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Inhibition of PARP overactivation protects acute kidney injury of septic shock

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Abstract. – OBJECTIVE: To evaluate pathological lesions in New Zealand white rabbits with acute kidney injury (AKI) of septic shock and to explore the potential role of poly (ADP-ribose) polymerase (PARP) in regulating AKI development.

MATERIALS AND METHODS: Endotoxic shock model in New Zealand white rabbits was first constructed. CVP (central venous pressure) was maintained at the baseline level by the saline administration. Rabbits were randomly assigned into sham group, LPS group, and LPS+3-AB group, respectively. Blood samples and kidney samples of rabbits were collected 4 h after LPS administration. Pathological kidney lesions were observed by HE (hematoxylin-eosin) staining and immunohistochemistry. Serum levels of renal damage markers (Scr, Cys-C, KIM-1, and NGAL) were detected by an automatic biochemical analyzer, immunoturbidimetry, and ELISA (enzyme-linked immunosorbent assay), respectively. Kidney energy metabolism changes (ATP, ADP, PCr, and NAD) were detected by HPLC (high performance liquid chromatography analysis). Western blot was conducted to detect protein expressions of NF-κB (nuclear factor-kappa B), TNF-α (tumor necrosis factor-α), ICAM-1 (intercellular cell adhesion molecule-1) and P-selectin in kidney tissues.

RESULTS: Significant pathological lesions in kidney tissues and higher pathological grade were seen in the LPS group. Multiple PARP-positive nuclei were found in renal tubular cells at the junction of renal cortex and renal cortex in the LPS group. Serum levels of Scr, KIM-1, NGAL, and Cys-C were remarkably higher in the LPS group than those of sham group. HPLC results showed decreased levels of ATP, ADP, PCr, and NAD in kidney cortex of LPS group compared with those of sham group. Western blot results suggested that protein expressions of NF-κB, TNF-α, ICAM-1, and P-selectin were remarkably upregulated in kidney tissues of LPS group. 3-AB pretreatment, the PARP inhibitor, remarkably alleviated pathological lesions and inflammation induced by AKI.

CONCLUSIONS: Inhibition of PARP overactivation alleviated pathological kidney lesions, improved kidney energy metabolism and inhibited inflammatory response resulted from AKI.

Key Words:
Septic shock, Acute kidney injury, PARP, Cell apoptosis.

Introduction

Severe infection and septic shock are the most common causes of acute kidney injury (AKI), accounting for about 50% of cases of acute renal failure (ARF)[1]. The incidence of AKI is positively correlated to the infection severity, accounting for 19% and 23% in patients with systemic infection and severe infection, respectively. In particular, the incidence of AKI is as high as 51% in septic shock patients[2,3]. AKI could further aggravate other organ damage, resulting in MODS (multiple organ dysfunction syndrome) and even death. The mortality of severely infected patients complicated by ARF is as high as 70%[4].

At present, the early treatments of septic shock are well studied and included early goal-directed therapy, blood glucose control, stress dose hormone therapy, application of active protein C, and sepsis bundle[5-7]. Currently, there are no effective methods for preventing and treating AKI. The potential mechanism of AKI induced by severe infection and septic shock has not been fully elucidated. Multiple pathogenic factors are involved in AKI, such as changes in renal hemodynamics and renal perfusion, kidney cell damage, inflammatory response, and immune regulation[8,9].

Overactivation of poly (ADP-ribose) polymerase (PARP) is the major cause for AKI. PARP exerts biological functions involving DNA protection, protein modification, nucleotide polymerization, and gene stability maintenance[10]. However, PARP overactivation resulted from multiple DNA damage leads to cell apoptosis and necrosis, thereafter participating in disease development[11]. In the past decade, researches[12-14] have confirmed

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that PARP overactivation is a key step in systemic infection, septic shock, and ischemia-reperfusion injury. Systemic infection and septic shock produce a large number of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to DNA damage, PARP overactivation, and cellular dysfunction. Some studies[15-17] have reported that inhibition of PARP overactivation can improve peripheral vascular reactivity, myocardial inhibition, intestinal mucosal barrier injury, liver and kidney function. Local blood flow and systemic vascular resistance are improved after inhibition of PARP overactivation, thereby elevating cardiac output and the overall survival of patients with ischemia/reperfusion injury[17,18]. However, whether inhibition of PARP overactivation could improve AKI caused by septic shock is rarely reported.

