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Original Article

Effect of ulinastatin on expression of MMP-9, TIMP-1 and TGF-β_1 in patients with bronchial asthma

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Abstract: *Objective:* The goal of this study was to investigate the effect of ulinastatin on the expression of matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1) and transforming growth factor-β_1 (TGF-β_1) in the treatment of bronchial asthma. *Methods:* A total of 120 patients with bronchial asthma were divided into the ulinastatin (n=60) and control groups (n=60) randomly. Patients in the control group received conventional anti-inflammatory symptomatic treatment, and patients in ulinastatin group were treated with ulinastatin for 7 consecutive days. The expression of MMP-9, TIMP-1, and TGF-β_1 in sputum and serum were measured, blood gas indexes were analyzed, and relief time of clinical symptom were observed. *Results:* At 7 days post-treatment with ulinastatin, the symptom of asthma were relieved, and the expression of MMP-9, TIMP-1 and TGF-β_1 in were decreased. Blood gas analysis showed that the blood gas indexes arterial partial pressure of oxygen (PaO_2), arterial partial pressure of carbon dioxide ($PaCO_2$) and PaO_2/fraction of inspiration oxygen (PaO_2/FiO_2) in the ulinastatin group were significantly higher than those in control group. The relief time of clinical symptoms, in the ulinastatin group was earlier, and the total clinical effective rate (93.3%) was significantly higher than that in control group (75.0%) ($p<0.05$). Furthermore, correlation analysis showed that MMP-9, TIMP-1 and TGF-β_1 levels were negatively correlated with PaO_2, PaO_2/FiO_2 and $PaCO_2$. *Conclusion:* Ulinastatin plays an important role in the treatment of patients with bronchial asthma possibly by improving blood gas indexes, alleviating clinical symptoms and decreasing the levels of MMP-9, TIMP-1, and TGF-β_1.

Keywords: Bronchial asthma, ulinastatin, MMP-9, TIMP-1, TGF-β_1

Introduction

Bronchial asthma is characterized by airway remodeling and this chronic inflammatory disease seriously affects patient health. Due to the increased incidence rate, more attention was paid on bronchial asthma in recent years [1]. Irreversible airflow obstruction and hormone resistance are the main factors for formation of refractory asthma [2]. Extracellular matrix (ECM) is the most basic and indispensable component of airway wall and alveoli. Various harmful factors stimulate the bronchial epithelial cells, cause damage to airway structural cells and result in proliferation of smooth muscle cell and ECM deposition. Furthermore, they eventually result in airway remodeling and irreversible airflow obstruction. Several studies have confirmed that a variety of inflammatory factor such as matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1) and transforming growth factor-β_1 (TGF-β_1) are involved in the airway wall fibrosis and airway hyper-responsiveness. Moreover, these inflammatory factors are reported to regulate the degradation and deposition processes of ECM [3-5]. In clinic, the main conventional treatment includes anti-inflammation, correction of acid-base imbalance, improvement of ventilation function and aerosol inhalation of glucocorticoids. However, the conventional treatment has limited efficacy for all the patients. Ulinastatin, as a broad-spectrum protease inhibitor, can inhibit the activity of hyaluronidase, hydrolase, plasmin, lipoprotein, and promotes the secretion of a variety of inflammatory cytokines and stabilize lysosomal membrane. Therefore, it is widely applied in the treatment of a variety of inflammatory diseases [6, 7]. However, whether ulinastatin can be used for the treatment of bronchial asthma remains poorly understood. The study aims to

investigate the clinical efficacy of ulinastatin in the treatment of bronchial asthma and its effect on the expression of MMP-9, TIMP-1, and TGF-β_1.

Materials and methods

Patients

A total of 120 patients with bronchial asthma in our hospital from February 2016 to February 2017 were enrolled in this study. According to *Global Initiative for Asthma,* all patients met the diagnostic and grading criteria (mild, moderate and severe). Patients who were complicated with serious dysfunction in heart, liver, kidney or other organs, pneumothorax, respiratory failure or other disease needing mechanical ventilation, and autoimmune diseases were excluded. Patients were randomly divided into the ulinastatin group (n=60) and the control group (n=60). There was no significant difference in age or gender between the two groups (p>0.05). All patients signed the informed consent and the study was approved by the Ethics Committee of Gansu Provincial Hospital.

Patients in the control group were treated with conventional treatment approaches, such as anti-inflammation, improvement of ventilation function, correction of acid-base imbalance and aerosol inhalation of glucocorticoids and β_2 receptor agonists (formoterol and salmeterol). Patients were treated with intravenous infusion of ulinastatin (manufacturer: Guangdong Techpool Bio-Pharma Co., Ltd.) (10 U, 3 times/day) in the ulinastatin group. Both groups were treated for 7 consecutive days.

