




Effect of ulinastatin combined with anti-infective therapy on systemic inflammatory response and oxidative stress response in patients with acute severe pneumonia

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ABSTRACT

Objective: To study the effect of ulinastatin combined with anti-infective therapy on systemic inflammatory response and oxidative stress response in patients with acute severe pneumonia.

Methods: A total of 50 patients with acute severe pneumonia who were treated in the hospital between September 2014 and December 2016 were collected and divided into control group and observation group according to the random number table method, 25 cases in each group. Control group received conventional therapy, and the observation group received ulinastatin combined with conventional therapy. The differences in inflammatory response and oxidative stress response in serum were measured before treatment and 10 d after treatment. **Results:** Before treatment, differences in serum levels of inflammatory response indexes and oxidative stress indexes were not statistically significant between the two groups. After treatment, serum IL-2, IL-6, PCT, HMGB1, CRP, IL-4, IL-10, IL-13, MDA, AOPP and ROS levels of both groups of patients were lower than those before treatment while SOD, T-AOC, GSH-Px and LHP levels were higher than those before treatment, and serum IL-2, IL-6, PCT, HMGB1, CRP, IL-4, IL-10, IL-13, MDA, AOPP and ROS levels of observation group were lower than those of control group while SOD, T-AOC, GSH-Px and LHP levels were higher than those of control group. **Conclusion:** Ulinastatin combined with anti-infective therapy can effectively reduce the systemic inflammatory response and oxidative stress response and optimize the overall condition in patients with acute severe pneumonia.

1. Introduction

Acute severe pneumonia belongs to clinical critically illness, patients mostly die of systemic inflammatory response syndrome (SIRS) or multiple organ dysfunction syndrome (MODS), and how to improve the survival rates and optimize the treatment outcome in such patients is the focus of clinical research[1,2]. Anti-infection is the basis of treatment of acute severe pneumonia, and the effective inhibition of systemic inflammatory response can inhibit the progress of the disease to a certain extent. Ulinastatin is a glycoprotein extracted from fresh human urine, which can inhibit the activity of multiple proteolytic enzymes and was used in treatment of acute pancreatitis and acute circulatory failure[3-5]. At present, many scholars have recommended ulinastatin for clinical adjuvant

treatment of patients with acute severe pneumonia, but there is not much discussion on the mechanism of action of ulinastatin. In the study, ulinastatin combined with anti-infection therapy was used for the treatment of patients with acute severe pneumonia, and the methods of ulinastatin to exert therapeutic effect were discussed from two aspects of inflammatory response and oxidative stress reaction, now reported as follows.

2. Information and methods

2.1 Case information

A total of 50 patients with acute severe pneumonia who were treated in the hospital between September 2014 and December 2016 were enrolled in the study, and the family members of the patients were informed of the study process and signed informed consent. According to the random number table method, the patients were

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divided into control group and observation group, 25 cases in each group. Control group included 13 men and 12 women that were 27-68 years old; observation group included 14 men and 11 women that were 30-72 years old. There was no significant difference in gender and age distribution between the two groups ($P > 0.05$).

2.2 Inclusion criteria

(1) In accordance with the diagnostic criteria of acute severe pneumonia; (2) without history of pulmonary inflammation 6 months prior to admission; (3) not receiving independent drug therapy before admission to the hospital; (4) completing the expected course of treatment and cooperating with the related inspection.

2.3 Exclusion criteria

(1) Combined with chronic obstructive pulmonary disease, chronic bronchitis and other pulmonary basic diseases; (2) combined with basic heart, liver and kidney insufficiency; (3) combined with serious autoimmune diseases; (4) combined with malignant tumor diseases.

2.4 Therapy

Control group received routine treatment for acute severe pneumonia, including sensitive antibiotics, glucocorticoid and nutritional support, etc. Observation group of patients received adjuvant ulinastatin therapy on the basis of conventional therapy, specifically as follows: ulinastatin (Guangdong Techpool Biopharma Co., Ltd., approved by H19990132) 200 000 U was dissolved in 50 mL of saline, which was by micro pump intravenous drip in 1 h, 2 times/d, for continuous 10 d.

