Original Article
Effects of dexmedetomidine pretreatment on TNF-α and IL-6, oxidative stress and myocardial apoptosis of rats after myocardial ischemia-reperfusion injury

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Abstract: Objective: This study aimed to investigate the effects of dexmedetomidine pretreatment on the levels of inflammatory cytokines TNF-α and IL-6 of rats after myocardial ischemia-reperfusion injury. Methods: 120 SPF SD male rats were randomly divided into control group (C group), model group (M group), pretreatment of dexmedetomidine group (PD group), PI3K broad-spectrum inhibitor LY 294002 pretreatment group (LY group) and the dexmedetomidine + LY 294002 pretreatment group (D&L group). Before the ischemia-reperfusion, the PD group was treated with intravenous drip of 6 μg/kg/h dexmedetomidine for 10 minutes, and followed by intravenous drip of 7 μg/kg/h dexmedetomidine for 15 minutes. The LY group was treated with intravenous injection of 0.3 mg/kg LY 294002. D&L group was treated with intravenous drip of LY 294002, and followed by dexmedetomidine administration at the same dose as before. After successful modelling, the left ventricular diastolic blood pressure, systolic blood pressure and heart rate were detected by electrophysiological signal recorder. Peripheral blood samples were collected from the abdominal aorta to detect related indexes of cardiac function (CK, CK-MB, cTnI). Finally, the rat's heart was cut out. TNF-α, IL-6, superoxide dismutase (SOD), malondialdehyde (MDA), Bax, Bcl-2 and phosphorylated protein kinase B (p-AKT) level in rat's myocardial tissue were detected by ELISA. The correlation between p-AKT and left ventricular diastolic blood pressure, systolic blood pressure, heart rate, CK, CK-MB, cTnI, TNF-α, IL-6, SOD, MDA, Bax, Bcl-2 was analyzed. Results: Dexmedetomidine pretreatment can effectively improve left ventricular diastolic blood pressure, systolic blood pressure, heart rate, CK, CK-MB, cTnI, TNF-α, IL-6, SOD, MDA, Bax, Bcl-2 and promote p-AKT levels (P < 0.05). LY 294002 holds the opposite effect with dexmedetomidine. It can reduce the protective effect of dexmedetomidine. p-AKT levels are associated with SOD and Bcl-2 levels (P < 0.05). Conclusion: Dexmedetomidine pretreatment can effectively reduce the myocardial cell inflammation and oxidative stress levels of rats after myocardial ischemia-reperfusion, decrease myocardial cell apoptosis, and protect cardiac function. This effect is related to the activation of PI3K/AKT signaling pathway.

Keywords: Dexmedetomidine, myocardial ischemia-reperfusion injury, TNF-α, IL-6, oxidative stress, apoptosis

Introduction
Cardiovascular disease is the primary cause of death and disability worldwide, accounting for 30% of global mortality and 10% of global disease burden [1, 2]. In 2005, there were 58 million deaths worldwide. 17 million of them died of cardiovascular disease, and 7.6 million of them died of coronary heart disease [3, 4]. The incidence of cardiovascular disease is still increasing due to the aging of population, unhealthy diet and rest habits [5].

Myocardial infarction is one of the five main manifestations of coronary heart disease. Thrombolytic therapy and interventional therapy are the main means to reduce myocardial ischemic injury. However, myocardial reperfusion can cause further damage to myocardial cells. Myocardial ischemia-reperfusion injury is also an important factor of poor prognosis in patients with coronary heart disease [6, 7]. With the development of therapeutic drugs and treatment means, myocardial reperfusion therapy has been continuously improved. However, it is still short of effective means to prevent myocardial ischemia-reperfusion injury [8]. In some research reports, the protective effect of dexmedetomidine on ischemia-reperfusion injury of tissue and organ has been increasingly
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recognized [9, 10]. In some studies, the role of different treatment way of dexmedetomidine is further analyzed. Liu et al. [11] reported that dexmedetomidine pretreatment has a good therapeutic effect on long-term inflammation caused by renal ischemia-reperfusion injury. Si et al. [12] also reported that dexmedetomidine pretreatment can reduce the apoptosis of renal tubular epithelial cells, play an anti-apoptotic effect, and reduce tissue damage. There are few reports about the effect of dexmedetomidine pretreatment on myocardial ischemia-reperfusion injury. Whether there is a same anti-inflammatory and anti-apoptotic effect still needs to be verified.

