Title

Imbalance of endogenous prostanoids in moderate-to-severe asthma

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Introduction

Asthma is a chronic inflammatory disease of the airways, which involves inflammatory mediators such as histamine, cysteinyl leukotrienes, platelet-activating factor and prostanoids. Prostaglandin (PG) D2, PGF2α, and thromboxane (TX) A2 have contractile effects on airway smooth muscle in vitro, and cause bronchoconstriction in asthmatic subjects when inhaled. These prostanoids may thus exert deleterious effects in the pathophysiology of asthma. In contrast, inhaled PGE2 attenuates allergen-induced early and late asthmatic responses, airway hyperresponsiveness, and inflammation characterized by the increased number of eosinophils. Inhaled PGE2 also protects against aspirin-induced exacerbation of asthma through mechanisms unrelated to its bronchodilatory activity. PGE2, when exogenously administered, may thus exert bronchoprotective and anti-inflammatory effects. Despite PGE2 is contractile via EP1 and EP3 receptor in mice and human, the net effect of PGE2 is therefore considered "inhibitory". Though a variety of cells have the capacity to release prostanoids in the asthmatic airways, the specific role of PGE2 remains to be elucidated.

Original article

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List of abbreviations used:

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2. Nickel H, et al. Inhaled PGE2 attenuates allergen-induced early and late asthmatic responses, airway hyperresponsiveness, and inflammation characterized by the increased number of eosinophils. Inhaled PGE2 also protects against aspirin-induced exacerbation of asthma through mechanisms unrelated to its bronchodilatory activity. PGE2, when exogenously administered, may thus exert bronchoprotective and anti-inflammatory effects. Despite PGE2 is contractile via EP1 and EP3 receptor in mice and human, the net effect of PGE2 is therefore considered "inhibitory". Though a variety of cells have the capacity to release prostanoids in the asthmatic airways, the specific role of PGE2 remains to be elucidated.
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. Previous studies have suggested that airway levels of some prostanoids are increased in subsets of asthmatic patients, such as smokers, 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 PGF$_2\alpha$ levels among the three groups, on the hypothesis that the ratio of contractile 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-naive 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. 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-naive 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).

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. Briefly, the subjects prem edicated with 200 mcg of salbutamol inhaled hyperonic (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 × 10$^7$/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, 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$_2$, 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.

**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 mild asthma.
mild asthma. The number of epithelial cells was significantly greater in patients with moderate-to-severe asthma than in those with mild asthma and in controls and but was similar in the latter two groups.

There was no significant difference in sputum levels of PGD$_2$, PGE$_2$, PGF$_{2\alpha}$, or TXB$_2$ among the three groups (Table 1).

**Relationship of sputum prostanoid levels with pulmonary function in asthmatic patients**

The relations between sputum levels of prostanoids and pulmonary function in the 60 asthmatic subjects are presented in Table 2. Sputum levels of PGF$_{2\alpha}$ and TXB$_2$ negatively correlated with FEV$_1$ and FEF$_{25-75}$. Sputum levels of PGD$_2$ negatively correlated with FEF$_{25-75}$. Sputum levels of PGE$_2$ were not related to any index of airflow obstruction.

Sputum epithelial cell number did not correlate with the sputum levels of PGD$_2$ ($r = -0.03$), PGE$_2$ ($r = -0.07$), PGF$_{2\alpha}$ ($r = 0.13$), or TXB$_2$ ($r = 0.08$) ($p > 0.1$ for all).

The sputum eosinophil number did not correlate with the sputum levels of PGD$_2$ ($r = -0.03$), PGE$_2$ ($r = -0.07$), PGF$_{2\alpha}$ ($r = 0.13$), or TXB$_2$ ($r = 0.08$) ($p > 0.1$ for all). The number of macrophages, neutrophils, or epithelial cells was unrelated to any of the prostanoid levels (data not shown). In the healthy controls, the sputum levels of prostanoids did not correlate with pulmonary function or sputum cell differentials (data not shown).

**Balance between PGE$_2$ and other prostanoids**

The levels of combined PGD$_2$, PGF$_{2\alpha}$, and TXB$_2$, and their ratio to PGE$_2$ levels, were calculated in each group to examine the net effect of these “contractile” and “inhibitory” prostanoids. The absolute levels of PGD$_2$, PGF$_{2\alpha}$, and TXB$_2$ combined did not differ among controls and two asthmatic groups ($p = 0.13$ by ANOVA). However, the ratio of these levels to PGE$_2$ levels differed among the three groups, and was highly significantly elevated in moderate-to-severe asthmatics as compared with controls or mild asthmatics, but was similar in the latter two groups (Table 1, Fig. 1). When the two asthmatic groups were combined, this ratio significantly correlated with sputum number of epithelial cells ($r = 0.39$, *p* = 0.0009).

