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Effect of dexmedetomidine and cholinergic anti-inflammatory pathways in myocardial ischemia-reperfusion injury

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Abstract: The aim of the current study was to determine the effect and mechanism underlying the cholinergic anti-inflammatory pathway of dexmedetomidine and the cholinergic anti-inflammatory pathway on myocardial ischemia-reperfusion injury by establishing a myocardial ischemia-reperfusion model in rats. Sixty healthy rats were randomly divided into 4 groups with 15 rats in each group. The first group was a sham operation group. The second group (myocardial ischemia-reperfusion model group [Ischemia-reperfusion injury (IRI)+S group]) was pre-treated with saline for 10 min before ischemia. The third group (myocardial ischemia reperfusion model with dexmedetomidine pre-treatment [IRI+Dex group]) received an intravenous injection of dexmedetomidine for 10 min before ischemia. The fourth group was the myocardial ischemia reperfusion model with dexmedetomidine pre-treatment and the disconnection of the vagus nerve group (IRI+Dex+V group). The serum creatine kinase (CK) and lactate dehydrogenase (LDH) levels were determined. The expression of B cell lymphoma/lewkmia-2 (bcl-2), BCL2-associated X (bax), caspase-3, α7 Nicotinic acetylcholine receptor (α-7 nR), and inhibitor of nuclear factor kappa-Bα (I-κB-α) in the myocardium was measured. Dexmedetomidine can significantly reduce the myocardial tissue injury induced by myocardial ischemia-reperfusion in rats. Dexmedetomidine may relieve myocardial ischemia-reperfusion injury by activating the cholinergic anti-inflammatory pathway.

Keywords: Dexmedetomidine, ischemia-reperfusion, inflammatory.

INTRODUCTION

Myocardial ischemia-reperfusion injury (MIRI) is a short-term cardiac muscular ischemia tissue damage. After reperfusion, myocardial ischemic injury becomes more severe. The phenomenon is common in patients undergoing heart surgery and coronary artery bypass. MIRI prevents the profit from reperfusion and is a major clinical problem (Mcguinness *et al*., 2016)**.** MIRI is often accompanied by oxidative stress, inflammation, and tissue injury, including the mechanism underlying reactive oxygen species (ROS) burst, calcium overload, myocardial energy metabolism disorders, neutrophil activation, inflammatory factor release, and apoptosis. MIRI is also the process of increasing the inflammatory response and persistent deterioration (Qu *et al*., 2019; Zhang *et al*., 2019; Marek-Iannucci *et al*., 2019; Ibarrola *et al*., 2019)**.** A study involving the protection of MIRI showed that cholinergic anti-inflammatory pathway (CAP) may be one of the protective mechanisms underlying MIRI (Sulaiman *et al*., 2012). CAP differs way from the traditional humoral immune inflammatory pathways. CAP plays a part in anti-inflammatory effects via the vagus nerve and acetylcholine immune regulation. Compared with the traditional humoral anti-inflammatory mechanism, CAP uses endogenous neural feedback regulation to regulate inflammatory response to reduce the role of inflammatory damage, which is characterized by more rapid and easier control, thus CAP has an advantage in regulating the inflammatory response of MIRI (Li *et al*., 2018; Kolgazi *et al*., 2013; Kawaguchi *et al*., 2011). In recent years, studies have shown that CAP may be one of the protective mechanisms underlying ischemia-reperfusion. When the body is stimulated by an exogenous source, the immune signal is transmitted to the nerve center of the brain. Then, the immune signal regulates the vagus nerves and promotes the release of the neurotransmitter, acetylcholine. Acetylcholine further binds to receptors in immune cells and activates or inhibits classic signaling pathways, such as STAT3/NF-κB. Consequently, acetylcholine regulates the release of IL-1/IL-6/IL-8/TNFα and has an anti-inflammatory effect (Reichert *et al*., 2011; Xu *et al*., 2017; Al-Sharea *et al*., 2017). Compared with the traditional humoral anti-inflammatory mechanism, CAP regulates the inflammatory response faster, is easier to control, and is more effective (Zang *et al*., 2008; Kamada *et al*., 2008; Franke *et al*., 2005). Some researchers have indicated that stimulation of the vagus nerve can effectively reduce the release of inflammatory cytokines and the inflammatory response, while severing the vagus nerve promotes the release of inflammatory factors and aggravates the inflammatory response. CAP can inhibit multiple pro-inflammatory cytokines at the same time, the anti-inflammatory effect is very extensive, and CAP can inhibit the inflammation of multiple organs, such as the heart, the liver, and kidneys (Xiang *et al*., 2014). The vagus nerve plays an anti-inflammatory role mainly by