Therefore, in order to provide experimental evidence for clinical prevention of myocardial ischemia-reperfusion injury, the effects of dexmedetomidine pretreatment on the levels of inflammatory cytokines TNF-α and IL-6 of rats after myocardial ischemia-reperfusion injury was studied.

Materials and methods

Research subject

120 SPF SD male rats were purchased from Beijing WeitongLihua Experimental Animal Technology Co., Ltd. (Strain code was 101, production license SCXK (Shanghai) 2017-0011). They were all fed with ordinary nutrient feed (Beijing Zhecheng Technology Co., Ltd.). The drinking water was autoclaved acidified water with a pH between 2.5 and 3. The average age of the rats was 21 ± 2 days; the average body weight was 264.1 ± 21.5 g; the feeding temperature was 18-22°C, and the relative humidity was 40%-70%. They were kept separately in the breeding box, and the pads were changed every morning and evening; the environmental noise should be less than 85 dB, ammonia concentration should not be more than 20 ppm, and ventilation should be carried out 8 to 12 times per hour; nest should be changed, cleaned and disinfected 1-2 times per week; noise should not be more than 60 dB, ammonia concentration should not be more than 14 ppm, ventilation should be carried out 15 times per hour, and the fluorescent lamps are cycled for 12 hours.

Modeling method

All rats were fed adaptably for 1 week, and then fasted overnight. They were free to drink water. According to random table number, 80 rats were selected to prepare a rat myocardial ischemia-reperfusion model [13]. 20 rats were placed into control group (group C) without surgery. 20 rats were placed into sham operation group (group S). Only the blood vessels were separated by thoracotomy. No ischemia and perfusion operations were performed. Rats were intraperitoneally injected with sodium pentobarbital (Sigma, St. Louis, USA, 40 mg/kg), and ventilated with a ventilator (ALC-V8, Shanghai, China). The sufficiency of anesthesia was examined by a tail clamp test before surgery. The thoracic cage was opened in the fourth intercostal space. The left anterior descending coronary artery (LAD) was freed. It was ligated at the left auricle junction 1-2 mm below the border of the lung cone. The LAD was clipped for 30 minutes and then opened for 120 minutes. According to random table number, 80 model rats were divided into model group (group M), dexmedetomidine pretreatment group (PD) group, PI3K broad-spectrum inhibitor LY 294002 pretreatment group (LY group) and dexmedetomidine + LY 294002 pretreatment group (group D&L). Dexmedetomidine was purchased from Nanjing Sairongrui Biotechnology Co., Ltd., and the item No. was T2524. LY 294002 was purchased from Shanghai Haoyang Biotechnology Co., Ltd., and the item No. was 440202-5MG. Before the ischemia-reperfusion, the group PD was treated with intravenous drip of 6 μg/kg/h dexmedetomidine for 10 minutes, and followed by intravenous drip of 7 μg/kg/h dexmedetomidine for 15 minutes. The LY group was treated with intravenous injection of 0.3 mg/kg LY 294002. D&L group was treated with intravenous drip of LY 294002, and followed by dexmedetomidine administration at the same dose as before.

Outcome measures

After successful rat modelling, the difference of left ventricular diastolic blood pressure, systolic blood pressure and heart rate was detected by electrophysiological signal recorder. Peripheral blood samples were collected from the abdominal aorta to detect related indexes of cardiac function (CK, CK-MB, cTnI). Finally, the rat’s heart was cut out. TNF-α, IL-6, superoxide dismutase (SOD), malondialdehyde (MDA), Bax, Bcl-2 and phosphorylated protein kinase B (p-AKT) level of rat myocardial tissue were detected by ELISA. The correlation between p-AKT and left ventricular diastolic blood pres-
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Detection method

The electrophysiological signal recorder (MP-150, Yawentai Trading (Beijing) Co., Ltd) was used to measure left ventricular diastolic blood pressure, systolic blood pressure and heart rate.

