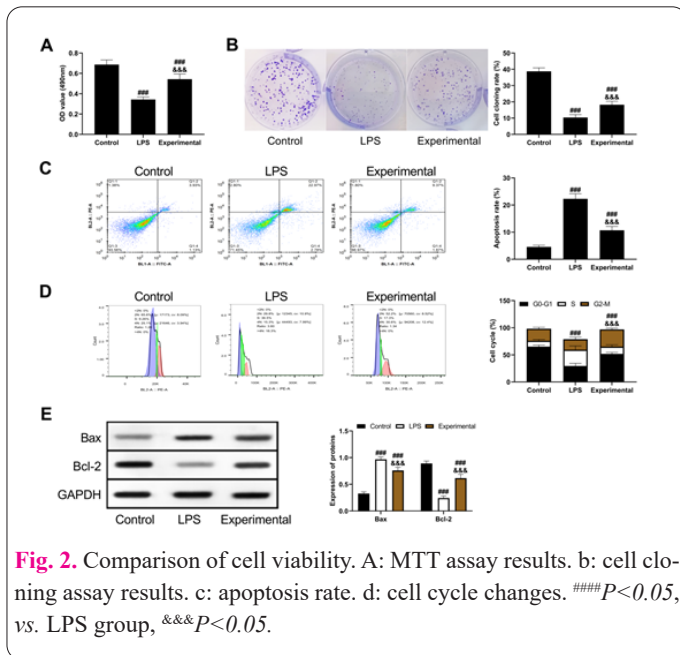
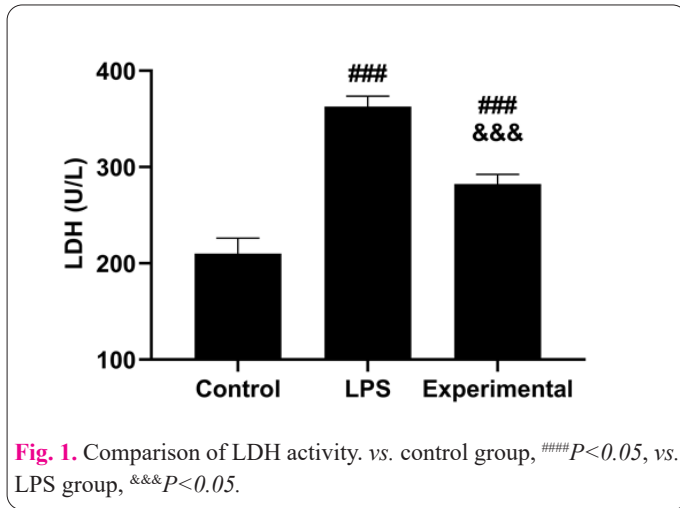
3. Results

3.1. Comparison of LDH activity

Through observation, the control group showed the lowest LDH activity; the LDH activity in the experimental group was lower than that in the LPS group (*P*<0.05, Fig 1).

3.2. Comparison of cell viability

As indicated by the results of MTT assay, the control group had the highest OD value, followed by the experimental group, and the LPS group had the lowest value (*P*<0.05). Similarly, the cloning rate in descending order was the control group, the experimental group, and the LPS group (*P*<0.05). For apoptosis, the control group had the lowest apoptosis rate, with most cells in the G0-G1 phase; the apoptosis rate in the experimental group was lower than that in the LPS group, with significantly prolonged G0-G1 phase (*P*<0.05). In addition, the control group showed lower Bax protein expression but higher Bcl-2 protein expression than the other two groups; com-



pared with the LBS group, the experimental group exhibited a lower expression of Bax but a higher expression of Bcl-2 ($P < 0.05$, Fig 2).

3.3. Comparison of mitochondrial damage and oxidative stress injury

The proportion of cells with decreased mitochondrial membrane potential in the LPS group was $(22.84 \pm 1.32)\%$, the highest among the three groups; the proportion was $(15.56 \pm 0.97)\%$ in the experimental group, higher than the control group ($P < 0.05$). In addition, the SOD level in the LPS group was the lowest among the three groups, while the MDA level was the highest; compared to the control group, the SOD level in the experimental group was lower and the MDA level was higher ($P < 0.05$, Fig 3).

3.4. Comparison of $\alpha 7nAChR$ expression

Compared to the control group, the other two groups exhibited significantly higher $\alpha 7nAChR$ protein expression, which was higher in the experimental group ($P < 0.05$). Similarly, the fluorescence intensity of $\alpha 7nAChR$ in the control group was lower than that in the other two groups; the intensity was (54.88 ± 2.69) in the experimental group, higher than that in the LPS group ($P < 0.05$, Fig 4).

