Effect of prostaglandin E_2 on eicosanoid release by human bronchial biopsy specimens from normal and inflamed mucosa

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Abstract

Background **– Eicosanoids such as prostaglandin E_2 (PGE_2), thromboxane A_2 (TXA_2), and peptidoleukotrienes (pLT) are known to be biologically highly active lipid mediators, especially in human lung epithelium. PGE_2 is thought to have mostly bronchoprotective effects, whereas pLT and TXA_2 are bronchoconstrictive. This study was undertaken to assess the release and interaction of eicosanoids in human bronchial biopsy specimens of normal and inflamed mucosa.**

Methods **– Bronchial biopsy specimens were obtained from 16 patients, seven controls without signs of inflammation and nine patients with severe inflammatory processes in the epithelium. The release of pLT, TXA_2 (measured as TXB_2), and PGE_2 was investigated using a "functional in vitro test" and the addition of several stimuli.**

Results **– Specimens incubated with arachidonic acid released higher amounts of pLT, TXB_2, and PGE_2 than unstimulated specimens. Preincubation with PGE_2 revealed significant inhibition of arachidonic acid-induced release of pLT and TXB_2 (> 50%). The inhibitory effect was higher in normal than in inflamed epithelium.**

Conclusions **– Exogenous PGE_2 has inhibitory effects on the release of pLT and TXB_2 in human bronchial biopsy specimens. This finding could explain the bronchoprotective effect of inhaled PGE_2 in normal subjects and asthmatic subjects as direct eicosanoid interactions. It also supports the concept of PGE_2 as a bronchoprotective endogenous substance. The complex effects of PGE_2 as a modulating mediator in inflammation may be worth investigating.**

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Keywords: eicosanoid metabolism, prostaglandin E_2, human mucosa.

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The epithelium of the respiratory tract has a remarkably complex and diverse structure consisting of various populations of cells, although the function of many of them remains uncertain.[1] Bronchial inflammation is known to be associated with pathological changes in the epithelium.[2 3] The epithelium is also a source of several highly biologically active substances such as cytokines[4] and eicosanoids.[5 6] Eicosanoids such as peptidoleukotrienes (pLT; for example, LTC_4, LTD_4 and LTE_4), thromboxane A_2 (TXA_2), and prostaglandin E_2 (PGE_2) are highly potent lipid mediators formed from metabolites of arachidonic acid.[7 8]

There are numerous cellular and tissue sources of eicosanoids including inflammatory cells such as basophil, neutrophil and eosinophil granulocytes, mast cells, macrophages, and monocytes.[9] Several groups of investigators have provided evidence that the qualitative and quantitative nature of eicosanoid release depends on the cell type as well as on the stimulus.[10–12] After allergen challenge the release of peptidoleukotrienes and thromboxane B_2 is increased,[13] and both eicosanoids cause bronchoconstriction in the respiratory tract.[14 15] Mucus secretion[16] and microvascular permeability are increased by peptidoleukotrienes. In contrast to the proinflammatory properties of peptidoleukotrienes and thromboxane B_2, prostaglandin E_2 is thought to protect against bronchoconstriction provoked by various stimuli.[17]

This study was undertaken to examine the role of PGE_2 on pLT and TXA_2 release using a "functional in vitro test" and native biopsy specimens of normal and inflamed human bronchial mucosa. This method was used to take into account the complex structure of the epithelium and to facilitate naturally occurring intracellular and transcellular eicosanoid metabolism. The eicosanoid release patterns of peptidoleukotrienes, thromboxane B_2 and prostaglandin E_2 were analysed in parallel using enzyme immuno assays.

Methods

SUBJECTS

Bronchial biopsy specimens were obtained from 16 patients aged 28–68 years during routine bronchoscopy. Indications for bronchoscopy included apparent abnormalities on the chest radiographs, inflammatory lung disease, and haemoptysis. Patients with a bleeding diathesis were excluded. None of the subjects received treatment with topical or systemic corticosteroids during the 10 days prior to the bronchoscopic examination. Bronchoscopic indications of mucosal inflammation were oedema and erythema, and the histopathological indication was granulocyte infiltration. Seven patients were examined to rule out a neoplasm. They had no inflammation, neither

on bronchoscopic nor on histopathological examination. This group of patients with normal epithelium served as controls. Nine patients showed signs of mucosal inflammation. Two further patients with allergic alveolitis to pigeon feathers were investigated for induced eicosanoid release using a specific allergen, immunoglobulin E, and immunoglobulin G.

