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Original Article

The effects of N-acetylcysteine on the Th1/Th2 ratio in elderly patients with chronic obstructive pulmonary disease

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Abstract: We assessed the effect of N-acetylcysteine treatment on the Th1/Th2 ratio in elderly patients with chronic obstructive pulmonary disease (COPD). Eighty patients with stage 3 and 4 COPD were randomly divided into an oral N-acetylcysteine group (1,200 mg per day) and a placebo group for 6 months. The number of acute exacerbations and their frequency and severity were recorded over the study period. In addition, serum levels of interferon γ and interleukin 4 were recorded at baseline and at 6 months, along with the Th1/Th2 ratio. Compared with the placebo group, the patients who received N-acetylcysteine experienced fewer and shorter acute exacerbations. N-acetylcysteine treatment increased interleukin 4 and Th2 levels, decreased interferon γ and Th1 levels, and restored the Th1/Th2 ratio after 6 months of treatment. Long-term oral administration of N-acetylcysteine may be beneficial in slowing the progression of COPD in elderly patients.

Keywords: Interferon γ, interleukin 4, acute exacerbation, Th1/Th2 ratio, COPD, N-acetylcysteine

Introduction

Chronic obstructive pulmonary disease (COPD) is a respiratory disease characterized by progressive severe airway limitation, which may affect patients' functioning and quality of life [1]. Patients with COPD exhibit immune dysfunction, and especially an imbalance in the Th1/Th2 cytokine ratio, leading to disease progression [2, 3]. Th1 cells can increase the numbers of eosinophils and neutrophils in airways while Th2 cells inhibit such an increase, maintaining balance. An imbalance in the Th1/Th2 ratio may cause acute exacerbation of COPD (AECOPD).

Studies have shown that oral administration of N-acetylcysteine (NAC) in large doses can reduce the acute exacerbation rate of patients with COPD [4-7] and improve their pulmonary functions and quality of life [4]. The therapeutic effect of NAC on COPD is believed to be its antioxidation and reduction of sputum viscidity and elasticity [8, 9], as it interferes with the activity of cytokines and alters the T-lymphocyte counts [10]. Only a few studies, however, have been published on the T-lymphocyte cell subsets in patients with COPD [2, 3]. We conducted a randomized, double-blind study to determine the effects of orally administered NAC on the Th1/Th2 balance and acute exacerbation in patients with COPD.

Materials and methods

General information

We recruited 80 patients with stage 3 and stage 4 COPD who were treated at Jinshan Hospital Affiliated to Fudan University between June 2016 and March 2017 (trial registry number: 2017-14-01). The patients were randomly divided into two groups of 40 (**Table 1**). The treatment group consisted of 25 male and 15 female patients aged 62-87 (mean age: 78.25 ± 6.44 years). The placebo group consisted of 24 male and 16 female patients aged 60-88 (mean age: 75.22 ± 7.48 years).

Inclusion and exclusion criteria

Inclusion criteria: All patients met the diagnostic criteria in the 2016 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (http://goldcopd.org/global-strategy-

Table 1. General patient characteristics

Group	Sex[1] (male/female)	Age[2] (years)	Smoking index[2]	FEV_1/predicted value %[2]	Acute exacerbation rate in past 1 year[2]	mMRC score[2]
Treatment (n = 34)	19/15	78.15 ± 6.82	367.6 ± 255.05	52.17 ± 15.90	2.06 ± 0.98	2.15 ± 0.78
Placebo (n = 29)	18/11	74.41 ± 7.12	365.5 ± 283.2	51.89 ± 15.74	1.66 ± 0.55	1.97 ± 0.78
p value	0.247	0.117	0.297	0.685	0.054	0.654

[1]Chi-squared test. [2]Single-factor variance analysis. FEV_1, forced expiratory volume in 1 second; mMRC, modified British Medical Research Council score.

Table 2. Patient immune indices

Group	Th1/$CD4^+$ T cells (%)	Th2/$CD4^+$ T cells (%)	Th1/Th2 ratio	T cell count	Interleukin 4 (pg/mL)	Interferon γ (pg/mL)
Treatment (n = 34)	14.66 ± 0.86	2.93 ± 0.45	5.15 ± 1.06	1408.15 ± 94.00	49.26 ± 24.38	127.65 ± 7.54
Placebo (n = 29)	14.29 ± 0.91	2.97 ± 0.32	4.88 ± 0.62	1441.45 ± 87.67	49.34 ± 26.84	126.31 ± 6.50
p value	0.098	0.741	0.226	0.156	0.855	0.458

