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Dexmedetomidine attenuates myocardial ischemia/reperfusion injury through regulating lactate signaling cascade in mice

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Abstract. – OBJECTIVE: The aim of this study was to investigate the role of dexmedetomidine (Dex) in lactate signaling cascade and myocardial ischemia/reperfusion (I/R) injury in mice.

MATERIALS AND METHODS: The left anterior descending of the coronary artery was ligatured for 30 min and then reperfused for 6 h to induce myocardial I/R injury in mice. Heart samples were collected and the levels of lactate, SOD and MDA were measured. Infarct size and myocardium were stained with triphenyltetrazolium chloride and TUNEL, respectively. In addition, the expression levels of MCT1, cytochrome c, cleaved caspase-9 and -3 were detected by Western blot.

RESULTS: The myocardial infarct size, lactate and MDA levels of the I/R group were significantly increased, whereas the SOD activity was decreased. However, Dex significantly reduced the myocardial infarct size, as well as lactate and MDA levels in contrast to the I/R group. Meanwhile, the SOD activity was remarkably increased. The expression levels of MCT1, cytochrome c, cleaved caspase-9 and -3 were significantly increased in the I/R group. In addition, Dex administration further increased the expression of MCT1, whereas decreased the expressions of cytochrome c, cleaved caspase-9 and -3 in contrast to the I/R group.

CONCLUSIONS: Dex elevated the expression of mitochondrial MCT1 and inhibited oxidative stress and the activation of mitochondria-dependent apoptosis in mice. This indicated that Dex attenuated myocardial I/R injury by regulating lactate signaling cascade.

Key Words:
Dexmedetomidine, Ischemia-reperfusion injury, Lactate, Reactive oxygen species, Apoptosis, Mitochondria.

Introduction

Myocardial ischemic injury refers to partial or complete acute obstruction of the coronary arteries. However, after ischemic myocardium restored to blood reperfusion, tissue damage progressively worsens. This may eventually lead to a series of pathophysiological changes, such as severe arrhythmia, myocardial stunning and energy metabolism disorder[1]. Multiple pathophysiological mechanisms are involved in myocardial ischemia/reperfusion (I/R) injury including calcium overload, oxidative stress, cell apoptosis and others[2].

Lactate is known as the end-product of glycolysis. Intracellular lactate shuttle is mediated by monocarboxylate transporter 1 (MCT1), which is located on the mitochondrial membrane[3,4]. Previous evidence has shown that lactate is a signaling molecule and regulates the generation of reactive oxygen species (ROS)[5]. Low levels of ROS can increase the expression of MCT1 and promote lactate metabolism in mitochondria[6]. However, under pathological conditions, lactate metabolic disorder results in an excessive generation of ROS, thereby leading to oxidative stress and the activation of mitochondria-dependent apoptosis[7]. Currently, it has been demonstrated that lactate signaling cascade is closely related to myocardial I/R injury[8]. Therefore, lactate signaling cascade can serve as a potential therapeutic target against myocardial I/R injury.

As a highly selective α_2-receptor agonist, dexmedetomidine (Dex) is widely used in the sedation of patients undergoing surgery and mechanical ventilation in ICU[9]. Dex has been reported to protect the heart from myocardial I/R injury by inhibiting NF-κB signaling pathway[10] and activating PI3K/Akt signaling pathway[11]. However, the exact role of Dex in lactate signaling cascade in the myocardial I/R injury has not yet been reported. Therefore, we established a myocardial I/R injury in mice, and explored the effect of Dex on lactate signaling cascade as well as the potential underlying mechanism.

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Materials and Methods

Ethics Statements

Male C57BL/6 mice (8-10 weeks old, weighing 20-30 g) were provided by the Experimental Animal Center of Jilin University (Jilin, China). Animal experiments were conducted following the NIH Guide for Laboratory Animals. All mice were kept at 22°C under 12:12 hour light/dark cycles, and were fed with standard mouse food and water. This investigation was approved by the Animal Ethics Committee of Jilin University Animal Center.

