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Dexmedetomidine protects against ischemia/reperfusion injury in rat kidney

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Abstract. – OBJECTIVES: Ischemia/reperfusion (I/R) injury in the kidney during perioperative period remains the leading cause of acute renal failure. The purpose of this experimental study was to determine the role of dexmedetomidine (Dex) on renal I/R injury in rats.

MATERIALS AND METHODS: Male Wistar rats, subjected to renal ischemia for 45 min, were either untreated or treated with dexmedetomidine 30 min prior to renal ischemia. A sham-operated group served as the control. Renal function [serum creatinine, blood urea nitrogen, serum Cystatin C and neutrophil gelatinase-associated lipocalin (NGAL)], histology, apoptosis and expression of the phosphorylations of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) were assessed.

RESULTS: The animals treated with dexmedetomidine improved renal functional recovery, especially reducing the level of serum Cystatin C and NGAL at early time after ischemia, attenuated histological lesions, reduced tubular epithelial apoptosis and inhibited the phosphorylation of JAK2 and its downstream molecule STAT3, contributing to ameliorating renal I/R injury.

CONCLUSIONS: Our data suggest that antiapoptosis effect contributes to the renoprotection of dexmedetomidine, via inhibiting JAK2/STAT3 signaling pathway partially.

Key Words:
Renal ischemic-reperfusion injury, Dexmedetomidine, Cystatin C, NGAL; JAK2/STAT3 signaling pathway

Introduction

The adverse effects of ischemia/reperfusion injury are now well known in both basic and clinical research fields. However, the morbidity and mortality rate from perioperative acute renal failure has been reported to change little and remained quite high since the past 30 years in high risk patients undergoing high risk surgery[1-3]. The evidence supports that I/R injury remains the first most common cause of inpatients' acute renal failure[4,5]. Renal ischemia/reperfusion injury secondary to prolonged reduction of blood flow is a prominent and common clinical problem and a complex syndrome involving several mechanisms, including renal vasoconstriction, tubular damage and glomerular injury[1,2]. The acute inflammatory response initiated by ischemia and reperfusion together is characterized by the induction of an inflammatory cytokine cascade, expression of adhesion molecules, and cellular infiltration, contributing to the impairment and the death of renal cells as a combination of both necrosis and apoptosis[6]. Therefore, aggressive protection and recognizing renal injury during its early stage after high risk surgery is likely to delay the deterioration of renal function and improve prognosis.

The most frequently used clinical markers of renal failure include creatinine, urea nitrogen and glomerular filtration rate (GFR) measured by dynamic methods. Among them, the golden markers for assessing acute renal dysfunction are serum creatinine and renal creatinine clearance rate. However, serum creatinine production changes significantly according to age, sex, muscle mass, dietary intake, and clearance by tubular secretion. There has evidence that serum creatinine is not a reliable biomarker of renal function to diagnose acute kidney injury (AKI) due to these limitations. Indeed, it rises slowly over 48 to 72 hours following renal dysfunction until steady-state equilibrium has been reached[7,8]. Recent studies reported that Cystatin C and NGAL (neutrophil gelatinase-associated lipocalin) were independent markers for early predicting acute kidney injury, prognosis and mortality. The production of Cystatin C or NGAL in the body is a stable process that is not altered by limitations as displayed above. Therefore, Cystatin C and NGAL may be more sensitive than creatinine in predicting renal tubular injury and severity of

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AKI[7,9]. Cystatin C and NGAL were used as biomarkers here in our study for early detection of acute renal injury induced by ischemia/reperfusion.

Although numerous studies have demonstrated that various protective methods may protect against renal impairment associated with ischemia/reperfusion during perioperation, renal I/R injury is still a major problem in surgical and anesthesia procedures involving the aorta operation[2]. Improving the ability of the kidney to tolerate I/R injury associated with surgery would have important implications. Dexmedetomidine (Dex), a highly selective α_2-adrenoreceptor agonist, has sedative, analgesic, sympatholytic and hemodynamic stabilizing properties. Previous studies demonstrate that it has renoprotective effects which can be abolished by α_2-adrenoreceptor antagonists[10,11]. It has been proven that the development of renal I/R injury is characterized by the activation of Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway which is involved in transducing signals for various cytokines and growth factors. Once the cytokines bind to their cognate receptor, STATs (STAT1, 2, 3, 4, 5a and 5b, 6) are activated by members of the JAK family of tyrosine kinase (JAK1, 2 and 3 and Tyk2). Of all the JAK/STAT pathways, JAK2 signaling through STAT3 are the best studied in diseases affecting the kidney[12,13]. Previous studies have shown that dexmedetomidine have the cytoprotective effect involving in extracellular regulated protein kinases (ERK) signaling pathway[14]. Moreover, interference with ERK and STAT signaling may play an important role in myocardial I/R injury[15]. However, the effect of dexmedetomidine on Cystatin C and NGAL, and the underlying mechanism of its renoprotection have not been elucidated by many researchers yet.