Subjects gave their signed consent after receiving both written and oral information. The study design was approved by the local ethic committee.

FIBREOPTIC BRONCHOSCOPY

Premedication was given with atropine 0.5 mg and codeine 15 mg subcutaneously. Topical anaesthesia of the mucosa was performed with lignocaine in a concentration of 2%. Midazolam, 3–5 mg, was given intravenously as a sedative. All bronchoscopic examinations were performed using a Pentax flexible fibreoptic bronchoscope by one experienced bronchoscopist using a transnasal approach. Six biopsy specimens were taken from the mucosa of the central bronchus of each patient for the functional biopsy test and divided into pieces with a fresh weight of 0.4–0.8 mg. One additional biopsy specimen was obtained for standard histological staining to confirm the macroscopic findings.

FUNCTIONAL BIOPSY TEST AND SAMPLE COLLECTION

The biopsy specimens were placed immediately into 1 ml of modified Hank's balanced salt solution (HBSS; 4°C) containing 50 mM L-serine for 20 minutes, then transferred either into 500 µl fresh HBSS (37°C) containing no stimuli in order to examine the basal release of pLT, TXB_2 and PGE_2, or into 500 µl fresh HBSS with one of the following additions: arachidonic acid (10 µM), acetylsalicylic acid (10 µM), caffeic acid (10 µM; Biomol, Hamburg, Germany), PGE_2 (10 µM), or rabbit anti-human immunoglobulin E (anti-IgE, dilution 1:100; DAKO Diagnostics, Hamburg, Germany). Specimens were incubated for 20 minutes at 37°C and continuously gassed with carbogen (95% O_2, 5% CO_2).

To test the inhibitory action of PGE_2, acetylsalicylic acid, and caffeic acid on arachidonic acid-induced eicosanoid release, biopsy specimens were preincubated as indicated in table 1, arachidonic acid was added and the specimens were further incubated for 20 minutes. Three biopsy specimens were then removed and the incubation medium was stored at –80°C for up to four weeks before being analysed by enzyme immuno assays. The stimulations were performed in triplicate for each patient. Each specimen was weighed twice using an ultrasensitive weighing machine. Unless otherwise indicated, chemicals were purchased from Sigma (Daisenhofen, Germany).

QUANTIFICATION OF EICOSANOID RELEASE

The release of immunoreactive pLT, TXB_2, and PGE_2 was quantified simultaneously for each sample in duplicate by highly sensitive and specific competitive enzyme immuno assays using monoclonal antibodies for pLT (clone 1A-LDR1),[18] TXB_2 (clone 4E-TBR1),[19] and PGE_2 (clone E2R1).[20] The monoclonal antibodies were a gift from Dr Reinke (Medical Clinic III, University of Erlangen-Nuremberg).