Since the pathogenesis of AKI still remains unclear, it is of great significance to study its underlying mechanism to improve the clinical outcomes. This research aims to investigate the effects of PARP on AKI-induced renal pathological lesions, which provides novel directions for treating AKI of septic shock.

Materials and Materials

Endotoxic Shock Model Construction

New Zealand white rabbits were kept in a fasting state overnight. Rabbits were positioned on the sterile table and anesthetized with intravenous injection of 20 mL/kg pentobarbital sodium (2%). A tracheotomy was performed, followed by a mechanical ventilation with 8-10 mL/kg of VT (tidal volume), 40 times/min of f (frequency), 0 mmHg of PEEP (positive end-expiratory pressure) and 30-50% of FiO_2 (fraction of inspiration O_2). VT and FiO_2 were adjusted according to the blood gas analysis during the animal experiment, so as to maintain over 100 mmHg of PaO_2 and 30-50 mmHg of $PaCO_2$. Catheterization was performed in the internal carotid and external jugular vein, and 2 mg/kg LPS was continuously injected into the central vein within 2 min. The endotoxic shock rabbit model was considered to be successfully constructed when rat MAP (mean arterial pressure) was decreased below 60 mmHg within 1 h of LPS administration. CVP (central venous pressure) was maintained at the baseline level by the saline administration.

30 New Zealand white rabbits were randomly assigned into sham group, LPS group, and LPS+3-AB group, with 10 rabbits in each group. All rabbits received tracheotomy and mechanical ventilation. Rabbits in sham group were administrated with isodose saline, whereas other rabbits received LPS administration. For rabbits in LPS+3-AB group, 15 mL/kg 3-aminobenzamide (3-AB) was injected 10 min prior to LPS administration within 2 min. This investigation was approved by the Animal Ethics Committee of Tianjin TEDA Hospital Animal Center.

Sample Collection

After LPS (or normal saline) administration for 4 h, 4 mL of blood sample collected from the right internal carotid artery was placed in the procoagulant tube and centrifuged for serum collection. Kidneys were harvested after rabbit sacrifice. Partial kidney tissue was preserved at -80°C and the remaining was fixed in 4% paraformaldehyde.

HE (Hematoxylin-Eosin) Staining

Kidney tissue was fixed in 4% paraformaldehyde for 6 h, which was then dehydrated by gradient ethanol, paraffin-embedded and sectioned in a 4-μm slice. Kidney tissue slice was used for HE staining (Boster, Wuhan, China) under a microscope for pathological evaluation in a double-blinded way.

Immunohistochemical Staining

Paraffin-embedded kidney tissues were dewaxed and hydrated, and 50 μL of anti-rabbit PARP (1:100) was added and incubated at room temperature for 1 h. After washing 3 times with PBS (phosphate-buffered saline), the corresponding secondary antibody was added and incubated at room temperature for another 1 h. DAB (diaminobenzidine) (Solarbio, Beijing, China) was then added to develop color for 5-10 min. Deionized water was used to rinse the residual DAB. The tissue was finally dehydrated, sealed and observed under a microscope.

Serum Renal Damage Markers Determination

Serum levels of Scr, Cys-C, KIM-1, and NGAL were detected by automatic biochemical analyzer, immunoturbidimetry, and ELISA (enzyme-linked immunosorbent assay) (R&D Systems, Minneapolis, MN, USA), respectively.

