ORIGINAL ARTICLE

THE PROTECTIVE EFFECT OF ULINASTATIN IN SEVERE SEPSIS. A MECHANISTIC APPROACH

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Abstract

The aim of this study was to investigate the effect of ulinastatin on endothelial polysaccharide coating and mortality in patients with severe sepsis and septic shock. Twenty-nine cases of sepsis and septic shock diagnosed from June 2015 to December 2018 were included in the study. According to their time of admission, the patients were randomly divided into ulinastatin group (Y, N = 13), which was subdivided in L15 group (APACHE II (Acute physiology and chronic health evaluation II) ≥ 15) (8 cases) and in S15 group (APACHE II < 15) (5 cases), and control group (N, N = 16), subdivided in L15 group (11 cases) and S15 group (5 cases). All patients were treated in the Intensive Care Unit (ICU). The ulinastatin group (Y) received 100,000 U ulinastatin as an intravenous infusion for a period of 8 h, while the control group received routine treatment. Temperature (T), respiratory rate (RR), heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), and pulmonary vascular permeability index (PVPI) at 0 h, 24 h and 72 h after admission were determined for each patient. Moreover, the blood samples were collected in order to evaluate the white blood cell count (WBC), lactate (Lac), C-Reactive Protein (CRP), procalcitonin (PCT), intercellular adhesion molecule (ICAM-1), Syndecan-1 (SDC-1), and systemic central venous oxygen saturation (ScVO$_2$). The results showed that the lactate levels after 72 h of treatment and the 28-day mortality rate of group Y significantly decreased compared to group N (p <0.018). In L15 subgroup, the lactate level and the 28-day mortality rate in group Y significantly decreased compared to group N (p < 0.018). In S15 subgroup, lactate level, PVPI, SDC-1 and The 28-day mortality rate significantly decreased in group Y compared to group N (p < 0.05). In conclusion, for sepsis patients with an APACHE II score ≥ 15, their structure of vascular endothelial polysaccharide coating may be affected by many factors, and the effect of ulinastatin is limited. For sepsis patients with an APACHE II score of < 15, ulinastatin may improve vascular permeability, reduce capillary leakage and improve microcirculation by protecting the structure of endothelial polysaccharide coating. In addition, ulinastatin may reduce the mortality of sepsis patients.

Rezumat

Scopul acestui studiu a fost de a investiga efectul ulinastatinei asupra endoteliului vascular, precum și asupra mortalității la pacienții cu sepsis sever și șoc septic. Au fost incluse în studiu douăzeci și nouă de cazuri cu sepsis și șoc septic diagnosticate în perioada iunie 2015 - decembrie 2018. Pacienții au fost împărțiți astfel: grupul ulinastatină (Y, N = 13), care a fost împărțit în subgrupul L15 (APACHE II (Acute physiology and chronic health evaluation II) ≥ 15) (8 cazuri) și subgrupul S15 (APACHE II <15) (5 cazuri) și respectiv grupul de control (N, N = 16), împărțit la rândul său în grupul L15 (11 cazuri) și grupul S15 (5 cazuri). Grupul ulinastatină (Y) a primit 100 000 U ulinastatină ca perfuzie intravenos timp de 8 ore, în timp ce grupul martor a primit tratament de rutină. S-au determinat următorii parametri: temperatura (T), frecvența respiratorie (RR), frecvența cardiacă (HR), tensiunea arterială medie (MAP), tensiunea venoasă centrală (CVP) și indicele de permeabilitate vasculară pulmonară (PVPI) la 0, 24 și 72 h după internare. De asemenea, s-au determinat numărul de leucocite, acidul lactic, proteina C reactivă, procalcitonină, molecula de adeziune intercelulară, Syndecan-1 și saturația sistemică de oxigen venos central. Rezultatele au arătat că ulinastatina poate îmbunătăți permeabilitatea vasculară, reduce scurgerile capilare și îmbunătățește microcirculația protejând structura endoteliului vascular. În plus, ulinastatina poate reduce mortalitatea pacienților cu sepsis.