TXB_2 and PGE_2 were coupled to bovine serum albumin (BSA) and LTD_4 was coupled to bovine thyroglobulin using 1-ethyl-3-(3-methylaminopropyl)carbodiimide HCl.[21] Polystyrene microtitre plates (Nunc, Wiesbaden, Germany) were coated with pLT, TXB_2, or PGE_2 conjugate for 18 hours (relevant conjugate in 15 mM $NaCO_3$/35 mM $NaHCO_3$, pH 9.6; 200 µl/well). Thereafter, remaining binding sites were blocked with 0.1% BSA in phosphate buffered saline (PBS) for 60 minutes. As standards, relevant eicosanoids were prepared in duplicate at seven different concentration levels (500 pg to 1 pg/well, in HBSS). Samples or standards (100 µl/well) were incubated for 18 hours at 4°C with monoclonal antibodies for pLT, TXB_2, or PGE_2 (100 µl/well of 1.8 µg, 2.3 µg, or 5 µg antibody, respectively, in 1 ml PBS-BSA 1%). Subsequently, biotinylated goat-anti mouse antibody was added (1:2000 diluted stock solution in PBS-BSA 0.1%; Amersham, Braunschweig, Germany; 200 µl/well for two hours at 37°C). Enzyme immuno assays for TXB_2 and PGE_2 were then incubated for one hour at 37°C with a streptavidin-biotin complex (coupled with horseradish peroxidase; 1:1000 diluted stock solution in PBS-BSA 0.1%, DAKO Diagnostics, Hamburg; 200 µl/well). The enzyme immuno assay for pLT was incubated for 30 minutes at 37°C with streptavidin-biotin complex followed by a series of incubations with modified tyramine-biotin conjugate[22] (0.1% (v/v) conjugate and H_2O_2 0.06 % in 50 mM Tris buffer, pH 8.5; 200 µl/well) for 15 minutes at 37°C, with streptavidin-biotin complex (diluted 1:750 in PBS-BSA 1%; 200 µl/well) for 30 minutes at 37°C, and finally in 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonate) solution as chromogenic substrate (1 mg in 1 ml 70 mM Na_2HPO_4/6 mM sodium perborate/40 mM citric acid, pH 4.5; 200 µl/well) for 45 minutes at room temperature. Absorption was measured at 405 nm (reference filter 490 nm). Each incubation step was followed by a washing step using PBS-Tween 0.1% (v/v). The detection limit was 1 pg/well for pLT and 3 pg/well for TXB_2 and PGE_2. The rate of recovery for pLT ($> 95\%$), TXB_2 ($> 98\%$), and PGE_2 ($> 98\%$) verified the accuracy of the assays. Intra-assay and interassay coefficients of variation for pLT were 7.4% and 8.9%, for TXB_2 were 4.9% and 7.2%, and for PGE_2 were 5.6% and 8.1%.

DATA ANALYSIS

The eicosanoid release values were normalised on the mean of the corresponding fresh weight. Results are expressed as arithmetic mean, standard deviation (SD), and standard error (SE). The Student's paired *t* test was used to compare the stimulated eicosanoid release within the subjects, and the Mann-Whitney

Table 1 Mean (SE) inhibitory effect of PGE_2 in comparison with specific enzyme inhibitors on arachidonic acid-induced or anti-IgE-induced eicosanoid release

		% inhibition of pLT release					*% inhibition of TXB_2 release*				
Source of epithelium	*n*	*PGE_2 (10 μM) + AA*	*PGE_2 (1 μM) + AA*	*PGE_2 (10 μM) + anti-IgE*	*CA (5 min) + AA*	*CA (10 min) + AA*	*PGE_2 (10 μM) + AA*	*PGE_2 (1 μM) + AA*	*PGE_2 (10 μM) + anti-IgE*	*ASA (5 min) + AA*	*ASA (10 min) + AA*
Normal	7	97 (1.8)	56 (2.4)*	74 (2.8)*	36 (2.5)	48 (1.7)	55 (1.5)	23 (1.7)*	43 (2.4)*	23 (1.7)	38 (1.4)
Inflamed	9	74 (2.7)	ND**	ND**	34 (1.9)	43 (2.1)	57 (1.9)	ND**	ND**	5 (0.4)	31 (1.8)
Allergic	2	79 (3.1)	ND**	67 (2.9)	33 (2.1)	46 (1.9)	56 (3.4)	ND**	59 (3.3)	8 (0.6)	28 (2.1)

* Only biopsy specimens of three patients were available for the functional biopsy test.
** No further biopsy specimens were available for testing quantitative PGE_2 effects.
PGE_2 = prostaglandin E_2; pLT = peptidoleukotriene; TXB_2 = thromboxane B_2; AA = arachidonic acid; CA = caffeic acid; ASA = acetylsalicylic acid.

