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**Original Research Article**

# Anisodamine combined with lidocaine improves healing of myocardial ischemia-reperfusion injury in rats via PI3K/Akt signaling pathway

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## Abstract

***Purpose:*** *To study the effects of anisodamine (Ad) combined with lidocaine (Ldc) on myocardial ischemia-reperfusion injury (MIRI) in rats, and its correlation with PI3K/AKT signaling pathway.*
***Methods:*** *A total of 70 healthy rats were randomly divided into S group, M group, Ad group, Ldc group, Ad + Ldc group, Ad + Ldc + LY group, and LY group. The cardiac hemodynamic indices in each group were determined, and the area of myocardial infarction measured. Serum biochemical indices were also determined. Furthermore, the protein expressions of p-Akt, T-Akt, Bcl-2, and Bax in myocardial cells were determined by Western blotting.*
***Results:*** *Compared with those in M group, Ad group, Ldc group, Ad + Ldc + LY group, and LY group, cardiac hemodynamic indices significantly improved, while the area of myocardial infarction was significantly reduced ($p < 0.01$). Furthermore, serum malondialdehyde (MDA) concentration but the activities of CK, CK-MB, TNF-α, and IL-6 declined, while the activities of superoxide dismutase (SOD), CAT and GSH-Px rose in Ad + Ldc group ($p < 0.01$). In Ad + Ldc group, p-Akt, T-Akt, and Bcl-2 increased, while Bax significantly decreased. Through comparison LY294002 significantly inhibited the protective effect of Ad combined with Ldc against MIRI in rats ($p < 0.01$).*
***Conclusion:*** *Anisodamine combination with lidocaine has a protective effect against MIRI in rats via PI3K/Akt signaling pathway, thus indicating that it is a potential therapeutic strategy for the management of myocardial ischemia-reperfusion.*

***Keywords:*** *PI3K/Akt pathway, Anisodamine, Lidocaine, Myocardial ischemia-reperfusion*



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## INTRODUCTION

Currently, myocardial infarction is the first threat to human life and health, and it is mainly manifested as myocardial necrosis caused by coronary artery occlusion. Myocardial necrosis is clinically treated with the recovery of blood flow, but myocardial reperfusion aggravates

myocardial injury [1]. It is currently believed in the medical field that myocardial ischemia-reperfusion benefits less from thrombolytic therapy for short-term myocardial ischemia or percutaneous coronary intervention [2-4]. The mechanism of myocardial ischemia-reperfusion is complex, and it is believed that oxygen-free radicals play an important role in association with the above symptoms [5,6]. Therefore, studying the mechanism of myocardial ischemia-reperfusion can provide a theoretical basis for the treatment of cardiovascular disease.

At present, lidocaine (Ldc) is mainly used for local anesthesia and arrhythmia treatment during operation, as well as the treatment of dizziness with the development of technology [7]. Studies have demonstrated that Ldc cannot only stabilize the cell membrane and reduce reperfusion edema and hypoxia but also raise cerebral vascular tension and inhibit the elevation of blood pressure, thereby preventing and treating cerebral edema. At the same time, the blood circulation in the brain tissues, vestibule, and other organs is significantly improved [8,9]. Besides, anisodamine (Ad) possesses many similar effects to Ldc. For example, it can regulate blood vessels and smooth muscles, thus dilating spastic vascular smooth muscles. It also accelerates or activates blood circulation thereby reducing blood viscosity [10]. Moreover, Ad promotes the reflux of lymphatic fluid, preventing cellular edema and enhancing oxygen circulation [11]. Despite the possible protective effects of Ad and Ldc against myocardial ischemia-reperfusion injury (MIRI), there are no reports on the treatment of MIRI using Ad combined with Ldc. In this study, therefore, the effects of Ad combined with Ldc on MIRI in rats through PI3K/Akt signaling pathway were investigated, and the correlation between its protective effect and PI3K/Akt signaling pathway was explored, so as to provide some theoretical bases for the treatment of myocardial infarction.

