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Research article

The study on role of endothelial cell autophagy in rats with sepsis-induced acute kidney injury

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ABSTRACT

Sepsis often causes acute kidney injury (AKI). Autophagy of renal tubular epithelial cells is considered a cytoprotective mechanism in septic AKI; however, the role of autophagy of renal endothelial cells is uninvestigated. The current study examined whether autophagy was induced by sepsis in renal endothelial cells and whether induction of autophagy in these cells attenuated the degree of AKI. Cecal ligation and puncture (CLP) was used as a model of sepsis in rats. Four experimental groups included: sham, CLP alone, CLP + rapamycin (RAPA), and CLP + dimethyl sulfoxide (DMSO), where RAPA was used as an activator of autophagy. CLP increased renal LC3-II protein levels with an additional transient increase by RAPA at 18 h. In addition, CLP induced autophagosome formation in renal endothelial cells had an additional increase induced by RAPA. Interestingly, the levels of bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI), an endothelial cell-specific protein in the kidney, were also increased by CLP, albeit it was transiently downregulated by RAPA at 18 h. Serum thrombomodulin increased and renal vascular endothelial (VE)-cadherin decreased following CLP, and these changes were attenuated by RAPA. The renal cortex exhibited and inflammatory tissue damage after CLP, and RAPA alleviated these histopathological injuries. The current findings indicate that autophagy was induced by sepsis in renal endothelial cells, and upregulation of autophagy in these cells alleviated endothelial injury and AKI. In addition, BAMBI was induced by sepsis in the kidney, which may play a role in regulating endothelial stability in septic AKI.

1. Introduction

Acute kidney injury (AKI) is a common and severe complication of sepsis. Septic AKI accounts for approximately 50% of all severe AKI cases in hospital and is associated with increased rates of morbidity and mortality [1]. Importantly, the pathological mechanisms of septic AKI are not well understood. Most early studies were focused on kidney hypoperfusion caused by global renal ischemia due to systemic hemodynamic derangements. However, an increasing body of recent evidence indicates that septic AKI can occur in the absence of reduced renal blood flow, and at least in the early phase of the disease, renal dysfunction largely results from interactive mechanisms involving inflammation, coagulation derangements, microvascular dysfunction, endothelial injury, and tubular epithelial cell damage [2,3]. Therefore, protecting renal endothelial cells from sepsis-induced injury may provide therapeutic benefits for the

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prevention or treatment of septic AKI.

Autophagy degrades and recycles damaged organelles and macromolecules to maintain cellular homeostasis. Investigations utilizing kidney-specific gene knockouts have demonstrated that autophagy plays an important role in the maintenance of kidney homeostasis under both physiological and pathological conditions [4]. Importantly, genetic or pharmacological upregulation of autophagy has been shown to alleviate inflammation and renal dysfunction in various animal models of sepsis-induced AKI [5–7]. In these studies, histological evidence revealed the induction of autophagy in tubular epithelial cells by sepsis, and further autophagy activation in these cells protected against tubular epithelial damage and AKI [5–7]. However, the role of endothelial cell autophagy in septic AKI remains undefined.

In this study, we investigated whether autophagy was induced in renal endothelial cells by sepsis and whether pharmacological induction of autophagy in these cells attenuated the degree of AKI. Cecal ligation and puncture (CLP) was used as a model of sepsis in rats. Autophagy was assessed based on the level of the autophagy biomarker membrane-bound microtubule-associated protein light chain 3 (LC3-II) and autophagosome formation was evaluated by transmission electron microscopy (TEM). Endothelial biomarkers and histopathological changes with TEM. Renal tissue damage was visualized with H&E staining.

2. Methods

2.1. CLP as a model of sepsis

Male Sprague-Dawley (SD) rats (n = 221, 8 weeks old, 200–250 g) were obtained from the animal facility at Beijing Friendship Hospital, affiliated with Capital Medical University (Beijing, China). The animals were reared in separate cages in the laboratory of barrier system without specific pathogens (SPF). All animals were housed in a constant temperature (22 ± 2 °C) and humidity (45 ± 5%) with a standard 12-h light–dark cycle. The rats were allowed free access to tap water and standard rodent laboratory food. Under such conditions, the experiment was carried out for a week. All animal studies were performed in accordance with the Chinese Code of Practice for the Care and Use of Animals for Scientific Purposes and received approval from the Institutional Animal Care and Use Committee of Beijing Friendship Hospital (Approval number: 18–1005). After one week of acclimation, the animals were anesthetized with 3% isoflurane and randomly divided into four groups: sham, CLP, CLP + rapamycin (RAPA), and CLP + dimethylsulfoxide (DMSO; vehicle control, the concentration of DMSO was 10%). CLP was performed with an 18-gauge double puncture and 100% cecal ligation following previously described procedures [8]. The sham group received the same operation except for the CLP. The CLP + RAPA group received RAPA (8 mg/kg) intraperitoneally immediately following the CLP procedure, and the CLP + DMSO group received the same volume of DMSO. Anesthesia was maintained with 1.5% isoflurane throughout the operation. After the operation was completed, all animals were transferred to metabolic cages and received subcutaneous fluid resuscitation (sterile saline, 5 mL/100 g body weight). At 3, 6, 12, 18, 24, and 48 h after the operation, blood (via the abdominal aorta) and urine samples were collected from six surviving rats in each group. The rats were sacrificed immediately after sample collection, and the contralateral renal cortex was harvested. The renal cortical tissues were stored at −80 °C until further analysis.