Keywords: ulinastatin; severe sepsis; vascular permeability; endothelial polysaccharide coating

Introduction

Currently, sepsis is one of the most frequently treated diseases in the Intensive Care Unit (ICU). In recent years, the incidence of sepsis has gradually increased, being one of the main risk factors for global mortality and critical diseases [1-3]. Bacterial resistance can be another risk factor for global mortality in sepsis patients [4]. Severe sepsis and septic shock are characterized by microcirculatory disturbance or hypovolemia, decreased peripheral vascular tension, myocardial depression, disruption of microcirculatory blood flow and cellular disturbance in oxygen utilization. In addition, changes in microcirculation may occur after the changes in body haemodynamics [5-7]. Microcirculation refers to a capillary network consisting

of small arteries, capillaries, and veins with diameters of less than 100 μm. The cell population in the microcirculation capillary network includes endothelial cells, smooth muscle cells (mainly in small arteries), leukocytes, erythrocytes, and platelets. Microcirculation can ensure the oxygen supply to tissues, promote the exchange of nutrients and metabolic wastes, and affect inflammation and coagulation. Due to the change in the level of angiotensin and the decrease in the level of heparan sulfate during the course of sepsis and septic shock, oxygen-free radicals are produced and can promote the formation of micro-circulatory thrombosis and the blockage of the microcirculatory system, resulting in the destruction of endothelial polysaccharide coating and a series of adverse reactions such as oedema [8]. Even if the normal state of microcirculation is restored after sepsis and septic shock, the persistence of pathological changes in the microcirculation still leads to poor prognosis.

Ulinastatin is a protease inhibitor that can scavenge oxygen free radicals, reduce the adhesion of leukocytes to vascular endothelial cells, and alleviate the level of inflammatory reactions in tissues and organs [9]. The purpose of this study was to investigate the effects of ulinastatin on the levels of endothelial polysaccharide degradation products and vascular permeability in sepsis patients. SDC-1 (Syndecan-1), ICAM-1 (intercellular adhesion molecule-1), and PVPI (pulmonary vascular permeability index) were used as observation indexes to evaluate the levels of plasma markers and vascular permeability at 0 h, 24 h, and 72 h after the sepsis patients were enrolled into the study.

Materials and Methods

Patients
This study included 29 patients with sepsis and septic shock treated in The Affiliated Hospital of HangZhou Normal University, China, between June 2015 and December 2018. Informed consent was signed by all patients or their families and this study was approved by the Ethics Committee of The Affiliated Hospital of HangZhou Normal University, China.

Inclusion criteria: Patients meeting the diagnostic criteria of SIRS (systemic inflammatory response syndrome), with at least two of the following symptoms: body temperature > 38°C or < 36°C, heart rate > 90 beats/min, breathing rate > 20 breaths/min or $PaCO_2$ < 32 mmHg, white blood cell count > $12*10^9$/L or < $4*10^9$/L (or immature granulocytes > 10%); patients with suspicious or definite infection foci; patients meeting the diagnostic criteria of severe sepsis or septic shock, i.e., hypotension caused by sepsis; patients with a level of lactic acid exceeding the normal level along with a urine volume less than 0.5 mL/kg/h for at least 2 h even after adequate fluid resuscitation; patients with acute lung injury not caused by pneumonia along with an oxygenation index less than 250 mmHg, acute lung injury induced by pneumonia and oxygenation index less than 200 mmHg; patients with a serum creatinine level > 2.0 mg/dL, platelet count < 100000 uL, and a coagulation disorder (international standardized ratio > 1.5).

Exclusion criteria: Patients with a history of diabetes mellitus; patients who were < 18 years old upon admission; patients with an expected hospital stay of ≤ 24 h; patients who have received chemotherapy within the past 2 weeks; pregnant or lactating women.

Grouping and treatment
According to their time of admission, the patients were randomly divided into ulinastatin group (Y, N = 13), which was subdivided in L15 group (APACHE II (Acute physiology and chronic health evaluation II) ≥ 15) (8 cases) and S15 group (APACHE II < 15) (5 cases), and control group (N, n = 16), which was subdivided in L15 group (11 cases) and S15 group (5 cases). All patients were treated in ICU and were monitored using a ECG-1210 digital electrocardiograph (Shenzhen Bangjian biological medical equipment Co., Ltd., China) for vital signs. The height and weight of each patient were measured using HGM-800 medical height and weight measuring instrument (Henan Shengyuan Industry Co., Ltd., China) and used to calculate the BMI (body mass index). APACHE II scoring was performed at 24 h after ICU admission.

