Beneficial effects of ulinastatin on gut barrier function in sepsis

Longyuan Jiang*, Lianhong Yang*, Meng Zhang, Xiangshao Fang, Zitong Huang, Zhengfei Yang & Tianen Zhou

Department of Emergency Medicine, The Memorial Hospital of Sun Yat-sen, Sun Yat-sen University, Guangzhou, PR China

Background & objectives: The gut contains some endogenous and exogenous microorganisms that can become potential pathogens of sepsis under certain circumstances. Therefore, the integrity and normal function of gut barrier is important for preventing the development of sepsis. The present study was designed to assess the effects of ulinastatin, a urinary trypsin inhibitor on gut barrier function and mortality in experimental sepsis.

Methods: Male Sprague-Dawley rats were subjected to ceacal ligation and puncture (CLP) or sham procedure. Rats were then treated with ulinastatin 50,000 U/kg/day or saline. The mortality rate was determined. Histology, apoptosis assays, and PCR were performed using ileum specimens at 3, 6, and 12 h following CLP. Serum levels of tumour necrosis factor α (TNF-α) and interleukin-6 (IL-6) were also measured at 0, 3, 6, and 12 h following CLP.

Results: Compared with the saline-treated CLP rats, the ulinastatin CLP rats had significantly increased survival time ($P<0.05$), lower histopathological scores of intestinal injury ($P<0.05$), reduced apoptosis detected by terminal deoxynucleotidyl transferase dUTP nick end labelling assay and caspase 3 activity ($P<0.01$). Moreover, RD-5 mRNA expression was significantly higher in ulinastatin-treated CLP animals than saline controls ($P<0.05$). These results suggested a preserved integrity and function of the gut barrier. Significantly lower plasma TNFα and IL-6 levels were detected in CLP rats with ulinastatin treatment, which contributed to increased survival time.

Interpretation & conclusions: Our results suggest that ulinastatin has a therapeutic potential to prevent gut barrier dysfunction in the early stage of sepsis, thereby improving the outcome of sepsis. Further studies need to be done to understand the mechanism of action of ulinastatin.

Key words Gut barrier - IL-6, sepsis - TNF-α - ulinastatin

Sepsis is an increasingly common and lethal condition among hospitalized patients and has become a leading cause of morbidity in severe illness, influencing millions of people worldwide and killing over one in four of those affected every year[1,2]. Conventional treatment for sepsis includes controlling the source of infection, antimicrobial therapy, and prevention of multiple organ failure due to sepsis. Recent strategies have targeted proinflammatory mediators such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), bradykinin,

---

*First two authors contributed equally to this paper

platelet-activating factor, elastase, nitric oxide (NO) and lipopolysaccharide (LPS), and activated protein C but their benefits on clinical outcome of septic patients remain controversial[3,4]. Only Drotrecogin alfa, a recombinant form of human activated protein C, can slightly improve the survival rate of patients with severe septic or septic shock [5]. Therefore, more effective therapies need to be developed for sepsis.

The gut contains endogenous and exogenous microorganisms that can sometimes become potential pathogens of sepsis, and it is also susceptive to ischaemia-reperfusion injuries due to sepsis[6]. Gut barrier integrity is an important defense against microbial pathogens. Its damage may lead to the escape of microbial pathogens from gut into body, inducing an exaggerated inflammatory response and consequently promoting multiple organ dysfunction and failure[7,8]. The pathogenesis of gut barrier dysfunction during sepsis is multifactorial, and involves mucosal hypoperfusion, intestinal epithelial atrophy and depletion of mucosal immune cells and some cytokines (*e.g.* TNF-α and IL-6, *etc.*)[6,9]. Therefore, novel interventions targeting gut barrier dysfunction and relevant pathogenic factors may reduce bacterial translocation and unfavourable inflammatory responses, thereby improving survival during sepsis.

Urinary trypsin inhibitors (UTI) have been shown to benefit hypoperfusion in lung transplantation[10,11] and haemorrhagic shock[12], and reduce endothelial dysfunction and leukocyte migration[13]. A clinical trial[14] showed that in addition to standard supportive care and antimicrobial therapy, treatment with combined ulinastatin, a UTI extracted from human urine, plus thymosin α₁ significantly increased the survival rate of patients with severe sepsis. However, the underlying mechanism of such benefits has not been systematically studied yet, especially the impacts on gut barrier function. We, therefore, conducted the present study using a rat model of sepsis induced by ceacal ligation and puncture (CLP) to investigate whether UTI has an effect on the outcome of sepsis and the underlying mechanisms. RNA expression of rat defensins-5(RD-5) was also measured. RD-5 is a native immune substance produced by Paneth's cells in the intestinal crypt, which is believed to play a role in gut barrier function[15].

