Original Article

Effects of leukotriene D_4 nasal challenge on bronchial responsiveness and inflammation in asthmatic patients with allergic rhinitis

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Background: In asthmatic patients with allergic rhinitis (AR), increased cysteinyl leukotrienes (CysLTs) production in the secretion of nasal mucosa has been associated with greater bronchial hyperresponsiveness (BHR) after nasal allergen challenge. However, the role of CysLTs in eliciting BHR after nasal allergen challenge has not been evaluated. The aim of this study is to evaluate the effect of LTD_4 nasal challenge on BHR and inflammation in asthmatic patients with AR.

Methods: In this self-controlled study, fifteen eligible consecutively recruited subjects underwent methacholine (Mch) bronchial provocation test before and 30 minutes after LTD_4 nasal provocation test. The cumulative concentration of LTD_4 inducing a 60% increase in nasal airway resistance (PC_{60}NAR) was calculated. The mean values of cumulative doses inducing a 20% decrease in forced expiratory flow in one second ($PD_{20}FEV_1$) for Mch before and after nasal challenge were compared. Fractional exhaled nitric oxide (FeNO), differential inflammatory cell counts in nasal lavage and induced sputum before and after nasal challenge were compared.

Results: House dust mites were the major allergens accounting for 10/15 (66.7%) of asthmatic patients with AR. The PC_{60}NAR for LT was $(8.39\pm3.48)\times10^{-3}$ mg·mL^{-1}. The $PD_{20}FEV_1$ before and after nasal challenge was 3.05±3.81 and 2.70±3.81 μmol, respectively (P=0.45). The percentages of eosinophils were (38.36±23.14)% and (45.70±24.86)% in nasal lavage, and (17.51±11.05)% and (24.29±16.52)% in induced sputum before and 24 hours after nasal challenge. The neutrophil counts were (60.64±23.14)% and (53.30±24.46)% in nasal lavage, and (53.83±23.27)% and (56.19±22.28)% in induced sputum before and 24 hours after nasal challenge. The values of FeNO were 40 [35] and 43 [30] ppb before and 24 hours after nasal challenge. No severe adverse effects were reported during the tests.

Conclusions: Although most asthmatic patients with AR were sensitive to LTD_4 nasal challenge, LTD_4 nasal provocation tests do not confer any major effect on BHR. LTD_4 might not play a vital role in eliciting bronchial responsiveness induced by nasal allergen challenge.

Keywords: Leukotriene D_4 (LTD_4); airway hyperresponsiveness; bronchial provocation; allergic rhinitis (AR); asthma

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Introduction

The prevalence of coexisting allergic rhinitis (AR) and asthma is increasing worldwide (1,2). AR, particular severe and persistent AR, facilitates the development and worsens of asthma control (3). Bronchial hyperresponsiveness (BHR) may have existed in patients with AR who had no clinical manifestation of asthma. BHR reportedly increased after nasal allergen challenges in patients with AR with or without coexisting asthma (4-7).

BHR has been associated with airway eosinophilia (8). Cysteinyl leukotrienes (CysLTs) are potent lipid mediators which recruit eosinophils and eosinophil progenitors from bone marrow to airways in allergic diseases (9,10). The increased production of CysLTs in the secretion of upper and lower airways has been shown to elicit BHR after nasal allergen challenges (8,9,11). However, the role of CysLTs in increasing BHR after nasal allergen challenge has not been evaluated.

The aim of this study was to evaluate the effects of LTD4 nasal challenge on BHR and airway inflammation in asthmatic patients with AR.

Methods

The study protocol (NCT01963741) was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University. Written informed consent was signed prior to the study.

Participants

Asthmatic patients with AR of either gender, aged 18 to 50 years were consecutively recruited from the First Affiliated Hospital of Guangzhou Medical University between March 2013 and April 2014. The participants tested positive to methacholine (Mch) bronchial provocation test, and had recurrent nasal symptoms (sneezing, nasal discharge, nasal blockage or itching) and lower airway symptoms (cough, breathlessness, chest tightness, wheezing, etc.) in the preceding year. All subjects also tested positive to at least one of the panel of aeroallergens by using skin prick testing. The diagnosis of AR and asthma were made according to the international guidelines (ARIA and GINA) (12,13). Patients who underwent immunotherapy, had acute upper or lower airway infection within the previous 4 weeks, had any other respiratory disease (e.g., bronchiectasis, chronic obstructive pulmonary disease), were during pregnancy or lactation, and currently smoking were excluded. Antihistamines, leukotrienes receptor antagonists and inhalation or systemic corticosteroids were withheld for at least 2 weeks prior to the study.

