Introduction

Sepsis, as one of the serious complications of trauma, shock, infection and major surgery in patients with clinical critical disease, can often lead to septic shock and multiple organ dysfunction (1, 2). Sepsis combined with acute kidney injury (AKI) is an important risk factor for the poor prognosis of patients with critical disease. Sepsis-induced AKI can promote and aggravate the damage of other organs, which in turn leads to death (3, 4). Increasing number of recent studies have shown that sepsis-induced AKI is affected by multiple factors. In addition to hemodynamic changes, changes in renal cell apoptosis, endotoxin-induced complex inflammation, and immune network responses also play important roles in this process (5, 6). However, the pathogenesis of sepsis-induced AKI is still unclear. Thomas (7) found that macrophage apoptosis is the main cause of immune function inhibition in sepsis. AKI can lead to increased levels of renal cell apoptosis, significantly increasing the mortality rate of patients. Sharma et al (8) also found that sepsis can activate autophagy, leading to a negative impact on the body. As a lysosome involved catabolic process, autophagy mainly acts on the recycle of intracellular macromolecules, removal of damaged organelles and clearance of intracellular pathogens, so as to maintain cell stability, ensure energy supply and protect cells. Autophagy can also assist antigen presenting cells to carry out antigen presentation. Autophagy is a linker between non-specific and specific immune system, and has important roles in infection control and immune steady-state maintenance. However, excessive activation brings damage to the body. Emergence of autophagy can affect the expression levels of autophagy-degrading substrate p62, autophagy constituent proteins LC3-II/I and autophagy-related protein Beclin1 (9-12). Autophagy activation and its role in AKI patients still have not been reported. Our study aimed to investigate the effects of autophagy on mice with sepsis-induced AKI so as to provide new insights into the pathogenesis and clinical treatment of AKI.

Materials and methods

Instruments and materials. Creatinine (Cr) assay kit and blood urea nitrogen (BUN) assay kit (R&D Systems, Inc., Minneapolis, MN, USA), 3-MA (Sigma-Aldrich; Merck

Abstract. This study investigated whether autophagy is activated after sepsis-induced acute kidney injury (AKI) and explored its biological role. Seventy-two normal C57 mice were randomly divided into sham operation group, cecal ligation and puncture (CLP) group and CLP+3-MA (autophagy inhibitor) group; 24 mice in each group. Mice in CLP and CLP+3-MA group were treated with cecal ligation to establish sepsis, while mice in sham operation group were treated with the same surgical operations, but not cecal ligation. Blood samples were collected from 12 mice of each group and the levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were measured. The pathological changes were observed. The remaining 12 mice in each group were kept and the survival rate was recorded. Changes in the expressions of autophagy-related proteins were detected by reverse transcription-semi-quantitative PCR and western blotting. The results revealed that the levels of Cr and BUN in CLP and CLP+3-MA group were significantly higher than those in sham operation group (P<0.05), and the levels of Cr and BUN in CLP+3-MA group were higher than those in CLP group (P<0.05). The pathological score of renal injury in CLP+3-MA group was significantly higher than that of CLP group (P<0.01). The expression levels of Beclin1 and LC3-II/I were significantly increased in CLP group compared to sham operation group (P<0.01), while the expression of p62 was decreased (P<0.01). After 3-MA treatment the expression levels of Beclin1 and LC3-II/I were decreased, compared with CLP group, but accumulation of p62 occurred, and the degree of renal injury was increased. In conclusion, AKI induced by sepsis in mice can induce apoptosis and activate autophagy. The activation of autophagy aggravates the renal injury in mice, which in turn inhibits AKI after sepsis.