Material & Methods

The present study was carried out at the Department of Animal Laboratory, Sun Yat-sen University, Guangzhou, Guangdong, PR China. Male Sprague-Dawley rats (n=92), weighing 140-220 g, were obtained from the Animal Center of Sun Yat-sen University. All rats were kept individually in polycarbonate cages with free access to standard rodent chow and tap water in a temperature-controlled environment (21 ± 2°C, lights on 0700 to 1900 h). The study protocol was approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University and conducted in accordance with institutional guidelines.

Experimental model of sepsis: Sepsis was induced by CLP as described previously[16], which is the most commonly used preclinical model for studying the process and the treatment of sepsis[17]. After anaesthetized by an intraperitoneal injection of sodium pentobarbital (45 mg/kg), a midline laparotomy was performed and a ligature was placed around the ceacum at approximately 70 per cent distal to the ileoceacal valve. The ceacum was then punctured twice with an 18-gauge needle and was squeezed to extrude the faecal material. A 4.0 silk thread was placed through each hole to avoid the blockage of the holes. Finally, the abdominal cavity was closed. After the surgery, the animals were placed in a metabolic cage, resuscitated with subcutaneous injection of saline (5 ml/100 g body weight/day). The sham-control animals were treated in an identical manner without the ceacal ligation and perforation.

The effect of ulinastatin on mortality rate: After CLP, 20 rats were randomized into two groups: the ulinastatin group (n = 10) administered with ulinastatin (Techpool Bio-Pharma Co., Ltd. China) 50,000 U/kg/day via *vena caudalis* and the saline control group (n = 10) administered with normal saline 50 ml/kg via *vena caudalis*. Rats were defined as mortal and sacrificed if they demonstrated any of the following characteristics: a moribund state, lateral recumbency, and/or hypothermia (rectal temperature <32 ◦C), *etc*.

The effects of ulinastatin on intestinal mucosal integrity and innate immunity: In a separate series of experiments, 72 rats were randomized into three groups: the ulinastatin CLP group, the saline CLP group, and the ulinastatin sham group (n=24 in each group). Each group was further divided into 3, 6, and 12 h subgroups according to the time after the surgical procedure (n=8 in each subgroup). The rats were treated with either ulinastatin 50,000 U/kg/day or normal saline 50 ml/kg via *vena caudalis*. Rats in each subgroup were anaesthetized with sodium pentobarbital (150 mg/kg), after which laparotomy was performed. A 5-cm segment of ileum was excised to detect the severity of intestinal mucosal damage and the expression of

α-RD-5. Thereafter, the upper lobe of the right lung in the 12 hours groups (n=6 each group) was excised and weighed. The lung specimen was dried in an oven at 80°C for 48 h, and the wet-to-dry weight ratio of the lung was calculated. Fasting blood sample (0.5 ml) was also collected from the tail vein at 0, 3, 6, and 12 h for the analyses of serum levels of TNF-α and IL-6 before the rats were sacrificed.

Biochemical tests: Plasma levels of TNF-α and IL-6 were measured using TNF-α ELISA kit (Shanghai Senxiong Biotech Industry Co., Ltd. China) and IL-6 ELISA kit (Shanghai Transhold Tech. Dev. Co. Ltd., China) according to the manufacturer's instructions.

Histological examination: Ileum specimens were fixed in 10 per cent neutral formalin for 24 h and then paraffin-embedded, sectioned (5 μm thick), and subjected to hematoxylin and eosin (H & E) staining. Intestinal mucosal morphological injury was observed under a light microscope (Olympus CX30, Japan) by a pathologist who was blind to the details of the study. Fifteen random fields of gut mucosa for each specimen were evaluated as reported previously[18]. Briefly, intestinal injury was scored as follows: grade 0, normal mucosal villous structure; grade 1, presence of subepithelial space at villous tips; grade 2, scattered epithelial denudation on villous tips; grade 3, denuded tips with exposed lamina propria and villous blunting; grade 4, epithelial shedding from both the apex and mid-region of the villi associated with shortened and widened villous structure; grade 5, complete destruction of villi and disintegration of lamina propria with ulceration.