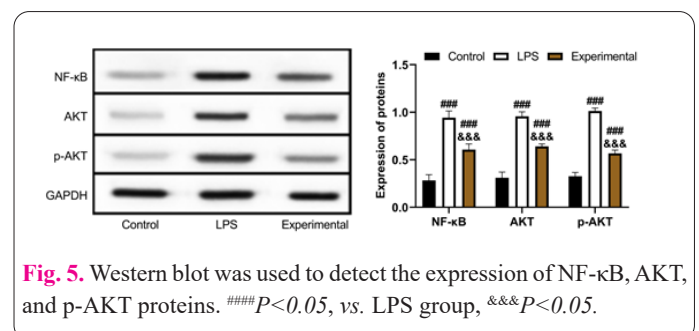
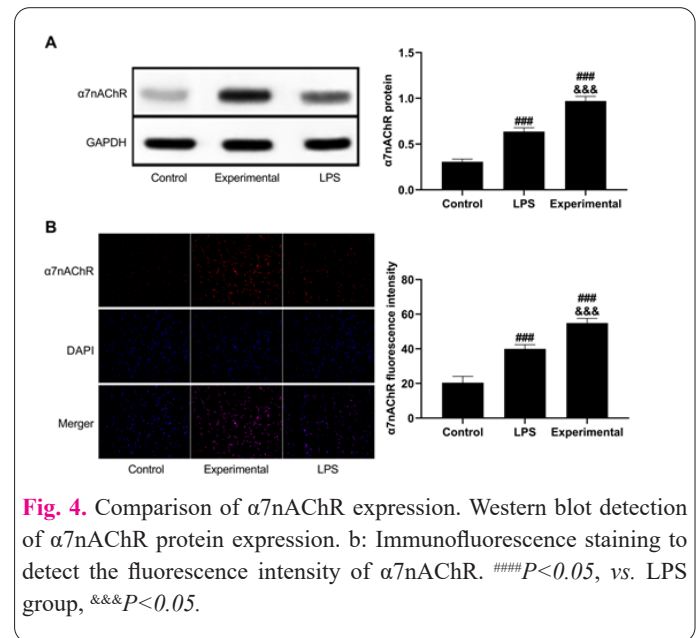
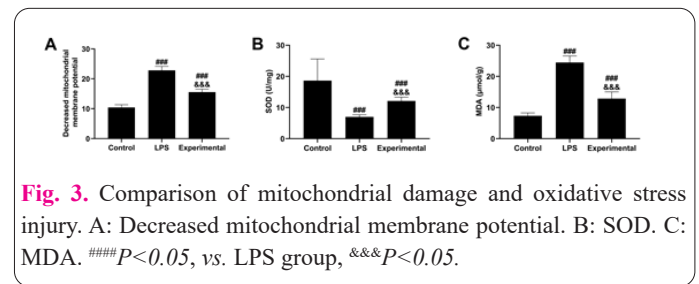
3.5. Comparison of NF- κ B/AKT pathway expression

According to the status of the NF- κ B/AKT pathway, the expression of NF- κ B, AKT and p-AKT protein in the experimental group was higher than that in the control group, but lower than that in the LPS group ($P < 0.05$, Fig 5).

4. Discussion

Cardiomyocyte apoptosis is an important mechanism of myocardial injury, and mitochondrial oxidative stress is essential in myocardial injury and protection [10]. In this study, it was found that Dex inhibited high sugar-induced cardiomyocyte apoptosis. This discovery holds significant potential for guiding the future treatment of various myocardial disorders.

Existing research has confirmed that high sugar promotes mitochondrial damage and cell apoptosis in cardiomyocytes [11]. Cardiomyocytes were treated with LPS, and the results showed notably reduced cell viability, considerably increased lactate dehydrogenase LDH level, and remarkably elevated proportion of cells with decreased mitochondrial membrane potential in the high su-



