Allergology International. 2008;57:429-436
DOI: 10.2332 allergolint.O-08-545

ORIGINAL ARTICLE

Correlation between the Prostaglandin D_2/E_2 Ratio in Nasal Polyps and the Recalcitrant Pathophysiology of Chronic Rhinosinusitis Associated with Bronchial Asthma

Tsuyoshi Yoshimura[1], Mamoru Yoshikawa[1], Nobuyoshi Otori[1], Shin-ichi Haruna[2] and Hiroshi Moriyama[1]

ABSTRACT

Background: The prevalence of patients with chronic rhinosinusitis (CRS) refractory to traditional therapy appears to be on the increase. In these cases, CRS tends to be associated with bronchial asthma (BA), especially, aspirin-intolerant asthma (AIA). On the other hand, arachidonic acid metabolites have been extensively investigated in the pathogenesis of BA. We sought to assess the role of prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2) in the recalcitrant pathophysiology of CRS.

Methods: Samples were prepared from the nasal polyps and mucosa of 40 patients undergoing endoscopic sinus surgery (ESS) at our hospital. The nasal polyp specimens obtained from the patients with CRS were divided into three groups, as follows: the CRS-AIA group, consisting of specimens obtained from patients with CRS complicated by AIA, the CRS-ATA group, consisting of specimens obtained from patients with CRS associated with aspirin-tolerant asthma (ATA), and the CRS-NA group, consisting of specimens obtained from CRS patients without BA. PGD_2 and PGE_2 were extracted from the specimens and quantified.

Results: The concentrations of PGD_2 were significantly higher in the nasal polyps of the CRS-ATA group. The concentrations of PGE_2 were lowest in the nasal polyps of the CRS-AIA group. The PGD_2/PGE_2 ratio was highest in the CRS-AIA group.

Conclusions: It has previously been reported that CRS complicated by AIA is most likely to be characterized by repeated remissions and relapses, and is thus the most intractable. We may therefore say that the PGD_2/PGE_2 ratio reflects the intractable nature of CRS.

KEY WORDS

aspirin-intolerant asthma, bronchial asthma, chronic rhinosinusitis, prostaglandin D_2, prostaglandin E_2

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory condition of the paranasal mucous membrane, characterized by the accumulation of eosinophils, fibroblasts, mast cells and goblet cells.[1-3] Nasal polyposis is frequently associated with other conditions, such as bronchial asthma (BA),[4-6] including aspirin-intolerant asthma (AIA).[7] Recently, although there has been an increase in the cure rates of patients with CRS, with the institution of low-dose long-term treatment with macrolide antibiotics and adoption of endoscopic sinus surgery (ESS),[8,9] there still remains a substantial number of patients with a poor prognosis.[10] Such patients with a poor prognosis have been found to include a high proportion of patients with lower respiratory tract disorders, such as BA.

BA is a chronic inflammatory disorder character-

[1]Department of Otorhinolaryngology, Jikei University School of Medicine, Tokyo and [2]Department of Otorhinolaryngology, Dokkyo Medical University, Tochigi, Japan.
Correspondence: Mamoru Yoshikawa, Department of Otorhinolaryngology, Jikei University School of Medicine, 3–25–8 Nishi-shimbashi, Minato-ku, Tokyo 105–8461, Japan.
Email: yoshikawa@jikei.ac.jp
Received 19 February 2008. Accepted for publication 30 June 2008.

Table 1 Patient clinical data

	Control	CRS-NA	CRS-ATA	CRS-AIA
N	7	14	12	7
Sex (F/M)	1/6	2/12	3/9	2/5
Age average	32.43 ± 10.26	44.14 ± 16.08	43.75 ± 14.42	48.43 ± 18.75
Age range	19–46	20–71	18–65	26–71
Total IgE	101.71 ± 81.41	100.25 ± 142.65	257.91 ± 258.73	206.86 ± 374.13
Peripheral blood eosinophils (%)	3.78 ± 2.56	2.57 ± 1.26	8.33 ± 4.44	13.39 ± 9.88

ized by mast cell and eosinophilic infiltration of the airways. BA can be categorized into two distinct types according to the presence or absence of aspirin sensitivity: aspirin-intolerant asthma (AIA) and aspirin-tolerant asthma (ATA). In particular, AIA is a distinct clinical syndrome affecting a significant proportion of adult patients with BA, in whom aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) precipitate bronchoconstriction.[11,12] Patients with AIA often have a particularly severe form of BA, associated with rhinorrhea and recurrent polyp formation.[11,12]

