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Original Research Article

Dexmedetomidine inhibits oxidative stress in sepsis-induced acute kidney injury in rats by regulating GSK-3 β/Nrf2/ARE axis

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Abstract

Purpose: *To determine the effect of dexmedetomidine on sepsis-mediated acute kidney injury (SAKI) in rats.*
Methods: *Twenty-four SD rats were randomly divided into blank untreated control group, model group given lipopolysaccharide at a dose of 10 mg/kg, dexmedetomidine (Dex) group, and glycogen synthase kinase-3 beta (GSK-3β) inhibitor group given sb216763. In addition, Dex and inhibitor groups received the same dose of lipopolysaccharide as model group. There were 10 rats in each group. Pathological changes in kidney tissue, and the levels of related oxidative stress indices were determined.*
Results: *Compared with the model group, granular degeneration of renal tubular epithelial cells was significantly reduced in Dex group, and the population of exfoliated cells decreased ($p < 0.05$). The levels of malondialdehyde (MDA) and reactive oxygen species (ROS) in dexmedetomidine and GSK-3β inhibitor groups were significantly reduced, while superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) activities were markedly raised, relative to model group ($p < 0.05$). Protein levels of p-GSK-3β and Nrf2 markedly were raised in dexmedetomidine and GSK-3β inhibitor groups ($p < 0.01$). After intervention with Dex and GSK-3β inhibitor, there were marked upregulations in mRNA and protein expression levels of heme oxygenase-1 (HO-1), when compared with model group ($p < 0.05$).*
Conclusion: *Dexmedetomidine ameliorates SAKI injury in rats via suppression of oxidative stress. Its mechanism of action may be related to the regulation of GSK-3β/Nrf2/ARE signaling pathway. This finding may be a novel approach to the clinical management of SAKI.*

Keywords: *Dexmedetomidine, GSK-3β/Nrf2/ARE signal pathway, Sepsis, AKI, Oxidative stress*

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INTRODUCTION

Sepsis refers to the systemic inflammatory response syndrome caused by infection, trauma and other factors which can cause secondary damage to multiple organs and tissues of the body. Sepsis is a serious threat to life: it is associated with high mortality, and it has become a major cause of death of non-cardiac patients in ICU [1]. In sepsis-induced acute stress, the

kidney is one of the most vulnerable target organs. A study showed that septic acute kidney injury (SAKI) occurred in 42 % of patients, making it an independent risk factor for poor prognosis in critically ill patients [2].

Previous studies have found that ischemia or perfusion insufficiency is a major contributor to the pathogenesis of SAKI, which is believed to be caused by renal tubular epithelial cell necrosis due to renal hemodynamic changes. In addition, endotoxin-mediated inflammatory response, renal cell apoptosis, and glomerular embolism are involved in the pathophysiological mechanism of SAKI [3]. In recent years, more and more studies have found that oxidative stress is also implicated in SAKI. During oxidative stress, large amounts of renin and angiotensin strongly constrict renal vessels, enhance ischemia and hypoxia of renal cell tissue, and cause renal dysfunction [4]. Dexmedetomidine (Dex), a novel α2 adrenergic receptor agonist widely used in anesthesia, intensive care and other aspects, exerts sedative, analgesic and sympathetic inhibitory effects [5]. Although it has been clinically found that dexmedetomidine exerts a protective effect against SAKI, the mechanism involved in this protective effect is not fully understood. This study was carried out to investigate anti-oxidative stress effect of Dex in SAKI in rats.

EXPERIMENTAL

Reagents and equipment

The chemicals and equipment employed, and the suppliers (in parenthesis) were: dexmedetomidine (Shanghai Jizhi Biochemical Technology Co. Ltd); lipopolysaccharide (Beijing Kairiji Biotechnology Co. Ltd); GSK-3β Inhibitor (Beijing Biolaibo Technology Co. Ltd); RIPA lysate (Harbin Xinhai Gene Testing Co., Ltd.); BCA protein concentration kit (Shanghai Kanglang Biotechnology Co. Ltd.); gel configuration kit (Shanghai Ruichu Biotechnology Co. Ltd); chemiluminescence kit (Shanghai Xinfan Biotechnology Co. Ltd.); TRIzol (Shanghai Yiyan Biotechnology Co. Ltd.); fluorescence quantitative PCR dyestuff (Beijing Biolaibo Technology Co., Ltd), and related antibodies (Wanjie Biotechnology Co. Ltd).

