Allergology International 66 (2017) 83–88

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Imbalance of endogenous prostanoids in moderate-to-severe asthma

Masaya Takemura [a], Akio Niimi [a, b, *], Hisako Matsumoto [a], Tetsuya Ueda [a], Masafumi Yamaguchi [a], Hirofumi Matsuoka [a], Makiko Jinnai [a], Kian Fan Chung [c], Michiaki Mishima [a]

[a] Department of Respiratory Medicine, Kyoto University, Kyoto, Japan
[b] Department of Respiratory Medicine, Allergy and Clinical Immunology, Nagoya City University, School of Medical Sciences, Nagoya, Japan
[c] Thoracic Medicine, National Heart & Lung Institute, Imperial College, London, UK

ARTICLE INFO

Article history:
Received 13 February 2016
Received in revised form
11 May 2016
Accepted 21 May 2016
Available online 12 July 2016

Keywords:
Asthma
Epithelial damage
Induced sputum
Prostaglandin
Thromboxane

List of abbreviations used:

PG	Prostaglandin
TX	Thromboxane
$FEF_{25-75\%}$	Mid-forced expiratory flow

ABSTRACT

Background: Inhalation studies suggested "protective" roles of exogenous prostaglandin E_2, but the clinical relevance of endogenous prostanoids in asthma is poorly known. The objective of this study is to measure sputum levels of prostanoids in asthmatic patients to correlate with clinical indices.
Methods: Mild ($n = 41$) or moderate-to-severe (19) asthmatics and 27 normal controls were examined for pulmonary function (FEV_1 and mid-forced expiratory flow), sputum cell differentials, and sputum levels of prostaglandins D_2, E_2, $F_{2\alpha}$, and thromboxane B_2 measured by sandwich enzyme immunoassay.
Results: Each prostanoid level did not differ among the three groups. Sputum number of bronchial epithelial cells was greater in moderate-to-severe asthmatics than in the other two groups, suggesting epithelial desquamation. Levels of prostaglandin $F_{2\alpha}$, D_2, and thromboxane B_2 positively correlated with the severity of airflow obstruction in the 60 asthmatic patients, whereas prostaglandin E_2 levels were unrelated to pulmonary function. The ratio of combined "contractile" prostanoids (prostaglandin D_2/prostaglandin $F_{2\alpha}$/thromboxane B_2) to prostaglandin E_2 was 2.5-fold greater in moderate-to-severe asthmatics than in controls ($p = 0.001$) or in mild asthmatics ($p = 0.0002$) but did not differ between the latter two groups. In the two asthmatic groups combined, this ratio positively correlated with the sputum number of epithelial cells. The combined "contractile" prostanoids levels positively correlated with prostaglandin E_2 levels in controls and in mild asthmatics but not in moderate-to-severe asthmatics.
Conclusions: An imbalance in production, breakdown, or both between prostaglandin E_2 and other prostanoids possibly due to epithelial damage may be involved in the pathogenesis of moderate-to-severe asthma.

Introduction

Asthma is a chronic inflammatory disease of the airways, which involves inflammatory mediators such as histamine, cysteinyl leukotrienes, platelet-activating factor and prostanoids.[1] Prostaglandin (PG) D_2, $PGF_{2\alpha}$, and thromboxane (TX) A_2 have contractile effects on airway smooth muscle *in vitro*,[2,3] and cause bronchoconstriction in asthmatic subjects when inhaled.[4,5] These prostanoids may thus exert deleterious effects in the pathophysiology of asthma. In contrast, inhaled PGE_2 attenuates allergen-induced early and late asthmatic responses, airway hyperresponsiveness, and inflammation characterized by the increased number of eosinophils.[6] Inhaled PGE_2 also protects against aspirin-induced exacerbation of asthma through mechanisms unrelated to its bronchodilatory activity.[7] PGE_2, when exogenously administered, may thus exert bronchoprotective and anti-inflammatory effects. Despite PGE_2 is contractile via EP1 and EP3 receptor in mice and human whereas relaxant via EP2 receptor in mice and human and EP4 in human,[8,9] the net effect of PGE_2 is therefore considered "inhibitory". Though a variety of cells have the capacity to release prostanoids in the asthmatic airways,[10] PGD_2, and its metabolite, 9α,11β-PGF_2 are primarily mast cell products,[11] while PGE_2 is primarily a product of epithelial cells.[1] Desquamation or damage of epithelial cells may be characteristic of more severe asthma.[12–14]

* Corresponding author. Department of Respiratory Medicine, Allergy and Clinical Immunology, Nagoya City University, School of Medical Sciences, Mizuho-ku, Nagoya 467-8601, Japan.
E-mail address: a.niimi@med.nagoya-cu.ac.jp (A. Niimi).
Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2016.05.013

The levels of endogenous prostanoids in the airway surface liquid of asthmatic patients and healthy controls have been examined in samples of bronchoalveolar lavage, sputum, and exhaled breath condensate.[15–21] Previous studies have suggested that airway levels of some prostanoids are increased in subsets of asthmatic patients, such as smokers,[19] but failed to show a relation to asthma severity or activity.

We measured sputum levels of PGD_2, PGE_2, $PGF_{2\alpha}$, and TXB_2 in a large number of nonsmoking, asthmatic patients and healthy controls. These levels were compared among patients with mild asthma, those with moderate-to-severe asthma, and controls, and were examined with respect to pulmonary function in the patients. We then compared the ratio of combined "contractile" prostanoids (PGD_2, $PGF_{2\alpha}$, and TXB_2), each of which was associated with airflow obstruction in the patients, to PGE_2 levels among the three groups, on the hypothesis that the ratio of constrictor to dilator prostanoids are increased in asthma dependent on asthma severity.

