Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells

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Background: Impaired braking of inflammatory cell cysteinyl leukotriene production by prostaglandin (PG) E_2 has been implicated in the pathogenesis of aspirin exacerbated airways disease, but the mechanism is obscure. PGE_2 acts via G-protein–coupled receptors, E-prostanoid $(EP)_{1\text{-}4}$, but there is little information on the expression of PGE_2 receptors in this condition.

Objective: To address the hypothesis that expression of 1 or more EP receptors on nasal mucosal inflammatory cells is deficient in patients with aspirin-sensitive compared with nonaspirin-sensitive polypoid rhinosinusitis.

Methods: By using specific antibodies, immunohistochemistry, and image analysis, we measured the expression of $EP_{1\text{-}4}$ in nasal biopsies from patients with aspirin-sensitive (n = 12) and nonaspirin-sensitive (n = 10) polypoid rhinosinusitis and normal controls (n = 9). Double-staining was used to phenotype inflammatory leukocytes expressing $EP_{1\text{-}4}$.

Results: Global mucosal expression of EP_1 and EP_2, but not EP_3 or EP_4, immunoreactivity was significantly elevated in aspirin-sensitive and nonaspirin-sensitive rhinosinusitis compared with controls ($P < .03$). This was attributable principally to elevated expression on tubulin$^+$ epithelial cells and Mucin 5 subtypes A and B (Muc-5AC$^+$) goblet cells. In contrast, the percentages of neutrophils, mast cells, eosinophils, and T cells expressing EP_2, but not EP_1, EP_3, or EP_4, were significantly reduced ($P \leq .04$) in the aspirin-sensitive compared with nonaspirin-sensitive patients.

Conclusion: The data suggest a possible role for PGE_2 in mediating epithelial repair in rhinitis and asthma. Because PGE_2 exerts a range of inhibitory actions on inflammatory leukocytes via the EP_2 receptor, its reduced expression in aspirin-sensitive rhinosinusitis may be partly responsible for the increased inflammatory infiltrate and production of cysteinyl leukotrienes that characterize aspirin-sensitive disease. (J Allergy Clin Immunol 2006;117:312-8.)

Key words: *Aspirin, rhinosinusitis, prostaglandin E_2, EP receptor, asthma*

Patients with idiosyncratic reactions to aspirin ingestion characteristically exhibit the clinical triad of aspirin sensitivity, asthma, and chronic polypoid rhinosinusitis.[1] A fundamental feature of aspirin sensitivity is excessive production of cysteinyl leukotrienes (CysLTs) in the steady state, which is further elevated after aspirin exposure.[2-5] The common feature of drugs that exacerbate symptoms in aspirin-sensitive patients is that they inhibit the enzyme COX-1, which mediates the formation of prostanoids. COX-1 inhibitors induce elevated local and systemic leukotriene release in these patients.[2,6,7]

Although certain prostanoids, particularly prostaglandin (PG) D_2, are regarded as having proinflammatory properties and contributing to bronchoconstriction in aspirin-sensitive asthma, there is good evidence that the prostanoid PGE_2 inhibits aspirin-induced phenomena. For example, in these patients, exogenous PGE_2 blocks aspirin-induced bronchoconstriction, elevated urinary leukotriene E_4 (LTE_4) concentrations, and release of CysLTs from peripheral blood leukocytes.[8,9] COX-1 inhibition, resulting in reduced PGE_2 production, thus provides 1 possible mechanism for COX-1 inhibitor-induced exacerbation of asthma and rhinitis, but the critical question is why this phenomenon is restricted to aspirin-sensitive patients and is not observed in all patients with asthma and chronic polypoid rhinosinusitis.

It has been suggested that aspirin-sensitive patients are particularly dependent on the tonic inhibition of CysLT generation by the action of PGE_2 on target cellular receptors.[10] This might reflect relative deficiency of PGE_2 production and/or abnormality of receptor expression and/or signaling. Studies addressing PGE_2 production in aspirin-sensitive patients are so far indefinitive. Aspirin-sensitive and tolerant patients with asthma had equivalent concentrations of PGE_2 in induced sputum.[11] Epithelial cell monolayers out grown from resected nasal polyps produced slightly less PGE_2 after 6 days of culture *in vitro* in aspirin-sensitive compared with tolerant patients.[12] Fibroblasts outgrown from bronchial biopsies (2-3 weeks

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Abbreviations used
CysLT: Cysteinyl leukotriene
DAB: 3,′3-Diaminobenzidine
EP: E-prostanoid
LTE_4: Leukotriene E_4
MBP: Major basic protein
PG: Prostaglandin
UK: United Kingdom

of culture) produced less PGE_2 in response to 18 hours of stimulation with cytomix in aspirin-sensitive compared with tolerant patients, although spontaneous production was equivalent.[13] In these *in vitro* studies, cells from aspirin-sensitive and tolerant patients were equally sensitive to suppression of PGE_2 production by exogenous COX-1 inhibitors. We have also shown[14] that COX-1 expression is not deficient in bronchial mucosal inflammatory leukocytes in aspirin-sensitive asthma.