Apoptosis assays: Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay to detect apoptosis was performed on paraffin-embedded ileum sections using the *in situ* Cell Death Detection kit (Roche, Switzerland). Samples were processed according to the manufacturer instructions. Apoptotic cells were enumerated by counting 100 epithelial cells in 10 randomly selected fields of ileal absorptive villi and crypts. Counting was done by an observer blinded to the experimental condition. A total of 1,000 epithelial cells were enumerated at each intestinal site and the apoptotic index was expressed as the percentage of apoptotic cells per 100 epithelial cells enumerated. For caspase-3 activity assay, proteins were homogenized and extracted from ileum tissues in the lysis buffer [20 mM Tris (*p*H 8), 137 mM NaCl, 10% glycerol, 1% NP-40, 1 mM phenylmethylsulphonyl fluoride, aprotinin at 10 μg/ml]. Protein extracts (50 μg) were incubated with 100μM Ac-DEVD-AMC caspase-3 specific fluorogenic substrate (Alexis Biochemicals, Switzerland) for 2 h in the caspase reaction buffer [1% NP-40, 20 mM Tris-HCl (*p*H 7.5), 137 mM NaCl, 10% glycerol]. Cleavage of the substrate was measured by spectrofluorometer at 380 nm excitation and 460 nm emission wavelengths[19].

Reverse transcriptase and polymerase chain reaction (RT-PCR)[20]: RT-PCR was performed to detect the expression of RD-5 mRNA. Ileum tissues were extracted to prepare total RNA using Trizol reagent (Invitrogen, California, USA), followed by the synthesis of cDNA using PTC-200 (MJ Research, USA). The primers and PCR conditions are shown in the Table. Finally,

Targeted genes or bacteria	Primers Beijing to embellish DE biotechnology development centre China	PCR condition
Table. PCR primers and conditions for targeted genes and bacteria		
RD-5 (Rat defensins-5)	Forward: 5′-ATCGGTGGCCTCAGAACTC-3′ Reverse: 5′-TCGCAGCCATTGAAGAAATT-3′.	94 °C for 3 min, 94 °C for 30 sec, 55 °C for 30 sec, and 72 °C for 30 sec, 33 cycles in total. The reaction mixtures were incubated for an additional 10 min at 72 °C and kept at 4°C.
GAPDH (Reduced glycereldehyde phosphate dehydrogenase)	Forward: 5'-AGACAGCCGCATCTTCTTGT-3′, Reverse: 5′-CTTGCCGTGGGTAGAGTCAT-3′;	House keeping genes for RD-5 and TEF3 (Trefoil Factor Family 3)
Escherichia coli[21]	BG-1: 5'-CTTTGCCTGGTTTCCGGCACCAGAA-3', BG-4: 5'-AACCACCGCACGATAGAGATTCGGG-3'.	94°C for 5 min, 94 °C for 1 min, 60°C for 30 sec, 72°C for 1 min, 72 °C for 10 min and final extension for 10 min at 4 °C, 30 cycles in total

10 μl of amplified product was applied to agarose gel electrophoresis and the results were detected by image analysator (UVP, GDS-8000pc, California, USA). The level of mRNA expression was normalized to reduced glyceraldehyde-phosphate dehydrogenase (GAPDH) mRNA.

Statistical analysis: The results are expressed as the means ± standard error of the mean (SEM). SPSS 16.0 software, USA was used for statistical analysis. The Kaplan–Meier analysis and log-rank test were used for the survival data. Biochemical data were analyzed by Student's t test for the comparisons between two groups and one-way or two-way ANOVA followed by Bonferonni test where appropriate for multiple comparisons. The chi-square test and Mann–Whitney test were applied to compare the gut mucosal injury score.

Results

Effects of ulinastatin on mortality rate in CLP-induced sepsis: The survival curves for CLP-induced septic rats delineated the benefit sustained from ulinastatin treatment. In the control group with saline treatment, 40 per cent rats died after 20 h and the remaining died within 50 h, while in the ulinastatin group, only 20 per cent rats died after 20 h and the remaining died within five days following the surgery, showing a significantly increased survival time compared with the control group (*P*<0.05) (Fig. 1).

Effect of ulinastatin on lung injury and plasma cytokines: After 12 h following CLP, pulmonary oedema was shown by a significant increase in wet-to-dry weight ratio of lung in the saline control group (5.4 ± 0.3) and in the ulinastatin group (5.2 ± 0.2) compared with that in the sham group (4.1 ± 0.2) (*P*<0.05). No significant difference was found between the saline group and the ulinastatin group, suggesting that ulinastatin could not prevent pulmonary oedema at the early stage of sepsis.

As shown in Fig. 2A and 2B, significantly (*P*<0.001) low plasma levels of TNFα and IL-6 were recorded in the sham CLP group at each time point of experiment. The levels were dramatically elevated from 3 to 6 hours and then reduced at 12 hours in the saline and the ulinastatin CLP groups. However, both TNFα and IL-6 levels were significantly lower in the ulinastatin CLP group than those in the saline CLP group (*P*<0.05).

