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Effect of dexmedetomidine on kidney injury in sepsis rats through TLR4/MyD88/NF-κB/iNOS signaling pathway

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Abstract. – OBJECTIVE: The aim of this study was to investigate the effect of dexmedetomidine (DEX) on kidney injury in sepsis rats through the Toll-like receptor 4 (TLR4)/myeloid differential protein-88 (MyD88)/nuclear factor-κB (NF-κB)/inducible nitric oxide synthase (iNOS) signaling pathway.

MATERIALS AND METHODS: A total of 30 Sprague-Dawley (SD) rats were randomly divided into three groups, including the control group (*n*=10), lipopolysaccharide (LPS)-induced acute kidney injury (AKI) group (model group, *n*=10) and DEX treatment group (DEX group, *n*=10). The model of sepsis was successfully established in rats. The levels of serum creatinine (Cr), blood urea nitrogen (BUN), serum interleukin-6 (IL-6), IL-1β, IL-10 and tumor necrosis factor-α (TNF-α) were detected *via* enzyme-linked immunosorbent assay (ELISA). The pathological changes in kidney tissues were detected *via* hematoxylin-eosin (HE) staining. Furthermore, the mRNA and protein expressions of TLR4, MyD88, NF-κB, and iNOS in the kidney were detected *via* fluorescence quantitative Polymerase Chain Reaction (PCR) and Western blotting, respectively.

RESULTS: Compared with the control group, rats in the model group showed significant kidney injury, markedly increased levels of serum Cr, BUN and pro-inflammatory cytokines, remarkably decreased the level of IL-10 ($p<0.05$), and significantly increased mRNA and protein expressions of TLR4, MyD88, NF-κB, and iNOS. In the DEX group, AKI was markedly improved, while the expressions of inflammatory cytokines were remarkably declined. Furthermore, the mRNA and protein expressions of TLR4, MyD88, NF-κB, and iNOS decreased significantly.

CONCLUSIONS: DEX has a protective effect on LPS-induced AKI, whose mechanism may be related to the inhibition of the TLR4/MyD88/NF-κB/iNOS pathway.

Key Words
Dexmedetomidine, Sepsis, Inflammatory factors, Kidney injury.

Introduction

Sepsis is the most common pathogenesis of severe acute kidney injury (AKI), whose morbidity and mortality rates remain high currently[1,2]. Systemic inflammatory response syndrome plays a key role in the occurrence and development of sepsis. Meanwhile, it is important pathogenesis leading to multiple organ dysfunction syndromes, such as AKI and acute lung injury[3,4].

Dexmedetomidine (DEX) is a kind of novel α adrenergic receptor agonist. Recent studies have shown that DEX mainly acts on the sympathetic nerve endings of the central nervous system and peripheral nervous system. Meanwhile, it can reduce the release of norepinephrine, with sedative, analgesic and anti-sympathetic nerve effects[5]. Previous studies[6-8] have demonstrated that DEX plays a positive role in alleviating organ injury and oxidative damage caused by sepsis.

In the present work, the model of lipopolysaccharide (LPS)-induced kidney injury was first established in rats. Furthermore, the regulatory effect of DEX on the Toll-like receptor 4 (TLR4)/nuclear factor-κB (NF-κB) signaling pathway was explored. Our findings might provide evidence for the anti-inflammatory molecular mechanism of DEX.

Materials and Methods

Animal Grouping and Treatment

A total of 30 specific pathogen-free male Sprague-Dawley (SD) rats weighing 200-220 g were purchased from the Ningbo SLAC Laboratory Animal Center. All rats were fed in the barrier system. This study was approved by the Laboratory Animal Welfare Ethics Committee.

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All SD rats were randomly divided into three groups, including the control group (n=10), LPS-induced AKI group (model group, n=10) and DEX treatment group (DEX group, n=10). The model of sepsis was established in rats *via* intraperitoneal injection of LPS (40 mg/kg) in the model group and DEX group. Meanwhile, DEX (80 mg/kg) was intragastrically administered every day since 4 d before modeling in the DEX group.