Measurement of the expression of MMP-9, TIMP-1 and TGF-β_1 in sputum and serum

Before and after treatment, 3 mL fasting venous blood was collected and centrifuged in the morning, and the supernatant was collected and stored in a refrigerator at -80°C. According to the sputum induction procedures of Pin et al. [8], 3-5 mL qualified sputum specimens were collected to prepare the homogeneous sputum solution. After centrifugation, the supernatant was collected and stored in the refrigerator at -80°C. Qualified sputum smears: more than 25 neutrophils and less than 1 squamous epithelial cell were detected under each low-power microscope. The expression of MMP-9, TIMP-1, and TGF-β_1 in sputum and serum were measured using a Tecan Sunrise full-automatic microplate reader (Tecan, Switzerland).

Detection of blood gas indexes in both groups

The extinction time of clinical symptoms, including dyspnea, wheezing rale, chest tightness and cough, in both groups were observed.

Evaluation criteria of therapeutic effects

During treatment, the clinical symptoms, body temperature and sleep quality in both groups were observed. (1) Remarkably effective: Clinical symptoms were disappeared completely, and body temperature was normal and sleep quality was restored. (2) Effective: Symptoms and signs were improved, and body temperature was close to normal. (3) Ineffective: Clinical symptoms and signs were similar to those before treatment and even aggravated. The total effective rates of both groups were recorded.

Statistical analysis

Statistical analysis was tested by Statistical Product and Service Solutions (SPSS) 19.0 software (IBM). Measurement data were presented as mean ± standard deviation (SD) and were compared by t test. Enumeration data were presented as n (%) and compared by Chi-square test. Correlation analyses were performed for the correlations among detection indexes. P<0.05 suggested that the difference was statistically significant.

Results

Basic characteristics

There were no significant differences in the basic characteristics (gender, disease severity, age and course of disease) between the ulinastatin and control groups (p>0.05) (**Table 1**).

Expression of MMP-9, TIMP-1 and TGF-β_1

On day 7 after treatment with ulinastatin, the symptoms of asthma were relieved and the expression of MMP-9, TIMP-1, and TGF-β_1 in sputum and serum were significantly decreased (p<0.05). The MMP-9/TIMP-1 ratio in the ulinastatin group was significantly higher compared with that in control group (p<0.05) (**Tables 2**, **3**).

Table 1. General conditions between the two groups of patients

Group	Gender		Disease severity			Age (years)	Course of disease (years)
	Male	Female	Mild	Moderate	Severe		
Ulinastatin group (n=60)	27 (45%)	33 (55%)	23 (38.3%)	18 (30%)	19 (31.7%)	41.32 ± 4.98	3.55 ± 0.51
Control group (n=60)	31 (51.7%)	29 (47.3%)	24 (40%)	19 (31.7%)	17 (28.3%)	42.03 ± 5.18	3.50 ± 0.46
χ^2 or t	0.534			0.159		1.077	0.076
p value	0.465			0.924		0.282	0.940

Table 2. MMP-9, TIMP-1, and TGF-β_1 expression in serum in both groups

Group		MMP-9	TIMP-1	TGF-β_1	MMP-9/TIMP-1
Ulinastatin group (n=60)	Before treatment	83.10 ± 17.79	173.59 ± 58.13	414.03 ± 100.11	0.55 ± 0.13
	After treatment	52.14 ± 4.41$^{\Delta,*}$	74.35 ± 29.83$^{\Delta,*}$	170.18 ± 45.56$^{\Delta,*}$	0.78 ± 0.30$^{\Delta,*}$
Control group (n=60)	Before treatment	84.54 ± 19.35	172.28 ± 56.38	410.35 ± 101.22	0.53 ± 0.12
	After treatment	63.44 ± 6.13$^{\Delta}$	128.77 ± 18.85$^{\Delta}$	309.21 ± 40.77$^{\Delta}$	0.60 ± 0.11$^{\Delta}$

Note: MMP-9: matrix metalloproteinase-9. TIMP-1: tissue inhibitor of metalloproteinase-1. TGF-β_1: transforming growth factor-β_1. $^{\Delta}p<0.05$ vs. corresponding before treatment group, $^{*}p<0.05$ vs. the control group after treatment.

