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Dexmedetomidine protects against lipopolysaccharide-induced early acute kidney injury by inhibiting the iNOS/NO signaling pathway in rats

Yongping Chen[a], Li Luan[a], Chaoran Wang[a], Manyu Song[a], Yuan Zhao[a], Yujie Yao[a], Haotian Yang[a], Biao Ma[a], Honggang Fan[a,b,*]

[a] College of Veterinary Medicine, Northeast Agricultural University, Harbin, 150030, PR China
[b] Heilongjiang Key Laboratory for Laboratory Animals and Comparative Medicine, Northeast Agricultural University, Harbin, 150030, PR China

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ABSTRACT

Increasing evidence has demonstrated that dexmedetomidine (DEX) possesses multiple pharmacological actions. Herein, we explored the protective effect and potential molecular mechanism of DEX on lipopolysaccharide (LPS)-induced early acute kidney injury (AKI) from the perspective of antioxidant stress. We found that DEX (30 μg/kg, i.p.) ameliorated the renal dysfunction and histopathological damage (tubular necrosis, vacuolar degeneration, infiltration of inflammatory cells and cast formation) induced by LPS (10 mg/kg). DEX also attenuated renal oxidative stress remarkably in LPS-induced early AKI, as evidenced by reduction in production of reactive nitrogen species, decreasing malondialdehyde levels, as well as increasing superoxide dismutase activity and glutathione content. DEX prevented activator protein-1 translocation, inhibited phosphorylation of I-kappa B (IκB) and activation of nuclear factor kappa B (NF-κB) in LPS-induced early AKI, as assessed by real-time quantitative polymerase chain reaction and protein levels of c-Jun, c-Fos, IκB and NF-κB. Notably, DEX pretreatment had the same effect as intraperitoneal injection of an inhibitor of inducible nitric oxide synthase inhibitor (1400W; 15 mg/kg), and inhibited the activity of renal inducible nitric oxide synthase (iNOS) and decreased the expression of iNOS mRNA and NO production. However, the protective effect of DEX on LPS-induced early AKI was reversed by the alpha 2 adrenal receptor (α_2-AR) inhibitor atipamezole, whereas the imidazoline receptor inhibitor idazoxan did not. Taken together, DEX protects against LPS-induced early AKI in rats by inhibiting the iNOS/NO signaling pathway, mainly by acting on α_2-ARs instead of IRs.

1. Introduction

Sepsis is a life-threatening syndrome caused by a dysfunctional response to infection [1]. In 2018, the World Health Organization reported that ~30 million people were affected by sepsis each year [2]. More than 60% of sepsis patients suffer from acute kidney injury (AKI) [3,4]. Sepsis-induced acute kidney injury (SAKI) is the main reason for a prolonged stay in hospital and increased mortality. One study involving 54 hospitals in 23 countries showed that the mortality prevalence of SAKI patients was 70.2% [5]. In the early stages of sepsis, the kidneys undergo histopathologic changes and dysfunction [6], but efficacious therapeutic drugs are not available for this disease stage. SAKI is associated with high morbidity and mortality, and causes admission to the intensive care unit (ICU) worldwide. Hence, it is very important to explore the potential mechanisms of early SAKI so that efficacious therapeutic drugs can be developed.

Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria. LPS is involved in the pathogenesis of SAKI. Infusion/injection of LPS has been used widely used as a model of experimental SAKI [7]. However, the pathogenesis of SAKI is extremely complex. Most reports on SAKI have focused on the inflammatory response. Therefore, understanding the pathogenesis and efficacious treatment of SAKI is still limited.

Recent studies have shown that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are participated in SAKI pathogenesis [8,9]. ROS have been reported to induce activation of nuclear factor-kappa B (NF-κB), a promoter of the synthesis of inducible nitric oxide synthase (iNOS) [10]. If sepsis occurs, iNOS is expressed in vascular endothelial cells, which induces high production of nitric oxide (NO) [11]. The latter inhibits the activity of antioxidant enzymes and increases oxidative stress [12]. Studies have shown that inhibition of iNOS activity can reduce oxidative stress in renal tubular cells [13]. In addition, Chen et al. demonstrated that LPS-induced AKI can be attenuated by inhibiting oxidative stress [14]. Therefore, antioxidation may

* Corresponding author. College of Veterinary Medicine, Northeast Agricultural University, Harbin, 150030, Heilongjiang Province, PR China.
E-mail address: fanhonggang2002@163.com (H. Fan).

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Abbreviations

DEX	dexmedetomidine
LPS	lipopolysaccharide
α_2-AR	alpha 2 adrenal receptor
IR	imidazoline receptor
AP-1	activator protein 1
NF-κB	nuclear factor kappa B
iNOS	inducible nitric oxide synthase
NO	nitric oxide
RNS	reactive nitrogen species
BUN	blood urea nitrogen
Scr	Serum creatinine
MDA	malondialdehyde
SOD	superoxide dismutase
GSH	glutathione
AT	atipamezole
IDA	idazoxan

be another important mechanism to protect LPS-induced early AKI, but its potential mechanism of action in not known.

Dexmedetomidine (DEX) is a highly selective alpha 2 adrenoceptor agonist (α_2-AR) and is used widely in the ICU. Accumulating evidence suggested that DEX has multiple pharmacological effects., including anti-inflammation [15], anti-apoptosis [16], sedation and no neurotoxicity [17,18]. Recently, DEX has been reported to ameliorate kidney damage by reducing oxidative stress [19]. DEX can also attenuated kidney injury by preventing NF-κB translocation [20]. Furthermore, DEX can alleviate neuropathic pain in chronic compression injury by suppressing iNOS activity [21]. Notably, DEX has been shown to inhibit neuronal expression of NOS by acting on the imidazoline receptors [22], which are distributed mainly on the surface of renal mitochondria. However, the potential antioxidant molecular mechanism of DEX in LPS-induced early AKI is not known. Moreover, whether the antioxidant effect of DEX on early AKI induced by LPS is mainly through the binding of α_2-ARs or imidazoline receptors (IRs) is not known.