Table 3. Acute exacerbation rates and duration

Group	Acute exacerbation rate	Acute exacerbation duration (days)
Treatment (n = 34)	0.74 ± 0.75*	4.62 ± 5.16*
Placebo (n = 29)	1.34 ± 0.67	8.34 ± 4.30
p value	0.010	0.004

*Compared with placebo group; $P < 0.05$.

diagnosis-management-prevention-copd20-16/). Characteristics of patients in stage 3: Severe or extremely severe airway limitation (percentage of forced expiratory volume in 1 second [FEV_1] accounting for predicted value: < 50%) and/or annual acute exacerbation rate ≥ 2 or at least one hospitalization for acute exacerbation; COPD assessment test (CAT) score < 10 or modified British Medical Research Council (mMRC) score of 0-1. Characteristics of patients in stage 4: Severe or extremely severe airway limitation (percentage of FEV_1 accounting for predicted value: < 50%) and/or annual acute exacerbation rate ≥ 2 or at least one hospitalization for acute exacerbation; CAT score ≥ 10 or mMRC score ≥ 2. Exclusion criteria: Age < 60 years; mechanical ventilation or long-term use of oxygen therapy (10 h or more per day); use of blood products, immunomodifiers, or immunosuppressors in the month preceding enrollment in the study; comorbidities such as immunodeficiency diseases, tuberculosis, malignant tumors, asthma, severe liver and kidney diseases, and heart disease.

Methods

Eighty patients with stage 3 or 4 COPD were randomly divided into two groups. Patients in the treatment group were treated with NAC orally for 6 months (Fluimucil, Hainan Zambon, 600 mg twice per day). Patients in the placebo group were given a placebo with other therapeutic measures. The placebo consisted of medical starch, cyclodextrin, and a small amount of flavor modifier, compressed to achieve the appearance and weight of the therapeutic drug. Patients in the two groups regularly inhaled a long-acting anticholinergic drug (tiotropium bromide, 18 μg per day) and received other symptomatic treatment during the 6-month period. The proportion of Th1 and Th2 in $CD4^+$ T cells in peripheral blood mononuclear cells was measured using flow cytometry. Concentrations of interleukin (IL) 4 and interferon (IFN) γ in serum were measured by ELISA. The flow cytometer used was a Gallios (Beckman Coulter, Brea, CA, USA). Human IL-4 and IFN-γ ELISA kits were provided by BD Biosciences (Franklin Lakes, NJ, USA). These indices were quantified at baseline and after 6 months of treatment. Blood samples were collected by a designated professional nurse who did not participate in the research project. Each patient contributed 4 mL of blood at 8:00 in the morning on an empty stomach. The patients were examined at the 3-month mark and at the end of the study and were contacted by telephone every two weeks. The times and duration of acute exacerbation were recorded, and the acute exacerbation events were classified.

Acute exacerbation was defined according to Anthonisen et al. [11]: Two of the three main symptoms (exacerbation of difficulty breathing, increase in sputum amount, and pus in spu-

Table 4. Incidence of acute exacerbation events

Group	Incidence of Class-I acute exacerbations	Incidence of Class-II and -III acute exacerbations
Treatment (n = 34)	0.35 ± 0.54	0.41 ± 0.56*
Placebo (n = 29)	0.48 ± 0.63	0.93 ± 0.80
p value	0.410	0.011

*Compared with placebo group; $P < 0.05$.

tum), or only one of these symptoms, combined with one minor symptom (upper respiratory infection, fever of unknown origin, and increased wheezing) for more than two days.

According to the GOLD guidelines, acute exacerbation can be divided into three classes. Patients in Class I exhibit AECOPD but no respiratory failure, and breathing frequency is 20-30 times/min without using accessory respiratory muscle groups, a change of consciousness, or an increase in partial pressure of carbon dioxide in arterial blood ($PaCO_2$). Hypoxemia in Class I patients can be treated with 28-35% oxygen administered by nasal catheter or Venturi mask. Patients in Class II are those with AECOPD and acute respiratory failure but no immediate danger to life, and a breathing frequency > 30 times/min using accessory respiratory muscle groups but without changes in consciousness. Hypoxemia in Class II patients can be treated with 25-30% oxygen, and $PaCO_2$ is 50-60 mmHg higher than the reference value. Patients in Class III are those in acute danger of respiratory failure, and who exhibit breathing frequency > 30 times/min with accessory respiratory muscle groups and acute changes in consciousness. Hypoxemia in Class III patients cannot be treated with oxygen, $PaCO_2$ is > 60 mmHg higher than the reference value, and the patient may exhibit acidosis (pH ≤ 7.25).