The patients in the ulinastatin group (Y) received routine treatment plus 100,000 U ulinastatin as intravenous infusion during a period of 8 h, while the patients in the non-ulinastatin group were given a routine treatment. According to the 2016 edition of International Guidelines for Sepsis and Septic Shock, early effective volume resuscitation, anti-microbial therapy, blood glucose control, enteral nutrition application, use of vasoactive drugs and other measures are recommended for sepsis patients. In addition to the treatment of systemic inflammatory response and organ dysfunction of sepsis patients, it is also necessary to treat and deal with other clinical symptoms of patients due to disease conditions. Meanwhile, strengthen the monitoring of the patients. When necessary, treat the patients with mechanical ventilation and continuous renal replacement therapy (CRRT).

Measurement of biochemical markers
Blood samples were collected at 0 h, 24 h and 72 h after admission to measure white blood cell count (WBC), lactate (Lac), C-Reactive Protein (CRP), and procalcitonin (PCT) using specific kits for each test (Guangzhou Yangpu Medical Technology Co., Ltd., China).

Blood samples were collected at 0 h, 24 h and 72 h after admission by disposable vacuum blood collection

(Shandong Aosaite Medical Equipment Co., Ltd., China). The supernatant of the blood samples was collected upon centrifugation and stored at -80°C. ELISA (enzyme-linked immunosorbent assay) was used to detect the levels of ICAM-1, SDC-1) and ScVO$_2$ using specific enzyme-free ELISA Kits (Shanghai Huyu Biotechnology Co., Ltd., China).

Measurement of vital signs

T (temperature) (Omron mc-872 (Omron Automation (China) Co., Ltd.), RR (respiratory rate) (JC15-OxiTop@ControlAN6-AN12 (Beijing Beixin Keyi Analytical Instrument Co., Ltd., China), HR (heart rate) (Changkun oximeter (Shenzhen Changkun Tech. Co., Ltd., China), MAP (mean arterial pressure) (PiCCO monitor (PULSION, German), CVP (central venous pressure) (Medifix (Belang Medical (Shanghai) International Trade Co., Ltd., China), and PVPI (pulmonary vascular permeability index) were collected from each patient at 0 h, 24 h and 72 h after admission. PVPI was monitored by PICCO (PULSION Corporation, USA).

Data processing and analysis

SPSS21.0 statistical software was used for data processing and analysis, emphasing small data approach [10]. Quantitative data that follows a normal distribution were expressed by mean ± standard deviation. Two independent samples t-test or rank-sum test were used to compare the two groups. Chi-square test was used to compare the rates in different groups.

Results and Discussion

Baseline characteristics in treatment (Y) and control (N) groups

There was no significant difference between group Y and group N (p > 0.05) in terms of their APACHE II scores, WBC, lactic acid, PVPI, PCT and CRP at admission. According to the blood samples collected at the time of admission, there was no significant difference between group Y and group N (p > 0.05) in terms of their SDC-1 and ICAM-1 levels at baseline (Table I), suggesting that the two groups of patients were comparable and balanced.

Table I

Baseline characteristics of the sepsis patients between treated and control group

Variable	Group		Statistics	P
	Y (n = 13)	N (n = 16)		
APACHEII	15.85 ± 4.04	19.13 ± 6.72	1.624	0.117
Age	68.85 ± 11.62	64.94 ± 10.47	0.952	0.350
BMI (kg/m^2)	24.24 ± 4.71	26.26 ± 6.54	0.933	0.359
CVP (mmHg)	8.00 ± 2.5	9.00 ± 3.0	0.665	0.506
T (°C)	38.20 ± 0.78	38.48 ± 0.97	0.831	0.413
WBC (10^9/L)	13.70 ± 4.92	12.47 ± 5.90	0.598	0.555
PCT (ng/mL)	2.14 ± 0.23	3.07 ± 1.7	0.526	0.599
CRP (mg/L)	189.65 ± 88.57	176.89 ± 114.06	0.330	0.744
ScVO$_2$ (%)	69.46 ± 5.75	66.63 ± 4.30	1.519	0.140
PVPI (%)	8.58 ± 5.52	10.81 ± 9.03	0.690	0.499
Lac (mmol/L)	2.55 ± 1.57	2.511 ± 1.03	0.069	0.945
ICAM-1 (pg/mL)	4627.87 ± 329.91	4895.03 ± 715.78	1.329	0.197
SDC-1 (pg/mL)	3204.10 ± 475.81	2899.46 ± 472.92	0.439	0.661