Whether or not PGE_2 production is impaired in aspirin sensitive patients, deficiency of PGE_2 braking of CysLT production may reflect deficiency of PGE_2 receptor expression. There are 4 G-protein–coupled PGE_2 receptors designated E-prostanoid $(EP)_{1\text{-}4}$.[15] Stimulation of EP_1 receptors results in activation of phosphatidyl inositol hydrolysis and elevation of the intracellular Ca^{++} concentration. EP_2 and EP_4 ligation increases intracellular cyclic adenosine monophosphate, whereas EP_3 ligation decreases it. Thus, there is plenty of scope for variability in the response of target cells to PGE_2 according to the spectrum of receptors they express. EP receptors are known to be expressed on a wide diversity of human cells.[15]

In this study, our aim was to measure expression of all 4 EP receptors in nasal biopsies from patients with aspirin-sensitive and nonaspirin-sensitive chronic polypoid rhinosinusitis and normal controls. We measured global expression of these receptors and then examined their expression on mucosal epithelial cells, fibroblasts, and inflammatory leukocytes. We hypothesized that inflammatory leukocytes show deficiency of expression of 1 or more EP receptors in aspirin-sensitive compared with aspirin-tolerant rhinosinusitis.

METHODS

Patients, controls, and nasal biopsies

Inferior turbinate nasal biopsy specimens were obtained from 22 patients with chronic rhinosinusitis, as defined by the presence of nasal polyps, and normal control subjects. These patients were a subset of those described in 2 previous studies.[16,17] Of the patients with rhinosinusitis, 12 were aspirin-sensitive and 10 were tolerant. Eleven of the 12 aspirin-sensitive patients, 7 of the 10 tolerant patients, and 7 of the 9 normal controls were atopic as defined by a positive skin prick test to 1 or more of a panel of 10 standard aeroallergens in the presence of positive (histamine) and negative (diluent) controls. Subjects' ages ranged from 25 to 69 years and were not significantly different in the 3 groups. None of the patients smoked, and none had taken oral or intranasal corticosteroids or CysLT antagonists for at least 1 month before the taking of the biopsies. The diagnosis of aspirin sensitivity was made as previously described.[16,17] If a patient had taken aspirin or a nonsteroidal anti-inflammatory drug at clinical dosages within the previous 6 months without any untoward reaction (exacerbation of rhinitis and/or asthma, urticaria/angioedema, anaphylaxis, nausea/vomiting/diarrhea within 4 hours after ingestion), the patient was considered aspirin-tolerant. All of the normal control subjects were tolerant by this definition. All other patients underwent bilateral intranasal challenge with incremental dosages of lysine aspirin in a single-blind, placebo-controlled fashion.[17] Reaction to the instillation was assessed subjectively with the use of symptom scores and objectively by means of acoustic rhinometry. A response was defined as reduction in nasal patency (in terms of minimal cross-sectional area and volume, as determined by rhinometry) of at least 25%. Such a response was usually accompanied by symptoms of rhinorrhea and sneezing within 60 minutes after instillation. The mean (±SD) threshold for these changes was 4.4 ± 3.8 mg (range, 2.0-16.0). None of the patients had any concurrent bronchoconstriction during the challenge. Aspirin tolerance was defined as recent (within 6 months) aspirin ingestion without resultant symptoms or absence of these changes in response to 16 mg intranasal aspirin. All patients who reported a history of clinical reactions to aspirin had a positive challenge response. The study was approved by the Ethics Committees of Guy's Hospital and the Royal National Throat, Nose and Ear Hospital. All patients provided written informed consent to participate.

Preparation of specimens

Processing of encoded inferior turbinate nasal biopsies was performed as previously described.[16,17] Briefly, nasal biopsies were embedded in optimum cutter temperature compound (Bayer, Basingstoke, United Kingdom [UK]), snap-frozen in precooled isopentane, and stored at −80°C until cryostat sectioning. Sections of nominal 10-μm thickness were freshly cut and thaw-mounted onto poly-L-lysine–coated slides (BDH, Poole, UK). After air-drying, slides were fixed in freshly prepared 4% paraformaldehyde (BDH) in PBS (pH 7.4) for 10 minutes, washed twice (10 minutes each) with 15% sucrose (Sigma, Poole, UK), rinsed in PBS, air-dried, covered with foil, and stored at −80°C until used.

Single immunohistochemistry and image analysis

Macrophages (CD68, clone #EBM11), mast cells (tryptase, clone #AA1), neutrophils (elastase, clone #NP57; Dako, High Wycombe, UK), T cells (CD3; Becton Dickinson, Cowley, Oxford, UK), and eosinophils (major basic protein [MBP], a kind gift from A. B. Kay, National Heart and Lung Institute, Imperial College, London, UK) were identified in biopsy sections by staining with optimised dilutions of these primary antibodies and developing with alkaline phosphatase antialkaline phosphatase as previously described.[18] The results were expressed as the numbers of individual immunoreactive cells per square millimeter of mucosa. $EP_{1\text{-}4}$ receptor immunoreactivity was detected by staining with specific antibodies against EP_1 (N-20), EP_2 (H-75), EP_3 (R-18), and EP_4 (C-18), purchased from Santa Cruz Biotechnology (Autogen Bioclear UK Ltd, Wiltshire, UK).[18] Staining was developed with the avidin-biotin complex previously described.[16,17] Optimal concentrations of all antibodies used were determined in pilot experiments (1:50 for anti EP_1, EP_2, EP_3, and EP_4). The signals were developed with Fast DAB (3,′3-diaminobenzidine; Sigma-Aldrich Ltd, Dorset, UK). After developing, the slides were washed in tap water for 10 minutes and counterstained with Mayer's hematoxylin (Sigma). Positive cells stained brown after