2.2. Western blot analysis

The renal cortical tissues were homogenized in pre-cooled radio-immunoprecipitation assay (RIPA) lysis buffer (Ruibio, Germany) and centrifuged at 13,000 rpm and 4 °C for 20 min. The clear supernatants were collected, and total protein concentrations of the supernatants were determined using the bicinchoninic acid (BCA) method. Proteins were separated using a 12–15% gel by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes. After blocking, the membranes were incubated with antibodies toward rat bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI; 1:1000, ab203070; Abcam, UK), LC3-II (1:1000, ab51520; Abcam), and vascular endothelial (VE)-cadherin (1:1000, ab166715; Abcam), at 4 °C overnight. After washing three times in tris-buffered saline with Tween 20 (TBS-T), the membranes were incubated with an appropriate horseradish peroxidase (HRP)-conjugated secondary antibody. The proteins were quantified by densitometric analysis using the Gel Image System 4.00 (Tanon, China). Data were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:1000, 5174; Cell Signaling Technology, USA).

2.3. ELISA

The urine samples were centrifuged at 1000×*g* (or 3000 rpm) for 15 min, and the supernatants were collected and stored at −20 °C or −80 °C until analysis. The blood samples (collected without anticoagulant) were allowed to stand at room temperature for 1–2 h, then centrifuged at 1000×*g* (or 3000 rpm) for 15 min. The serum supernatants were collected and stored at −20 °C or −80 °C until analysis. The urine protein levels, as well as the serum soluble thrombomodulin (sTM) and creatinine levels were determined using ELISA kits in accordance with manufacturer's instructions (urine protein E02U0025, serum sTM E02S0222, serum creatinine E02C0629; Shanghai Lanji Biotechnology Co. Ltd., China). Plates were read using the Multiskan Mk3 Microplate Reader (Thermo Fisher, USA).

2.4. H&E staining

Hematoxylin and eosin (H&E) staining was used to evaluate histopathological changes of the renal cortex. Renal cortical tissues,

approximately 3 mm × 3 mm × 3 mm in size, were fixed in 2.5% glutaraldehyde for 24 h. The fixed tissues were immersed in 0.01 mol/L PBS overnight, dehydrated with a conventional ethanol gradient, embedded in paraffin, and sectioned. The tissue sections were stained with H&E, dried, and sealed with a neutral resin. The sections were subsequently examined under a light microscope. The histopathological injuries of the glomerulus were evaluated by a pathologist blinded to the experimental conditions. The injuries were scored from 0 to 4 based on the grade of glomerular telangiectasia, interstitial edema, inflammatory cell infiltration, congestion, hemorrhage, and tissue necrosis as follows: 0, normal; 1, minimal injury; 2, mild injury; 3, moderate injury; 4, severe injury. Five sections from each group were selected for evaluation. The injury score of each section was determined using 10 randomly selected fields of view [9].

2.5. Transmission electron microscopy

Transmission electron microscopy (TEM) was used to detect the formation of autophagosomes and visualize the morphological changes of renal vascular endothelial cells. Renal cortical tissues, approximately 1 mm × 1 mm × 1 mm in size, were placed in fixation solution for electron microscopy (Ruibio) for 2–4 h at 4 °C. After washing three times with 0.1 mmol/L PBS for 15 min each, the samples were fixed using 1% citric acid in 0.1 mmol/L phosphate buffer (pH 7.4) for 2 h at room temperature. The samples were subsequently dehydrated, infiltrated, embedded, and cut into 60–80 nm sections using a microtome. The tissue slices were double stained with uranium and lead, dried overnight at room temperature, and subjected to TEM then for analysis (Hitachi, Japan). As to analysis briefly, a minimum of 8–10 random fields (to minimize unintended sampling bias) were examined at 2500 × magnification for

