Dexmedetomidine pretreatment alleviates ischemia-reperfusion injury-induced inflammatory reaction via inhibition of TLR-4/NF-κB signaling pathway

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Abstract

Purpose: To study the effect of dexmedetomidine on ischemia-reperfusion injury (IRI)-induced inflammatory response, as well as its underlying mechanism of action.

Methods: Three groups of healthy adult Sprague Dawley (SD) rats (mean weight, 275 ± 10 g): control, IRI and treatment groups were used. With the exception of control group, ligation was performed on left anterior descending coronary arteries for 30 min and blood perfusion was restored within 100 min to establish IRI. Control group rats were without left ligation. Rats in control and IRI groups received normal saline intraperitoneally 30 min prior to surgery, while the treatment group received 100 μg/kg dexmedetomidine via intraperitoneal injection 30 min before operation. Infarct volume was determined using triphenyl tetrazolium chloride (TTC) staining. IL-6 and TNF-α levels of myocardial tissues and serum were determined using enzyme-linked immunosorbent assay (ELISA). Western blotting was used to determine protein expressions of NF-κB and TLR-4 in myocardial tissues.

Results: Pretreatment with dexmedetomidine markedly decreased infarct volume caused by IRI (p < 0.05). Serum and myocardial TNF-α and IL-6 were significantly upregulated in IRI group, relative to control group, but were downregulated by pretreatment with dexmedetomidine (p < 0.05). There was marked upregulation of NF-κB and TLR-4 proteins in IRI rats, relative to untreated rats (p < 0.05). Dexmedetomidine also down-regulated the expressions of these proteins (p < 0.05).

Conclusion: Pretreatment with dexmedetomidine alleviates IRI-induced inflammatory reaction via suppression of TLR-4/NF-κB signaling pathway. This finding provides a basis for large-scale clinical trials with dexmedetomidine.

Keywords: Myocardial ischemia-reperfusion injury, Dexmedetomidine, Pretreatment, Inflammatory response

INTRODUCTION

Cardiac ischemia is common among patients undergoing non-cardiac surgery. Blood perfusion of myocardial ischemic infarction site is immediately restored after surgery to avoid aggravating myocardial damage in the ischemic area. This phenomenon is called myocardial ischemia-reperfusion injury (MIRI) [1], and it is common in patients with heart disease...
undergoing either cardiac surgery, myocardial infarction thrombolysis or non-cardiac surgery. The precise molecular mechanism underlying the pathogenesis of MIRI remains unknown. However, calcium overload, oxygen free radicals, and activation of neutrophils have been postulated to play key roles [2]. In the 1980s, a concept of myocardial ischemic preconditioning was proposed, which is now the generally accepted mechanism for endogenous protection, but it is limited in clinical application, since it cannot provide explanation for the process of myocardial damage. This has prompted studies in search of drugs that can produce pre-adapted effects without organ damage [3,4].

Dexmedetomidine, an emerging selective α2 receptor agonist, is a widely used anesthetic [5]. Studies have shown that it maintains hemodynamic stability in patients with cardiovascular disease during the preoperative period. Dexmedetomidine reduces the levels of inflammatory factors and occurrence of myocardial ischemia in experimental animals, an indication that it plays an important role in alleviating IRI-induced myocardial inflammation [6]. However, there are controversies surrounding the protective mechanism of dexmedetomidine in MIRI [7]. Studies have shown that NF-κB and TLR-4 are closely related to inflammatory response in MIRI [8]. This study investigated the influence of dexmedetomidine on IRI–induced inflammatory response, and the underlying mechanism.

**EXPERIMENTAL**

**Materials**

Dexmedetomidine injection was purchased from Jiangsu Hengru Medical Co. Ltd. Chloral hydrate. Mouse ventilator was obtained from Shanghai Alcott Biotechnology Co. Ltd. Triphenyltetrazole chloride (TTC). Evan blue dye was product of Sigma (USA), while ELISA kits were purchased from Beijing Zhongshan Jinqiao Co. Ltd. Phosphate-buffered saline (PBS) and centrifuge were purchased from Leica (Germany). This research received approval from Animal Ethics Committee of our institution (approval no. 0182183) and was conducted in line with the NIH regulations [9].

**Experimental rats and grouping**

Three groups of healthy adult SD rats (mean weight = 275 ± 10 g): control, IRI and treatment groups were used (14/group). They were housed in metal cages under standard conditions of animal care, and were given standard feed and water ad libitum. They were kept under 12-h light/12 h photoperiod, and maintained at temperatures between 25 °C, and relative humidity of 48 – 60 %. They were given routine disinfection and anesthesia with pentobarbital, and were connected to ECG and respiratory ventilator. With the exception of control group, the left-side anterior descending coronary arteries were ligated for 30 min, and blood perfusion was restored within 100 min to establish IRI. The sham-operated group was without left ligation. Rats in the control and IRI sham-operated groups received normal saline intraperitoneally 30 min before surgery, while the treatment group received 100 μg/kg bwt dexmedetomidine via intraperitoneal injection 30 min before operation.