Detection of Indexes via Fluorescence Quantitative Polymerase Chain Reaction (PCR)

Kidney tissues of rats were ground in liquid nitrogen, followed by extraction of total RNA using 1 mL of TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration of extracted RNA was measured by a spectrophotometer. According to the instructions of TaKaRa (Otsu, Shiga, Japan), 1 μg RNA was taken for reverse transcription. Subsequently, obtained complementary deoxyribose nucleic acid (cDNA) was stored at -20°C for use. The mRNA level of each index was detected according to the instructions of All-in-One™ qPCR Mix kit (MedChem Express, Monmouth Junction, NJ, USA). The relative expression level of mRNA was calculated as follows: 2-ΔCt [ΔCt = Ct (target gene) - Ct (GAPDH)]. Primer sequences used in this study were shown in Table I.

Detection of Protein Expression Levels of TLR4, MyD88, NF-κB and iNOS in Kidney Tissues via Western Blotting

40 μg proteins were loaded, separated and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After sealing with 5% skimmed milk at room temperature for 1 h, the membranes were incubated with primary antibodies of TLR4, MyD88, NF-κB and iNOS (1:1000, Proteintech, Rosemont, IL, USA). After washing with Tris-Buffered Saline and Tween (TBST; Sigma-Aldrich, St. Louis, MO, USA), the membranes were incubated with the corresponding horseradish peroxidase-labeled (HRP) secondary antibodies (1:5000, Shanghai Beyotime Biotechnology Co., Ltd., Shanghai, China). Immunoreactive bands were visualized using the enhanced chemiluminescence (ECL) detection system (Bio-Rad, Hercules, CA, USA), and gray values were analyzed using a gel analyzer. The ratio of the gray value of the target protein to that of the corresponding internal reference indicated the relative content of the target protein.

Detection of Serum Indexes

Creatinine (Cr) and blood urea nitrogen (BUN): whole blood was drawn and immediately anti-coagulated with ethylenediaminetetraacetic acid (EDTA)-2K. Subsequently, Cr and BUN were automatically detected using the Coulter-JT full-automatic blood routine detector.

Inflammatory factors: whole blood was placed at 4°C for 2 h, followed by centrifugation at 3000 rpm for 15 min. The supernatant was taken and stored in a low-temperature refrigerator at -80°C for use. Finally, the enzyme-linked immunosorbent assay (ELISA; Boster, Wuhan, China) was performed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 16.0 software package (SPSS, Chicago, IL, USA) was used for all statistical analysis. Enumeration data were expressed as mean ± standard deviation. Student's t-test or nonparametric Wilcoxon signed rank test was adopted for comparison of indexes between the two groups. $p<0.05$ was considered statistically significant.

Table I. RT-PCR primer sequences.

Gene	Primer sequence	Size (bp)
TLR4	F 5'-3' GTGGAAGTTGAACGAATGGA R 5'-3' TGGATGATGTTGGCAGCA	391
MyD88	F 5'-3' TCGCGCATCGGACAAACG R 5'-3' GCAATGGACCAGACACAGGT	228
NF-κB	F 5'-3' AGTTGAGGGGACTTTCCCAGGC R 5'-3' GATTCGAGTATTAGTTCATGGA	132
iNOS	F 5'-3' TCAGAAGCAGAATGTGACCA R 5'-3' TACATGCTGGAGCCGAGGCCAAA	95
GAPDH	F 5'-3' TGCACCACCAACTGCTTAGC R 5'-3' GGCATGGACTGTGGTCATGAG	