Key words: sepsis, acute renal injury, autophagy, apoptosis
Experimental animals and grouping. Seventy-two male C57 mice, weighing 22-25 g, were purchased from Guangdong Medical Laboratory Animal Center [certificate no. SCXK (Guangdong) 2013-0015]. The animals were raised for 1 week following the circadian rhythm in quiet environment at 25°C with free access to water and food to adapt the feeding environment. The 72 C57 mice were randomly divided into three groups (24 mice in each group): CLP group (cecal ligation), sham operation group (same operation as sepsis group except cecal ligation), CLP+3-MA group (intraperitoneal injection of 3-MA saline solution at a dose of 10 mg/kg before cecal ligation). Peripheral blood of 12 mice of each group was taken at 12 and 24 h after modeling, and the kidneys were taken 24 h after operation (13). The remaining 12 mice in each group were fasted for 12 h before surgery to avoid obstructions of intestinal tract. The mice were anesthetized with isoflurane (RWD Life Science, Shenzhen, China). After anesthesia, hair of the mouse abdomen was removed by hair removal cream, and abdomen was opened to find the cecum. One-third of the cecum was ligated with 6-0 suture. Needle puncture with 18 gauge needle was performed and a little intestinal content was squeezed out. Then cecum was put back to the abdominal cavity without squeezing out intestinal contents to contaminate the surgical incision. Mice were injected with a small amount of saline to supplement body fluids. The incision was sutured and disinfected with iodophor (13). After waking up, the mice were reared in a cage with free access to food and water.

Sample collection and preparation. Peripheral blood was extracted from mice of each group at 12 and 24 h after surgery. Blood samples were centrifuged at 2,100 x g for 15 min at 4°C to collect the supernatant. The supernatant was stored in a refrigerator at -80°C. After blood extraction, the right kidney was rapidly collected and fixed in 10% formalin for pathological examination.

Establishment of sepsis model. Mice in each group were fasted for 12 h before surgery to avoid obstructions of intestinal tract. The mice were anesthetized with isoflurane (RWD Life Science, Shenzhen, China). After anesthesia, hair of the mouse abdomen was removed by hair removal cream, and abdomen was opened to find the cecum. One-third of the cecum was ligated with 6-0 suture. Needle puncture with 18 gauge needle was performed and a little intestinal content was squeezed out. Then cecum was put back to the abdominal cavity without squeezing out intestinal contents to contaminate the surgical incision. Mice were injected with a small amount of saline to supplement body fluids. The incision was sutured and disinfected with iodophor (13). After waking up, the mice were reared in a cage with free access to food and water.

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with a secondary antibody (1:5,000) for 2 h. After that, the membranes were washed 3 times with TBST, 5 min for each time. The solutions A and B of ECL kit (EMD Millipore, Burlington, MA, USA) were added with a ratio of 1:1 to detect the signal. The results were scanned and ImageJ (National Institutes of Health, Bethesda, MD, USA) software was used to analyze gray values.

Statistical analysis. The data in this study are expressed as mean ± standard deviation. Data were processed using SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA). Comparisons between two groups were performed by t-test and comparisons among multiple groups were performed by variance analysis. Test for homogeneity of variances was performed. If the variance was homogeneous, Bonferronic method was used for comparison between two groups. If the variance was heterogeneous, multiple comparisons were performed using Dunnett’s T3 method. Kaplan-Meier method was used for survival rate analysis and a log-rank test was performed for comparison. P<0.05 was considered to indicate a statistically significant difference.

Results

Changes in renal function in mice. Levels of Cr and BUN in the urine of each group were detected by Cr and BUN detection kit. As shown in Figs. 1 and 2, the levels of Cr and BUN in CLP and CLP+3-MA group were significantly higher than those in sham operation group (P<0.05), and the levels of Cr and BUN in CLP+3-MA group were significantly higher than that in CLP group (P<0.05), n=6. Cr, creatinine.

Pathological changes of renal tissue in mice. Pathological changes of renal tissue samples were observed under an optical microscope. No sepsis was observed in sham operation group, the tissue structure was clear and the structures of renal tubules and glomerulus were normal. Telangiectasia and severe congestion were observed in renal tissue sections of the CLP and CLP+3-MA group, obvious edema was observed in renal tubular epithelial cells, and granule-like degeneration or vacuolar degeneration was observed in the tissues (Fig. 3A). The tissues were scored using the same scoring criteria. The results showed that the scores of sepsis and CLP+3-MA group were significantly higher than that of sham operation group (P<0.01), and the score of CLP+3-MA group was significantly higher than that of the sepsis group (P<0.01) (Fig. 3B and Table II).