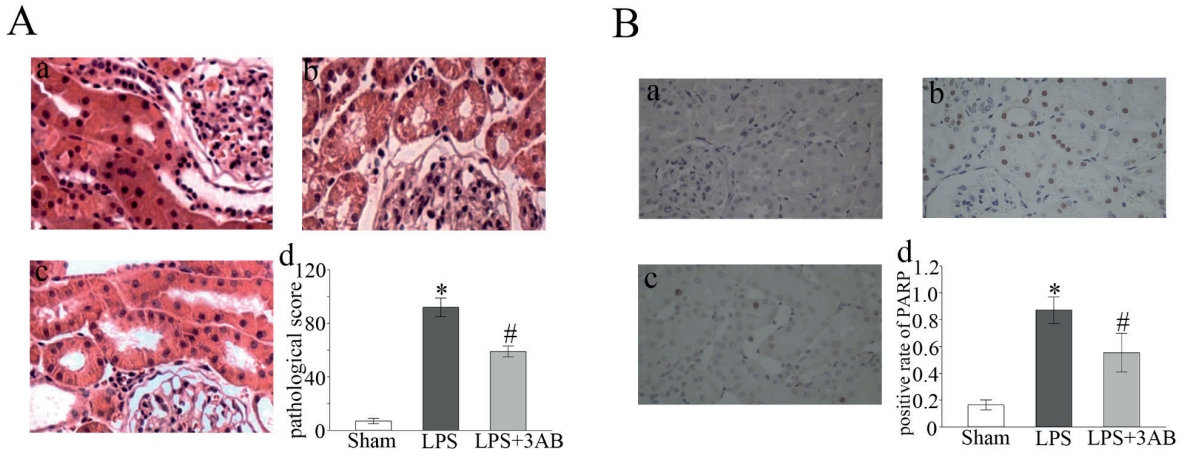

Figure 1. ***A,*** HE staining of kidney tissue in New Zealand white rabbits. ***A-a,*** HE staining of kidney tissue in sham group. ***A-b,*** HE staining of kidney tissue in LPS group. ***A-c,*** HE staining of kidney tissue in LPS+3-AB group. ***A-d,*** Pathological grade in sham group, LPS group, and LPS+3-AB group. *$p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with LPS group. ***B,*** Immunohistochemical staining of PARP in kidney tissue in New Zealand white rabbits (Magnification: 400×). ***B-a,*** PARP expression in kidney tissue of sham group. ***B-b,*** PARP expression in kidney tissue of LPS group. ***B-c,*** PARP expression in kidney tissue of LPS+3-AB group. ***B-d,*** Comparison of positive rate of PARP in sham group, LPS group, and LPS+3-AB group. *$p<0.05$, compared with sham group; $^{\#}p<0.05$, compared with LPS group (Magnification: 400×).

HPLC (High Performance Liquid Chromatography Analysis) of Kidney Energy Metabolism

200 mg kidney tissue was incubated in pre-cooled 0.42 M perchloric acid solutions and prepared for kidney homogenate. After centrifugation, the supernatant was adjusted to pH 7.0 and maintained in the ice bath for 10 min. The supernatant was preserved at -80°C, followed by the detection of ATP, ADP, PCr, and NAD levels by HPLC.

Western Blot

The total protein of kidney tissues was extracted by RIPA (radioimmunoprecipitation assay) and quantified by BCA (bicinchoninic acid) method. The protein expression levels of NF-Kb (nuclear factor-kappa B), TNF-α (tumor necrosis factor-α), ICAM-1 (intercellular cell adhesion molecule-1) and P-selectin were detected based on the standard protocols of Western blot.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for data analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$). The comparison of measurement data between different groups was conducted using one-way ANOVA test, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

Pathological Observation of Rabbit Kidney Tissues

Clear nephron structure, normal glomerular morphology, and complete tubular structure were observed in kidney tissues of sham group (Figure 1A-a). However, there were significant pathological lesions in kidney tissues of LPS group, including destructed nephron structure, contracted glomerulus, various infiltration of inflammatory cells, microthrombus formation in the glomerular capillary, serious degeneration and necrosis of renal tubular epithelial cells, edema and hemorrhage of renal interstitium (Figure 1A-b). Pathological lesions were remarkably alleviated in LPS+3-AB group, indicating that 3-AB, the PARP inhibitor, could suppress LPS-induced kidney injury (Figure 1A-c). The pathological grade was remarkably higher in LPS group compared with that of sham group. LPS+3-AB group showed lower pathological grade than that of LPS group (Figure 1A-d).

