Effects of imipenem combined with low-dose cyclophosphamide on the intestinal barrier in septic rats

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Abstract. Anti-infection therapy combined with immunotherapy is one of the important research approaches for treating sepsis. However, the combination of anti-infection and immunotherapy therapeutic agents may have an adverse effect on intestinal barrier function. In the present study, it was hypothesized that imipenem combined with low-dose cyclophosphamide (CTX) could improve the sepsis survival rate compared with imipenem treatment alone. In addition, the alterations in the intestinal barrier were investigated and the possible mechanisms of altering intestinal barrier function in septic rats treated with imipenem combined with low-dose CTX or imipenem alone were explored. To investigate the effect of imipenem combined with low-dose CTX on the intestinal barrier, the markers of histopathology, intestinal permeability, intestinal epithelial apoptosis, cytokines interleukin (IL)-6, IL-10 and tumor necrosis factor (TNF)-α, and tight junction proteins zonula occludens (ZO)-1, occludin and claudin-2, were quantitatively and qualitatively evaluated. The results indicated that imipenem combined with low-dose CTX significantly improved the survival rate of rats compared with imipenem alone (P<0.05). However, no significantly difference between the treatment with imipenem combined with low-dose CTX and imipenem treatment alone was indicated with regard to histopathology, intestinal permeability, intestinal epithelial apoptosis and the expression of claudin-2, ZO-1 and TNF-α. However, imipenem combined with low-dose CTX significantly reduced IL-6 and IL-10 expression and significantly increased occludin expression compared with imipenem alone (P<0.05). It was concluded that imipenem combined with low-dose CTX could improve the survival rate of rats with sepsis compared with rats treated with imipenem alone. The present findings suggest that imipenem combined with low-dose CTX may cause damage to the intestinal barrier function and the mechanism may be associated with a reduction in IL-10 expression.

Introduction

Sepsis is a series of complex disease processes that results from infections (1). Approximately 750,000 patients are infected with sepsis each year and over 225,000 of these patients die from the condition in the United States (2). However, the prevalence of sepsis has increased by 5% annually (3,4). To date, anti-infective therapeutic agents and supportive care have been considered effective therapeutic strategies against sepsis. However, sepsis and septic shock remain to be primary causes of fatality in intensive care units (1). Subsequently, innovative therapeutic schedules that are different from traditional strategies are required. With a breakthrough in immunology and a clearer pathophysiology of sepsis, immunotherapy is considered to become a promising therapeutic strategy (5). However, the clinical applications of immunostimulatory agents such as interferon (INF)-γ (6) and anti-inflammatory components such as interleukin (IL)-1 receptor antagonist (7) have not yet been used successfully utilized. Researchers have recently identified that an antimicrobial combined with low-dose cyclophosphamide (CTX) can improve the survival of mice in a sepsis murine model (8). Notably, the use of CTX to treat infectious diseases is not novel.

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Wei *et al* (9) suggested that low-dose CTX elicits a protective effect on acute lung injury in sepsis rats and Waymack and Alexander (10) demonstrated that low-dose CTX extends the survival time of burned and fatal virus-infected guinea pigs. As an immunosuppressor, CTX has been widely used in tumor and autoimmunity disease therapy. However, CTX produces various complications, including neutrophil granulocytopenia and end-organ damage (8,11). Notably, intestinal barrier dysfunction is a common complication of antimicrobial agents and high-dose CTX (12-15).

The intestinal tract serves a pivotal role in the progression of diseases, including inflammatory bowel disease (16) and hepatitis (17), and contains large quantities of endogenous and exogenous bacteria and toxins (18). The intestinal mucosal barrier between the gut and the body is influenced by intestinal cells and tight junction structures (19). The role of the intestinal mucosal barrier is to prevent intestinal flora and toxins from entering the blood circulation. Under unfavorable conditions, including cytotoxic conditions, the intestinal mucosal barrier may become dysfunctional, thus causing the translocation of intestinal bacterial microflora or toxins, which can therefore infect other organs; such occurrences may then promote sepsis and result in multiple organ dysfunction and failure (20-22). However, the treatment of intestinal barrier function may yield improved outcomes. It is not known whether imipenem combined with low-dose CTX is related to intestinal barrier in sepsis.

In the present study, it was hypothesized that imipenem combined with low-dose cyclophosphamide could improve the sepsis survival rate compared with imipenem alone. In addition, the intestinal barrier function was examined and the possible mechanisms influencing intestinal barrier function were explored.

Materials and methods

Animals. All animal experiments were approved and supervised by the Experimental Animal Centre Committee of Shihezi University (Shihezi, China). A total of 116 healthy male Sprague-Dawley (SD) rats aged 8-10 weeks and weighing 150-250 g (experimental animal production license no. XJYK0011, 2011; Laboratory Animal Centre of Xinjiang Medical University, Urumqi, China) were prepared for the experiments. Rats had free access to food and water for 2 days under experimental conditions (12-h light/dark cycle; 22±1˚C; humidity, 45%) and were subsequently subjected to 12 h of fasting. All rats used in experiments were maintained in a special cage with no more than 8 rats per cage. The rats were randomly divided into sham, cecal ligation and puncture (CLP), imipenem and combination treatment (imipenem with CTX) groups using the number table method (23) prior to the surgery.