Construction of the Myocardial I/R Injury Model in Mice

Mice were first anesthetized with pentobarbital (50 mg/kg, intraperitoneal injection), followed by intubation and ventilation. A left horizontal incision was made at the third intercostal space. To induce I/R injury, an 8-0 silk suture was tied around both the left anterior descending (LAD) and a silicon tube. Subsequently, myocardial ischemia was measured by the electrocardiograph performance (ST-elevated). Sham-operated mice underwent the same surgical procedure without ligation. After 30 min of ischemia, the silicon tube was removed to induce reperfusion for 6 h. Totally five groups were set in this study, including 1) the sham group; 2) the I/R group, 0.5 mL saline was injected intraperitoneally at the start of reperfusion; 3) the I/R + Dex5 group, 5 μg/kg Dex was injected intraperitoneally at the start of reperfusion; 4) the I/R + Dex10 group, 10 μg/kg Dex was injected intraperitoneally at the start of reperfusion; 5) the I/R + Dex20 group, 20 μg/kg Dex was injected intraperitoneally at the start of reperfusion. Each group had 10 mice (n=10). After reperfusion, heart samples were harvested and stored at -70°C for subsequent experiments.

Assessment of Myocardial Infarct Size

Heart sections were first stained with 1% triphenyltetrazolium chloride at 37°C for 20 min. Then the sections were fixed with 4% paraformaldehyde at room temperature for 8 h. Infarcted myocardium was carefully separated from the non-infarcted myocardium and weighed. Infarct size was expressed as the percentage of ischemic risk area.

Measurement of Lactate, SOD and MDA Levels in Myocardium

The enzyme-labeled immunosorbent assay was used to measure the levels of lactate, SOD and MDA in the myocardium. After 6 h of reperfusion, left ventricular tissues were homogenized, centrifuged, and then transferred to EP tubes. The following steps were performed according to the instructions of the detection kit (Jiancheng Bioengineering Research, Nanjing, China).

TUNEL Assay

Cell apoptosis was detected by an *in situ* cell death detection kit (Roche, Basel, Switzerland). Heart sections were stained with the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) reaction mixture and Converter-POD, followed by observation under a microscope (Eclipse, Nikon, Tokyo, Japan). Apoptotic nuclei showed brown, while normal nuclei showed blue. The number of TUNEL-positive nuclei was expressed as the percentage of total nuclei.

Western Blot

Western blot was used to determine the expression levels of cytoplasmic and mitochondrial proteins extracted from I/R myocardium. Briefly, extracted proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Billerica, MA, USA). Then the membranes were incubated with primary antibodies of anti-MCT1, anti-cytochrome c, anti-cleaved caspase 9 and anti-cleaved caspase 3 at 4°C overnight. After washing with Tris-Buffered Saline and Tween 20 (TBST), the membranes were incubated with the HRP-conjugated secondary antibody at room temperature for 2 h. Western blot detection kit and Image J software (NIH) were used to measure the blot signal and density.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 Software (IBM, Armonk, NY, USA) was used for all statistical analysis. Experimental results were expressed as means ± standard deviations (SD). One-way analysis of variance (ANOVA) was used to compare the differences among different groups, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was confirmed statistically significant.

Results

Effect of Dex on Myocardial Infarct Size

As shown in Figure 1, compared with the sham group, myocardial infarct size in the I/R group

