Evaluation of eicosanoids in nasal lavage as biomarkers of inflammation in patients with allergic rhinitis

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Abstract

Introduction: Cysteinyl leukotrienes (cys-LTs), 8-isoprostane and prostaglandin E2 (PGE2) constitute fundamental mediators in allergic inflammation; therefore we wanted to determine the utility of PGE2, 8-isoprostane and cys-LT levels in nasal lavage as biomarkers of allergic inflammation.

Material and methods: Twenty-one patients with allergic rhinitis (AR) were included on the basis of a positive history of AR symptoms and positive results of skin prick tests to grass pollen allergens. The main exclusion criteria were: uncontrolled asthma, nasal polyps, respiratory infection, tuberculosis, neoplastic and autoimmune diseases, current smoking and immunotherapy. Both outside the pollen season and at the height of the pollen season, total nasal symptom score (TNS-4) was evaluated and the levels of cys-LTs, 8-isoprostane and PGE2 were measured in nasal lavage fluid (NALF).

Results: Natural allergen stimulation resulted in a significant increase of TNS-4 (p < 0.001) and nasal eosinophilia (p < 0.001). The concentration of PGE2 dominated in the NALF outside the pollen season and decreased significantly at the height of natural exposure (p < 0.01). In contrast, lower baseline concentrations of cys-LTs and 8-isoprostane increased significantly upon allergen stimulation (p < 0.05). There was a significant correlation between mean concentration of PGE2 and eosinophil number in NALF (r = 0.67, p = 0.0439).

Conclusions: The NALF concentrations of cys-LTs and 8-isoprostane change simultaneously with TNS-4 and nasal eosinophilia. However, due to the lack of any significant correlation, their utility as markers of allergic rhinitis should be warily considered. The decrease of PGE2 concentration in NALF which correlated with nasal eosinophilia may participate in escalation of allergic inflammation and needs further evaluation.

Key words: nasal lavage, allergic rhinitis, leukotrienes, prostaglandins, eicosanoids, biomarkers.

Introduction

There have been many attempts to find non-invasive and simple to execute methods for evaluation of respiratory diseases. Determining the concentration or activity of biological markers in materials obtained from patients or people with risk factors was used in the diagnosis of sarcoidosis, interstitial lung diseases, lung cancer or chronic obstructive pulmonary disease (COPD) [1–4]. Finding specific and sensitive markers of the disease would allow rapid diagnosis, assessment of the disease stage,
and estimation of the response to treatment. Unfortunately, in allergic rhinitis such specific markers of airways inflammation have not yet been clearly defined.

However oxidative stress is increased [5] and cyclooxygenase-2 (COX) and 5-lipoxygenase (5-LOX) are overexpressed in the inflammatory milieu [6, 7]. Therefore we hypothesized that 8-isoprostane, prostaglandin E2 (PGE2) and cysteinyl leukotrienes (cys-LTs), which constitute the end products of nonenzymatic and enzymatic metabolism of arachidonic acid, would be detectable in nasal lavage collected from patients with allergic rhinitis and could serve as markers of allergic inflammation in the nasal mucosa.

In this study we assessed the levels of PGE2, cys-LTs and 8-isoprostane in the nasal lavage of patients with seasonal allergic rhinitis at baseline, outside the pollen season and during natural allergen stimulation, at the height of the pollen season. In addition we wanted to assess the relationship between the levels of PGE2, cys-LTs, 8-isoprostane and the symptom score as well as nasal eosinophilia in patients with seasonal allergic rhinitis.

Material and methods

In this single center, open study, we enrolled 21 patients with seasonal allergic rhinitis (AR). Allergic rhinitis was diagnosed in accordance with the allergic rhinitis and its impact on asthma guidelines [8]. All patients had to have at least a two-year history of pollinosis due to the sensitization to grass pollen allergens with the positive skin prick test (SPT) result and high concentration of IgE specific to grass pollen. Well-controlled asthma only with short acting inhaled beta agonists did not constitute an exclusion criterion. The main exclusion criteria comprised: asthma treated with inhaled or systemic glucocorticosteroids, topical or systemic treatment with glucocorticosteroids due to other disease, nasal polyps, respiratory tract infection for at least 4 weeks preceding the study, breast feeding, pregnancy, tuberculosis, neoplastic and autoimmune diseases, current smoking and specific allergen immunotherapy.