## EXPERIMENTAL

### Materials

A total of 70 healthy male Wistar rats weighing 220 - 250 g were purchased from Tianjin Jinyao Amino Acid Co., Ltd. (Tianjin, China). Superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), catalase (CAT), creatine kinase (CK), creatine kinase isoenzyme (CK-MB), tumor necrosis factor-α (TNF-α), LY294002, and interleukin-6 (IL-6) were purchased from Sigma (St. Louis, MO, USA). Western blotting rabbit anti-B-cell lymphoma-2 (Bcl-2) polyclonal antibody and rabbit anti-Bcl-2 associated X protein (Bax) polyclonal antibody were products from Abcom (Cambridge, MA, USA). Western blotting protein assay kit and chemiluminescence assay kit were purchased from Thermo Fisher Scientific (Waltham, MA, USA), and nitrocellulose membrane was obtained from Bio-Rad (Hercules, CA, USA).

### Establishment of rat model of MIRI

Under sterile conditions, the rats were anesthetized *via* intraperitoneal injection of pentobarbital sodium (50 mg/g) and fixed on a super clean bench. The neck of each rat was cut open using a scalpel, and the right common carotid artery was isolated for later use. The trachea was exposed and connected to the ventilator (60 - 70 times/min), and the electrodes were inserted subcutaneously into limbs and connected to the biological functional system for electrocardiography. Then the skin to the left of the sternum was cut, the thoracic cavity was cut open between the left $4^{th}$ and $5^{th}$ intercostal space, and the pericardium was also cut to expose the heart. The heart surface was punctured using the non-invasive silk thread at 2 mm below the left auricle, and the left coronary artery was ligated to induce myocardial ischemia for 30 min, followed by reperfusion for 90 min. After the operation, whitening of local myocardial tissues, ST-segment elevation, and T wave height indicated the successful establishment of the myocardial ischemia model, while reddening of myocardial tissues in the ischemic region and obvious ST-segment depression by over 50 % indicated the successful establishment of reperfusion model. This study was approved by the Animal Ethics Committee of Anhui University of Traditional Chinese Medicine Animal Center (19-AH-no.16). All procedures were conducted in accordance with the 'Animal Research: Reporting in vivo Experiments guidelines 2.0' [12].

### Rat grouping

The 70 healthy male Wistar rats were randomly divided into 7 groups containing ten rats each: (1) sham operation group (S group): thoracotomy without ligation of the left coronary artery, and intraperitoneal injection of a certain amount of normal saline 1 h before operation. (2) MIRI group (M group): intraperitoneal injection of a certain amount of normal saline 1 h before ischemia, ischemia for 30 min, and reperfusion for 90 min. (3) Ad pretreatment group (Ad group): intraperitoneal injection of Ad (30 mg/L) 1 h before ischemia, ischemia for 30 min, and reperfusion for 90 min. (4) Ldc pretreatment group (Ldc group): intraperitoneal injection of Ldc (10 mg/L) 1 h before ischemia, ischemia for 30

min, and reperfusion for 90 min. (5) Ad + Ldc pretreatment group (Ad + Ldc group): intraperitoneal injection of Ad (30 mg/L) and Ldc (10 mg/L) 1 h before ischemia, ischemia for 30 min and reperfusion for 90 min. (6) Ad + Ldc pretreatment + LY294002 group (Ad + Ldc + LY group): intraperitoneal injection of Ad (30 mg/L), Ldc (10 mg/L) and LY (0.3 mg/L) 1 h before ischemia, ischemia for 30 min and reperfusion for 90 min. (7): PI3K/Akt inhibitor LY294002 group (LY group): intraperitoneal injection of LY (0.3 mg/L) 1 h before ischemia, ischemia for 30 min, and reperfusion for 90 min.