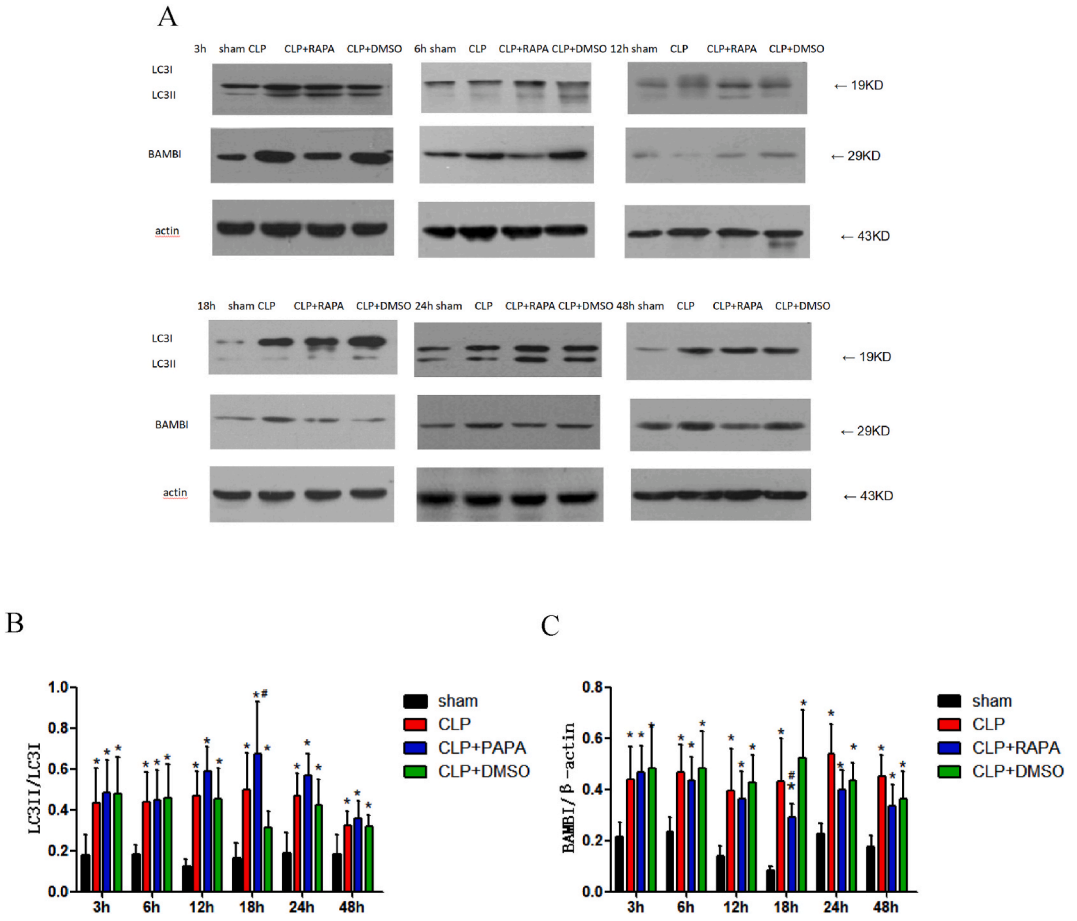

Fig. 1. The renal cortex LC3II and BAMBI levels by Western blot analysis. (A) Representative gel images of Western blot analysis for renal cortex levels of LC3II and BAMBI in sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the CLP operation. (B) The renal cortex LC3II/LC3I levels in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6, *P < 0.05 vs. sham, (Tukey's correction and Dunnet's correction) #P = 0.045 (Dunnet's correction) (C) The renal cortex BAMBI levels in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6, *P < 0.05 vs. sham, (Tukey's correction and Dunnet's correction) #P = 0.036 (Tukey's correction) vs. CLP + DMSO. Abbreviations: BAMBI, bone morphogenetic protein and activin membrane-bound inhibitor; CLP, cecal ligation and puncture; DMSO, dimethylsulfoxide; LC3-II, membrane-bound microtubule-associated protein light chain 3; RAPA, rapamycin (potent inducer of autophagy).

the number of autophagosomes or autolysosomes. The median ± interquartile per 8 images from each rat was calculated and the data from different groups were compared (CLP (n = 5) versus CLP + RAPA (n = 5)) at the time course of 24 h after operation.

2.6. Animal survival

The percentages of surviving animals in each group at 3, 6, 12, 18, 24, and 48 h after the operation were recorded. Apnea for more than 1 min was considered to indicate death.

2.7. Statistical analysis

Survival analysis was performed using the Kaplan-Meier method, and the survival data were compared using the log-rank test. All other data are presented as mean ± standard deviation (SD). For comparison of normally distributed data, an analysis of variance (ANOVA) was used if the data met the assumption of homogeneity of variance, while a Welch ANOVA was used if the data violated the assumption. The observation values of the samples did not conform to normal distribution and the variances were uneven among the groups, so one-way ANOVA could not be used, so the Kruskal-Wallis H test was used. The number of autophagosomes or autolysosomes were expressed as median ± interquartile and Mann-Whitney test was used for analysis. A P-value less than 0.05 was deemed statistically significant. All statistical analyses were performed using SPSS 19.0 software.

3. Results

3.1. CLP increased LC3II/LC3I in the renal cortex with additional induction by RAPA

To evaluate autophagic activity in the renal cortex, the protein levels of LC3-II, a common biomarker for autophagy, were determined using Western blot analysis (Fig. 1A). CLP increased the levels of LC3II/LC3I in the renal cortex from 3 to 48 h after the