PARP Was Overactivated in AKI

PARP-positive nuclei were barely seen in kidney tissues of sham group (Figure 1B-a), whereas multiple PARP-positive nuclei were found in renal tubular cells at the junction of renal cortex and renal cortex in LPS group (Figure 1B-a, b and d). On the contrary, LPS+3-AB group showed fewer PARP-positive nuclei in renal

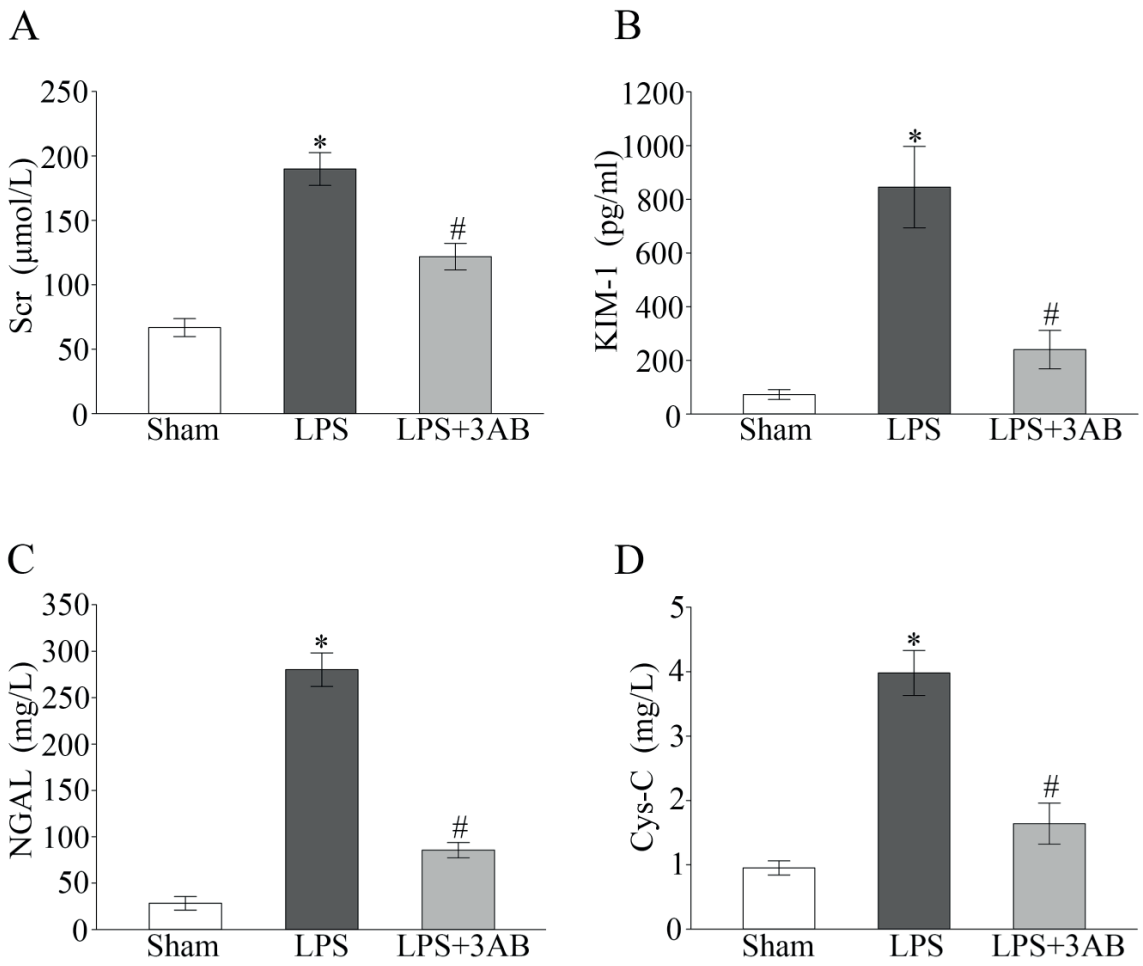

Figure 2. Comparison of serum renal damage markers determination among the three groups. ***A,*** Serum level of Scr in sham group, LPS group, and LPS+3-AB group. ***B,*** Serum level of KIM-1 in sham group, LPS group, and LPS+3-AB group. ***C,*** Serum level of NGAL in sham group, LPS group, and LPS+3-AB group. ***D,*** Serum level of Cys-C in sham group, LPS group, and LPS+3-AB group. *$p<0.05$, compared with sham group; #$p<0.05$, compared with LPS group.

tubular cells compared with those of LPS group (Figure 1B-b, c and d).

Comparison of Serum Renal Damage Markers Determination Among Three Groups

Serum levels of Scr, KIM-1, NGAL, and Cys-C were remarkably higher in LPS group than those of sham group. However, LPS+3-AB group showed lower levels of serum renal damage markers in comparison with those of LPS group (Figure 2).