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regulating the release of acetylcholine (Wilhelm *et al*., 2005). Acetylcholine is synthesized by nerve endings, and is combined with the acetylcholine receptors produced by immune cells, such as macrophages, and thus plays a role in regulating signaling pathways (Gilhus and Verschuuren, 2015). The primary cholinergic receptor for CAP is α7nAChR, which is expressed by macrophages, lymphocytes, and endothelial cells. Activation of α7nAChR can inhibit the NF-κB pathway and decrease the release of IL-1/IL-8 and TNFα (Mantz *et al*., 2011).

Dexmedetomidine is a new type of highly selective α2-adrenoceptor agonist that is widely used in the intensive care unit and clinical anesthesia (Yatabe *et al*., 2016). Studies have shown that dexmedetomidine has protective effects on lung and kidney injury, reduces cell apoptosis, and inhibits inflammatory response (Vincent *et al*., 2013; Peng *et al*., 2013). In recent years, studies have shown that dexmedetomidine also has protective effects on heart injury, including reducing myocardial ischemia-reperfusion injury, stabilizing the heart rhythm, and reducing complications of cardiac surgery (Chen *et al*., 2014; Lin and Knowlton, 2014; Riquelmea *et al*., 2016). Dexmedetomidine excites the vagus nerve, slows the heart rate, and also has an anti-inflammatory effect. The pharmacologic properties are very similar to the anti-inflammatory effect of CAP on the nerve center. Therefore, this study was based on the model of MIRI in rats to explore the effects of CAP on MIRI and the intervention effect of dexmedetomidine.

MATERIALS AND METHODS

Experimental animals

Wistar male rats were purchased from the Medical School of Nanchang University Laboratory Animal Science Department. The rats were the same age, weighed 200-250 g, and had standard feeding under normal conditions.

Animal model of acute MIRI

Sixty healthy rats were randomly divided into four groups with 15 rats in each group. The first group was the sham operation group (sham group). The second group was the myocardial ischemia-reperfusion model (IRI+S group) and were pre-treated with intravenous saline for 10 min. The third group was the myocardial ischemia- reperfusion model with dexmedetomidine pre-treatment (IRI+Dex group) and were pre-treated with an intravenous injection of 10 mg/kg dexmedetomidine for 10 min. The fourth group was the myocardial ischemia-reperfusion model with dexmedetomidine pre-treatment and severed vagus nerve group (IRI+Dex+V group). The IRI+Dex+V group was pre-treated with an intravenous injection of 10 mg/kg of dexmedetomidine for 10 min, then the vagus nerve was severed. All rats were fasted for12 h before surgery and were not limited to drinking water. The rats were anesthetized with an intraperitoneal injection of 2 % pentobarbitone sodium (50 mg/kg) before surgery, the left femoral artery was intubated to record blood pressure, and the electrodes were inserted into the subcutaneous electrodes to guide the ECG. Heart ischemia was induced by coronary artery ligation, reperfusion was performed after 30 min, and the reperfusion time was 180 min. The blood pressure was reduced by 20 mmHg and the ECG revealed an elevation of the S-T segment consistent with ischemia. Reperfusion success was indicated by the gradually restoration of blood pressure and normalization of the ECG tracing. Serum and left ventricular myocardium were collected after reperfusion.