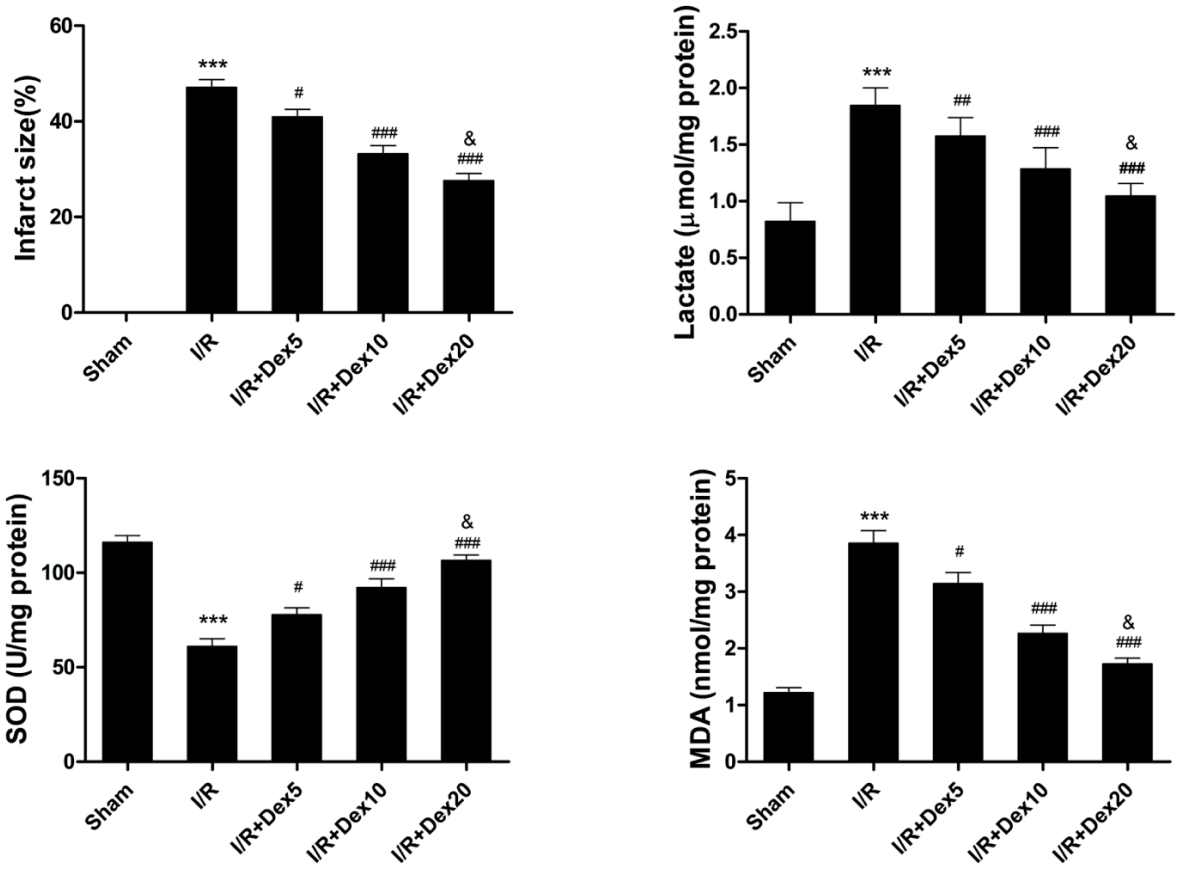

Figure 1. Effect of Dex on infarct size, lactate, SOD and MDA. ***$p<0.001$ versus the sham group; #$p<0.05$, ##$p<0.01$ and ###$p<0.001$ versus the I/R group; &$p<0.05$ versus the I/R+Dex10 group.

was significantly increased ($p<0.001$). 5, 10, 20 μg/kg of Dex administration all significantly reduced the myocardial infarct size in contrast to the I/R group ($p<0.05$; $p<0.001$; $p<0.001$, respectively). Moreover, myocardial infarct size in the Dex (20 μg/kg)-treated group was less than that of the Dex (10 μg/kg)-treated group ($p<0.05$).