Therefore, this study observed the effect of dexmedetomidine on biomedical indicators, tissue necrosis and cell apoptosis, especially (for the first time, to our knowledge) the effect on Cystatin C and NGAL, key biomarkers for early detection of acute renal injury, and the expression of the phosphorylation of JAK2 and STAT3 in renal I/R injury of rat model experiments.

Materials and Methods

Animal preparation

Male Wistar rats (220 to 310 g) obtained from the Animal Experiment Centre of our institution were housed in standard conditions. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University. The animals were allowed free access to standard rodent chow and water until 12 h prior to surgery when food (but not water) was removed. All procedures described here had prior approval from the Institute Animal Ethics Committee.

All animals were anesthetized with pentobarbital sodium (65 mg/kg, i.p.) and anesthesia was maintained by supplementary intraperitoneal injections of the same anesthetic. Animals were prepared surgically for renal I/R as described previously[3,4]. In all rats, a rectal probe was inserted to monitor body temperature, which was maintained at 38 ± 1°C by a heating blanket. A midline laparotomy was performed and bilateral renal pedicles were carefully isolated. Middle abdomen incision was closed in two layers and covered with antibiotic ointment after surgery. The animals were allowed to recover from anesthesia, remaining 48 hours in a controlled-environment room with food and water freely available. At the end of all experiments, animals were subjected to euthanasia by an overdose of pentobarbital sodium.

Experimental protocol

Animals were randomly divided into three groups in this study. Rats in the sham-operated group (n = 8) underwent sham surgery only. Rats in groups ischemia/no-Dex (n = 8) and ischemia/Dex (n = 8) were subjected to bilateral renal ischemia for 45 min by clamping both renal pedicles with non-traumatic vascular clamps. Renal ischemia was judged by the color change, and renal blood flow was measured by Doppler before and after clamping. Rats in sham-operated and ischemia/no-Dex group received saline via intraperitoneal injection and rats in the ischemia/dex group received dexmedetomidine (25 g/kg, i.p.) 30 min before renal ischemia. Blood samples were obtained from the tail vein at 0, 12, 24, and 48 h after reperfusion. Serum creatinine, blood urea nitrogen, serum Cystatin C and NGAL were measured as biochemical indicators of impaired glomerular function[7,9,16]. Tissue samples were received from the kidneys of three groups at 48 h after reperfusion for histological examination and apoptosis assay.

Measurement of biochemical parameters

Blood samples (1 ml) that were collected via the tail vein at 0, 12, 24, and 48 h after reperfusion were placed in the refrigerator at 4°C for 20 min and centrifuged (6000 rpm for 3 min) to separate serum. These serum samples were assayed immediately or stored at –20°C until further study. Serum creatinine, blood urea nitrogen, serum Cystatin C and NGAL were used as indicators of impaired renal function. Cystatin C (Rat Cystatin C ELISA kits; Bioporto Diagnostics, Gentofte, Denmark) and NGAL (Rat NGAL ELISA kits; Bioporto Diagnostics, Gentofte, Denmark) assay employed the quantitative sandwich enzyme immunoassay technique.

Histological examination and apoptosis assay

For histological examination, tissue samples from the kidneys in group's sham-operated, ischemia/no-Dex, and ischemia/Dex were removed at 48 h after reperfusion. They were bisected along the long axis, cut into 3 equal-sized slices, and fixed with 10% buffered-formalin solution overnight. After dehydration and embedding in paraffin, 4-μm sections were stained with hematoxylin and eosin. Renal injury (acute tubular necrosis) was graded on a scale of 0 to 4, as described by Jablonski et al[17]: 0 = normal kidney; 1 = minimal damage (< 5% involvement of the cortex or outer medulla); 2 = mild damage (5-25% involvement of the cortex or outer medulla); 3 = moderate damage (25-75% involvement of the cortex or outer medulla); 4 = severe damage (> 75% involvement of the cortex or outer medulla).