Hence, based on the pharmacological properties of DEX, we investigated the protective effects of DEX on LPS-induced early AKI and the molecular mechanism of inhibition of the AP-1/NF-κB/iNOS/NO signaling pathway. We also used receptor antagonists alone or in combination to regulate the α_2-ARs and IRs, and explored the pharmacodynamic targets of DEX.

2. Materials and methods

2.1. Reagents and antibodies

DEX was obtained from Wuhan Belka Biomedical Co., Ltd. (Wuhan, China). Escherichia coli LPS (serotype 055: B5) was purchased from Sigma Co., Ltd. (Beijing, China) and diluted in saline. Inducible nitric oxide synthase inhibitor (1400W), alpha 2 adrenal receptor (α_2-AR) inhibitor atipamezole (AT), imidazoline receptor inhibitor idazoxan (IDA) were provided by Selleck Co. Ltd. (Shanghai, China). The kits for detecting malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, glutathione (GSH) concentration, iNOS activity and NO content were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Kidney injury molecule 1 (KIM-1) detection kit and RNS assay kit were purchased from Shanghai Enzyme Biotechnology Co., Ltd. (Shanghai, China). LightCycler 480II was purchased from Roche, USA. Primary antibodies against c-Jun, c-Fos, IκB were from Wanlei biotechnology Co. Ltd. (Shenyang, China); rabbit anti-phospho-NF-κB p65 was from Bioss biotechnology Co., Ltd. (Beijing, China). Antibodies against GAPDH, β-Tubulin and PCNA were purchased from Cell Signaling Technology Inc. (MA, USA). All secondary antibodies were obtained from ZSGB-BIO Co., Ltd. (Beijing, China). RIPA, PMSF, Nuclear and Cytoplasmic Protein Extraction Kit and BCA protein assay kit were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China).

2.2. Animals and treatments

Forty-two adult male Sprague Dawley (SD) rats, weighing 180–220 g, were obtained from Experimental Animal Centre of Harbin Medical University (Harbin, China). The rats were acclimated for one week in the laboratory of Northeast Agricultural University (20 ± 2 °C) with a 12 h light/dark cycle. Standard rodent chow and tap water were available ad libitum. All experimental procedures in the present study were approved by the Ethical Committee for Animal Experiments of Northeast Agricultural University, Harbin, China.

Table 1
Primer sequence of the genes were tested in the present study.

Gene	Accession number	Primer sequence (5'-3')
GAPDH	XM_216453	Forward: AGTGCCAGCCTCGTCTCATA Reverse: GATGGTGATGGGTTTCCCGT
c-Jun	NM_021835	Forward: CAGCCGCCGCACCACTTG Reverse: TCCGCCTTGATCCGCTCCTG
c-Fos	XM_234422	Forward: CGCAGAGCATCGGCAGAAGG Reverse: TTCTCGTCTTCAAGTTGATCTGTCTCC
NF-κB	XM_238994	Forward: GGCCATATGTGGAGATCATTGAGCAG Reverse: GCGTCTTAGTGGTATCTGTGCTTCTC
iNOS	XM_220732	Forward: TCTGTGCTAATGCGGAAGGTCATG Reverse: TTGTCACCACCAGCAGTAGTTGTTC

Rats were randomly divided into seven groups (n = 6): control, LPS, 1400W + LPS, DEX + LPS, AT + DEX + LPS, IDA + DEX + LPS and AT + IDA + DEX + LPS. The procedure for the LPS-induced acute kidney injury model was performed according to previous studies [23]. LPS group rats were intraperitoneally (i.p.) injected with LPS (10 mg/kg). In the control group, rats were i.p. injected with an equal volume of physiological saline. In the 1400W + LPS group and the DEX + LPS group, rats were i.p. injected with 1400W (15 mg/kg) and DEX (30 μg/kg), respectively. LPS was administered to both groups 30 min later. Rats in ATI + DEX + LPS group and IDA + DEX + LPS group were injected with ATI (250 μg/kg, i.p.) and IDA (1.5 mg/kg, i.p.) respectively. The operation was the same as that in DEX + LPS group 30 min later. ATI + IDA + DEX + LPS group rats were given ATI and IDA by i.p. injection. After 30 min, the operation was conducted according to DEX + LPS group.

Four hours after the last treatment, all rats were sacrificed to collect blood, urine and kidney samples.