Arachidonic acid metabolites have been extensively investigated in the pathogenesis of BA. Cysteinyl leukotrienes (CysLTs), strong proinflammatory factors generated from arachidonic acid through the 5-lipoxygenase pathway, have been recognized as the key mediators of an asthmatic attack.[13] CysLTs are components of the slow-reacting substance of anaphylaxis (SRS-A) and have been shown to have profound effects on the airways,[14,15] e.g., able to induce airway smooth muscle contraction and vasodilatation, enhance vascular permeability, and alter the remodeling process in asthma.[16,17] Prostaglandin D_2 (PGD_2) is a potent bronchoconstrictor and its effect is mediated by the TP receptor.[18] Therefore, PGD_2 may be an important mediator of airway smooth muscle contraction in an immediate asthmatic reaction.[19] Furthermore, it has recently been reported from experiments using knockout mice that PGD_2 exacerbates asthma.[20,21] In contrast, prostaglandin E_2 (PGE_2) protects airways against inflammation and promotes normal airway function.[22,23] On the other hand, PGE_2 enhances immunoglobulin (Ig) E production. PGE_2 promotes IL-4 production from Th2 cells, suppresses IFN-γ production from Th1 cells, and promotes IgE production from B cells.[24]

As described above, a large number of reports have been published concerning the involvement of arachidonic acid metabolites in the pathophysiology of BA. It seems probable that arachidonic acid metabolites are among the factors responsible for the intractable course of CRS as well. Taking into account this possibility, additionally there have been recent reports that PGD_2 and PGE_2 are closely involved in airway inflammation[25-27] and that the balance between PGD_2 and PGE_2 production determines the severity of inflammation in various inflammatory conditions.[28-30] Consequently, we undertook the present study in which patients with CRS associated with BA were divided according to whether they had ATA or AIA, and the amounts of PGD_2 and PGE_2 in the nasal polyps were compared between patients with intractable CRS complicated by ATA and those with CRS complicated by AIA.

METHODS

SUBJECTS

Nasal polyp tissue specimens were obtained at the time of surgery from subjects (n = 33) referred to Jikei University Hospital for ESS. To examine the role of the arachidonic acid metabolites in the pathogenesis of CRS, the specimens were obtained from CRS patients classified into 3 subgroups, as follows: CRS without BA (CRS-NA group; n = 14), CRS associated with ATA (CRS-ATA group; n = 12), and CRS associated with AIA (CRS-AIA group; n = 7). In addition, specimens from 7 controls were also examined (5 middle turbinate mucosa specimens obtained from patients with non-allergic rhinitis and 2 middle meatus mucosa specimen obtained from a blowout fracture patient). Informed consent was obtained from all of the patients prior to their enrollment in the study, and the study was approved by the ethics committee at the Jikei University Hospital. The clinical data of the patients are summarized in Table 1. Before the ESS, measurements of the serum IgE levels and peripheral blood eosinophil counts, as well as sinus computed tomographic (CT) studies, were performed for each patient.

CLINICAL ASSESSMENT

The diagnosis of CRS was based on the typical symptoms (nasal congestion, dysosmia, etc.) as assessed from the documented medical history, the presence of endoscopically visible nasal polyps arising from the middle nasal meatus, and involvement of the ethmoidal and maxillary sinuses as visualized in limited CT scans of the paranasal sinuses.

The diagnosis of asthma was made at the Department of Pulmonology, Jikei University Hospital, based on the documented clinical history of the typical symptoms. The diagnosis of AIA was based on a history of asthma exacerbation, nasal congestion, and

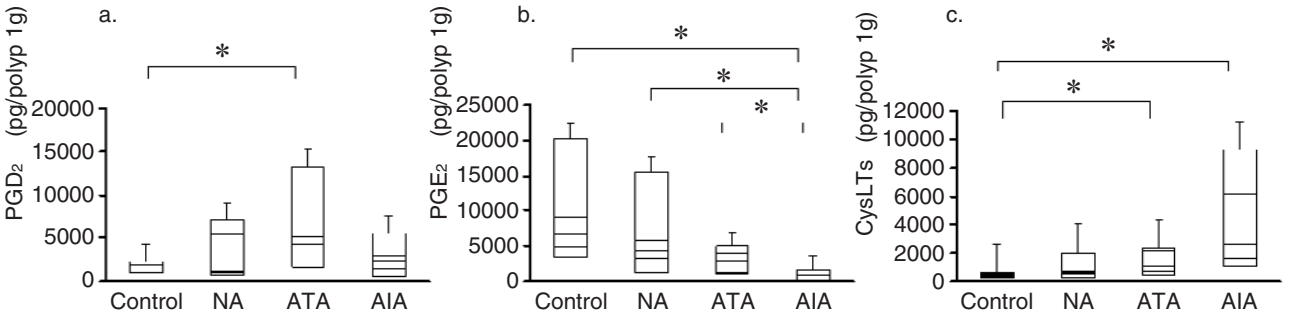

Fig. 1 PGD_2 (**a**), PGE_2 (**b**) and CysLTs (**c**) concentrations in the nasal polyps. Between-group comparisons were performed by Mann-Whitney's *U*-test with Bonferroni correction (*$p < 0.05$).

or rhinorrhea after the ingestion of aspirin or other nonsteroidal anti-inflammatory drugs.