The study was approved by the ethics committee of Jinshan Hospital Affiliated to Fudan University, and all patients signed informed consent forms before beginning treatment.

Statistical methods

Data are represented as mean ± standard deviation and compared using the Chi-squared test. SPSS 19.0 (IBM SPSS, Armonk, NY, USA) was used for calculations. One-way analysis of variance was used to compare measurements between groups, and paired *t*-tests and correlation analysis were used for intra-group comparisons. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Eighty patients with stage 3 and 4 COPD were enrolled in the study between September 2016 and March 2017. Seventeen patients with acute exacerbation were disenrolled from the study owing to immune system dysfunction. Sixty-three patients completed the study (34 patients in the treatment group and 29 patients in the placebo group). Baseline patient characteristics, pulmonary parameters, quality of life scores, proportion of Th1 and Th2 cells, Th1/Th2 ratios, total T cell counts, and IL-4 and IFN-γ concentrations in peripheral blood did not differ significantly between the two groups (**Tables 1** and **2**).

Acute exacerbation rates, duration, and severity

Patients receiving NAC had a mean acute exacerbation rate of 0.74 ± 0.75, with a mean duration of 4.62 ± 5.16 days. Patients receiving a placebo had a mean acute exacerbation rate of 1.34 ± 0.67, with a mean duration of 8.34 ± 4.30 days. Both the rate and duration of the acute exacerbation in the treatment group were lower than in the placebo group ($P < 0.05$; **Table 3**). Patients in the treatment group experienced a total of 32 acute exacerbations (18 Class-I AECOPD events, 10 Class-II events, and four Class-III events). Patients in the placebo group experienced a total of 53 acute exacerbations (16 Class-I AECOPD events, 28 Class-II events, and nine Class-III events). Patients in the placebo group experienced more Class-II and -III acute exacerbations ($P < 0.05$; **Table 4**).

Serum IL-4 and IFN-γ levels

Table 5 shows that serum IL-4 levels in the patients who received NAC increased significantly over the study period ($P < 0.05$), while the levels in patients who received a placebo did not differ over time. The IFN-γ levels decreased in both groups, but the decrease was only statistically significant in the treatment group ($P < 0.05$). **Table 6** shows the correlation of age and sex with serum IL-4 and

Table 5. Serum interleukin (IL) 4 and interferon (IFN) γ levels in patients

Group	Treatment (n = 34)		Placebo (n = 29)	
	Baseline	After 6 months	Baseline	After 6 months
IL-4 (pg/mL)	49.26 ± 24.38	72.26 ± 14.36*,&	49.34 ± 26.84	46.72 ± 12.17#
IFN-γ (pg/mL)	127.65 ± 7.54	76.88 ± 19.97*,&	126.31 ± 6.50	119.00 ± 24.82#

*$P < 0.05$ before and after treatment; #$P > 0.05$ before and after treatment; &$P < 0.05$ before and after treatment and in comparison with the placebo group.

Table 6. Correlation of age and sex with serum interleukin (IL) 4 and interferon (IFN) γ levels in patients after 6 months of treatment

	Treatment (n = 34)		Placebo (n = 29)	
	IL-4 (pg/mL)	IFN-γ (pg/mL)	IL-4 (pg/mL)	IFN-γ (pg/mL)
Age (Pearson's correlation coefficient)	0.407	0.786	0.215	0.513
Sex (Spearman's correlation coefficient)	0.215	0.242	0.348	0.273

Table 7. Th1/Th2 levels and ratio in patients

Group	Treatment (n = 34)		Placebo (n = 29)	
	Baseline	After 6 months	Baseline	After 6 months
Th1/CD4+ T cells (%)	14.66 ± 0.86	8.77 ± 2.63*,&	14.29 ± 0.91	13.46 ± 2.81#
Th2/CD4+ T cells (%)	2.93 ± 0.45	4.41 ± 1.27*,&	2.97 ± 0.32	3.28 ± 0.99#
Th1/Th2	5.15 ± 1.06	2.24 ± 1.22*,&	4.88 ± 0.62	4.36 ± 1.34#

*$P < 0.05$ before and after treatment; #$P > 0.05$ before and after treatment; &$P < 0.05$ before and after treatment and in comparison with the placebo group.

IFN-γ levels after 6 months of treatment. No correlation was observed in either group.