2.3. Preparation of serum, urine supernatant and renal parameters

Collected blood and urine were rested at room temperature for 20 min, then centrifuged at 3000 g for 10 min at 4 °C. The KIM-1 content was determined using assay kit according to the manufacturer's instructions. Blood urea nitrogen (BUN) and serum creatinine (Scr) levels were measured using a UniCel DxC800 Synchron chemistry system (Bekman, USA). The ratio of BUN to Scr was calculated according to the following formula:

$$BUN/Scr = (BUN * 2.8)/(Scr/88.4)$$

2.4. Histopathological analysis of kidney

Part of the kidney tissue was fixed in 10% formalin solution, then cut into 3 mm pieces, embedded in paraffin, and cut into 4–5 μm

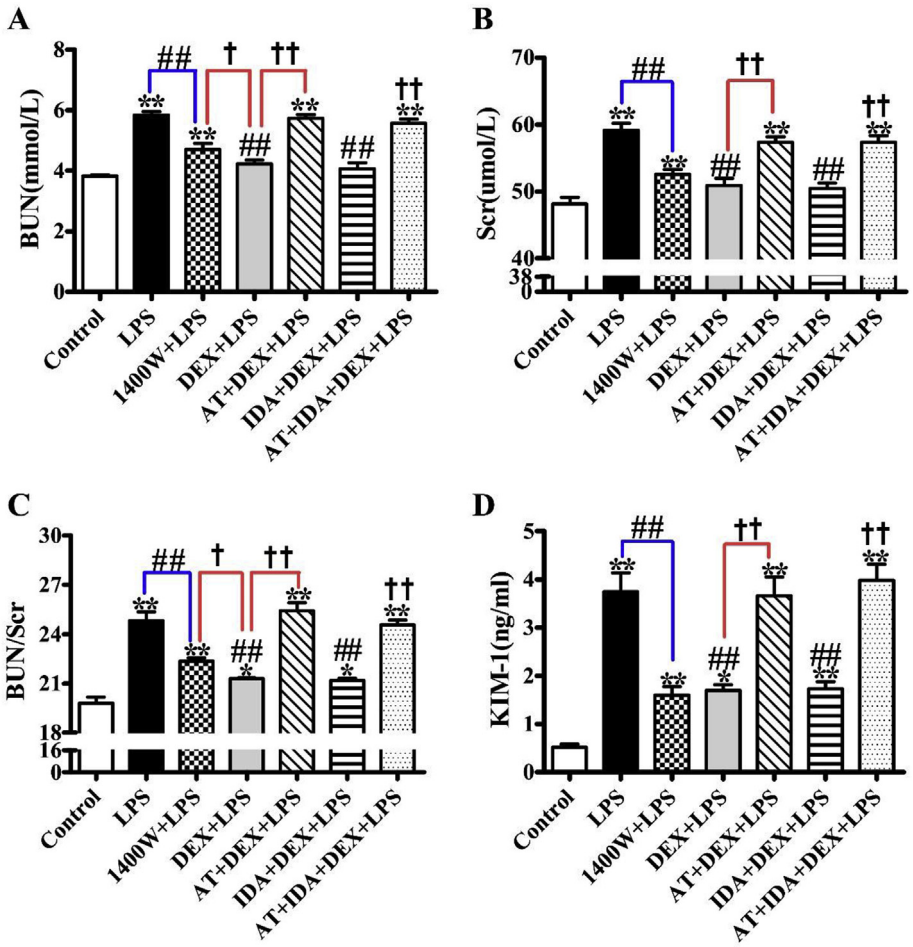

Fig. 1. Effects of DEX on renal function and KIM-1 level in urine. (A) BUN, (B) Scr, (C) BUN/Scr, and (D) KIM-1 levels were detected. Data were presented as mean ± SEM (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ vs control group. $^{\#\#}P < 0.01$ vs LPS group. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs DEX + LPS group.

sections. All sections were stained with hematoxylin and eosin (H&E) and examined by a light microscope (TE2000, Nikon, Japan). An observer who was unclear about the experimental group evaluated the sections at 400x magnification. Five non-continuous fields of the renal cortex and medulla were assessed in each section. The semi-quantitative evaluation of kidney injury is as follows [24]: no injury (0); mild: < 25% (1); moderate: < 50% (2); severe: < 75% (3); and very severe: > 75% (4).

2.5. ELISA assay

Kidney tissue was mixed with 9 vol of PBS and then ground at low temperature to prepare 10% homogenate. After centrifugation at 3000 g for 10 min at 4 °C, the supernatant was used to measure the level of GSH, MDA, NO, RNS and the activity of SOD, *iNOS*. All procedures were performed as described in the assay kit.

2.6. Real-time PCR analysis

Total RNA in renal tissue was extracted with TRIzol reagent. Then reverse transcription of mRNA was performed using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) as described previously [25]. The primers (Table 1), synthesized by Shanghai Bioengineering Co., Ltd. (Shanghai, China), were designed using Primer 5.0 and verified by Blast. qRT-PCR was performed using LightCycler 480II. In this experiment, the response system of 10 μL was used and GAPDH was used as the internal reference for relative quantitative analysis of gene mRNA expression level. Relative quantification was performed according to $2^{-\Delta\Delta Ct}$ method [26,27].

2.7. Western blot analysis

Frozen renal tissues (100 mg) were adequately lysed with RIPA buffer (1 ml) supplemented with PMSF (10 μl) and prepared into homogenized. The supernatant was collected after centrifugation at 12000 g for 10 min at 4 °C. Cytoplasmic and cytoplasmic proteins were extracted with Nuclear and Cytoplasmic Protein Extraction Kit. Protein concentration was determined by BCA protein assay kit according to manufacturer's instructions. Total protein (30 μg) were loaded onto SDS-PAGE gel for electrophoresis and transferred to PVDF membrane as described previously [28,29]. After blocking for 2 h in 5% skim milk TBST powder at room temperature, membranes were incubated overnight in antibody dilutions with anti-antibody at 4 °C. The antibodies used in this study include c-Jun (1:750), c-Fos (1:500), IκB (1:500), P-IκB (1:500), P-NF-κB p65 (1:300), GAPDH (1:1000), β-Tubulin (1:1000) and PCNA (1:1000). They were washed with TBST and then incubated in TBST solution with appropriate concentration of secondary antibody for 2 h. The immune-reactive protein bands were captured using Amersham Imager 600 software (GE, USA) and quantified with ImageJ software.