CK, CK-MB and cTnl were tested by automatic animal biochemical analyzer (99-92524-00, ID-EXX Maine Biological Products Trading (Shanghai) Co., Ltd.).

TNF-α, IL-6, SOD, Bax, Bcl-2, and p-AKT kits were purchased from Shanghai Jingkang Biotechnology Co., Ltd., and the Cat. No. was JLC2062, JLC1721, JLC2390, JLC183, JLC183, and JLC1794, respectively. MDA kits were purchased from Shanghai Guduo Biotechnology Co., Ltd., and the item No. was GD-BN1921.

Statistical analysis

SPSS19.0 (Asia Analytics Formerly SPSS China) was used. The measurement data was expressed as % and the ratio was compared by the χ² test. The enumeration data was expressed as mean ± standard deviation (mean ± sd). The t test was used for comparison between the two groups. The analysis of variance was used for comparison among groups. The LSD test was used for post hoc testing. Pearson was used to analyze the correlation between p-AKT and left ventricular diastolic blood pressure, systolic blood pressure, heart rate, CK, CK-MB, cTnl, TNF-α, IL-6, SOD, MDA, Bax, Bcl-2. P < 0.05 meant statistically significant.

Results

Difference analysis of left ventricular diastolic blood pressure, systolic blood pressure and heart rate in rats

There were dramatic differences in left ventricular systolic pressure, diastolic blood pressure and heart rate among the 6 groups of rats (P < 0.05). There was no difference in left ventricular systolic pressure, diastolic blood pressure and heart rate between the C group and the S group (P > 0.05). The systolic blood pressure and heart rate of M group, PD LY group and D&L group were lower than those of C group and S group (P < 0.05). The diastolic blood pressure was higher than that of C group and S group (P < 0.05). The systolic blood pressure and heart rate of M group, PD LY group and D&L group were lower than those of group PD group, and the diastolic blood pressure was lower than that of D&L group (P < 0.05) (Figure 1).

Differential expression of CK, CK-MB and cTnl in peripheral blood of rats

There was statistical significance in CK, CKMB and cTnl among the 6 groups of rats (P < 0.05). There was no difference in CK, CK-MB and cTnl between the C group and the S group (P > 0.05). The CK, CK-MB and cTnl in peripheral blood of M group, PD group, LY group and D&L group were higher than those of C group and S group.
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The CK, CK-MB and cTnI in peripheral blood of PD group and the D&L group were lower than those of LY group (P < 0.05). The CK, CK-MB and cTnI in peripheral blood of PD group were lower than those of D&L group (P < 0.05) (Figure 2).

Figure 2. Differential expression of CK, CK-MB, and cTnI in peripheral blood of rats. *Indicated that P < 0.05 for comparison with C group, #indicated that P < 0.05 for comparison with S group, $indicated that P < 0.05 for comparison with M group, &indicated that P < 0.05 for comparison with group PD group, @indicated that P < 0.05 for comparison with group LY group.

Differential expression of TNF-α and IL-6 in myocardial tissue of rats

There was statistical significance in TNF-α and IL-6 among the 6 groups of rats (P < 0.05). The TNF-α and IL-6 in the myocardial tissues of S group, M group, PD group, LY group and D&L group were lower than that of C group (P < 0.05), and the MDA was higher than that of C group (P < 0.05). The SOD in myocardial tissue of M group, PD group and D&L group was higher than that of LY group (P < 0.05), and MDA was lower than that of LY group (P < 0.05). The SOD in myocardial tissue of PD group and D&L group was lower than that of M group (P < 0.05). The TNF-α and IL-6 in the myocardial tissue of PD group were lower than those of the D&L group (P < 0.05) (Figure 3).