QUANTIFICATION OF ARACHIDONIC ACID METABOLITES

The nasal polyp tissue specimens were homogenized in ethanol to extract the lipid fraction, the arachidonic acid metabolites were stabilized, and the cellular protein was removed. After centrifugation of the precipitated proteins, the supernatants were dried, resuspended in 80% ethanol and loaded on to a C18 Sep-Pak column (Waters, Milford, MA). This column was rinsed with ultra pure water followed by ethyl acetate, and the arachidonic acid metabolites were then eluted with ethanol/water (90 : 10). PGD_2, PGE_2 and CysLTs were quantified using a high-sensitivity competitive enzyme immunoassay kit (EIA kit; Cayman Chemical, Ann Arbor, MI, USA), in accordance with the manufacturer's directions. The EIA is a competitive sandwich enzyme-linked immunosorbent assay technique, and the data were determined in comparison with standards for arachidonic acid metabolites. Data were expressed as picograms of PGD_2, PGE_2 and CysLTs per gram of nasal polyp tissue in the original homogenate.

IMMUNOHISTOCHEMICAL ANALYSIS

Monoclonal antibodies against human eosinophil cationic protein (EG2: Pharmacia, Uppsala, Sweden) and mast cell tryptase (Chemicon International, Temecula, CA, USA), CD68 (Dako Cytomation, Glostrup, Denmark) were used for the immunohistochemical analysis. Immunostaining with the avidin-biotin complex was performed as described previously.[31] In brief, deparaffinized sections were initially incubated with 3% H_2O_2 in methanol for 15 minutes to quench the endogenous peroxidase activity, washed twice with TBS (Dako Cytomation) and incubated overnight at 4℃ with the primary antibody specific for EG2, mast cell tryptase or CD68; they were then washed and incubated for 30 minutes with Envision/AP® (Dako, Cambridgeshire, UK) or Envision+® (Dako). After being washed again with TBS, color development was conducted using Fast Red (Sigma, Poole, UK) or diaminobenzidine (DAB; Dako) as the chromogen for signal visualization. Using this method, the cells expressing tryptase or CD68 were visualized in brown color (using DAB) while those expressing EG2 were red in color (using Fast Red). Negative controls were prepared by replacing the primary antibodies with isotype immunoglobulin.

Cells in the coded biopsy sections were counted by an investigator blinded to the protocol. The cells showing positive immunostaining in the nasal submucosal region where the highest cellular infiltration was seen were counted under a microscope. Then, the average number of positive cells in three high-power fields (200× or 400×) was determined.

STATISTICAL ANALYSIS

All the statistical analyses were nonparametric. Between-group comparisons were performed by the nonparametric Mann-Whitney's *U*-test with Bonferroni correction. Correlation analysis was performed using Spearman's rank correlation coefficient Differences (two-sided) were considered significant when the *P* value was less than 0.05.

RESULTS

PGD_2, PGE_2 AND CysLTs CONCENTRATIONS IN THE NASAL POLYPS

The concentrations of PGD_2, PGE_2 and CysLTs determined in the nasal polyps are summarized in Figure 1. The concentrations of PGD_2 were significantly higher in the nasal polyps of the CRS-ATA group as compared with those in the control specimens ($P = 0.035$, Fig. 1a). The concentrations of PGD_2 tended to be higher in the nasal polyps of the CRS-ATA group than in those of the CRS-AIA group, but this difference was not statistically significant.

The concentrations of PGE_2 were highest in the control specimens and lowest in the nasal polyps of the CRS-AIA group (Fig. 1b). Comparison of the concentrations of PGE_2 in the nasal polyps of the two patients groups (CRS-ATA and CRS-AIA) divided according to the type of BA associated with CRS (ATA

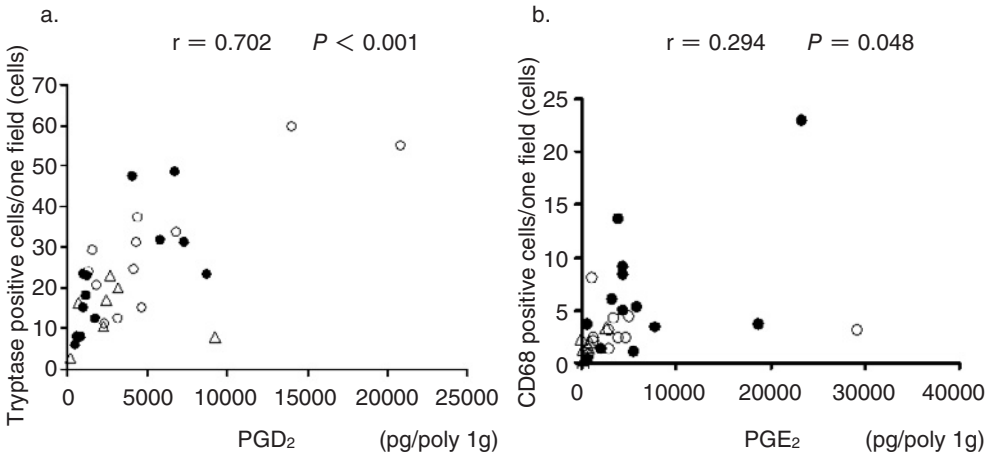

Fig. 2 Correlation between the concentration of PGD_2 and the number of tryptase-positive cells (**a**), PGE_2 and the number of CD68-positive cells (**b**). Correlation analysis was performed using Spearman's rank correlation coefficient (●: CRA-NA, ○: CRS-ATA, △: CRS-AIA).