Th1 and Th2 levels and ratio

In patients receiving NAC, the proportion of Th1 in $CD4^+$ T cells decreased over time and in comparison with the proportion in patients who received a placebo; the proportion of Th2 in $CD4^+$ T cells increased over time and in comparison with the proportion in patients who received a placebo. The Th1/Th2 ratio in patients receiving NAC decreased over time and in comparison with the proportion in patients who received a placebo. In patients who received a placebo, no significant differences were observed in any of these indices over the study period (**Table 7**). We did not find any correlation between age or sex and Th1 and Th2 levels and ratio (**Table 8**).

Discussion

Chronic airway inflammation is a hallmark of COPD pathogenesis [12]. The inflammatory cells distribute into different areas; neutrophilic granulocytes tend to infiltrate the airways, while lymphocyte infiltration occurs mainly in the airway wall. Th lymphocytes, mainly Th1 and Th2, modulate COPD airway inflammation. Th1 cells can produce cytokines such as IL-2 and IFN-γ, contributing to macrophage activation, cellular immunity, and delayed-type hypersensitivity. Th2 cells secrete IL-4, stimulating humoral immunity and regulating allergic disease. IFN-γ and IL-4 levels are an indirect measure of Th1 and Th2 function, respectively. Impairment of the Th1/Th2 balance can lead to abnormal immune responses and a pathological state.

Airway inflammation has been shown to be mainly related to Th1 cells in COPD. IFN-γ secreted by Th1 cells is negatively related to patients' FEV_1, showing that the more severe the disease, the more active the Th1 cells may be. IFN-γ secreted by Th1 cells may induce the production and release of matrix metalloproteinase (MMP) 9 and 12, to degrade the extracellular matrix in pulmonary parenchyma. IFN-γ can also inhibit α-antitrypsin, impairing the protease/antiprotease balance and hydrolysis of elastin [13]. Th2 cells mainly secrete IL-4 and other cell factors that can inhibit cell-mediated immune responses, so Th1 and Th2 balance each other. When Th1 function increases and Th2 function decreases, the result is increased MMP secretion, elastase activation, extracellular matrix degradation, and elastin hydrolysis, leading to pulmonary emphysema. Therefore, impairment of the Th1/Th2 balance may progress COPD. Moreover, the amelioration of airway inflammation has been shown to be related to recovery of the Th1/Th2 balance [14], so

Table 8. Correlation of age and sex with Th1/Th2 levels and ratio in patients after 6 months of treatment

	Treatment (n = 34)			Placebo (n = 29)		
	Th1/CD4$^+$ T cells (%)	Th2/CD4$^+$ T cells (%)	Th1/Th2	Th1/CD4$^+$ T cells (%)	Th2/CD4$^+$ T cells (%)	Th1/Th2
Age (Pearson's correlation coefficient)	0.810	0.067	0.144	0.721	0.072	0.131
Sex (Spearman's correlation coefficient)	0.054	0.182	0.051	0.231	0.163	0.152

restoring the Th1/Th2 balance can prevent the progression of COPD.

High doses of NAC have been reported to reduce the frequency of acute exacerbations in patients with COPD [4, 15, 16]. NAC is a strong antioxidant and expectorant. Its mechanism of action to reduce and eliminate phlegm is the dissolution of mucoproteins, reducing the glutinousness of phlegm, increasing the physiological running capacity of cilia and the stomach-lung vagal reflex, and enhancing airway activity to transport and scavenge wastes. NAC can reduce elastic proteinase activity and prevent excessive elastin hydrolysis, balancing the protease/antiprotease system and maintaining the elasticity of the pulmonary alveolus. NAC can also increase the levels of glutathione, reducing chronic damage to the lungs.

We treated elderly COPD patients in stages 3 and 4 with 1,200 mg NAC for 6 months and found that NAC treatment decreased Th1 levels and increased Th2 levels, restoring the Th1/Th2 balance. These results are consistent with those reported by Zhao et al. [16], indicating that NAC can effectively reduce inflammatory reactions and restore the Th1/Th2 balance.

Moreover, we found that compared with the placebo group, the NAC group experienced lower numbers and durations of acute exacerbations and a lower incidence rate of Class-II and -III AECOPD. This is consistent with the results reported by Tse et al. [4] and Zheng et al. [17], suggesting that long-term oral administration of NAC can reduce acute exacerbations and heavy AECOPD events and shorten their duration. Further study, however, will be required to determine whether the NAC-corrected Th1/Th2 ratio in COPD patients is a cause or an effect of decreased acute exacerbations.

In conclusion, long-term administration of 1,200 mg NAC improved the condition of elderly stage 3 and 4 COPD patients, restoring the Th1/Th2 balance and reducing acute exacerbations. Longer-term clinical observation is required to monitor the benefits of NAC treatment.

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Disclosure of conflict of interest

None.

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