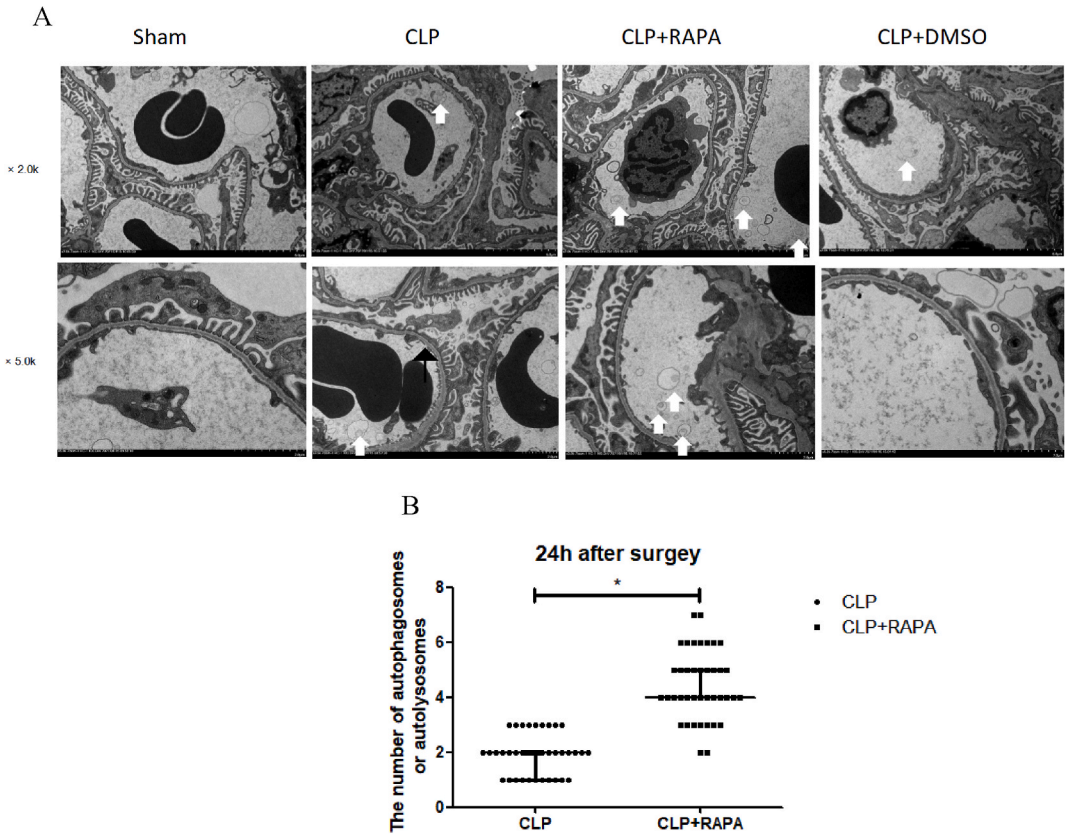

Fig. 2. (A) Representative TEM images of renal cortex endothelial cells from the CLP and CLP + RAPA groups at 24 h after the CLP operation. Representative images at × 2.0 k and × 5.0 k magnification. White arrows indicate autophagosomes or autolysosomes with double-layer membranes. (B)The number of autophagosomes or autolysosomes were expressed as the median ± interquartile range. Data were analyzed for statistical significance using the Mann-Whitney *U* test (*P = 0.000; n = 5 in each group). Abbreviations: CLP, cecal ligation and puncture; RAPA, rapamycin (potent inducer of autophagy); TEM, transmission electron microscopy.

operation (Fig. 1B, $P < 0.05$). The LC3II/LC3I level peaked at 18 h, and then declined. RAPA further increased LC3II/LC3I levels compared to DMSO at 18 h after CLP (Fig. 1B, $P = 0.045$). At other time points, RAPA slightly increased the LC3-II level, but the differences did not reach statistical significance (Fig. 1B).

3.2. CLP upregulated BAMBI in the renal cortex, which was downregulated by RAPA

The transforming growth factor beta (TGFβ) and Wnt/β-catenin pathways are key regulators of AKI pathogenesis [10,11]. BAMBI is a membrane-bound pseudo-receptor that influences both TGFβ and Wnt/β-catenin signaling [12,13]. Given that BAMBI expression in the kidney is restricted to endothelial cells in the glomerulus and renal blood vessels [14], it was considered that BAMBI may play a role in regulating renal endothelial function/stability in septic AKI. The Western blot analysis revealed that CLP increased BAMBI protein levels in the renal cortex from 3 to 48 h after the operation (Fig. 1C, $P < 0.05$), and these levels were downregulated by RAPA at 18 h (Fig. 1C, $P = 0.036$). Since the BAMBI protein is regulated by autophagy-mediated degradation [14], its downregulation by RAPA was likely attributed to upregulated degradation through autophagy. However, RAPA had no significant effects on BAMBI at any other time points (Fig. 1C), indicating that the downregulation of BAMBI by RAPA was only transient.

3.3. CLP increased autophagy in renal endothelial cells with additional induction by RAPA

To determine whether CLP induced autophagy in renal endothelial cells, TEM was used to examine the formation of autophagosomes in these cells. Autophagosomes encircled by double-layer membranes were detected in renal endothelial cells of the CLP but not the sham group at 24 h after the operation (Fig. 2A). In addition, the CLP + RAPA group had a higher number of autophagosomes compared to the CLP + DMSO group (Fig. 2B,$P = 0.000$). These findings demonstrated that CLP induced autophagy in renal endothelial cells with an additional induction by RAPA.