Methods

Study design

This was a cross-sectional study. To investigate the role of endogenous prostanoids in asthma, we measured sputum levels of PGD_2, PGE_2, $PGF_{2\alpha}$, and TXB_2 in steroid-naïve asthmatic patients to correlate with clinical indices.

Subjects

Sixty asthmatic and 27 healthy subjects including members of our hospital staff, from whom adequate sputum samples were obtained, were studied between March 2002 and June 2005. Asthma was diagnosed according to the Global Initiative for Asthma.[22] The inclusion criteria of asthmatic patients were as follows: symptomatic but without exacerbations during the previous one month, no history of aspirin-sensitive asthma or nasal polyps, and taking only short-acting inhaled beta-2 agonists as needed. They were steroid-naïve asthmatics or those who had been given inhaled corticosteroid but voluntary discontinued it for more than one month before presentation to our clinic. For patients who fulfilled the entry criteria, sputum induction was performed followed by asthma therapy including inhaled corticosteroid. After the minimal medication required to maintain control had been determined, the severity of asthma was subsequently evaluated and classified as mild for 41 patients (steps 1 and 2) and moderate-to-severe for 19 patients (steps 3 and 4).[22]

During the month before the study, no subject, including control, had a respiratory tract infection, or had taken any anti-leukotriene drugs, thromboxane synthase inhibitors or receptor antagonists, cyclooxygenase inhibitors, or angiotensin-converting-enzyme inhibitors. All participants were lifetime nonsmokers, and had no evidence of COPD.

The study was approved by our Institutional Review Board (the ethics approval number: E-715), and written informed consent was obtained from all subjects.

Induced sputum production and processing

Sputum induction and processing were performed as described.[23–25] Briefly, the subjects premedicated with 200 mcg of salbutamol inhaled hypertonic (3%) saline solution for 15 min, delivered by an ultrasonic nebulizer (MU-32, Azwell, Osaka, Japan). Patients were then asked to try to cough sputum into a plastic petri dish. No significant bronchoconstriction was observed during the procedure.

All adequate plugs of sputum were separated from saliva and were weighed. The plugs were treated with 0.1% dithiothreitol (DTT) (Sputasol™, OXOID, Hampshire, UK), 2 times the weight of the sputum sample. The samples were then treated with the same volume of Dulbecco's phosphate buffered saline. After centrifugation at 1000 g for 10 min, the supernatants were stored at −80 °C.

The cell pellet was resuspended in PBS solution. The total cell count, excluding squamous cells, was determined with a standard hemocytometer and expressed as cells $\times 10^5$/g wet weight sputum. Then the cells were centrifuged and stained by the May-Grünwald-Giemsa method. Cell differentials were determined by counting at least 400 non-squamous cells.

Measurement of sputum levels of inflammatory mediators

As we previously described,[25] concentrations of PGD_2, PGE_2, $PGF_{2\alpha}$, and TXB_2 in the sputum supernatant were measured with the use of commercially available sandwich enzyme immunoassay kits (PGE_2: Amersham Biosciences, NJ, USA; PGD_2-methoxime, $PGF_{2\alpha}$, TXB_2: Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturers' instructions. Duplicate measurements were averaged for analysis. Coefficient of variation for the duplicate measurements was 3.9 (2.1–5.8) % for PGE_2. Because PGD_2 and TXA_2 are both relatively unstable compounds, we measured PGD_2-methoxime (PGD_2-MOX) and TXB_2, stable derivatives of PGD_2 and TXA_2,[18,25,26] respectively. The detection limit was 40 pg/ml for PGE_2, 8 pg/ml for $PGF_{2\alpha}$, 3.1 pg/ml for PGD_2-MOX, and 13 pg/ml for TXB_2. The results were presented as per gram of sputum.

Pulmonary function

Pre-bronchodilator values of FEV_1, and mid-forced expiratory flow ($FEF_{25-75\%}$), were measured using a spirometer (Chestac-65V™, Chest, Tokyo, Japan) before sputum induction.[27]

Statistical analysis

Data are expressed as medians (25th–75th percentiles), and analyzed with the StatView 5.0 program (SAS Institute, Cary, NC, USA). The Mann–Whitney U-test or Fisher's exact probability test was performed to compare two groups. Comparison of three groups was made by the Kruskal–Wallis test followed by Mann–Whitney U-test, ANOVA followed by Fisher's PLSD test, or chi-square test as appropriate. Spearman's rank correlation test was used to analyze correlations. P values < 0.05 were considered statistically significant.

Results

Characteristics and outcome of asthmatic patients and control subjects

The characteristics of the control subjects and the two asthmatic groups are shown in Table 1. Age differed among the three groups, and the controls were significantly younger than both asthmatic groups. The distribution of sex, duration of asthma, total IgE levels, and prevalence of atopy did not differ between patients with mild asthma and those with moderate-to-severe asthma. FEV_1 and $FEF_{25-75\%}$ differed significantly among the three groups, and between each pair of the three groups.

Sputum total cell count differed among the three groups but not between any pair of groups. As compared with controls, the number of eosinophils was significantly increased in patients with mild asthma and in those with moderate-to-severe asthma, and the number of macrophages was significantly decreased in patients with