2.8. Statistical analysis

All data were expressed as mean ± standard error means (SEM). Statistical analysis was performed by one-way ANOVA. Data were analyzed with the PASW Statistics 18 software (SPASS, IL, USA).

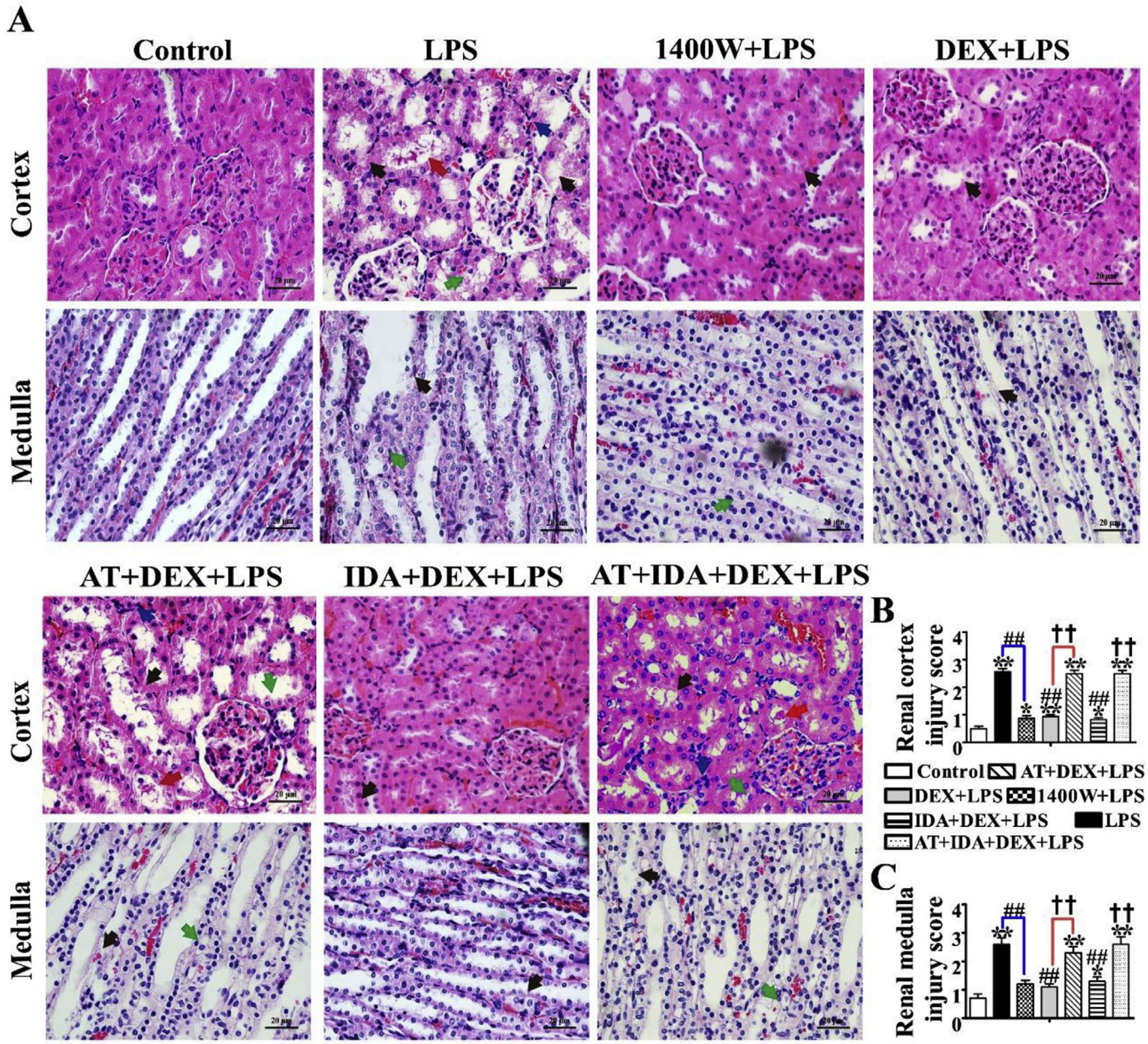

Fig. 2. Effects of DEX on LPS-induced renal histopathology. (A) Representative H&E-stained paraffin sections of renal cortex and medulla in control group, LPS group, DEX + LPS group, AT + DEX + LPS group, IDA + DEX + LPS group and AT + IDA + DEX + LPS group (magnification 200x, bars = 20 μm). Black arrow: tubular necrosis; green arrow: vacuolar degeneration; blue arrow: inflammatory cell infiltration; red arrow: formation of casts. Histopathological scores of renal cortex (B) and medulla (C) in rats. Data were presented as mean ± SEM (n = 6). $^{*}p < 0.05$, $^{**}P < 0.01$ vs control group. $^{\#\#}P < 0.01$ vs LPS group. $^{\dagger\dagger}P < 0.01$ vs DEX + LPS group.

GraphPad Prism 5 (San Diego, California) was used to made graphs. Values with $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of DEX on renal function and KIM-1 level in urine

Blood urea nitrogen (BUN) and serum creatinine (Scr) are the main indicators of renal function. The BUN:Scr ratio is very important for evaluation of renal injury [30]. Hence, we investigated the effects of LPS and DEX on levels of BUN, Scr and the BUN:Scr ratio. Levels of BUN, Scr and the BUN:Scr ratio in the LPS group were increased significantly compared with those in the control group ($P < 0.01$). Interestingly, concentrations of BUN and Scr were both within the normal range. However, after DEX treatment, levels of the indicators mentioned above were attenuated significantly ($P < 0.01$, Fig. 1A–C).