3.4. CLP induced renal endothelial injury, which was transiently reversed by RAPA

Serum sTM is a biomarker for endothelial injury and an independent predictor for the development of septic AKI [15]. Using ELISA, higher levels of serum sTM were detected in the operation groups compared to the sham group at 18 h (Fig. 3C, $P < 0.05$). VE-cadherin, an endothelial-specific cell-cell adhesion protein, plays a central role in endothelial permeability and vascular stability [16]. The extracellular domain of VE-cadherin can be cleaved during inflammatory processes and shed into the circulation as soluble VE-cadherin (sVE-cadherin). A recent report has shown that shedding of sVE-cadherin is associated with the severity of sepsis-induced

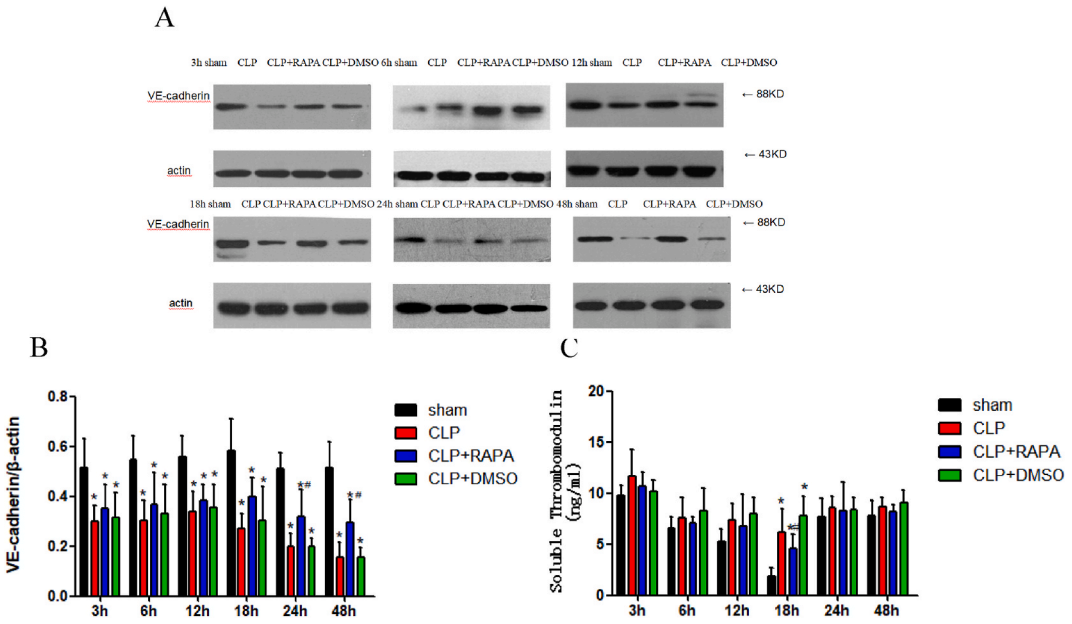

Fig. 3. The renal cortex VE-cadherin levels and serum sTM levels by Western blot analysis and ELISA. (A)Representative gel images of Western blot analysis for renal cortex VE-cadherin in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the CLP operation. (B) The renal cortex VE-cadherin levels in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6, *P < 0.05 vs. sham, $^{\#}P < 0.05$ vs. CLP + DMSO. 24 h, $^{\#}P = 0.04$ (Tukey's correction); 48 h, $^{\#}P = 0.028$ (Tukey's correction).(C) The serum sTM levels in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6, *P < 0.05 vs. sham, $^{\#}P < 0.05$ vs. CLP + DMSO. $^{\#}P = 0.047$ (Dunnet's correction). Abbreviations: CLP, cecal ligation and puncture; DMSO, dimethylsulfoxide; ELISA, enzyme-linked immunosorbent assay; RAPA, rapamycin (potent inducer of autophagy); sTM, soluble thrombomodulin; VE, vascular endothelial.

AKI [17]. In this study, Western blot analysis revealed decreased VE-cadherin levels (Fig. 3A) in the renal cortex from 3 to 48 h after CLP (Fig. 3B, $P < 0.05$), suggesting an increase in shedding of sVE-cadherin caused by renal endothelial injury. RAPA *transiently* reversed the changes in serum sTM and renal VE-cadherin (Fig. 3C and B, $P < 0.05$).

3.5. RAPA alleviated the glomerulus injury scores in CLP-induced AKI, which had no significant effect on renal fucnation

H&E staining of the renal cortex revealed dilated and damaged glomerular lobules with inflammatory cell infiltration at 24 h after CLP (Fig. 4A). Compared with the CLP + DMSO group, the CLP + RAPA group exhibited reduced glomerular telangiectasia, congestion, and inflammatory cell infiltration. The glomerulus injury scores based on histopathological changes observed with H&E staining. $n = 5$, (Fig. 4B,*$P < 0.05$ vs. sham, #$P = 0.004$ (Kruskal-Wallis H Test) vs. CLP + DMSO. These results indicated that *RAPA alleviated the glomerulus injury scores in CLP-induced AKI.* To assess kidney function, serum creatinine and urine protein levels were determined using ELISA. CLP increased serum creatinine from 3 to 48 h after the operation (Fig. 5A, $P < 0.05$), but had no significant effect on urine protein levels at any given time point (Fig. 5C). RAPA showed no significant effects on serum creatinine or urine protein (Fig. 5A–D). In addition, RAPA did not significantly change the survival rates of the animals following the induction of sepsis (Fig. 6).