Sepsis model establishment. According to the previously published method, the rat model of sepsis was used with CLP (20,24). After weighing, the rats were intraperitoneally injected with 1% pentobarbital (30 mg/kg; Merck KGaA, Darmstadt, Germany) for anaesthetization. The animals were placed in the supine position on a workstation and along the white line of the abdomen an ~2-cm longitudinal incision was made under full anesthesia after routine disinfection.

Approximately 2/3 of the caecum was ligated using a 4-0 silk suture and the caecum was punctured twice in different places using 21 needles (24,25). A small amount of stool was extruded and the abdomen was close using a 4-0 suture. Pre-warmed saline (37˚C) was injected into the subcutaneous tissue with fluid resuscitation following surgery. The rats under sham operation were exposed and the caecum was removed following laparotomy without ligation and puncture. The animals were monitored every 30 min post-surgery. The rats that demonstrated unimproved lethargy or moribund behavior were euthanized.

Imipenem and CTX administration. Imipenem was obtained from the Merck KGaA. For survival studies, imipenem (120 mg/kg) was intraperitoneally injected 6 h post-CLP and every 12 h for a total of 7 days. For intestinal barrier function analysis, imipenem (120 mg/kg) was intraperitoneally injected 6 h post-CLP and twice every 12 h thereafter. CTX was purchased from the Sigma-Aldrich (Merck KGaA). For survival studies, CTX (10 mg/kg, dissolved in saline) was intraperitoneally injected 6 h post-CLP and every 12 h for a total of 7 days into animals treated with or without imipenem. For intestinal barrier function analysis CTX (10 mg/kg, dissolved in saline) was intraperitoneally injected 6 h post-CLP and twice every 12 h thereafter into animals treated with or without imipenem.

Intestinal histopathology. At 24 h following imipenem or CTX administration, small-intestine tissue (~10 cm) above the ileocecal valves was collected, repeatedly flushed with PBS and placed in 10% formalin (40˚C; 24-48 h). The intestinal tissue was sliced, fixed, deparaffinized by soaking in xylene for 15 min at room temperature, dehydrated in 100% alcohol for 10 min, 95% alcohol for 10 min, 85% alcohol for 3 min, 75% alcohol 3 min at room temperature and stained with hematoxylin and eosin (20% Harris for 10 min and 0.5% eosin for 1 min) at room temperature prior to observation under a light microscope (magnification, x100).

Intestinal permeability. Intravenous injection of 120 mg/ml fluorescein isothiocyanate-conjugated-dextran (FD-70; 0.5 ml, molecular mass 70 kDa, Sigma-Aldrich; Merck KGaA) was administered approximately ~6 h prior to the sacrifice. Blood samples were centrifuged at 12,000 x g for 4 min at 4˚C, and the plasma was diluted with an equal volume of PBS (pH 7.4). The excitation wavelength of 480 nm and the emission wavelength of 520 nm were used to analyze fluorescence in a microplate reader.

Intestinal epithelial apoptosis. Apoptosis of epithelial cells was analyzed using the Terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling (TUNEL) assay and the number of apoptotic cells was quantified. Intestinal tissues were fixed, embedded in paraffin wax, and cut into 5-μm sections. The TUNEL apoptosis assay kit (Sigma-Aldrich; Merck KGaA) was used for the experiments according to the manufacturer's instructions. The apoptotic cells stained with brown nuclei were analyzed under an optical microscope (magnification, x400).

Cytokine measurement. Rats were anesthetized and fixed on the operating table. The abdominal cavity was opened to
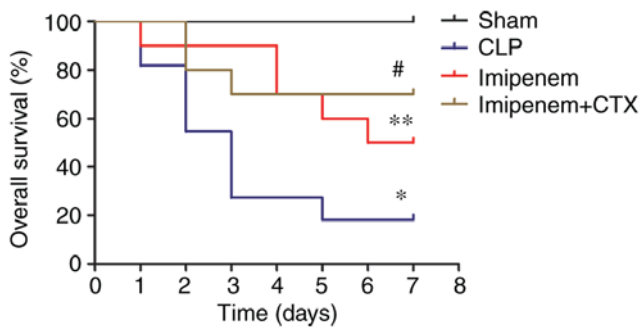
Figure 1. Survival distributions of rats in each group. The survival rates of septic rats were significantly lower compared with the sham group. The survival rate was significantly increased in septic mice that received imipenem treatment and combination treatment. Furthermore, the combination treatment group exhibited a higher survival rate at day 7 compared with the imipenem group. *P<0.05 vs. sham group; **P<0.05 vs. CLP group; #P<0.05 vs. imipenem group. CLP, cecal ligation and puncture; CTX, cyclophosphamide.
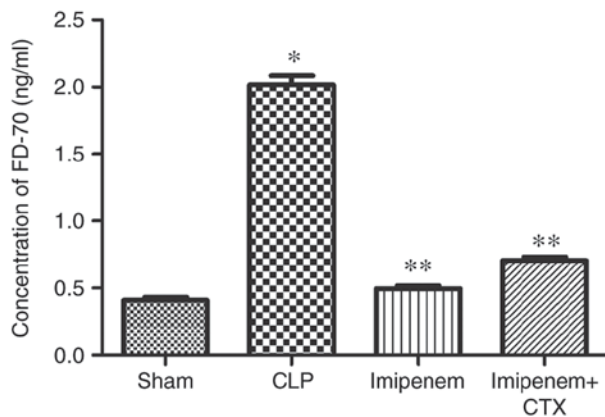

