

## Original Research Article

# Resveratrol protects against sepsis-induced acute kidney injury in mice by inducing Klotho-mediated apoptosis inhibition

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### Abstract

**Purpose:** To investigate the mechanism of resveratrol protection against sepsis-induced acute kidney injury in mice.

**Methods:** A sepsis-induced acute kidney injury model was established in mice by cecal ligation and puncture (CLP). Sixty healthy male ICR mice were randomly divided into the sham operation (sham) group, sepsis-induced acute kidney injury model (CLP) group, CLP + low-dose (20 mg/kg) resveratrol treatment (CLP + ResL) group, CLP + high-dose (40 mg/kg) resveratrol treatment (CLP + ResH) group and CLP + Klotho (0.01 mg/kg) treatment (CLP + Klotho) group. All mice were administered treatment on the day after surgery and once every 24 h for 3 days. Various serum biochemical parameters and protein expressions were evaluated.

**Results:** After CLP, the levels of serum creatinine (Scr) and blood urea nitrogen (BUN) increased and the pathology was exacerbated. The protein and mRNA expression levels of Klotho and Bcl-2 decreased, while those of Bax and Caspase-3 increased ( $p < 0.05$ ). After resveratrol and Klotho protein intervention, Scr and BUN levels recovered, and pathological changes were alleviated. The protein and mRNA expression levels of Klotho and Bcl-2 increased, while those of Bax and Caspase-3 decreased. The conditions of the mice in CLP + ResH group and the CLP + Klotho group improved more significantly than those of the mice in the CLP + ResL group ( $p < 0.05$ ).

**Conclusion:** Resveratrol upregulates the expression of endogenous Klotho to exert its antiapoptotic effects, which can protect the kidneys of mice against sepsis-induced acute kidney injury. Thus, the compound has potentials for development for protection against acute kidney injury.

**Keywords:** Sepsis, Acute kidney injury, Resveratrol, Klotho, Apoptosis

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## INTRODUCTION

Sepsis is a systemic inflammatory response syndrome (SIRS) caused by severe infection [1].

The kidneys are some of the most vulnerable organs to sepsis [2]. Early prevention and treatment of acute kidney injury (AKI) in sepsis is of great clinical importance for reducing the high

mortality rate of sepsis and improving the prognoses of septic patients [3].

The Klotho protein was first discovered in 1997 by the Korean scientist Kuro-O through anti-aging research. Klotho is a unidirectional transmembrane protein encoded by the Klotho gene. The hormone-like secretory form of Klotho plays a major role in AKI. Decreased Klotho protein levels may play an important role in the occurrence and progression of AKI and may be useful as new markers for AKI diagnosis and prognosis [4]. Klotho has different protective effects against AKI induced by ischemia and poisoning. In a previous study, a mouse model of sepsis-induced AKI was established by the cecal ligation and puncture (CLP) method, and human proximal curved renal tubular epithelial cells (HK-2 cells) were treated with lipopolysaccharide (LPS) to establish a sepsis-induced AKI cell model. The expression of Klotho decreased after the completion of model induction. Acute Klotho protein deficiency is also present in sepsis-induced AKI. Therefore, restoration of endogenous Klotho protein expression may have a protective effect against sepsis-induced AKI [5]. Resveratrol, which is similar to polygonin, is an anthraquinone terpenoid compound with the molecular formula  $C_{14}H_{12}O_3$  and a molecular weight of 228.25. Resveratrol is known to have antioxidative, free radical-scavenging and antiaging properties as well as antitumor, anti-mutation effects, hepatoprotective, estrogen-like, bone metabolism-affecting, neuroprotective, bacteriostatic, anti-inflammatory, antiviral and immunoregulatory effects. Furthermore, it can enhance insulin sensitivity [6].

A previous study has shown that Klotho protein levels are acutely deficient and that Klotho expression is reduced in sepsis-induced AKI. Restoration of endogenous Klotho expression may have a protective effect against sepsis-induced AKI [5]. Resveratrol may also have a protective effect against sepsis-induced AKI. However, whether the protective effect of resveratrol is related to the protein expression of Klotho remains unclear. Thus, the present study was conducted to investigate the protective effect of resveratrol against sepsis-induced AKI and to observe whether the effects of resveratrol are related to upregulation of Klotho protein expression.

## EXPERIMENTAL

### Animals

Sixty specific pathogen-free healthy male ICR mice with body weights of  $30 \pm 2$  g (28 - 32 g)

were provided by the Laboratory Animal Center of Wenzhou Medical University (Laboratory Animal Production License: SCXK (Zhejiang) 2015-0001) (Laboratory Animal Use License: SYXK (Zhejiang) 2015-0009). Normal feeding, artificial 12-h light/dark cycles, a relative humidity of approximately 45 %, and a constant temperature of 25 °C were provided, and the animals were adaptively fed for 1 week with standard pellets.

### Ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Wenzhou Medical University approved the experiment (approval no: wyd2019-0472), and also followed international guidelines for animal studies.

### Reagents and drugs

Resveratrol was purchased from Shanghai Yuanye Biological Company. Klotho protein was purchased from Abcam. The Bcl-2, Bax, Caspase-3, and  $\beta$ -actin antibodies as well as horseradish peroxidase (HRP)-labeled secondary antibodies were purchased from Cell Signaling. Hydrogen peroxide solution (3 %), goat serum blocking solution, polymerized HRP-labeled secondary antibodies, and diaminobenzidine (DAB) were purchased from Beijing Zhongshan Jinbridge. Radioimmunoprecipitation assay (RIPA) lysis buffer was purchased from Biyuntian. A bicinchoninic acid (BCA) protein assay kit was purchased from Pierce.

Polyvinylidene difluoride (PVDF) membranes were purchased from Millipore Corporation. An enhanced chemiluminescence (ECL) kit was purchased from Pierce. TRIzol reagent was purchased from Invitrogen Corporation. A Fermentask1622 RevertAid™ First Strand cDNA Kit was purchased from Thermo Scientific Corporation. Power SYBR Green PCR Master Mix (2×) was purchased from Applied Biosystems Corporation. The primers were synthesized by Shanghai Shenggong Corporation.

A 7500 quantitative PCR instrument was purchased from Applied Biosystems Corporation. A blood creatinine detection kit (sarcosine oxidase method) and urea nitrogen detection kit (urease method) were purchased from Nanjing Jiancheng Institute of Biological Engineering Corporation. All other reagents were domestically acquired and were of analytical grade.

### Establishment of sepsis-induced AKI mouse model (CLP model)

Healthy male ICR mice, aged 6 - 8 weeks and weighing 28 - 32 g, were given standard pellets and water ad libitum. They were then injected intraperitoneally with 2 % sodium pentobarbital. After being anesthetized, the mice were fixed in the supine position on a plate, and the abdominal area was disinfected with 75 % alcohol three times with cotton balls. A longitudinal median incision of 1.0 cm was made in the abdomen with ophthalmic scissors, and the peritoneum was cut open to fully expose the abdominal cavity.

The cecum was dissected with ophthalmic tweezers, and the end of the cecum was located. The cecum was ligated 1.5 - 2 cm from the end with 4-0 surgical sutures. A 22 needle (black #5 syringe needle) was used to penetrate the cecum twice between the ligation site and the distal end of the cecum, and a small amount of intestinal content was extruded. The cecum was returned to the abdominal cavity, and the fascia and skin were routinely sutured. After the operation, 1 mL of sterile normal saline preheated at 37 °C was injected subcutaneously into the relaxed part of the back of each mouse for resuscitation. In the sham operation group, only cecal exposure, handling, closure and resuscitation were performed. The cecum was neither ligated nor perforated. Modeling success was judged on the basis of the following criteria: no death within 6 h of CLP but the presence of standard symptoms of laboratory sepsis, including erection of hair, diarrhea, reduced activity, and malaise. Mortality reached a peak at 24 h and was 40 - 60 % at 72 h, indicating that the model was successfully established. Moreover, pathological changes in the kidneys of the mice were observed to determine whether AKI was present. The results of the preliminary experiment confirmed that the sepsis-induced AKI model was successfully established.

### Animal grouping

Sixty mice were randomly divided into the sham operation (sham) group, sepsis-induced AKI model (CLP) group, CLP + low-dose resveratrol treatment (CLP + Res<sup>L</sup>) group, CLP + high-dose resveratrol treatment (CLP + Res<sup>H</sup>) group, and CLP + Klotho treatment (CLP + Klotho) group, with 12 mice in each group.

### Drug administration

Resveratrol was prepared as a uniform suspension with normal saline at concentrations of 2 and 4 mg/mL, and the mice were administered 20 and 40 mg/kg intragastrically. All the groups were given approximately the same dose of drug at a dose volume of 10 mL/kg. All

mice underwent intragastric administration every 24 h for 3 days beginning on the day after surgery: CLP + Res<sup>L</sup> group; 20 mg/kg (2 mg/mL) and CLP + Res<sup>H</sup> group; 40 mg/kg (4 mg/mL). In the CLP + Klotho group, recombinant murine  $\alpha$ -Klotho protein (0.01 mg/kg) was intraperitoneally injected once every 24 h for 3 days beginning 1 h after CLP according to a previously described method [5,7]. The sham group and the CLP group were intragastrically and intraperitoneally injected with equal doses of normal saline every day, respectively.