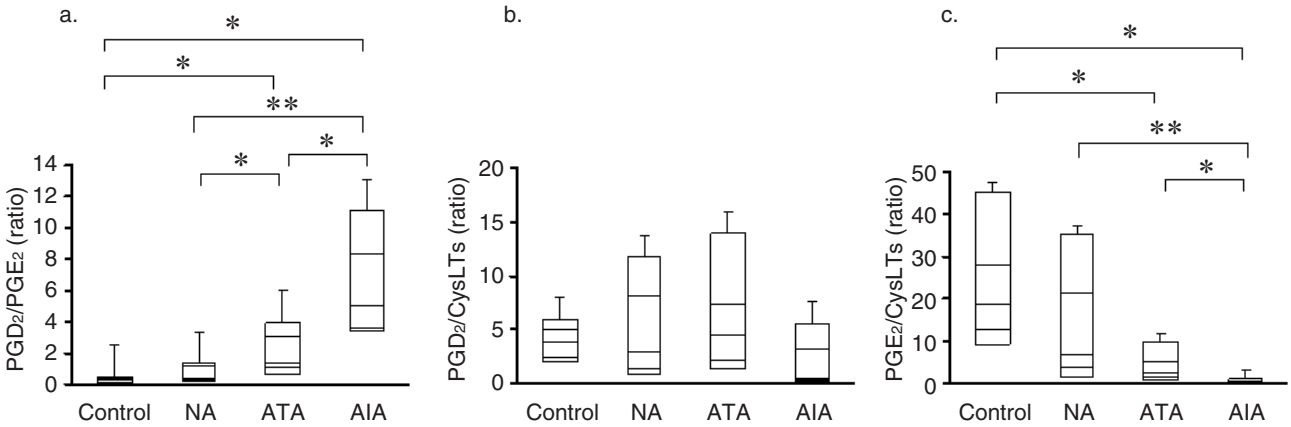

Fig. 3 Balance among the PGD_2, PGE_2, CysLTs concentrations in the nasal polyps. PGD_2/PGE_2 ratio (**a**), PGD_2/CysLTs ratio (**b**) and PGE_2/CysLTs ratio (**c**). Between-group comparisons were performed by Mann-Whitney's *U*-test with Bonferroni correction (*$p < 0.05$, **$p < 0.01$).

and AIA, respectively) revealed that the concentration of PGE_2 was lower in the CRS-AIA group than in the CRS-ATA group ($P = 0.024$).

The concentrations of CysLTs were significantly higher in the nasal polyps of the CRS-AIA group than in the control specimens ($P = 0.037$, Fig. 1c). The same parameter tended to be higher in the CRS-AIA group than in the CRS-ATA group, although this difference was not statistically significant.

EXPRESSION OF MAST CELL TRYPTASE AND CD68 IN THE NASAL POLYPS

Next, the mast cells and monocyte macrophages in the nasal polyps were counted by means of immunohistochemical staining and their counts were analyzed in relation to the levels of expression of the arachidonic acid metabolites (Fig. 2). The results revealed that the number of mast cell tryptase-positive cells was strongly correlated with the concentrations of PGD_2 (r = 0.702, $P < 0.001$; Fig. 2a). According to the analysis in each group, the number of mast cell tryptase-positive cells was correlated with the concentrations of PGD_2 in the CRS-NA group ($P < 0.001$) and the CRS-ATA group ($P = 0.029$). The number of CD 68-positive cells was correlated with the concentrations of PGE_2 in the nasal polyps (r = 0.294, $P = 0.048$; Fig. 2b). According to the analysis in each group, the number of CD68-positive cells was correlated with the concentrations of PGE_2 in the CRS-NA group ($P = 0.044$).