To ascertain if LPS induced AKI, we measured urinary levels of kidney injury molecule (KIM)-1, which is a sensitive indicator of AKI and can reflect early renal tubular injury in AKI specifically [31,32]. The KIM-1 level in the LPS group was significantly higher than that in the control group ($P < 0.01$, Fig. 1D), suggesting that the model of LPS-induced AKI had been established. However, DEX pretreatment reduced the KIM-1 concentration in urine markedly ($P < 0.01$, Fig. 1D).

Interestingly, levels of BUN, SCR, BUN:SCR ratio and KIM-1 were significantly higher in the AT + DEX + LPS group and AT + IDA + DEX + LPS group compared with those in the DEX group ($P < 0.01$), but levels of these indicators were not increased in the

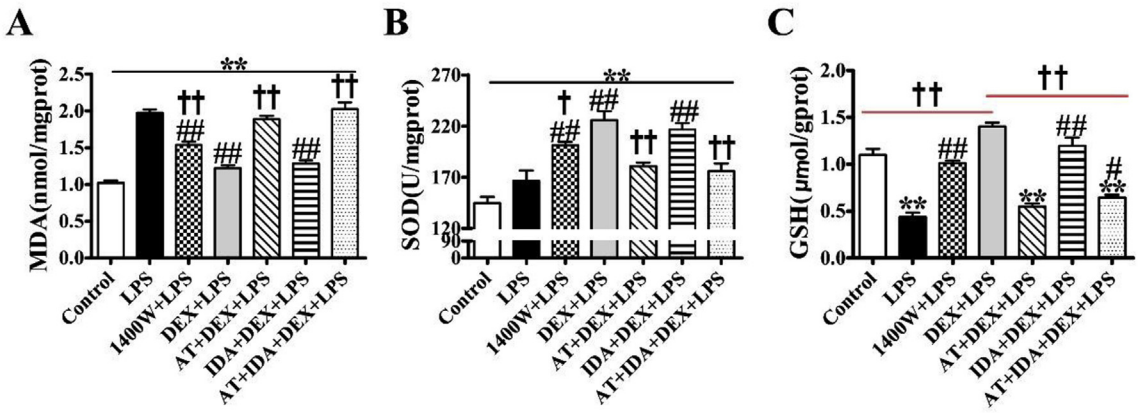

Fig. 3. DEX reduces renal oxidative stress induced by LPS. (A) MDA level, (B) SOD activity and (C) GSH concentration were measured. Data were presented as mean ± SEM (n = 6). $^{**}P < 0.01$ vs control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs LPS group. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs DEX + LPS group.

IDA + DEX + LPS group or 1400W + LPS group (Fig. 1A–D).

3.2. Effects of DEX on LPS-induced renal histopathology

Histopathological changes and injury scores can reflect kidney injury visually. Hematoxylin and eosin (H&E) staining revealed a normal structure of the renal cortex and medulla in the control group (Fig. 2A). In contrast, the pathological changes in the LPS group manifested mainly as tubular necrosis, vacuolar degeneration, infiltration of inflammatory cells, and cast formation. However, the pathologic damage induced by LPS in the renal cortex and medulla was ameliorated significantly by DEX and 1400W ($P < 0.01$, Fig. 2B and C). Interestingly, the effect of DEX on LPS-induced renal histopathology was reversed by the α_2-AR inhibitor AT. Specifically, tubular necrosis, vacuolar degeneration, casts, and infiltration of inflammatory cells were observed in the AT + DEX + LPS group, and AT + IDA + DEX + LPS group. Abnormalities in the renal cortex and medulla of rats in the IDA + DEX + LPS group were not observed (Fig. 2A–C).

3.3. DEX reduces renal oxidative stress induced by LPS

We measured the overall levels of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) in kidney tissues. We found that DEX not only reduced MDA content significantly ($P < 0.01$, Fig. 3A), but also increased SOD activity ($P < 0.01$, Fig. 3B) and the GSH level ($P < 0.01$, Fig. 3C). AT reversed these changes wrought by DEX upon MDA, SOD, and GSH significantly, but IDA did not (Fig. 3A–C). Interestingly, the effect of 1400W pretreatment upon MDA, SOD and GSH was identical to that of DEX, but significantly different from that of DEX ($P < 0.05$).

3.4. Effects of DEX on the AP-1/NF-κB signaling pathway

To investigate the protective molecular mechanism of DEX upon LPS-induced AKI, mRNA levels of c-Jun, c-Fos and NF-κB and their protein expression levels were measured. We also measured expression of the proteins related to IκB, phosphorylated (P) -IκB, nuclear P-NF-κB and cytosolic P-NF-κB. mRNA levels of c-Jun (Fig. 4A), c-Fos (Fig. 4B) and NF-κB (Fig. 4H) and expression of the proteins of c-Jun (Fig. 4C), c-Fos (Fig. 4D), IκB, P-IκB (Fig. 4E), nuclear P-NF-κB (Fig. 4F), and cytosolic P-NF-κB (Fig. 4G) in the LPS group were increased significantly compared with those in the control group ($P < 0.01$), whereas DEX weakened these increases significantly. Notability, AT inhibited the protective effect of DEX, showing that levels of all the indicators mentioned above were increased significantly compared with those of the DEX group ($P < 0.01$, Fig. 4A–H). However, expression of the mRNA and protein of c-Jun, c-Fos and NF-κB in the IDA + DEX + LPS group was not significantly different from that in the DEX + LPS group (Fig. 4A–H).