**Table 1**

Characteristics and outcome of asthmatic patients and healthy controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy control (n = 27)</th>
<th>Asthma Mild (n = 41)</th>
<th>Moderate-to-severe (n = 19)</th>
<th>p Values for 2 or 3 Groups</th>
<th>Control vs mild</th>
<th>Control vs Moderate-to-severe</th>
<th>Mild vs Moderate-to-severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>30 (25–33.8)</td>
<td>48 (32.8–66.8)</td>
<td>57 (38–63.8)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Sex (M/F)</strong></td>
<td>17/10</td>
<td>22/22</td>
<td>8/11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Duration of asthma (yr)</strong></td>
<td>1.5 (0.25–6.5)</td>
<td>2.5 (0.75–7.0)</td>
<td>2.2 (2.1–2.3)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Log IgE (IU/ml)</strong></td>
<td>Not tested</td>
<td>2.2 (1.7–2.7)</td>
<td>2.2 (2.1)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Atopy/Non-atopy</strong></td>
<td>Not tested</td>
<td>30/11</td>
<td>15/4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>FEV$_1$ (%pred)</strong></td>
<td>98 (92–107)</td>
<td>91 (83–100)</td>
<td>75 (64–84)</td>
<td>&lt;0.0001</td>
<td>0.012</td>
<td>0.0001</td>
<td>0.0009</td>
</tr>
<tr>
<td><strong>FEF$_{25-75}$ (%pred)</strong></td>
<td>84 (76–96)</td>
<td>61 (45–84)</td>
<td>43 (34–48)</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

**Table 2**

Relationship between sputum levels of prostanoids and pulmonary function in 60 asthmatic patients.

<table>
<thead>
<tr>
<th>Prostanoid</th>
<th>FEV$_1$ (%predicted)</th>
<th>FEF$_{25-75}$. (%predicted)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>PGE$_2$ (ng/g)</td>
<td>0.15</td>
<td>0.26</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ (ng/g)</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td>PGD$_2$ (ng/g)</td>
<td>0.56</td>
<td>0.06</td>
</tr>
<tr>
<td>TXB$_2$ (ng/g)</td>
<td>0.27</td>
<td>0.05</td>
</tr>
</tbody>
</table>

FEF$_{25-75}$. mid-forced expiratory flow; PC, prostaglandin; TX, thromboxane.
Potential pathophysiologic roles of prostanoids in asthma have been extensively studied.1,7,10,12–18 PGD2, PGF2α, and TXA2 induce smooth muscle cell contraction and hyperplasia, as well as mucous hypersecretion in human airway preparations.2,3 In contrast, PGE2 inhibits various inflammatory events, including mast cell degranulation, leukotriene B4 production by macrophages, and eosinophil activation.29 Mice deficient in prostaglandin receptors have also been used for in vivo studies. After ovalbumin sensitization and challenge, PGD2 receptor-deficient mice failed to develop Th2 cytokines production, eosinophil infiltration, and airway hyperresponsiveness.30 In sharp contrast, mice lacking PGE2 (EP3) developed much more pronounced inflammation after ovalbumin than wild-type mice or mice deficient in other PGE2 receptor subtypes. An EP3-selective agonist suppressed the inflammation in wild-type mice.31 Other study demonstrated that EP4 receptor knock-out mice had an enhanced cellular inflammation.32 Furthermore, cell-based assays using human monocytes or eosinophils showed an inhibition of cytokine release or cyclic AMP production via activation of the EP4 receptor, suggesting an endogenous anti-inflammatory role for PGE2 acting on the EP4 receptor.33,34 In asthmatic subjects, synthase inhibitors or receptor antagonists of TX attenuate airway eosinophilia and hyperresponsiveness.32