COMPARISON OF THE ARACHIDONIC ACID METABOLITES

The PGD_2/PGE_2 ratio, serving as an indicator of the balance between PGD_2 and PGE_2, was compared among the different groups (Fig. 3a). The PGD_2/PGE_2 ratio was found to be the highest in the CRS-AIA group, followed by that in the CRS-ATA group,

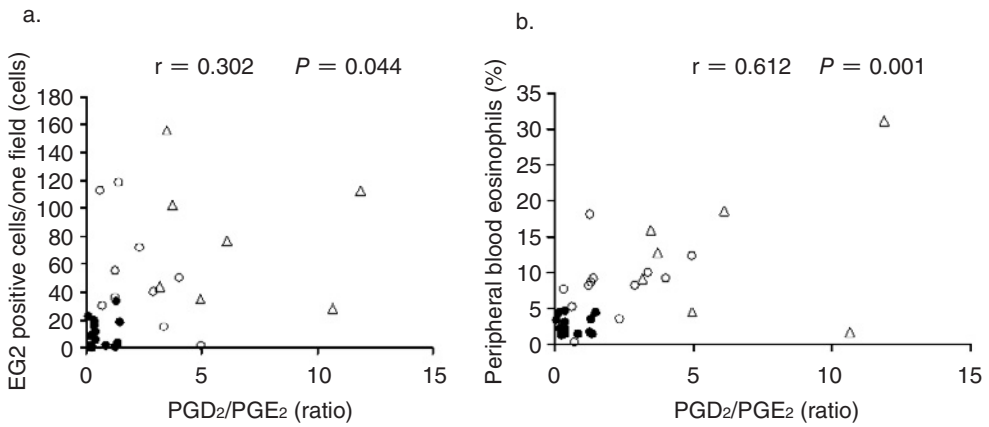

Fig. 4 Correlations between the PGD_2/PGE_2 ratio and the number of EG2-positive cells (**a**) and the percentage of eosinophil in peripheral blood (**b**). Correlation analysis was performed using Spearman's rank correlation coefficient (●: CRA-NA, ○: CRS-ATA, △: CRS-AIA).

and lowest in the CRS-NA group. Furthermore, the PGD_2/CysLTs ratio (serving as an indictor of the balance between PGD_2 and the CysLTs) and the PGE_2/CysLTs ratio (serving as an indicator of the balance between PGE_2 and the CysLTs) were also compared among the different groups, as shown in Figure 3b, 3c, respectively. There was no significant difference in the PGD_2/CysLTs ratio. On the other hand, the PGE_2/CysLTs ratio was the highest in the CRS-NA group followed by that in the CRS-ATA group, and lowest in the CRS-AIA group.

COMPARISON OF THE PGD_2/PGE_2 RATIO AND EOSINOPHILS IN THE NASAL POLYPS

Figure 4a shows the data concerning the correlation between the PGD_2/PGE_2 ratio and the number of EG2-positive cells in the nasal polyps. The PGD_2/PGE_2 ratio was positively correlated with the number of EG2-positive cells in the nasal polyps ($r = 0.302$; $P = 0.044$). According to analysis in each group, this ratio was not correlated with the number of EG2-positive cells in the nasal polyps although a tendency was observed. Figure 4b shows the data concerning the correlation between the PGD_2/PGE_2 ratio and the differential eosinophil count in the peripheral blood. The PGD_2/PGE_2 ratio was positively correlated with the peripheral blood differential eosinophil count ($r = 0.612$; $P = 0.001$). According to analyses in each group, this ratio was not correlated with the number of EG2-positive cells in the peripheral blood though a tendency was observed.

DISCUSSION

From the findings in this study, we provided evidence, for the first time, of a significant elevation of the concentration of PGD_2 in the nasal polyps of patients with CRS complicated by ATA (CRS-ATA). Furthermore, we found that the PGD_2/PGE_2 ratio was markedly elevated in the nasal polyps of patients with CRS complicated by AIA (CRS-AIA). Previous studies have reported a strong correlation between the severity of BA and the concentrations of PGD_2.[19,32,33] In contrast, it is generally believed that CRS with BA is associated with a poor prognosis. Some reports have suggested that similar pathogenetic mechanisms might be involved in BA and CRS in patients with CRS complicated by BA.[4-6,10] Based on these observations, we analyzed the PGD_2 and PGE_2 concentrations in the nasal polyps of patients with intractable CRS complicated by BA.

The CRS patients from whom the nasal polyp specimens were collected were divided into three groups: (1) CRS-NA (CRS patients without BA), (2) CRS-ATA (CRS patients with complicating aspirin-tolerant (no aspirin sensitivity) asthma) and (3) CRS-AIA (CRS patients with complicating aspirin-intolerant (aspirin-sensitive) asthma). The PGD_2 concentrations were highest in the nasal polyps of the CRS-ATA group (Fig. 1a). This result endorses the view that there might be a close correlation between the pathophysiology of BA and PGD_2, and suggests that PGD_2 might be involved in the intractable course of CRS. Based on this finding, we checked the expression of tryptase of mast cell origin in the nasal polyps by means of immunohistochemical analysis. This analysis revealed a positive correlation between the tryptase-positive cell count and the nasal polyp PGD_2 concentration (Fig. 2a). This finding suggested that PGD_2 production is elevated in nasal polyps showing intense mast cell infiltration. The PGD_2 concentration tended to be lower in the polyps of the CRS-AIA group than in those of the CRS-ATA group, although this difference was not statistically significant (Fig. 1 a). In previous reports, the pathogenesis of AIA was attributed to downregulation of cyclooxygenase (COX)-2, and it was shown that COX-2 downregula-