Effect of ulinastatin on the intestinal mucosal integrity and RD-5 expression: In the sham CLP group, normal
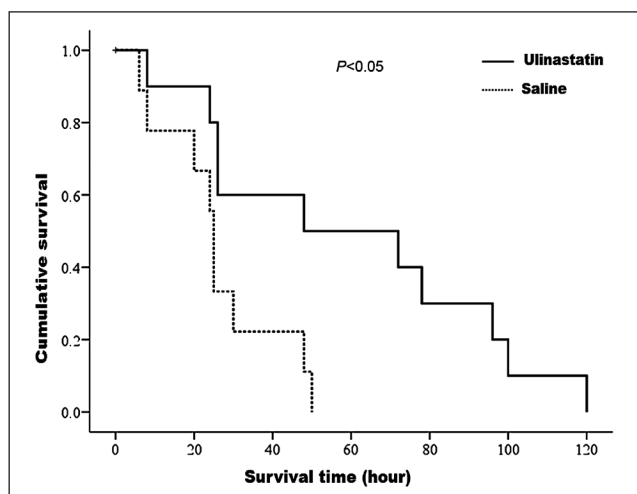

Fig. 1. Ulinastatin treatment prolongs the survival time of septic rats. There was a significant difference in survival time between the rats treated with ulinastatin and saline controls. (*P*<0.05). n=10 per group.

intestinal histology was seen, showing distinct mucosal structures and complete villi. Paneth cells could be seen clearly in the crypts. In the saline CLP group,
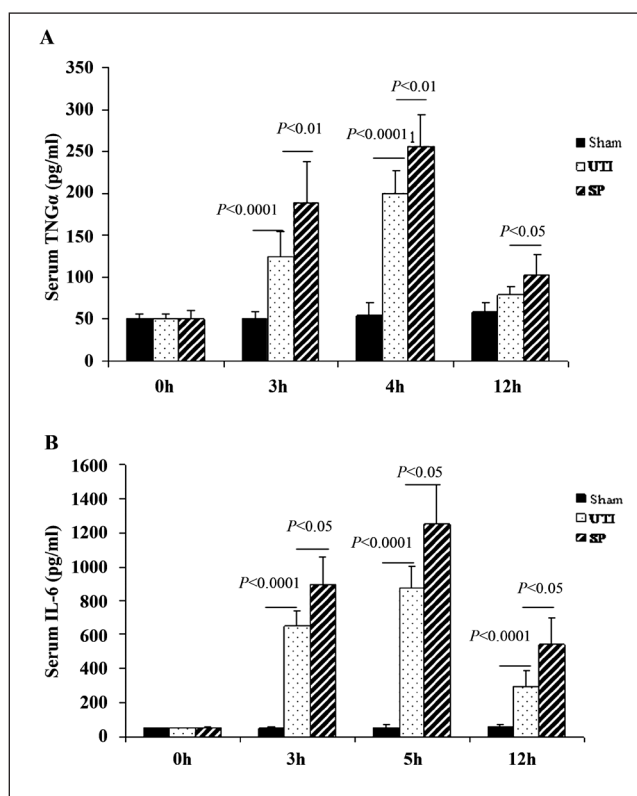

Fig. 2. The effect of UTI on plasma cytokine levels. **A:** Changes of plasma TNFα with treatment; **B:** Changes of plasma IL-6 with treatment. SP, saline; UTI, ulinastatin. Data are mean ± SEM, n = 6 per group.

mucosal injury was serious and demonstrated loss of villi, hemorrhage, ulceration, bleeding of the lamina propria, inflammatory infiltration and loss of glandular architecture. Paneth cells were hardly seen in the crypts. However, in the ulinastatin group, mucosal injury was not as serious as the septic group. Paneth cells could be seen in some crypts (Fig. 3A). As shown in Fig. 3B, the histopathological score of intestinal injury was significantly ($P<0.05$, <0.001) lower in septic rats with ulinastatin treatment than saline controls from 3, 6 and 12 h after CLP. Significantly less apoptosis in the ulinastatin CLP group was detected by TUNEL assay (Fig. 4A and 4B) and caspase 3 activity (Fig. 4C) ($P<0.001$), suggesting that ulinastatin can improve intestinal cell survival in septic rats.