### Specimen collection and processing

The mice were sacrificed 3 days after modeling. The mice were anesthetized with 1 % pentobarbital sodium, and the eyes removed for blood collection. The serum was collected by centrifugation at 2500 rpm for 10 min and stored at -80 °C for renal function analysis. The kidneys were exposed and removed through a ventral midline incision, and the renal capsules were carefully removed. The kidney tissue was divided into three parts: one part of the kidney tissue was washed with ice-cold normal saline, cut into four pieces, quickly transferred to liquid nitrogen, and then stored at -80 °C for Western blot and real-time PCR analysis; one part of the renal tissue was cut into four pieces, fixed in 4 % paraformaldehyde, embedded in routine dehydrated paraffin, and then subjected to hematoxylin and eosin (H & E) and periodic acid-Schiff (PAS) staining and immunohistochemistry. The final part of the renal tissue was fixed in 2.5 % glutaraldehyde for electron microscopy examination.

### Measurement of renal function

Serum creatinine (Scr) and blood urea nitrogen (BUN) were evaluated according to a previous method [5].

### Histopathological examination

Pathological changes in renal tissue were observed by H & E and PAS staining under a light microscope while the formation of apoptotic bodies was observed by transmission electron microscopy. Mouse kidney tissues were collected 3 days after CLP and sectioned. The tissue sections were fixed and stained with H & E and PAS. Images at 400 $\times$  magnification were acquired using a biological imaging microscope (BX53, Olympus, Japan). The specific methods are described in a previous study [5]. Electron microscopy images of the proximal tubule epithelial cells in the renal cortices of mice were

observed under a Hitachi H7500 electron microscope (Hitachi, Japan).

### Determination of protein expression levels of Klotho, Bcl-2, Bax and Caspase-3

The renal cortical tissues of mice were collected, preserved at -80 °C and subjected to Western blotting. The specific methods of the Western blot analysis are described in previous paper [5]. Image Lab 3.0 software (Bio-Rad) was used for exposure, and the gray value was analyzed.

### Immunohistochemical analysis of Klotho and caspase-3 protein expression

The specific methods used for immunohistochemistry (IHC) are described in a previous report [5]. The integrated optical densities (IODs) of the Klotho and Caspase-3 proteins in renal tissue were determined with Image-Pro Plus 6.0 software.

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

Primer 5.0 software was used to design specific primers for Klotho, Bcl-2, Bax and Caspase-3, and the primer sequences are shown in Table 1. The  $\beta$ -actin was used as an internal reference, and the primers were synthesized by Shanghai Shengong Company. Mouse kidney tissue was removed from liquid nitrogen. The RNA was extracted from the kidney tissue using TRIzol reagent, and the absorbance value was measured at 260/280 nm to analyze the purity and concentration of the RNA. The RNA was reverse-transcribed into cDNA using a RevertAid™ First Strand cDNA Kit (25 °C for 5 min, 42 °C for 60 min, and 70 °C for 5 min). Finally, Power SYBR Green PCR Master Mix (2x) was used for real-time quantitative PCR. The PCR procedure was 50 °C for 2 min for one cycle; 95 °C for 10 min for one cycle; and 95 °C for 15 s and 60 °C for 60 s for 40 cycles.

The reliability of the PCR results was evaluated by dissociation curve analysis. The results were analyzed by the  $\Delta\Delta C_t$  method. The  $2^{-\Delta\Delta C_t}$  value is used to represent the relative expression level of the target gene, where  $\Delta\Delta C_t$  was calculated using eqn 1.

$$\Delta\Delta C_t = \{C_t \text{ target gene (test sample)} - C_t \text{ internal reference (test sample)}\} - \{C_t \text{ target gene (corrected sample)} - C_t \text{ internal reference (corrected sample)}\} \dots\dots\dots (1)$$

**Table 1:** Primer sequences

Primer	Direction	Sequence (5'- 3')
Klotho	Forward	TCACTGGGTCAATCTCTGGA
	Reverse	CGCCCTAAACTTGTCTCATCGT
Bcl-2	Forward	GGTGGTGGAGGAACTCTTCA
	Reverse	GAGCAGCGTCTTCAGAGACA
BAX	Forward	TGAAGACAGGGGCCTTTTGT
	Reverse	AATTCCGCCGAGACTCTCG
Caspase-3	Forward	TGACTGGAAAGCCGAAACTC
	Reverse	GCAAGCCATCTCCTCATCA

### Statistical analysis

Statistical Package for the Social Sciences (SPSS) 23.0 software was used for statistical analysis. The measurement data are expressed as the mean  $\pm$  standard deviation. One-way ANOVA was used to compare values in different groups. The differences between groups were tested by the LSD test. The *P*-value was 2-sided, and *P* < 0.05 was considered to indicate statistical significance.