tion leads to an increase in the production of CysLTs and decrease in the production of PGE_2.[34-37] In the present study also, significantly lower PGE_2 concentrations (Fig. 1b) and significantly higher CysLTs concentrations (Fig. 1c) were found in the polyps of the CRS-AIA group. We may therefore estimate that COX-2 downregulation is responsible for the reduction of the PGD_2 concentrations in the nasal polyps of patients with CRS-AIA. When the correlation of the PGE_2 with the number of infiltrating cells as determined by immunohistochemical analysis was examined, a positive correlation was noted between the PGE_2 concentration and the CD68-positive cell count (Fig. 2b). On the basis of these results, we may estimate that the PGE_2 production is primarily undertaken by macrophages.

It has recently been reported that the PGD_2/PGE_2 balance is closely involved in various inflammatory conditions.[28,29,38] In the present study also, the PGD_2/PGE_2 ratio was in the order of CRS-AIA group > CRS-ATA group > CRS-NA group (Fig. 3a). That is, this ratio was higher in cases with intractable CRS that was considered to be likely to be characterized by repeated cycles of remission and relapse (e.g., CRA-AIA and CRS-ATA). Furthermore, a positive correlation was noted between the PGD_2/PGE_2 ratio and the nasal polyp EG2-positive cell count (Fig. 4a) and between the PGD_2/PGE_2 ratio and the percentage of eosinophils in the peripheral blood (Fig. 4b), suggesting that the PGD_2/PGE_2 ratio may serve as an indicator of the severity of CRS, as is the case with tissue and peripheral eosinophil counts. However, it is unclear why this ratio correlated with the percentage of eosinophils in the peripheral blood. In previous reports, PGD_2 induced the rapid release of eosinophils from bone marrow though PGE_2 induced apoptosis in developing eosinophils through inducible nitric oxide synthase (NOS) and inhibited the responses of bone-marrow myeloid progenitors to GM-CSF and of eosinophil precursors to IL-5.[39-41] We considered that high PGD_2 and low PGE_2 levels might increase eosinophils in the peripheral blood accordingly. In this connection, Pierzchalska *et al.*[42] reported a study involving stimulation of fibroblasts (cultured after collection from bronchial biopsy specimens) with IL-1β, TNF-α or LPS and analysis of the expression of PGE_2 and PGD_2 in vitro, in which they demonstrated that the PGE_2/PGD_2 ratio was significantly lower in these fibroblasts as compared with those in the fibroblasts isolated from non-asthmatic patients. In contrast to the design of their study, our study was designed to quantify the expression of PGD_2 and PGE_2 throughout all tissues containing cells capable of producing them. Therefore, the data from this study may be viewed as reflecting clinical cases more faithfully. Regarding the pathophysiology of CRS, Okano *et al.*[30] analyzed the nasal polyp levels of PGD_2 synthase (PGDS) and PGE_2 synthase (PGES) in patients with CRS, based on the contention that an increase in PGD_2 and decrease in PGE_2 can aggravate eosinophil-induced airway inflammation. Our study differs from theirs in that our study involved the quantification of arachidonic acid metabolites in the nasal polyps by EIA instead of analysis of the respective syntheses. Furthermore, by dividing CRS complicated by asthma into two types (a type with aspirin sensitivity and a type without aspirin sensitivity), the present study demonstrated a closer correlation of the PGD_2/PGE_2 ratio with CRS-AIA (CRS complicated by asthma with aspirin sensitivity) than with CRS-ATA. Furthermore, both the PGD_2/CysLTs ratio (Fig. 3b) and the PGE_2/CysLTs ratio (Fig. 3c) were lower in the CRS-AIA group than in the CRS-ATA group. These results seem to be attributable to the greater downregulation of COX-2 in these cases, as described above.

In the analysis of the association with predisposition to allergy, no significant correlation was noted between the PGD_2/PGE_2 ratio and the serum total IgE level (data not shown). These results suggest that the PGD_2/PGE_2 ratio may be independent of atopic status.

Some investigators reported that paranasal sinus CT scans tend to reveal more severe lesions in cases of CRS with intense eosinophil infiltration (e.g., cases of CRS-ATA and CRS-AIA).[43] If this previous finding is taken into consideration with the results of the present study, it seems likely that the PGD_2/PGE_2 ratio correlates positively with the radiological severity of CRS. In the previous report, a significant positive correlation was noted between the haemopoietic-type PGDS (h-PGDS) levels and the radiological severity of sinusitis, while a significant inverse correlation was noted between the microsomal PGES-1 (m-PGES-1) levels and the severity.[30] However, the PGD_2/PGE_2 ratio were not correlated with radiological severity of sinusitis in this study (P = 0.189; data not shown). One possible reason for this finding is that in cases with intense eosinophil infiltration (e.g., CRS-AIA group and CRS-ATA group), lesions are often confined to the ethmoid sinus or the surrounding area and the CT score is not always high.

