Article

Atomization Inhalation of Terbutaline and Budesonide Efficiently Improved Immunity and Lung Function of AECOPD Patients

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Chronic obstructive pulmonary disease (COPD) is a syndrome of chronic progressive airflow limitation as a result of chronic inflammation of the airways and lung parenchyma. COPD patients always have airway hyperreactivity (AHR), so how to reduce AHR becomes the key purpose of clinical treatment. It is hypothesized that combined inhalation of corticosteroids and β_2-agonists can reduce the AHR in COPD. In this study, atomization inhalation of budesonide and terbutaline plus conventional therapies was applied to treat AECOPD (acute exacerbation of chronic obstructive pulmonary disease) patients for two weeks. The results showed that additional inhalation of budesonide and terbutaline could upregulate serum IL-2 levels, the percentages of $CD3^+$ T and $CD4^+$ T cells, and CD4/CD8 ratio, and decrease eosinophils and serum CRP level more efficiently than conventional treatment in patients with AECOPD. And the lung function of the atomization inhalation group was improved more obviously after the treatment compared with the conventional treatment group. Thus, atomization inhalation of terbutaline and budesonide can control AECOPD effectively, and has wide clinical perspective in controlling and preventing the exacerbation of COPD. *Cellular & Molecular Immunology*. 2008;5(4):287-291.

Key Words: AECOPD, T lymphocyte subset, IL-2, eosinophil, C-reactive protein

Introduction

Chronic obstructive pulmonary disease (COPD) is a syndrome of chronic progressive airflow limitation which occurs as a result of chronic inflammation of the airways and lung parenchyma, and is at most partially reversible (1). Recently, the morbidity and mortality of COPD have risen in China and there are about one million people dying of COPD per year. In America, COPD is the fourth lethal cause. By 2020, COPD will be the third leading cause of mortality and the fifth leading cause of disability worldwide (2, 3). COPD brings much damage to human health; what is more, COPD has not been controlled well yet.

It is generally considered that chronic airway inflammatory damages the airway wall and influences airway wall repair and remodeling, thus causing airflow limitation. The development and aggravation of airway inflammation to infection, harmful gas, abnormal particles, etc., lead to the imbalance of inflammatory cell network, including neutrophils, lymphocytes, eosinophils and other inflammatory cells, and cytokine network, all of which contribute to airway hyperreactivity (AHR) (4, 5). So, it is extremely urgent to investigate how to reduce the AHR of COPD patients.

Lots of clinical studies showed that COPD patients treated with long-acting β_2-agonists and inhaled corticosteroids (ICSs) had better lung function and improved symptom control than those treated with these two kinds of medicines alone (6-10). The global initiative for chronic obstructive lung disease (GOLD) (11) points out that COPD is a preventable and curable disease, and advocates atomization inhalation of corticosteroids and β_2-agonists together to treat COPD, which brings new hope to the clinic practice. We hypothesized that combined inhalation of corticosteroids and β_2-agonists might reduce the AHR in COPD and investigated the underlied mechanism.

Materials and Methods

Study population

Forty patients (20 male and 20 female) hospitalized in our hospital and definitely diagnosed as AECOPD (acute exacerbation of chronic obstructive pulmonary disease) (stage II or III) were adopted. The criteria for inclusion were:

1) diagnosis is consistent with the standard of the GOLD of 2006;

2) no application of glucocorticosteroid systematically in 6

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Received Jun 26, 2008. Accepted Jul 20, 2008.

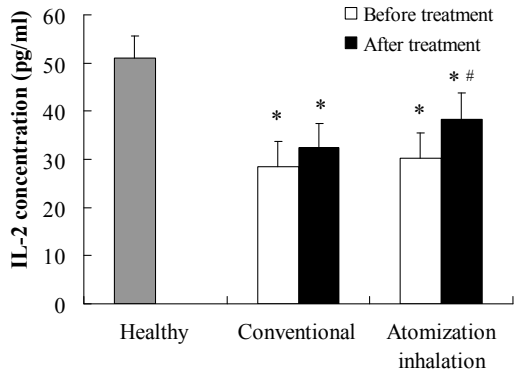

Figure 1. Atomization inhalation of terbutaline and budesonide upregulated serum IL-2 levels. The sera were collected and IL-2 levels were measured by ELISA. *$p < 0.05$ *vs* healthy group; #$p < 0.05$ *vs* conventional group after treatment.

months;

3) no systemic infection in 2 weeks before the study;

4) no application of β_2-receptor agonist or antihistamine drugs in 24 hours;

5) no any other chronic heart or lung disease or endocrine disease;

6) no need of mechanical ventilation;

7) voluntary to take part in the study.