Figure 2. Intestinal permeability of each group according to the FD-70 concentration detected by fluorescence analysis. *P<0.05 vs. sham group; **P<0.05 vs. CLP group. CLP, cecal ligation and puncture; CTX, cyclophosphamide; FD-70, fluorescein isothiocyanate-conjugated-dextran.

expose the abdominal aorta. Approximately 10 ml of blood was extracted from the abdominal aorta and placed into 2-ml EP tubes. Blood samples were centrifuged at 2,000 x g for 15 min and the plasma was removed into 2-ml EP tube. Interleukin (IL)-6 (cat. no. F15870), IL-10 (cat. no. F15900) and tumor necrosis factor (TNF)-α (cat. no. F3768; all Shanghai Westang Bio-tech Co., Ltd.) serum levels were detected using an ELISA according to the manufacturer's instructions.

Tight junction protein expression analysis. Approximately 120 mg of intestinal tissue was extracted and placed in liquid nitrogen for 2 min. Total protein was extracted using radioimmunoprecipitation assay buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) at a ratio of 10 mg tissue to 100 μl buffer. The turbid liquid was placed in an ultra-high-speed centrifuge with at 12,000 x g for 20 min at 4°C and the resulting solution was transferred to the 2-ml EP tubes. This procedure was repeated three times. The protein content was determined using the bicinchoninic acid method (Beyotime Institute of Biotechnology, Haimen, China). Western blotting was performed as previously described (13,26). Samples were incubated with 4X SDS-PAGE

loading buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) in a boiling water bath for 5 min. Equal amounts of total proteins (30 μg/lane) were separated using 10% SDS-PAGE gels. Proteins were transferred to polyvinylidene difluoride membranes and blocked using 5% skimmed milk powder for 2 h at room temperature. Membranes were subsequently incubated with primary antibodies occludin (1:5,000; cat. no. ab167161), claudin-2 (1:500; cat. no. ab53032; both Abcam, Cambridge, UK), ZO-1 (1:500; cat. no. sc-33725; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and β-actin (1:100; cat. no. ab8226) at 4°C overnight, followed by horseradish peroxidase-linked anti-rabbit IgG (1:10,000; cat. no. ab6734; both Abcam) secondary antibody for 2 h at room temperature. The films were then stored in a dark room and a chemiluminescent peroxidase substrate (cat. no. 34094; Thermo Fisher Scientific, Inc.) was applied according to the manufacturer's instructions. Proteins were detected using a chemiluminescent system and visualized using a gel imaging system (ChemiDoc Touch, Bio-Rad Laboratories, Inc., Hercules, CA, USA). The results were analyzed using intensity quantification software (ImageLab 5.2, Bio-Rad Laboratories, Inc.).

Statistical analysis. All values are represented as mean ± standard deviation of at least three independent experiments. The log-rank test was used to statistically analyze differences in survival between the experimental groups. Multiple group comparisons were performed with using one-way analysis of variance followed by an LSD post hoc test to compare differences between the two groups. If data were not normally distributed, the Kruskal-Wallis non-parametric test was used to compare differences among multiple groups followed by the Dunn-Bonferroni post hoc test to compare differences between two groups. The test level was α=0.05, and P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using GraphPad Prism software (Version 5; GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 20.0 (IBM Corp., Armonk, NY, USA).

Results

Effect of imipenem combined with CTX on survival. To evaluate the effect of CTX combined with antimicrobial drugs, the survival rates for all the groups were assessed following treatment. The CLP group had an 81.18% survival rate on day 1 and an 18.18% survival rate on day 7. Notably, on day 2 the mortality was the highest. As previously reported (8), septic rats treated with imipenem exhibited a significantly higher 7-day survival rate (50%) compared with the CLP group (P<0.05). The combination treatment group exhibited a 100% survival rate on day 1 and a 70% survival rate on day 7, which was significantly higher than that of imipenem group (P<0.05; Fig. 1).