The death of tubular epithelial cells was detected by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining. 3-4 m-thick paraffin sections were deparaffinized and washed with phosphate buffered saline (PBS), then treated with proteinase K at 37°C for 15 minutes. Sections were incubated with a mixture of nucleotides and TdT (Terminal deoxynucleotidyl Transferase) enzyme for 60 min at 37°C. The sections were washed with PBS and subsequently incubated with anti-digoxygenin conjugated to horseradish peroxidase for 30 minutes at 37°C. They were then stained by the substrate diaminobenzidine (DAB). As a negative control, sections were incubated in the absence of TdT enzyme. Apoptosis was also evaluated using previously defined morphological criteria[18]. These criteria include eosinophilic cytoplasm, cytoplasmic shrinkage, nuclear fragmentation, nuclear chromatin condensation, membrane-bound cellular blebbing and formation of apoptotic bodies.

Morphological assessment and apoptosis assay were performed by two pathologists who were unaware of the treatment which the animals had received.

Western blotting analysis

Frozen renal tissue from each animal was crushed and lysed, subsequently homogenized, then centrifuged at 12000 rpm for 20 min. The extracted proteins were separated by SDS-PAGE and electrophoretically transferred on to nitrocellulose membranes (Hybond, Amersham Biosciences, Little Chalfont, UK). The membranes were incubated with antibodies against p-JAK2, p-STAT3 (1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and β-actin (1:5000; Abcam, Cambridge, MA, USA) at 4°C and then incubated with respective horseradish peroxidase coupled secondary antibodies (1:5000 dilution; Jackson Immunoresearch Laboratories, West Grove, PA, USA). The protein bands were detected by an enhanced chemiluminescent detective system (Amersham Biosciences UK Ltd., Little Chalfont, UK) and were quantified using the Quantity One software package (Bio-Rad Laboratories, UK).

Statistical analysis

All values are expressed as mean ± SD. Statistical analysis was carried out using the SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Biochemical parameters were analyzed by analysis of variance (ANOVA) followed by Bonferroni *post hoc* test where appropriate. Histopathologic examination was analyzed by the Kruskal-Wallis test. A value of $p < 0.05$ was considered statistically significant.

Results

Effect of dexmedetomidine on renal dysfunction caused by I/R

Compared to sham-operated animals, those animals which underwent renal I/R exhibited a significant elevation in serum creatinine and blood urea nitrogen concentrations at 24, 48 h and a significant elevation in serum concentration of Cystatin C and NGAL at 12, 24 and 48 h after reperfusion ($p < 0.05$), reflecting a significant degree of renal dysfunction. In comparison, dexmedetomidine administration decreased the level of serum creatinine and blood urea nitrogen at 24, 48 h after reperfusion, also reduced the level of serum Cystatin C and NGAL at 12, 24, 48 h after reperfusion ($p < 0.05$) (Figures 1 A and B, 2 A and B).