In addition, 20 samples (11 male and 9 female) of our academy were included in the healthy control group, with the average age of 63.32 ± 3.08 years old.

Study design

Forty samples definitely diagnosed as AECOPD (stage II or III) were randomly divided into two groups (20 samples for each group), 9 male and 11 female in the conventional group, with the average age of 66.4 ± 6.48 years old; and 11 male and 9 famale in the atomization inhalation group, with the average age of 65.1 ± 5.83 years old. The conventional group took routine therapies containing antibiotics, doxofylline, apophlegmatisant and antitussive. The atomization inhalation group received routine therapies and additional atomization inhalation of budesonide suspl containing 0.25 mg budesonide (Astrazeneca) and 3 ml 0.9% isotonic NaCl, and terbutaline containing 2 mg terbutaline (Chengdu Arima Pharmaceutical Co. Ltd) and 3 ml 0.9% isotonic NaCl, twice a day. The treatment period lasted for two weeks.

ELISA

Venous blood was collected into sterile EDTA-containing tubes and serum was collected after centrifugation. The serum IL-2 level was measured by the double-antibody sandwich ELISA kits from Hysen-on Technology Co. Ltd.

Flow cytometric analysis

Anti-CD3 conjugated with PE-Cy5, anti-CD4 conjugated with FITC and anti-CD8 antibodies conjugated with PE were purchased from Becton Dickson. Whole blood (100 μl) was incubated with saturating concentrations of the mAbs for 15 min at room temperature. Thereafter, the cells were lysed by RBC lysis buffer for 15 min at room temperature. Cells were washed with PBS and analyzed using EPICS ALTRA (Beckman Coulter).

Eosinophil count

Venous blood was collected and the amounts of eosinophil were examined by the full automatic hemocyte analyzer (Beckman Coulter).

CRP level determination

The serum CRP level was measured using scatter nephelometry (BN Prospec).

Lung function measurement

The lung function was measured by lung function Detector (SensorMedics V6200).

Statistical analysis

The data were expressed as mean ± SD. The statistical analysis was performed by the Student's *t* test using SPSS 11.5 and PEMS 3.1. The difference between each group was considered statistically significant when *p* value was less than 0.05.

Results

Atomization inhalation of terbutaline and budesonide upregulated serum IL-2 levels efficiently

The serum IL-2 level of the AECOPD patients was lower

Table 1. The percentages of T lymphocyte subsets before and after treatment

Group		CD3 (%)	CD4 (%)	CD8 (%)	CD4/CD8
Atomization inhalation	Before treatment	54.78 ± 3.90[a]	26.11 ± 4.64[a]	27.23 ± 4.25	0.98 ± 0.36[a]
	After treatment	65.92 ± 5.89[a, b, c]	37.97 ± 5.47[a, b, c]	27.37 ± 3.33	1.43 ± 0.28[a, b, c]
Conventional	Before treatment	54.86 ± 4.24[a]	25.66 ± 3.63[a]	28.30 ± 4.57	0.95 ± 0.26[a]
	After treatment	60.86 ± 5.20[a, b]	33.02 ± 3.89[a, b]	27.21 ± 4.13	1.24 ± 0.28[a, b]
Healthy		69.48 ± 3.84	42.12 ± 3.64	26.48 ± 2.82	1.61 ± 0.22

[a]$p < 0.05$ *vs* healthy group; [b]$p < 0.05$ *vs* before treatment; [c]$p < 0.05$ *vs* conventional group after treatment.