Figure 3. Differential expression of TNF-α and IL-6 in myocardial tissue of rats. *Indicated that P < 0.05 for comparison with C group, #indicated that P < 0.05 for comparison with S group, &indicated that P < 0.05 for comparison with M group, $indicated that P < 0.05 for comparison with group PD group, @indicated that P < 0.05 for comparison with group LY group.

Differential expression of SOD and MDA in myocardial tissue of rats

There was statistical significance in SOD and MDA among the 6 groups of rats (P < 0.05). The SOD in myocardial tissues of S group, M group, PD group, LY group and D&L group was lower than that of C group (P < 0.05), and the MDA was higher than that of C group (P < 0.05). The SOD in myocardial tissue of M group, PD group, LY group and D&L group was lower than that of S group (P < 0.05), and MDA was higher than that of S group (P < 0.05). The SOD in myocardial tissue of PD group, PD group and D&L group was higher than that of LY group (P < 0.05), and MDA was lower than that of LY group (P < 0.05). The SOD in myocardial tissue of PD group and D&L group was higher than that of M group (P < 0.05), and MDA was lower than that of M group (P < 0.05). The SOD in myocardial tissue of PD group was higher than that of LY group (P < 0.05), and MDA was lower than that of C group (P < 0.05). The SOD in myocardial tissue of D&L group was higher than that of LY group (P < 0.05), and MDA was lower than that of LY group (P < 0.05).

Differential expression of Bax and Bcl-2 in myocardial tissue of rats

There was statistical significance in Bax and Bcl-2 among the 6 groups of rats (P < 0.05). The Bax and Bcl-2 in the myocardial tissues of S group, M group, PD group and D&L group were lower than those of LY group (P < 0.05). The Bcl-2 in the myocardial tissue of M group, PD group, LY group and D&L group was lower than that of C group (P < 0.05), and the MDA was higher than that of C group (P < 0.05). The SOD in myocardial tissue of M group, PD group and D&L group was higher than that of LY group (P < 0.05), and MDA was lower than that of LY group (P < 0.05). The SOD in myocardial tissue of PD group and D&L group was higher than that of M group (P < 0.05), and MDA was lower than that of M group (P < 0.05). The SOD in myocardial tissue of PD group was higher than that of LY group (P < 0.05), and MDA was lower than that of LY group (P < 0.05).

Differential expression of Bax and Bcl-2 in myocardial tissue of rats

There was statistical significance in Bax and Bcl-2 among the 6 groups of rats (P < 0.05). The Bcl-2 in the myocardial tissues of S group,
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There was statistical significance in p-AKT among the 6 groups of rats (P < 0.05). There was no difference in the p-AKT levels of the myocardial tissues among the paired comparison of C group, S group, LY group and D&L group (P < 0.05). The p-AKT level in myocardial tissue of M group and PD group was higher than that of LY group (P < 0.05), and Bax was lower than that of C group (P < 0.05) (Figure 6).

Correlation analysis

Pearson correlation analysis showed that p-AKT level was associated with SOD and Bcl-2 (P < 0.05), but was not associated with left ventricular diastolic blood pressure, systolic blood pressure, heart rate, CK, CK-MB, cTnI, TNF-α, IL-6, MDA, and Bax (P > 0.05) (Figure 7).

Discussions

Reperfusion therapy can effectively reduce the size of ischemic myocardial infarction, but isch-
Embolia-reperfusion injury has been an important clinical problem [14, 15]. In order to provide experimental basis for clinical prevention of myocardial ischemia-reperfusion injury, the protective effect of dexmedetomidine pretreatment on the cardiac dysfunction of myocardial ischemia rats was analyzed in this study.