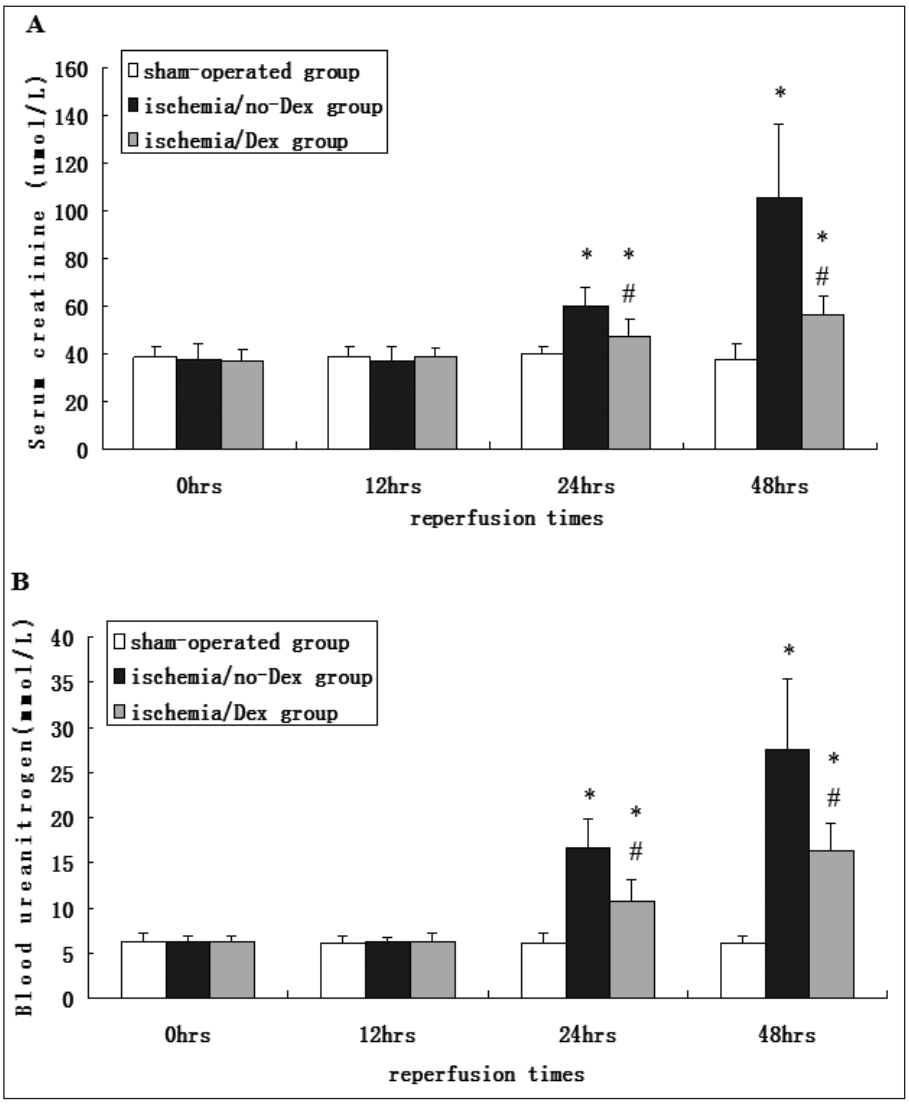

Figure 1. Effect of dexmedetomidine on renal function. Serum creatinine and blood urea nitrogen at 0, 12, 24, and 48 h after reperfusion from sham-operated, ischemia/no-Dex and ischemia/Dex groups (all n = 24). Dexmedetomidine attenuated a significant increase of serum creatinine **(A)** and blood urea nitrogen **(B)** after renal ischemia/reperfusion. Data are expressed as mean ± SD. $^{*}p < 0.05$ compared with sham-operated group; $^{\#}p < 0.05$ compared with ischemia/no-Dex group.

Effects of dexmedetomidine on histologic alterations caused by renal I/R

The sham-operated group did not show any morphological changes (Figure 3A). Renal I/R caused marked renal alteration in renal histology compared with kidneys taken from sham-operated animals. Specifically, this included widespread degeneration tubular architecture, tubular dilation, tubular cell swelling, cellular vacuolization, pyknotic nuclei, severe tubular necrosis and luminal congestion (Figure 3B). By contrast, renal section obtained from rats that were administrated of dexmedetomidine before ischemia significantly attenuated severe damage of renal tissue morphology (Figure 3C). The histopathological score of the rats' renal in all groups are presented in Figure 3D. The scores of the kidneys from animals in ischemia/no-Dex and ischemia/Dex groups were higher than those in sham-operated group ($p < 0.01$). However, the scores of the kidneys from rats in ischemia/Dex group were lower than those in ischemia/no-Dex group ($p < 0.05$).