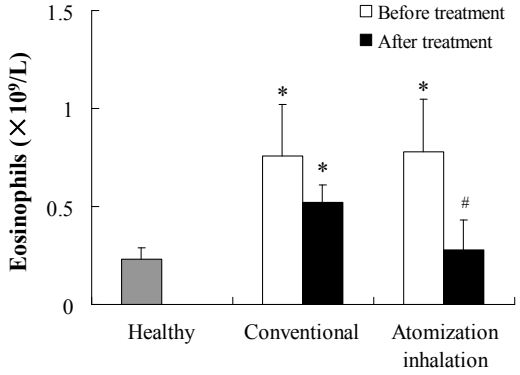

Figure 2. Atomization inhalation of terbutaline and budesonide decreased the number of eosinophils efficiently. Venous blood was collected and the amounts of eosinophil were examined by the full automatic hemocyte analyzer. *$p < 0.05$ *vs* healthy group; #$p < 0.05$ *vs* conventional group after treatment.

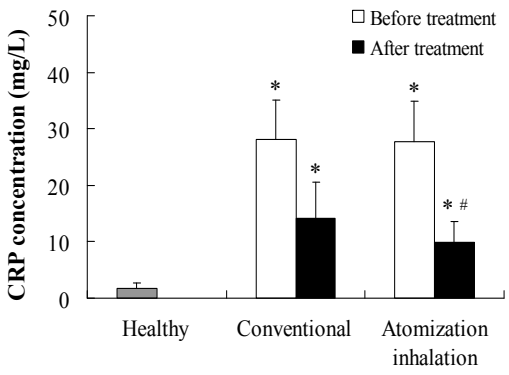

Figure 3. Atomization inhalation of terbutaline and budesonide decreased serum CRP level efficiently. The sera were collected and CRP levels were measured using scatter nephelometry. *$p < 0.05$ *vs* healthy group; #$p < 0.05$ *vs* conventional group after treatment.

than that of healthy control. After treatment, the levels of IL-2 were upregulated in both atomization inhalation group and conventional group, and more obviously in the atomization inhalation group. However, it was still lower than that of healthy control group ($p < 0.05$, Figure 1).

The percentages of $CD3^+$ and $CD4^+$ T cells were increased obviously in atomization inhalation group

The percentages of $CD3^+$ T cells and $CD4^+$ T cells, and the ratio of CD4/CD8 cells of the AECOPD patients were lower than those of healthy control samples ($p < 0.05$, Table 1). These data were increased after treatment but still lower than those of healthy control ($p < 0.05$). Compared with conventional treatment, the effect of additional atomization inhalation of terbutaline and budesonide was obviously stronger ($p < 0.05$). However, there was no difference in the percentages of $CD8^+$ T cells before and after medical treatment (Table 1).

Atomization inhalation of terbutaline and budesonide decreased the number of eosinophils efficiently

The number of eosinophils was larger in AECOPD patients than that in healthy control. After treatment, eosinophil counts were decreased in both conventional and atomization inhalation groups. But it was much lower in the atomization inhalation group than that in conventional group ($p < 0.05$, Figure 2).

Atomization inhalation of terbutaline and budesonide decreased serum CRP level efficiently

After treatment, the higher levels of serum CRP in AECOPD patients were decreased in both the conventional group and atomization inhalation group, but the CRP levels were still higher than that of healthy control ($p < 0.05$, Figure 3). However, additional atomization inhalation of terbutaline and budesonide downregulated serum CRP level more efficiently ($p < 0.05$, Figure 3).

Lung function was improved obviously in atomization inhalation treatment

Compared with the conventional group, the lung function of the atomization inhalation group was improved more obviously after the treatment ($p < 0.05$, Table 2). But the FEV1 level, the percentage of FEV1/predicted value, and the ratio of FEV1/FVC were still lower than those of the healthy control group ($p < 0.05$, Table 2).

Discussion

COPD is a chronic condition characterized by progressive

Table 2. The lung function before and after treatment

Group		FEV1 (L/s)	FEV1/predicted value (%)	FEV1/FVC (%)
Atomization inhalation	Before treatment	1.14 ± 0.50^{a}	51.05 ± 9.70^{a}	49.75 ± 10.6^{a}
	After treatment	$1.90 \pm 0.52^{a, b, c}$	$64.00 \pm 8.14^{a, b, c}$	$63.70 \pm 10.84^{a, b, c}$
Conventional	Before treatment	1.15 ± 0.45^{a}	50.10 ± 9.66^{a}	48.15 ± 12.69^{a}
	After treatment	$1.55 \pm 0.43^{a, b}$	$55.70 \pm 9.18^{a, b}$	$55.65 \pm 11.74^{a, b}$
Healthy		3.49 ± 0.96	104.05 ± 11.41	87.00 ± 6.21

[a]$p < 0.05$ *vs* healthy group; [b]$p < 0.05$ *vs* this group before treatment; [c]$p < 0.05$ *vs* conventional group after treatment.

airflow limitation that is at most partially reversible and its underlying pathophysiology is complex. In this study, we found that improved lung function was obtained when additional atomization inhalation of terbutaline and budesonide was applied to treat COPD patients compared with traditional treatment.