Table 3. MMP-9, TIMP-1 and TGF-β_1 expressions in sputum in both groups

Group		MMP-9	TIMP-1	TGF-β_1	MMP-9/TIMP-1
Ulinastatin group (n=60)	Before treatment	68.14 ± 15.36	205.11 ± 103.35	485.81 ± 87.76	0.42 ± 0.22
	After treatment	46.13 ± 5.66$^{\Delta,*}$	79.18 ± 41.15$^{\Delta,*}$	249.77 ± 38.13$^{\Delta,*}$	0.79 ± 0.46$^{\Delta,*}$
Control group (n=60)	Before treatment	67.73 ± 13.55	201.14 ± 101.17	188.43 ± 85.56	0.44 ± 0.19
	After treatment	56.35 ± 7.12$^{\Delta}$	100.38 ± 49.98$^{\Delta}$	373.65 ± 62.26$^{\Delta}$	0.60 ± 0.38$^{\Delta}$

Note: MMP-9: matrix metalloproteinase-9. TIMP-1: tissue inhibitor of metalloproteinase-1. TGF-β_1: transforming growth factor-β_1. $^{\Delta}p<0.05$ vs. corresponding before treatment group, $^{*}p<0.05$ vs. the control group after treatment.

Table 4. Correlation analysis of MMP-9, TIMP-1 and TGF-β_1 with blood gas analysis indexes

Index		MMP-9 (serum)	TIMP-1 (serum)	TGF-β_1 (serum)	MMP-9 (sputum)	TIMP-1 (sputum)	TGF-β_1 (sputum)
PaO_2	r	-0.324	-0.182	-0.134	-0.138	-0.222	-0.170
	p	<0.001	0.001	<0.001	0.002	<0.001	0.006
$PaCO_2$	r	-0.153	-0.135	-0.132	-0.131	-0.166	-0.208
	p	0.030	0.040	0.047	0.003	0.007	0.002
PaO_2/FiO_2	r	-0.205	-0.237	-0.182	-0.140	-0.255	-0.221
	p	0.007	<0.001	0.010	0.002	<0.001	<0.001

Correlation analysis of MMP-9, TIMP-1 and TGF-β_1 with blood gas analysis indexes

Correlation analysis showed that MMP-9, TIMP-1, and TGF-β_1 levels in serum and sputum of patients after treatment with ulinastatin were negatively correlated with PaO_2, PaO_2/FiO_2, and $PaCO_2$ (**Table 4**).

Blood gas analysis indexes

Blood gas analysis (PaO_2, $PaCO_2$ and PaO_2/FiO_2) showed that the blood gas indexes in the ulinastatin group on day 7 after treatment were significantly higher than those in control group except $PaCO_2$ ($p<0.05$), indicating that ulinastatin can effectively improve pulmonary ventilation function in asthmatic patients (**Table 5**).

Comparison of extinction time of clinical symptoms and clinical efficacy

The relief time (days) of clinical symptoms (dyspnea, wheezing rale, chest tightness and cough) in the ulinastatin group was earlier compared with control group, and the total clinical effective rate (93.3%) was significantly higher than that in control group (75.0%) is shown in **Tables 6** and **7**.

Discussion

The pathogenesis of bronchial asthma is very complicated, and the main cause is cytokines and chemokines. They can damage airway structural cells, and it evenly lead to the airway hyper-responsiveness, airflow obstruction and

Table 5. Comparison of blood gas analysis indexes between the two groups of patients

Group	Group	Before treatment	Day 7
PaO_2 (mmHg)	Ulinastatin group	53 ± 6	120 ± 13$^{\Delta,*}$
	Control group	56 ± 7	99 ± 12$^{\Delta}$
$PaCO_2$ (mmHg)	Ulinastatin group	33 ± 5	43 ± 4*
	Control group	34 ± 5	40 ± 3
PaO_2/FiO_2 (mmHg)	Ulinastatin group	165 ± 24	385 ± 65$^{\Delta,*}$
	Control group	167 ± 23	279 ± 61$^{\Delta}$

Note: $^{\Delta}p<0.05$ vs. corresponding before treatment group, $^{*}p<0.05$ vs. the control group after treatment.

Table 6. Comparison of relief time (days) of clinical symptoms between the two groups

Group	Dyspnea	Wheezing rale	Chest tightness	Cough
Ulinastatin group (n=60)	3.99 ± 0.83$^{\Delta}$	4.45 ± 0.46$^{\Delta}$	4.12 ± 0.57$^{\Delta}$	5.03 ± 0.98$^{\Delta}$
Control group (n=60)	5.78 ± 1.05	5.98 ± 0.78	6.03 ± 0.77	7.35 ± 1.03
t	11.77	10	12.56	15.26
p value	<0.0011	<0.0011	<0.0011	<0.0011

Note: $^{\Delta}p<0.0001$: compared with control group.