**Myocardial infarct volume**

Myocardial infarct volume was measured using TTC staining method [10]. The cervical vein of each rat was rapidly injected and stained with 2 ml of 2 % Evan blue dye, followed by heart excision. Myocardial tissues along the long axis of the left ventricle were immersed in TTC solution. Then, they were divided into blue (normal myocardial tissue), red (ischemic myocardial tissue), and pale (infarcted myocardial tissue). The volume of infarction areas was calculated using Image J software.

**Evaluation of tumor necrosis factor-α and interleukin-6 in serum**

Blood (3 ml) was drawn from the right jugular vein, subjected to centrifugation at 1,500 rpm, and the resultant serum was cryopreserved in Eppendorf tube. The respective ELISA kits were used for assay of TNF-α and IL-6.

**Determination of TNF-α and IL-6 in myocardial tissues**

At the end of reperfusion, rat myocardial tissue was digested with trypsin and the resultant cell suspension was clarified through centrifugation at 4 °C at 8000 g for 0.5 h. The supernatant was collected in a centrifuge tube, and the protein was denatured in a boiling water bath, and refrigerated. The amounts of the two cytokines in the supernatant were assayed with their respective kits.

**Western blotting**

Protein expressions of NF-κB and TLR-4 in myocardial tissues were determined using...
Western blotting [11]. Cell suspension resulting from trypsinization of myocardial tissue was rinsed 2 times in PBS, and lysed with cold RIPA buffer tainted with protease inhibitor. The resultant lysate was centrifuged at 15,000 rpm for 15 min at 4 °C. The protein was quantified with BCA assay kit. A portion of total cell protein (30 μg) was subjected to SDS-PAGE. The protein bands were then transferred to a fixed polyvinylidene fluoride membrane at 110 V and 90 °C for 120 min. Subsequently, non-fat milk (5 %) in TBS-T was added, with gentle shaking at 37 °C, and incubated to block non-specific binding of the blot. Incubation of the blots was performed overnight at 4 °C with primary antibodies of rabbit polyclonal anti-NF-κB, TLR-4 and GAPDH at a dilution of 1 to 1000, after which it was incubated with HRP-bound secondary antibody for 1.5 h. The blot was developed using an X-ray film. Grayscale analysis of the bands was done with image J processing software. The respective protein expression levels were normalized to that of GAPDH standard.

Statistical analysis

The data are presented as mean ± SEM, and were statistically analyzed with Statistical Package for the Social Sciences (18.0). Group comparison was done with χ² test. Statistical significance was fixed at p < 0.05.

RESULTS

Myocardial infarct volume

As presented in Table 1 and Figure 1, pretreatment with dexmedetomidine significantly reduced the infarct volume due to IRI (p < 0.05).

TNF-α and IL-6 levels in myocardial tissue

As shown in Table 2, there were marked upregulations of myocardial tissue TNF-α and IL-6 in IRI rats, relative to control rats, but were markedly downregulated by pretreatment with dexmedetomidine (p < 0.05).

Table 1: Myocardial infarct size (n = 14, %)

| Group | Myocardial infarct size (%) |
|-------|-----------------------------|
| Control | 0.00 ± 0.00 |
| IRI    | 56.34 ± 4.66 |
| Treatment | 50.16 ± 4.00 |
| T      | 3.765 |
| p      | 0.001 |

*p < 0.05, relative to IRI group

Serum levels of TNF-α and IL-6

Serum TNF-α and IL-6 were markedly upregulated in IRI rats, relative to control rats, but were markedly downregulated by pretreatment with dexmedetomidine (p < 0.05).

Table 2: Myocardial levels of IL-6 and TNF-α

| Group | N | TNF-α (pg/mL) | IL-6 (pg/mL) |
|-------|---|--------------|--------------|
| Control | 14 | 174.96 ± 22.58 | 130.44 ± 16.49 |
| IRI    | 14 | 610.34 ± 22.59 | 340.29 ± 17.44 |
| Treatment | 14 | 280.36 ± 25.38 | 206.25 ± 12.43 |

*p < 0.05, vs untreated rats; #p < 0.05, vs IRI rats

Expressions of NF-κB and TLR-4 proteins in myocardial tissue

There were upregulated expressions of NF-κB and TLR-4 proteins in IRI rats, relative to control rats (p < 0.05). Prior treatment with dexmedetomidine down-regulated the expressions of these proteins (p < 0.05; Figure 2).