2.5 Observation indexes

Before and after treatment, 3-5 mL of fasting cubital venous blood was collected from two groups of patients between 6:00 am-8:00 am, anti-coagulated with heparin sodium (Sichuan Deebio Pharmaceutical Co., Ltd., approved by H51021989), let stand at room temperature for stratification and then centrifuged at low speed to get upper serum, which was cryopreserved in deep hypothermia

environment for test. Enzyme-linked immunosorbent assay method was used to determine serum levels of pro-inflammatory/anti-inflammatory factors and oxidation/anti-oxidation indicators; pro-inflammatory indicators included interleukin-2 (IL-2), interleukin-6 (IL-6), procalcitonin (PCT) and high mobility group B1 (HMGB1) and C-reactive protein (CRP); anti-inflammatory indicators included interleukin-4 (IL-4), interleukin-10 (IL-10) and interleukin-13 (IL-13); the oxidation indexes included malondialdehyde (MDA), advanced oxidation protein product (AOPP) and reactive oxygen species (ROS); anti-oxidation indicators included superoxide dismutase (SOD), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px) and lipid hydroperoxide (LHP).

2.6 Statistical processing

Pro-inflammatory indicators, anti-inflammatory indicators, oxidation indicators and anti-oxidation indicators belonged to measurement data and were in terms of mean \pm standard deviation (Mean \pm SD), comparison within group before and after treatment was by paired test and comparison between groups was by grouping test. Statistical software was SPSS 25.0 and statistics $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1 Pro-inflammatory indexes

Comparison of serum pro-inflammatory indexes IL-2 (pg/mL), IL-6 (pg/mL), PCT (mg/mL), HMGB1 (ng/mL) and CRP (mg/L) levels between two groups of patients before and after treatment was as follows: before treatment, serum IL-2, IL-6, PCT, HMGB1 and CRP levels were not significantly different between the two groups ($P < 0.05$). After treatment, serum IL-2, IL-6, PCT, HMGB1 and CRP levels of both groups of patients were significantly lower than those before treatment ($P < 0.05$), and serum IL-2, IL-6, PCT, HMGB1 and CRP levels of observation group were significantly lower than those of control group ($P < 0.05$), shown in Table 1.

Table 1.

Comparison of serum IL-2, IL-6, PCT, HMGB1 and CRP levels between two groups of patients.

Groups	n	Time	IL-2	IL-6	PCT	HMGB1	CRP
Control group	25	Before treatment	27.83 \pm 3.49	309.26 \pm 45.71	30.47 \pm 4.51	12.63 \pm 1.78	72.15 \pm 8.36
		After treatment	18.68 \pm 2.11 [*]	214.75 \pm 25.39 [*]	18.12 \pm 2.12 [*]	7.15 \pm 0.86 [*]	40.82 \pm 6.41 [*]
Observation group	25	Before treatment	27.61 \pm 3.25	308.97 \pm 42.68	30.49 \pm 4.21	12.59 \pm 1.84	72.38 \pm 8.71
		After treatment	11.53 \pm 1.72 ^{**}	119.62 \pm 13.45 ^{**}	7.04 \pm 0.86 ^{**}	2.63 \pm 0.34 ^{**}	23.73 \pm 3.07 ^{**}

Note: compared with indexes of same group before treatment, ^{*} $P < 0.05$; compared with indexes of control group after treatment, ^{**} $P < 0.05$.

Table 2.

Comparison of serum IL-4, IL-10 and IL-13 levels between two groups of patients (pg/mL).

Groups	n	Time	IL-4	IL-10	IL-13
Control group	25	Before treatment	48.29 \pm 6.18	35.47 \pm 5.32	28.36 \pm 3.41
		After treatment	30.36 \pm 4.52 [*]	20.18 \pm 2.64 [*]	15.23 \pm 1.88 [*]
Observation group	25	Before treatment	48.17 \pm 5.93	35.29 \pm 5.46	28.31 \pm 3.24
		After treatment	16.23 \pm 2.19 ^{**}	9.64 \pm 1.03 ^{**}	6.12 \pm 0.78 ^{**}

Note: compared with indexes of same group before treatment, ^{*} $P < 0.05$; compared with indexes of control group after treatment, ^{**} $P < 0.05$.

Table 3.

Comparison of serum MDA, AOPP and ROS levels between two groups of patients.

