Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma

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Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma. K. Sladek, A. Szczeklik. ©ERS Journals Ltd 1993.

ABSTRACT: In order to examine the hypothesis that in aspirin-induced asthma (AIA) cyclooxygenase inhibition is associated with enhanced release of leukotrienes (LTs), we measured urinary leukotriene E_4 (LTE_4) and 11-dehydro-thromboxane B_2 (TXB_2) (as a measure of cyclooxygenase production) following challenge with oral aspirin or inhaled methacholine, in 10 AIA patients. We also determined serum tryptase and eosinophilic catonic protein (ECP) levels, in order to evaluate mast cell and eosinophil activation.

Urinary LTE_4 excretion was increased sevenfold 4–6 h after aspirin challenge, while 11-dehydro-TXB_2 decreased gradually reaching 50% baseline levels 24 h after challenge ($p<0.05$). This was accompanied by a significant fall in blood eosinophil count at 6 h, and a tendency to a rise in ECP. The intensity of both LTE_4 and 11-dehydro-TXB_2 responses depended on the dose of aspirin used ($p<0.001$, analysis of variance (ANOVA)). The accompanying maximum fall in forced expiratory volume in one second (FEV_1) was not correlated with peak LTE_4 levels. In contrast to aspirin, methacholine challenge producing comparable bronchial obstruction, did not alter eicosanoid excretion or serum tryptase or ECP levels.

In a separate study, lysine-aspirin inhalation challenge was performed in seven AIA patients, four of whom had responded with a rise in serum tryptase to oral aspirin challenge. Challenge with inhaled aspirin led to similar bronchoconstriction as with oral challenge, but non-respiratory symptoms such as scarlet flush or rhinorrhea were absent, and serum tryptase levels remained unchanged.

This study demonstrates that, in AIA, overproduction of LTs is accompanied by cyclooxygenase inhibition (measured as a fall in TXB_2), and that the magnitude of leukotrienes' response is related to the dose of aspirin used. During the reaction to aspirin, blood eosinophil count falls, and both eosinophils and mast cells frequently show signs of activation. These cells may be the source of LTs as well as other mediators responsible for additional skin and nasal symptoms in aspirin-precipitated attacks of asthma.
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In about 10% of adults with asthma, but rarely in asthmatic children, aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) precipitate asthma attacks. This distinct clinical syndrome is called aspirin-induced asthma (AIA) [1, 2]. Inhibition of cyclooxygenase appears to initiate a chain of reactions, leading to asthma attacks in intolerant patients [3]. What follows at the biochemical level remains largely unknown. Since their discovery [4], leukotrienes have been indicated as possible mediators of aspirin-precipitated attacks, but it was only recently that this notion gained some clinical support [5, 6]. In allergic asthma, as opposed to AIA, inhibition of cyclooxygenase has no effect on the course of clinical reaction, *i.e.* allergen-precipitated bronchoconstriction, and on allergen-stimulated release of leukotrienes [7]. In AIA, the origin of leukotrienes remains unknown. Mast cells and eosinophils, involved in asthmatic bronchoconstriction, have the capacity to synthesize substantial quantities of peptidoleukotrienes [8, 9].

In the present study, we examined the hypothesis that cyclooxygenase inhibition in AIA is associated with enhanced release of leukotrienes. We measured the appearance of a thromboxane A_2 (TXA_2) metabolite (11-dehydro-thromboxane B_2 (TXB_2), and leukotriene E_4 (LTE_4), in urine of AIA subjects undergoing aspirin or methacholine provocation. We also determined serum tryptase and eosinophilic cationic protein (ECP) levels, in order to evaluate mast cell and eosinophils activation in AIA.

Material and methods

Subjects

Studies were performed in 10 nonsmoking asthmatic patients (three males and seven females) with AIA, age range 32–50 yrs. The duration of asthma was from 5–15 yrs, on average 10 yrs. Three of the patients were atopic to common aeroallergens. All patients were in stable clinical condition (table 1).

proper study. Methacholine inhalation test [11] was carried out using a DeVilbiss nebulizer model 646 (DeVilbiss Co, Somerset, PA, USA). Twenty four hours after aspirin provocation each subject underwent methacholine challenge to evaluate possible changes in airway reactivity.

Pulmonary function was assessed by measuring FEV_1, forced vital capacity (FVC) and forced mid-expiratory flow ($FEF_{25-75\%}$) at baseline, and then every 30 min until

Table 1. – Characteristics of the AIA patients studied

Patient		Age	Sex	Atopy	Aspirin Threshold mg		Mean chronic daily oral dose prednisone
					Oral	Inhaled	
No	Init	yrs					mg
1	K.G.	37	F	+	80	(-)	10
2	K.B.	34	F	-	60	(-)	5
3	B.R.	30	F	-	20	(-)	5
4	S..Z.	42	F	+	160	(-)	-
5	K.J.	37	F	+	80	(-)	5
6	K.H.	48	F	-	20	1	-
7	G.H.	50	F	-	100	2	-
8	K.L.	47	M	-	160	2	10
9	G.J.	38	M	-	150	2	10
10	W.J.	33	M	-	150	2	-
11	D.J.	45	F	+	(-)	1	5
12	S.G.	32	F	-	(-)	2	5

Patients No. 11 and 12 underwent only lysine-aspirin inhalation challenge. AIA: aspirin-induced asthma.