## RESULTS

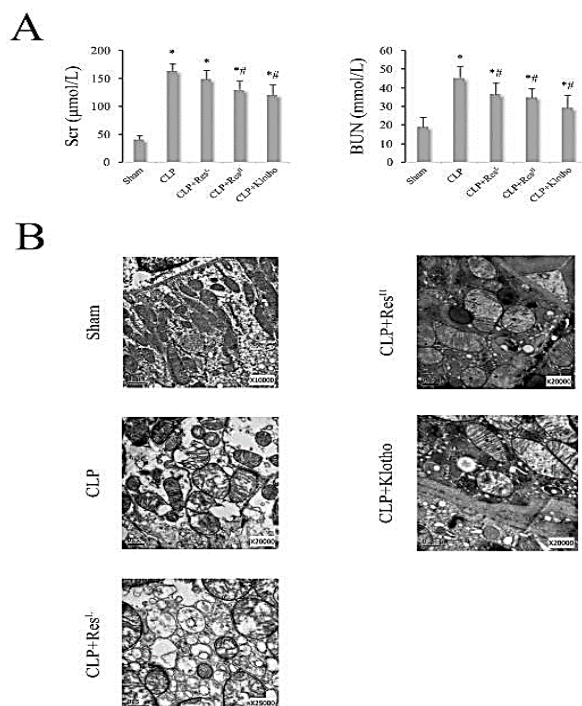
### Changes in renal function

After the CLP operation, Scr and BUN levels were significantly increased, leading to the deterioration of renal function. Compared with those in the sham group, Scr and BUN levels were significantly increased in the CLP group, CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group (\**p* < 0.05). Compared with those in the CLP group, the Scr and BUN levels in the CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group were decreased, and there were statistically significant differences in the CLP + Res<sup>H</sup> group and CLP + Klotho group (#*p* < 0.05). These findings confirmed that treatment with resveratrol or Klotho had beneficial effects on renal function and that high-dose resveratrol had a better effect than low-dose resveratrol (Figure 1 A).

### Changes in renal tissue ultrastructure

The ultrastructure of the proximal convoluted tubule microvilli was dense and slender and were arranged regularly in the sham group. The mitochondria were rod-like without obvious swelling, the mitochondrial cristae were clear and regular, the endoplasmic reticulum was not expanded, and the matrix was uniform and rich. After CLP, mitochondrial swelling, vacuolation, microvillus disorder, mitochondrial outer membrane damage, blurred or broken mitochondrial cristae, unclear mitochondrial boundaries, matrix solidification, fuzzy internal

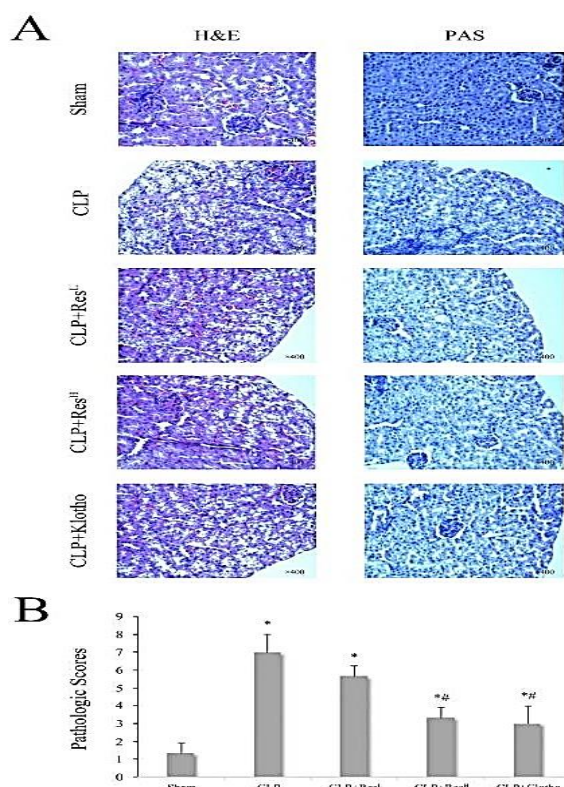
structures, and endoplasmic reticulum expansion were observed. After resveratrol and Klotho intervention, the pathological changes in renal tubule ultrastructure in the CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group were alleviated (Figure 1 B).



**Figure 1:** Changes in renal function and ultrastructural changes in renal tissue in mice (A). Images showing renal function and the effects of resveratrol and the Klotho protein on Scr and BUN levels in mice in the different groups. \* $P < 0.05$  vs. sham group; # $p < 0.05$  vs. CLP group. (B). Electron microscopy images showing the ultrastructure of proximal tubule epithelial cells in the renal cortices