Effect of imipenem combined with CTX on intestinal permeability. To assess whether CTX causes intestinal barrier dysfunction, the intestinal permeability to FD-70 was examined. Results indicated that the septic rats achieved significantly higher serum levels of FD-70 compared with the sham group (P<0.05; Fig. 2). Compared with the CLP group, the expression of FD-70 in the imipenem group and combination treatment group was significantly reduced (P<0.05). Notably, the serum
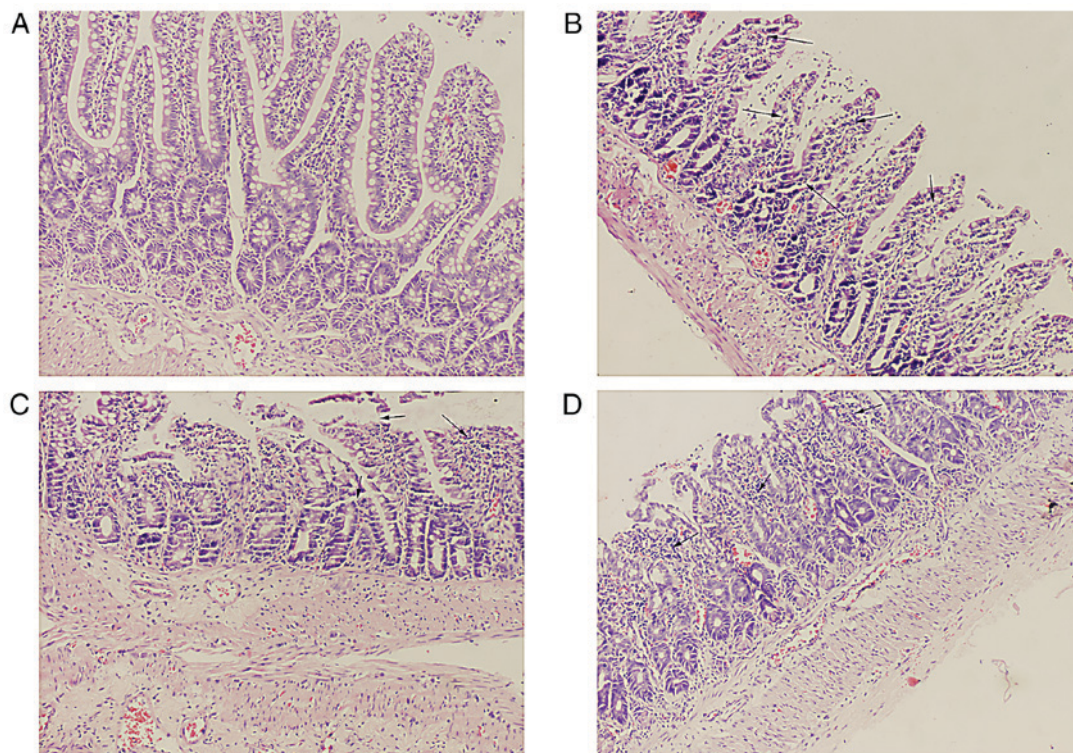
Figure 3. Pathological examination of tissue by H&E staining. H&E staining images of each group (magnification, x100). (A) Sham, (B) CLP, (C) imipenem and (D) combination treatment groups were indicated. CLP, cecal ligation and puncture.

FD-70 levels in the combination treatment group were increased compared with that of the imipenem group (P>0.05).

Effect of imipenem combined with CTX on intestinal tissue integrity. Histopathologically stained sections were used to measure the effect of imipenem combined with CTX on intestinal mucosal integrity (Fig. 3). No notable inflammatory cells were observed in the sham group. Furthermore, the intestinal goblet cells were not damaged and no intestinal villi were ruptured in the sham operation group (Fig. 3A). The CLP group demonstrated clear inflammatory cell infiltration and locally necrotic areas compared with the sham group (Fig. 3B). In the combination treatment and imipenem groups, regular epithelial appearance with intestinal epithelial infiltration and mild alteration was observed (Fig. 3C and D). However, no distinction between the combination treatment and the imipenem groups was observed with regard to intestinal tissue pathology.

Effect of imipenem combined with CTX on intestinal epithelial apoptosis. Apoptotic cells were stained brown and analyzed under a light microscope. Few brown-stained cells were observed in the sham operation group (Fig. 4A). The number of apoptotic cells was markedly increased in the CLP group (Fig. 4B). Imipenem and combination treatment markedly reduced the number of intestinal apoptotic epithelial cells (Fig. 4C and D). No marked difference in the number of apoptotic epithelial cells was noted between the imipenem group and the combination treatment group.

Effect of imipenem combined with CTX on tight junction protein expression. Results indicated that there was no significant difference in the expression level of ZO-1 protein between groups (Fig. 5). Notably, the protein expression levels of occludin and claudin-2 in intestinal tissue were significantly decreased in the CLP group compared with the sham group (P<0.05; Figs. 6 and 7). Compared with the CLP group, the expression of occludin in the imipenem group was significantly decreased (P<0.05). By contrast, its expression was significantly increased in the combination treatment group (P<0.05; Fig. 6). As indicated in Table I, a significant increase in occludin protein expression was indicated in the combination treatment group compared with the imipenem group (P<0.05). Furthermore, results indicated that claudin-2 expression was significantly decreased in the imipenem and combination treatment groups compared with the CLP group (P<0.05; Fig. 7). However, there was no significant difference between the imipenem group and the combination treatment group (Table I; Fig. 7).

