Original Article

Effect of Inhaled Budesonide on Interleukin-4 and Interleukin-6 in Exhaled Breath Condensate of Asthmatic Patients

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Abstract

Background: Studies of interleukin (IL)-4 and IL-6 in the exhaled breath condensate (EBC) of asthmatic patients are limited. This study was to determine the effect of inhaled corticosteroid (ICS) treatment on IL-4 and IL-6 in the EBC of asthmatic patients.
Methods: In a prospective, open-label study, budesonide 200 μg twice daily by dry powder inhaler was administered to 23 adult patients with uncontrolled asthma (mean age 42.7 years) for 12 weeks. Changes in asthma scores, lung function parameters (forced expiratory volume in 1 s [FEV_1], peak expiratory flow [PEF], forced expiratory flow at 50% of forced vital capacity [FEF_{50}], forced expiratory flow at 75% of forced vital capacity, maximum mid-expiratory flow rate) and the concentrations of IL-4 and IL-6 in EBC were measured.
Results: Both asthma scores and lung function parameters were significantly improved by ICS treatment. The mean IL-4 concentration in the EBC was decreased gradually, from 1.92 ± 0.56 pmol/L before treatment to 1.60 ± 0.36 pmol/L after 8 weeks of treatment ($P < 0.05$) and 1.54 ± 0.81 pmol/L after 12 weeks of treatment ($P < 0.01$). However, the IL-6 concentration was not significantly decreased. The change in the IL-4 concentration was correlated with improvements in mean FEV_1, PEF and FEF_{50} values (correlation coefficients −0.468, −0.478, and −0.426, respectively).
Conclusions: The concentration of IL-4 in the EBC of asthmatic patients decreased gradually with ICS treatment. Measurement of IL-4 in EBC could be useful to monitor airway inflammation in asthmatics.

Key words: Asthma; Exhaled Breath Condensate; Inhaled Corticosteroids; Interleukin-4; Interleukin-6

Introduction

The majority of asthmatics can be effectively controlled by well-chosen treatment. However, as asthma is a chronic disease with variable symptoms, treatment should not only be individualized but also timed appropriately. In clinical practice, asthma may be exacerbated by a decrease in the dosage of a patient's antiasthma medication or by inappropriate discontinuation of treatment. As modifying the therapeutic regimen based on the patient's pulmonary function parameters and clinical symptoms cannot always be expected to maintain the optimum therapeutic benefit, finding better parameters to indicate asthma control is a current focus of asthma studies.

The most frequently used methods for studying the status of pulmonary diseases are invasive. Among noninvasive methods, fractional exhaled nitric oxide (FeNO) is the best-studied inflammatory marker. FeNO levels are elevated in asthma and show a dose-dependent reduction with inhaled corticosteroid (ICS) therapy.[1] FeNO is biomarker for asthma phenotyping and management.[2] However, FeNO measurement requires expensive instrumentation.

The use of exhaled breath condensate (EBC) is another relatively new noninvasive technique for assessing inflammatory markers. Various markers of airway

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inflammation and oxidative stress such as cytokines, eicosanoids, nitric oxide, urates, and hydrogen peroxide have been shown to be increased in EBC of patients with asthma.[3-7] The method of collecting EBC is easy, and the cost is low. Interleukin (IL-4), a T-helper type 2 (Th2) proxy cytokine, play a crucial role in the development of atopic disease including asthma, was a widely used marker of asthma.[6,8,9] IL-6, a cytokine produced by inflammatory cells is also produced by primary lung epithelial cells in response to a variety of different stimuli including allergens.[10] However, recent studies have shown it may be a potential wide-ranging contributor to asthma as well as other pulmonary disease where the lung epithelium is damaged.[11]

However, most of the previous studies were cross-sectional in design, the aim of this prospective study was to determine the effect of ICS therapy on two inflammatory cytokines, IL-4, and IL-6, in the EBC of patients with uncontrolled asthma, and explore the correlation between changes in each measurement.