Effect of Dex on Lactate, SOD, and MDA Levels

As shown in Figure 1, compared with the sham group, the levels of lactate and MDA were significantly increased in the I/R group ($p<0.001$; $p<0.001$, respectively), whereas the SOD activity was markedly decreased ($p<0.001$). Meanwhile, 5, 10, 20 μg/kg of Dex administration significantly reduced lactate and MDA levels in contrast to the I/R group ($p<0.05$; $p<0.01$; $p<0.001$, respectively), whereas the SOD activity was remarkably increased ($p<0.05$; $p<0.001$; $p<0.001$, respectively). Moreover, the levels of lactate and MDA in the Dex (20 μg/kg)-treated group were both significantly lower than those of the Dex (10 μg/kg)-treated group ($p<0.05$; $p<0.05$, respectively). However, SOD activity of the Dex (20 μg/kg)-treated group was higher than that of the Dex (10 μg/kg)-treated group ($p<0.05$).

Effect of Dex on Myocardial I/R-Induced Apoptosis

As shown in Figure 2, compared with the sham group, the apoptotic rate of the infarcted myocardium was significantly increased in the I/R group ($p<0.001$). Meanwhile, 20 μg/kg Dex administration reduced the apoptotic rate in contrast to the I/R group ($p<0.001$).

Effect of Dex on Lactate Signaling Cascade

As shown in Figure 3, compared with the sham group, the expression level of MCT1 on the mitochondrial membrane was significantly increased

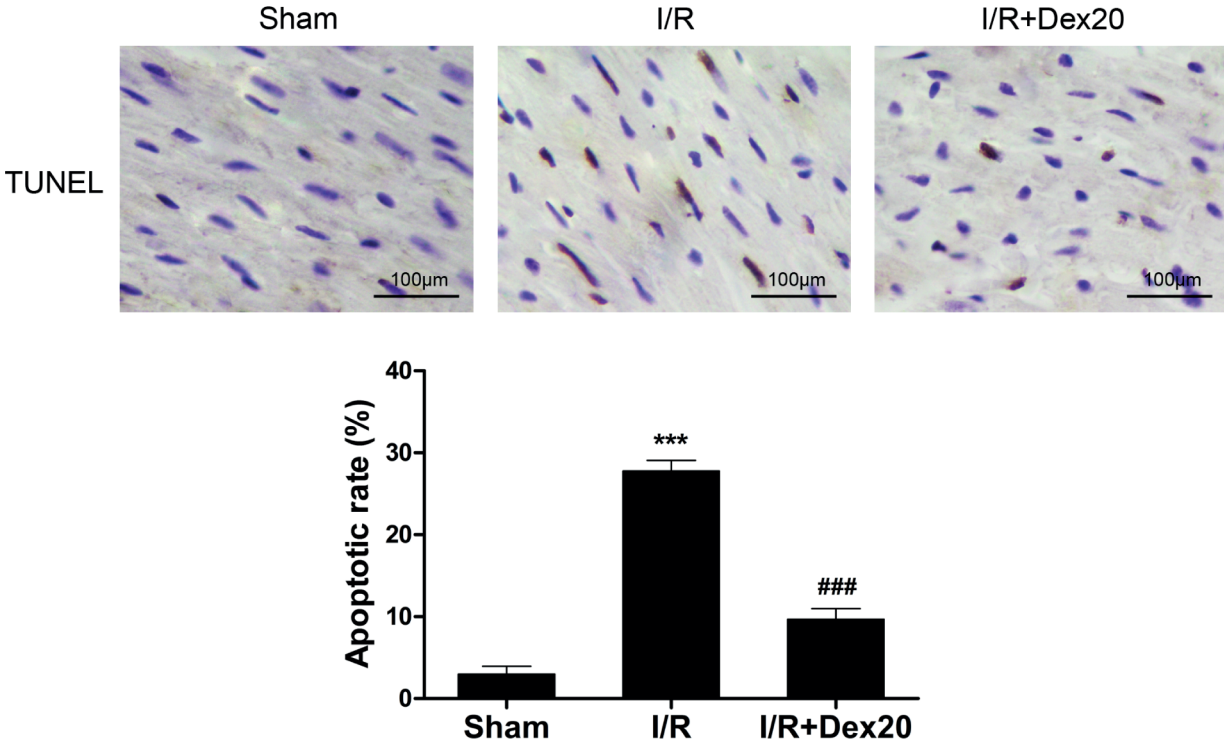

Figure 2. Effect of Dex on myocardium apoptosis. ***p<0.001 versus the sham group; ###p<0.001 versus the I/R group.

