ORIGINAL ARTICLE

EFFECT OF ULINASTATIN COMBINED WITH HAEMODIALYSIS ON SEVERE ACUTE PANCREATITIS

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Abstract

This study aimed to explore the clinical efficacy of ulinastatin and haemodialysis in the treatment of severe acute pancreatitis (SAP). 60 SAP patients treated in the *Binzhou Central Hospital* in Shandong Province, China, during June 2015 and December 2016 were randomly selected as the research subjects in this study and divided into a conventional treatment group A and an ulinastatin combined with haemodialysis treatment group B. The clinical data of the patients were compared and the blood amylase, urine amylase, serum urea nitrogen, serum lactose, serum creatinine levels, CD4+, CD8+, interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) ratio were compared after treatment. The levels of serum amylase, urinary amylase, serum creatinine, serum urea nitrogen and serum lactose of group B decreased with the increase of treatment time and showed a significant difference when compared with the conventional treatment group ($p < 0.05$); IL-1 and TNF-α levels decreased, CD4+, CD8+ and the ratio of both increased gradually. The improvement of the clinical symptoms such as fever, abdominal pain and swelling in group B was shorter than in group A ($p < 0.05$). The cure rate of group B was about 50% and its total effective rate was about 93.3% while those of group A was about 26.7% and 53.3% ($p < 0.05$). Ulinastatin combined with haemodialysis has a good clinical effect on severe acute pancreatitis.

Rezumat

Obiectivul prezentului studiu a fost de a analiza eficacitatea clinică a asocierii ulinastatinei cu hemodializa în tratamentul pancreatitei acute severe (PAS). În studiu au fost incluși 60 de pacienți cu PAS tratați în perioada iunie 2015 - decembrie 2016 în *Binzhou Central Hospital* din provincia Shandong, China. Aceştia au fost împărțiți în 2 loturi: lotul A care a primit tratament convențional și lotul B la care s-a administrat ulinastatină în asociere cu hemodializă. Au fost comparate datele clinice ale pacienților și au fost analizate valorile amilazei serice, amilazei urinare, ureei, lactozei, creatininei serice și a CD4+, CD8+, IL-1 (interleukina-1), TNF-α (factorul de necroză tumorală alfa) la diferite intervale de tratament. Valorile amilazei serice, a amilazei urinare, a ureei, lactozei și creatininei serice la lotul B au scăzut odată cu creșterea perioadei de tratament în comparație cu lotul de tratament convențional ($p < 0.05$). Valorile IL-1 și TNF-α au scăzut, iar CD4+, CD8+ și raportul ambelor au crescut gradual, diferența fiind semnificativă în comparație cu lotul A. Simptomele clinice precum febra și durerea abdominală s-au îmbunătățit semnificativ la lotul B comparativ cu lotul A ($p < 0.05$). Rata de vindecare la lotul B a fost de aproximativ 50%, iar rata de eficacitate totală a fost de aproximativ 93,3% în comparație cu lotul A unde procentele au fost de 26,7%, respectiv 53,3% ($p < 0,05$). Ulinastatinul în combinație cu hemodializa este eficient în tratamentul pancreatitei acute severe.

Keywords: ulinastatin, haemodialysis, SAP, clinical efficacy

Introduction

Severe acute pancreatitis (SAP) is an integral part of acute pancreatitis, accounting for about 20% of the incidence rate of acute pancreatitis [11]. At the same time, SAP is also one of the commonly seen clinical diseases characterized by fast progression, complications, poor prognosis and high mortality rate which can lead to various organ disorders, causing the failure of the organism to maintain normal physiological activities, and can be life-threatening. Chronic alcohol consumption can cause pancreatitis accompanied by severe psychoses and hallucinations, requiring differential diagnosis [16] or can be associated with non-alcoholic fatty liver [7]. In advanced stages of gastric adenocarcinoma [8], colon and colorectal cancers [24] acute pancreatitis may be a symptom. Septic complications with pathogenic germs in pancreatitis are frequent and severe [2]. Antibiotic treatment in pancreatitis selects bacterial resistance and increases the severity of systemic fungal infections from *Aspergillum* or *Fusarium* genus [18]. Hence, it is one of the critical acute abdominal diseases to which special attention has been paid in the intensive care unit [5]. Severe acute pancreatitis is mainly caused by cholelithiasis (gall-stones), excessive drinking and overeating [13]. Early diagnosis and treatment is very important in the treatment of SAP since it can improve the body environment and help the body to form a good