**Determination of hemodynamics**

The hemodynamic indices in each group were detected to observe the postoperative changes in rats. First, the cervical lymph nodes were removed, so that the right common carotid artery could be isolated. Then, an arterial catheter was inserted into the left ventricle. The rats were fixed, and the left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and maximal rate of the increase/decrease of left ventricular pressure (± $dp/dt_{max}$) were determined using a physiological recorder.

**Measurement of myocardial infarction area**

The area of myocardial infarction was measured using the method reported in most literature. First, the center of the infarction region was located, the heart was cut in the direction from the apex to the base of the heart parallel to the atrioventricular groove, and the left ventricle was cut into pieces. The myocardial tissues were incubated with a mixed solution of phosphate-buffered saline (PBS) (pH = 7.4) and 1 % of 2,3,5-triphenyl tetrazolium chloride at 37 °C for 20 min, and stained. The infarction region was pale, while normal myocardial tissues were red in colour. Then the non-stained non-ischemic region and red-stained ischemic region were separated using a scalpel. The area of red-stained myocardial infarction (Am) was determined in a vessel with buffer and computed using Eq 1.

$$Am = (Ai/Tlv)100 \quad \text{……} \quad (1)$$

where Ai = area of the infarction region and Tlv = total area of the left ventricle.

**Assessment of biochemical indices**

After reperfusion for 90 min, 4 mL of blood was drawn and centrifuged at 3000 rpm and 4 °C for 15 min. The supernatant was kept in a refrigerator at -20 °C. Then the serum levels of MDA, SOD, CK, CK-MB, CAT, GSH-Px, TNF-α, and IL-6 were detected according to the instructions of the kits.

**Determination of protein expressions**

(1) Sodium dodecyl sulphate (SDS) polyacrylamide gel was prepared as follows: The glass plate was placed as required, the separation gel was prepared and solidified at room temperature, and then the spacer gel was also prepared. Finally, the comb was inserted. (2) The protein concentration in 10 - 50 μg of samples was measured using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). (3) The protein samples were added with 1/4 of 5 × loading buffer and shaken evenly in a shaker, followed by denaturation *via* heating at 100 °C for 5 min. (4) The electrophoresis was performed at 80 V for 30 min and then at 120 V until there was a loading buffer at the bottom of the separation gel, and then the protein was transferred onto a membrane at 100 V for 60 min. (5) Bovine serum albumin (BSA) blocking buffer (5 %) was used as a blocking reagent. The protein was sealed at room temperature for 60 min and incubated with primary antibodies (p-Akt, Akt, Bcl-2, and Bax) at an appropriate concentration at 4 °C for 12 - 24 h on the shaker. The membrane was washed with 1 × phosphate buffered saline-tween (PBST) 3 times. (6) The protein was incubated again with an appropriate amount of secondary antibodies at room temperature for 60 min in the shaker, followed by the same operation as (5). After pretreatment, the protein expression was observed *via* chemiluminescence.

**Statistical analysis**

The SPSS analysis software (version 26.0) was used for the processing of the experimental data obtained. Experimental data are expressed as mean ± standard deviation (SD). Univariate analysis was performed for the data analysis in different groups. $P < 0.05$ suggested a significant difference.

## RESULTS

**Effect of Ad combined with Ldc on hemodynamics and infarction area**

The M group had significantly decreased LVSP, + $dp/dt_{max,}$ and -$dp/dt_{max}$ ($p < 0.01$), and significantly increased LVEDP compared with the S group ($p < 0.01$). Compared with the M group, Ad group, Ldc group, and the Ad + Ldc group had significantly increased LVSP, + $dp/dt_{max}$ and