Mouse survival rate. The mice were reared in the same environment after surgery, and the survival rates at the 4th and 7th day after surgery were recorded. As shown in Fig. 4 and Table III, the survival rates of mice in CLP and CLP+3-MA group were significantly lower than that in sham operation group (P<0.01), and the survival rate of mice in CLP+3-MA group was significantly higher than that in CLP group (P<0.01).

mRNA expression detected by reverse transcription-semi-quantitative PCR. The expression of autophagy-related genes
was detected by reverse transcription-semi-quantitative PCR. As shown in Fig. 5, the expression levels of autophagy-related genes Beclin1 and LC3-II were significantly increased in CLP group compared with the sham group (P<0.01), while the expression of p62 was significantly decreased (P<0.01). With 3-MA treatment, the expression levels of autophagy-related genes Beclin1 and LC3-II were decreased compared with CLP group (P<0.01 and P<0.05, respectively), but the expression level of p62 was increased (P<0.01).

Western blotting was used to detect the expression of related proteins. Western blotting was used to detect the expression of autophagy-related proteins. As shown in Fig. 6, the expression levels of autophagy-related proteins Beclin1 and LC3-II were significantly increased in CLP group compared with that in the sham group (P<0.01), while the expression level of p62 protein was significantly decreased (P<0.05). With 3-MA treatment, the expression of autophagy-related proteins Beclin1 and LC3-II were decreased compared with CLP group (P<0.01 and P<0.05, respectively), but the expression level of p62 was increased (P<0.01).

Table II. Pathological changes of renal tissues in each group of mice.

| Groups          | 12 h     | 24 h     |
|-----------------|----------|----------|
| Sham operation  | 0.68±0.19| 0.71±0.16|
| Sepsis          | 1.78±0.26| 1.86±0.19|
| 3-MA            | 1.95±0.21| 2.06±0.27|

*P<0.01, compared with sham operation group; *P<0.01, compared with sepsis group.
Figure 5. Reverse transcription-semi-quantitative PCR to detect the expression of autophagy-related genes. The expression levels of the autophagy-related genes (A and C) Beclin1 and (A and D) LC3-II were significantly increased in CLP group compared with those in the sham group (**P<0.01), while (A and B) the expression level of p62 was significantly decreased (*P<0.05). Compared with CLP group, the expression levels of (A and C) Beclin1 and (A and D) LC3-II were decreased in CLP+3-MA group (**P<0.01, *P<0.05), while (A and B) the expression level of p62 was increased (*P<0.05, **P<0.01), n=12.

Figure 6. Western blotting to detect the expression of autophagy-related proteins. The expression levels of autophagy-related proteins (A and C) Beclin1 and (A and D) LC3-II/I were significantly increased in CLP group compared with the sham group (**P<0.01), while (A and B) the expression level of p62 protein was significantly decreased (*P<0.05). Compared with CLP group, the expression levels of (A and C) Beclin1 and (A and D) LC3-II/I protein were decreased in CLP+3-MA group (**P<0.05, *P<0.01), while (A and B) the expression level of p62 protein was increased (*P<0.01, **P<0.01), n=12.
LC3-II was inhibited; compared with that in CLP group the expression levels were decreased (P<0.05 and P<0.01, respectively), but the expression level of p62 protein was increased in CLP+3-MA group (P<0.01).

**Discussion**

Sepsis can lead to multiple organ failures. Pathophysiological changes of sepsis are complex, and the specific pathogenesis is still unclear, which in turn brings great obstacles to the clinical treatment (14). AKI is a common complication of severe sepsis and is an important factor for the death of patients (15). At present, the basic research on sepsis mainly includes in vivo and in vitro experiments. The use of animal models can effectively simulate the pathological process of sepsis, which provides reference for elucidating the pathogenesis of sepsis in human (16). In this study, cecal puncture was used to establish the mouse model of AKI induced by sepsis. The size of the selected puncture needle and the number of punctures can significantly affect the stability of the model. The position and number of the punctures in experiment were strictly in accordance with the standard to ensure the consistency of the experimental results. Infusion was performed after surgery to simulate the onset of clinical sepsis.