Effect of imipenem combined with CTX on the expression of plasma cytokines. IL-6, IL-10 and TNF-α expression levels were significantly increased in the CLP group compared with the sham group (P<0.05; Figs. 8-10). Compared with the CLP group, the expression levels of IL-6, IL-10 and TNF-α in the imipenem and combination treatment groups were decreased significantly (P<0.05; Figs. 8-10). Combination treatment significantly decreased the expression levels of IL-6 (P<0.05; Fig. 8) and IL-10 (P<0.05; Fig. 9) and increased the expression level of TNF-α (P>0.05; Fig. 10) compared with imipenem alone.

Discussion

With the exception of antibiotics and fluid resuscitation, there are still no effective treatments for sepsis. The concept of treating sepsis with immunosuppressant has recently
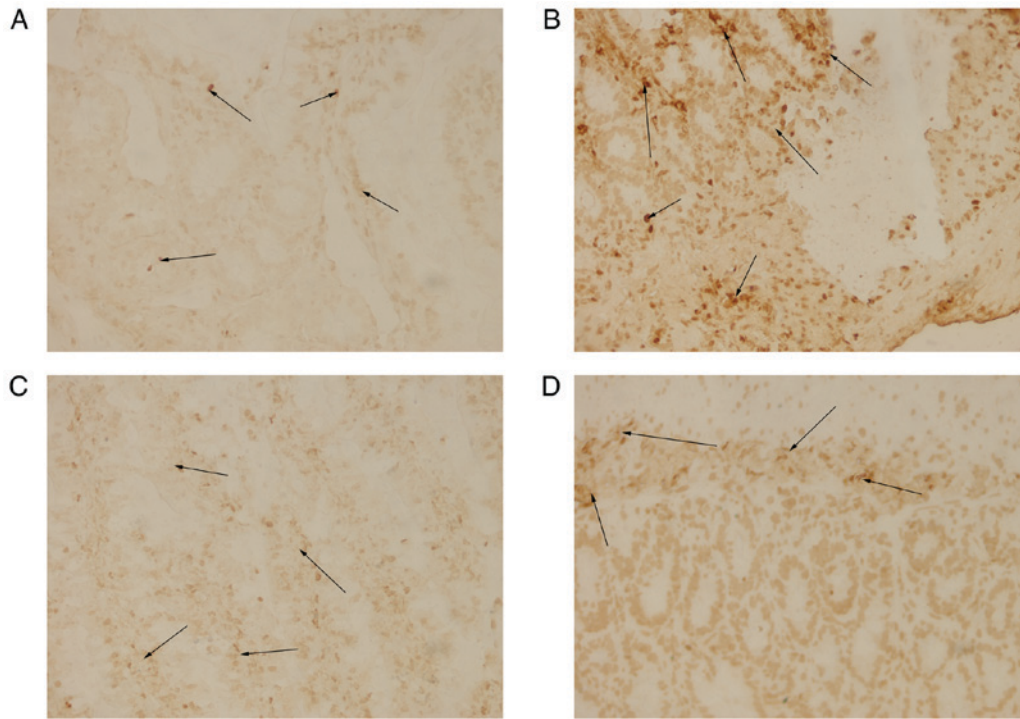
Figure 4. TUNEL staining of the intestine of each group. TUNEL-positive cells were identified according to brown stained nuclei (magnification, x400). Arrows highlight the apoptotic nucleus. (A) Sham, (B) CLP, (C) imipenem and (D) combination treatment groups were indicated. TUNEL, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling; CLP, cecal ligation and puncture.
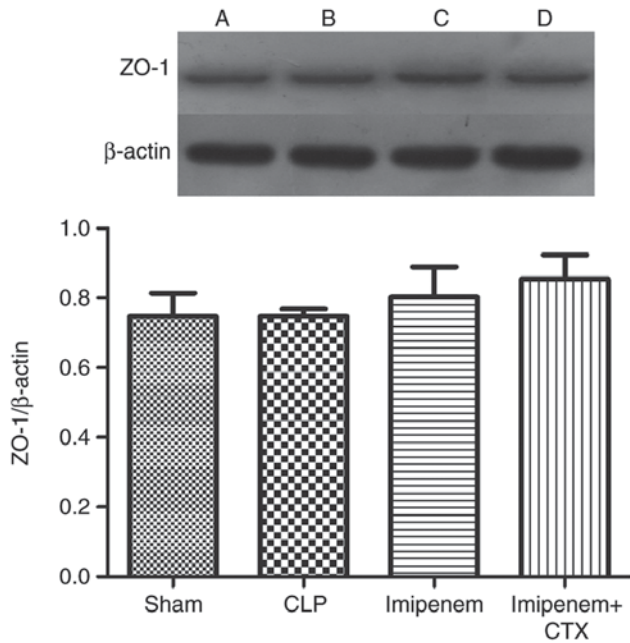

