

# DEXMEDETOMIDINE AMELIORATES ISCHEMIA/REPERFUSION-INDUCED ACUTE KIDNEY INJURY BY INHIBITING ENDOPLASMIC RETICULUM STRESS VIA THE NRF2 SIGNALING PATHWAY

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**Abstract: Objective** To investigate the effect of dexmedetomidine (Dex) on the expression of nuclear factor Erythroid 2-related factor 2 (Nrf2) and the activation of endoplasmic reticulum stress (ERS) in renal tissues during ischemia/reperfusion (I/R). **Methods** C57BL/6 mice were selected and divided into Sham group, I/R group, Dex group and (Dex+I/R) group. Serum urea nitrogen (BUN) and creatinine (Cr) were detected, and the expressions of Nrf2, ER chaperone BiP (GRP78) and C/EBP homologous protein (CHOP) were detected by qPCR. **Results** Compared with the Sham group, the serum levels of BUN and Cr were significantly elevated in I/R group, meanwhile, the expressions of Nrf2, GRP78 and CHOP in renal tissues were also significantly increased ( $P < 0.05$ ). Dex pretreatment significantly lowered serum levels of BUN and Cr, as well as inhibited intracellular expressions of GRP78 and CHOP, while obviously increased intracellular Nrf2 expression in renal tissues of I/R-treated mice ( $P < 0.05$ ). **Conclusions** Dex has a protective effect on renal I/R injury, its mechanism may be related to the activation of Nrf2 expression and further inhibit the activation of ERS-related apoptotic pathways.

**Keywords:** dexmedetomidine, ischemia/reperfusion, Nrf2, endoplasmic reticulum stress

## INTRODUCTION

AKI is a usual clinical complication characterized by an a sudden decrease in glomerular filtration rate. Despite significant advances in supportive care, including renal replacement therapy, the five-year mortality after AKI remains close to 50%[1]. Ischemia/reperfusion (I/R) is a major cause of AKI, which can occur in many clinical settings, including partial nephrectomy, renal transplantation, heart surgery and hypoperfusion[2]. Several signaling pathways that may be potential targets to I/R injury, such as apoptosis, necrosis, inflammatory cell infiltration, release of reactive oxygen species (ROS) and active mediators, have been investigated[3, 4]. However, the molecular mechanism of renal I/R injury is still unclear, and various therapeutic methods have little effect on it. Therefore, in-depth study of the potential mechanism of renal I/R injury remains an urgent clinical problems to be solved. Previous studies have shown that apoptosis and necrosis mainly occur in tubular epithelial cells following ischemic AKI[5]. Necrosis and apoptosis are the most important modes of cell death in AKI, especially apoptosis is the main mode of renal cell

death during acute ischemia, which is closely related to oxidative stress, inflammatory response and tissue damage caused by reperfusion[6, 7]. In addition to cell death receptor pathway and mitochondrial pathway, endoplasmic reticulum stress (ERS)-related apoptotic pathway was considered as a new apoptotic pathway and has attracted more and more attention. ERS is believed to be the early or initial response of cells to stimulation or injury, it is an important inducement of cell apoptosis.

Our previous studies have confirmed that cell apoptosis induced by over-activation of ERS plays a vital role in renal I/R injury, however no clinical drugs have been found to effectively inhibit ERS activation currently. Dexmedetomidine (Dex), a highly selective  $\alpha$ -2 adrenoceptor agonist, is commonly used as a clinical sedative in anesthesia and ICU[8]. In vivo and vitro studies, researcher have found that Dex can play a protective role in I/R injury of organs, such as liver[9], lung[10], heart[11], kidney[12] and intestinal[13], which may related to its properties of antioxidant, anti-inflammatory and anti-apoptosis, but its specific mechanism has not been clarified. This study aims to clarify the effect of

Dex on I/R-induced AKI and its related mechanism, so as to provide new ideas for the development of effective drugs to treat AKI.

## MATERIALS AND METHODS

### Materials

8-10 weeks old, weighing 20–25g, C57BL/6 mice were obtained from Medical Experimental Animal Center of Guangdong Province (China). Trizol reagent were purchased from Invitrogen. ReverTra Ace qPCR RT Master Mix and SYBR® Green Realtime PCR Master Mix were purchased from TOYOBO. Dex was provided by Jiangsu Hengrui Medicine Co., Ltd.(Jiangsu, China).