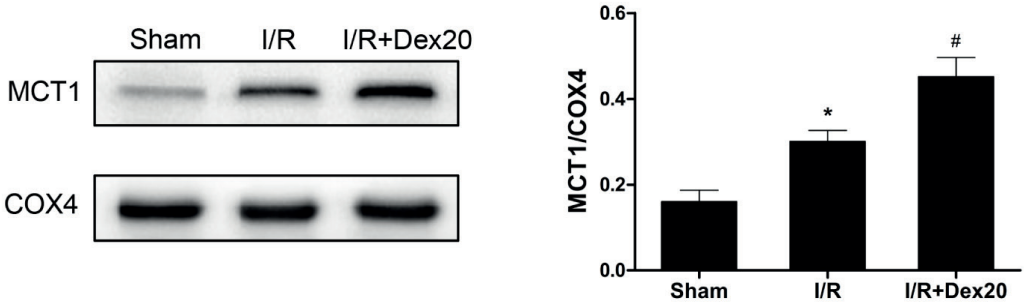

Figure 3. Effect of Dex on the expression of MCT1. *p<0.05 versus the sham group; #p<0.05 versus the I/R group.

in the I/R group (p<0.05). 20 μg/kg Dex administration further increased the expression level of MCT1 in contrast to the I/R group (p<0.05).

As shown in Figure 4, compared with the sham group, the expression levels of cytochrome c, cleaved caspase-9 and -3 were markedly increased in the I/R group (p<0.01; p<0.01; p<0.01, respectively). Meanwhile, 20 μg/kg Dex administration significantly decreased the expressions of cytochrome c, cleaved caspase-9 and -3 in contrast to the I/R group (p<0.05; p<0.05; p<0.05, respectively).

Discussion

The focus of this study was to investigate the cardioprotective effect of Dex on myocardial I/R injury in mice. Our results demonstrated that Dex administration protected the heart from myocardial I/R injury, as confirmed by the reduction in infarct size. This protective effect was associated with the regulation of lactate signaling cascade, including elevating the expression of mitochondrial MCT1, inhibiting oxidative stress and suppressing the activation of mitochondria-dependent apoptosis.

As the end-product of glycolysis, lactate can be transported into mitochondria by mono-carboxylate transport 1 (MCT1) protein[3]. Brooks et al[12] first proposed the hypothesis of intracellular lactate shuttle mechanism. They demonstrated that MCT1 was localized on the mitochondrial membrane of rodent and human hearts[13,14]. Under physiological or pathological conditions, glycolysis can be enhanced and may result in excessive production of lactate. Meanwhile, the expression of MCT1 compensatory increases to alleviate intracellular acidification. In the rat heart failure model, both Johannsson et al[15] and Evans et al[16] have found that the increased expression of MCT1 on the mitochondrial membrane is closely associated with advanced lactate influx. Similarly, Xu et al[17] have also found that in patients with atrial fibrillation, the expression of MCT1 is upregulated alone with the increase of lactate concentration in atrial tissues. Conversely, Martinov et al[18] have reported that inhibiting the expression of MCT1 can aggravate myocardial I/R injury in mice. In accordance with these findings, our results showed that the expression of mitochondrial MCT1 and the level of lactate in the infarcted myocardium were both significantly increased. Moreover, Dex administration could further enhance the expression of mitochondrial MCT1 and reduce the level of lactate in infarcted myocardium. Taken together, intracellular lactate shuttle mediated by MCT1 exhibited an inimitable effect on the development of myocardial I/R injury.