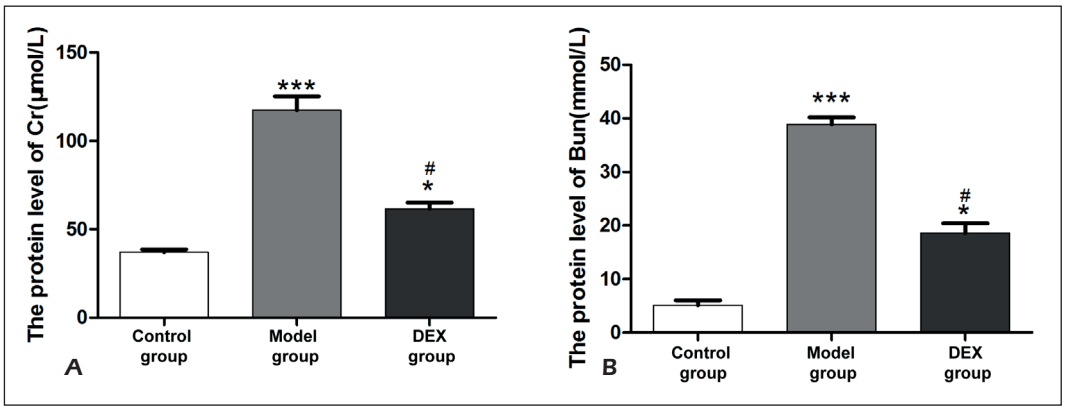

Figure 1. Serum levels of Cr and BUN in rats detected via ELISA. **A**, Expression of serum Cr in each group. **B**, Expression of serum BUN in each group. *p<0.05 & ***p<0.001 *vs.* control group, #p<0.05 *vs.* model group.

Results

Serum Indexes of Kidney Function Detected via ELISA

The levels of Cr and BUN in the model group increased significantly compared with those of the control group [(123.15±13.52) *vs.* (42.51±8.24), (38.41±8.21) *vs.* (6.27±2.54)], showing statistically significant differences (p<0.05). Moreover, the levels of Cr and BUN in the DEX group (54.29±12.84) were markedly declined compared with the model group (17.22±6.46; p<0.05; Figure 1).

Effects of DEX on the Levels of Serum IL-6, IL-1β, TNF-α and IL-10 in Rats

The levels of IL-6, IL-1β and TNF-α were significantly up-regulated, while IL-10 was markedly declined in the model group when compared with the control group [(14.23±3.71) *vs.* (7.64±1.81), (227.73±25.72) *vs.* (33.27±8.27), (195.62±11.42) *vs.* (51.21±6.42), (101.53±17.74) *vs.* (124.37±17.88)], and the differences were statistically significant (p<0.05). Moreover, the levels of IL-6, IL-1β and TNF-α were significantly declined [(10.62±0.92), (122.51±25.81) and (103.32±4.13)], while IL-10 (184.33±8.93) was remarkably up-regulated in the DEX group compared with those in the model group (p<0.05; Table II).

Morphology of Kidney Tissues Detected via HE Staining

In the control group, kidney tissues had clear texture, with no significant inflammatory response and edema. In the model group, there were tubular dilatation, glomerular inflammatory response and severe edema. Compared with the model group, the pathological changes in kidney tissues were significantly improved in the DEX group (Figure 2).

Expression Levels of Indexes in Kidney Tissues in Each Group Detected via Real Time-Polymerase Chain Reaction

The mRNA expressions of TLR4, MyD88, NF-κB and iNOS were detected *via* fluorescence quantitative Polymerase Chain Reaction. As shown in Figure 3, the mRNA expressions of TLR4, MyD88, NF-κB and iNOS in the model group increased significantly when compared with the control group (p<0.05). However, their expressions were markedly declined in the DEX group than the model group (p<0.05). The above results suggested that DEX could significantly inhibit the TLR4/MyD88/NF-κB/iNOS signaling pathway.

Table II. Effects of DEX on levels of serum IL-6, IL-1β, TNF-α, and IL-10 in rats.

Group	IL-6	IL-1β	TNF-α	IL -10
Control group	7.64±1.81	33.27± 8.27	51.21±6.42	124.37±17.88
Model group	14.23±3.71*	227.73± 25.72**	195.62±11.42**	101.53±17.74*
DEX group	10.62± 0.92*#	122.51±25.81*#	103.32± 4.13*#	184.33±8.93*#

*p<0.05, **p<0.01 & ***p<0.001 *vs.* control group, #p<0.05 *vs.* model group.