Glucocorticosteroids (GCSs), which are also known as corticosteroids, are anti-inflammatory medicines. GCSs are effective in reducing airway and systemic inflammation. GCSs can inhibit the aggregation and activation of multiple inflammatory cells, including neutrophils, macrophages, lymphocytes, eosinophils, etc., the hyperplasy and damage of the epithelial cells, and even the thickening of the basement membrane. They regulate the release of CRP, leukotriene, etc., degrade the vasopermeability and the formation of new vessels, and reduce edema and exudation, thus reducing the airway inflammatory, relieving the airflow limitation, and finally reversing the AHR. However, long-term systemic GCS therapy is associated with inevitably serious side effects. At present, ICS treatment has been widely used in clinic practice (12-17). ICS can reach a higher drug concentration in the local target region, but only small amount goes into the bloodstream and can be rapidly removed from the body. So, it has less systemic side effects normally. In addition, ICS raises the chemotherapeutic index (the ratio of safety and efficiency). Budesonide is a new generation GCS with high anti-inflammatory activity and reduced systemic side effects and it is widely used to treat COPD in clinical practice.

β_2-agonist is one of the main drugs to control the symptoms of COPD patients, which can prevent/reduce the airflow limitation and relieve dyspnea quickly when inhaled regularly. Terbutaline is an immediate-effect β_2-agonist. It can stabilize the membrane of mast cells and relax bronchial smooth muscles, thus relieving dyspnea of the patients quickly and improve the respiration. Furthermore, terbutaline inhibits the hyperplasy of contractile fiber cells and the release of inflammatory mediators, and inhibits the adhesion, aggregation and activity of neutrophils. Terbutaline can also reduce tissue hypoxia, and prevent or reduce the cardiovascular disease complications of the patients with COPD.

Both GOLD guide of 2006 (11) and ATS/ERS (18) recommended combined atomization inhalation of cortico-steroids and β_2-agonists to treat COPD. Corticosteroids may regulate β_2-receptor function by enhancing expression of the receptor and inhibiting β_2-receptor downregulation, and upregulate the transcription of the anti-inflammatory genes. Meanwhile, the β_2-receptors can activate glucocorticosteroid receptor (GR), and enhance the combination of GCS and GR, through which playing their anti-inflammatory role. In the study, the treatment group got an additional treatment by applying combination of budesonide and terbutaline on the basis of routine treatment. After two-week treatment, the results showed that the level of serum IL-2 in the atomization inhalation group was significantly higher than those before treatment and the conventional group. The percentages of $CD3^+$ T cells and $CD4^+$ T cells, and the ratio of CD4/CD8 cells were also increased much more in the atomization inhalation group than conventional group. At the same time, when atomization inhalation was used in the treatment, the amount of eosinophils and serum CRP levels of AECOPD patients were decreased significantly compared with conventional group. Although all these values were improved compared with the status before treatment, they did not get back to the normal range. Even though, the lung function of the atomization inhalation group was improved more obviously after the treatment compared with the conventional group. While the FEV1 level, the percentage of FEV1 accounting for the predicted value, and the percentage of FEV1/FVC were still lower than those of the healthy control group. The results showed that combined inhalation was more effective in improving the immune function and the lung function and reducing the inflammatory response and AHR compared with the traditional therapy. Although the symptoms of COPD patients have been relieved, the immune function is stilled inhibited and inflammatory response and AHR still exist, and the patients should receive continuing treatment.

In conclusion, combined inhalation of terbutaline and budesonide can reduce AHR, improve the lung function, and regulate the immunological disfunction. It has great importance in controlling symptoms, improving ventilatory function of the lungs, and improving the immunity and quality of life. What is more, it has little systemic or local side effects, and has wide clinical perspective in treating AECOPD and good effect in stable stage patients.

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