Groups	n	Time	MDA	AOPP	ROS
Control group	25	Before treatment	15.38±1.92	8.36±0.92	605.27±74.18
		After treatment	8.15±0.94 [*]	5.18±0.62 [*]	342.84±45.19 [*]
Observation group	25	Before treatment	15.41±1.98	8.41±0.93	602.18±75.22
		After treatment	3.27±0.46 ^{*#}	1.49±0.15 ^{*#}	120.66±15.91 ^{*#}

Note: compared with indexes of same group before treatment, ^{*}*P*<0.05; compared with indexes of control group after treatment, [#]*P*<0.05.

Table 4.

Comparison of serum SOD, T-AOC, GSH-Px and LHP levels between two groups of patients.

Groups	n	Time	SOD	T-AOC	GSH-Px	LHP
Control group	25	Before treatment	34.28±4.51	5.39±0.72	40.28±5.61	212.37±25.83
		After treatment	46.17±5.62 [*]	8.26±0.91 [*]	52.17±6.23 [*]	304.82±42.57 [*]
Observation group	25	Before treatment	35.07±4.27	5.46±0.78	41.39±5.27	210.84±24.61
		After treatment	78.59±9.23 ^{*#}	14.19±1.85 ^{*#}	71.88±9.53 ^{*#}	482.62±58.93 ^{*#}

Note: compared with indexes of same group before treatment, ^{*}*P*<0.05; compared with indexes of control group after treatment, [#]*P*<0.05.

3.2 Anti-inflammatory indexes

Comparison of serum anti-inflammatory indexes IL-4, IL-10 and IL-13 levels between two groups of patients before and after treatment was as follows: before treatment, serum IL-4, IL-10 and IL-13 levels were not significantly different between the two groups (*P*<0.05). After treatment, serum IL-4, IL-10 and IL-13 levels of both groups of patients were significantly lower than those before treatment (*P*<0.05), and serum IL-4, IL-10 and IL-13 levels of observation group were significantly lower than those of control group (*P*<0.05), shown in Table 2.

3.3 Oxidation indexes

Comparison of serum oxidation indexes MDA (μmol/L), AOPP (μmol/L) and ROS (μmol/L) levels between two groups of patients before and after treatment was as follows: before treatment, serum MDA, AOPP and ROS levels were not significantly different between the two groups (*P*<0.05). After treatment, serum MDA, AOPP and ROS levels of both groups of patients were significantly lower than those before treatment (*P*<0.05), and serum MDA, AOPP and ROS levels of observation group were significantly lower than those of control group (*P*<0.05), shown in Table 3.

3.4 Anti-oxidation indexes

Comparison of serum anti-oxidation indexes SOD (U/L), T-AOC (U/mL), GSH-Px (g/L) and LHP (μmol/L) levels between two groups of patients before and after treatment was as follows: before treatment, serum SOD, T-AOC, GSH-Px and LHP levels were not significantly different between the two groups (*P*<0.05). After treatment, serum SOD, T-AOC, GSH-Px and LHP levels of both groups of patients were significantly higher than those before treatment (*P*<0.05), and serum SOD, T-AOC, GSH-Px and LHP levels of observation group were significantly higher than those of control group (*P*<0.05), shown in Table 4.

4. Discussion

Patients with acute severe pneumonia are seriously ill, the basic pathological change is the local inflammation of the lungs caused by the pathogen infection, systemic inflammatory response is formed after inflammation expands, and pathogenic bacteria enter into the blood and form bacteremia/septicopyemia, it will damage important viscera function, and patients may die in the short term with the disease progression[6,7]. Positive anti-infection therapy is the basis for the treatment of acute severe pneumonia, it can inhibit the continuous action of pathogenic bacteria, but it is weak in reversing the massively existing inflammatory mediators and the related viscera function damage caused by them. Ulinastatin belongs to the broad-spectrum trypsin inhibitor, it has been proven to be able to inhibit the activity of many kinds of proteases and lipid hydrolases, and realize the effects such as stabilizing cell membrane and lysosome membrane and inhibiting excessive release of inflammatory mediators, and it has been successfully applied in the treatment of serious infectious diseases such as acute pancreatitis[8,9]. In the research, ulinastatin and conventional anti-infective drugs were used together in clinical treatment for patients with acute severe pneumonia, and the curative effect and mechanism of action were elaborated from two aspects of systemic inflammation and oxidative stress.