### Animals and treatment

All study protocols were approved by the Institutional Animal Care and Use Committee of Guangzhou Medical University. All mice were housed with 12h light/dark cycles at room temperature, and allowed free access to standard laboratory diet and distilled water. Mice were divided into 4 groups by random number table method: Sham operation group (Sham group), I/R group, Dex+I/R (10ug/kg, i.p., 1h before renal ischemia) group and Dex group. The mice treated with I/R were undertook bilateral renal pedicle

**Table 1** Primer sequences of genes.

Gene	Forward	Reverse
GRP78	CTATTCCTGCGTCGGTGTGT	GCAAGAACTTGATGTCCTGCT
CHOP	CCCTCGCTCTCCAGATTCCAGTC	TCGTTCTCCTGCTCCTTCTCCTTC
Nrf2	AAGCACAGCCAGCACATTCTCC	TGACCAGGACTCACGGGAAGCTTC
GAPDH	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA

### Statistical analysis

Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL) software. Multiple comparisons among different groups were analyzed using one-way ANOVAs followed by Tukey post hoc comparisons. Quantitative data are presented as mean±SD.  $p < 0.05$  was considered as statistically significantly different.

## Results

### Dex significantly improved renal function of I/R-treated mice

To some extent, the changes of Cr and BUN could indirectly reflect the degree of kidney injury. The serum levels of Cr and BUN in I/R group were significantly higher than those in Sham group, however, Dex pretreatment 1 hour before ischemia could significantly reduce the serum levels of Cr and BUN (Fig. 1A and 1B).

### Dex obviously inhibited the expression of ERS-related genes

GRP78 and CHOP are key effectors of ERS-related apoptotic pathways, and the increased expression of GRP78 and CHOP suggest the activation of ERS.

clamping for 45 min and reperfusion for 24h. The mice in Sham group and Dex group were identical to the surgery manner without renal pedicle occlusion. Blood samples were collected at the corresponding time points, then mice were sacrificed and the kidneys were obtained for further examinations.

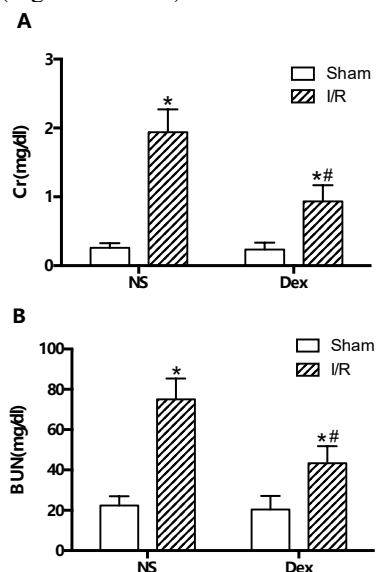
### Assessment of renal function

The blood samples were obtained from orbits. Serum urea nitrogen (BUN) and creatinine (Cr) were measured with an automatic biochemical analyzer (Epoch Chemray 240, Shenzhen, China) to evaluate renal function.

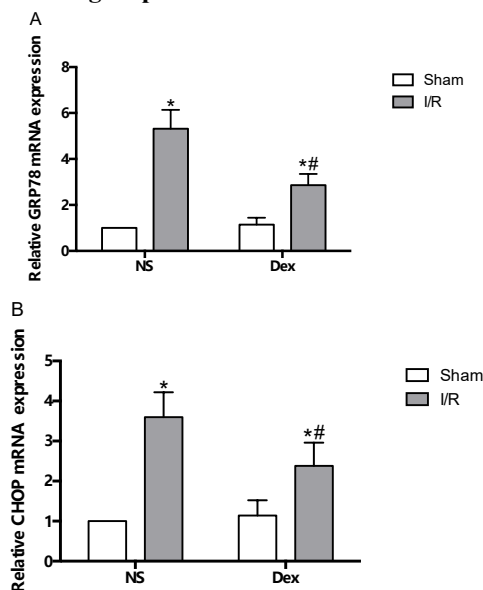
### Quantitative Real-Time PCR (qPCR)

The total RNA in renal tissues were extracted with Trizol reagent. The quality and concentration of RNA were detected with Nanodrop-1000 spectrophotometer. ReverTra Ace qPCR RT Master Mix was used for reverse transcription. SYBR® Green Realtime PCR Master Mix and Roche LightCycler 1.1 qPCR were used to quantitatively analyze the changes of ER chaperone BiP (GRP78) mRNA, C/EBP homologous proteins (CHOP) mRNA and nuclear factor Erythroid 2-related factor 2 (Nrf2) mRNA. GAPDH was used as housekeeping gene. GCAAGAACTTGATGTCCTGCT

Compared with the Sham group, the expressions of GRP78 and CHOP in renal tissues of mice with I/R treatment were significantly increased, and Dex pretreatment 1 hour before ischemia could obviously inhibit the expressions of GRP78 and CHOP in renal tissues (Fig. 2A and 2B).