Methods

Study design and patients

Uncontrolled asthmatics aged 24–58 years were enrolled in this prospective, open-label study. Uncontrolled asthma was diagnosed according to GINA 2006. On entering the study, all patients had demonstrated a ≥12% and ≥200 ml increase in forced expiratory volume in 1 s (FEV_1) in response to 400 μg inhaled albuterol (salbutamol), and none was receiving a corticosteroid or anti-leukotriene drug. Treatment with budesonide dry powder inhaler (Pulmicort Turbuhaler, AstraZeneca, China) 200 μg BID was administered for 12 weeks. The patients attended the hospital clinicon 4 occasions: On day 1, and at the end of weeks 4, 8, and 12 of treatment. All patients were allowed to use albuterol via a metered dose inhaler as required during the study. At all clinic visits, EBC samples were obtained for measurement of IL-4 and IL-6 concentrations. Symptom scores and short-acting β_2-agonist (SABA) use during 24-h periods were assessed at each visit.

Outcome measures included changes in asthma scores, lung function parameters, and concentrations of IL-4 and IL-6 in EBC and the relationships between these parameters were analyzed. The protocol was approved by the Peking University First Hospital ethics committee and written consent was obtained from each patient before the commencement of the study.

Measurement of asthma symptoms scores

Every subject evaluated symptoms and recorded the ratings on dairy cards. Symptoms (dyspnea, cough, and wheeze) were evaluated by using a 0–3-point scale as other studies.[12,13] Dyspnea was scored as: 0 = none (no dyspnea), 1 = mild (dyspnea on exertion), 2 = moderate (dyspnea on slight activity), and 3 = severe (dyspnea on rest). Cough was scored as: 0 = none (no cough), 1 = mild (intermittent cough), 2 = moderate (frequent cough without disturbed sleep), and 3 = severe (frequent cough with disturbed sleep). Wheezes on chest auscultation were recorded by the investigators at each follow-up and scored as: 0 = none (no wheeze), 1 = mild (a little bilateral wheeze), 2 = moderate (some bilateral wheeze), 3 = severe (widespread bilateral wheeze). Asthma scores for each patient were determined by the summation of the above scores at each visit. In addition, the patients recorded on their diary cards the daily use of inhaled albuterol.

Measurement of interleukin-4 and interleukin-6 in exhaled breath condensate

EBC was collected for 15 min by means of a cooled (0°C) condenser, as described previously.[14] A self-made EBC collection was used similarly as van Beurden used,[15] mainly including a collection tube, two-way nonrebreathing valve, the collection tube was constructed with eight bubble-wall glass ball, the collecting vessel was cooled with ice. While wearing a nose clip, patients breathed tidally through a mouthpiece connected to a two-way nonrebreathing valve. The two-way valve and tubing served as a trap to minimize the possibility of salivary contamination.

After collection, EBC was stored at −80°C until analysis. IL-4 and IL-6 were assayed by hypersensitivity enzyme-linked immunosorbent assay (ELISA) kits (IL-4, Bender Medsystems Company, Austria; IL-6, Cayman Company, USA). The intra-assay and inter-assay variability was ≤10%. The detection limit of the assay was 0.1 pg/ml and 3.9 pg/ml.

Lung function testing

Before lung function tests at each follow-up visit, patients were asked to refrain from using their SABA inhalers for at least 6 h. Lung function parameters measured were: FEV_1, peak expiratory flow (PEF), forced expiratory flow at 50% of forced vital capacity (FEF_{50}), forced expiratory flow at 75% of forced vital capacity (FEF_{75}), and maximum mid-expiratory flow rate (MMEF).

Statistical analysis

All data were expressed as means ± standard deviation (SD) of the mean. Levels of each measurement at pretreatment, week 4 of treatment, week 8 of treatment, and week 12 of treatment were compared. When data distribution was normal, repeated measures were used for statistical analysis. When data were abnormal distribution, the Friedman test was used for the ordinal variable. The Pearson correlation coefficient was used to assess relationships between IL-4 or IL-6 concentrations in EBC and asthma scores, FEV_1 and PEF values. A $P < 0.05$ was considered statistically significant.

Results

Study population characteristics

A total of 23 adult asthmatics (8 male; 15 female) of mean age 42.7 ± 10.0 years (range 24–58 years) with

moderate-to-severe asthma participated in the study. Their baseline (pretreatment) characteristics are shown in Table 1. None of the patients was receiving regular treatment with inhaled or oral corticosteroids or anti-leukotriene drugs, and none had a history of upper respiratory tract infection for at least 4 weeks before the study.