Table 7. Comparison of clinical efficacy between the two groups (%)

Group	Markedly effective	Effective	Ineffective	Patient efficiency
Ulinastatin group (n=60)	32 (53.3)	24 (40.0)	4 (6.6)	56 (93.3)$^{\Delta}$
Control group (n=60)	25 (41.7)	20 (33.3)	15 (325.0)	45 (75.0)
χ^2		7.592		7.566
p value		0.02		0.006

Note: $^{\Delta}p<0.01$: compared with the control group.

airway remodeling. In addition, it is also associated with the interaction between immune-inflammatory injury and neural mechanism [9].

In the last few years, ulinastatin has been widely used in the treatment of acute pancreatitis and severe infection. It has been gradually applied in other inflammatory diseases in recent years [10, 11]. It was pointed out that ulinastatin can produce inflammatory mediator antagonism, inhibit platelet aggregation and adhesion, which can not only stabilize lysosomal membrane, but also scavenge oxygen free radicals, and inhibit the release of inflammatory mediators [12, 13]. MMP-9 is demonstrated to be involved in the airway and pulmonary remodeling as a major rate-limiting enzyme that regulates ECM metabolism. In addition, it can also act on fibroblasts and smooth muscle cells, thus activating matrix growth factors and causing ECM deposition. TIMP-1 regulates the renewal of ECM by specifically inhibiting MMP-9 activity. Furthermore, TGF-β_1 can stimulate airway inflammation and immune response, and it can lead to airway epithelial fibrosis, airway wall thickening and decreased lung function through stimulating proliferation of airway smooth muscle cells [5]. Moreover, it can destroy the metabolic balance of ECM and ultimately resulting in the formation of irreversible airway obstruction [14].

Belleguic et al. showed that expression of MMP-9 and TIMP-1 in sputum and serum in patients with bronchial asthma was sig-nificantly increased, and the MMP-9/TIMP-1 ratio was decreased [15-17]. It was suggested that the MMP-9 level in asthmatic patients was increased, followed by the compensatory increased level of TIMP-1 which is involved in ECM degradation and deposition. In addition, previous studies demonstrated that the expression of TGF-β_1 in sputum and serum in patients with bronchial asthma was significantly higher than those in normal control group [18, 19]. Consistent with these, this study found that on day 7 after treatment with ulinastatin, the symptoms of asthma were relieved, and the expression of MMP-9, TIMP-1, and TGF-β_1 in sputum and serum were decreased, indicating that ulinastatin can inhibit the release of local and systemic inflammatory factors (MMP-9, TIMP-1, and TGF-β_1), thus ameliorating the inflammation in patients with bronchial asthma. These data together suggest that ulinastatin can inhibit trypsin and plasmin and down-regulate the expressions of proMMP-1 and proMMP-9 [20]. Blood gas analysis can not only reflect the lung function status, but also pro-

vide an important reference for clinical treatment. In this study, it was shown that compared with those in control group, the blood gas indexes (PaO_2, $PaCO_2$ and PaO_2/FiO_2) were higher, the relief time of clinical symptoms (dyspnea, wheezing rale, chest tightness and cough) was earlier, and the total clinical effective rate was significantly higher in ulinastatin group on day 7 after treatment. Serum inflammatory factors reflects systemic inflammatory conditions, and sputum from the deep airway of patients with asthma can directly reflect the release extent of airway local inflammatory factors. Correlation analysis showed that MMP-9, TIMP-1, and TGF-β_1 levels in serum and sputum were negatively correlated with PaO_2, PaO_2/FiO_2, and $PaCO_2$, indicating that the abnormal blood gas might be resulting from the abnormal expression of MMP-9, TIMP-1, and TGF-β_1 levels.

Conclusion

In conclusion, ulinastatin displays a significant efficacy in treatment of patients with bronchial asthma by improving blood gas indexes, alleviating clinical symptoms and decreasing the levels of MMP-9, TIMP-1, and TGF-β_1.

Disclosure of conflict of interest

None.

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References

[1] Ortega VE and Torgerson DG. On a collision course: the electronic medical record and genetic studies of asthma. Am J Respir Crit Care Med 2017; 195: 412-414.