Table 2 Eicosanoid release after specific allergen challenge in two patients with allergic alveolitis

	Mean (SE) eicosanoid release (pg/mg fresh weight)		
Allergen challenge	*pLT*	*TXB_2*	*PGE_2*
Basal	0.97(0.04)	0.30(0.05)	3.60(0.6)
Arachidonic acid	1.63(0.08)	3.36(0.07)	10.69(0.3)
Pigeon feathers	1.21(0.07)	1.23(0.05)	6.18(0.3)
Anti-IgE	2.18(0.11)	1.15(0.04)	4.12(0.09)
Anti-IgG	0.42(0.02)	0.1 (0.002)	0.01(0.002)

pLT = peptidoleukotrienes; TXB_2 = thromboxane B_2; PGE_2 = prostaglandin E_2.
Two patients with allergic alveolitis to pigeon feathers were investigated. From each patient three biopsy specimens were taken for measurement of basal and arachidonic acid (10 μM)-induced eicosanoid release, and two biopsy specimens for allergen challenge.

test was used to determine the significance of differences in normal and inflamed tissue. A p value of < 0.05 was regarded as statistically significant.

Results

EICOSANOID RELEASE PATTERNS IN NORMAL AND INFLAMED EPITHELIUM

The biopsy specimens of normal epithelium released significantly larger amounts of basal and arachidonic acid-induced peptidoleukotrienes ($p < 0.05$) than specimens of inflamed epithelium (fig 1A). The basal release of TXB_2 did not differ significantly between normal and inflamed epithelium, but arachidonic acid-induced release of TXB_2 was significantly higher in inflamed epithelium ($p < 0.01$; fig 1B). The basal and arachidonic acid-induced release of prostaglandin E_2 was higher ($p < 0.05$) in inflamed than in normal epithelium (fig 1C). Biopsy specimens released significantly larger amounts of eicosanoids upon stimulation with arachidonic acid ($p < 0.01$) (fig 1).

EFFECT OF PGE_2 ON pLT AND TXB_2 RELEASE COMPARED WITH CYCLO-OXYGENASE AND LIPOXYGENASE INHIBITORS

The inhibitory effect of PGE_2 on the release of pLT and TXB_2 in normal epithelium was compared with the lipoxygenase inhibitor caffeic acid[23] and the cyclo-oxygenase inhibitor acetylsalicylic acid.[24] Two different preincubation times were chosen for the enzyme inhibitors. Significant inhibition of pLT (48%; $p < 0.01$) or TXB_2 (38%; $p < 0.05$) release by the enzyme inhibitors was achieved using the longer preincubation time (10 minutes). PGE_2 was more potent than the enzyme inhibitors in inhibiting pLT and TXB_2 release using five minute preincubation. PGE_2 inhibited pLT and TXB_2 release quantitatively in normal epithelium and in mucosa from patients with allergic alveolitis (table 1). Furthermore, eicosanoid release in biopsy specimens from the two patients with allergic alveolitis was allergen specific and mediated by immunoglobulin E but not by immunoglobulin G (table 2).

EFFECT OF PGE_2 ON pLT AND TXB_2 RELEASE IN NORMAL AND INFLAMED EPITHELIUM

Basal release of pLT and TXB_2 in biopsy specimens from controls was reduced by preincubation with PGE_2 ($p < 0.05$; 23% and 29%, respectively) but no such inhibition was seen in inflamed epithelium (fig 2). Preincubation with PGE_2 inhibited arachidonic acid-induced pLT release in both normal (97%; $p < 0.01$) and inflamed epithelium (74%; $p < 0.01$) and similarly inhibited TXB_2 release ($p < 0.05$; 55% and 57%, respectively; fig 3). Furthermore, the release of pLT and TXB_2 induced by anti-IgE was significantly inhibited by PGE_2 ($p < 0.05$; 67% and 58%, respectively) (table 1).