After obtaining written consent from each participant, the patients were asked to withhold any anti-allergic therapy for 2 weeks prior to the study (a 2-week run-in) and were supplied with the diaries. The patients were to record the symptoms every day, with particular consideration of the symptoms of allergic rhinitis. Then, at the first visit still outside the pollen season, the diaries were evaluated, physical examination and spirometry were performed, and nasal lavage was collected. Next the patients were asked to fill in their diaries during the pollen season when the predicted concentration of pollen in the air was the highest: 2 weeks before, at the height of the pollen season, and 2 weeks after the peak of the pollen season. The course of the pollen season (start, the height, and termination) was predicted on the basis of retrospective analysis of the grass pollen concentration in the air during the last 5 pollen seasons.

At the height of the pollen season, the patients underwent physical examination, and nasal lavage (NAL) was collected. The characteristics of the study population are presented in Table I.

The study protocol was approved by the local Ethics Committee.

Collection of nasal lavage

Collection of nasal lavage was performed as previously described [9]. Briefly, a syringe with gum rubber serving as a seal was connected to one nostril, and 5 ml of sterile, isotonic NaCl at room temperature was instilled into the nasal cavity. The patients were asked to sit motionless with the head bent to the chest for 5 min. Then, fluid was aspirated to the syringe by gentle suction. The procedure was repeated in the opposite nostril.

Lavage was centrifuged (10 min, 300xg, room temperature) and the supernatant was collected for further analysis. The cells were counted with the light microscope and the numbers of eosinophils were presented as a percentage of total cell count.

Leukotrienes

The lavage concentration of cysteinyl leukotrienes (cys-LTs) was examined using an enzyme immunoassay (EIA) kit (Cayman Chemical, MI, USA) as previously described [2]. The minimum detectable concentration was 13 pg/ml [10].

8-Isoprostanee

8-Isoprostane concentration in nasal lavage was determined with the EIA kit (Cayman Chemical, MI, USA) as previously presented [3] with an antiserum which had 100% cross-reactivity with 8-isoprostane, 0.77% with prostaglandin F1, 0.66% with prostaglandin F3, and 0.31% with prostaglandin E1. The detection limit of the assay was 5 pg/ml.
Prostaglandin E₂

To measure the concentration of prostaglandin E₂ in nasal lavage the EIA kit (Cayman Chemical, MI, USA) was used with an antiserum which had 100% cross-reactivity with PGE₂, 43% with PGE₃, 18.7% with PGE₁, and 0.1% each with PGF₂α, PGA₁, and PGA₂. The minimum detection limit was 1.5 pg/ml.

Lung function test

Pulmonary functions were measured in all participants at the screening visit, outside the pollen season, with a computer-assisted spirometer (Lung Test 1000, Mes Dymek, Poland) according to American Thoracic Society guidelines [11]. The best value of three maneuvers was noted and expressed as a percentage of the predicted value. The methacholine challenge test was performed as previously described [12], and the concentration of methacholine which produced a 20% fall of forced expiratory volume in 1 s (FEV₁) was calculated (PC₂₀). Patients with PC₂₀ lower than 8 mg were considered to have bronchial hyperresponsiveness.

Symptom score

Both outside the pollen season and during the symptomatic period evoked by natural allergen stimulation, the patients were asked to write down AR symptoms every day. The symptoms included nasal itching, sneezing, nasal blockage and rhinorrhea, each graded 0–3 (0 no symptoms, 1 mild symptoms with minimal inconvenience, 2 moderate symptoms, and 3 severe symptoms that interfere with everyday activity and/or sleep). Then, the combined result which sums the scores of each symptom was calculated and presented as the total nasal symptom score (TNS-4). The maximum TNS-4 was 12.

Skin prick tests and specific IgE

Skin prick tests were performed with a commercially available set of allergens (Allergopharma J. Ganzer KG, Reinbeck, Germany), with histamine (10 mg/ml) as a positive control and diluent as a negative one, while the serum concentration of IgE to grass pollen was determined using the Pharmacia UniCap System according to the manufacturer’s protocol (Pharmacia & Upjohn, Diagnostics AB). IgE levels had to be higher than 1.5 kU/l. The sensitivity for detection was 0.15 kU/l.