Changes in endothelial polysaccharide coating and perfusion index in treatment (Y) and control (N) groups after treatment

As shown in Figure 1, the levels of lactic acid in group Y significantly decreased compared to group N (1.17 ± 0.24 *vs.* 1.71 ± 0.48 mmol/L) at 72 h after treatment (p = 0.001). There was no significant difference between the two groups in terms of the levels of PVPI, SDC-1 and ICAM-1 (p > 0.05).
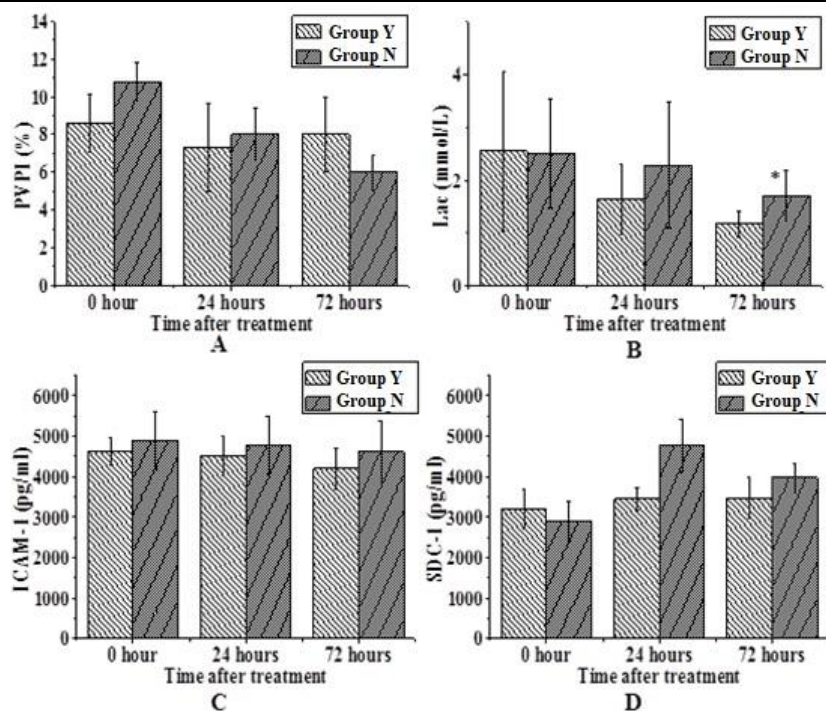
Figure 1.
Changes in endothelial polysaccharide coating and perfusion index after treatment in group Y and group N
(A: PIPV; B: Lac; C: ICAM-1; D: SDC-1), * $p < 0.05$ compared with group Y

Mortality level in treatment (Y) and control (N) groups after 28 days of treatment
In Figure 2, the 28-day mortality of group Y and group N were 0% and 31%, respectively ($p < 0.05$).

There was no significant difference between the two groups in terms of the length of hospitalization in ICU ($p > 0.05$).
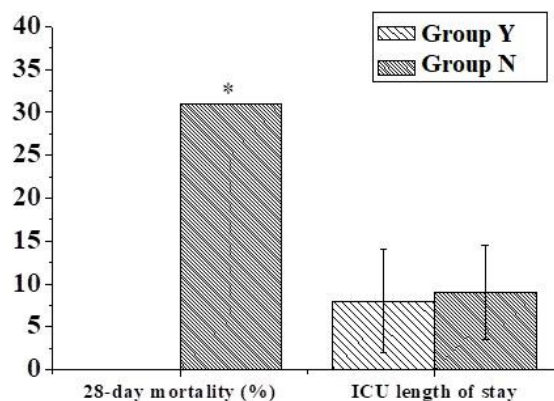