Figure 5. Expression levels of the ZO-1 in each group. (A) Sham, (B) CLP, (C) imipenem and (D) combination treatment groups were indicated. CLP, cecal ligation and puncture; ZO-1, zonula occludens-1; CTX, cyclophosphamide.
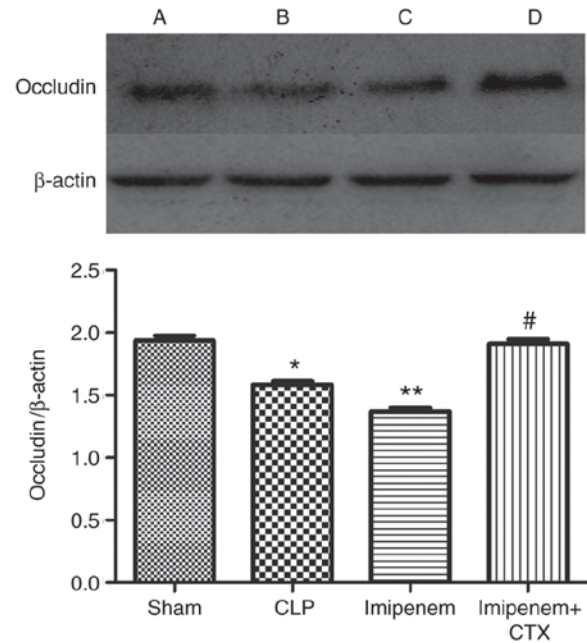

Figure 6. Expression levels of the occludin in each group. (A) Sham, (B) CLP, (C) imipenem and (D) combination treatment groups were indicated. *$P < 0.05$ vs. sham group; **$P < 0.05$ vs. CLP group; #$P < 0.05$ vs. imipenem group. CLP, cecal ligation and puncture; CTX, cyclophosphamide.

become a promising approach. Some researchers revealed that imipenem combined with low-dose CTX improved the survival rate of mice with sepsis (8,9). In their study, the early excessive inflammatory response to sepsis caused an excessive utilization of inflammatory factors, which resulted in immunosuppression. CTX is an immunosuppressant that inhibits

the innate immune response and reduces the hyperinflammatory response of sepsis, which reduces the damage to the body itself (8). In the present study, a sepsis rat model was successfully replicated based on a previous report (24). The survival rate was significantly increased following imipenem treatment and combination treatment groups. Consistent with

Table I. Expression of the tight junction proteins in the intestine of each group.

Group	ZO-1	Occludin	Claudin-2
Sham	0.7469±0.0385	1.9370±0.0172	0.3135±0.0103
CLP	0.7470±0.0123	1.5816±0.0140[a]	0.2696±0.0078[a]
Imipenem	0.8022±0.0500	1.3698±0.0123[b]	0.2187±0.0075[b]
Imipenem+CTX	0.8548±0.0397	1.9117±0.0156[c]	0.2075±0.0107[b]

Each value was relative to the expression of β-actin. [a]P<0.05 vs. sham; [b]P<0.05 vs. CLP group; [c]P<0.05 vs. imipenem group. CLP, cecal ligation and puncture; CTX, cyclophosphamide; ZO-1, zonula occludens-1.

previous reports, the present results suggested that imipenem combined with low-dose CTX may improve the mortality of sepsis compared with imipenem alone (70 vs. 50%). Notably, combination treatment improved the 7-day survival rate of sepsis rats.

It is not clear whether the exacerbation of intestinal barrier dysfunction cause serious complications, including multiple organ dysfunction syndrome (27,28). To investigate the effect of imipenem combined with low-dose CTX on the intestinal barrier function, the quantitative and qualitative markers that affected the function of the intestinal barrier were evaluated. Consistent with our previous findings (unpublished data), the present results suggested that the intestinal permeability increased significantly in septic rats and this was improved in the rats treated with imipenem. Compared with the CLP group, intestinal permeability was also improved in the combination treatment group. However, the intestinal permeability of rats was higher in the combination treatment group compared with that in the imipenem monotherapy group, which suggested that the combination treatment may further damage intestinal barrier function. In our previous study, a combination treatment of imipenem, normal saline and CTX significantly improved the survival rate of septic rats (unpublished data). Conversely, the results of previous study revealed that the combination therapy of imipenem, normal saline and CTX is beneficial for intestinal barrier. Notably, previous studies have identified many factors can affect intestinal permeability (29-31). The present study further analyzed the possible mechanisms involved in changes in intestinal barrier function.