The others were spectrophotometer (Beijing Jinda Sunshine Technology Co. Ltd); high-speed, low-temperature centrifuge (Sichuan Shuke Instrument Co. Ltd); high-pressure steam sterilizer (Shanghai Yuyan Scientific Instrument Co. Ltd); tissue slicer (Shanghai Yuyan Scientific Instrument Co. Ltd); inverted microscope (Beijing Jiayuan Xingye Technology Co. Ltd); vertical electrophoresis tank (Shanghai Sangsun Biotechnology Co. Ltd); vortex oscillator (Guangzhou Haohan Instrument Co. Ltd); enzyme label (Beijing Anmag Trading Co. Ltd); real-time fluorescence quantitative system (Xi 'an Tianlong Technology Co. Ltd), and electrophoresis apparatus (Shanghai Sangsun Biological Technology Co. Ltd).

Animals and specimens

Twenty-four healthy male SD rats weighing 220 ~ 240 g were supplied by Beijing Vitong Lihua Experimental Animal Technology Co. Ltd, and were raised in the Animal Experimental Center of our hospital at temperature of 22 ~ 24 ℃, and relative humidity of 50 ~ 60 %. The rats were allowed *ad libitum* access to feed and water. After adaptive feeding for one week, they were used for the experiment. The rats were assigned to 4 groups: blank control, model, dexmedetomidine, and GSK-3β inhibitor groups (n= 10 each). This research received approval (no. 20190873) from the Animal Ethical Committee of The Fourth Affiliated Hospital of Gansu University of Chinese Medicine, and was conducted according to “Principles of Laboratory Animal Care” [6].

Blank control rats were intraperitoneally given lipopolysaccharide (10 mg/kg). Rats in the model group received dexmedetomidine (30 μg/kg), in addition to lipopolysaccharide intraperitoneally, while those in GSK-3β inhibitor group were given SB216763 at a dose of 10 mg/kg, in addition to lipopolysaccharide (10 mg/kg). Four hours after drug administration, the rat kidneys were excised under isoflurane anesthesia.

Morphological examination of tissues

Tissue lesions in kidney tissue of rats were determined histologically. Samples of kidney tissue of rats in each group were taken from both sides. The right kidney was stored at -80℃, while the left kidney tissue was sliced into pathological sections with surgical scissors. After washing with PBS, the tissues were subjected to formaldehyde fixation and paraffin embedding. Routine sections (about 4-μm thick) were sliced using a histological microtome, and the slides were stained with hematoxylin-eosin, followed by examination under an inverted microscope to identify pathological changes.

Determination of stress indices

After centrifugation, the supernatant from tissue homogenate was subjected to assay of levels of

superoxide dismutase (SOD), while serum malondialdehyde (MDA) was estimated with barbital method. Reactive oxygen (ROS) was estimated in single cell suspension using DCFH-DA (10 μM). The fluorescence intensity of renal tissue cells in each group was measured at 525 nm in a fluorescein micrometer. Glutathione (GSH) levels were determined with microenzyme label assay, while catalase (CAT) activity was assayed with UV method.

Determination of protein expression levels

Protein expression levels were determined with Western blot assay. Total protein was extracted from renal tissue with RIPA buffer and protein quantification was carried out using BCA kit. Equal amounts of protein (30-μg portions) were separated with SDS-PAGE, followed by transfer onto PVDF membranes which were then sealed by incubation with 5 % skimmed milk powder at laboratory temperature for 2 h, prior to incubation overnight at 4℃ with the primary antibodies P-GSK-3β, GSK-3β, Nrf2, HO-1 and NQO1.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Real-time quantitative PCR method was used to detect the expression of related genes: Total RNA was extracted with TRIzol reagent, and its content and integrity were determined. The RNA samples were reverse-transcribed to cDNA, and the cDNA was amplified using RT-PCR, with GAPDH as the internal reference gene. The CT values of each target gene were obtained. The PCR protocol was: 95 ℃ and pre-denaturation for 10 min, 95 ℃ for 15 sec, 60 ℃ for 45 sec, 72 ℃ for 2 min, and a total of 40 cycles at 4 ℃. The relative expression of each gene was quantified using the $2^{-\triangle\triangle CT}$ method. The primers and sequences used are listed in Table 1.

Table 1: Primers and sequences used in PCR

Gene	F/R	Sequence of primers
HO-1	Forward	ATCGTGCTCGCATGTAAC
	Reverse	GAGCTCCTCAAACAGCTCAA
NQO1	Forward	CAGCGGCTCCATGTACT
	Reverse	GACCTGGAAGCCACAGAAG
GAPDH	Forward	ATGACCCCTTCATTGACCTCA
	Reverse	GAGATGACCCTTTTGGCT

Statistical analysis

All data were analyzed with SPSS 21.0 software package. Measurement data that conformed to normal distribution are presented as mean ± SD. Multi-group data comparison was done with ANOVA, while Student-Newman-Keuls-Q (SNK-Q) test was used for paired comparison. Statistical significance of differences were assumed at $p < 0.05$.