After model establishment, the degree of renal injury was evaluated by measuring serum Cr and BUN levels. Results showed that serum Cr and BUN levels in CLP group were significantly higher than those in Sham operation group, indicating the successfully established mouse sepsis model. 3-MA is a commonly used autophagy inhibitor that inhibits autophagy activity in vivo and is often used as a tool for studying the physiological significance of autophagy activity in the body (17). 3-MA treatment before surgery can reduce damage to renal function in mice with sepsis-induced AKI, indicating that the activation of autophagy plays a negative role in sepsis-induced AKI, and can be protective through the inhibition of autophagy. The expression of autophagy-related proteins was detected by reverse transcription-semi-quantitative PCR and western blotting. Results showed that the expression levels of autophagy-related proteins Beclin1 and LC3-II/I were significantly increased and the expression level of autophagy substrate p62 was decreased in CLP group compared to sham operating group, indicating that sepsis can activate the autophagy in the body. With 3-MA treatment, the expression levels of Beclin1 and LC3-II/I were decreased and the expression level of p62 was increased, compared to the CLP group, and thus the activation of autophagy in sepsis was further confirmed by this inhibitor. At the same time, compared with mice in CLP group, renal injury of CLP+3-MA group was less serious, and the survival rates of CLP+3-MA group at the 4th and 7th day after surgery were significantly higher than those of CLP group, indicating that the inhibition of autophagy can protect the body from AKI induced by sepsis. Autophagy in sepsis can be activated by a variety of cell stresses and the activated autophagy has protective effects on the body. Macdonald et al (18) found that macrophage apoptosis would occur after sepsis, leading to the dysfunction of the immune system. Macrophage apoptosis can also cause the production of inflammatory factors, leading to immunological suppression. Both autophagy and apoptosis present in sepsis-induced AKI. Autophagy and apoptosis share the same regulatory factors, and there is a dynamic balance between apoptosis and autophagy in the body (19,20). Our study still has some limitations. The activation of autophagy and apoptosis in sepsis-induced AKI was not deeply studied. The mechanism of the functions of autophagy and apoptosis was not elucidated. These will be the focus of future studies.

In summary, autophagy is activated in sepsis-induced AKI and autophagy activation brings damage to the body. The survival rate of patients with sepsis-induced AKI can be increased by inhibiting autophagy. This study provides a new direction for the clinical treatment of sepsis.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Authors' contributions**

YW and YZ assisted with PCR and western blotting. LW and LM were responsible for the establishment of the sepsis model. GC and YLZ contributed to ELISA and renal tissue injury scoring. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee of Xuzhou Municipal Hospital Affiliated to Xuzhou Medical University (Xuzhou, China).

**Patient consent for publication**

Not applicable.

**Competing interest**

The authors declare that they have no competing interests.

**References**

1. Endo A, Okamura M, Yoshikawa S, Otomo Y and Morio T: Multilateral functional alterations of human neutrophils in sepsis: From the point of diagnosis to the seventh day. Shock 48: 629-637, 2017.
2. Arulkumaran N, Sixma ML, Jentho E, Ceravola E, Bass PS, Kellum JA, Unwin RJ, Tam FWK and Singer M: Sequential analysis of a panel of biomarkers and pathologic findings in a resuscitated rat model of sepsis and recovery. Crit Care Med 45: e821-e830, 2017.

3. Wang N, Mao L, Yang L, Zou J, Liu K, Liu M, Zhang H, Xiao X and Wang K: Resveratrol protects against early polymicrobial sepsis-induced acute kidney injury through inhibiting endoplasmic reticulum stress-activated NF-κB pathway. Oncotarget 8: 36449-36461, 2017.

4. Cai ZY, Sheng ZX and Yao H: Pachymic acid ameliorates sepsis-induced acute kidney injury by suppressing inflammation and activating the Nrf2/HO-1 pathway in rats. Eur Rev Med Pharmacol Sci 21: 1924-1931, 2017.

5. Martin-Löeches I, Muriel-Bombín A, Ferrer R, Artigas A, Sole-Violan J, Lorente L, Andaluz-Ojeda D, Prina-Mello A, Herrán-Monge R, Suberviola B, et al; GRECIA group: The protective association of endogenous immunoglobulins against sepsis mortality is restricted to patients with moderate organ failure. Ann Intensive Care 7: 44, 2017.