Statistical analysis

For normally distributed data, values were expressed as the mean ± standard error of the mean, whereas for data which were not normally distributed, the values were presented as the median with 25th and 75th percentiles. A Wilcoxon test was used to compare paired results that were not normally distributed, and correlations between variables were evaluated by Spearman’s test. With a sample size of 21 and α = 0.05 as well as a sigma value adequate to each parameter assessed in the study, the power of the test reached 100%. Values of p < 0.05 was considered significant (GraphPad Prism 5, San Diego, CA).

Results

The levels of cys-LTs were below the detection limit in 4 patients, whereas the concentration of 8-isoprostane was below the detection limits in 1 patient outside the pollen season and in 5 patients at the height of the pollen season. Prostaglandin E₂ was not detected in 3 unexposed patients.

Outside the pollen season the concentration of PGE₂ was the highest in nasal aspirate and the concentration of 8-isoprostane was the lowest when compared with other mediators (Table II, Figure 1).

Concentrations of cys-LTs and 8-isoprostane in nasal lavage increased significantly at the height of the pollen season (p < 0.05 for both cys-LTs and 8-isoprostane), whereas the levels of PGE₂ significantly decreased during the pollen exposure (p < 0.01) (Table II, Figure 1), and then it was detectable only in 10 patients.

The cellular profile of nasal lavage was as follows: neutrophils 21.6 ±4.67%, macrophages 0%,

|                | cys-LTs | PGE₂ | 8-isoprostane | Eosinophils (%) |
|----------------|---------|-------|---------------|-----------------|
|                | A       | B     | A             | B               | A       | B       | A       | B      |
| AR patients    |         |       |               |                 |         |         |         |        |
|                | 98.14 ±13.4 | 153.1 | 159.9 ±27.9 | 91.95 ±25.15 | 32.14   | 43.39   | 6.91 ±0.79 | 10.76 |
|                | 78      | ±20.1 | ±27.9        | ±3.6           | ±5.0    | ±0.79   | ±1.28    |
|                | (36.5–153.5) |       | (0–200)      |                |         |         |          |

*p < 0.001.
lymphocytes 6.6 ±1.4%, monocytes 0.05 ±0.05%, 
eosinophils 6.9 ±0.79%, basophils 0, epithelium 
65.5 ±5.7%. Natural stimulation with grass pollen 
resulted in a prompt increase in the number of na-
sal eosinophils (p < 0.001) (Table II).

There was a strong positive correlation be-
tween PGE 2 in nasal lavage and the number of 
eosinophils at the height of the pollen season 
(r = 0.67, p = 0.039). We did not find any correlation 
for cys-LTs or for 8-isoprostane (r = 0.106, 
p = 0.68; r = 0.13, p = 0.61 for cys-LTs and 8-isopros-
tane respectively) (Table III).

We found no correlation between the values of 
FEV1 and concentrations of eicosanoids.

Finally, natural pollen exposure resulted in a sig-
ificant increase of the mean TNS-4 score (3.04 ±2.23, 5.65 ±2.97, p < 0.001 for baseline and the 
hop of the pollen season respectively); however, 
we did not find any correlation between TNS-4 and 
eicosanoid concentrations.

Discussion

The nasal mucosa is the first protective barri-
er of the body providing a platform for the first line immunologic responses. Being metabolically 
active, mucosal epithelium has been shown to 
regulate repair and contribute to inflammatory 
processes. In this context, the epithelium releases 
a package of growth factors, adhesion molecules 
and pro-inflammatory cytokines [13] as well as 
potent anti-inflammatory mediators such as ni-
tric oxide (NO) and PGE2. Inflammatory cells (eo-
sinophils, lymphocytes, mast cells) that reside in 
or infiltrate the mucous membrane intensify the 
allergic response and through rapidly released 
(histamine) or synthesized mediators (cytokines, 
metabolites of arachidonic acid) produce severe 
symptoms of rhinitis that may significantly dete-
riorate patients’ quality of life [14]. Since allergic 
rhinitis is the most common allergy in urbanized 
countries, there is a great need for new, specific 
and validated biomarkers that are predictive of 
allergic inflammation severity in the airway epi-
thalmium and could help to determine treatment.

Although the measurement of NO in the air ex-
haled orally may serve as a useful marker in air-
way inflammation monitoring in asthma [15, 16], 
up till now there are no standardized and reliable 
methods for NO measurement in the nose [17]. 
Furthermore, function tests that measure nasal 
patency or volumes may identify nasal congestion, 
which is one, selected symptom of allergic 
rhinitis, which poorly reflects the intensity of in-
flammation [18]. In this context, metabolites of 
arachidonic acid seemed to be potential markers 
of inflammation in allergic rhinitis.