Asthma scores

The asthma scores were improved by ICS treatment [Table 2]. After 4 weeks of budesonide treatment, dyspnea, cough, wheeze, and SABA use had already improved significantly compared with pretreatment values. However, there were no significant differences between the values recorded after 4 weeks, 8 weeks, and 12 weeks of treatment.

Pulmonary function parameters

Lung function data recorded before and after budesonide therapy are shown in Table 3. All of the lung function parameters measured (FEV_1, FEV_1/forced vital capacity, PEF, FEF_{50}, FEF_{75} and MMEF) were significantly improved after 8 and 12 weeks of treatment, and FEV_1, PEF and FEF_{50} were significantly improved after 4 weeks. Most of the parameters continued to improve during the study.

Table 1: Clinical characteristics of the study population before treatment

Characteristics	*n* = 23
Age (years)	42.7 ± 10.0
Height (cm)	156.1 ± 31.2
Weight (kg)	66.8 ± 11.1
Gender (male/female)	8/15
Wheeze score	1.30 ± 1.06
Cough score	1.43 ± 0.99
Daily SABA use*	1.74 ± 3.33
Asthma score	5.13 ± 4.50
FEV_1 (% predicted)	79.01 ± 17.89
FEV_1/FVC (%)	65.86 ± 10.28
PEF (% predicted)	85.39 ± 24.14
FEF_{50} (% predicted)	41.43 ± 21.17
FEF_{75} (% predicted)	37.23 ± 21.61
MMEF (% predicted)	42.69 ± 22.46

Values are means ± SD, except for gender, which are patient numbers. *Times of daily use of 100 μg salbutamol. FEV_1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; FEF_{50}: Forced expiratory flow at 50% of FVC; FEF_{75}: Forced expiratory flow at 75% of FVC; MMEF: Maximum mid-expiratory flow rate; PEF: Peak expiratory flow; SABA: Short-acting β_2-agonist; SD: Standard deviation.

Table 2: Asthma scores before and after budesonide treatment (*n* = 23)

Scores	Pretreatment	Week 4 of treatment	Week 8 of treatment	Week 12 of treatment
Dyspnea score	1 (0, 3)	0 (0, 2)*	0 (0, 3)	0 (0, 3)*
Cough score	1 (0, 3)	0 (0, 3)†	0 (0, 3)*	0 (0, 3)*
Wheeze score	0 (0, 2)	0 (0, 0)*	0 (0, 2)*	0 (0, 2)
SABA use	0 (0, 14)	0 (0, 5)*	0 (0, 6)	0 (0, 4)*
Asthma score	4 (0, 18)	0 (0, 7)†	1 (0, 10)	1 (0, 8)†

Values are medians (minimum, maximum). *P<0.05; †P<0.01 compared with pretreatment. SABA: Short-acting β_2-agonist.

Interleukin-4 and interleukin-6 concentrations in exhaled breath condensate

Mean (±SD) concentrations of the inflammatory markers IL-4 and IL-6 measured in the patients' EBC are shown in Table 4. There was a significant reduction in the IL-4 concentration after 8 weeks of budesonide treatment, and there was a further reduction at the end of 12 weeks of treatment. However, concentrations of IL-6 were not significantly decreased during the study.

Relationships between interleukin-4 concentrations and asthma scores and pulmonary function parameters

Correlation analyses showed there were significant correlations between the changes in IL-4 concentration in EBC and improvements in mean FEV_1, PEF, and FEF_{50} values (correlation coefficients −0.468, −0.478, and −0.426, respectively) after 4 weeks of treatment. However, no relationship was found between IL-4 concentrations in EBC and asthma scores.

Discussion

Collection and analysis of EBC are a noninvasive technique which may play an important role in diagnosing asthma, monitoring its severity, and achieving symptom control.[3,4] Asthma is characterized by an increase of Th2 and a decrease of Th1 cells.[16] In this study, we have shown that IL-4, a Th2 cytokine, is gradually decreased in EBC during treatment with inhaled budesonide, achieving significantly lower concentration compared with the pretreatment value after 12 weeks of treatment ($P < 0.01$). However, IL-6, another inflammatory marker in chronic airway disease, was not significantly decreased in EBC after budesonide treatment.