Comparison of Kidney Energy Metabolism Among Three Groups

HPLC results showed decreased levels of ATP, ADP, PCr, and NAD in kidney cortex compared with those of sham group. PARP inhibition remarkably elevated ATP, ADP, and NAD levels. However, PCr level was not altered by 3-AB pretreatment (Figure 3).

PARP Inhibition Alleviated AKI Via Suppressing NF-κB Pathway

Western blot results suggested that protein expressions of NF-κB, TNF-α, ICAM-1, and P-selectin were remarkably upregulated in kidney tissues of LPS group. LPS+3-AB group showed remarkably downregulated levels of NF-κB, TNF-α, ICAM-1, and P-selectin in kidney tissues than those of LPS group (Figure 4).

Discussion

PARP is a non-histone chromosomal protein that is widely presented in eukaryotic cells[19]. Toxin-

induced gap or fragmentation of DNA strands may activate PARP[20]. The activated PARP stimulates the production of ADP-ribose and nicotinamide by cleavage of the coenzyme I. ADP-ribose subsequently transfers to the glutamate residue of the receptor protein, thus producing macromolecule homopolymers containing over 200 ADP-ribose. PARP participates in various biological processes, including chromatin depolymerization, DNA replication and repair, gene regulation, cell differentiation, and apoptosis[21-24].

Our study found overactivation of PARP in kidney tissue during endotoxin shock. Immunohistochemistry confirmed that PARP was highly expressed in renal tubular cells after LPS administration for 4 h. Histopathological results and serum detection both confirmed kidney cell damage and PARP overactivation, indicating PARP is involved in AKI of septic shock. 3-AB pretreatment remarkably alleviated pathological lesions of rabbit kidney.

Studies[25,26] have shown that PARP activation is closely related to cell death. In 1985, Berger[27] first proposed that overactivation of PARP promotes cell necrosis. Overactivated PARP consumes a large amount of NAD^+ and ATP, eventually exhausting energy for programmed apoptosis. Further studies[28-30] proved that PARP overactivation leads to cell necrosis or apoptosis depending on the degree of ATP consumption. Therefore, necrosis or apoptosis of the renal tubules in AKI is mainly associated with the