It has been demonstrated that cys-LTs, 8-isopros-
tane and PGE2 found in the airways of AR 
patients [19, 20] contribute to the progression 
and modulation of the allergic response. Cys-LTs 
are potent proinflammatory lipid mediators which 
increase nasal blood flow and escalate leukocyte 
accumulation, their activation and survival at the 
site of inflammation [21]. 8-Isoprostane origi-
nates as a dominant secondary product of lipid 
peroxidation, and its measurement reflects quan-
titatively the activity of oxidative stress in the 
inflammatory milieu [22, 23]. Contrariwise, PGE2,
which is both a pro- and an anti-inflammatory me-
diator in allergic inflammation, is believed to exert 
a suppressive function [24]. Prostaglandin E2, not 
only inhibits leukocytechemotaxis and release of 
mediators but also enhances epithelial cell surviv-
al [21], regulates production of proinflammatory 
cytokines [25] and up-regulates some anti-inflammatory 
cytokines, including IL-10 [26]. Prostaglan-
din E2 regulates angiogenesis [27] and relaxes air-
way smooth muscle [28]. When inhaled, it protects 
against bronchoconstriction [29].

Cys-LTs and 8-isoprostan concentrations were 
found to be elevated in exhaled breath condens-
sate (EBC) and bronchoalveolar lavage fluid (BALF)
in patients with asthma, lung cancer [30], and sarcoidosis [4], as well as in EBC [31] and nasal lavage [31, 32] in patients with seasonal allergic rhinitis. Prostaglandin E, was detected in EBC and BALF of patients with sarcoidosis [30], chronic obstructive pulmonary disease [19] and lung cancer [5, 6, 27], and in nasal lavage in patients with allergic rhinitis [20].

Our study demonstrates that cys-LTs, 8-isoprostane and PGE, are detectable in nasal lavage and their concentrations significantly vary during natural allergen stimulation. While baseline status is associated with low NAL concentrations of 8-isoprostane and cys-LTs and high concentration of PGE, natural allergen stimulation reverses this state, increasing cys-LTs and 8-isoprostane simultaneously reducing PGE.

Inflammatory cells within the nasal mucosa of AR patients are in an activated state and have the capability of generating leukotriene mediators that are also of potential relevance to symptom generation. The immediate nasal response raises cys-LTs and 8-isoprostane levels in nasal lavage fluids [33], which is accompanied by deterioration of nasal symptoms. The patient’s recovery is then paralleled by a decline of NAL concentrations of lipid mediators [34]. Our study also showed that the intensification of symptoms was accompanied by an increase of cys-LTs and 8-isoprostane levels in NAL, whereas the asymptomatic period was associated with a significant decrease of both mediators. A noteworthy fact is that allergen stimulation resulted in a significant decrease of PGE levels, whereas a lack of stimulation restored PGE concentrations. Our study has demonstrated this for the first time. It is in contrast with the results of Sugimoto’s study [20], where nasal provocation tests resulted in a significant increase of NAL PGE levels. Furthermore, simulation with nonspecific irritants (cold, dry air) resulted in a significant increase of PGE in healthy subjects [35]. However, Koren did not find such an increase in patients stimulated with ozone [36]. The results of these studies may support a dual role of PGE in inflammation. Since natural stimulation with an allergen resulted in a decrease of PGE, the same time as severe symptoms and nasal eosinophilia, it is plausible that the impairment of biosynthesis of this prostaglandin could be an additional cause of exacerbation.

In conclusion, as we have demonstrated, natural allergen stimulation increases both cys-LT and 8-isoprostane concentrations in NAL as well as nasal eosinophilia and the symptom score in AR patients. The decrease in concentration of PGE, which possesses anti-inflammatory properties, at the height of the pollen season may constitute another background for an outbreak of inflammation and strengthen AR symptoms. As we did not find any significant positive correlation between the levels of 8-isoprostane, cys-LTs in NAL and values of the TNS-4 score and nasal eosinophilia, the usefulness of measurements of these lipid mediators as indices of inflammation severity should be considered warily. Furthermore, targeting the PGE-dependent signals in the airways should be better evaluated, since it may constitute another new therapeutic strategy.

Acknowledgments

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