- $dp/dt_{max}$ ($p < 0.01$), and decreased LVEDP, and the area of myocardial infarction ($p < 0.01$). Compared with the Ad + Ldc group, the Ad + Ldc + LY group had obviously decreased LVSP, + $dp/dt_{max}$ and - $dp/dt_{max}$ ($p < 0.05$), and increased LVEDP and the area of myocardial infarction ($p < 0.05$). Compared with the Ad + Ldc group, the Ad group and Ldc group had obviously decreased LVSP, + $dp/dt_{max}$ and - $dp/dt_{max}$ ($p < 0$ .05), and obviously increased LVEDP and an area of myocardial infarction ($p < 0.05$), indicating that Ad combined with Ldc is superior to Ad and Ldc alone. Compared with those in the M group, LVSP, + $dp/dt_{max}$, - $dp/dt_{max}$, LVEDP and the area of myocardial infarction showed obvious changes in the LY group. The above findings suggest that the PI3K/Akt signaling pathway inhibitor LY294002 can inhibit the protective effect of Ad combined with Ldc against MIRI in rats (Figure 1 and Table 1).

**Effect of Ad combined with Ldc on serum MDA, SOD, GSH-Px, and CAT**

The M group had significantly higher MDA concentration, and significantly lower activities of SOD, CAT, and GSH-Px than the S group, showing significant differences ($p < 0.01$). The Ad group, Ldc group, and Ad + Ldc group had significantly lower MDA concentration, and higher activity of SOD, CAT, and GSH-Px than the M group, showing significant differences ($p < 0.01$). The Ad + Ldc + LY group and LY group had significantly higher MDA concentration, and significantly lower activities of SOD, CAT, and GSH-Px than the Ad + Ldc group ($p < 0.01$). Compared with the Ad + Ldc group, the Ad group and Ldc group had significantly decreased activities of SOD, CAT, and GSH-Px, and significantly increased MDA concentration ($p < 0.01$). It can be seen that Ad combined with Ldc is superior to Ad and Ldc alone. There were no significant differences in SOD, CAT, MDA, and GSH-Px between the LY group and M group, indicating that LY294002 can inhibit the effects of Ad and Ldc on the above indices (Table 2).

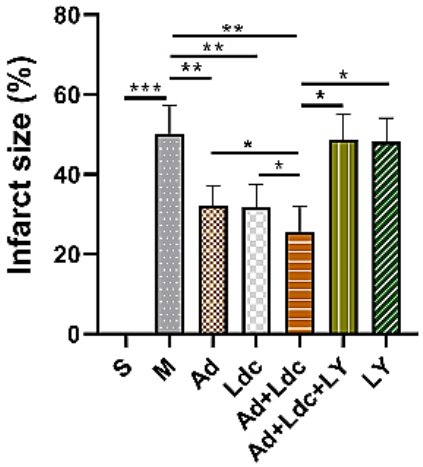


**Figure 1:** Myocardial infarction area in each group. *$P < 0.05$ vs. Ad + Ldc, **$p < 0.01$ vs. M, ***$p < 0.001$ M vs S

**Table 1:** Effect of Ad combined with Ldc on hemodynamics and infarction area in MIRI rats (n = 10, mean ± SD)

| Group | LVSP (mmHg) | LVEDP (mmHg) | +$dp/dt_{max}$ (mmHg/s) | -$dp/dt_{max}$ (mmHg/s) | Infarction area (%) |
|---|---|---|---|---|---|
| S | 138.2±8.9 | 5.3±0.2 | 4103.2±178.5 | -3456.2±187.2 | - |
| M | 89.2±8.6[a] | 13.2±0.5[a] | 2303.3±218.5[a] | -1975.2±179.2[a] | 50.2±7.2[a] |
| Ad | 112.3±7.8[bc] | 9.3±0.3[bc] | 3214.6±225.1[bc] | -2321.2±231.1[bc] | 32.2±4.8[bc] |
| Ldc | 108.3±8.7[bc] | 8.9±0.6[bc] | 3463.1±215.8[bc] | -2235.1±210.1[bc] | 31.8±5.7[bc] |
| Ad+Ldc | 126.8±7.6[b] | 7.2±0.2[b] | 3826.2±238.4[b] | -2783.2±198.4[b] | 25.4±6.6[b] |
| Ad+Ldc+LY | 94.5±8.8[c] | 12.1±0.7[c] | 2412.2±198.5[c] | -2019.3±231.5[c] | 48.5±6.2[c] |
| LY | 92.3±8.4[c] | 11.6±0.5[c] | 2385.1±228.5[c] | -2130.1±188.2[c] | 47.9±5.6[c] |