Table 3: Effect of dexmedetomidine on TNF-α and IL-6 concentrations

| Group | n | TNF-α (pg/mL) | IL-6 (pg/mL) |
|-------|---|--------------|--------------|
| Control | 14 | 159.99 ± 18.52 | 100.41 ± 16.37 |
| IRI    | 14 | 622.37 ± 22.53 | 340.13 ± 29.28 |
| Treatment | 14 | 241.34 ± 10.18 | 161.22 ± 12.09 |

*p < 0.05, vs untreated rats, #p < 0.05, vs IRI rats

Figure 2. Effect of dexmedetomidine on NF-κB and TLR-4. *p < 0.05, vs untreated rats, #p < 0.05, vs IRI group
DISCUSSION

Cardiopulmonary resuscitation, organ transplantation, thrombolysis and bypass reperfusion ischemic tissues or organs with blood after surgery, in an attempt to repair damaged structures. However, in some organs with high oxygen demand such as brain and heart, reperfusion after ischemia may aggravate structural damage and dysfunction, and even lead to irreversible damage of tissues or organs. Myocardial necrosis is the most serious consequence of reperfusion after ischemia.

Dexmedetomidine, a highly selective α2 receptor agonist, has high affinity for α2 adrenergic receptor. In addition to its sedative, analgesic and antispasmodic effects, it inhibits the release of catecholamines, reduces sympathetic nerve function, and maintains hemodynamic stability without causing respiratory depression [12]. Pretreatment with dexmedetomidine has been shown to confer protection on MIRI [13].

In this study, the myocardial infarct volume of the treatment group was significantly reduced, when compared with that of IRI group. This is in agreement with previous reports, an indication that pretreatment with dexmedetomidine may alleviate MIRI. Studies have shown that IRI is a congenital immune response in which the complement system erroneously attacks own tissue antigen [14]. Ischemic myocardial reperfusion is characterized by the release of high amounts of inflammatory factors and overactivation of the inflammatory response in which TNF-α has a major role, which not only directly damages myocardial cells, but also causes the release of large amounts of inflammatory factors. On the one hand, this causes micro-arterial spasm, thereby aggravating damaged tissue ischemia, and on the other hand, increases vascular permeability, thereby exacerbating inflammatory cell infiltration and tissue edema [15]. Interleukin-6 (IL-6) plays a pivotal role in inflammatory response. It has been suggested that IL-6 overexpression occurs in areas where inflammatory cells accumulate, leading to increase in complications after myocardial damage. This is an index which reflects body stress and severity of tissue cell damage [16]. Therefore, suppression of the synthesis and liberation of TNF-α and IL-6 is key in reducing inflammation and MIRI.

The results of this study showed that myocardial and blood TNF-α and IL-6 concentrations were markedly increased in IRI group, when compared with untreated rats, but were significantly reduced by pretreatment with dexmedetomidine. These results suggest that large amounts of inflammatory factors may be released during IRI, and that dexmedetomidine may lower inflammatory reaction induced by TNF-α and IL-6.

The TLR-4/NF-κB signaling route has been shown to be involved in the pathogenesis of IRI [17]. Toll-like receptors are expressed in immune cells and cardiomyocytes, and activate the innate immune response [18]. Toll-like receptor-4 (TLR-4) activates downstream signal transduction pathways by specifically binding pathogen-associated molecular mode, thereby performing a vital function in the onset of inflammation-related responses. The transcription factor NF-κB, a major regulator of responses associated with the immune system and inflammation, is ubiquitously expressed in eukaryotic cells. Toll-like receptor-4 (TLR-4) activates NF-κB after interaction with pathogen-associated molecular signals, which in turn induce downstream signal transduction and promote the initiation of inflammatory responses [19]. Studies have shown that ischemia-reperfusion rapidly activates myocardial transcription factor NF-κB, blocks its expression and shrinks the area of myocardial infarction in rats. Over-activation of NF-κB is responsible for the death of myocardial cells after ischemia-reperfusion. It has been suggested that blockage of TLR-4/NF-κB signal route may be an important target for the treatment of IRI [20]. In this study, the protein expressions of NF-κB and TLR-4 in myocardial tissue were significantly upregulated in IRI group, an indication that reperfusion injury after ischemia may be accompanied by over-activation of the TLR-4/NF-κB signal route. It is likely that dexmedetomidine inhibits the TLR-4/NF-κB signal route.

CONCLUSION

Pretreatment with dexmedetomidine alleviates IRI-induced inflammatory reaction via suppression of TLR-4/NF-κB signal route. This finding provides a basis for large-scale clinical trials with dexmedetomidine.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

This work was done by the authors named in this article, and the authors accept all liabilities.
resulting from claims which relate to this article and its contents. Kai Xiong designed the study and interpreted the results. Haiping Zhang, Zhiying Wu, Nanhuo Han, Lu Xu, Kai Xiong collected data and drafted the manuscript. Haiping Zhang performed the experiments.

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