None had a history of respiratory tract infection, or relevant aspirin or allergen exposure for 4 weeks before the study. At the time of study, forced expiratory volume in one second (FEV_1) exceeded 70% of predicted values. All patients were on chronic therapy with inhaled beclomethasone, and six were also receiving oral prednisone at a dose of 5 or 10 mg per day (tables, patients 1–10). Throughout the study, the steroid treatment remained unchanged. Patients stopped any inhaled or oral adrenergic agents, oral theophylline and antihistamines for at least 8, 48 and 96 h respectively, before the beginning of the study.

Atopy was diagnosed if: 1) skin prick-test was positive with at least one of the aeroallergens: mixed grass pollen, tree pollen, cat fur, dog hair, feathers, house dust, *Dermatophagoides pteronyssinus* (Bencard Allergy Service, Brentford, Middlesex, UK); and 2) a significant concentration of a specific immunoglobulin E (IgE) was detected in plasma (IgE FAST-Plus test, 3M Diagnostic System Inc., Santa Clara, CA, USA).

Design of study

Each subject was studied at the same time in the morning, on two separate occasions, at least 14 days apart. On each occasion, the patient was challenged with either inhaled methacholine or oral aspirin in a random order. Oral provocation test with aspirin was performed as described previously [2, 10], using a threshold dose resulting in a >20% fall in FEV_1. This dose was predetermined for each subject at least 4 weeks before the

the sixth hour, and was repeated at 8 and 24 h after the challenge.

Urine for analysis of LTE_4 and the enzymatic metabolite of TXA_2, 11-dehydro-TXB_2, was collected within 2 h before the challenge, and at 2, 4, 6, 8, 10, 14 and 24 h after aspirin or methacholine challenge. Blood for determination of eosinophil count, and tryptase and ECP plasma concentration, was taken at baseline, and then hourly for the 6 h, and at 12 and 24 h after challenges.

Additionally, inhalation aspirin challenge was performed in seven AIA patients on a separate occasion (table 1) [12]. Four of these subjects, had a previously significant increase in serum tryptase levels following oral aspirin challenge. We measured serum tryptase, ECP and eosinophil count before lysine-aspirin inhalation, and then every 30 min for 4 h following at least 20% a fall in FEV_1 produced by aspirin.

All patients participated in the study on a voluntary basis and gave signed informed consent to a protocol approved by the Ethics Committee of the University School of Medicine in Cracow.

Methacholine challenge

Methacholine (Mch) challenge was performed using the method of TOWNLEY and HOPE [11]. Aerosol was generated by a DeVilbiss 646 nebulizer using oxygen at a flow rate of 9 $l \cdot min^{-1}$. The dosimeter, with a manually-operated valve for 1.2 s, delivered 0.5 ± 0.02 $ml \cdot min^{-1}$ (mean±SD) of the aerosol. Measurements of FEV_1, FVC

and FEF$_{25-75\%}$ were made by pneumotachograph (Pneumo-screen). When pulmonary function after placebo inhalation (solvent of methacholine solution at the same pH) was near baseline value, the subjects inhaled five breaths of doubled, increasing concentrations of methacholine aerosol at 5 min intervals. The first concentration was 0.15 mg·ml^{-1}. The challenge was carried out until FEV$_1$ fell by 20% of baseline values or until the highest concentration of methacholine (50 mg·ml^{-1}) was reached. Methacholine (Provocholine, Roche Laboratories, Nutley, NJ, USA) was dissolved freshly on the study day, in phosphate-buffered saline (PBS) to pH 7.4. Measurements of FEV$_1$ were made at 1 and 3 min after completion of the inhalation of each concentration. The log-linear dose response curve was analysed by interpolation, to give the estimated concentration required to cause a fall in FEV$_1$ by 20% of baseline, (PC$_{20}$Mch).

Inhalation aspirin challenge

Inhalation aspirin challenge was performed as described by PHILLIPS et al. [12]. Lysine-aspirin containing 900 mg of lysine-acetylsalicylate (Aspegic, Synthelabo Pharma, Lausanne, Switzerland) with 100 mg of glycine was made up freshly on each challenge day with sterile water, to produce lysine-aspirin solution (360 mg·ml^{-1}) at a concentration of 200 mg·ml^{-1} of acetylsalicylic acid. The lysine-aspirin solution was diluted in 0.9% sodium chloride to produce a range of increasing, doubled concentrations, from 11.25–180 mg·ml^{-1}. A placebo solution had the same pH and concentration of lysine and glycine. Aerosol of these solutions was generated by a DeVilbiss 646 nebulizer connected to an oxygen flow of 9 l·min^{-1}. The dosimeter with a manually operated valve for 1.2 s, delivered 0.5±0.02 ml·min^{-1} of the aerosol. When pulmonary function after placebo inhalation was near baseline value, the subjects inhaled five breaths of increasing concentration of lysine-aspirin aerosol, beginning with a concentration of 11.25 mg·ml^{-1} (0.5 mg of lysine-aspirin for five breaths), until FEV$_1$ fell by 20% from baseline values. Measurements of FEV$_1$, FVC and FEF$_{25-75\%}$ were made at 15 min intervals for 60 min after inhalation of each concentration, and then hourly for 4 h after the concentration of lysine-aspirin inhalation which produced at least a 20% fall in FEV$_1$.