Systemic inflammatory response is the initial cause of acute severe pneumonia aggravation, pro-inflammatory/anti-inflammatory factor expression imbalance is the fundamental mechanism, excessive generation of pro-inflammatory mediators can reactively increase the synthesis of anti-inflammatory mediators, but patients' overall state of inflammation is aggravated when the synthesized anti-inflammatory mediators cannot completely inhibit the role of pro-inflammatory mediators[10,11]. The anti-inflammatory effect of ulinastatin is mainly related to the following aspects: (1) inhibiting the release of various pro-inflammatory mediators such as the interleukins and tumor necrosis factor α ; (2) preventing the interaction between inflammatory factors and white blood cells and preventing the hyperactivation of white blood cells; (3) reducing the damage of white blood cells to important organ tissues[12,13].

IL-2, IL-6, PCT, HMGB1 and CRP are all pro-inflammatory factors whose roles have been confirmed in different research reports; IL-4, IL-10 and IL-13 have anti-inflammatory effects, their contents can reactively increase in the early stage of inflammatory reaction, but the increase is not as high as that of the pro-inflammatory factors[14]. In this study, differences in serum levels of above pro-inflammatory/anti-inflammatory factors were compared between two groups of patients before and after treatment, and it was found that compared with those before treatment, serum pro-inflammatory factors IL-2, IL-6, PCT, HMGB1 and CRP contents of both groups of patients decreased and anti-inflammatory factors IL-4, IL-10 and IL-13 contents also decreased after treatment, showing that both treatments are helpful to reduce the systemic inflammatory response; further compared with the control group, the observation group were with lower serum levels of pro-inflammatory factors IL-2, IL-6, PCT, HMGB1 and CRP as well as anti-inflammatory factors IL-4, IL-10 and IL-13 after treatment, indicating that adjuvant ulinastatin therapy on the basis of anti-infective therapy can more effectively inhibit the extent of systemic inflammatory response in patients with acute severe pneumonia, and the specific mechanism is directly related to the pharmacological effects of ulinastatin.

Massive release of inflammatory mediators and systemic inflammatory response can both directly cause oxidation/anti-oxidation system imbalance, and it is mainly associated with the massive ROS generation after inflammatory mediators injure the tissue organs, which will promote lipid peroxidation and lead to the production of more oxidative metabolites, including MDA and AOPP[15,16]. The anti-oxidation system is attacked under excessive oxidative stress, and the contents of SOD, T-AOC, GSH-Px, LHP and other markers that represent the body's antioxidant capacity reduce[17]. Animal studies suggest that ulinastatin has oxygen free radical-scavenging and anti-oxidation effects; in vitro cell research has also confirmed that ulinastatin can inhibit the release of free radicals out of neutrophils. Therefore, it is speculated that anti-oxidative stress is one of the important mechanisms for it to treat various inflammatory diseases. In this study, the differences in serum levels of above oxidation/anti-oxidation indexes were compared between two groups of patients before and after treatment, and it was found that compared with those before treatment, serum oxidation indexes MDA, AOPP and ROS contents of both groups of patients decreased while anti-oxidation indexes SOD, T-AOC, GSH-Px and LHP contents increased after treatment, indicating that both kinds of treatments can optimize the oxidation/anti-oxidation balance to different extent; further compared with the control group, the observation group were with lower serum contents of oxidation indexes MDA, AOPP and ROS, and higher contents of anti-oxidation indexes SOD, T AOC, GSH-Px and LHP after treatment, confirming that the adjuvant ulinastatin therapy can reduce the patients' systemic oxidative stress, and this is also the important mechanism for it to exert therapeutic effect.

Thus, ulinastatin combined with anti-infective therapy for patients with acute severe pneumonia can effectively suppress the systemic inflammatory response and oxidative stress, is expected to optimize the treatment outcome, and is worthy of popularization and application in clinical practice in the future.