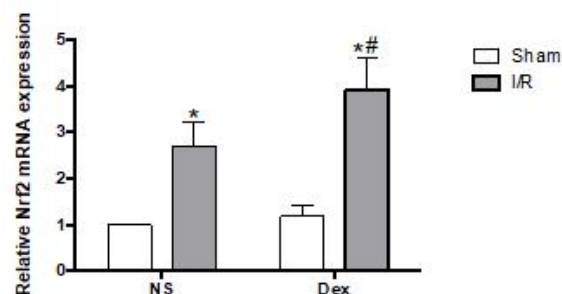
**Figure 1. Dex significantly improved renal function of I/R-treated mice. A. Serum Cr level of mice in different groups. B. Serum BUN level of mice in different groups. Data are the mean values  $\pm$  SD (n=5). \*p < 0.05 vs Sham group, #p < 0.05 vs I/R group.**



**Figure 2. Dex obviously inhibited the expression of ERS-related genes in I/R-treated mice. A. GRP78 expression in mice of different groups. B. CHOP expression in mice of different groups. Data are the mean values  $\pm$  SD (n=5). \*p < 0.05 vs Sham group, #p < 0.05 vs I/R group.**

#### *Dex markedly improve the Nrf2 expression of I/R-treated mice*

Nrf2 is one of the main regulators of endogenous antioxidant defense. The expressions of Nrf2 was analyzed by qPCR. Compared with the Sham group, the Nrf2 expression in renal tissues of I/R-treated mice was significantly increased, and Dex pretreatment 1 hour before ischemia further improved the Nrf2 expression in renal tissues of I/R-treated mice (Fig. 3)



**Figure 3. Dex markedly improve the Nrf2 expression of I/R-treated mice. Nrf2 expression in mice of different groups. Data are the mean values  $\pm$  SD (n=5). \*p < 0.05 vs Sham group; #p < 0.05 vs I/R group.**

## DISCUSSION

As previous studies have found that oxidative stress induced by renal I/R injury is an important inducer of ERS activation and renal pathological injury, We detected the expressions of GRP78 and CHOP, two key genes of ERS-related apoptosis pathway. Under physiological conditions, the ERS sensing protein (PERK, IRE1, ATF6) binds to ER chaperone GRP78 and remains inactive. When ERS occurs, these proteins dissociate from GRP78, activate downstream signaling pathways to inhibit protein translation, up-regulate molecular chaperones to enhance protein folding, promote the degradation of unfolded or misfolded proteins, promote cell homeostasis, and reduce cell damage[14]. Appropriate ERS have a self-defense function to protect cells. However, excessive or severe ERS activates apoptotic signaling pathways such as CHOP, caspase12 and JNK[15, 16]. CHOP, as a pro-apoptotic transcription factor, is the most distinctive regulator of the ERS transition to apoptosis and plays a key role in ERS response. In this study, we found that I/R significantly increased the expression of GRP78 and CHOP in renal tissues, while Dex pretreatment significantly inhibited the expression of GRP78 and CHOP, which suggesting that Dex may weaken I/R-induced ERS activation.

Nrf2 is one of the main regulators of endogenous antioxidant defense. In the case of oxidative stress, Nrf2 promotes the expression of a variety of antioxidant genes (including enzyme/non-enzyme antioxidant genes), as well as enters the nucleus through translocation and binds to nuclear antioxidant reaction elements (AREs), regulating the transcription of downstream target genes. Nrf2 plays a major role in maintaining cell redox homeostatic state. Activation of Nrf2 may be a potential therapeutic target for organ I/R injury[17, 18]. Recent studies have suggested that Dex reduces oxidative stress injury, which may be related to the activation of Nrf2 expression. Dex may reduce acute lung injury by regulating the expression of Nrf2 and its downstream pathway target genes to improve endogenous antioxidant capacity and reduce acute lung injury[19]. In this study, we tested the Nrf2 expression in renal tissues of mice in different groups, the results show that I/R can activate Nrf2 expression in renal tissue, while Dex pretreatment was helpful to further enhance Nrf2 expression, suggesting that Dex may enhance the antioxidant capacity of cells, which may be related to the protective effect of Dex on renal tissue.

In summary, Dex has a protective effect on renal I/R injury, its mechanism may be related to the activation of Nrf2 expression and further inhibit the activation of ERS-related apoptotic pathways. This study provides a theoretical basis for the correct selection of clinical drugs and the protection of kidney under the clinical background of renal I/R injury.