[a]$p < 0.01$ *vs.* S group, [b]$p < 0.01$ *vs.* M group, [c]$p < 0.05$ *vs.* Ad + Ldc group

**Table 2:** Effects of Ad combined with Ldc on serum MDA, SOD, GSH-Px, and CAT in MIRI rats (n = 10, mean ± SD)

| Group | MDA (μmol/L) | SOD (U/mL) | CAT (U/mg) | GSH-Px (U/mg) |
|---|---|---|---|---|
| S | 10.2±1.5 | 132.5±20.3 | 63.2±4.5 | 20.2±2.2 |
| M | 20.4±3.1[a] | 83.2±6.5[a] | 8.3±2.5[a] | 12.2±1.2[a] |
| Ad | 15.2±2.8[bc] | 101.3±14.3[bc] | 32.6±3.1[bc] | 15.2±2.1[bc] |
| Ldc | 14.5±2.6[bc] | 105.9±10.6[bc] | 34.1±2.8[bc] | 15.7±2.8[bc] |
| Ad+Ldc | 11.8±2.3[b] | 117.2±12.2[b] | 49.2±3.4[b] | 17.2±1.4[b] |
| Ad+Ldc+LY | 17.5±2.8[c] | 91.1±7.7[c] | 9.2±1.5[c] | 13.3±2.5[c] |
| LY | 18.3±2.4[c] | 87.6±8.4[c] | 8.7±1.6[c] | 12.9±3.2[c] |

[a]$p < 0.01$ *vs.* S group, [b]$p < 0.01$ *vs.* M group, [c]$p < 0.05$ *vs.* Ad + Ldc group

### Effect of Ad combined with Ldc on serum CK, CK-MB, TNF-α and IL-6

Compared with that in the S group, the activity of CK, CK-MB, TNF-α, and IL-6 was significantly enhanced in the M group, and there were significant differences ($p < 0.01$). Compared with that in the M group, the activity of CK, CK-MB, TNF-α, and IL-6 declined in the Ad group, Ldc group, and Ad + Ldc group, and there were significant differences ($p < 0.01$). Compared with that in the Ad + Ldc group, the activities of CK, CK-MB, TNF-α and IL-6 significantly rose in the Ad + Ldc + LY group and LY group ($p < 0.01$), and the same is true in the Ad group and Ldc group ($p < 0.01$). It can be seen that the Ad combined with Ldc is better than the Ad and Ldc alone. No statistically significant differences were found in CK, CK-MB, TNF-α, and IL-6 between the LY group and the M group, suggesting that LY294002 suppressed the effects of Ad and Ldc on the above indices. To sum up, it is confirmed through comparison of the biochemical indices, hemodynamics, and myocardial infarction area in each group that Ad combined with Ldc has a better effect than Ad and Ldc alone. Therefore, only Ad + Ldc group was studied in subsequent experiments (Table 3).

### Effect of Ad combined with Ldc on phosphorylation level of Akt

The protein expression of p-Akt and phosphorylation level of Akt (p-Akt/T-Akt) remarkably rose in the M group when compared with those in the S group ($p < 0.05$). Compared with those in the M group, the protein expression of p-Akt and p-Akt/T-Akt significantly increased in the Ad + Ldc group ($p < 0.01$). In Ad + Ldc + LY group, the protein expression of p-Akt and p-Akt/T-Akt remarkably declined ($p < 0.01$). The above results demonstrate that Ad combined with Ldc may be able to activate the PI3K/Akt signaling pathway, thereby exerting a protective effect against MIRI in rats (Figure 2).