Lactate has been confirmed as a signaling molecule that can regulate the generation of ROS and related signaling cascade. Low level of lactate can promote ROS generation and increase MCT1 expression[19]. However, a high level of lactate may result in acidosis, oxidative stress and mitochondria-dependent apoptosis[17,20]. In this study, we found that the lactate level was significantly increased in infarcted myocardium alone with upregulated MCT1 expression, remarkable oxidative stress and activated mitochondria-dependent apoptosis. Our results indicated that lactate signaling cascade was closely associated with myocardial I/R injury. In addition, Dex administration significantly inhibited oxidative stress, reduced the release of cytochrome c, and down-regulated

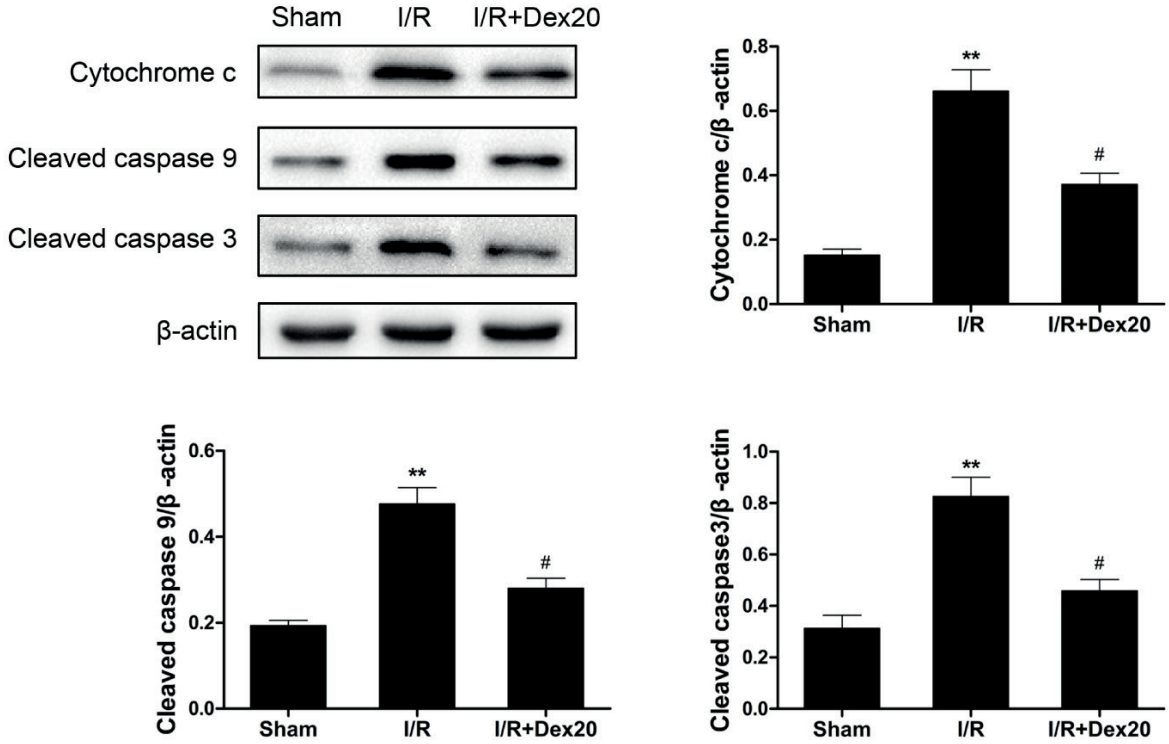

Figure 4. Effect of Dex on the expression of cytochrome c, cleaved caspase-9 and -3. **$p<0.01$ versus the sham group; #$p<0.05$ versus the I/R group.

the expression of cleaved caspase-9 and -3 in infarcted myocardium.

Conclusions

We showed that Dex protected against myocardial I/R injury by regulating lactate signaling cascade, including enhancing the expression of mitochondrial MCT1, alleviating oxidative stress, and inhibiting mitochondria-dependent apoptosis. This suggested that lactate signaling cascade might be a potential therapeutic target for myocardial I/R injury. However, further researches are still needed to investigate the precise mechanism *in vitro*.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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