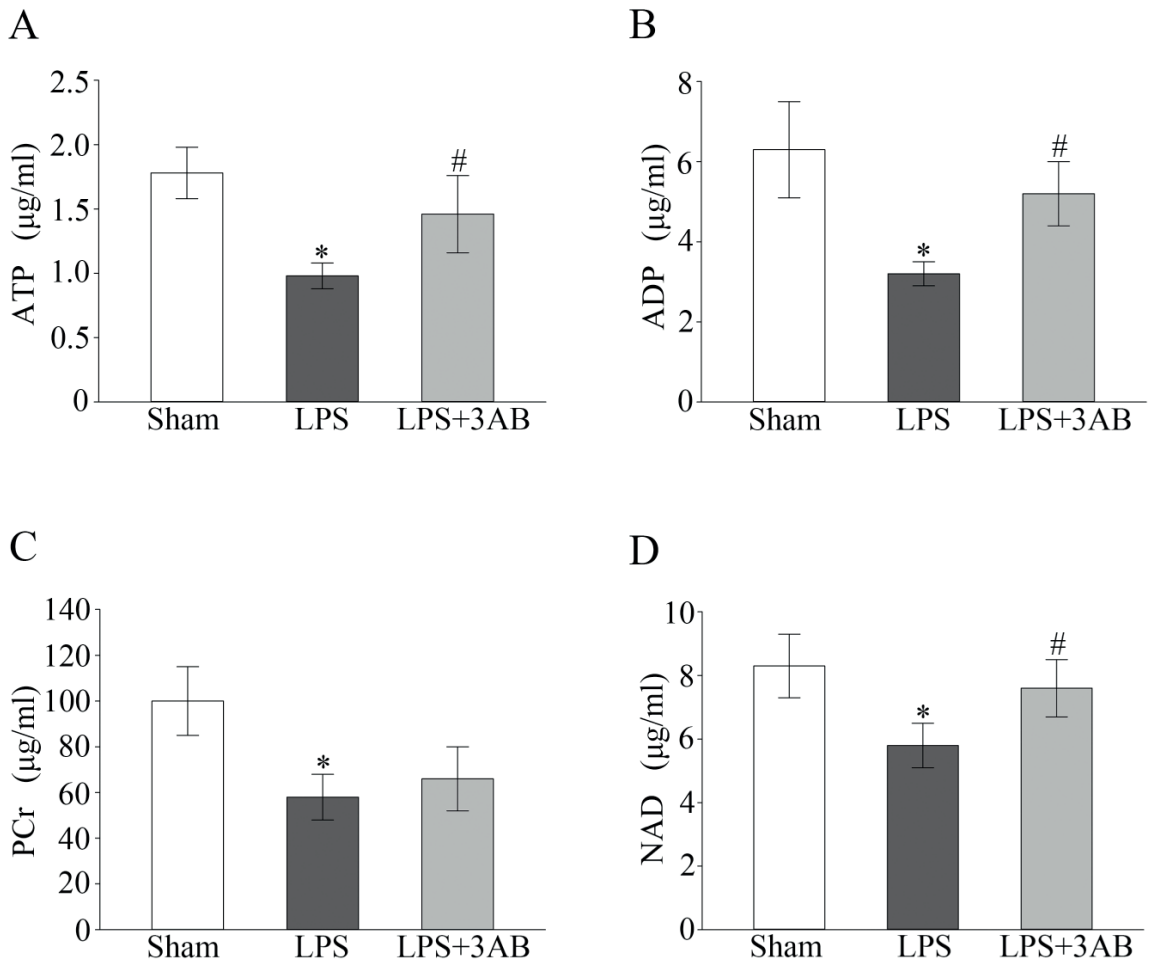

Figure 3. Comparison of kidney energy metabolism among the three groups. ***A,*** ATP level in sham group, LPS group, and LPS+3-AB group. ***B,*** ADP level in sham group, LPS group, and LPS+3-AB group. ***C,*** PCr level in sham group, LPS group, and LPS+3-AB group. ***D,*** NAD level in sham group, LPS group, and LPS+3-AB group. *$p<0.05$, compared with sham group; #$p<0.05$, compared with LPS group.

reduction level of energy metabolism. Our work detected kidney energy metabolism by HPLC. The data showed that levels of ATP, ADP, PCr, and NAD were remarkably decreased in LPS group, but they were not depleted. HE staining did not show significant necrosis of renal tubular cells. As a result, we considered that the apoptosis of tubular cells may be the major performance of septic shock-induced AKI.

PARP exerts its transcriptional regulatory function by modifying chromosomal-related proteins, including NF-κB, AP-1, and MAPK[31,32]. NF-κB can also regulate expressions of many pro-inflammatory proteins, including cytokines (IL-1, IL-6, TNF-α, IL-2, IL-8) and adhesion molecules (ICAM-1, VCAM-1, P-selectin)[33,34]. Accumulating evidence showed that PARP level is associated with inflammatory responses in the development of various acute and chronic inflammatory diseases.

PARP activates NF-κB, thereafter improving the sensitivity of cells (especially endothelial cells) to oxygen free radicals produced during inflammatory response[35,36]. In the present report, Western blot