Dexmedetomidine administration reduced tubular apoptosis

To confirm that dexmedetomidine is indeed required for anti-apoptosis action, a TUNEL assay was used (Figure 4A, B and C). Apoptosis-assessed morphology was not seen in the animals of sham-operated group. In comparison, kidneys from animals that were subjected to renal I/R showed extensive nuclear changes consistent with apoptotic cell death. They were exhibited more severe tissue damage and much more TUNEL-positive cells than those in

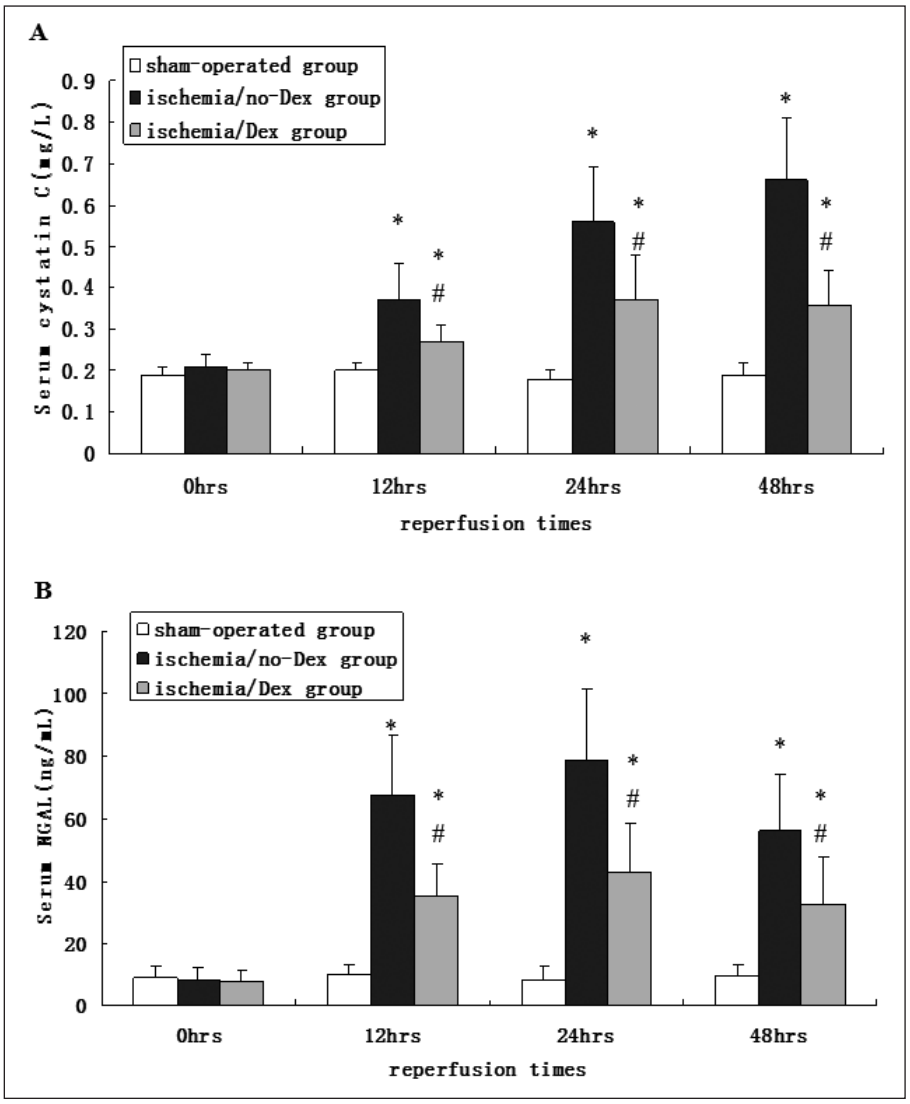

Figure 2. Effect of dexmedetomidine on renal function. Serum Cystatin C ***(A)*** and NGAL ***(B)*** at 0, 12, 24, and 48 h after reperfusion from sham-operated, ischemia/no-Dex and ischemia/Dex groups (all n = 24). Dexmedetomidine blunted a significant increase of serum Cystatin C and NGAL after renal ischemia/reperfusion. Data are expressed as mean ± SD. $^{*}p < 0.05$ compared with sham-operated group; $^{\#}p < 0.05$ compared with ischemia/no-Dex group.

ischemia/Dex group. In the renal tissue sections of ischemia/Dex group, only scattered TUNEL-positive cells were observed; thus, it demonstrated the less degree of apoptosis for dexmedetomidine pretreated animals.

The effect of dexmedetomidine on JAK2 and STAT3

To verify that dexmedetomidine protects against renal I/R injury via inhibiting JAK2/STAT3 signaling pathway, we performed western blot to analyse p-JAK2 and p-STAT3. In the kidney of sham-operated rats, there is a low grade of p-JAK2 and p-STAT3. The expression of p-JAK2 and p-STAT3 proteins increased significantly in the ischemia/no-Dex and ischemia/Dex groups ($p < 0.05$ vs. the sham group). They were inhibited by dexmedetomidine treated prior ischemia in the ischemia/Dex group ($p < 0.05$ vs. the ischemia/no-Dex group) (Figure 5).