Myocardial infarction area detection

After rat myocardial reperfusion was completed, the coronary artery was blocked again and 0.15% Evans blue was injected into the left ventricle to stain the left ventricle without infarction. After 20 min, the rat was sacrificed and the heart was removed by caesarean section. After freezing the cardiac tissues, necropsy of the venturi blue-dyed area was the normal area, the ischemic area was not shaded, and the pale area of tissue was the necrosis area. Image-pro Plus 6.0 was used to analyze the image and the total and infarct areas were measured. The percentage of myocardial infarction was the infarction area compared with the total area.

Determination of LDH and CK in serum

Blood was collected from orbital venous plexus, and 2 mL of blood collection vessel containing sodium citrate and EDTA-K2 anticoagulant was used. The blood was thoroughly mixed, and the upper plasma was collected after centrifuged at 3 000 r/min for 15 min. The creatine kinase (CK) and lactate dehydrogenase (LDH) levels were determined using a colorimetric method, and the content of serum CK and LDH was calculated according to the sample instruction of the kit.

Determination of IL-1, IL-6, TNF-α, and HMGB1 levels in serum

Blood was collected from orbital venous plexus, and 2 mL of blood collection vessel containing sodium citrate and EDTA-K2 anticoagulant was used. The blood was thoroughly mixed, and the upper plasma was collected after centrifuged at 3 000 r/min for 15 min. The levels of Il-1, Il-6, TNF-α, and HMGB1 in serum, were determined by enzyme-linked immunosorbent assay (ELISA). The methods refer to the kit instructions. The diluted standard products and samples were added to 100 μL/well, sealed with sealing-plate adhesive paper, and incubated for 120 min. The antibody was added to 100 μL/well. The enzyme conjugate was added to the 100 μL/ well and incubated for 30 min. Then, the color agent was added to 100 μL/well, and incubated at 37^{o}C. The Optical density (**OD**) value of the wavelength at 450 nm was measured 5 min after mixing with the end solution of 50 μL/well.

Protein extraction and western blotting

The total protein was extracted. The protein concentration was detected using a protein concentration detection kit. The sample amount was 50 μg of protein. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was carried out. After electrophoresis, the protein was transferred to nitrate fiber membranes at a constant voltage for 90 min. After the transfer was completed, the first antibody was added, then incubated for 4 hours, and the second antibody was added to the milk containing rabbit second antibody. After incubation at room temperature for 1 h, the final chromogenic solution was added in a dark chamber. Quantitative analysis of Western protein was carried out by Image J software.

Ethical approval

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University.

STATISTICAL ANALYSIS

The experimental data were analyzed using SPSS16.0 software. The T-test was used to analyze the differences of LDH and CK between groups. A chi-square test was used to compare the sample rate. All of the experimental data are expressed as the mean ± SD. A *P*-value < 0.05 was considered to be a statistically significant.

RESULTS

Area of myocardial infarction

The myocardial infarction area of the four groups after reperfusion was determined. The myocardial infarction area of the ischemia reperfusion group was significantly greater than the sham operation group ($P < 0.05$). Dexmedetomidine treatment significantly reduced the area of myocardial infarction in rats. After vagotomy, the myocardial infarction area of rats treated with dexmedetomidine was not significantly decreased (*P*<0.05, fig. 1).

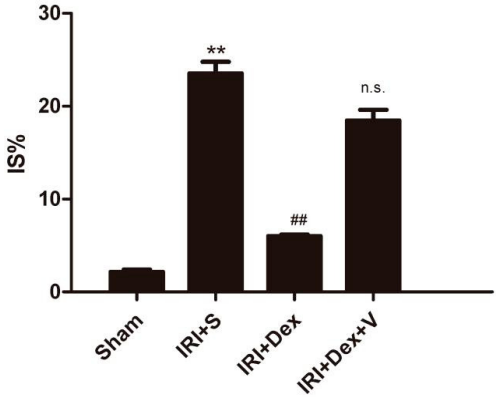
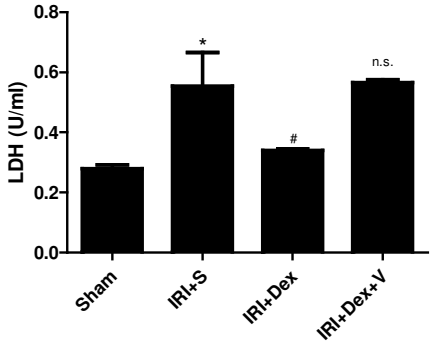


Fig. 1: Comparison of myocardial infarction area in four groups of rats.