### Effect of Ad combined with Ldc on Bcl-2 and Bax protein expressions

According to Western blotting results, the M group had greatly decreased Bcl-2 protein expression and Bcl-2/Bax and greatly increased Bax protein expression when compared with the S group. Compared with the M group, the Ad + Ldc group had greatly increased Bcl-2 protein expression and Bcl-2/Bax ($p < 0.01$), and greatly decreased Bax protein expression ($p < 0.01$). The Bcl-2 protein expression and Bcl-2/Bax were notably lower ($p < 0.01$), while the Bax protein expression was notably higher in the Ad + Ldc + LY group and LY group than those in Ad + Ldc group ($p < 0.01$). Bcl-2, Bax, and Bcl-2/Bax had no obvious differences in the Ad + Ldc + LY group and LY group compared with those in the M group ($p > 0.05$). The above findings suggest that LY294002 inhibited the ability of Ad combined with Ldc to raise Bcl-2 protein expression and lower Bax protein expression. To sum up, Ad combined with Ldc can activate the PI3K/Akt signaling pathway, thereby resisting myocardial apoptosis after myocardial ischemia-reperfusion in rats (Figure 3).

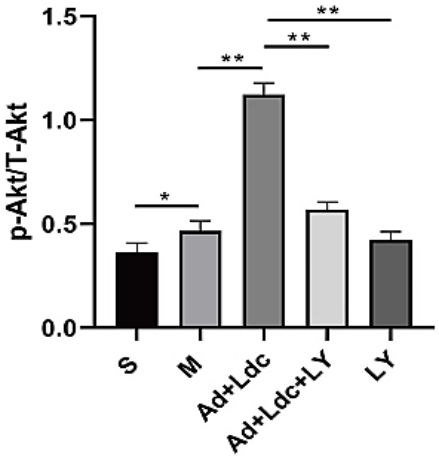


**Figure 2:** Protein expressions of P-Akt and T-Akt in each group. ***P* < 0.01 vs. Ad + Ldc, **p* < 0.05 M vs S

**Table 3:** Effect of Ad combined with Ldc on serum CK, CK-MB, TNF-α and IL-6 in MIRI rats (n = 10, mean ± SD)

| Group | CK (U/L) | CK-MB (U/L) | TNF-α (ng/L) | IL-6 (ng/L) |
|---|---|---|---|---|
| S | 1083.2±101.7 | 1032.5±120.8 | 33.2±3.5 | 2.7±0.2 |
| M | 3020.5±306.1[a] | 3202.2±312.5[a] | 132.3±22.5[a] | 7.2±0.4[a] |
| Ad | 2015.2±192.8[b] | 2101.3±214.3[b] | 92.6±12.1[b] | 5.2±0.1[b] |
| Ldc | 2114.5±182.6[b] | 2005.9±210.6[b] | 84.1±11.8[b] | 5.7±0.3[b] |
| Ad+Ldc | 1511.8±152.3[b] | 1417.2±182.2[b] | 69.2±8.4[b] | 3.9±0.4[b] |
| Ad+Ldc+LY | 2917.5±222.8[c] | 2991.1±197.7[c] | 129.2±11.5[c] | 7.1±0.5[c] |
| LY | 2896.3±231.4[c] | 3087.6±215.4[c] | 118.7±10.6[c] | 6.9±0.2[c] |