Airway levels of endogenous prostanoids have been examined in asthmatic patients.15–21,34 Bronchoalveolar levels of PGD2, 9α,11β-PGF2α, and PGF2β, but not of TXB2, 6-keto-PGF1α, or PGE2 were higher in 15 asymptomatic patients than in 12 normal controls, but were unrelated to either airway obstruction or hyperresponsiveness.15 Sputum levels of PGD2, PGE2, PGF2α, and TXB2 were similar in 17 asthmatic patients (8 receiving inhaled corticosteroids) and 10 normal controls,17 and failed to correlate with disease severity or FEV1 values in 10 aspirin-tolerant patients or 13 aspirin-intolerant patients.11 Exhaled breath condensate levels of PGD2, PGE2, PGF2α, and TXB2 levels were similar between 15 steroid-naive asthmatics and 12 healthy controls.18 Kostikas et al.19 have examined PGE2 levels in breath condensate of smoking and nonsmoking mild asthmatics (n = 15 for each) and healthy controls (n = 10 for each). PGE2 levels were higher in asthmatic smokers than in the other two groups, but no difference was found among the latter. The increased PGE2 levels in the asthmatic smokers were
attributed to the activation of airway macrophages by cigarette smoke. We have demonstrated, for the first time to our knowledge, contrasting characteristics of endogenous prostanoïds in asthmatic patients, by correlating their sputum levels and pulmonary function. These are consistent with previous in vitro, animal, and human studies, that showed a contractile property of PdG2, PGE2α, and TXB2 but not PGE2, which may even be dilatory.5,28,30,31 Our study showed that airway obstruction was greater in moderate-to-severe asthmatics than in controls and mild asthma, and each sputum contractile prostanoïd level correlated with indices of airway obstruction. However, the combined contractile prostanoïd levels were not different among the three groups. It is difficult to answer this discrepancy but it may be due to the inhomogeneous distribution of sputum levels of contractile prostanoïds irrespective to asthma severity and the factors other than prostanoïds such as airway remodeling. Arguably, both “contractile” and “inhibitory” prostanoïds may be active in the airway asthma and their net effects should be considered. Wenzel et al. measured the bronchoalveolar lavage fluid levels of bronchoconstrictor prostanoïds (PdG2, TXB2) and bronchoprotectors (6-keto-PGF1α, PGE2) before and after allergen challenge in asthmatic patients. They demonstrated that the ratio of combined PdG2 and TXB2 to combined PGE2 and 6-keto-PGF1α increased greater than 5-fold in asthmatics after allergen challenge although the underlying mechanism was not referred.35 In the present study, the ratio of combined PdG2/PGE2α/TXB2 levels to PGE2 levels was significantly higher in moderate-to-severe asthmatics than in controls or mild asthmatics, although the levels of each prostanoïd were similar in the three groups. The levels of combined PdG2/PGE2α/TXB2 positively correlated with PGE2 levels in the controls and in the mild asthmatic group, but not in the moderate-to-severe asthmatic group. The effects of “deleterious” prostanoïds may be counterbalanced by the synthesis of “inhibitory” prostanoïd PGE2 in mild asthmatics, but such mechanism might be deficient in more severe disease. Previous studies have shown that the number of epithelial cells in bronchoalveolar lavage or sputum of asthmatic patients is elevated as compared with healthy controls,2,14 correlates with the severity of disease14 and airway responsiveness,12 and responds to intervention in parallel with clinical improvement.13 These indicate the presence, and pathophysiolegie relevance, of epithelial desquamation or damage in asthma. In our study, the sputum epithelial cell number was increased in moderate-to-severe asthmatics as compared with controls and mild asthmatics, and positively correlated with the PdG2+/PGE2α+/TXB2/PGE2 ratio in the two asthmatic subgroups combined. Epithelial damage as suggested in the latter group might be responsible for this imbalance. The effect of proinflammatory stimuli on PGE2/PGE2 production by bronchial fibroblasts of aspirin-tolerant and -intolerant asthmatic patients has been examined.36 Both prostanoïds were increasingly produced, but PGE2/PGE2 concentration ratio elevated significantly less in aspirin-tolerant patients, a severer phenotype of asthma, than in aspirin-tolerant patients.1 This imbalance in the prostanoïd production by fibroblasts may also explain our results, but fibroblasts could not be addressed by our sputum study. Other investigator also demonstrated a dysregulation of PGE2 production from alveolar macrophage from severe asthmatics.38 We found no correlation between the sputum PGE2 levels and the number of sputum macrophages in moderate-to-severe asthmatics, but cannot exclude the possibility that PGE2 synthesis of alveolar macrophage is downregulated. Our asthmatic patients were older than controls. It was very difficult to obtain an age-matching control group. To our knowledge, there is no evidence that prostanoïd production or metabolism could be affected by age. Indeed, our patients and controls showed no correlation between age and prostanoïd levels (data not shown). Ex vivo production of eicosanoïds is unlikely to have influenced our results, because treatment of sputum with agents blocking ex vivo production and breakdown of prostanoïds dose not affect their concentrations.10 In the decades, evidence has been collected demonstrating the role of prostanoïds in asthma. Recent studies have shown each prostanoïd receptor has multiple functions resulting in opposing effects, such as deleterious and inhibitory outcomes. Therefore, the current study supports the view that an imbalance of endogenous prostanoïds may play an important role in the pathophysiology of asthma. Our findings provide some insight into the mechanisms of development of severe asthma and may suggest consideration of a new therapeutic strategy of asthma.

Conflict of interest

AN has received research grants from Teijin Pharma, Astellas Pharma, and Chugai Pharmaceutical, and lecture fee from Astellas Pharma, AstraZeneca, GlaxoSmithKline, Kyorin Pharmaceutical. The rest of the authors have no conflict of interest.

Authors’ contributions

MT, AN had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. MT, H Matsumoto, and AN contributed to the study concept and design; MT, H Matsumoto, TU, MY, H Matsuo, MJ contributed to the data acquisition and analysis; MT, AN, KFC, and MM contributed to drafting the manuscript and interpretation of the data, and MT, AN, KFC, and MM contributed to the final approval of the manuscript.

References