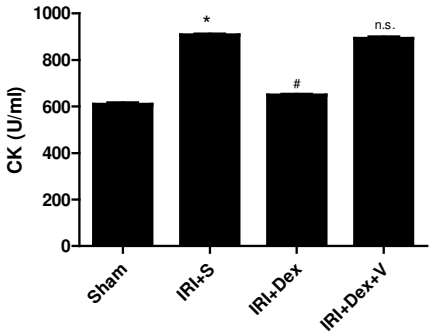
**compared with the sham group, *P*<0.05. ## compared with the IRI+Dex+V group, *P*<0.05. There was no difference in the myocardial infarction area between the IRI+S and IRI+Dex+V groups, *P*>0.05.

Serum LDH and CK levels

The serum LDH and CK levels were determined in four groups of rats after reperfusion. The serum LDH and CK levels in the other groups were significantly higher than the sham operation group (*P*<0.05), while dexmedetomidine treatment significantly reduced the levels of LDH and CK (*P*<0.05), suggesting that dexmedetomidine treatment can reduce the damage of membranes and capillaries caused by ischemia reperfusion. After vagotomy, dexmedetomidine treatment of rats did not decrease the serum LDH and CK levels significantly (*P*<0.05, fig. 2).



A.



B.

Fig. 2: Comparison of serum LDH and CK levels in four groups of rats.

**compared with the sham group, *P*<0.05. ## compared with the IRI+Dex+V group, *P*<0.05. There was no difference in serum LDH and CK levels between the IRI+S and IRI+Dex+V groups, *P*>0.05.

Expression of inflammatory factors

The levels of cell inflammatory factors were detected in the four groups of rats after reperfusion. Ischemia-reperfusion increased the levels of IL-1, IL-6, TNF-α, and HMGB1 significantly (*P*<0.05), while dexmedetomidine treatment significantly reduced the levels of IL-1, IL-6, TNF-α, and HMGB1 (*P*<0.05, fig. 3). Thus, dexmedetomidine effectively reduced the inflammatory response induced by myocardial ischemia-reperfusion. After vagotomy, dexmedetomidine treatment did not