microcirculation by inhibiting pancreatic enzymes and activating cytokines [1]. Zerem E. [22] showed that the treatment of severe acute pancreatitis needs prolonged intervention to ensure a better demarcation and pressure rise (delay, drainage and debridement) has a positive effect on the treatment of the disease. Wang G. *et al.* [20] used ulinastatin and gabexate in the treatment of severe acute pancreatitis and found that both drugs are effective in alleviating the clinical symptoms of the disease. Ulinastatin is a broad-spectrum enzyme inhibitor which can inhibit trypsin, reduce the degree of damage produced by trypsin to pancreatic tissues and control the disease progression [17]. Persistent haemodialysis is a relatively common way to remove slowly the solutes and water from the body [14]. In this study, ulinastatin was combined with persistent haemodialysis and used in the treatment of severe acute pancreatitis. The aim of this study was to investigate their clinical efficacy.

Materials and Methods

Patients. 60 patients who were diagnosed with SAP in the Binzhou Central Hospital in Shandong Province, China during June 2015 - December 2016 were selected to participate in the study. The diagnostic criteria are in line with the related criterion in *Guidelines for the Diagnosis and Treatment of Acute Pancreatitis in China* (2013) [21], as follows: upper abdominal acute, sudden, sustained, severe pain, mostly with back radiation; blood amylase activity 3 times higher than normal; more than 48 hours of persistent single organ or multiple organ dysfunction. The exclusion criteria [10] are as follows: clinically suspected cases; patients with onset time longer than 3 days or dead patients; special cases, such as pregnant women, patients with localized complications; patients with respiratory diseases, cardiovascular or cerebro-vascular diseases or related medical history; patients allergic to ulinastatin; patients that did not agree to receive relevant experimental treatment; patients with chronic pancreatitis acute attack.

All the included patients were randomly divided into a conventional treatment group A and the ulinastatin combined with haemodialysis treatment group B, 30 patients in each group. Approved by the Ethics Committee of the hospital, this study has been granted the consent of the included patients and their families, who have signed a relevant informed consent.

Reagents and instruments. The main reagents and instruments were as follows: ulinastatin (Guangdong Tianpu Biochemical Pharmaceutical Co., Ltd. China); NaCl (Beijing Kangpuhuiwei Technology Co., Ltd. China); haemodialysis catheter (Nanjing Ningchuang Medical Equipment Co., Ltd. China); blood purifier (Fresenius Medical Care AG & Co Germany); polysulfone membrane filter (Fresenius Medical Care AG & Co Germany); heparin (Shanghai Jingke Chemical Technology Co., Ltd. China); centrifuge (Beckman Coulter United States); human CD4 molecule (CD4) ELISA kit (Suzhou Keming Biotechnology Co., Ltd. China), rat CD8 molecule (CD8) ELISA kit (Suzhou Keming Biotechnology Co., Ltd. China), mouse interleukin 1 (IL-1) ELISA kit (Suzhou Keming Biotechnology Co., Ltd. China) and human tumour necrosis factor alpha (TNF-[alpha]) ELISA kit (Suzhou Keming Biotechnology Co., Ltd. China).

Methods for detection: Dry chemical method was used to detect blood amylase and urine amylase using a Biochemical Analyzer (Beckman Coulter, United States). Urease - wave colorimetric method was applied to detect serum urea nitrogen and lactose detection kit was used to detect serum lactose according to instructions. Creatininase amidohydrolase method was applied to detect serum creatinine levels.

Conventional treatment. The conventional treatment for patients in group A was as follows: the patients were deprived from food and water and nutritional supplements were injected into their peripheral veins; the secretion of trypsin was inhibited; antibiotics were used for preventing infections; fluid infusion was performed to maintain the normal level of water, electrolytes and acid-base in the body; the functions of important organs were monitored.

Ulinastatin combined with haemodialysis treatment. Firstly, 200,000 U of ulinastatin were dissolved in 250 mL of 0.9% NaCl solution, which was then intravenously injected to the patients in group B after they received conventional treatment. The instillation lasted for 2 hours, which was performed twice a day. Three days later, the amount of ulinastatin was adjusted according to the changes. Secondly, vein intubation was performed and a sterile haemodialysis catheter was used to form a vascular pathway. Through the continuous intravenous - venous haemodialysis method of treatment, the blood flow was controlled at 250 mL/min. The haemodialysis treatment was continued for 2 - 3 days with a blood purifier and a polysulfone membrane filter. Then, femoral vein catheterization was carried out and heparin was applied for anticoagulation. The first dose of unfractionated heparin was of 1000 IU, followed by 10 IU/(kg·h), both of which injected through the vein, with a displacement flow of 50 mL/(kg·h).