RD-5 mRNA expression was maintained at similar levels in the sham ulinastatin group during the experiment. In the saline CLP group, RD-5 mRNA expression was gradually reduced from 3 to 12 h after CLP (0.43 ± 0.03 vs. 0.24 ± 0.02, $P<0.05$), which was significantly lower than that in the sham group at 3, 6, and 12 h ($P<0.001$). In the ulinastatin CLP group, RD-5
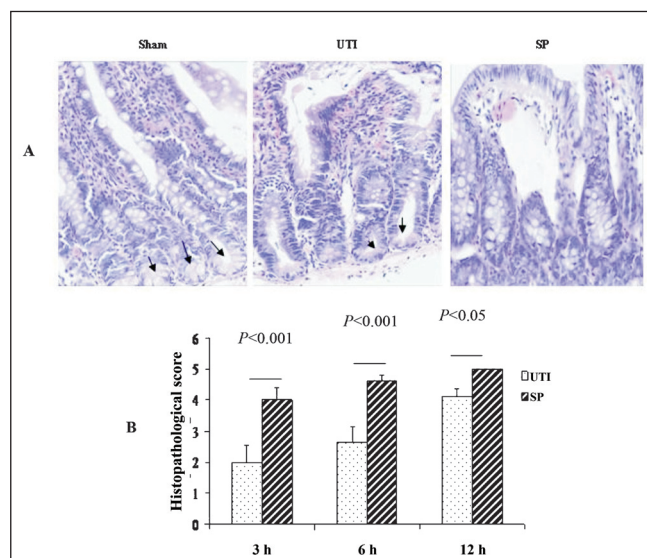

Fig. 3. Ulinastatin treatment improves the intestinal mucosal integrity of septic rats. **A:** HE staining. In the sham group, there were distinct mucosal structures and complete villi. Paneth cells could be seen clearly in the crypts (arrows). In the UTI group, mucosal injury was not as serious as the saline controls. Paneth cells could be seen in some crypts. In the saline control group, mucosal injury was serious with loss of villi, hemorrhage, ulceration, bleeding of the lamina propria, inflammatory infiltration and loss of glandular architecture. Paneth cells were hardly seen in the crypts. Scale bar=100 μm. **B:** the histopathological score of intestinal injury was significantly lower with UTI treatment than the saline controls. SP, saline; UTI, ulinastatin. Data are mean ± SEM, n=6 per group.
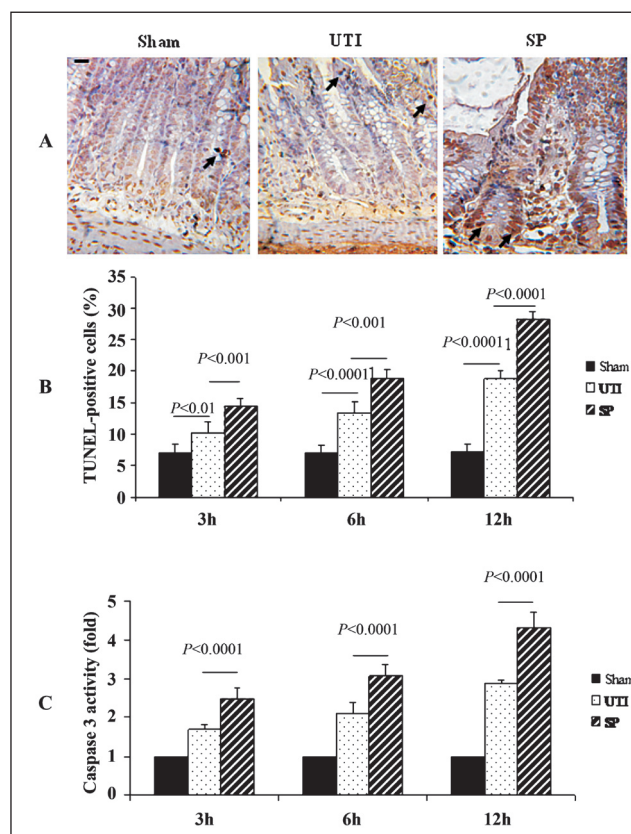

Fig. 4. Ulinastatin improved intestinal cell survival in sepsis. **A:** Reduced apoptosis in septic rats with ulinastatin treatment was detected by TUNEL assay. Arrows indicate TUNEL positive nuclei (brown). Scale bar = 100μm. **B:** The bar chart summarizes the number of apoptotic nuclei in sham and septic rats with or without UTI treatment. **C:** The bar chart shows caspase 3 activity in sham and septic rats with or without UTI treatment. SP, saline; UTI, ulinastatin. Data are mean ± SEM, n = 6 per group.

mRNA expression was also gradually decreased from 3 to 12 h (0.58 ± 0.04 vs. 0.39 ± 0.03, $P<0.05$). Its level at each time point was significantly lower than that in the sham group ($P<0.05$) but significantly higher than that in the saline controls ($P<0.05$).

All these data indicated favourable effects of ulinastatin on the structural integrity and function of the gut barrier.