[2] Profita M, Gagliardo R, Di Giorgi R, Bruno A, Riccobono L, Bonanno A, Bousquet J and Vignola AM. In vitro effects of flunisolide on MMP-9, TIMP-1, fibronectin, TGF-beta 1 release and apoptosis in sputum cells freshly isolated from mild to moderate asthmatics. Allergy 2004; 59: 927-932.

[3] Vignola AM, Kips J and Bousquet J. Tissue remodeling as a feature of persistent asthma. J Allergy Clin Immunol 2000; 105: 1041-1053.

[4] Mattos W, Lim S, Russell R, Jatakanon A, Chung F and Barnes PJ. Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids. Chest 2002; 122: 1543-1552.

[5] Lasky JA and Brody AR. Interstitial fibrosis and growth factors. Environ Health Perspect 2000; 108: 751-762.

[6] Zhou L, Lan H, Zhou Q, Tang XJ, Zhu D, Yue J and Liu B. Continuous infusion of high-dose ulinastatin during surgery does not improve early postoperative clinical outcomes in patients undergoing radical lung cancer surgery: a pilot study. Thorac Cancer 2016; 7: 581-587.

[7] Fan H, Zhao Y, Zhu JH, Ye JH and Yao XQ. Ulinastatin and thymosin a1 therapy in adult patients with severe sepsis: a meta-analysis with trial sequential analysis of randomized controlled trials. Iran J Public Health 2016; 45: 1234-1235.

[8] Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE and Dolovich J. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992; 47: 25-29.

[9] Agache I and Akdis CA. Endotypes of allergic diseases and asthma: an important step in building blocks for the future of precision medicine. Allergol Int 2016; 65: 243-252.

[10] Jiang XM, Hu JH, Wang LL, Ma C, Wang X and Liu XL. Effects of ulinastatin on global ischemia via brain pro-inflammation signal. Transl Neurosci 2016; 7: 158-163.

[11] Jia LJ, Yi L, Yang ZX, Wang SP, Li G and Zhu X. [Preventive effects of ulinastatin on acute respiratory distress syndrome]. Beijing Da Xue Xue Bao Yi Xue Ban 2016; 48: 672-679.

[12] Wang LP, Huang X, Kong GQ, Xu HX, Li JK, Hao D, Wang T, Han SS, Han CL, Sun YY, Liu XY and Wang XZ. Ulinastatin attenuates pulmonary endothelial glycocalyx damage and inhibits endothelial heparanase activity in LPS-induced ARDS. Biochem Biophys Res Commun 2016; 478: 669-675.

[13] Wang J, Chen X, Su L, Zhu Z, Wu W, Zhou Y. Suppressive effects on cell proliferation and motility in gastric cancer SGC-7901 cells by introducing ulinastatin in vitro. Anticancer Drugs 2016; 27: 651-659.

[14] Suzuki R, Miyazaki Y, Takagi K, Torii K and Taniguchi H. Matrix metalloproteinases in the pathogenesis of asthma and COPD: implications for therapy. Treat Respir Med 2004; 3: 17-27.

[15] Vignola AM, Riccobono L, Mirabella A, Profita M, Chanez P, Bellia V, Mautino G, D'accardi P, Bousquet J and Bonsignore G. Sputum metalloproteinase-9 tissue inhibitor of metalloproteinase-1 ratio correlates with airflow obstruction in asthma and chronic bronchitis. Am J Respir Crit Care Med 1998; 158: 1945-1950.

[16] Belleguic C, Corbel M, Germain N, Lena H, Boichot E, Delaval PH and Lagente V. Increased release of matrix metalloproteinase-9 in the plasma of acute severe asthmatic patients. Clin Exp Allergy 2002; 32: 217-223.
[17] Higashimoto Y, Yamagata Y, Iwata T, Okada M, Ishiguchi T, Sato H, Masuda M and Itoh H. Increased serum concentrations of tissue inhibitor of metalloproteinase-1 in COPD patients. Eur Respir J 2005; 25: 885-890.
[18] Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, Hamid Q. Eosinophil-associated TGF-beta(1) mRNA expression and airways fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1997; 17: 326-333.
[19] Joseph J, Benedict S, Badrinath P, Wassef S, Joseph M, Abdulkhalik S and Nicholls MG. Elevation of plasma transforming growth factor beta 1 levels in stable nonatopic asthma. Ann Allergy Asthma Immunol 2003; 91: 472-476.
[20] Imada K, Ito A, Kanayama N, Terao T and Mori Y. Urinary trypsin inhibitor suppresses the production of interstitial procollagenase proMMP-1 and prostromelysin 1 proMMP-3 in human uterine cervical fibroblasts and chorionic cells. FEBS Lett 1997; 417: 337-340.