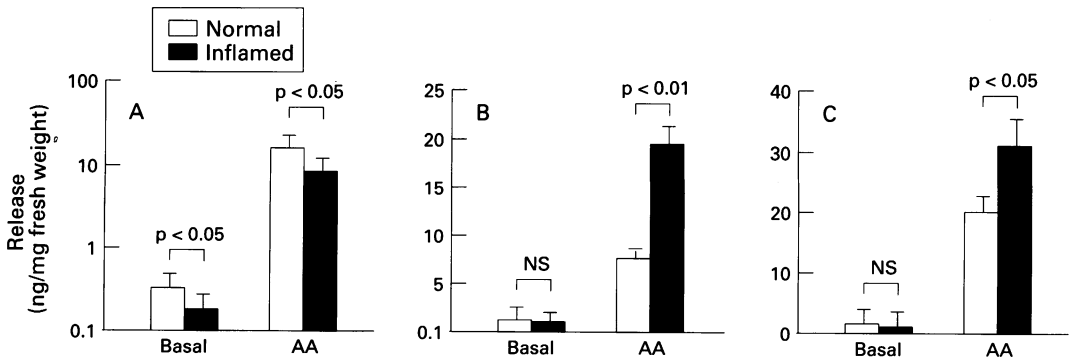

Figure 1 Eicosanoid release pattern in normal and inflamed epithelium. Basal and arachidonic acid (AA, 10 μM)-induced release of (A) peptidoleukotrienes (pLT), (B) thromboxane B_2 (TXB_2), and (C) prostaglandin E_2 (PGE_2) in biopsy specimens from seven normal subjects and nine patients with inflamed mucosa. Results are given as mean values, bars indicating SE.

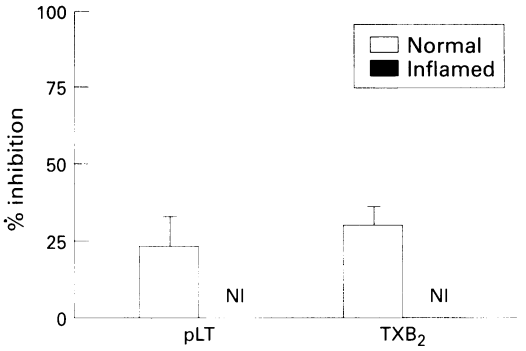

Figure 2 Effect of prostaglandin E_2 (PGE_2) on basal release of peptidoleukotrienes (pLT) and thromboxane B_2 (TXB_2). Biopsy specimens from seven normal subjects and nine patients with inflamed mucosa were incubated with PGE_2 (10 μM). Results are presented as % inhibition of pLT and TXB_2 release by PGE_2 compared with the corresponding basal eicosanoid release, bars indicating SE. NI = no inhibition.

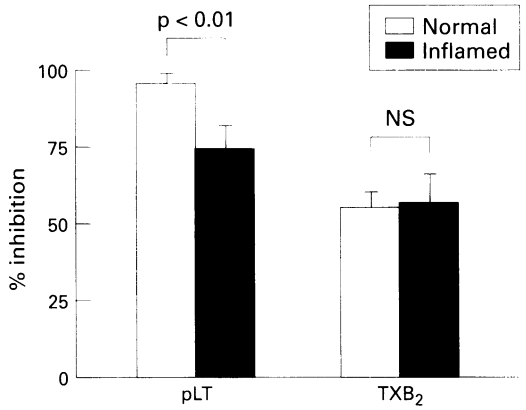

Figure 3 Effect of prostaglandin E_2 (PGE_2) on arachidonic acid-induced release of peptidoleukotrienes (pLT) and thromboxane B_2 (TXB_2). Biopsy specimens from seven normal subjects and nine patients with inflamed mucosa were preincubated with PGE_2 (10 μM) for five minutes. Results are presented as % inhibition of pLT and TXB_2 release by PGE_2 compared with arachidonic acid (10 μM)-induced eicosanoid release, bars indicating SE.

Discussion

Intensive research on eicosanoid metabolism in respiratory tract epithelium has shown that epithelial cells release high levels of eicosanoids. Several studies have attributed the release of single eicosanoids to specific cell types.[25] Transcellular eicosanoid metabolism has also been described.[26] To take into account these naturally occurring intercellular mechanisms we used intact unhomogenised tissue specimens.