Discussion

The present study demonstrated that in a CLP model of sepsis, ulinastatin significantly improved the survival time. It also significantly reduced mucosal apoptosis, histopathological scores of intestinal injury, and plasma levels of TNF-α and IL-6 but significantly increased RD-5 mRNA expression in rats with sepsis, thereby minimizing the damage to both structure and
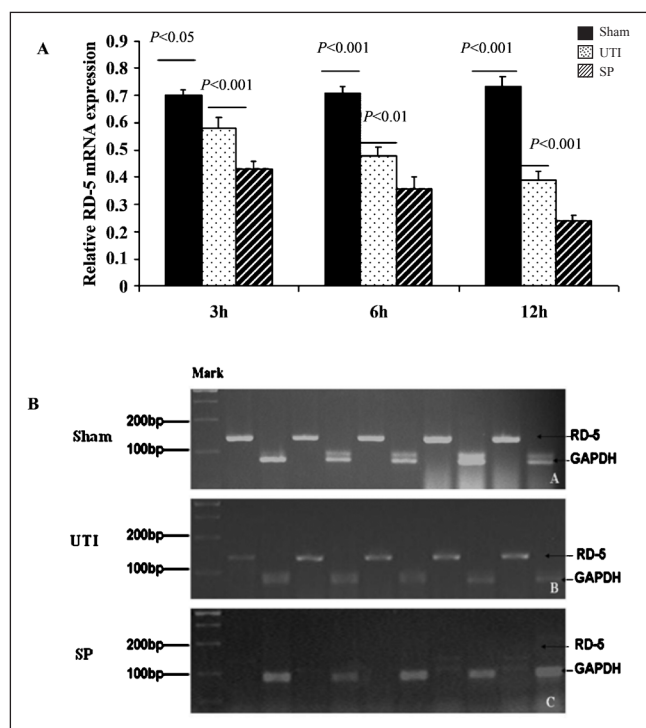
Fig. 5 A and **B.** RD-5 mRNA expressions in intestinal tissue. RD-5 mRNA expression in septic rats was significantly reduced compared with the sham group. However, the level in the UTI group was still higher than that in the saline controls. SP, saline; UTI, ulinastatin. Data are mean ± SEM, n = 6 per group.

function of the gut barrier. Ulinastatin has been shown to effectively decrease intestinal mucosal apoptosis and inhibited bacterial translocation[22]. In another study, one dose of ulinastatin administration immediately after CLP not only suppressed the levels of pro-inflammatory cytokines such as TNF-α and IL-6 but also markedly enhanced the levels of anti-inflammatory cytokines such as IL-10 and IL-13 within the early stage of sepsis[23].

The structural alteration of gut barrier in sepsis is multifactorial. Due to its anatomical characteristics of microcirculation, the gut mucosa is vulnerable to hypoxia induced by systematic sepsis. Ulinastatin has been found to improve ischaemia-reperfusion damage in multiple organs[11,24,25]. We, therefore, speculate that the beneficial effect of ulinastatin on the gut barrier integrity may be partly through its effect against hypoxic damage due to sepsis on one hand, and on the other hand, the beneficial effect of ulinastatin on reducing serum IL-6 and TNF-α levels may also help to protect the mucosal integrity. This was also supported by the evidence about the adverse effects of IL-6 and TNF-α

on intestinal mucosa. It has been reported that deletion of IL-6 in mice resultes in less gut barrier dysfunction after haemorrhagic shock and resuscitation, suggesting that IL-6 is essential for the development of gut barrier dysfunction[26]. Also, the intestinal permeability is reduced in septic IL-6 knockout mice[27]. TNF-α has also been reported to play a role on gut mucosal atrophy after severe burns[28]. Therefore, ulinastatin may protect gut barrier via multiple pathways, which prevents further development of sepsis and contributes to the favourable outcome in survival.

The role of the immune system in the pathogenesis of sepsis has been widely documented. Overproduction of TNF-α and IL-6 has been shown to correlate with poor outcome in patients with severe sepsis and septic shock[29], thus the suppression of TNF-α and IL-6 will be beneficial for the outcome of sepsis. Our findings are consistent to previous studies. It was reported that patients with sepsis treated with ulinastatin and thymosin $α_1$ had reduced serum TNF-α and IL-6 levels and a lower mortality rate[14]. But using LPS to induce inflammation in UTI deficient mice and wild-type littermates, Inoue *et al*[30] demonstrated that UTI deficient mice had enhanced pro-inflammatory cytokine and chemokine expression as well as more serious organ injuries after LPS challenge compared with wild-type littermates, suggesting that UTI can downregulate pro-inflammatory cytokines.