3.5. DEX inhibits LPS-induced renal iNOS mRNA transcription and NO production

We wished to explore further the potential molecular mechanism of DEX against LPS-induced renal oxidative stress. Hence, we measured the activity of iNOS, the level of iNOS mRNA and the content of NO in renal tissue, and found them to be significantly higher in the LPS group than those in the control group ($P < 0.01$, Fig. 5), whereas DEX treatment reversed these effects significantly. Interestingly, the inhibitor 1400W attenuated iNOS activity and decreased the level of NO significantly, but did not reduce expression of iNOS mRNA. In addition, iNOS activity, expression of iNOS mRNA, and NO level in the AT + DEX + LPS group and AT + IDA + DEX + LPS group were increased distinctly compared with those in the DEX + LPS group ($P < 0.01$), but there was no significant difference between the IDA + DEX + LPS group and DEX group.

3.6. DEX attenuates LPS-induced renal RNS production

Compared with the control group, the RNS level in the LPS group was increased markedly. After DEX treatment, the increase in the RNS level was attenuated. In addition, the RNS level in the 1400W + LPS group was significantly lower than that of the LPS group ($P < 0.01$, Fig. 6). However, AT pretreatment reversed this effect of DEX inhibiting RNS production. Renal levels of RNS in the AT + DEX + LPS group and AT + IDA + DEX + LPS group were significantly higher than those in the DEX + LPS group ($P < 0.01$). Notably, there was no significant difference in the RNS level between the IDA + DEX + LPS group and that in the DEX + LPS group.

4. Discussion

Endotoxins are a common cause of sepsis [33]. LPS, as the main component of endotoxins, has been reported to be involved in the pathological process of sepsis [34]. Therefore, based on previous studies [35], an acute model of sepsis was established by intraperitoneal injection of LPS (10 mg/kg body weight) for 4 h. We found that the BUN concentration in the LPS group was 1.5 -times higher than that in the control group, and that Scr concentration was 1.2-times higher than that in the control group, but both were within the normal range. A possible reason is that, in early-stage AKI, the glomerular filtration rate is ≥ 50% of the normal value, and BUN and SCr concentrations do not increase rapidly, and are susceptible to renal or extrarenal factors. Also, the Scr concentration does not reflect early kidney damage [36].