Figure 2.
Mortality in group Y and group N after 28 days of treatment, * $p < 0.05$ compared with group Y

Baseline characteristics of L15 patients in treatment (Y) and control group (N)
As shown in Table II, the blood samples collected from the L15 patients in group Y and group N within 6 h of admission showed no significant difference in baseline data of WBC, lactic acid, PCT, CRP, SDC-1 and ICAM-1 ($p > 0.05$). According

to the PICCO indicators measured within 6 h of admission, there was also no significant difference in L15 patients of group Y and group N in terms of their baseline PVPI data ($p > 0.05$), suggesting that the two groups of the patients were comparable and balanced.

Table II

Comparison of baseline characteristics of L15 patients in group Y and group N

Variable	Group		Statistics	p
	Y (n = 8)	N (n = 11)		
Age	70.50 ± 9.72	64.27 ± 12.41	1.177	0.255
BMI (kg/m^2)	25.06 ± 5.06	26.00 ± 7.51	0.305	0.764
CVP (mmHg)	8.75 ± 2.43	9.46 ± 4.06	0.435	0.669
T (°C)	38.10 ± 0.72	38.58 ± 1.16	1.037	0.314
WBC (10^9/L)	14.68 ± 4.50	12.39 ± 6.65	0.839	0.413
PCT (ng/mL)	1.68 ± 0.73	3.62 ± 0.52	0.661	0.509
CRP (mg/L)	203.69 ± 98.39	175.34 ± 124.48	0.533	0.601
ScVO$_2$ (%)	70.00 ± 7.27	66.18 ± 4.98	1.363	0.191
PVPI (%)	12.17 ± 4.96	13.02 ± 2.44	0.155	0.880
Lac (mmol/L)	2.36 ± 1.37	2.47 ± 0.85	0.217	0.831
ICAM-1 (pg/mL)	2128.07 ± 724.35	2899.46 ± 286.17	0.331	0.741
SDC-1 (pg/mL)	4531.43 ± 311.08	4864.35 ± 725.95	1.211	0.242

Changes of endothelial polysaccharide coating and perfusion index in the L15 patients of treatment (Y) and control (N) groups after treatment

As shown in Figure 3, the lactic acid level in group Y was significantly lower than that in group N (1.09 ± 0.20 *vs*. 1.57 ± 0.49 mmol/L) at 72 h after treatment (p = 0.018). There was no significant difference between the two groups in terms of SDC-1, ICAM-1 and PVPI (p > 0.05).

The mortality of the L15 patients in group Y and group N after 28 days of treatment is depicted in Figure 4.
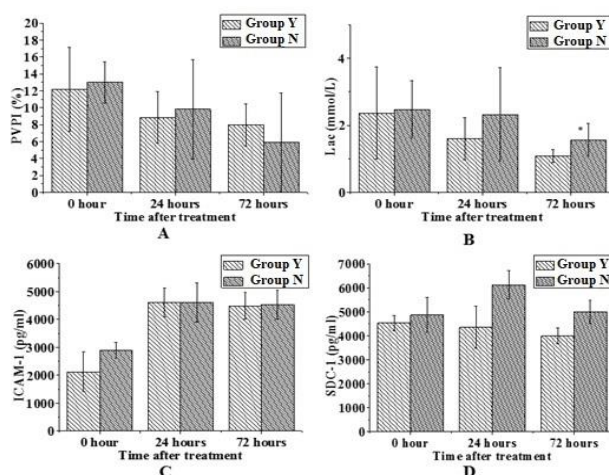

Figure 3.

Changes of endothelial polysaccharide coating and perfusion index in the L15 patients of group Y and group N after treatment (A: PIPV; B: Lac; C: ICAM-1; D: SDC-1), * p < 0.05 compared with group Y
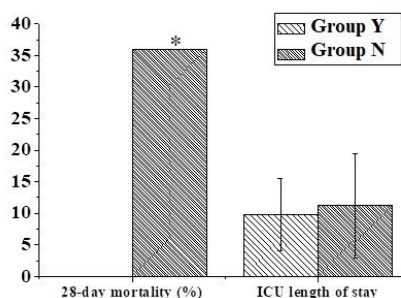

Figure 4.