Biochemical assays. The levels of serum amylase, urea nitrogen, lactose, IL-1 and TNF-α as well as the ratio of CD4+ and CD8+ were reviewed on the 1st, 3rd and 5th day after the initiation of treatment. The specific steps were as follows: (1) 5 mL of peripheral venous blood was collected and centrifuged at 2500 r/min at 25°C for 15 min. After the removal of the suspended matter and standing and solidification, the supernatant was collected. (2) CD4+, CD8+, IL-1,

TNF-α and other inflammatory factors were detected according to the instructions of the kits. (3) If the patient's SPA-related symptoms and signs completely disappeared, and the auxiliary detection was normal, he was cured; if the patient's SPA-related symptoms and signs were alleviated but not disappeared and the auxiliary detection showed basic recovery, the treatment was effective; if the patient's SPA-related symptoms and signs and the auxiliary detection showed no obvious changes, the treatment was ineffective.

Total effective rate = (cure + effective)/total number.

Statistical methods. SPSS17.0 was applied for statistical analysis of the experimental data; the measurement data was evaluated by t-test; the data format is $\bar{x}$ (average) ± a (standard deviation); the count data was analysed with chi square test and the test standard was set to be 0.05. For $p < 0.05$ data were considered significant.

Results and Discussion

Clinical data of patients. From Table I, we can see that there were no statistical differences in the gender, age, onset time and pathogenesis between the two groups of patients ($p > 0.05$), suggesting that the basic situation of the two groups of patients was similar.

Table I

Patients' clinical data sheet

Group	Gender (male: female)	Age	Weight	Onset time	Pathogenesis			
					cholangitis-originated	Overeating	Alcohol	Other
Group A	19:11	53.3 ± 3.6	76.9 ± 8.4	29.4 ± 2.3	12	10	6	2
Group B	18:12	53.8 ± 3.3	77.7 ± 8.2	29.9 ± 2.1	13	8	7	2
p value	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05

Comparison of test indicators. As shown in Figure 1, there were statistical differences regarding the blood and urine amylase and serum creatinine between group A and group B in the same time period ($p < 0.05$). As shown in Figure 2, there were statistical differences in serum urea nitrogen and lactose between group A and group B in the same time period ($p < 0.05$). Meanwhile, with the increase of treatment time, serum amylase, urine amylase, serum creatinine, urea nitrogen and lactose decreased, suggesting that severe acute pancreatitis was to some extent cured after ulinastatin and continuous blood treatment.

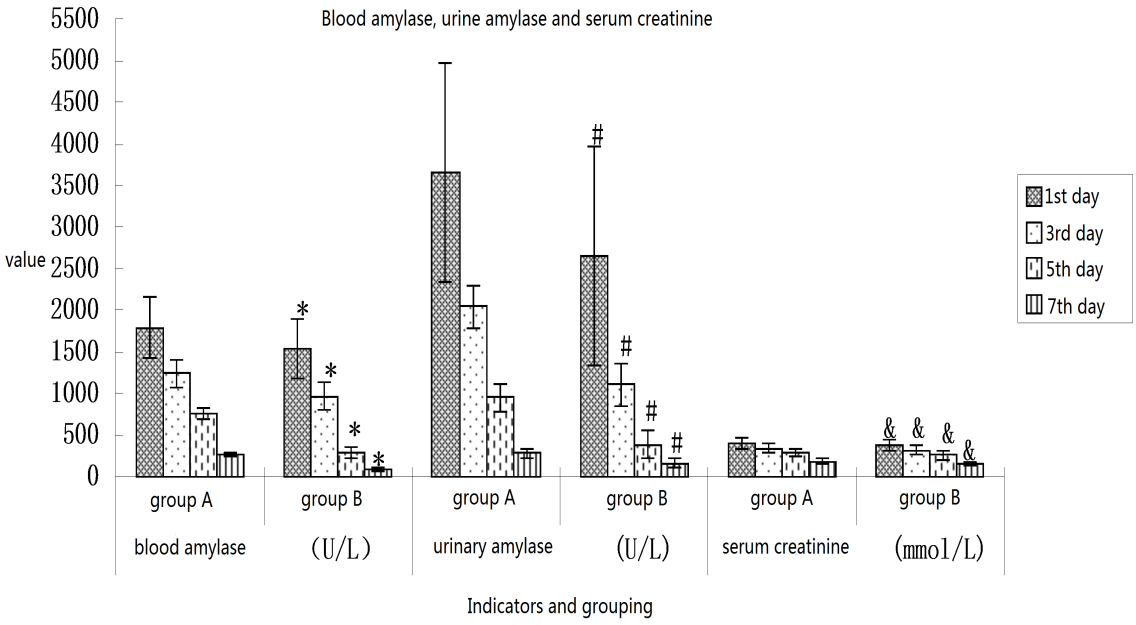

Figure 1.