RESULTS

Pathological changes in renal tissues

The structures of renal tubules and glomeruli in blank group were intact and clear, and no obvious pathological changes were observed. In the model group, the granules of renal tubule epithelial cells were degenerated, the structure of renal tubule was seriously damaged, and there were many protein tubule types. However, granule degeneration of renal tubular epithelial cells in the dexmedetomidine group was markedly decreased, relative to model group, and there were fewer exfoliated cells. In the GSK-3β inhibitor group, the granule degeneration of renal tubular epithelial cells was significantly reduced, with reduced infiltration of renal interstitial inflammatory cells. These results are shown in Figure 1.

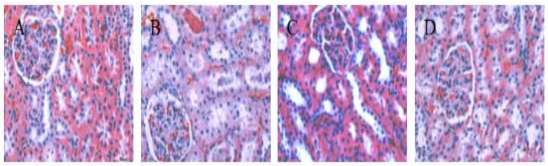

Figure 1: Pathological lesions in renal tissues of rats in (A) blank, (B) model, (C) dexmedetomidine, and (D) GSK-3β inhibitor groups

Oxidative stress indices in renal tissue of rats

Table 1 shows that, compared with blank rats, MDA and ROS levels in kidney tissue of model rats were significantly raised, while the levels of SOD, GSH and CAT were significantly decreased ($p < 0.01$). However, levels of MDA and ROS were significantly decreased in dexmedetomidine group and GSK-3β inhibitor group, while SOD, GSH and CAT levels were markedly raised, relative to model rats.

P-GSK-3β and Nrf2 protein levels in renal tissues

As presented in Table 2 and Figure 2, kidney protein levels of p-GSK-3β and Nrf2 in model rats were up-regulated, relative to the blank control ($p < 0.01$). In dexmedetomidine and GSK-3β inhibitor groups, the protein contents of p-GSK-3β and Nrf2 were significantly higher than those of the model group.

Table 1: Levels of oxidative stress indices in renal tissue

Group	MDA (nmol/mL)	SOD (U/mg protein)	ROS (ng/L)	GSH (μmol/L)	CAT (U/mg protein)
Blank	1.50±0.01	110.25±15.47	386.50±56.39	21.47±2.58	138.41±10.70
Model	2.67±0.18*	47.32±8.96*	492.30±20.78*	14.26±2.89*	50.22±3.69*
Dexmedetomidine	1.61±0.07#	152.02±25.74#	390.27±35.02#	26.96±1.41#	150.30±15.47#
GSK-3β inhibitor	1.80±0.12#	140.20±26.82#	405.74±50.86#	24.33±1.63#	146.20±16.07#
F	130.80	30.95	8.01	36.63	87.02
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

*$P < 0.05$, vs blank; #$p < 0.05$, vs model

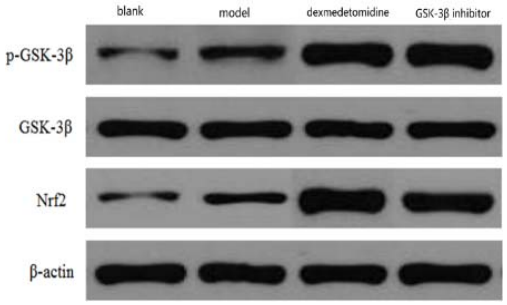

Figure 2: Kidney protein expression levels of p-GSK-3β and Nrf2.

Table 2: p-GSK-3β/GSK-3β ratio and Nrf2 in renal tissues of rats (mean ± SD, n = 6)

Group	p-GSK-3β/GSK-3β	Nrf2
Blank	0.41±0.10	0.24±0.05
Model	0.59±0.12*	0.35±0.08*
Dexmedetomidine	0.83±0.16#	0.92±0.13#
GSK-3β inhibitor	0.85±0.14#	0.75±0.10#
F	15.17	64.74
P-value	<0.001	<0.001

*$P < 0.05$, vs blank; #$p < 0.05$, vs model

Renal protein and mRNA levels of HO-1 and NQO1

There were higher mRNA and protein concentrations of HO-1 and NQO1 in rat renal tissue in model group than in blank control ($p < 0.05$). In dexmedetomidine and GSK-3β inhibitor groups, mRNA and protein levels of HO-1 and NQO1 were markedly higher than those in the model group. These data are shown in Figure 3 and Table 3.