In the present study, ulinastatin–treated CLP rats had less reduction in RD-5 mRNA expression compared with saline controls. As a native immune substance produced by Paneth cells in intestinal crypts, RD-5 has a key role against microorganisms including bacterium, eumycete, spirochaeta pallida and some tunic virus[31]. The reduction in RD-5 mRNA and protein levels would result in increase of bacterium existing in the enteric cavity[9]. We did not measure RD-5 protein level or detect the distribution of RD-5 by immunochemistry; hence it is difficult to determine whether this treatment will lead to an increase of RD-5 in protein level. Even so, further study about the underlying mechanism is still warranted. The histological examination in the present study has shown concomitant severe damage of mucosa and a reduced number of Paneth cells in saline controls, suggesting that the decrease of RD-5 mRNA expression is at least partly due to the loss of surface epithelium as a consequence of sepsis. The increased RD-5 mRNA expression in ulinastatin-treated rats may result from the recovery of mucosal injury.

In the present study, ulinastatin did not completely prevent pulmonary oedema. This may be due to the short period of treatment, in which the benefit of ulinastatin on pulmonary oedema could not be achieved within 12 h. Therefore, our result on this aspect is different from the previous study[32], which assessed the efficacy of ulinastatin on pulmonary injury after 24-48 h following smoking inhalation.

The present study had some limitations. Other cytokines that may be involved in the development of sepsis such as IL-1, IL-10, high mobility group Box-1 (HMGB-1), *etc*. were not measured. Because cytokines were measured within 12 hours after CLP, HMGB-1level may not change as a late mediator of sepsis[33]. In addition, concomitant antibiotic treatment was not given or compared with other treatments such as anti-TNF antibody as our aim in the present study was to assess the efficacy of monotherapy with ulinastatin.

In conclusion, ulinastatin showed a therapeutic potential to prevent gut barrier dysfunction in the early stage of sepsis, thereby improving the outcome of sepsis. The underlying mechanisms may partly relate to the prevention of excessive inflammatory response. Further studies need to be done to understand the mechanism completely.

Acknowledgment

References

1. Martin GS. Sepsis: the future is bright. *Crit Care Med* 2006; *34* : 2484-5.

2. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, *et al*. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008; *36* : 296-327.

3. Dietrich W, Busley R, Kriner M. High-dose aprotinin in cardiac surgery: is high-dose high enough? An analysis of 8281 cardiac surgical patients treated with aprotinin. *Anesth Analg* 2006; 103 : 1074-81.

4. Lazar HL, Bao Y, Tanzillo L, O'Gara P, Reardon D, Price D, *et al*. Aprotinin decreases ischemic damage during coronary revascularization. *J Card Surg* 2005; *20* : 519-23.

5. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, *et al*. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001; *344* : 699-709.

6. Kanwar S, Windsor AC, Welsh F, Barclay GR, Guillou PJ, Reynolds JV. Lack of correlation between failure of gut barrier function and septic complications after major upper gastrointestinal surgery. *Ann Surg* 2000; *231* : 88-95.

7. Suliburk J, Helmer K, Moore F, Mercer D. The gut in systemic inflammatory response syndrome and sepsis. Enzyme systems fighting multiple organ failure. *Eur Surg Res* 2008; *40* : 184-9.

8. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002; *415* : 389-95.

9. Wilson CL, Ouellette AJ, Satchell DP, Ayabe T, Lopez-Boado YS, Stratman JL, *et al*. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 1999; *286* : 113-7.

10. Flaujac C, Pouard P, Boutouyrie P, Emmerich J, Bachelot-Loza C, Lasne D. Platelet dysfunction after normothermic cardiopulmonary bypass in children: effect of high-dose aprotinin. *Thromb Haemost* 2007; *98* : 385-91.

11. Ren B, Wu H, Zhu J, Li D, Shen Y, Ying R, *et al*. Ulinastatin attenuates lung ischemia-reperfusion injury in rats by inhibiting tumor necrosis factor alpha. *Transplant Proc* 2006; *38* : 2777-9.

12. Royston D, Levy JH, Fitch J, Dietrich W, Body SC, Murkin JM, *et al*. Full-dose aprotinin use in coronary artery bypass graft surgery: an analysis of perioperative pharmacotherapy and patient outcomes. *Anesth Analg* 2006; *103* : 1082-8.

13. Eser O, Kalkan E, Cosar M, Buyukbas S, Avunduk MC, Aslan A, *et al*. The effect of aprotinin on brain ischemic-reperfusion injury after hemorrhagic shock in rats: an experimental study. *J Trauma* 2007; *63* : 373-8.

14. Li Y, Chen H, Li X, Zhou W, He M, Chiriva-Internati M, *et al*. A new immunomodulatory therapy for severe sepsis: Ulinastatin Plus Thymosin {alpha} 1. *J Intensive Care Med* 2009; *24* : 47-53.

15. Condon MR, Viera A, D'Alessio M, Diamond G. Induction of a rat enteric defensin gene by hemorrhagic shock. *Infect Immun* 1999; *67* : 4787-93.