An elevation of IL-4 concentrations in EBC in asthmatics has been reported in other studies,[8,9] and higher concentrations were noted in patients with persistent asthma receiving high-dose ICS than those receiving low-dose ICS.[17] However, most of the studies were cross-sectional in design. Our study has shown that along with improvements in asthma symptom scores and lung function parameters, the concentration of IL-4 in EBC of uncontrolled asthmatic patients was decreased during the 12 weeks of budesonide treatment and that the IL-4 concentration in EBC was correlated with changes in FEV_1 values. Two prospective studies had shown significant reductions in IL-4 concentrations in EBCin asthma adults after ICS treatment, but there were some differences from our study.[18,19] Matsunaga *et al*. only measured IL-4 and lung function before and 12 weeks after inhaled steroid therapy, finding changes in FEV_1 values were significantly associated with reductions.[18] Carpagnano *et al*. reported that IL-4 concentrations in EBC started to decrease significantly after treatment with inhaled steroids for 2 months to further fall

Table 3: Lung function parameters measured before and after budesonide treatment (n = 23)

Week of treatment	FEV_1 (L)	FEV_1/FVC (%)	PEF (L/s)	FEF_{50} (L/s)	FEF_{75} (L/s)	MMEF (L/s)
0	2.21 ± 0.71	65.86 ± 10.28	5.58 ± 1.98	1.62 ± 0.84	0.49 ± 0.30	1.27 ± 0.72
4	2.39 ± 0.69†	69.85 ± 9.91†	6.34 ± 1.69†	1.81 ± 0.88*	0.52 ± 0.36	1.41 ± 0.80
8	2.41 ± 0.40*	70.53 ± 8.62†	6.29 ± 1.91†	1.96 ± 0.93†	0.61 ± 0.39*	1.56 ± 0.86†
12	2.43 ± 0.73†	70.17 ± 9.11†	6.31 ± 1.70†	2.01 ± 1.01†	0.65 ± 0.43†	1.62 ± 0.91†

Values are means ± SD. *P<0.05; †P<0.01 compared with pretreatment. FEV_1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; FEF_{50}: Forced expiratory flow at 50% of forced vital capacity; FEF_{75}: Forced expiratory flow at 75% of forced vital capacity; MMEF: Maximum mid-expiratory flow rate; PEF: Peak expiratory flow; SD: Standard deviation.

Table 4: Concentrations of IL-4 and IL-6 in the exhaled breath condensate of asthmatic patients (n = 23)

Week of treatment	IL-4 (pmol/L)	IL-6 (pmol/L)
0	1.92 ± 0.56	8.45 ± 1.93
4	1.79 ± 0.44	7.97 ± 1.93
8	1.60 ± 0.36*	8.23 ± 1.63
12	1.54 ± 0.81†	7.74 ± 2.17

Values are means ± SD. *P<0.05; †P<0.01 compared with pretreatment. IL; Interleukin; SD: Standard deviation.

after 6 months, while that of IL-6 did not, but no correlation was observed with lung function parameters, the reason may be that the patients enrolled were mild persistent asthma with FEV_1 ≥80% predicted.[19] Using a logistic regression model, Robroeks *et al*. found that IL-4 was the most contributory inflammatory marker for indicating a diagnosis of asthma, and that it was also useful for assessing asthma control.[4]

Our study has some limitations. First, the measurement of EBC markers might be useful in clinical practice, but obviously, would not be available immediately, due to the lack of appropriate standardization and the absence of reference values. We used commercial ELISA kit, for example, hypersensitive ELISA kit was used for IL-4 test, but the levels of IL-4 and IL-6 were a little different from other studies due to different ELISA kit.[8,9] Second, we did not enroll a controlled group of healthy volunteers, the normal ranges of IL-4 and IL-6 in the EBC have not been established. Thus, although we did not compare changes of IL-4 in EBC of our patients with a normal control group, the findings of this study suggest that IL-4 may be used as a marker for monitoring airway inflammation in patients with asthma and that it may also be of value for assessing asthma control.

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Conflicts of interest

There are no conflicts of interest.

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