Study design

After screening, participants attended the research center on 3 consecutive days. During the first visit, anterior rhinoscopy, fractional exhaled nitric oxide (FeNO), nasal lavage, spirometry and Mch bronchial provocation test (Jaeger, Germany), and induced sputum (30 minutes after bronchial provocation test, when forced expiratory flow in one second (FEV_1) restored to baseline after inhalation of 200 μg salbutamol) were performed. In the second visit, participants underwent FeNO measurement, LTD4 nasal challenge, Mch bronchial provocation test (30 minutes after LTD4 nasal challenge), nasal lavage, and induced sputum (30 minutes after the bronchial provocation test when FEV_1 recovered to pre-challenge level following inhalation of 200 μg salbutamol). In the third visit (24 hours after nasal challenge test), all subjects underwent FeNO, nasal lavage, spirometry, and induced sputum tests.

Measurements

Measurements were taken indoors with the room temperature between 20 and 25 ℃ and a constant humidity in the morning (8:00–12:00).

Prior to nasal challenge, there was a 30-minute acclimatization period for each participant. LTD4 nasal challenge tests were performed as described previously (14). Briefly, the diluents $\{[4–16]\times10^{-3}mg\cdot mL^{-1}\}$ were delivered via nasal spray in a step-wise manner with the rate of increase in nasal airway resistance (NAR) and induced symptom scores as the measurement outcomes (15). NAR was measured with an active anterior rhinomanometry by using rhinomanometer (Jaeger, Germany) according to international guideline (16). The procedures were terminated in case of a 60% increase in NAR and/or a composite symptom score reached to greater than 3 points (0 point for sneezing <3; 1 point for the score of 3–5; 2 points for the score of >5; 0 point for no rhinorrhea; 1 point for mild (<1 mL) rhinorrhea; 2 points for abundant (>1 mL) rhinorrhea; 0 point for no pruritus; 1 point for mild pruritus (palate, eyes or throat); 2 points for severe pruritus (conjunctivitis, cough, urticaria or difficult breathing) was reached or until the use of the final concentration of LTD_4

Table 1 Characteristics of the study population

Patients	Gender	Age (yr)	FEV_1%pred	NAR ($Pa \cdot s^{-1} \cdot cm^{-3}$)	Duration (yr)	
					AR	Asthma
1	F	24	77.3	0.20	12	2
2	F	26	80.1	0.17	6	3
3	F	18	80.7	0.23	5	10
4	F	27	90.2	0.15	1	1
5	F	49	78.0	0.29	40	25
6	M	38	70.4	0.25	4	3
7	F	18	93.2	0.15	9	1
8	F	43	80.8	0.22	33	10
9	F	44	73.3	0.20	24	8
10	F	28	71.2	0.26	1	1
11	F	28	78.3	0.20	8	5
12	M	30	86.8	0.17	0.5	10
13	F	32	91.8	0.19	22	25
14	F	34	84.0	0.23	7	5
15	M	20	76.9	0.24	8	0.5
M ± SD	3/12	30.6±9.5	80.9±7.1	0.21±0.04	12.0±12.1	7.3±8.0

FEV_1, forced expiratory flow in one second; NAR, nasal airway resistance; AR, allergic rhinitis.

diluent.

FeNO was measured by using NIOX MINO (Aerocrine, Sweden) according to the American Thoracic Society (ATS) guideline (17); lung function and Mch bronchial provocation tests were performed (Jaeger, Germany) according to ATS/European Respiratory Society (ERS) guidelines (18,19). All instruments met the standard guidelines of ATS/ERS and were calibrated each day.

Nasal lavage was performed with the patient's head forward. A 10 mL saline solution was injected into the nostrils and the lavage fluid was recovered. The nasal lavage fluid was immediately centrifuged, and cells were re-suspended for cytology staining. Differential cells counts were performed on haematoxylin-eosin stained slides. Sputum was induced by 3–5% hypertonic saline which was nebulized for 20 minutes. Cell viability was determined using the trypan blue staining approach. Samples with cell viability >70% and squamous cell <20% were considered of adequate quality. Differential inflammatory cells counts were performed by counting 400 cells on haematoxylin-eosin slides.

Statistical analysis

Statistical analysis was performed by using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Data with normal distribution were expressed as mean ± standard deviation ($\bar{x}$±s); otherwise median (interquartile range). Paired *t*-tests and one-way ANOVA tests were performed for comparison of the differences of $PD_{20}FEV_1$-Mch, FeNO, and inflammatory cells counts in nasal lavage and sputum before and after nasal challenges.