16. Parker SJ, Watkins PE. Experimental models of gram-negative sepsis. *Br J Surg* 2001; *88* : 22-30.

17. Doi K, Leelahavanichkul A, Yuen PS, Star RA. Animal models of sepsis and sepsis-induced kidney injury. *J Clin Invest* 2009; *119* : 2868-78.

18. Huang CY, Hsiao JK, Lu YZ, Lee TC, Yu LC. Anti-apoptotic PI3K/Akt signaling by sodium/glucose transporter 1 reduces epithelial barrier damage and bacterial translocation in intestinal ischemia. *Lab Invest* 2011; *91* : 294-309.

19. Hyoh Y, Ishizaka S, Horii T, Fujiwara A, Tegoshi T, amada M, Arizomo N. Activation of caspases in intestinal villus epithelial cells of normal and nematode infected rats. *Gut* 2002; *50* : 71-7.

20. Mastroianmi JR, Ouellette AJ. Alpha-defensinsin enteric innate immunity: functional Paneth cell alpha-defensins in mouse colonic lumen. *J Biol Chem* 2009; *284* : 27848-56.

21. Ponnala L. Detecting slow-translating regions in *E. coli. Int J Bioinform Res Appl* 2010; *6* : 521-30.

22. Li KY, Jiang LY, Zhang M, Zhong M, Xie WZ. Effects of ulinastatin on gut mucosal apoptosis and bacterial translocation

in rats with sepsis. *Nan Fang Yi Ke Da Xue Xue Bao* 2008; *28* : 1244-6.

23. Cao YZ, Tu YY, Chen X, Wang BL, Zhong YX, Liu MH. Protective effect of Ulinastatin against murine models of sepsis: Inhibition of TNF-alpha and IL-6 and augmentation of IL-10 and IL-13. *Exp Toxicol Pathol* 2012; *64* : 543-7.

24. Kim SJ, Yoo KY, Jeong CW, Kim WM, Lee HK, Bae HB, *et al*. Urinary trypsin inhibitors afford cardioprotective effects through activation of PI3K-Akt and ERK signal transduction and inhibition of p38 MAPK and JNK. *Cardiology* 2009; *114* : 264-70.

25. Li XK, Suzuki H, Kimura T, Kawabe A, Uno T, Harada Y. Ulinastatin, a protease inhibitor, attenuates intestinal ischemia/ reperfusion injury. *Transplant Proc* 1994; *26* : 2423-5.

26. Yang R, Han X, Uchiyama T, Watkins SK, Yaguchi A, Delude RL, *et al*. IL-6 is essential for development of gut barrier dysfunction after hemorrhagic shock and resuscitation in mice. *Am J Physiol Gastrointest Liver Physiol* 2003; *285* : G621-9.

27. Wang Q, Fang CH, Hasselgren PO. Intestinal permeability is reduced and IL-10 levels are increased in septic IL-6 knockout mice. *Am J Physiol Regul Integr Comp Physiol* 2001; *281* : R1013-23.

28. Spies M, Chappell VL, Dasu MR, Herndon DN, Thompson JC, Wolf SE. Role of TNF-alpha in gut mucosal changes after severe burn. *Am J Physiol Gastrointest Liver Physiol* 2002; *283* : G703-8.

29. Damas P, Ledoux D, Nys M, Vrindts Y, De Groote D, Franchimont P, *et al*. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. *Ann Surg* 1992; *215* : 356-62.

30. Inoue K, Takano H, Shimada A, Yanagisawa R, Sakurai M, Yoshino S, *et al*. Urinary trypsin inhibitor protects against systemic inflammation induced by lipopolysaccharide. *Mol Pharmacol* 2005; *67* : 673-80.

31. Bastian A, Schafer H. Human alpha-defensin 1 (HNP-1) inhibits adenoviral infection in vitro. *Regul Pept* 2001; *101* : 157-61.

32. Qiu X, Ji S, Wang J, Li H, Xia T, Pan B, *et al*. The therapeutic efficacy of Ulinastatin for rats with smoking inhalation injury. *Int Immunopharmacol* 2012; *14* : 289-95.

33. Chavan SS, Huerta PT, Robbiati S, Valdes-Ferrer SI, Ochani M, Dancho M, *et al*. HMGB1 mediates cognitive impairment in sepsis survivors. *Mol Med* 2012; *18* : 930-7.

Reprint requests: Dr Longyuan Jiang, Department of Emergency Medicine, The Memorial Hospital of Sun Yat-sen,
　　　　　Sun Yat-sen University, 107# West Yanjiang Road, Guangzhou, Guangdong, 510120, PR China
　　　　　e-mail: jly1964@vip.163.com