Clinical indicators of blood and urine amylase and serum creatinine

* – indicates that the blood amylase of group A and group B in the same time period showed significant differences, $p < 0.05$;

\# – indicates that the urine amylase of group A and group B in the same time period showed significant differences, $p < 0.05$;

& – indicates that the serum creatinine of group A and group B in the same time period showed significant differences, $p < 0.05$

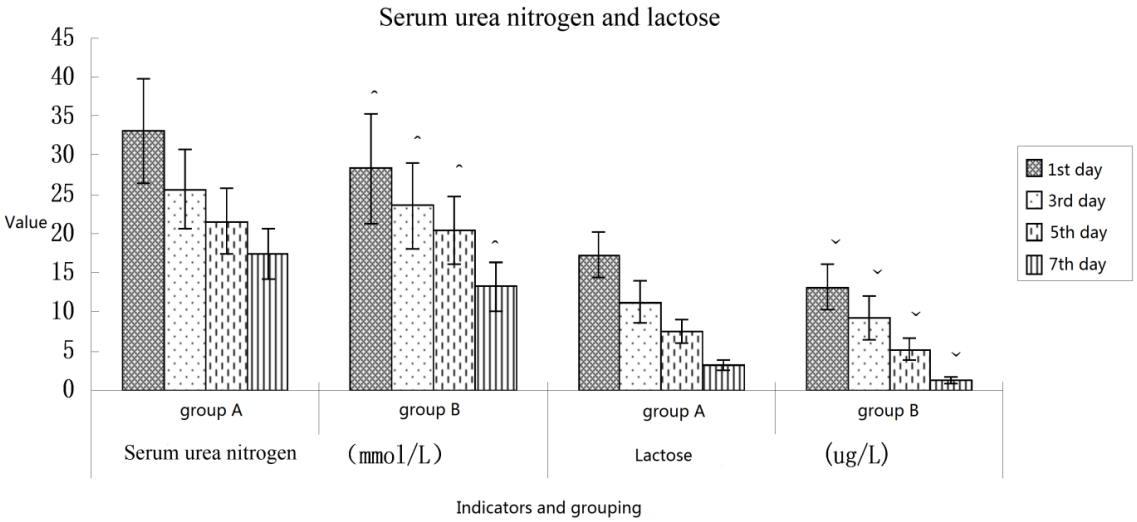

Figure 2.

Clinical indicators of serum urea nitrogen and lactose

^ – indicates that the serum urea nitrogen of group A and group B in the same time period showed significant differences, $p < 0.05$;
ˇ – indicates that the serum lactose of group A and group B in the same time period showed significant differences, $p < 0.05$

Comparison of inflammatory cytokines. As shown in Figure 3, there were significant differences between the T lymph subgroup at the 3rd day of treatment and the 1st day of treatment ($p < 0.05$). There were also significant differences between the T lymph subgroup at the 5th day and 1st day of treatment and the 5th day and 3rd day of treatment ($p < 0.05$). The T lymph subgroup in the seventh day was also statistically significant compared with the previous three ($p < 0.05$). With the increase in treatment time, CD4+ and CD8+ levels were constantly improving, so was the ratio of the two parameters, while the levels of inflammatory factors IL-1 and TNF-α decreased with time and the differences were also statistically significant ($p < 0.05$). The T lymphocyte levels in group B were significantly higher than those in group A ($p < 0.05$). The levels of IL-1 and TNF-α were lower in group B and there were statistical differences ($p < 0.05$).