gar-treated cardiomyocytes. These results confirmed that induction with LPS could trigger mitochondrial damage in cardiomyocytes, exacerbate oxidative stress damage, and promote apoptosis, which is consistent with the results of previous similar research [12]. Dex alleviates LPS-induced acute lung injury in rats by blocking oxidative stress, mitochondrial dysfunction, and cell apoptosis [13], highlighting the important potential of Dex in protecting against various types of cell damage. In this study, cells in the experimental group treated with Dex showed significantly decreased LDH levels, enhanced cell viability, and reduced apoptosis, suggesting that Dex effectively reversed the acceleration of cardiomyocyte apoptosis induced by LPS and alleviated the resulting damage. Liu XR also proved in their study that Dex can improve H₂O₂-induced cardiomyocyte apoptosis in neonatal rats by regulating the endoplasmic reticulum stress pathway [14], which supports the results of our experiment. In research regarding the pharmacological mechanisms of Dex, researchers have found that Dex alleviates the damage to the enzyme system activity and the respiratory chain enzyme activity in mitochondria, as well as the neuronal damage and autophagy in the hippocampus caused by neuronal oxidative stress [15]. Similarly, the proportion of cells with decreased mitochondrial membrane potential in the experimental group was evidently lower than that in the model group, along with elevated SOD levels and reduced MDA levels, indicating that Dex can also reverse myocardial mitochondrial damage induced by LPS and improve oxidative stress response. As it is widely known, the pathological changes mentioned above also serve as the basis for diseases such as DM-induced cardiomyopathy and myocardial infarction, and the positive effects demonstrated by Dex establish its significant role in improving myocardial disorders in the future.

Furthermore, as mentioned earlier, the impact of Dex on myocardial cells may be related to $\alpha 7$ nAChR. Through our testing, $\alpha 7$ nAChR was notably up-regulated in the LPS group in comparison with the control group, confirming the association between elevated $\alpha 7$ nAChR levels and LPS-induced myocardial cell injury. Moreover, the expression of $\alpha 7$ nAChR was further elevated in the experimental group, proving that the effects of Dex on cardiomyocytes were achieved by the activation of $\alpha 7$ nAChR. $\alpha 7$ nAChR is an essential component of the cholinergic nervous system; when cholinergic neurons in the central nervous are activated by inflammation, information is transmitted to the efferent vagus nerve, so that acetylcholine is released, which interacts with $\alpha 7$ nAChR on immune cells to block the production of pro-inflammatory cytokines [16]. Therefore, the cholinergic anti-inflammatory pathway serves as a mechanism for negative feedback regulation of inflammation, which is up-regulated during the early stages of injury but suppressed as the inflammatory response intensifies [17]. It has been reported that the cholinergic anti-inflammatory pathway inhibits the release of inflammatory cytokines in experimental models of sepsis and ischemia-reperfusion injury, thereby suppressing excessive inflammatory response [7, 18]. Additionally, Dex activates the efferent vagus nerve by enhancing the activity of the parasympathetic nervous system, thereby suppressing the pro-inflammatory cytokine biosynthesis and release [19].

The NF- κ B/AKT signaling pathway is crucial in the

inflammatory injury in the body, and LPS can activate the NF- κ B/AKT signaling pathway to promote the release of inflammatory cytokines [20]. Previous studies on the effects of Dex have also highlighted the role of the NF- κ B/AKT pathway [21]. Here, we observed that the NF- κ B/AKT pathway was obviously activated in the LPS group, in line with previous findings. The team of Lu Z suggested in their study that sustained activation of $\alpha 7$ nAChR improves the anti-inflammatory system within the body and inhibits the expression of the NF- κ B/AKT pathway [22]. Lower expression of NF- κ B, AKT, and p-AKT proteins in the experimental group also confirmed that Dex inhibited the NF- κ B/AKT pathway through $\alpha 7$ nAChR, exerting its anti-inflammatory and antioxidant effects.

However, due to the lack of *in vivo* samples, the distribution and expression of $\alpha 7$ nAChR in myocardial tissues were not determined by immunohistochemistry, it could not be confirmed whether Dex acts through other subunits of nAChR. Second, the actual clinical dosage of Dex is different from that in cell experiments, and its clinical effect requires further verification. In the future, we will conduct a more in-depth and comprehensive analysis as soon as possible to address these limitations.

5. Conclusion

Through the activation of $\alpha 7$ nAChR, Dex inhibited the expression of NF- κ B/AKT signaling pathway, reversed LPS-induced cardiomyocyte apoptosis, and ameliorated oxidative stress and mitochondrial damage, which is of great value in the treatment of myocardial disorders in the future. However, more experiments are needed to further verify the protective mechanism of Dex on cardiomyocytes, so as to lay a solid foundation for its clinical application.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Weiwei Li conceived and designed this study; Xiangyun Li performed the experiments; Baozeng Chen collected important background information; Mingjing Feng and Changlu Zheng performed the statistical analysis and edited the manuscript; Mingjing Feng and Changlu Zheng made equal contributions to this work. All authors read and approved the final submitted manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

Not applicable.

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