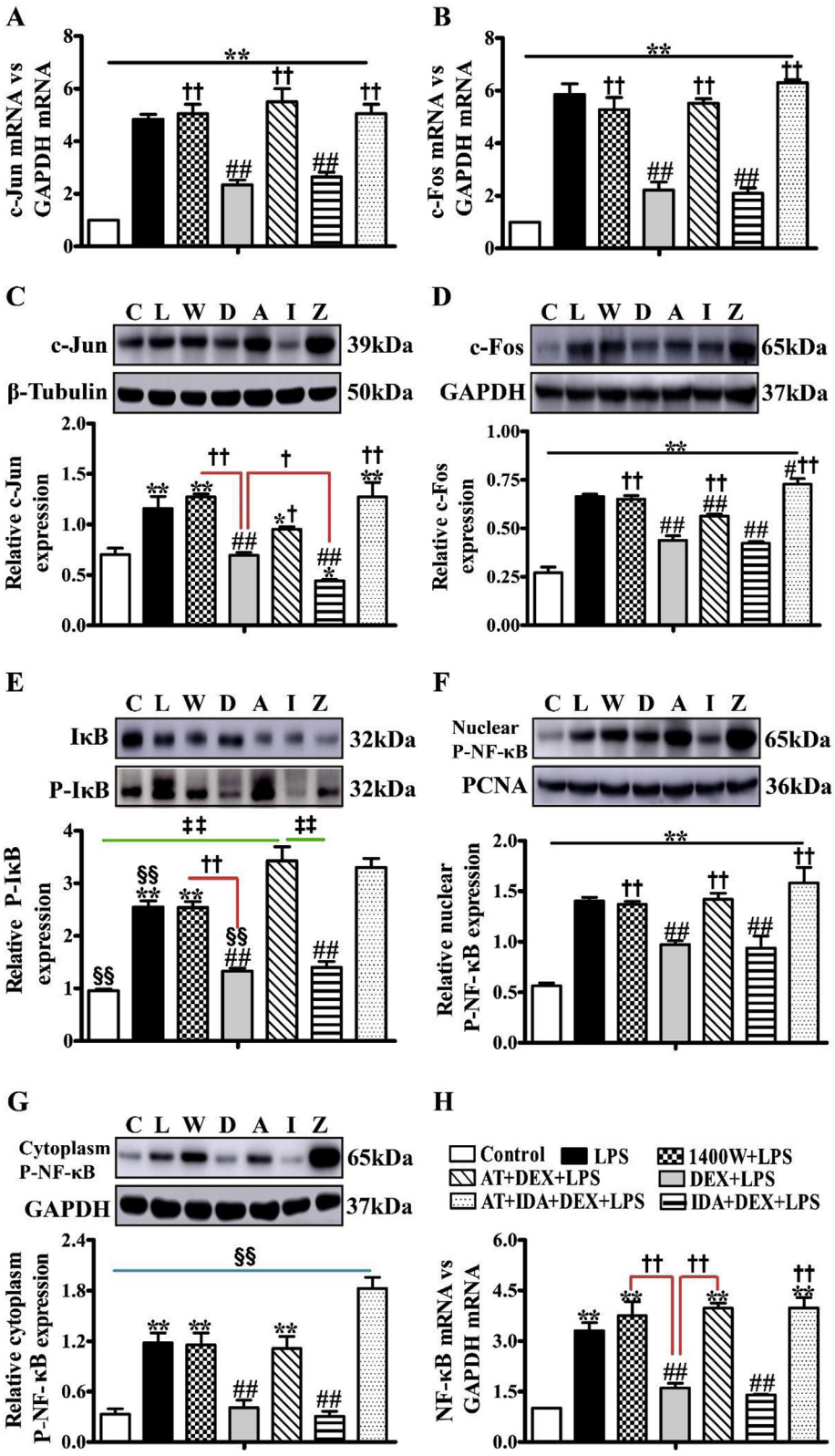

Fig. 4. Effects of DEX on the AP-1/NF-κB signaling pathway. Real-time PCR to evaluate the mRNA levels of c-Jun (A), c-Fos (B) and NF-κB (H) were determined by real-time PCR. Protein expression in c-Jun (C), c-Fos (D), P-IκB (E), nuclear P-NF-κB (F), and cytoplasm P-NF-κB (G). C, L, W, D, A, I and Z respectively represent the control group, LPS group, 1400W + LPS group, DEX + LPS group, AT + DEX + LPS group, IDA + DEX + LPS group and AT + IDA + DEX + LPS group. Data were presented as mean ± SEM (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ vs control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs LPS group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs DEX + LPS group. $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$ vs AT + DEX + LPS. $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ vs AT + IDA + DEX + LPS.

Therefore, we calculated the BUN:Scr ratio, which reflects the extent of impaired renal function. The present study indicated that LPS induced the impairment of renal function, which was ameliorated remarkably by DEX. Moreover, KIM-1 content, an early biomarker of AKI [37], was reduced significantly after DEX treatment. In addition, the histology of the renal cortex and medulla provided further evidence that DEX attenuated LPS-induced early AKI.

In recent years, it has been recognized that LPS-induced AKI is associated with a weakened antioxidant defense system [38]. Indeed, in the present study, the MDA level was increased significantly, and SOD activity and GSH content were decreased markedly, in our LPS-induced AKI model. Interestingly, the levels of MDA, SOD and GSH were restored significantly after DEX treatment. However, the effects of DEX on renal function, histopathology, MDA level, SOD activity and GSH

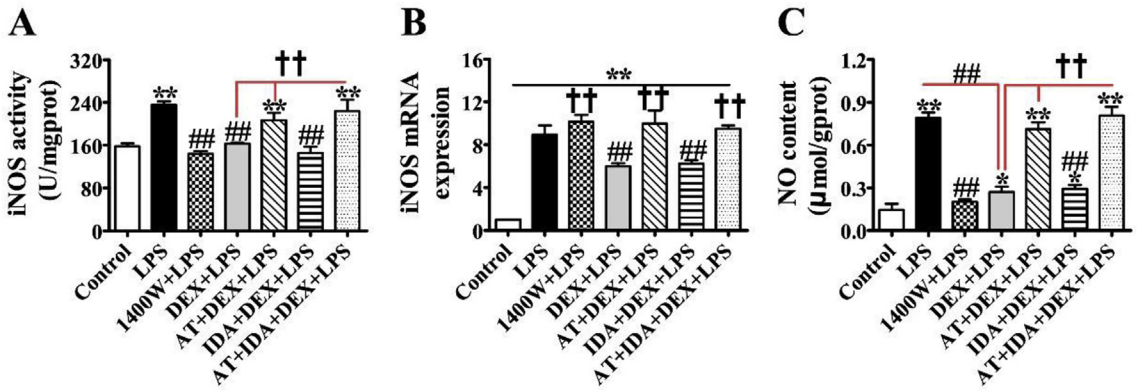

Fig. 5. DEX inhibits LPS-induced renal iNOS mRNA transcription and NO production. (A) Renal iNOS activity. (B) Renal iNOS mRNA expression. (C) NO content. Data were presented as mean ± SEM (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ vs control group, $^{\#\#}P < 0.01$ vs LPS group, $^{\dagger\dagger}P < 0.01$ vs DEX + LPS group.

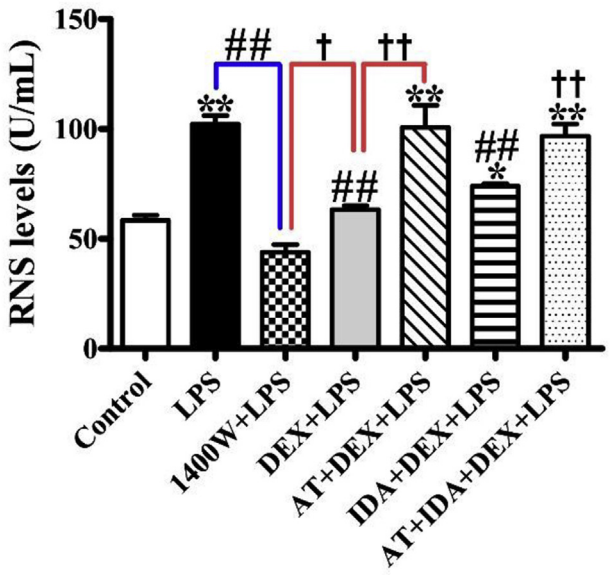

Fig. 6. DEX attenuates LPS-induced renal RNS production. The level of RNS was evaluated. Data were presented as mean ± SEM (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ vs control group, $^{\#\#}P < 0.01$ vs LPS group, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs DEX + LPS group.

content in LPS-induced early AKI were reversed by the α_2-AR inhibitor AT, but not by the IR inhibitor IDA. These results suggest that DEX protects against LPS-induced AKI may by moderating oxidative stress injury, which is related to α_2-ARs.