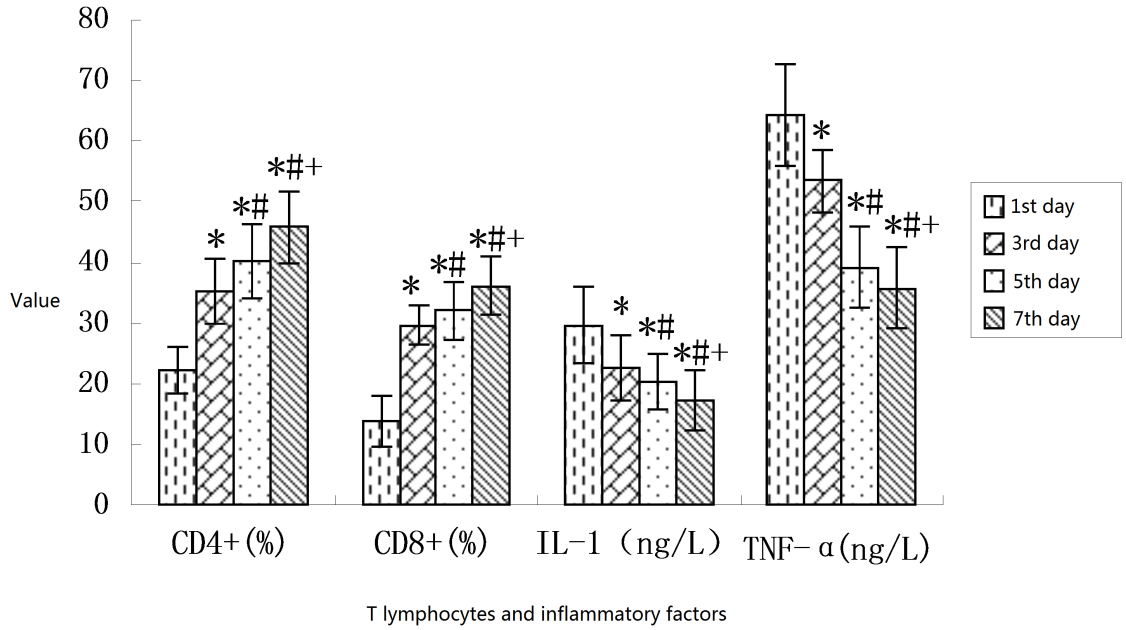

Figure 3.

The T lymph subgroup and inflammatory factors of the experimental group at each time period

* – $p < 0.05$ compared with the first day; # – $p < 0.05$ compared with the 3rd day; + – $p < 0.05$ compared with the 5th day

Comparison of remission time of clinical symptoms and efficacy. After treatment, the clinical symptoms of both patients groups A and B were alleviated. The recovery time of fever, abdominal pain and bloating

in group B was shorter than in group A ($p < 0.05$), indicating that the combination of ulinastatin and haemodialysis had an effective effect on the clinical symptoms of SPA and could shorten the treatment time. The cure rate of group B was about 50% and its total effective rate was about 93.3% while those of group A was about 26.7% and 53.3%, which showed statistically significant differences ($p < 0.05$).

There are many causes for SAP and the imbalance of the body's inflammatory factors is a primary issue which aggravates the disease [4]. Until now, the root cause of its high mortality remains unclear. The clinical symptoms of SAP are mainly abdominal pain, fever, nausea and vomiting [19]. Pancreatitis may be complicated by fluid effusion in the pleural cavity, with infectious aetiology [3].

The commonly treatment methods for SAP are the surgical treatment and non-surgical treatment [12]. The surgical treatment includes pancreatic pseudocyst drainage while the non-surgical treatments are mainly from the Chinese medicine pharmacological treatment with nutritional support. In this study, the non-surgical treatment was adopted. The clinical indicators are as follows: serum amylase, urinary amylase, urea nitrogen, lactose, serum creatinine, IL-1 and TNF-α levels, CD4+, CD8+ as well as the ratio of CD4+ and CD8+. CD4+ cells are assisted T lymphocytes and CD8+ play a mediating role in cytotoxicity. Therefore, the balance of both plays an important role in the normal immune response of human cells. In addition, their ratio can reflect the immune status of the body; the reduction of the ratio means that the immune function of the body is damaged. TNF-α is an important cytokine in the pathogenesis of SAP and has an induced effect on the expression of inflammatory factors. Moreover, it can activate the body cascade reaction and leads to pancreatic tissue necrosis and other organ damage [15]. IL-1, released by macrophages, and TNF-α can stimulate each other, which can aggravate the organ damage. In addition, IL-1 has a stimulating effect on other inflammatory mediators [9]. Zhang XB *et al.* [23] combined the Chinese and pharmacological treatment methods and found that their combination had a great effect on the treatment of SAP. Chen Y *et al.* [6] used dexamethasone and ulinastatin for the treatment of SAP and the results showed that the multiple organ dysfunction syndrome caused by SAP was effectively treated and the mortality was reduced.

Conclusions

In this study, ulinastatin and continuous haemodialysis were combined for the treatment of SAP. The results showed that IL-1 and TNF-α levels continued to decline while CD4+, CD8+ and the ratio of both gradually increased after treatment, indicating that the combination treatment could effectively inhibit the body's inflammatory response and help the inflammatory factors to reach a balance. Meanwhile, it could effectively alleviate the symptoms of patients, reduce urine amylase and blood amylase levels and thus proving a good clinical effect.

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