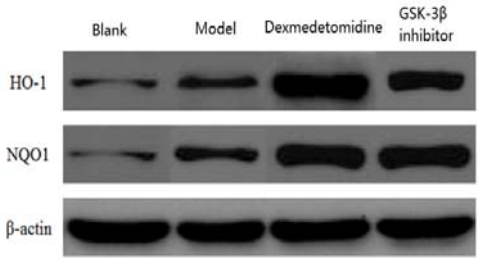

Figure 3: Protein levels of HO-1 and NQO in renal tissues in each group

DISCUSSION

The pathogenesis of sepsis is not yet fully understood. However, AKI is one of its major complications. The kidney is the major regulator of water and electrolyte metabolism under normal physiological conditions. It performs an important role of maintaining cellular electrolyte balance and protecting lung function during sepsis. The incidence of SAKI is on the rise worldwide, and may be as high as 51 - 66.9 % in sepsis patients, with mortality up to 70 % [7]. The balance between oxygen acquisition and consumption affects the oxygenation status of renal tissues. Sepsis gives rise to renal microvascular dysfunction and oxygen balance disorders, leading to intracellular ROS overload, cell damage, and organ dysfunction [8].

Table 3: Quantified mRNA and protein expressions of HO-1 and NQO1 in kidney tissues of rats (mean ± SD, n = 6)

Group	HO-1		NQO1	
	mRNA	*Protein*	*mRNA*	*Protein*
Blank	1.00±0.17	0.23±0.03	1.00±0.09	0.21±0.10
Model	1.11±1.51	0.41±0.06*	1.05±0.13	0.39±0.16*
Dexmedetomidine	7.78±1.23#	0.82±0.15#	4.87±1.26#	0.57±0.20#
GSK-3β inhibitor	5.96±1.04#	0.58±0.10#	3.86±1.17#	0.59±0.18#
F	57.88	40.95	31.30	7.02
P-value	<0.001	<0.001	<0.001	0.002

Oxidative stress injury is an important early marker of renal tubule injury caused by sepsis, and it is closely related to reduced renal perfusion area. Although the treatment methods of SAKI are varied, the treatment outcome is still unsatisfactory, and mortality rate is still high.

The pathophysiology of SAKI is very complex: it is has been closely linked to renal ischemia reperfusion injury, nitric oxide theory, cell apoptosis theory, endothelin effect, and direct endotoxin injury [9]. Alpha 2 (α2) adrenergic receptor is widely distributed in organs, tissues and cells. Dexmedetomidine, a frequently-used sedative drug, has the same efficacy as α2 general adrenoceptor agonist. It exerts a variety of biological effects such as anti-sympathetic stimulatory, antioxidant, immune-regulatory, anti-inflammatory and anti-apoptotic effects. Thus, it has a significant advantage in the treatment of sepsis and organ protection [10,11].

Studies outside China have shown that dexmedetomidine strengthens the body's oxidative stress defenses by reducing the activity of nitric oxide synthase, reducing the release of carbon monoxide, suppressing ROS levels, and increasing the concentrations of SOD and GSH [12].

In this study, the model rats were treated with dexmedetomidine, and it was found that the granularity of renal tubular epithelial cells was markedly decreased, relative to model group, with a small amount of exfoliated cells, suggesting that dexmedetomidine significantly reversed renal damage caused by LPS. Furthermore, compared with the model group, the levels of MDA and ROS were markedly reduced, while the SOD, GSH and CAT levels were markedly raised in dexmedetomidine group, suggesting that dexmedetomidine reduced oxidative stress damage to kidney by increasing SOD concentration, thereby exerting a protective effect.

In recent years, it has been found that GSK-3β is involved in acute liver injury caused by oxidative stress. Therefore, it was speculated that GSK-3β might be involved in SAKI [3]. Mammalian cells contain GSK-3β which regulates a variety of signaling proteins and transcription factors. In a variety of cells, GSK-3β is involved in the regulation of the movement of Nrf2 in and out of the nucleus. As a transcription factor that regulates oxidative response, Nrf2 is involved in oxidative stress and metabolism of exogenous proteins. It binds to ARE in cells to form the Nrf2/ARE signaling pathway involved in activation of the expression of the antioxidant proteins HO-1 and NQO1, and enhancement of the antioxidant capacity of cells [14-17].

This present research has shown that under the influence of dexmedetomidine and GSK-3β inhibitor, the protein expression levels of p-GSK-3β and Nrf2, as well as the mRNA and protein expressions of HO-1 and NQO1 were significantly increased, when compared with the model group. These results suggest that dexmedetomidine inhibits the expression of GSK-3β protein, upregulates the expression of Nrf2, and activates the downstream antioxidant protective genes, thereby enhancing the ability of the body to combat oxidative stress.

CONCLUSION

Dexmedetomidine ameliorates SAKI-induced damage in rats by decreasing oxidative stress via a mechanism involving the regulation of GSK-3β/NRF2/ARE signaling pathway. This finding may provide a new strategy for the treatment of SAKI in humans.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Chengjing Si and Yan Jing designed the study, supervised the data collection, and analyzed the data. Yan Jing interpreted the data and prepared the manuscript for publication. Li Yao, Weicui Du, Jia Liu, Rongrong Yang, Wanchang Zhou, Xiaolin Xu, Jingjing Cao and Lichao Zhang supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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