In conclusion, the concentrations of the PGD_2 were significantly higher in the polyps obtained from patients with CRS-ATA as compared with those in the control specimens. The concentrations of PGE_2 were highest in the control specimens, followed by those in the CRS-ATA group, and lowest in the CRS-AIA group. The PGD_2/PGE_2 ratio was highest in the CRS-AIA group, followed by that in the CRS-ATA group, and lowest in the CRS-NA group. These findings suggest that the PGD_2/PGE_2 ratio may be an indicator of the severity of CRS as one of the alternatives to the eosinophilic infiltration. Clinically, CRS complicated by AIA is known to be characterized by repeated cycles of remission and relapse and to be highly intrac-

table. Because such cases had the highest nasal polyp PGD_2/PGE_2 ratio in the present study, we may say that the PGD_2/PGE_2 ratio reflects the intractable nature of CRS very well. Treatment of intractable CRS may be possible if the increase of PGD_2 and decrease of PGE_2 in such cases can be controlled.

ACKNOWLEDGEMENTS

We thank Ms. Hiromi Katsuyama and Ms. Nao Aida of the Jikei University School of Medicine for their skillful technical assistance. This study was supported by a research fund from the Taiho Pharmaceutical Co, Ltd., Japan.

REFERENCES

1. Borish L. Sinusitis and asthma: entering the realm of scientific study. *J Allergy Clin Immunol* 2002;**109**:606-8.
2. Kaliner MA, Osguthorpe JD, Fireman P *et al*. Sinusitis, bench to bedside. Current findings, future directions. *J Allergy Clin Immunol* 1997;**99**(6 Pt 3):S829-48.
3. Hamilos DL, Leung DYM, Wood R *et al*. Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF-α. *Am J Respir Cell Mol Biol* 1996;**15**:443-50.
4. Rachelefsky GS, Spector SL. Sinisitis and asthma. *J Asthma* 1990;**27**:1-3.
5. Slavin RG. Complications of allergic rhinitis: implications for sinusitis and asthma. *J Allergy Clin Immunol* 1998;**101**(2 Pt 2):S357-60.
6. Lamblin C, Tillie-Leblond I, Darras J *et al*. Sequential evaluation of pulmonary function and bronchial hyperresponsiveness in patients with nasal polyposis: a prospective study. *Am J Respir Crit Care Med* 1997;**155**:99-103.
7. Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999;**28**:717-22.
8. Drake-Lee AB. Medical treatment of nasal polyps. *Rhinology* 1994;**32**:1-4.
9. Stammberger H. Surgical treatment of nasal polyps: past, present, and future. *Allergy* 1999;**53**:7-11.
10. Vennto SI, Ertamo LO, Hytonen ML *et al*. Nasal polyposis: clinical course during 20 years. *Ann Allergy Asthma Immunol* 2000;**85**:209-14.
11. Samter M, Beers RF Jr. Intolerance to aspirin. Clinical studies and consideration of its pathogenesis. *Ann Intern Med* 1968;**68**:975-83.
12. Stevenson DD. Diagnosis, prevention, and treatment of adverse reactions to aspirin and nonsteroidal anti-inflammatory drugs. *J Allergy Clin Immunol* 1984;**74**:617-22.
13. Leff AR. Role of leukotrienes in bronchial hyperresponsiveness and cellular responses in airways. *Thorax* 2000;**55**(Suppl 2):S32-7.
14. Funk CD. Prostaglandins and leukotrienes, advances in eicosanoid biopsy. *Science* 2001;**294**:1871-5.
15. Borish L. The role of leukotrienes in upper and lower airway inflammation and the implications for treatments. *Ann Allergy Asthma Immunol* 2002;**88**:16-22.
16. Holgate ST, Peters-Golden M, Panettieri RA, Henderson WR Jr. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodering. *J Allergy Clin Immunol* 2003;**1111**(Suppl):S18-34.
17. Vignola AM. Effects of inhaled corticosteroids, leukotriene receptor antagonists, or both, plus long-acting beta2-agonists on asthma pathophysiology: a review of the evidence. *Drugs* 2003;**63**:35-51.
18. Coleman RA, Sheldrick RL. Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. *Br J Pharmacol* 1989;**96**:688-92.
19. Awaed JA, Morrow JD, Roberts LJ. Detection of the major urinary metabolite of prostaglandin D_2 in the circulation: demonstration of elevated levels in patients with disorders of systemic mast cell activation. *J Allergy Clin Immunol* 1994;**93**:817-24.
20. Matsuoka T, Hirata M, Tanaka H *et al*. Prostaglandin D_2 as a mediator of allergic asthma. *Science* 2000;**287**:2013-7.
21. Fujitani Y, Kanaoka Y, Aritake K, Uodome N, Okazaki-Hatake K, Urada Y. Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D_2 synthase transgenic mice. *J Immunol* 2002;**168**:443-9.
22. Pavord ID, Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E_2. *Lancet* 1995;**345**:436-8.
23. Kleeberger SR, Freed AN. Prostanoids. In: Busse W, Holgate S (eds). *Asthma and Rhinitis.* Blackwell Scientific Publications, 1995;825-37.
24. Gomi K, Zhu FG, Marshall JA. Prostaglandin E_2 selectively enhanced IgE mediated production of IL-6 and GM-CSF by mast cells through an EP1/EP3 dependent mechanism. *J Immunol* 2000;**165**:6545-52.
25. Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. *Immunol Today* 1991;**12**:349-52.
26. Sladek K, Sheller JR, FitzGerald GA, Morrow JD, Roberts LJ 2nd. Formation of PGD_2 after allergen inhalation in atopic asthmatics. *Adv Prostaglandin Thromboxane Leukot Res* 1991;**21A**:433-6.
27. Murray JJ, Tonnel AB, Brash AR *et al*. Release of prostaglandin D_2 into human airways during acute antigen challenge. *N Engl J Med* 1986;**315**:800-4.
28. Cipollone F, Fazia M, Lezzi A *et al*. Balance between PGD synthase and PGE synthase is a major determinant of atherosclerotic plaque instability in humans. *Arterioscler Thromb Vasc Biol* 2004;**24**:1259-65.
29. Muraki T, Fujimori K, Ishizaka M *et al*. Effect of interleukin-1beta and prostaglandin E_2 on prostaglandin D synthase production in cultivated rat leptomeningeal cells. *J Cereb Blood Flow Metab* 2004;**23**:409-18.
30. Okano M, Fujiwara T, Yamamoto M *et al*. Role of prostaglandin D_2 and E_2 terminal synthases in chronic rhinosinusitis. *Clin and Exp Allergy* 2006;**36**:1028-38.
31. Nasser SM, Pfister R, Christie PE *et al*. Inflammatory cell populations in bronchial biopsies from aspirin-sensitive asthmatic subjects. *Am J Respir Crit Care Med* 1996;**153**:90-6.
32. Murrary JJ, Tonnel AB, Brash AR *et al*. Release of prostaglandin D_2 into human airways during acute antigen challenge. *N Engl J Med* 1986;**315**:800-4.
33. Hardy CC, Robinson C, Tattersfield AE, Holgate ST. The bronchoconstrictor effect of inhaled prostaglandin D_2 in normal and asthmatic men. *N Engl J Med* 1984;**311**:209-13.
34. Baenkler HW, Schafer D, Hosemann W. Eicosanoids from biopsy of normal and polypous nasal mucosa. *Rhinology* 1996;**34**:166-70.
35. Kowalski ML, Pawliczak R, Wozniak J *et al*. Differential metabolism of arachidonic acid in nasal polyp epithelial