### Pathological changes

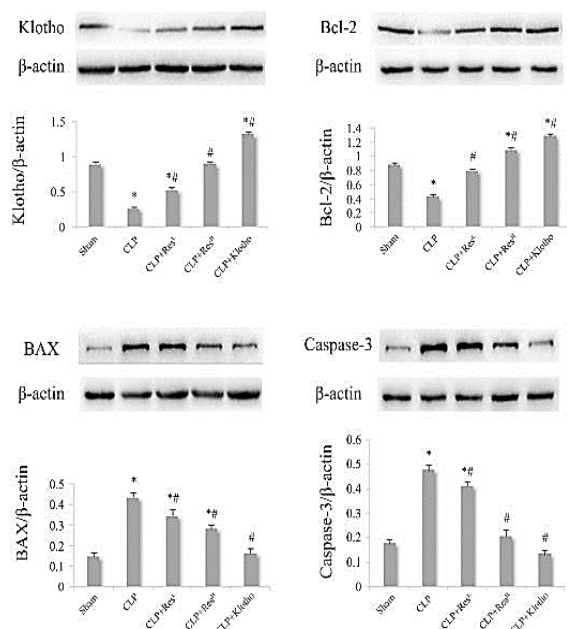
The H & E and PAS staining showed vacuolar degeneration, renal tubule brush border epithelial cell exfoliation, basement membrane exposure and interstitial inflammatory cell infiltration in the kidneys of the mice in each group after CLP. These changes were not obvious in the sham group. After resveratrol and Klotho intervention, the pathological changes in renal tubules in CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group were alleviated. Semiquantitative scores were calculated for epithelial vacuolar degeneration, basement membrane exposure, and interstitial inflammatory cell infiltration. No, mild, moderate, and severe lesions were indicated by scores of 0, 1, 2, and 3 points, respectively. The pathological scores of each group were evaluated (Figure 2).



**Figure 2:** Pathological changes in renal tissue in mice in the different groups (A). Images of H & E and PAS-stained kidney tissues. (B). Semiquantitative pathological scores in the different groups. \* $P < 0.05$  vs. sham group; # $p < 0.05$  vs. CLP group

### Protein expressions

Western blotting showed that the protein expression of Klotho and Bcl-2 was decreased after CLP, while that of Bax and Caspase-3 was increased after CLP. After treatment with resveratrol and Klotho, the protein expression of Klotho and Bcl-2 increased, while that of Bax and Caspase-3 decreased. Both directional changes were alleviated compared with those in the CLP group. Compared with that in the sham group, the protein expression of Klotho and Bcl-2 in CLP group, CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group was significantly decreased, while the protein expression of Bax and Caspase-3 was significantly increased, and some differences were statistically significant (partly \* $p < 0.05$ ). After resveratrol and Klotho intervention, compared with that in the CLP group, the protein expression of Klotho and Bcl-2 was increased, while the protein expression of Bax and Caspase-3 was decreased in the CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group. Some differences were statistically significant (# $p < 0.05$ ) (Figure 3).



**Figure 3:** Protein expression (Semiquantitative Western blots) of Klotho, Bcl-2, Bax and Caspase-3. \* $P < 0.05$  vs. sham group; # $p < 0.05$  vs. CLP group

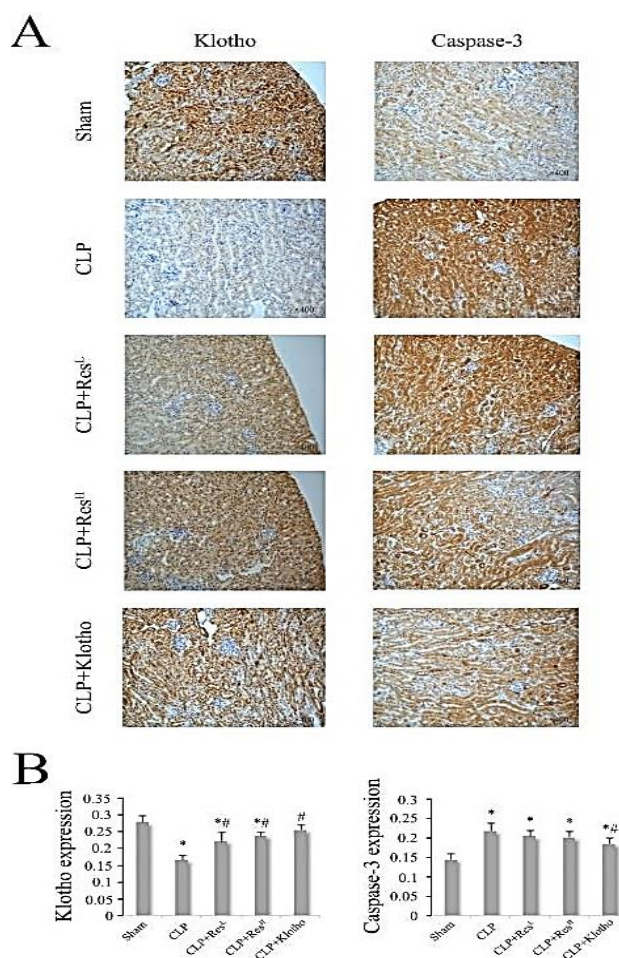
### Distribution and expression of Klotho and Caspase-3 proteins

Immunohistochemistry showed that the Klotho and Caspase-3 proteins were mainly distributed in the tubular cytoplasm of mice. Compared with that in the sham group, Klotho protein expression was significantly decreased in the CLP group, CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group, while Caspase-3 protein expression was significantly increased (\* $p < 0.05$ ). These findings indicated that Klotho levels were decreased and that Caspase-3 levels were increased after CLP. After resveratrol and Klotho intervention, compared with that in the CLP group, the protein expression of Klotho was increased in the CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group, while the protein expression of Caspase-3 was decreased. The decreases in Klotho expression in the CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group and the increase in Caspase-3 expression in the CLP + Klotho group were significant (# $p < 0.05$ ; Figure 4).