The underlying molecular mechanism by which DEX exerts antioxidant activity in LPS-induced early AKI is incompletely understood. The literature suggests that LPS binds to lipopolysaccharide binding protein (LBP) and leukocyte differentiation antigen (CD14) on the cell membranes to form a LPS-LBP-CD14 triple complex, which is transduced into the cell by the transmembrane action of toll-like receptor (TLR4), thereby activating AP-1 and NF-κB signaling pathways [39,40]. At rest, AP-1 is present mainly exists in the form of c-Jun homodimer [41]. Concomitantly, NF-κB binds to the NF-κB inhibitory protein (IκB) and is present in the cytosol in an inactive form. However, if stimulated by LPS, IκB kinase (IKK) is activated, which promotes IκB phosphorylation, and results in ubiquitination and proteasomal degradation of IκB, thereby releasing NF-κB and transferring it to the nucleus [42,43]. AP-1 is transformed from homodimer to heterogeneous c-Jun and c-Fos [44]. In the present study, DEX attenuated the mRNA and protein expression of c-Jun and c-Fos induced by LPS significantly. DEX also inhibited IκB phosphorylation, weakened the expression of NF-κB mRNA, and blocked activation of NF-κB, as evidenced by a reduction of protein expression of P-NF-κB in the nucleus and cytoplasm. Collectively, these results demonstrated that DEX attenuates LPS-induced early AKI possibly by inhibiting AP-1 and NF-κB signaling pathways.

NF-κB [45] and AP-1 [46] have been reported to possess recognition sites for the iNOS mRNA promoter. After the cascade amplification of NF-κB and AP-1 signaling pathways induced by LPS, the transcription level of iNOS gene was improved, resulting in substantial production of iNOS [47]. Unexpectedly, in the current study, iNOS activity and expression of iNOS mRNA in renal tissue were increased markedly after LPS injection. However, DEX attenuated the increase in iNOS activity and expression of iNOS mRNA significantly. To explore further if iNOS is an important factor in LPS-induced renal oxidative stress, we blocked iNOS transcription with the iNOS inhibitor 1400W. We found that 1400W pretreatment improved renal function, attenuated the KIM-1 level, alleviated histological damage of the renal cortex and medulla significantly, decreased the MDA concentration, enhanced SOD activity, and increased GSH content. Thus, suppression of iNOS transcription may be an important protective mechanism for DEX against LPS-induced early AKI.

Notably, increased activity of iNOS leads to excessive production of NO in organisms, thereby reducing vasodilation and causing hypotension [48]. In addition, NO can inhibit the activity of antioxidant enzymes [49] and increase oxidative stress in organisms [12]. NO is a free radical, so excessive production of NO inhibits oxidative phosphorylation and reduces oxygen consumption [50]. NO can also interact with other ROS to form more toxic active substances (e.g., peroxide-nitrite anions) to cause damage to DNA, proteins and cell membranes, thereby resulting in increased mitochondrial permeability [51]. In the present study, DEX suppressed the production of NO significantly. In addition, 1400W pretreatment reduced NO content in renal tissue significantly. Our results suggest that DEX protects against LPS-induced early AKI possibly by inhibiting iNOS transcription and thereby attenuating NO production.

Increasing evidence has demonstrated that NO is an important component of RNS [52]. Excessive NO can cause RNS to be produced in large quantities, leading to damage due to lipid peroxidation [53]. However, studies have shown that oxidative stress can activate NF-κB [54]. The latter is transferred to the nucleus, and iNOS is transcribed to produce iNOS, NO, and RNS, which induce further oxidative stress, causing the body to enter a “vicious circle” and aggravate kidney damage. In the present study, DEX and 1400W reduced the level of RNS significantly. These results suggest that RNS inhibition may be a molecular mechanism by which DEX attenuates oxidative stress in LPS-induced early AKI. Notably, oxidative stress activates the inflammatory pathway that, in turn, promotes the production of oxidizing substances [55]. LPS induces AKI by activating oxidative stress and inflammation, but whether oxidative stress occurs first is not known, and requires

further research.

Studies have revealed that DEX attenuates kidney damage by inhibiting the inflammatory response in an α_2-AR dependent manner [56]. Furthermore, DEX has been reported to exert an analgesic effect in combination with IRs [22]. DEX is an agonist of α_2-ARs and IRs [57]. However, whether DEX has a protective role by binding α_2-ARs or IRs in LPS-induced early AKI is not known. The present study was the first to explore if DEX improves LPS-induced AKI through α_2-ARs or IRs. Our results showed that inhibition of ARs alone had the same effect as double antagonism of ARs and IRs, thereby reversing the effects of DEX on AP-1, NF-κB, iNOS, NO and RNS in LPS-induced renal tissue. However, inhibition of IRs alone was not effective. In brief, DEX ameliorated LPS-induced early AKI by binding to α_2-ARs rather than IRs.

5. Conclusion

Our results revealed that DEX protects against LPS-induced early AKI possibly by binding to α_2-ARs, inhibiting IκB phosphorylation, preventing NF-κB activation, down-regulating expression of NF-κB mRNA, and blocking AP-1 translocation. These actions would reduce iNOS activity, decrease expression of iNOS mRNA, attenuate NO production, lower the level of RNS, and enhance the antioxidant stress system. This present study illuminated the potential protective molecular mechanism of DEX in early AKI from the perspective of oxidative stress, and provides useful evidence for application of DEX as treatment for early AKI.

Conflicts of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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