4. Discussion

Septic AKI is a disease of complex etiology. Recent evidence suggests that a key event in the early stage of kidney dysfunction is tubular epithelial cell damage in response to inflammation associated with peritubular microvascular dysfunction [18]. Thus, renal endothelial cell injury induced by sepsis is an early pathological hallmark of AKI. In this study, using CLP as a sepsis model in rats, it was found that sepsis induced autophagy in renal endothelial cells, and the further induction of autophagy in these cells by RAPA alleviated sepsis-induced renal endothelial damage and AKI. These findings support the hypothesis that an upregulation of autophagy in renal endothelial cells may provide therapeutic benefits in sepsis-induced AKI.

By recycling degraded macromolecules for energy metabolism or anabolism, autophagy serves as a mechanism for survival in response to starvation. In pathological conditions, autophagy can exhibit cytoprotective effects against cell injury induced by oxidative stress or inflammation [19]. However, excessive autophagy can lead to unwanted cell death [20]. The role of autophagy in septic AKI appears to be somewhat controversial [21]. In lipopolysaccharide (LPS)-induced AKI mice, an early study by Wu and colleagues showed that autophagy was induced in the renal cortex in a dose- and time-dependent manner, and pharmacological inhibition of autophagy alleviated LPS-induced AKI and inflammation [22]. Matsuda et al.reported that endothelial autophagy protects glomeruli from oxidative stress and maintains the integrity of glomerular capillaries. Enhancing endothelial autophagy may provide a novel therapeutic approach to minimizing glomerular diseases [23]. However, in a later study by Mei et al., pharmacological or genetic

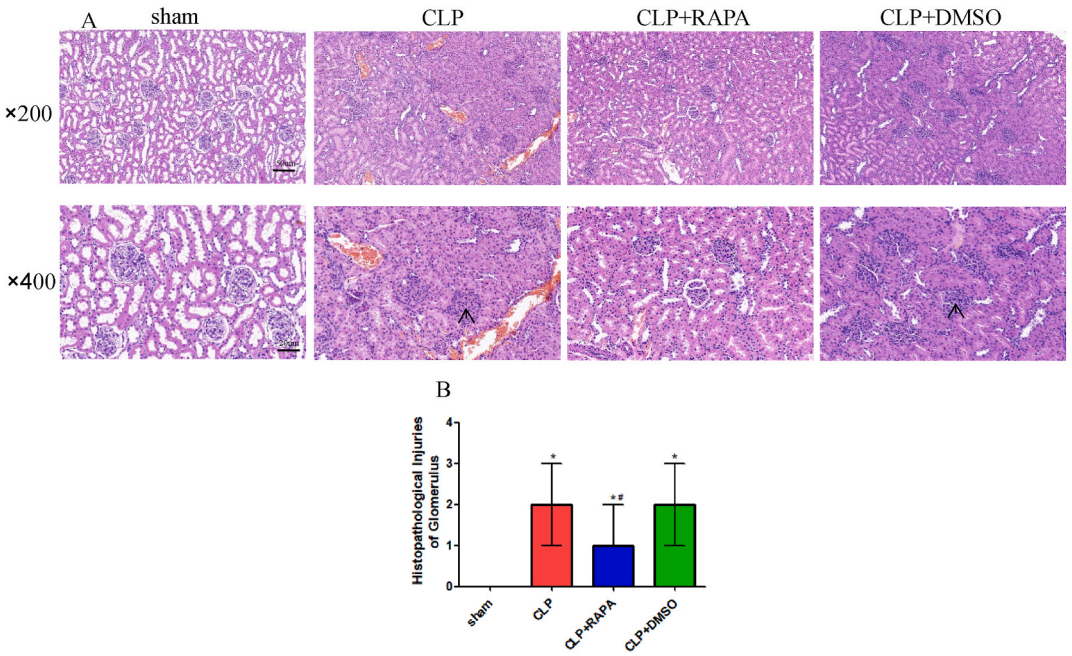

Fig. 4. Glomerulus injuries in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 24 h after the operation evaluated by H&E staining. (A) Representative H&E staining images at × 200 and × 400 magnification. Black arrows indicate the swelling of endothelial cells. (B) The glomerulus injury scores based on histopathological changes observed with H&E staining. $n = 5$, *$P < 0.05$ vs. sham, #$P = 0.004$ (Kruskal-Wallis H Test) vs. CLP + DMSO. Abbreviations: CLP, cecal ligation and puncture; DMSO, dimethylsulfoxide; H&E, hematoxylin and eosin; RAPA, rapamycin (potent inducer of autophagy).