Intestinal mucosal permeability includes transcellular and paracellular permeability (32). Transcellular permeability is primarily impacted by mucosal cells and intrinsic layer cells in the intestine whereas paracellular permeability is impacted by the tight junction structure of intestinal endothelial cells (33). Consistent with our previous study (unpublished data), intestinal injury influencing transcellular permeability was evident in the rats with sepsis. However, treatment with imipenem markedly improved intestinal injury. Furthermore, intestinal epithelial apoptosis analysis indicated the number of apoptotic cells was increased in the CLP group in the present study. Notably, the number of apoptotic cells was diminished in the imipenem group compared with CLP group. However, no significant differences in intestinal tissue injury or apoptosis of intestinal epithelium cells were identified between the imipenem combined with CTX compared with the imipenem group.
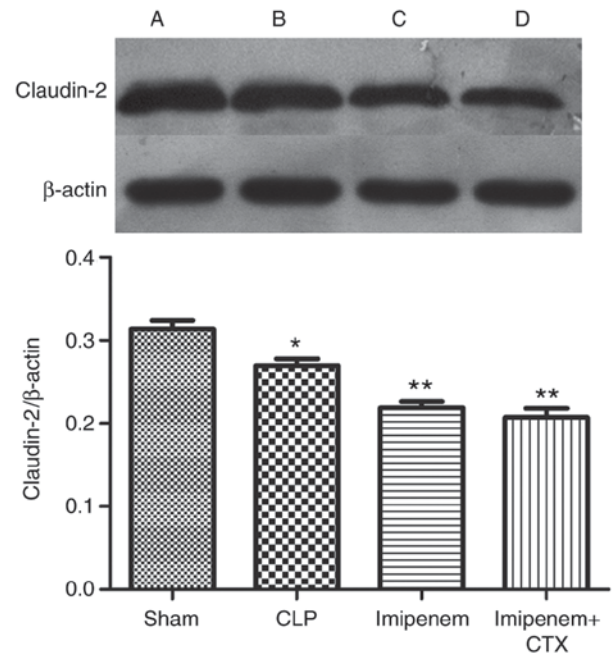

Figure 7. Expression levels of claudin-2 in each group. (A) Sham, (B) CLP, (C) imipenem and (D) combination treatment groups were indicated. *P<0.05 vs. sham group; **P<0.05 vs. CLP group. CLP, cecal ligation and puncture; CTX, cyclophosphamide.

Intestinal tight junctions regulate paracellular permeability. Notably the intestinal tight junction proteins comprise of the ZO family, the claudin family and occluding (13). The combination therapy of imipenem, normal saline and CTX significantly increased expression of occludin and reduced expression of FD-70 compare with combination therapy of imipenem and CTX (unpublished data). However, in the present study, no significant differences the expression levels of ZO-1 protein were observed between groups. Consistent with previous results (34), the present findings revealed that the expression of occludin decreased in the intestines of the rats with sepsis. It is interesting to note that imipenem treatment resulted in decreased occludin expression and that combined treatment of imipenem with CTX increased occludin expression. Therefore, according to the present results, there is no reason to believe that occludin is responsible for the increased permeability observed in the combination treatment group. Claudin-2, which is primarily present in intestinal tissue, is member of the claudin family. In the present study, results indicated that the expression of claudin-2 was reduced
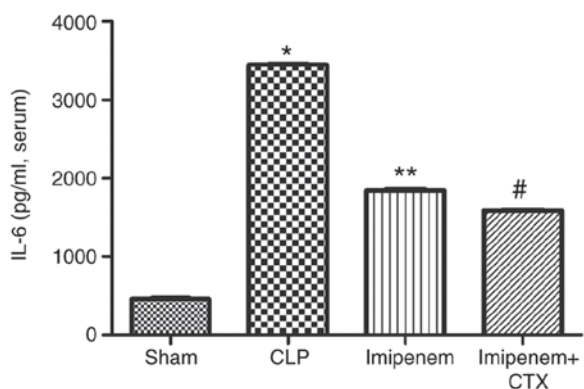
Figure 8. IL-6 expression in the serum of each group. *P<0.05 vs. sham group; **P<0.05 vs. CLP group; #P<0.05 vs. imipenem group. CLP, cecal ligation and puncture; IL, interleukin; CTX, cyclophosphamide.