References

- [1] Ohta R, Shimabukuro A. Rural physicians' scope of practice on remote islands: A case report of severe pneumonia that required overnight artificial airway management. *J Rural Med* 2017; **12**(1): 53-55.
- [2] Xiao B, Wang M, Hu X, Li J, Wang F, Sun J. Antibiotic de-escalation principle in elderly patients with chronic obstructive pulmonary disease complicated with severe pneumonia. *Exp Ther Med* 2017; **13**(4): 1485-1489.
- [3] Yu Z, Rayile A, Li Y, Zhao Q, Zhang X. Ulinastatin alleviated Lps-induced injury of cardiac micro vascular endothelial cell via NF- κ B pathway. *Pak J Pharm Sci* 2017; **30**(3(Suppl.)): 983-988.
- [4] Li X, Su L, Zhang X, Zhang C, Wang L, Li Y, et al. Ulinastatin downregulates TLR4 and NF-kB expression and protects mouse brains against ischemia/reperfusion injury. *Neurol Res* 2017; **39**(4): 367-373.
- [5] Tao Z, Hu FQ, Li CF, Zhang T, Cao BZ, Cui LQ. Effect of ulinastatin, a human urinary protease inhibitor, on heatstroke-induced apoptosis and inflammatory responses in rats. *Exp Ther Med* 2017; **13**(1): 335-341.
- [6] Liu C, Feng M, Zhu J, Tao Y, Kang M, Chen L. Severe pneumonia due to *Nocardia otitidiscaviarum* identified by mass spectroscopy in a cotton farmer: A case report and literature review. *Medicine (Baltimore)* 2017; **96**(13): e6526.
- [7] Mizgerd JP. Pathogenesis of severe pneumonia: advances and knowledge gaps. *Curr Opin Pulm Med* 2017; **23**(3): 193-197.
- [8] Liu DH, Yao YT, Li LH, Huang CM. Effects of ulinastatin on in vitro storage lesions of human red blood cells. *Clin Lab* 2017; **63**(4): 833-838.
- [9] Li X, Su L, Zhang X, Zhang C, Wang L, Li Y, et al. Ulinastatin downregulates TLR4 and NF-kB expression and protects mouse brains against ischemia/reperfusion injury. *Neurol Res* 2017; **39**(4): 367-373.
- [10] Li W, An X, Fu M, Li C. Emergency treatment and nursing of children with severe pneumonia complicated by heart failure and respiratory failure: 10 case reports. *Exp Ther Med* 2016; **12**(4): 2145-2149.
- [11] Chen C, Shi L, Li Y, Wang X, Yang S. Disease-specific dynamic biomarkers selected by integrating inflammatory mediators with clinical informatics in ARDS patients with severe pneumonia. *Cell Biol Toxicol* 2016; **32**(3): 169-184.
- [12] Luo Y, Che W, Zhao M. Ulinastatin post-treatment attenuates lipopolysaccharide-induced acute lung injury in rats and human alveolar epithelial cells. *Int J Mol Med* 2017; **39**(2): 297-306.
- [13] Wang KY, Yang QY, Tang P, Li HX, Zhao HW, Ren XB. Effects of ulinastatin on early postoperative cognitive function after one-lung ventilation surgery in elderly patients receiving neoadjuvant chemotherapy. *Metab Brain Dis* 2017; **32**(2): 427-435.
- [14] Ma L, Li C, Wang S, Wang J, Shao R, Hang C, et al. Ulinastatin ameliorates gastrointestinal injury sustained in a 2-hit porcine model of septic shock. *Am J Emerg Med* 2016; **34**(8): 1497-1504.
- [15] Li C, Ma D, Chen M, Zhang L, Zhang L, Zhang J, et al. Ulinastatin attenuates LPS-induced human endothelial cells oxidative damage through suppressing JNK/c-Jun signaling pathway. *Biochem Biophys Res Commun* 2016; **474**(3): 572-578.
- [16] Wang X, Xue Q, Yan F, Liu J, Li S, Hu S. Ulinastatin protects against acute kidney injury in infant piglets model undergoing surgery on hypothermic low-flow cardiopulmonary bypass. *PLoS One* 2015; **10**(12): e0144516.
- [17] Lin B, Liu Y, Li T, Zeng K, Cai S, Zeng Z, et al. Ulinastatin mediates protection against vascular hyperpermeability following hemorrhagic shock. *Int J Clin Exp Pathol* 2015; **8**(7): 7685-7693.