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Intraintestinal administration of ulinastatin protects against sepsis by relieving intestinal damage

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ABSTRACT

Background: Intravenous administration of ulinastatin (UTI), a broad spectral protease inhibitor, has been used on an experimental basis with severe sepsis patients in Asia. However, the effects of intraintestinal administration of UTI on intestinal and multiple organ damage in sepsis have not been reported.

Materials and methods: In this study, we established a sepsis model in rats using cecal ligation and puncture and compared the effects of intraintestinal administration of UTI through an artificial fistula of duodenum and intraperitoneal administration of UTI on the pathophysiological changes of sepsis.

Results: It was found that intraintestinal administration of UTI (1) significantly improved the survival of septic rats, (2) significantly reduced the serum levels of tumor necrosis factor-α, interleukin-1β, interleukin-6 as well as intestinal injury biomarkers diamine oxidase, D-lactic acid, and fluorescein isothiocyanate-dextran 4, and (3) significantly reduced intestinal microscopic and ultrastructural damage of septic rats. In addition, the protective effects of intraintestinal administration of UTI were significantly better than those of intraperitoneal administration of UTI.

Conclusions: Overall, the present study for the first time revealed that intraintestinal administration of protease inhibitor UTI could reduce systemic inflammatory responses and multiple organ dysfunction in rats with sepsis by inhibiting autodigestion of intestinal wall due to proteases and provided new research ideas and experimental evidences for treatment of sepsis by intraintestinal administration of UTI.

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Introduction

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection,[1] with main clinical manifestations of inflammatory response imbalance, coagulation disorders, and immune dysfunction.[2,3] Although the pathophysiological mechanism of sepsis is better understood[4,5] and some progress has been made in therapeutic intervention[6] in recent years, the incidence of sepsis is still increasing,[7] and the mortality rate of patients with sepsis is still as high as 30%-50%.

Ulinastatin (UTI), an acidic glycoprotein purified from human urine or blood, has broad inhibitory activity of proteinases including trypsin, chymotrypsin, elastase, and so forth.[8] Currently, it is mainly used to treat peripheral circulatory failure and pancreatitis through intravenous route.[9,10]

The intestine is one of important effector organs for systemic sepsis. Its barrier function impairment plays a critical role in sepsis development.[11] Studies have shown that in sepsis, intestinal hypotension, hypoperfusion, and hypoxia could damage tight junctions between intestinal epithelial cells and other cells,[12] elevated intestinal permeability,[13] and increased invasion of intestinal bacteria and endotoxin into the intestinal blood or lymphatic vessels could cause gut derived infections.[14,15] In recent years, researchers have proposed the gut autodigestion theory[16] and believed that sepsis was caused by resultant imbalance of systemic inflammatory responses and multiorgan dysfunction. Due to the presence of intestinal barrier dysfunction induced by intestinal ischemia and hypoxia, hypotension, and hypoperfusion, proteinases such as trypsin could digest bowel wall, resulting in infiltration or leakage of trypsin, translocation of intestinal flora and endotoxin, imbalance of systemic inflammatory responses, and multiorgan dysfunction. However, whether direct intraintestinal administration of UTI could reduce intestinal damage of patients with sepsis and subsequently improve the pathophysiological process of sepsis has not been reported.

In this study, we, for the first time, directly administered UTI through duodenal fistulation to inhibit protease activity and explored its potential therapeutic effects on septic rats. The results showed that intraintestinal administration of UTI could improve intestinal permeability, attenuate intestinal damage, inhibit systemic inflammatory response, reduce multiple organ dysfunction, and increase survival of rats with sepsis. Moreover, the protective effects of intraintestinal administration of UTI are more obvious than those of intraperitoneal administration of UTI. These findings provided new ideas and experimental evidences for treatment of sepsis using a classic medicine.

Materials and methods

Animals and reagents

Male Sprague Dawley rats weighing 220-240 g were purchased from the Experimental Animal Center of Central South University. All experiments were approved by the Animal Ethical Committee, Xiangya School of Medicine, Central South University and performed strictly following the guidelines of the Animal Care and Use Committee of Xiangya School of Medicine, Central South University.

Rat tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) ELISA kits were purchased from Boster (Wuhan, China); serum diamine oxidase (DAO) ELISA kit was from Uscnk (Wuhan, China); serum D-lactic acid detection kit was from BioVision (San Francisco, USA); fluorescein isothiocyanate-dextran 4 (FD-4) was from Sigma (St. Louis, MO). UTI was from Techpool Bio-pharma Co, Ltd (Guangzhou, China).

Animal grouping and treatment

Sixty Sprague Dawley rats were randomly assigned into sham, cecal ligation and puncture (CLP), CLP and intraperitoneal administration of UTI (CLP + Uip) and CLP and intraintestinal administration of UTI (CLP + Uii) groups and subjected to duodenal fistula surgery and intubation. After that, rats in all groups except the sham group underwent cecal ligation and puncture to establish a septic rat model. At 2, 6, and 12 h after successful modeling, rats in CLP + Uip and CLP + Uii group were given 100,000 units/kg intraperitoneally or intraintestinally through duodenum cannula, respectively, whereas rats in CLP and sham groups were given isometrical saline intraintestinally through duodenum cannula and intraperitoneally. All the treatments were randomized and blinded.

Preparation of septic rat model by CLP

Rats were reared in strict accordance with standard procedures. In detail, rats were subjected to water and diet fasting 8 h before surgery and anesthetized by intraperitoneally given 30 mg/kg of 10% chloral hydrate. After skin preparation and disinfection, a 2.0-cm incision was made along the ventral midline to free duodenum. Then, a stoma was made at 1.5 cm away from the pylorus to implant a catheter and covered afterward using purse-string suture. The catheter was extended subcutaneously to and fixed on the back skin for intraintestinal drug administration. The mesentery and cecum were freed. The cecum was ligated using 4-0 sutures within the cecal haemal arches at 1.0 cm away from bind end of the cecum, and the blind end of the cecum was perforated once using a 20 gauge needle. After a small amount of cecal contents were extruded, the cecum was returned to its original position, and the abdomen was closed layer by layer. Rats were then treated with fluid resuscitation and antibiotics by subcutaneous injection of 50-mL/kg normal saline and 25 mg/kg imipenem, and returned to the cages, placed in a 25°C environment and free to water and diet. In addition, rats were subcutaneously injected 0.1-mg/kg morphine for postoperative analgesia every 6 h for 48 h. To avoid differences in surgical procedure, all surgeries were performed by the same person. A group of