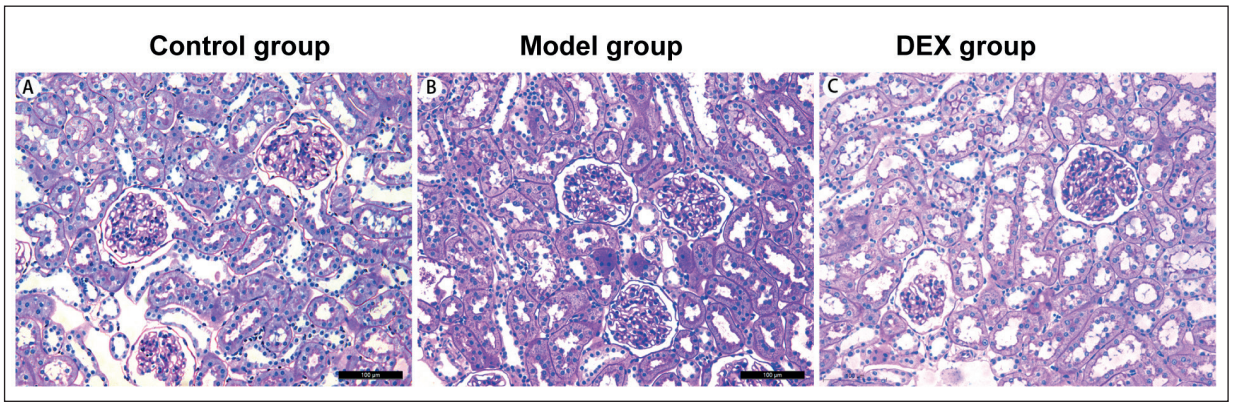

Figure 2. Kidney tissue morphology detected via HE staining (Magnification × 40).

Signaling Pathway-Related Indexes Detected via Western Blotting

As shown in Figure 4, the protein expressions TLR4, MyD88, NF-κB and iNOS were remarkably elevated in the model group when compared with the control group ($p<0.05$). However, their protein expressions were significantly declined in the DEX group than the model group ($p<0.05$).

Discussion

Sepsis is one of the major causes of death in severe patients. During the process, LPS binds to endotoxin receptors to activate a series of cell signal transduction pathways, promotes NF-κB expression and release inflammatory factors such as TNF-α and IL-6 in the body. This may eventually induce severe systemic inflammatory response syndrome, leading to multiple organ injury. Currently, AKI is one of the major complications caused by sepsis[9,10].

TLR4 is an important subtype of the TLR family, which is widely expressed in a variety of human cells. At present, at least 10 members have been found in the human TLR family. They are mainly distributed in endothelial cells, macrophages, neutrophils, astrocytes, etc[11,12]. According to current studies, TLR4 is related to the recognition of various microbial pathogens by host cells. Ligands of TLR4 include bacterial LPS, heparan sulfate, fibronectin EDA, hyaluronic acid and fibrinogen. Meanwhile, endogenous activators of TLR4 can also be released by damaged tissues and necrotic cells[13,14]. There are pattern recognition receptors for TLR4 pathogens on the surface