cells cultured from aspirin-sensitive and aspirin-tolerant patients. *Am J Respir Crit Care Med* 2000;**161**:391-8.
36. Perez-Novo CA, Watelet JB, Claeys C, Van Cauwenberqe P, Bachert C. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. *J Allergy Clin Immunol* 2005;**115**:1189-96.
37. Picado C, Fernandez-Morata JC, Juan M *et al*. Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1999;**160**:291-6.
38. Cao H, Xiao L, Park G *et al*. An improved LC-MS/MS method for the quantification of prostaglandins E (2) and D (2) production in biological fluids. *Anal Biochem* 2008; **372**:41-51.
39. Schratl P, Royer JF, Kostenis E *et al*. The role of the prostaglandin D2 receptor, DP, in eosinophil trafficking. *J Immunol* 2007;**179**:4792-9.
40. Jones CP, Paula Neto HA, Assreuy J, Vargaftig BB, Gaspar Elsas MI, Elsas PX. Prostaglandin E2 and dexamethasone regulate eosinophil differentiation and survival through a nitric oxide- and CD95-dependent pathway. *Nitric Oxide* 2004;**11**:184-93.
41. Gaspar Elsas MI, Joseph D, Lintomen L *et al*. Murine myeloid progenitor responses to GM-CSF and eosinophil precursor responses to IL-5 represent distinct targets for downmodulation by prostaglandin E (2). *Br J Pharmacol* 2000;**130**:1362-8.
42. Pierzchalska M, Szabó Z, Sanak M, Soja J, Szczeklik A. Deficient prostaglandin E_2 production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin-induced asthma. *J Allergy Clin Immunol* 2003;**111**: 1041-8.
43. Kountakis SE, Arango P, Bradley D, Wade ZK, Borish L. Molecular and cellular staging for the severity of chronic rhinosinusitis. *Laryngoscope* 2004;**114**:1895-905.