***Note:*** [a]*P* < 0.01 *vs.* S group, [b]*p* < 0.01 *vs.* M group, [c]*p* < 0.05 *vs.* Ad + Ldc group

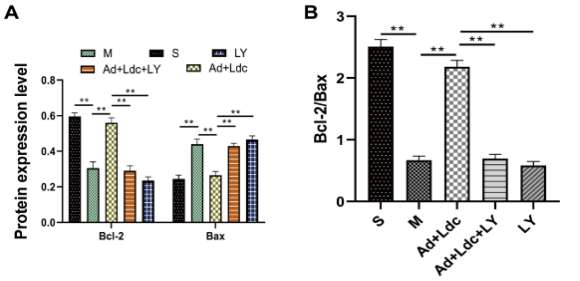


**Figure 3:** Protein expressions of Bcl-2 and Bax in each group. (A) Protein expressions of Bcl-2 and Bax in each group. (B) Bcl-2/Bax in each group. ***p* < 0.01 vs. Ad + Ldc

## DISCUSSION

It is thought currently that the kinase pathway able to relieve MIRI is mainly composed of PI3K/Akt and extracellular signal-regulated kinase (ERK). After activation, PI3K/Akt and ERK pathways remarkably reduced MIRI. According to studies, PI3K/Akt and ERK pathways can be activated *via* preconditioning and postconditioning by reperfusion occurs, confirming their similar protective mechanisms of them [13]. As a signal transduction pathway in cells, PI3K/Akt can regulate cell activation, glucose transport, and protein synthesis, remove oxygen free radicals in cells, promote cell proliferation, and reduce neutrophil activity and aggregation. Besides, it can inhibit MIRI [14]. LVEDP, LVSP, +dp/dt$_{max}$, and -dp/dt$_{max}$ are generally used to detect the left ventricular systolic and diastolic functions. This study confirmed that LY294002 can abolish the ability of Ad combined with Ldc to improve left ventricular dysfunction. A study has shown [15] that in the case of myocardial ischemia, the energy supply will be reduced, and there will be a dysfunction of oxygen-free radical scavenger enzymes (CAT, GSH-Px, and SOD), weakening oxygen free radical scavenging and increasing oxygen free radicals. Besides, the MDA activity can be detected to determine the content of oxygen free radicals, and it also indicated the degree of lipid peroxidation. Therefore, the serum CAT, GSH-Px, SOD, and MDA are generally detected, so as to prove whether the body is able to scavenge oxygen free radicals, and the myocardial cell injury can be evaluated via the detection of the activity of myocardial enzymes [16]. This study also indicates that LY294002 can inhibit the effects of Ad and Ldc on the above indexes. Overall, Ad + Ldc can remove oxygen free radicals, reduce the myocardial enzymes content and diminish inflammation through the activation of the PI3K/Akt signaling pathway, thereby exerting a protective effect against MIRI. Myocardial apoptosis is related to MIRI, which determines the degree of myocardial injury. Therefore, increasingly more scholars are currently studying how to protect myocardial cells by reducing apoptosis [17,18]. Membrane receptor signals can be transmitted into cells *via* the PI3K/Akt signaling pathway, in which Bcl-2 and Bax are the key recognition sites of the PI3K/Akt signaling pathway and key members in the Bcl-2 family [19-21]. In this study, LY294002 can inhibit the ability of Ad combined with Ldc to raise Bcl-2 protein expression and lower Bax protein expression.

## CONCLUSION

The combination of anisodamine with lidocaine activates PI3K/Akt signaling pathway, thereby resisting myocardial apoptosis after myocardial ischemia-reperfusion in rats. Thus, this combination therapy is a potential strategy for the management of myocardial ischemia-reperfusion.

## DECLARATIONS

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Key Discipline Established by Zhejiang Province and Jiaxing City Jointly--Pain Medicine (2019-ss-Styx), Key Discipline of Anesthesiology of Jiaxing City (2019-zc-06), Construction Project of Anesthesiology Discipline Special Disease Center in Zhejiang North Region (201524) and Jiaxing Key Laboratory of Neurology and Pain Medicine.

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***Ethical approval***

None provided.

***Availability of data and materials***

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

***Conflict of Interest***

No conflict of interest associated with this work.

***Contribution of Authors***

We declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors. Shouyi Wang and Jiahao Chen contributed equally to this work.



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