28-day mortality and ICU length of stay in L15 patients in groups Y and N, * p < 0.05 compared with group Y

Baseline characteristics of the S15 patients in treatment (Y) and control groups (N)
As shown in Table III, the blood samples collected from the S15 patients in group Y and group N within 6 h of admission showed no significant difference in baseline data of WBC, lactic acid, PCT, CRP,

SDC-1 and ICAM-1 ($p > 0.05$). According to the PICCO indicators measured within 6 h of admission, there was also no significant difference in S15 patients of group Y and group N in terms of their baseline PVPI data ($p > 0.05$), suggesting that the two groups of patients were comparable and balanced.

Table III

Comparison of baseline characteristics of the S15 patients in group Y and group N

Variable	Group		Statistics	P
	Y (n =5)	N (n = 5)		
Age	66.20 ± 15.01	66.40 ± 4.72	0.028	0.978
BMI (Kg/m^2)	22.92 ± 4.27	26.82 ± 4.30	1.439	0.188
CVP (mmHg)	8.40 ± 1.95	8.80 ± 1.30	0.381	0.713
T (℃)	38.36 ± 0.92	38.24 ± 0.24	0.283	0.790
WBC (10^9/L)	12.12 ± 5.66	12.64 ± 4.45	0.160	0.877
PCT (ng/mL)	2.34 ± 1.82	1.37 ± 0.87	0.104	0.917
CRP (mg/L)	167.20 ± 74.60	180.32 ± 100.13	0.235	0.820
ScVO$_2$ (%)	68.60 ± 2.30	67.60 ± 2.41	0.671	0.521
PVPI (%)	4.28 ± 1.76	8.60 ± 4.04	2.193	0.060
Lac (mmol/L)	1.50 ± 0.65	2.10 ± 0.95	0.838	0.402
ICAM-1 (pg/mL)	4782.18 ± 329.87	4962.50 ± 771.68	0.481	0.644
SDC-1 (pg/mL)	3204.10 ± 988.07	1723.86 ± 1731.31	0.943	0.346

Changes of vascular endothelial polysaccharide coating and perfusion index in the S15 patients of treatment (Y) and control (N) groups after treatment
As shown in Figure 5, the lactic acid levels in group Y significantly decreased compare to group N (1.3 ± 0.25 *vs.* 2.02 ± 0.27mmol/L) at 72 h after the treatment ($p = 0.003$). At 72 h after treatment PVPI level and

SDC-1 level in group Y significantly decreased compared to group N (4.28 ± 1.75 *vs.* 12.16 ± 4.95%, $p = 0.008$), and (984.35 ± 311.58 *vs.* 3588.30 ± 572.76 pg/mL, $P = 0.049$), respectively. There was no significant difference between group Y and group N in terms of ICAM-1 levels after treatment ($p > 0.05$).
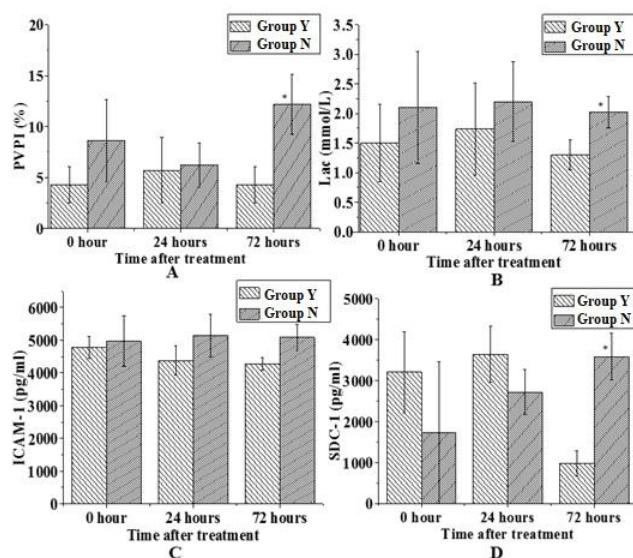

Figure 5.
Changes of vascular endothelial polysaccharide coating and perfusion index in the S15 patients of group Y and group N after treatment (A: PIPV; B: Lac; C: ICAM-1; D: SDC-1). * $p < 0.05$ compared with group Y

Mortality of the S15 patients in group Y and group N after 28 days of treatment
Figure 6 suggested that the 28-day mortality rate of group N was significantly increased compared to group Y ($p < 0.05$).