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## REFERENCES

1. Ueda N, Kaushal GP, Shah SV. Apoptotic mechanisms in acute renal failure. *The American journal of medicine* 2000, **108**(5): 403-415.
2. Choi YJ, Zhou D, Barbosa ACS, Niu Y, Guan X, Xu M, Ren S, Nolin TD, Liu Y, Xie W. Activation of Constitutive Androstane Receptor Ameliorates Renal Ischemia-Reperfusion-Induced Kidney and Liver Injury. *Molecular pharmacology* 2018, **93**(3): 239-250.
3. Friedewald JJ, Rabb H. Inflammatory cells in ischemic acute renal failure. *Kidney international* 2004, **66**(2): 486-491.
4. Kieran NE, Rabb H. Immune responses in kidney preservation and reperfusion injury. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research* 2004, **52**(5): 310-314.
5. Shi Y, Melnikov VY, Schrier RW, Edelstein CL. Downregulation of the calpain inhibitor protein calpastatin by caspases during renal ischemia-reperfusion. *American journal of physiology Renal physiology* 2000, **279**(3): F509-517.
6. Price PM, Safirstein RL, Megyesi J. The cell cycle and acute kidney injury. *Kidney international* 2009, **76**(6): 604-613.
7. Daemen MA, van 't Veer C, Denecker G, Heemskerk VH, Wolfs TG, Clauss M, Vandenamee P, Buurman WA. Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. *The Journal of clinical investigation* 1999, **104**(5): 541-549.
8. Weerink MAS, Struys M, Hannivoort LN, Barends CRM, Absalom AR, Colin P. Clinical Pharmacokinetics and Pharmacodynamics of Dexmedetomidine. *Clinical pharmacokinetics* 2017, **56**(8): 893-913.
9. Chen Z, Ding T, Ma CG. Dexmedetomidine (DEX) protects against hepatic ischemia/reperfusion (I/R) injury by suppressing inflammation and oxidative stress in NLRC5 deficient mice. *Biochemical and biophysical research communications* 2017, **493**(2): 1143-1150.
10. Jiang L, Li L, Shen J, Qi Z, Guo L. Effect of dexmedetomidine on lung ischemia-reperfusion injury. *Molecular medicine reports* 2014, **9**(2): 419-426.
11. Yoshikawa Y, Hirata N, Kawaguchi R, Tokinaga Y, Yamakage M. Dexmedetomidine Maintains Its Direct Cardioprotective Effect Against Ischemia/Reperfusion Injury in Hypertensive Hypertrophied Myocardium. *Anesthesia and analgesia* 2018, **126**(2): 443-452.
12. Li Q, Chen C, Chen X, Han M, Li J. Dexmedetomidine attenuates renal fibrosis via  $\alpha$ 2-adrenergic receptor-dependent inhibition of cellular senescence after renal ischemia/reperfusion. *Life sciences* 2018, **207**: 1-8.
13. Zhang XY, Liu ZM, Wen SH, Li YS, Li Y, Yao X, Huang WQ, Liu KX. Dexmedetomidine administration before, but not after, ischemia attenuates intestinal injury induced by intestinal ischemia-reperfusion in rats. *Anesthesiology* 2012, **116**(5): 1035-1046.
14. Shu S, Zhu J, Liu Z, Tang C, Cai J, Dong Z. Endoplasmic reticulum stress is activated in post-ischemic kidneys to promote chronic kidney disease. *EBioMedicine* 2018, **37**: 269-280.
15. Shen X, Zhang K, Kaufman RJ. The unfolded protein response--a stress signaling pathway of the endoplasmic reticulum. *Journal of chemical neuroanatomy* 2004, **28**(1-2): 79-92.
16. Lai E, Teodoro T, Volchuk A. Endoplasmic reticulum stress: signaling the unfolded protein response. *Physiology (Bethesda, Md)* 2007, **22**: 193-201.
17. Tonelli C, Chio IIC, Tuveson DA. Transcriptional Regulation by Nrf2. *Antioxidants & redox signaling* 2018, **29**(17): 1727-1745.
18. Zhang R, Xu M, Wang Y, Xie F, Zhang G, Qin X. Nrf2-a Promising Therapeutic Target for Defending Against Oxidative Stress in Stroke. *Molecular neurobiology* 2017, **54**(8): 6006-6017.
19. Chen Y, Bian W, Xu B. Pretreatment with dexmedetomidine alleviates lung injury in a rat model of intestinal ischemia reperfusion. *Molecular medicine reports* 2020, **21**(3): 1233-1241.