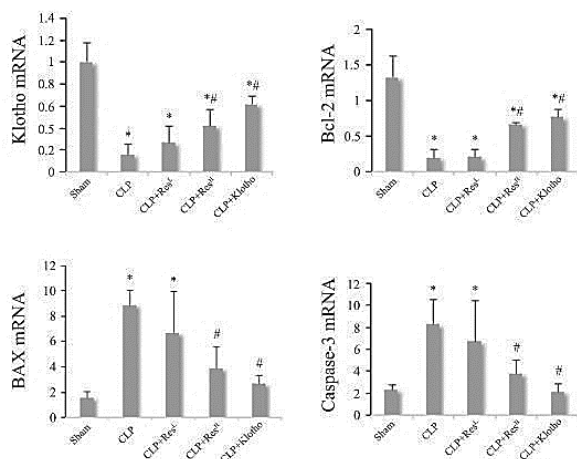
### MRNA expressions of Klotho, Bcl-2, Bax and Caspase-3

Quantitative RT-PCR showed that the mRNA expression of Klotho and Bcl-2 was decreased after CLP, while that of Bax and Caspase-3 was increased after CLP. After resveratrol and Klotho protein intervention, the mRNA expression of Klotho and Bcl-2 was increased, and the mRNA

expression of Bax and Caspase-3 was decreased. Both of the directional changes were alleviated compared with those in the CLP group. Compared with that in the sham group, the mRNA expression of Klotho and Bcl-2 in the CLP group, CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group was significantly decreased, while the mRNA expression of Bax and Caspase-3 was significantly increased. Some differences were statistically significant (\* $p < 0.05$ ). After resveratrol and Klotho intervention, compared with that in the CLP group, the mRNA expression of Klotho and Bcl-2 was increased in the CLP + Res<sup>L</sup> group, CLP + Res<sup>H</sup> group and CLP + Klotho group, while the mRNA expression of Bax and Caspase-3 was decreased. There were statistically significant differences compared with the sham and CLP groups (# $p < 0.05$ ; Figure 5).



**Figure 4:** Distribution and expression of the Klotho and Caspase-3 proteins in the kidney tissues. (A). Immunohistochemical images showing Klotho and Caspase-3 protein expression. Images at 200x magnification (B). Semiquantitative immunohistochemical staining among the different groups. \* $P < 0.05$  vs. sham group; # $p < 0.05$  vs. CLP group



**Figure 5:** The mRNA expression (semiquantitative PCR) of Klotho, Bcl-2, Bax and Caspase-3. \* $p < 0.05$  vs. sham group; # $p < 0.05$  vs. CLP group

## DISCUSSION

A mouse model of sepsis-induced AKI was successfully established by CLP. After the CLP operation, the levels of Scr and BUN increased, and renal function deteriorated. The H & E and PAS staining showed vacuolar degeneration and abscission of the epithelial cells in the brush borders of the renal tubules, basement membrane exposure, interstitial inflammatory cell infiltration, and exacerbation of pathological scores, all of which indicated successful modeling. Furthermore, the protective effect of resveratrol and Klotho protein against sepsis (CLP)-induced AKI in mice was investigated and the relationship between resveratrol and endogenous Klotho protein expression was observed. The role of resveratrol in sepsis-induced AKI has not been well studied.

Resveratrol is known to significantly reduce mortality and alleviate AKI in septic mice by reducing endoplasmic reticulum stress, inhibiting the NF- $\kappa$ B pathway and alleviating the inflammatory response [8,9]. Resveratrol can play a therapeutic role in sepsis-induced AKI mice by activating the SIRT1/3 pathway [10]. Resveratrol also promotes the deacetylation of NF- $\kappa$ B P65 by upregulating SIRT1, thereby alleviating sepsis-induced AKI [11].

A previous study showed that administration of both 0.01 mg/kg recombinant Klotho protein to a mouse model of sepsis-induced AKI *in vivo* and 0.4  $\mu$ g/mL recombinant Klotho protein to a cell model of sepsis-induced AKI *in vitro* alleviated acute renal dysfunction and kidney injury and partly restored endogenous Klotho protein expression, playing a protective role in the kidney

[5]. Other studies have reported that recombinant  $\alpha$ -Klotho is safe and effective and has the potential to prevent and treat the progression of AKI to chronic kidney disease (CKD) and to block the progression of uremic cardiomyopathy [12]. Yamamoto *et al* [13] showed that Klotho protein supplementation 30 min after ischemic renal injury can alleviate AKI, indicating that Klotho can prevent the decline in renal function.