SDC-1 and SDC-4 play an essential role in the intimal hyperplasia caused by vascular injury. In addition, SDC-1 and SDC-4 can prevent intimal thickening by regulating the growth and migration of vascular smooth muscle cells [11-14].
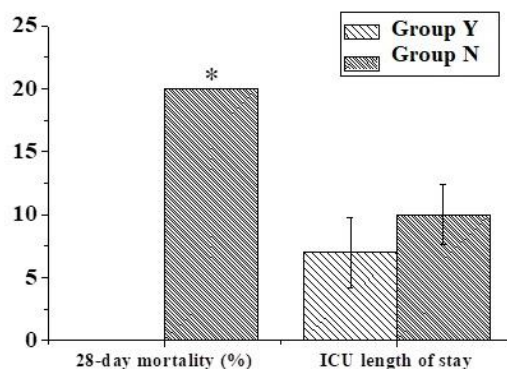
Figure 6.
28-day mortality and ICU length of stay in S15 patients in groups Y and N, * $p < 0.05$ compared with group Y

Interestingly, the expression of SDC-1 on the surface of vascular endothelial cells is enhanced during tumorigenesis, wound healing and inflammation [15-17]. Therefore, SDC-1 can be used as a marker of polysaccharide encapsulation damage [18]. ICAM, also known as CD54, is a representative molecule of immunoglobulin-like adhesion molecules. In the normal physiological state, endothelial cells can also express ICAM-1 at a low level. However, when stimulated by TNFα (Tumor necrosis factor-α), interferon, LPS (lipopolysaccharide), oxides and IL-1, endothelial cells rapidly increase ICAM-1 expression in a few hours and they can bind to the surface ligands on leukocytes, and determining an adhesion reaction between neutrophils and endothelial cells. Such adhesion aggravates the inflammation injury induced by leukocytes and promotes the adhesion of leukocytes to the surface of endothelial cells, thus influencing the formation of small emboli in blood vessels, blocking capillaries, and further aggravating microcirculation disorders [19-22]. The results of this study showed that the SDC-1 level in group Y was decreased compared to group N at 72 h after treatment when the APACHE II score of the patients was < 15, indicating that ulinastatin determined a protective effect on the structure of vascular endothelial polysaccharide coating. In the L15 group, the levels of SDC-1, ICAM-1 and PVPI in group Y were not significantly different compared to group N, indicating that the endothelial polysaccharide coating structure in critically ill patients was severely damaged and could not be restored by ulinastatin alone.

Due to the damage of endothelial polysaccharide coating, the increase in capillary permeability, the release of inflammatory factors and the exudation of alveolar capillaries in sepsis patients, the volume of extravascular lung water (EVLW) is increased in sepsis patients [23]. PVPI can adequately reflect the permeability of pulmonary capillaries and the value of PVPI was not increased over an increased blood

volume [24]. It was also found that in the S15 group, the 72 h value of PVPI in group Y was significantly decreased compared to group N, and the 72 h level of SDC-1 in group Y was also lower than that in group N, indicating that ulinastatin can protect the vascular endothelium and reduce capillary leakage. The results of our study is in agreement with the results of the study of Yun *et al*. [25] that showed that early intervention of ulinastatin can significantly improve the coagulation function of sepsis in young rats and significantly reduce mortality. However, in our study there was no significant difference between the two groups in terms of their mortality, which may be related to the small sample size used.

Conclusions

Ulinastatin may protect the structure of endothelial polysaccharide coating in sepsis patients with an APACHE II score of < 15, thereby improving vascular permeability, reducing capillary leakage, and improving microcirculation. Ulinastatin may also reduce the mortality of sepsis patients. Due to the small number of subjects in this study, the effect of infusion of blood products on plasma colloid osmotic pressure and tissue leakage was not analysed and discussed.

Conflict of interest

The authors declare no conflict of interest.

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