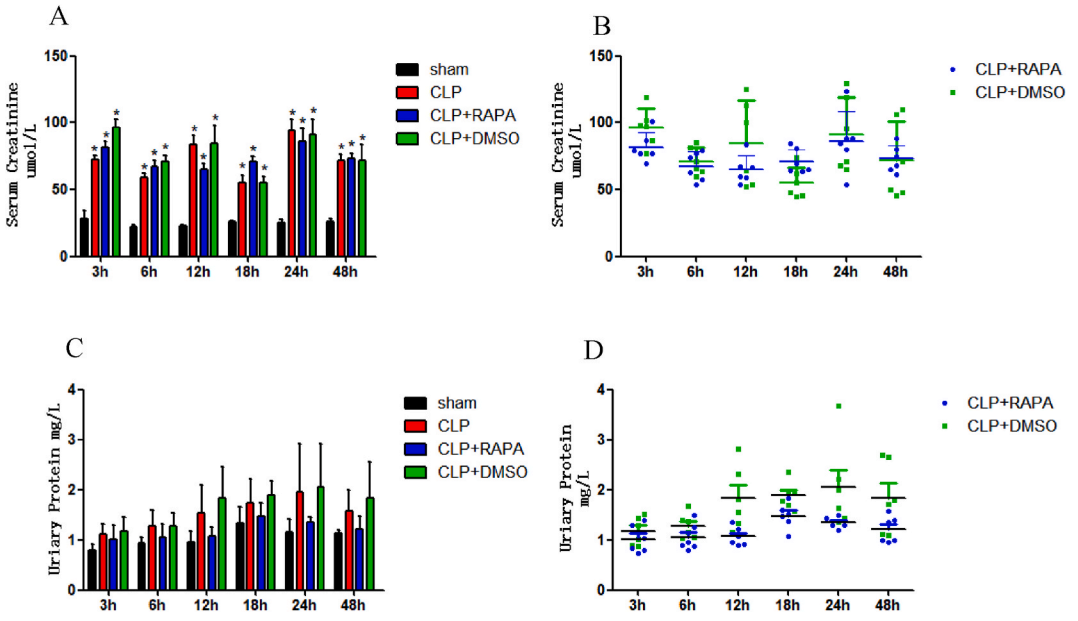

Fig. 5. The serum creatinine and urine protein levels by ELISA (One-way ANOVA test were used). (A). The serum creatinine levels in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6, *P < 0.05 vs. sham. (B). The serum creatinine levels in the CLP + RAPA and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6. Data from each individual rat are shown. (C) The urine protein levels in the sham, CLP, CLP + RAPA, and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6. (D) The urine protein levels in the CLP + RAPA and CLP + DMSO groups at 3, 6, 12, 18, 24, 48 h after the operation. n = 6. Data from each individual rat are shown. Abbreviations: CLP, cecal ligation and puncture; DMSO, dimethylsulfoxide; ELISA, enzyme-linked immunosorbent assay; RAPA, rapamycin (potent inducer of autophagy).

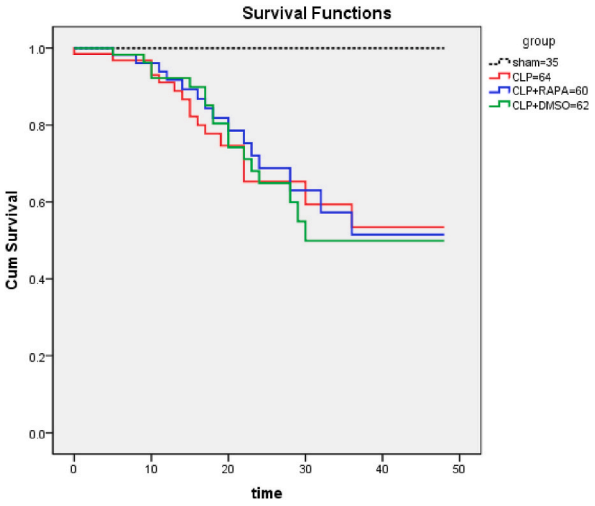

Fig. 6. The Kaplan-Meier survival curves of the sham, CLP, CLP + RAPA, and CLP + DMSO groups. The number of rats used was 221. Abbreviations: CLP, cecal ligation and puncture; DMSO, dimethylsulfoxide; RAPA, rapamycin (potent inducer of autophagy).

ablation of autophagy exacerbated LPS-induced AKI in mice [24]. In our study, we found that CLP induced renal endothelial injury, which was transiently reversed by RAPA. These controversial findings might be due to differences in model construction and the reagent/dose used in autophagy suppression. In CLP-induced AKI mice, most studies have focused on the role of autophagy in renal tubular epithelial cells. It was found that autophagy in tubular epithelial cells transiently increased, and then declined within 8–24 h after the induction of sepsis [5,7]· RAPA upregulated the level of autophagy in these cells and alleviated renal tubular injury induced by CLP [5,7]. Given the central role of renal endothelial dysfunction in the early phase of septic AKI, the current study evaluated the level of autophagy in renal endothelial cells of rats with CLP-induced AKI. This was accomplished by measuring the changes in LC3-II levels and by monitoring autophagosome formation by TEM. It was found that CLP increased autophagy in renal endothelial cells, with an

additional induction by RAPA at 18 h after the operation. RAPA transiently reversed changes in s-TM and VE-cadherin for endothelial injury and alleviated renal endothelial fenestration induced by CLP. Elevated levels of s-TM in the blood are markers of severe endothelial cell damage [25]. Katayama showed that s-TM predicts an increased risk of AKI in patients with sepsis [26]. Itenov et al. revealed that s-TM levels predict a reduction in the chance of AKI recovery in hospitalized patients. These results support the hypothesis that disintegration of renal endothelial cells is a key factor in the development of refractory AKI [27]. RAPA transiently reversed the changes in serum sTM may suggest that autophagy has a transient protective effect on endothelial cell injury and may has a effect on the recovery from AKI in later stages.