The results of the current study showed that both low-dose and high-dose resveratrol reduced Scr and BUN levels, improved renal function, partially ameliorated the pathological changes in sepsis-induced AKI, and alleviated tubulointerstitial lesions, especially high-dose resveratrol. The improvement was more obvious in the high-dose group than in the low-dose group, with a significant difference between groups. After Klotho protein treatment, kidney function was improved, pathological changes were alleviated, and protective therapeutic effects were observed. These effects were statistically significant and were consistent with those in previous studies. Therefore, it became necessary to investigate whether there is a connection between resveratrol and Klotho. Additional results indicated that Klotho expression was decreased after CLP, as shown by immunohistochemistry, Western blotting, and quantitative real-time PCR, while Klotho was increased (at both the mRNA and protein levels) after resveratrol intervention, indicating that resveratrol could restore endogenous Klotho expression.

Resveratrol induced the same effect as exogenous administration of the Klotho protein and had the same renoprotective effect. The next question that needed attention was to find out the mechanism by which resveratrol upregulates endogenous Klotho expression. Resveratrol is known to reduce septic organ damage by affecting apoptosis. Chen *et al* [14] reported that resveratrol can reduce apoptosis in macrophages, reduce the expression of iNOS, Bcl-2 and Bcl-xL in LPS-induced macrophages and reduce sepsis-induced AKI. An *et al* [15] confirmed that resveratrol reduces cardiomyocyte apoptosis during sepsis by activating SIRT 1. Studies have also shown that resveratrol can inhibit LPS-induced alveolar epithelial cell apoptosis by activating the SIRT 1 pathway to reduce organ damage [16]. Klotho may also exert a renoprotective effect by inhibiting apoptosis. Studies have shown that upregulation of the Klotho protein can play a protective role in cisplatin-induced AKI through antioxidant and antiapoptotic effects [17].

In umbilical vein endothelial cells, Klotho protein inhibits apoptosis by affecting oxidative stress in the endoplasmic reticulum via the PI3K/Akt pathway [18]. Klotho also inhibits osteoblast apoptosis through the NF- $\kappa$ B pathway [19]. Therefore, both resveratrol and Klotho have the ability to inhibit apoptosis, but is there a correlation between resveratrol and Klotho? Only one report of such a relationship is available. It suggests that resveratrol can enhance the expression of the antiaging gene Klotho in the kidney by activating the signaling pathway mediated by the transcription factor 3/c-Jun complex [20].

Further results of the current study showed that after CLP, Bcl-2 expression decreased, while Bax and Caspase-3 expression increased, indicating that CLP activated apoptosis. In contrast, after low-dose and high-dose resveratrol intervention, Bcl-2 expression increased, while Bax and Caspase-3 expression decreased (at both the mRNA and protein levels). The results showed that resveratrol reduced the activation of apoptosis, especially in the high-dose intervention group. Exogenous application of Klotho had an effect similar to that of resveratrol. The expression of Bcl-2 increased and the expression of Bax and Caspase-3 decreased (at both the mRNA and protein levels) after Klotho protein administration, which suggested that the Klotho protein also had antiapoptotic effects. Therefore, resveratrol can increase endogenous Klotho expression induced AKI.

## CONCLUSION

The findings of this study using mouse model of CLP-induced sepsis with AKI show that endogenous Klotho expression reduces after CLP operation, and consequently activates the apoptotic pathway. Resveratrol and Klotho administration upregulates the expression of endogenous Klotho and has antiapoptotic effects. Therefore, this treatment has a renoprotective effect in mice with sepsis-induced AKI, and has potentials for development as a strategy for the management of sepsis-induced AKI.

## DECLARATIONS

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None provided

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### Ethical approval

None provided.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done 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. Yu Chen and Xinxin Chen were the main researchers; they planned the study, wrote the protocol, performed all the experiments, and contributed to writing this manuscript. Xiaoting Ye and Shasha Jin helped with animal experiments. Weixia Huang and Chaosheng Chen helped with technical advice and statistical analysis. Xinxin Chen critically reviewed and revised the manuscript. All authors approved the final manuscript.

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## REFERENCES

1. Shaw PJ, Feske S. Regulation of lymphocyte function by ORAI and STIM proteins in infection and autoimmunity. *J Physiol* 2012; 590(17): 4157-4167.
2. Schrier RW, Wang W. Acute renal failure and sepsis. *N Engl J Med* 2004; 351(2):159-169.