Discussion

Our study provides evidence that dexmedetomidine protected the kidney against ischemia/reperfusion injury in a rat model. Dexmedetomidine as a single intraperitoneal bolus injection prior to renal ischemia significantly attenuated tubular dysfunction and injury caused by severe ischemia (45 min) and reperfusion in the animals. It decreased the levels of biochemical indicators in the plasma, preserved tissue morphology; reduced the numbers of apoptosis and inhibited the phosphorylation of JAK2 and STAT3 in the kidney induced by I/R injury. In

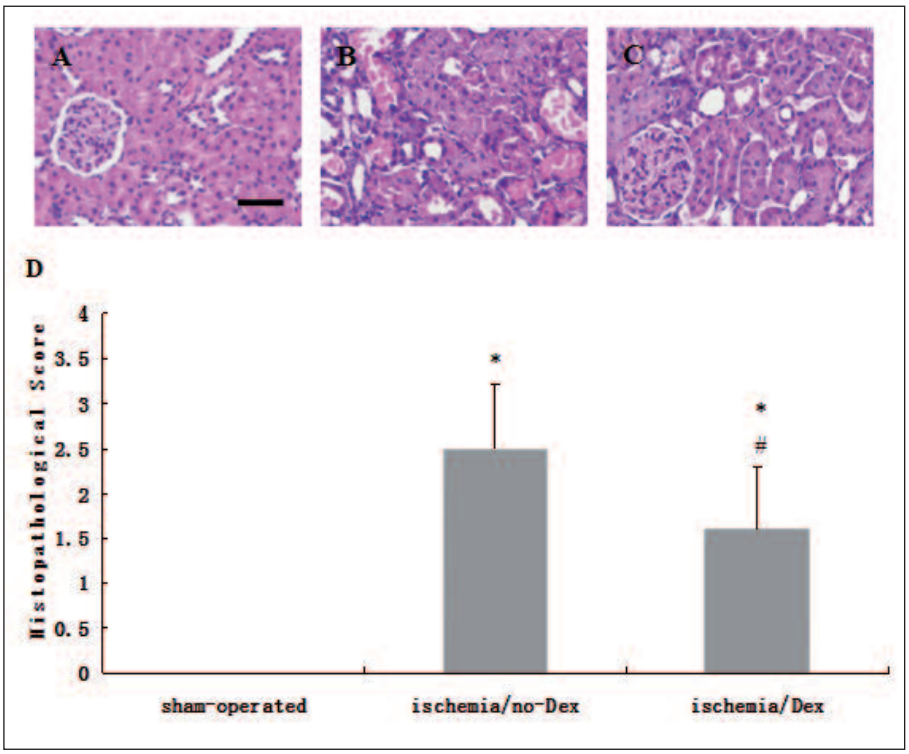

Figure 3. Effect of dexmedetomidine on I/R-induced renal injury. Microscopic findings of kidneys after reperfusion at 48 h from sham-operated **(A)**, ischemia/no-Dex **(B)** and ischemia/Dex **(C)** groups. Histopathologic examination was performed using hematoxylin and eosin. Renal sections from ischemia/no-Dex rats showed more severe lesions of tubular necrosis than those from sham-operated and ischemia/Dex rats. Administration of dexmedetomidine before ischemia significantly attenuated severe damage of renal tissue morphology. Semi-quantitative assessment of the histological lesions based on tubular necrosis **(D)**. Bar = 100 μm. Values represent scores±SD. $^{*}p < 0.01$ compared with sham-operated group; $^{\#}p < 0.05$ compared with ischemia/no-Dex group. Magnification × 200.

this study, we have reported (for the first time, to our knowledge) the effect of dexmedetomidine on Cystatin C and NGAL, which are involving in early determining renal dysfunction, and the activations of JAK2 and STAT3 proteins.