Results

A total of 18 subjects were enrolled in this study, 3 subjects withdrew due to their unwillingness to complete the LTD4 nasal challenge or Mch bronchial provocation tests. Fifteen asthmatic patients with AR (male/female: 3/12 cases) completed the study (*Table 1*).

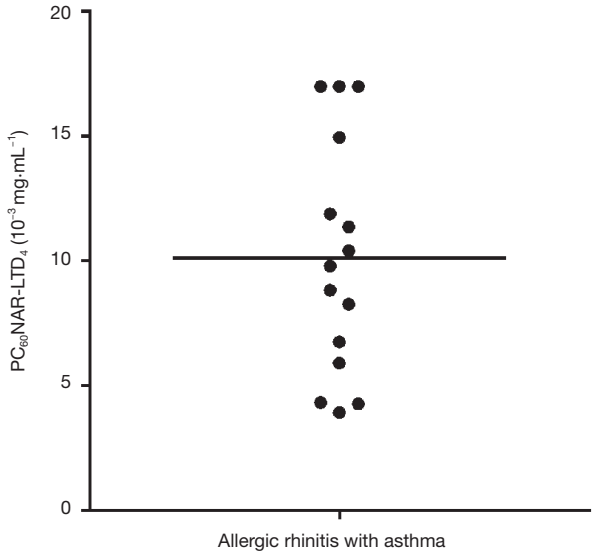

Figure 1 Distribution of $PC_{60}NAR\text{-}LTD_4$. Three subjects who tested negative to LTD_4 provocation were assigned a value of 17.00×10^{-3} mg·mL^{-1} for $PC_{60}NAR\text{-}LTD_4$. NAR, nasal airway resistance; LTD_4, leukotriene D_4.

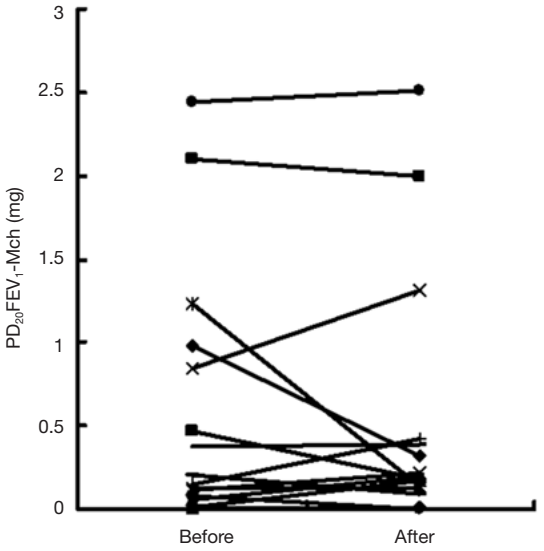

Figure 2 Distribution of $PD_{20}FEV_1$-Mch before and after LTD_4 nasal provocation. The mean $PD_{20}FEV_1$-Mch decreased non-significantly after LTD_4 nasal challenge (P>0.05). $PD_{20}FEV_1$, 20% decrease in forced expiratory flow in one second; LTD_4, leukotriene D_4; Mch, methacholine.

Table 2 Spirometric parameters before and after nasal LTD_4 provocation

Parameters	Before	After	P
FVC (pred%)	89.25±8.94	88.43±9.39	0.31
FEV_1 (pred%)	80.20±8.50	79.84±8.82	0.76
PEF (pred%)	85.43±14.27	83.38±14.62	0.15
MMEF (pred%)	52.69±16.01	51.22±15.74	0.44
MEF50%	53.90±14.83	53.13±14.78	0.70
MEF25%	53.79±20.46	51.07±19.00	0.22
$PD_{20}FEV_1$ (μmol)	3.05±3.81	2.70±3.81	0.45

FEV_1, forced expiratory flow in one second; LTD_4, leukotriene D_4; MMEF, maximum mid-expiratory flow; MEF50%, maximal expiratory flow at 50%; MEF25%, maximal expiratory flow at 25%; $PD_{20}FEV_1$, 20% decrease in FEV_1.

Dust mites were the major category of allergens tested in this study (10/15 of subjects were sensitive to house dust mite). In total, 80% (12/15) of subjects tested positive to LTD4 nasal challenge with a mean $PC_{60}NAR$ of $(8.39\pm3.48)\times10^{-3}$ mg·mL^{-1} (*Figure 1*) and induced symptom score of 0.75±1.29. The distribution of $PD_{20}FEV_1$-Mch before and after LTD_4 nasal challenges are shown in *Figure 2*. $PD_{20}FEV_1$-Mch decreased non-significantly after LTD_4 nasal challenges (3.05±3.81 *vs.* 2.70±3.81 μmol, P=0.45). There were no significant changes in FEV_1, maximum mid-expiratory flow (MMEF), maximal expiratory flow at 50% (MEF50%), and maximal expiratory flow at 25% (MEF25%) before and after LTD_4 nasal challenges (*Table 2*).