Similar to previous findings in mice that underwent CLP [5,7], RAPA attenuated histopathological changes in the renal cortex induced by the CLP procedure. We specifically investigated the role of endothelial cells and the change of autophagy which may add new insight to the current understanding.

AKI is an independent risk factor for the development of chronic kidney disease (CKD) [28]. TGF-β, a key profibrotic growth factor, is activated during AKI and drives the transition of AKI to CKD [10]. Wnt/β-catenin signaling is also upregulated in AKI; however, its functions in AKI are somewhat controversial. It appears to play a beneficial role in AKI, but is considered to promote CKD progression by stimulating tubulointerstitial fibrosis [11]. The pseudo-receptor BAMBI has been shown to downregulate TGF-β and upregulate Wnt/β-catenin signaling [12,13]. Since the expression of BAMBI in the kidney is restricted to endothelial cells [14], we speculated a regulatory role of BAMBI in endothelial function/stability during AKI. In this study, BAMBI expression in the renal cortex was induced in response to CLP, and RAPA transiently reduced renal BAMBI in CLP-stimulated rats. Because BAMBI is regulated by autolysosomal degradation at the protein level [14], its reduction by RAPA could likely be attributed to upregulated degradation mediated by autophagy.

BAMBI acts as a pseudo-receptor in the TGF-β signal transduction pathway, and its negative feedback regulation of this pathway has been widely confirmed [29]. Numerous studies have shown that TGF-β is involved in the development of sepsis and plays an important role in down-regulating the production of inflammatory factors. Studies by McCartney-Francis et al. and others found that in TGF-β gene-deficient mice stimulated with lipopolysaccharide (LPS), expression of the pro-inflammatory factors and their receptors increased significantly, leading to uncontrolled inflammatory response 6. BAMBI blocks the TGF-β pathway, which aggravates the progression of sepsis [30]. We found that the levels of BAMBI in the CLP + RAPA group were statistically lower than those in the CLP + DMSO group at 18 h. Therefore, the decreased levels of BAMBI that degraded by autophagy may attenuate the progression of sepsis. However, the regulation of BAMBI by autolysosome degradation in renal endothelial cells, as well as its effect on renal endothelial integrity and the development of sepsis-induced AKI requires further investigation.

Similar to previous findings [31], elevated levels of serum creatinine were detected after CLP, confirming renal dysfunction in these animals. Although RAPA substantially attenuated CLP-induced renal tissue damage, it showed no significant effect on serum creatinine levels. The reasons for this effect were not clear but it may be partially due to the rather transient induction of autophagy by RAPA. Urine protein levels, another marker for kidney function, showed no significant changes up to 48 h after CLP. This finding was not surprising since protein levels in the urine can show delayed changes following kidney injury [32]. However, it would be interesting to monitor this marker for a longer period after the induction of sepsis.

This work was a preliminary study on the role of renal endothelial autophagy in septic AKI. In future studies, the measurement of inflammatory factors and the use of autophagy inhibitors will be included during *in vivo* experiments. Additionally, *in vitro* experiments with renal endothelial cells will be performed. What is more is that the limitations of this study is that only LC3 expression was determined as a marker for autophagy. Only male was examined in this study.

We need to mention that the interventions that we use are not endothelial cell specific, and there is no comparison made with other cell types, so it is hard to get a sense of how important the endothelial cell response is in the overall context of the septic shock.

Septic AKI is a complex pathophysiological process involving many aspects, not only involving the changes of cells and even organelles. Autophagy is a self-protective mechanism. At present, there are relatively many studies, such as pyroptosis, as we all know, rapamycin is currently used as an immunosuppressant in clinic. Whether rapamycin can play a protective role in septic AKI still has a long way to go, which is worthy of further study.

5. Conclusion

In this study, sepsis induced autophagy in renal endothelial cells, and the upregulation of autophagy in these cells alleviated the glomerulus injury scores in CLP-induced AKI, which had no significant effect on renal fucnation. The expression of BAMBI in the kidney was induced in response to sepsis and was regulated at the protein level by autophagy-mediated degradation. BAMBI may play a role in regulating renal endothelial stability in sepsis-induced AKI.

Author contribution statement

Ran Pang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lei Dong: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jingfeng Liu: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools.

Xiaojun Ji, Haizhou Zhuang: Conceived and designed the experiments.

Meili Duan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

No data was used for the research described in the article.

Declaration of interest's statement

The authors declare no competing interests.

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