It has been reported that dexmedetomidine has the renoprotective effect against ischemia/reperfusion injury[10,11]. Our data also showed that dexmedetomidine improves renal function recovery, reduces the number of apoptotic tubular epithelial cells and attenuates renal tissue necrosis and histological lesions. However, the effects of dexmedetomidine on early biomarkers are still rare and the mechanism of its renoprotective action remains unknown.

Serum creatinine is not a reliable biomarker of renal function to diagnose acute kidney injury due to some limitations such as age, sex, muscle mass, dietary intake, and clearance by tubular secretion[7,8]. The production of Cystatin C and NGAL in the body are not altered by limitations as displayed above, more sensitive than creatinine in predicting renal tubular injury and severity of AKI and were independent markers for ear-

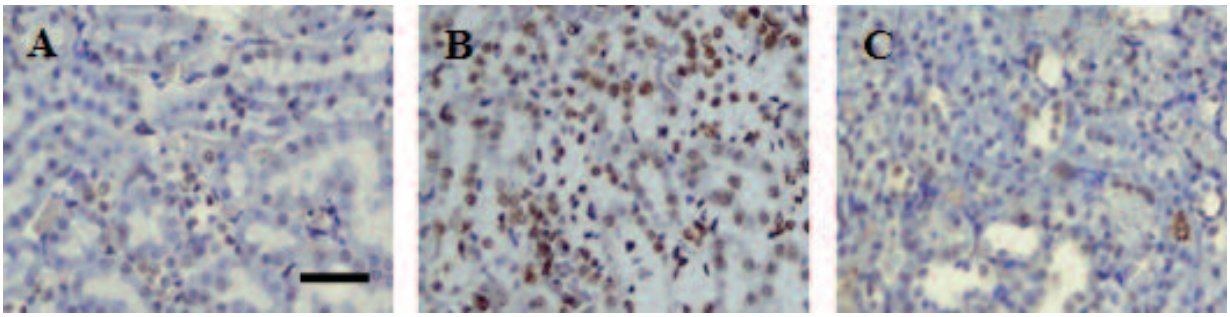

Figure 4. Effect of dexmedetomidine pretreatment on I/R-induced cell apoptosis. Apoptosis was evaluated by TUNEL staining of kidney sections after 48 h of reperfusion. Apoptosis-assessed morphology was not observed in the animals of sham-operated group **(A)**. Increased number of TUNEL-positive cells were seen in ischemia/no-Dex group at 48 h after reperfusion **(B)**. In ischemia/Dex group, only scattered TUNEL-positive cells were observed, demonstrating the less degree of apoptosis for dexmedetomidine pretreated animals **(C)**. Bar = 100 μm. Magnification × 200.

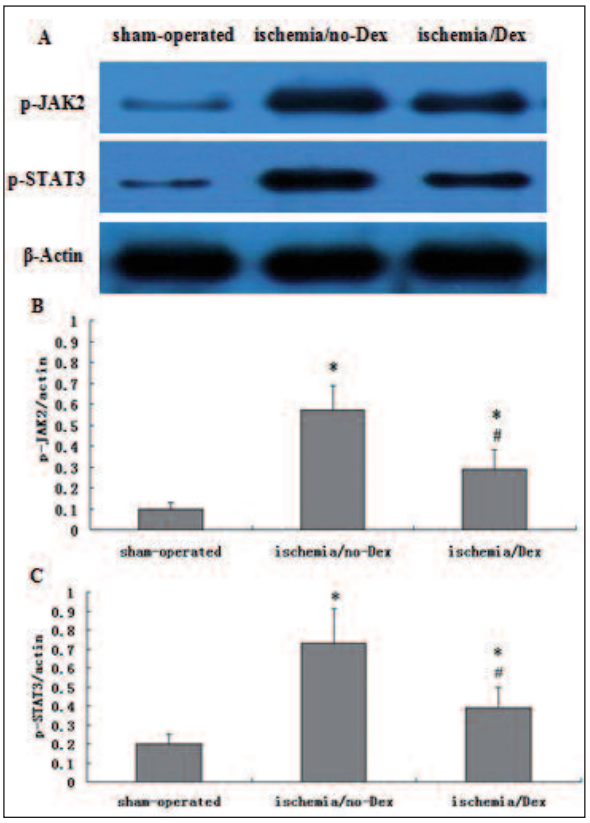

Figure 5. Dexmedetomidine inhibited the phosphorylation of JAK2 and STAT3 proteins. Representative Western blots for the phosphorylation of JAK2 and STAT3 of the kidneys were detected after 48 h of renal I/R in the sham-operated, ischemia/no-Dex and ischemia/Dex groups **(A)**. Densitometry analysis of Western blots for the ratio of p-JAK2/actin **(B)** and p-STAT3/actin **(C)**. Values represent scores±SD. *$p < 0.05$ compared with sham-operated group; #$p < 0.05$ compared with ischemia/no-Dex group.