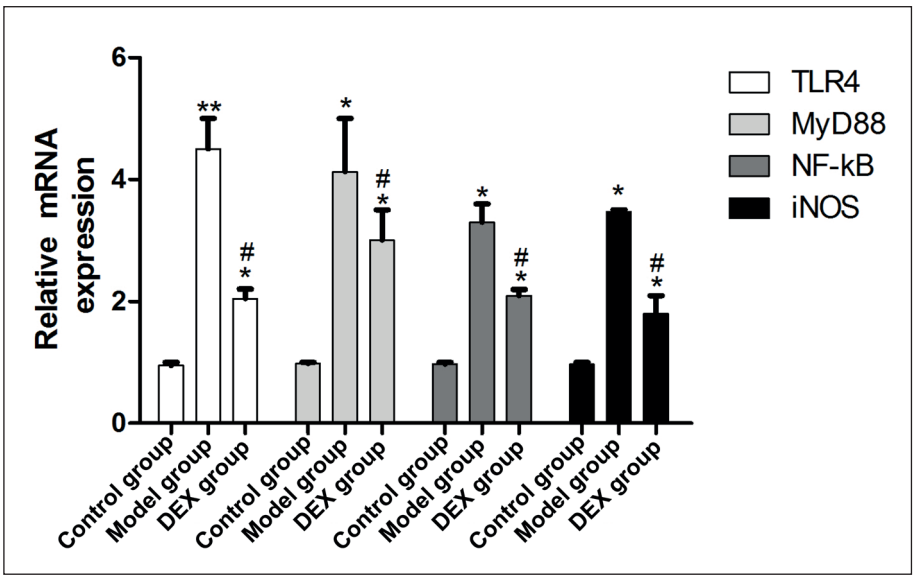

Figure 3. Expression levels of indexes in kidney tissues in each group detected via RT-PCR.

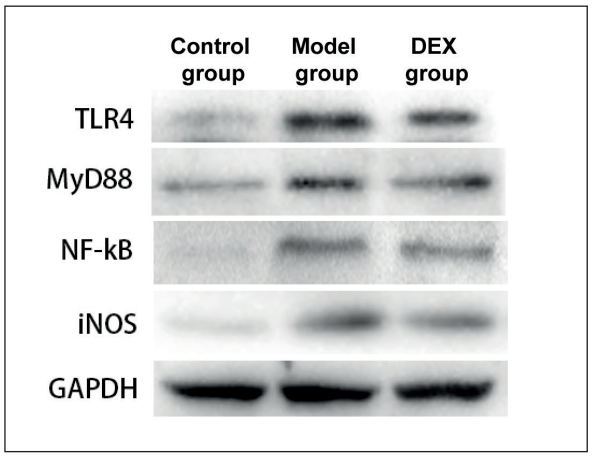

Figure 4. TLR4/MyD88/NF-κB/iNOS signaling pathway-related indexes detected via Western blotting. Note: the protein expressions of TLR4, MyD88, NF-κB and iNOS increased significantly in the model group when compared with those in the control group ($p<0.05$). However, their expressions were markedly declined in the DEX group when compared with the model group ($p<0.05$).

of renal tubular epithelial cells. It has been found that they can recognize the exogenous homologous ligand LPS, stimulate the signal transduction into cells through TLR4, and activate the transcription factor NF-κB. Ultimately, this may initiate the expression of inflammatory cascade effectors[15]. MyD88 is a member of the adaptin family[16], which can be recruited by TLR4. Once activated, it triggers signaling cascade and activates NF-κB in the intracellular domain, leading to transcriptional expression of inflammation-related genes. This can ultimately result in a massive release of inflammatory factors and inflammatory response[17,18]. Overall, the TLR4/MyD88/NF-κB/iNOS signaling pathway is a pathway that promotes an inflammatory response in the kidney.

In the present work, it was found that DEX could significantly inhibit the protein expressions of LPS-induced TLR4 and MyD88. This indicated that DEX might inhibit MyD88 by down-regulating TLR4 protein expression, thereby suppressing NF-κB activation and iNOS protein expression. Ultimately, the production of inflammatory factors was inhibited. Wu et al[19] have shown that, consistent with the present study, DEX can reduce the expressions of TNF-α and IL-6 in sepsis rats and lower the mortality rate. High dose DEX can inhibit the up-regulation of macrophage inflammatory factors and cellular molecules in rats with sepsis-induced sepsis[20]. In the present study, DEX was continuously pumped every day since 4 d before injection of LPS in rats. Subsequent results demonstrated that there was only a small amount of inflammatory cell infiltration in kidney tissues, and the glomerular structure was clear. Moreover, the content of IL-6, IL-1β and TNF-α and the expressions of TLR4, MyD88, NF-κB and iNOS in kidney tissues were significantly declined in the DEX group when compared with those in the model group. These findings all indicated that DEX could inhibit the early inflammatory response in AKI induced by LPS. The underlying mechanism might be related to the TLR4 signaling pathway.

Conclusions

We showed that DEX represses the production of inflammatory mediators by inhibiting the TLR4/MyD88/NF-κB/iNOS signaling pathway, thereby alleviating the inflammatory response. Furthermore, other inflammatory signaling pathways are worthy of studies in the future.

Disclosure of interest

The authors declare that they have no conflict of interest.

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