The results of this study showed that compared with the model group, the degree of rat's cardiac dysfunction was markedly reduced after dexmedetomidine pretreatment. The analysis of myocardial tissue inflammation and stress response also showed that the level of IL-6, TNF-α and MDA in the myocardium tissues of rat were decreased, while the SOD level was increased. The inflammatory response and stress response were controlled. The related protein of anti-apoptosis in myocardial tissue, Bcl-2, was also increased. The apoptosis-promoting protein, Bax level was decreased. It indicated that dexmedetomidine can control the inflammation and stress response level after myocardial reperfusion in rats, reduce myocardial cell apoptosis, and play a protective role in cardiac function. Dexmedetomidine is a highly selective α2 adrenergic agonist. Its anti-inflammatory and anti-stress effects have been reported in many studies. Study of Zeng et al. [16] reported that oxidative stress and inflammation were usually associated with ischemia-reperfusion, and that dexmedetomidine can reduce cerebral ischemia-reperfusion injury by alleviating oxidative stress and inflammation. This effect has also been demonstrated in other surgical procedures, such as abdominal surgery.
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hysterectomy and heart surgery [17, 18]. Moreover, inhibition of apoptosis can prevent organ dysfunction caused by ischemia-reperfusion injury [19]. Combined with their research, it is believed that dexmedetomidine has a certain preventive effect on ischemia-reperfusion injury.

The mechanism of action by which dexmedetomidine pretreatment protects cardiac function has also been explored. In many studies, the protective role of PI3K/AKT signaling pathway in ischemia-reperfusion injury has been reported [20, 21]. Cheng et al. [22] also reported in the study that dexmedetomidine posttreatment can reduce rabbit’s cardiac ischemia-reperfusion injury by depending on PI3K/Akt signaling pathway and activating GSK-3β. The results of this study showed that the degree of impaired cardiac function in rats was increased after the use of PI3K inhibitor. What’s more, compared with dexmedetomidine alone, the curative effect of dexmedetomidine combined with PI3K inhibitor was lower. Therefore, it is believed that PI3K pathway is one of the mechanisms of actions by which dexmedetomidine prevents myocardial ischemia-reperfusion injury in rats. The expression level of p-AKT in each group of rats was further analyzed. It was found that dexmedetomidine can activate PI3K/AKT signaling pathway and promote p-AKT expression in myocardial tissue. The activation effect of dexmedetomidine on AKT was greatly reduced after blocking PI3K. However, the correlation analysis showed that the p-AKT level in myocardial tissue was not correlated greatly with cardiac function, inflammatory response and stress response. p-AKT levels were only associated with SOD and Bcl-2. It is speculated that the preventive effect of dexmedetomidine on myocardial ischemia-reperfusion injury is not only achieved by activating the PI3K/AKT signaling pathway. It requires to be validated by more tests.

The other limitation of this study is that the animal model was used. In order to shorten the experiment duration, before establishing a model of myocardial ischemia-reperfusion, a model of coronary atherosclerosis was not established. There was difference in the pathological changes of myocardial ischemia-reperfusion injury in clinical practice, which requires to be validated by more clinical experiments. A signal molecule downstream of PI3K was only validated. The inclusion of indicators such as cardiac function and inflammation was not comprehensive enough. The detection of apoptosis was carried out through apoptosis-related proteins. For the accuracy and credibility of the results, a more intuitive picture interpretation may be required. It is hoped that more relevant researches could be further provided to prove our results.

In summary, dexmedetomidine pretreatment can effectively reduce the myocardial cell inflammation and oxidative stress levels of rats after myocardial ischemia-reperfusion, decrease myocardial cell apoptosis, and protect cardiac function. This effect is related to the activation of PI3K/AKT signaling pathway.

Ten animals died prior to the experimental endpoint, which was attributable to three possible reasons. First, anesthesia was too deep for two animals at the beginning in sham group. Second, mechanical ventilation injured the lungs of three animals during the process of ischemia/reperfusion in I/R and DEX groups. Finally, during the procedure of surgical operation the heart function was severely compromised in five animals in DEX, DEX/YOH and YOH groups.

Disclosure of conflict of interest

None.

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