3. Vincent JL, Atalan HK. Epidemiology of severe sepsis in the intensive care unit. *Br J Hosp Med (Lond)* 2008; 69(8): 442-443.
4. Hu MC, Shi M, Zhang J, Quinones H, Kuro-o M, Moe OW. Klotho deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective. *Kidney Int* 2010; 78(12): 1240-1251.
5. Chen XX, Tong H, Chen Y, Chen CS, Ye JJ, Mo QF, Zhao GJ, Hong GL, Zheng CF, Lu ZQ. Klotho ameliorates sepsis-induced acute kidney injury but is irrelevant to autophagy. *Onco Targets Ther* 2018; 11: 867-881.
6. Nonomura S, Kanagawa H, Makimoto A. Chemical constituents of polygonaceous plants. I. Studies on the components of ko-j o-kon. (*Polygonum cuspidatum* Sieb. Et zucc. *Yakugaku zasshi* 1963; 83: 988-990.
7. Li XX, Huang LY, Peng JJ, Liang L, Shi DB, Zheng HT, Cai SJ. Klotho suppresses growth and invasion of colon cancer cells through inhibition of IGF1R-mediated PI3K/AKT pathway. *Int J Oncol* 2014; 45(2): 611-618.
8. Luo CJ, Luo F, Bu QD, Jiang W, Zhang W, Liu XM, Che L, Luan H, Zhang H, Ma RX et al. Protective effects of resveratrol on acute kidney injury in rats with sepsis. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2020; 164(1): 49-56.
9. Wang N, Mao L, Yang L, Zou J, Liu K, Liu M, Zhang H, Xiao X, Wang K. Resveratrol protects against early polymicrobial sepsis-induced acute kidney injury through inhibiting endoplasmic reticulum stress-activated NF-kappaB pathway. *Oncotarget* 2017; 8(22): 36449-36461.
10. Xu S, Gao Y, Zhang Q, Wei S, Chen Z, Dai X, Zeng Z, Zhao KS. SIRT1/3 Activation by Resveratrol Attenuates Acute Kidney Injury in a Septic Rat Model. *Oxid Med Cell Longev* 2016; 2016: 7296092.
11. Gan Y, Tao S, Cao D, Xie H, Zeng Q. Protection of resveratrol on acute kidney injury in septic rats. *Hum Exp Toxicol* 2017; 36(10): 1015-1022.
12. Hu MC, Shi M, Gillings N, Flores B, Takahashi M, Kuro OM, Moe OW. Recombinant alpha-Klotho may be prophylactic and therapeutic for acute to chronic kidney disease progression and uremic cardiomyopathy. *Kidney Int* 2017; 91(5): 1104-1114.
13. Yamamoto M, Clark JD, Pastor JV, Gurnani P, Nandi A, Kurosu H, Miyoshi M, Ogawa Y, Castrillon DH, Rosenblatt KP et al. Regulation of oxidative stress by the anti-aging hormone klotho. *J Biol Chem* 2005; 280(45): 38029-38034.
14. Chen L, Yang S, Zumbun EE, Guan H, Nagarkatti PS, Nagarkatti M. Resveratrol attenuates lipopolysaccharide-induced acute kidney injury by suppressing inflammation driven by macrophages. *Mol Nutr Food Res* 2015; 59(5): 853-864.
15. An R, Zhao L, Xu J, Xi C, Li H, Shen G, Zhang W, Zhang S, Sun L. Resveratrol alleviates sepsis-induced myocardial injury in rats by suppressing neutrophil accumulation, the induction of TNFalpha and myocardial apoptosis via activation of Sirt1. *Mol Med Rep* 2016; 14(6): 5297-5303.
16. Liu X, Shao K, Sun T. SIRT1 regulates the human alveolar epithelial A549 cell apoptosis induced by *Pseudomonas aeruginosa* lipopolysaccharide. *Cell Physiol Biochem* 2013; 31(1): 92-101.
17. Ko JW, Shin NR, Jung TY, Shin IS, Moon C, Kim SH, Lee IC, Kim SH, Yun WK, Kim HC et al. Melatonin attenuates cisplatin-induced acute kidney injury in rats via induction of anti-aging protein, Klotho. *Food Chem Toxicol* 2019; 129: 201-210.
18. Cui W, Leng B, Liu W, Wang G. Suppression of Apoptosis in Human Umbilical Vein Endothelial Cells (HUVECs) by Klotho Protein is Associated with Reduced Endoplasmic Reticulum Oxidative Stress and Activation of the PI3K/AKT Pathway. *Med Sci Monit* 2018; 24: 8489-8499.
19. Liang X, Li B, Huang Q, Liu D, Ma H. Klotho prevents DEX-induced apoptosis in MC3T3-E1 osteoblasts through the NF-kappaB signaling pathway. *Biochem Biophys Res Commun* 2018; 507(1-4): 355-361.
20. Hsu SC, Huang SM, Chen A, Sun CY, Lin SH, Chen JS, Liu ST, Hsu YJ. Resveratrol increases anti-aging Klotho gene expression via the activating transcription factor 3/c-Jun complex-mediated signaling pathway. *Int J Biochem Cell Biol* 2014; 53: 361-371.