In this study human bronchial specimens were found to have different basal and arachidonic acid-induced eicosanoid release patterns depending on the inflammatory status of the epithelium. Specimens of normal epithelium released higher basal levels of pLT, TXB_2, and PGE_2 than inflamed mucosa, but after stimulation with arachidonic acid the inflamed tissue released more TXB_2 and PGE_2 than normal tissue. Incubation with PGE_2 resulted in a mild degree of inhibition of basal pLT and TXB_2 release in normal epithelium but not in inflamed tissue. When biopsy specimens were stimulated with arachidonic acid or anti-IgE, PGE_2 inhibited pLT release most effectively and, to a smaller extent, TXB_2 release in both normal and inflamed epithelium. Prostaglandin E_2 inhibition of arachidonic acid-induced pLT and TXB_2 release was more potent than specific cyclo-oxygenase and 5-lipoxygenase inhibitors. Bronchial biopsy specimens from the two patients with allergic alveolitis released higher levels of eicosanoids than those of the control group. The release was allergen specific and the mechanism was mediated by immunoglobulin E rather than by immunoglobulin G.

Peptidoleukotrienes and thromboxane A_2 are known to be potent bronchoconstrictors.[14 15] The bronchoprotective effects of PGE_2 inhaled by normal and asthmatic subjects using variable antigen challenge have been published. Only slight side effects such as initial cough and retrosternal soreness were observed, which rapidly subsided as inhalation of PGE_2 continued. The inhibitory effect of PGE_2 was assumed to occur on the neuronal pathway by inhibition of afferent and efferent mediator release.[17 27] Inhibition of leukotriene B_4 release by PGE_2 has also been reported for activated human neutrophils. The action of PGE_2 was shown to be associated with increased levels of cyclic AMP, implying that prostaglandin E was acting at the level of the prostaglandin receptor.[28] Furthermore, leukotriene B_4 is known to activate 5-lipoxygenase.[29] Using arachidonic acid in the functional biopsy test, PGE_2 inhibited pLT release to a greater extent in normal (97%) than in inflamed epithelium (74%), whereas TXB_2 was inhibited similarly in both. There are some parallels between this functional biopsy test and clinical studies in which inhaled PGE_2 provoked bronchodilatation in normal subjects. However, no changes could be shown in patients with asthma,[30] which is associated with inflamed bronchial epithelium.[2 3] Thus, the reduced effectiveness of PGE_2 on inflamed epithelium could be due to altered prostaglandin receptor expression and/or activity or PGE_2 uptake. Further studies are to be done in this area.

As seen in our study, eicosanoids were released in an allergen specific manner in patients with allergic alveolitis to pigeon feathers. Specific inhibition of cyclo-oxygenase by aspirin was shown to have a bronchoprotective effect in asthmatic subjects which was explained by inhibition of bronchoconstrictive PGD_2 and TXA_2 synthesis.[31 32] As shown in our study, arachidonic acid-induced TXB_2 release was significantly higher in inflamed epithelium and could be inhibited by PGE_2, but we have found no publication describing the inhibition of TXB_2 release by PGE_2. This effect remains to be clarified. The inhibition of this potent and quantitative predominant bronchoconstrictor, TXA_2, is most likely to cause beneficial effects to patients as bronchoprotective effects were shown using thromboxane receptor antagonists.[33] Furthermore, the inhibition of leukotriene synthesis by specific 5-lipoxygenase inhibitors and the use of a specific LTD_4 receptor antagonist caused bronchoprotection.[34] The probability of slowing down leukotriene biosynthesis by PGE_2 has been also proposed[35] and modulation of cytokine production by PGE_2 is known.[36]

Our data, which provide additional aspects on the action of PGE_2 as a bronchoprotective mediator, in combination with the results of other studies on the effects of inhaled PGE_2, can be explained in several ways: (1) modulation of cytokine and neuronal mediator release, (2) inhibition of chemotactic leukotriene B_4 synthesis and therefore no activation of 5-lipoxygenase by leukotriene B_4, and/or (3) inhibition of synthesis of bronchoconstrictive peptidoleukotrienes and TXA_2 (measured as TXB_2).

In conclusion, this study has shown that PGE_2 has inhibitory effects on the release of bronchoconstrictive pLT and TXA_2, measured as TXB_2. This supports the transcellular action of eicosanoids and the hypothesis of bronchoprotective effects of PGE_2. The efficiency of PGE_2 is modulated by the inflammatory status of the tissue. The use of human biopsy specimens in a functional test is a good method for investigating these effects.

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