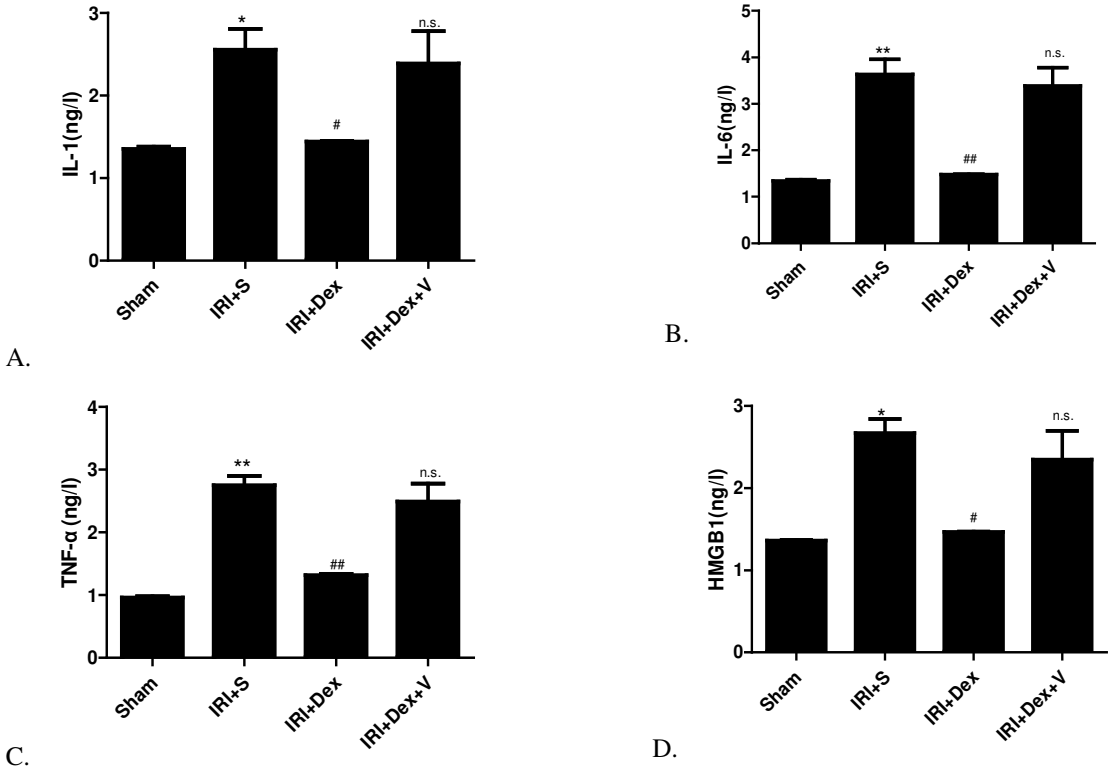


Fig. 3: Comparison of the levels of IL-1, IL-6, TNF-α, and HMGB1 in four groups of rats.
** compared with the sham group, $P<0.05$. ## compared with the IRI+Dex+V group, $P<0.05$. There was no difference in the levels of IL-1, IL-6, TNF-α, and HMGB1 between the IRI+S and IRI+Dex+V groups, $P>0.05$.

significantly reduce the levels of IL-1, IL-6, TNF-α, and HMGB1 (P<0.05, fig. 3).

Level of cardiomyocyte apoptosis

Apoptosis-related proteins in myocardial ischemia-reperfusion were detected, including BCL2-Associated X (Bax), B cell lymphoma/lewkmia-2 (Bcl-2), and caspase-3. Compared with the IRI+S group, the expression of Bax and caspase-3 in the IRI+Dex group was significantly decreased ($P < 0.05$), while the expression of Bcl-2 was significantly increased (P<0.05). Compared with the IRI+S group, there was no significant difference in the expression of Bax and caspase-3 in the IRI+Dex+V group, and the expression of Bcl-2 was increased ($P<0.05$, fig. 5). Bcl-2 expression in the IRI+Dex+V group was lower than the IRI+Dex group ($P<0.05$, fig. 4).

Dexmedetomidine activates the cholinergic signaling pathway

Expression of classic apoptosis pathway inhibitor of nuclear factor kappa-Bα (I-κB-α) and p65 was detected in rat myocardial tissue. Compared with the IRI+S and IRI+Dex+V groups, the expression of α7nAChR in the IRI+Dex group was significantly increased ($P<0.05$), the expression of I-κB-α was up-regulated, and the expression of p65 was down-regulated. The expression of α7nAChR in the IRI+Dex+V group was higher than the IRI+S group ($P<0.05$, fig. 5).

DISCUSSION

MIRI has become a major obstacle to the recovery of patients with myocardial ischemic disease. The present mechanism includes ROS, calcium overload, myocardial energy metabolism disorders, apoptosis, neutrophil activation, and inflammatory factors. The process of MIRI is the process of worsening of the inflammatory response. At present, the main protection measures for the treatment of MIRI includes the application of calcium channel agents, such as oxygen-free radical scavengers, and many of these drugs are still in the animal experimental stage; the clinical effect and the underlying mechanism remain to be proved.