The eosinophil counts were (38.36±23.14)% and (45.70±24.86)% in nasal lavage, and (17.51±11.05)% and (24.29±16.52)% in induced sputum before and 24 hours after nasal challenge. No significant correlation was found between the changes in sputum eosinophil counts and the changes in $PD_{20}FEV_1$ for Mch after nasal challenge (Pearson correlation: r=–0.418, P=0.155). There were no significant changes of inflammatory cell counts in nasal lavage and induced sputum before and after LTD_4 nasal challenge (P>0.05). The values of FeNO, eosinophil counts and neutrophil counts in nasal lavage and induced sputum before and after LTD_4 nasal challenges are shown in *Table 3*.

Discussion

In this study, most subjects (80%) tested positive to LTD_4 nasal challenge with the nasal responsiveness (increased nasal resistance and more prominent symptoms) consistent

Table 3 FeNO and inflammatory cell counts in nasal lavage and sputum on three consecutive days

Variables	D 1	D 2	D 3	P
FeNO (ppb)	40 [35]	42 [26]	43 [30]	0.93
Nasal lavage (%)				
Eos	38.36±23.14	42.38±37.44	45.70±24.86	0.32
Neu	60.64±23.14	57.13±36.94	53.30±24.46	0.39
Induced sputum (%)				
Eos	17.51±11.05	20.57±15.51	24.29±16.52	0.51
Neu	53.83±23.27	60.72±24.35	56.19±22.28	0.69

FeNO, nitric oxide.

with those reported in previous studies (20,21), which supported the theory that CysLTs play a vital role in the pathogenesis of AR. Moreover, it has also been proven that LTD_4 effectively elicited bronchospasm in asthmatic patients in an attempt to identify leukotriene responsiveness subtypes (22,23). AR and asthma is a continuum of the inflammation involving one common airway (2), and the patients with coexisting AR and asthma might be the ideal candidates for initiating anti-leukotriene therapy (24,25).

LTD_4 nasal provocation tests were safe for asthmatics, because LTD_4 would mostly be deposited in the upper airways when properly performed. In our study, no significant differences were found in spirometric parameters (FEV_1, MMEF, MEF50% and MEF25%) before and after LTD_4 nasal challenges, which was consistent with the previous findings (26). Our results showed that the $PD_{20}FEV_1$-Mch decreased non-significantly after LTD_4 nasal challenges. Corren and his colleagues have reported that nasal challenge with allergen might induce an increase in BHR to Mch compared to that of placebo (6). Our data suggested that although CysLTs play a vital role in the pathogenesis of AR and asthma, it may not be capable of increasing the BHR after nasal allergen challenge. Neurogenic inflammation, nasal-bronchial reflex and other factors may have accounted for the increase in BHR after nasal allergen challenges (27).

CysLTs are potent lipid mediators which recruit inflammatory cells in AR and asthma (9,28). In the present study, the percentages of eosinophils in sputum and nasal lavage increased non-significantly after nasal LTD_4 challenge. It also has been reported that, compared with allergens, LTD_4 inhalation challenge did not increase the number of sputum eosinophils in asthmatic patients (28). Several reasons may have accounted for the BHR after nasal allergen challenge. First, the season of nasal challenge or natural allergen exposure may have activated the airway inflammatory cells, as reported by Marcucci *et al.* (26). Second, the significant local nasal inflammation leading to a generalized systemic immune stimulation is essential for the increase in bronchial responsiveness. Third, the provocative agents or allergens may have stimulated the inflammatory cells after nasal challenge (29). Apart from these, the percentages of inflammatory cells may vary with time after nasal challenges, however, in this study the inflammatory cells have not been measured continually at different time points after nasal challenge.

There were several limitations of this study. First, the sample size might not be sufficiently powered for comparisons of all individual parameters. Second, the inflammatory mediators in nasal secretions were not assessed at different time points after nasal challenges. Third, we lacked a control group of patients with AR without asthma which could be useful to investigate how LTD_4 nasal challenge impacts on airway inflammatory cell counts.

In conclusion, although the majority of asthmatic patients with AR tested positive to LTD_4 nasal challenge, no remarkable difference in BHR could be observed after nasal challenge. LTD_4 might not have elicited an increase in bronchial responsiveness following nasal allergen challenge. Eosinophil recruitment after LTD_4 nasal challenge needs to be further studied in asthma.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (No. NCT01963741) and written informed consent was obtained from all patients.

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