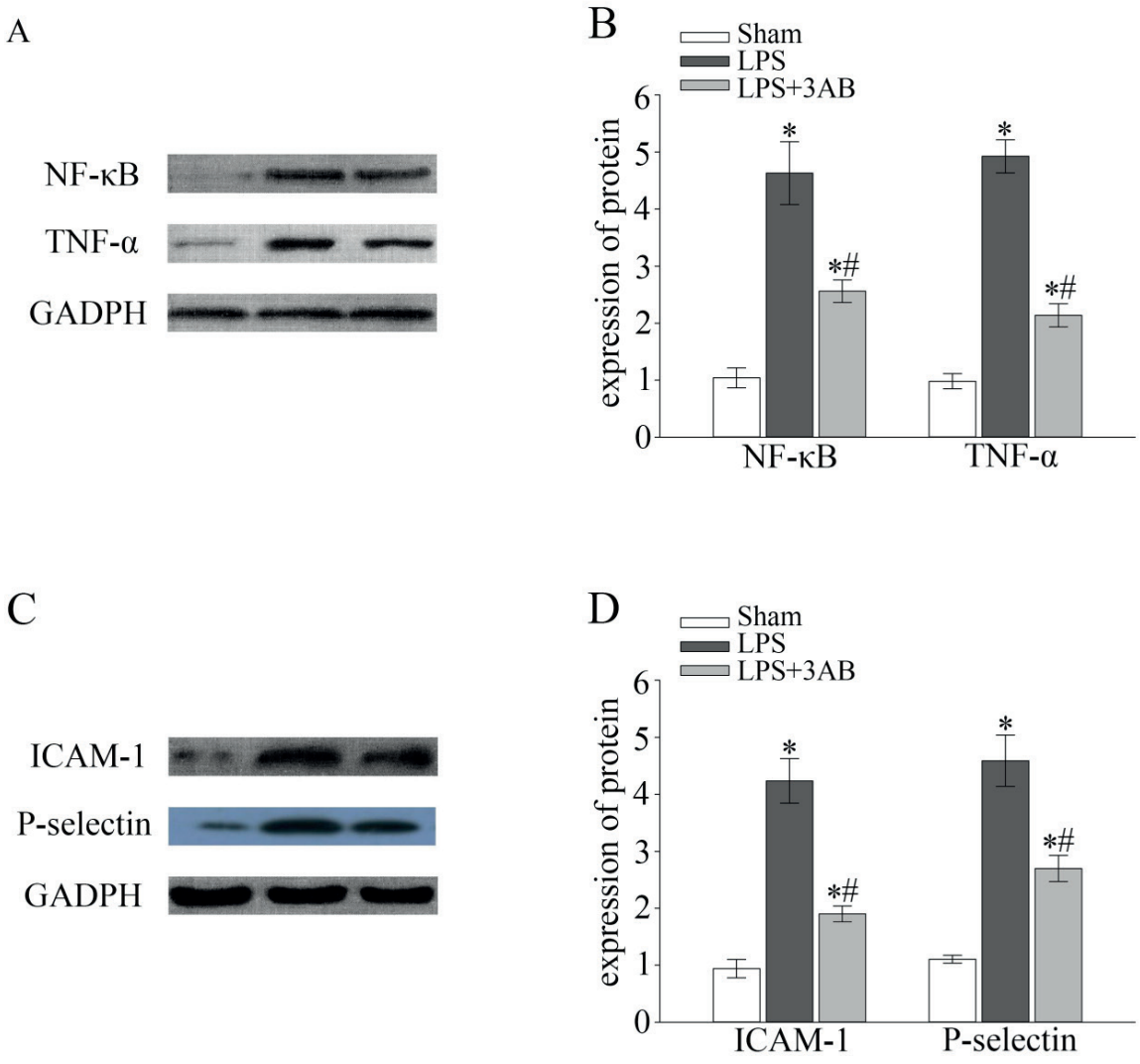

Figure 4. Comparison of NF-κB pathway-related genes in kidney tissues. ***A,*** Protein expressions of NF-κB and TNF-α in kidney tissues of sham group, LPS group, and LPS+3-AB group. ***B,*** Comparison of protein expressions of NF-κB and TNF-α in kidney tissues of sham group, LPS group, and LPS+3-AB group. ***C,*** Protein expressions of ICAM-1 and P-selectin in kidney tissues of sham group, LPS group, and LPS+3-AB group. ***D,*** Comparison of protein expressions of ICAM-1 and P-selectin in kidney tissues of sham group, LPS group, and LPS+3-AB group. *$p<0.05$, compared with sham group; #$p<0.05$, compared with LPS group.

results indicated that protein expressions of NF-κB, TNF-α, ICAM-1, and P-selectin were upregulated in LPS group compared with those of sham group. 3-AB pretreatment remarkably downregulated their expression levels, indicating PARP inhibition could reduce levels of transcription factors and inflammatory mediators in renal tissues.

In summary, PARP overactivation decreased energy metabolism in kidney tissue, leading to kidney damage and apoptosis. Inhibition of PARP overactivation can significantly reduce AKI of septic shock *via* improving kidney energy metabolism and inhibiting inflammatory response.

Conclusions

We found that the inhibition of PARP overactivation alleviated pathological kidney lesions, improved kidney energy metabolism and inhibited inflammatory response resulted from AKI.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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