Dexmedetomidine is a highly selective α2- adrenergic receptor agonist. Dexmedetomidine is widely used in the intensive care unit (ICU) and clinical anesthesia. It has been reported that dexmedetomidine has a protective effect on the lungs, kidneys, and other organs. Dexmedetomidine can also reduce cell apoptosis and inflammation (Xu *et al*., 2013). In recent years, a number of studies have shown that dexmedetomidine has a protective effect on heart injury, including maintaining a stable rhythm and reducing the MIRI. Dexmedetomidine can reduce the complications of cardiac surgery. We constructed a model of myocardial ischemia-reperfusion in rats and found that dexmedetomidine pre-treatment of

rats reduced the myocardial tissue injury caused by ischemia-reperfusion, reduced apoptosis, significantly reduced serum LDH activity of CK, and significantly decreased the serum pro-inflammatory factor levels (IL-1, IL-6, TNFα, and HMGB1). Thus, dexmedetomidine decreased the inflammation induced by myocardial ischemia-reperfusion.

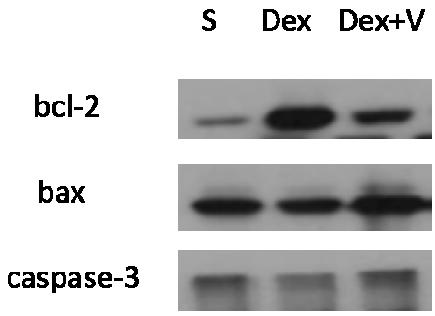


Fig. 4: Expression of the apoptosis-related protein in myocardial tissue.

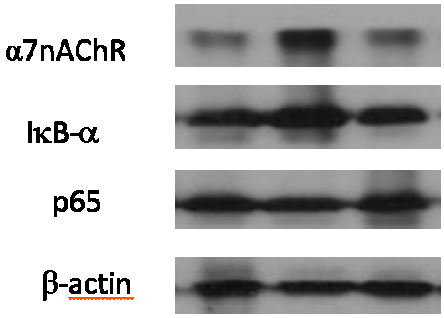


Fig. 5: Expression of the cholinergic signaling pathway-related proteins in the myocardium.

Previous studies have shown that apoptosis plays an important role in myocardial ischemia-reperfusion. During ischemia-reperfusion, the expression of the apoptotic factor, Bax, is increased and the caspase cascade is activated. This study confirmed that dexmedetomidine inhibits the expression of the apoptosis-promoting factor, Bax, and promotes the expression of the anti-apoptotic factor, Bcl-2, during myocardial ischemia-reperfusion, and reduced the level of caspase-3, suggesting inhibition of apoptosis. This finding is consistent with a previous study (Zhu *et al*., 2016). Dexmedetomidine can excite the vagus nerve, slow the heart rate, and have an anti-inflammatory effect. These pharmacologic properties are very similar to the cholinergic anti-inflammatory pathway (Mantz *et al*., 2011; Pasupathy and Homer-Vanniasinkam, 2005). In addition, dexmedetomidine, as a central sedative analgesic drug, targets the central nervous system. Therefore, in the experimental model of myocardial ischemia-reperfusion in rats, we designed the group with the treatment of dexmedetomidine and severed the vagus nerve. After severing the vagus nerve, despite treatment dexmedetomidine, the original anti-inflammatory effect was not achieved. The expression of tissue morphology, LDH and CK activity, or proinflammatory cytokines and apoptotic factors was not significantly changed in the control group. We hypothesize that the anti-inflammatory effect of dexmedetomidine may be related to CAP. Thus, we determined the expression of I-κB-α and p65 in rat myocardial tissue. The IRI+Dex group significantly up-regulated the expression of α7nAChR, up-regulated I-κB-α, and down-regulated p65.

In summary, the current research showed that myocardial ischemia-reperfusion with dexmedetomidine treatment significantly reduced MIRI and reduced the inflammatory response and apoptosis. After vagotomy, the anti-inflammatory effect of dexmedetomidine disappeared. This study suggests that dexmedetomidine can be used as a treatment to protect the myocardium from ischemia and reperfusion injury during cardiac surgery. The myocardial protective mechanism of dexmedetomidine may be associated with the vagus nerve, which provides the basis for further research.

CONCLUSION

Taken together, dexmedetomidine can reduce the myocardial tissue injury induced by myocardial ischemia-reperfusion. Dexmedetomidine may relieve myocardial ischemia-reperfusion injury by activating the cholinergic anti-inflammatory pathway.

ACKNOWLEDGMENTS

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