Figure 9. IL-10 expression in the serum of each group. *P<0.05 vs. sham group; **P<0.05 vs. CLP group; #P<0.05 vs. imipenem group. CLP, cecal ligation and puncture; IL, interleukin; CTX, cyclophosphamide.
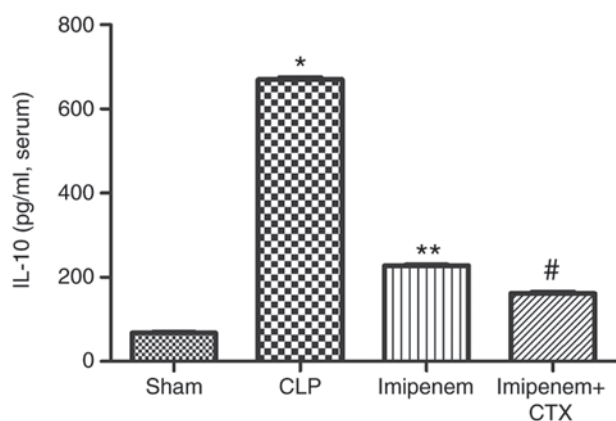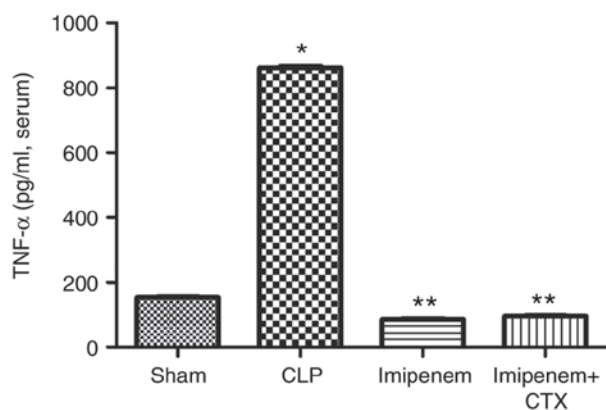

Figure 10. TNF-α expression in the serum of each group. *P<0.05 vs. sham group; **P<0.05 vs. CLP group. CLP, cecal ligation and puncture; TNF, tumor necrosis factor; TX, cyclophosphamide.

in the imipenem and combination treatment groups. However, there was no significant difference between the imipenem and combination treatment groups. Notably, the combination treatment group exhibited increased intestinal permeability compared with the imipenem group. It was therefore inferred that intestinal permeability may not be independently affected

by the occludin tight junction. Therefore, these findings suggest that combination treatment can increase intestinal permeability, which may be associated with factors other than occludin.

Cytokines are also a factor involved in intestinal barrier function, particularly IL-6, IL-10 and TNF-α. Previous studies have indicated that TNF-α (35) and IL-6 (36) increase intestinal permeability and IL-10 (37) exerts a protective effect on the function of the intestinal barrier. In the present study, imipenem treatment decreased IL-6, IL-10 and TNF-α expression levels. Inconsistent with our previous study (unpublished data), the combined treatment of imipenem and CTX further reduced IL-6 and IL-10 expression. Therefore, it was inferred that the increase in intestinal permeability in the combination treatment group may be due to the decrease in IL-6 and IL-10. In addition, various studies have identified that mice lacking IL-10 exhibit a significant increase in intestinal permeability but the exact mechanisms remain clear (38-40). Taken together, the present findings suggest a decrease in IL-10 may increase intestinal permeability. It is interesting that the expression of IL-6 in the combination treatment group is also reduced, which is not consistent with previous studies (36,41). Therefore, the possible mechanisms of this change were we further explored. Wang indicated that increased intestinal permeability during sepsis was regulated by an interaction between IL-6 and IL-10 and that treatment with IL-10 may prevent the increase in mucosal permeability during sepsis in IL-6-/- mice (42). They found that IL-6 did not directly affect the intestinal mucosa but did suppress the expression of IL-10. Therefore, a reduction in IL-6 may be associated with the increase in intestinal permeability in present study. In the present study, imipenem combined with low-dose CTX significantly reduced the expression of IL-10. It was suggested that there was a slight increase in intestinal permeability in the combined-treatment group due to the decrease in IL-10 expression level. In light of this, combination therapy may have a potential effect on increased intestinal permeability by inhibiting the expression of IL-10.

The present study exhibited some limitations. Firstly, converse to previous studies, male SD rats were used as the experimental animals; different species or sexes may elicit different effects on study results. Additionally, the present study focused on the CTX-induced intestinal mucosal injury without further investigating its effects on cellular immune function. Furthermore, the present study consisted of a 24-h investigation without the dynamic monitoring of the relevant indicators, including intestinal permeability, intestinal epithelial apoptosis, IL-6, IL-10, TNF-α, and tight junction proteins, which will be further explored in subsequent studies. In the present study, the tight junction protein expression levels were implicated in intestinal permeability; however, other mechanisms could be involved that were not discussed. Finally, previous studies suggested a dose-dependent damaging effect of CTX on intestinal mucosa (13). The present findings also implied that low-dose CTX has a potential effect on the intestinal barrier dysfunction. However, these results should be investigated further to determine whether long-term low-dose CTX treatment causes intestinal injury.

In conclusion, a rat model of sepsis was successfully replicated in the present study. Imipenem combined with low-dose

CTX improved the survival rate of rats with sepsis compared with treatment with imipenem alone. Taken together, the present findings suggest that imipenem combined with low-dose CTX has the potential to damage to the intestinal mucosal barrier and the mechanism may be associated with reduced IL-10.

Availability of data and materials

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

Authors' contributions

PG performed all animal experiments and revised the manuscript. S-WZ was a major contributor in writing the manuscript and performed the statistical analysis W-JZ and FW jointly designed the study. JZ and J-TD performed and guided animal experiments. J-DW revised the statistical analysis. STT and J-TY performed survival experiments. All authors have read and approved the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Shihezi University (Shihezi, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have competing interests.

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