ly predicting acute kidney injury[7,9]. According to our result, the level of serum creatinine and blood urea nitrogen increased in later time (24 and 48 h) after reperfusion, whereas the level of serum Cystatin C and NGAL significantly elevated in earlier time (12 h) after reperfusion in the animals subjected to renal I/R. This result is consistent with the studies that have been reported[7-9]. We found dexmedetomidine reduced serum Cystatin C and NGAL at earlier time (12 h) and serum creatinine and blood urea nitrogen at later time (24 and 48 h) after reperfusion. We infer that dexmedetomidine treated prior to ischemia protects renal function and starts the protective action in early time after renal ischemia.

Acute kidney injury induced by I/R is an experimental and clinical syndrome characterized by major attenuation in glomerular filtration rate, extensive glomerular impairment, severe tubular damage, tubular cell necrosis, and signs of tubular obstruction with cell debris[3,18]. Kocoglu et al[10] made a histopathologic study about the effect of dexmedetomidine on I/R injury in the rat kidney. They found that dexmedetomidine preserved normal glomeruli and slight edema of the tubular by reducing renal histological alterations induced by I/R. Gu et al[11] investigated apoptosis assay of renal tissue in a rat model with I/R injury, finding better histopathological structure and less degree of cell apoptosis in dexmedetomidine treated animals. Consistent with the evidence of previous studies described above, our study showed that dexmedetomidine significantly inhibited severe damage of renal tissue morphology and apoptosis contributing to less function impairment, tubular damage, tubular cell necrosis, tubular cell apoptosis and better histopathological structure against I/R injury. These evidences indicate that dexmedetomidine has the cytoprtective effect against renal I/R injury.

Numerous evidences show that dexmedetomidine activates presynaptic α_2 adrenergic receptors of peripheral and central of sympathetic norepinephrine neurons[19-22], subsequently attenuates the stress response induced by surgery and reduces plasma catecholamines concentrations[23,24]. There also may be direct vascular effects of dexmedetomidine in the renal tissue. It decreases the sympathetically mediated presynaptic release of norepinephrine in the kidney[25]. Secondary to these actions, dexmedetomidine attenuates the detrimental effect of vasoconstriction induced by circulating and local norepinephrine. Dexmedetomidine also may cause considerable redistribution of cardiac output, maintain renal blood flow and glomerular filtration[26,27], provide aqueous diuresis and inhibit vasopressin secretion[28]. In combination, cytoprotecive action, improved glomerular filtration and increased diuresis induced by dexmedetomidine result in enhancing renal function, causing less change of histopathological structure and making kidney recovered from I/R injury rapidly.

In this study, we further investigated the molecule mechanism of dexmedetomidine's renoprotective effect. Renal ischemia and reperfusion procedure is associated with the activation of JAK/STAT pathway[29]. The phosphorylation of JAK2 is induced in the kidney after renal ischemia, accompanied with the activation of downstream molecule STAT3[19,29]. It has been proven that AG490 is a selective JAK2 inhibitor which ameliorates renal renal function and his-

tology[19]. Interestingly, our data showed that dexmedetomidine significantly inhibited the phosphorylation of JAK2 and STAT3, indicating that its renoprotection was at least partially dependent on suppressing the activation of JAK2/STAT3 signaling pathway induced by renal I/R. Dexmedetomidine maybe act the same point as AG490.

Conclusions

Dexmedetomidine significantly reduces the I/R-mediated increases in the serum levels of biochemical markers, inhibits apoptosis and directly protects renal tissue against I/R injury. We conclude for the first time that dexmedetomidine protects renal function in early period after I/R by detecting the biomarkers, Cystatin C and NGAL, and its role of renoprotection maybe involve in inhibiting JAK2/STAT3 signaling pathway.

Acknowledgements

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Conflict of interest

The Authors declare that they have no conflict of interests.

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