6. Wollborn J, Schlegel N and Schick MA: Phosphodiesterase 4 inhibition for treatment of endothelial barrier and microcirculation disorders in sepsis. Anaesthesist 66: 347-352, 2017 (In German).

7. Thomas H: Sepsis: Bile acids promote inflammation in cholestasis-associated sepsis. Nut Rev Gastroenterol Hepatol 14: 324-325, 2017.

8. Sharma D, Farahbakhsh N, Shastri S and Sharma P: Biomarkers for diagnosis of neonatal sepsis: A literature review. J Matern Fetal Neonatal Med 31: 1646-1659, 2018.

9. Park SY, Shrestha S, Youn YJ, Kim JK, Kim SY, Kim HJ, Park SH, Ahn WG, Kim S, Lee MG, et al: Autophagy primes neutrophils for neutrophil extracellular trap formation during sepsis. Am J Respir Crit Care Med 196: 577-589, 2017.

10. Crowell KT, Soybel DI and Lang CH: Inability to replete white adipose tissue during recovery phase of sepsis is associated with increased autophagy, apoptosis, and proteasome activity. Am J Physiol Regul Integr Comp Physiol 312: R388-R399, 2017.

11. Jin L, Batra S and Jayaseelan S: Deletion of Nlrp3 augments survival during polymicrobial sepsis by decreasing autophagy and enhancing phagocytosis. J Immunol 198: 1253-1262, 2017.

12. Shi X, Yang F, Zheng YN, Zhang H, Wang XX, Shao GJ and Lai XL: Metabolomic approach for the identification of therapeutic targets of erythropoietin against sepsis in rat models. Eur Rev Med Pharmacol Sci 20: 537-546, 2016.

13. Deng M, Huang L, Bing N, Wang N, Zhang Q, Zhu C and Fang Y: β-asarone improves learning and memory and reduces acetyl cholinesterase and Beta-amyloid 42 levels in APP/PS1 transgenic mice by regulating Beclin-1-dependent autophagy. Brain Res 1652: 188-194, 2016.

14. Salah FS, Ebbinghaus M, Muley YY, Zhou Z, Al-Saadi KR, Pacyna-Gengelbach M, O’Sullivan GA, Betz H, König R, Wang ZQ, et al: Tumor suppression in mice lacking GABARAP, an Atg8/LC3 family member implicated in autophagy, is associated with alterations in cytokine secretion and cell death. Cell Death Dis 7: e2205, 2016.

15. Zheng YH, Xiong B, Deng YY, Lai W, Zheng SY, Bian HN, Liu ZA, Huang ZF, Sun CW, Li HH, et al: Effects of allogeneic bone marrow mesenchymal stem cells on polarization of peritoneal macrophages in rats with sepsis. Zhonghua Shao Shang Za Zhi 33: 217-223, 2017 (In Chinese).

16. Huan JN: Recognizing prevention and treatment of burn sepsis with the concept of holistic integrative medicine. Zhonghua Shao Shang Za Zhi 33: 196-199, 2017 (In Chinese).

17. Greninger AL and Hess JR: Clostridium perfringens sepsis masquerading as a hemolytic transfusion reaction. Transfusion 57: 1112, 2017.

18. Macdonald SPJ, Bosio E, Neil C, Arends G, Burrows S, Smart L, Brown SGA and Fatovich DM: Resistin and NGAL are associated with inflammatory response, endothelial activation and clinical outcomes in sepsis. Inflamm Res 66: 611-619, 2017.

19. Liu W, Guo J, Mu J, Tian L and Zhou D: Rapamycin protects sepsis-induced cognitive impairment in mouse hippocampus by enhancing autophagy. Cell Mol Neurobiol 37: 1195-1205, 2017.

20. Wan SX, Shi B, Lou XL, Liu JQ, Ma GG, Liang DY and Ma S: Ghrelin protects small intestinal epithelium against sepsis-induced injury by enhancing the autophagy of intestinal epithelial cells. Biomed Pharmacother 83: 1315-1320, 2016.

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