LTE$_4$ assay

4-hydroxy-TEMPO (Aldrich Chemical Co., Milwaukee, WI, USA) was added to samples of urine, which were then adjusted to pH 9.0 with NaOH, and stored at -70°C until analysed [13]. LTE$_4$ was measured in urine samples by radio-immunoassay, after extraction on C18 Prep-Sep column (Fisher Sci, Fair Lawn, NJ, USA), followed by purification and separation on high-performance liquid chromatography (HPLC) [13]. After centrifugation, freshly thawed urine supernatant was acidified to pH 5.4 and then applied to the C18 Prep-Sep cartridge, preconditioned with H$_2$O, MeOH and 0.1% ammonium acetate buffer at pH 5.4, containing 1 mM disodium ethylene-diaminetetra-acetic acid (EDTA). After washing the col-

umn with H$_2$O to remove the polar material, leukotrienes were eluted with MeOH, and the sample was evaporated to dryness under nitrogen. The residue was reconstituted with 50 μ l of mobile phase (MeOH:0.1% ammonium acetate buffer, 80:20, at pH 5.4) and injected via Rheodyne injector (Rheodyne Inc., Cotati, CA, USA) onto a reverse-phase C18 column (Spherisorb ODS-2, 5 μ m, 250×4 mm, Pharmacia LKB Instrument, Uppsala, Sweden), protected by guard cartridge (ODS-2, 5 μ m, 10×4 mm, Pharmacia LKB Instrument, Sweden). The flow rate generated by the HPLC gradient pump (HPLC Gradient Pump 2249, Pharmacia LKB Instrument, Uppsala, Sweden) was 1 ml·min^{-1} and the effluent was monitored by Variable Wavelength Monitor at a 280 nm wavelength (VWM 2141 Detector, Pharmacia LKB Instrument, Uppsala, Sweden). The fractions, having the same elution time as radiolabelled [^{3}H]LTE$_4$ (Amersham International plc, Aylesbury, UK) were collected at 0.5 min intervals, evaporated under nitrogen and dissolved in radio-immunoassay buffer (0.05 M phosphate buffer pH 7.4 containing 0.14 M NaCl and 0.01% gelatin; Amersham International plc, Aylesbury, UK). Leuko-triene recovery was determined using [14,15(n)-^{3}H]LTC$_4$ (20–60 Ci·mmol^{-1}; Amersham International plc, Aylesbury, UK) added to urine samples before extraction. Immunoreactive LTE$_4$ concentrations was measured by radio-immunoassay, using a commercial kit (Amersham International plc, Aylesbury, UK), which utilized a monoclonal leukotriene C$_4$/D$_4$/E$_4$/F$_4$ antibody with the cross-reactivity 100%/181.8%/92.7%/121.3%, respectively.

11-dehydro-thromboxane B$_2$ assay

The enzymatic metabolite of TXA$_2$, 11-dehydro-TXB$_2$, was measured by radio-immunoassay in urine sample [14, 15]. Freshly thawed urine sample was extracted on an Amprep C2 column (C2 Amprep columns; Amersham International plc, Aylesbury, UK), preconditioned with MeOH and H$_2$O, then washed with H$_2$O, 10% ethanol, and hexane, and eluted with methyl formate. The sample was subsequently dried under nitrogen and exposed to 0.05 M Tris/HCl radio-immunoassay buffer in pH 7.4 (Amersham International plc, Aylesbury, UK) for 24 h at 25°C, to ensure that the 11-dehydro-TXB$_2$ was in the open chain form. The concentration of 11-dehydro-TXB$_2$ in a sample was calculated using commercial radio-immunoassay kit (Amersham International plc, Aylesbury, UK).

Pulmonary function tests

Pulmonary function tests were performed on a flow-integrating computerized pneumotachograph (Pneumo-screen, E. Jaeger, Wuerzburg, Germany).

Tryptase and ECP assay

Tryptase and ECP serum concentrations were measured by radio-immunoassay using commercial kits (Pharmacia Diagnostic AB, Uppsala, Sweden). Venous blood

samples (3 ml) were collected into a glass tube and allowed to clot at 37°C for 1 hr. Serum was then separated by centrifugation at 1,350 g for 10 min, and put into a test tube for radio-immunoassay procedure. Concentrations of tryptase and ECP were quantified against a freshly prepared standard curve.

Eosinophil count

Eosinophils were counted using a light microscope, after dissolving whole blood sample in Pilot's solution as described previously [16].

Results

At baseline, there were no differences in FEV_1 on any of the challenge days (2.93 ± 0.17 l on aspirin, and 2.90 ± 0.15 l on methacholine) (table 2). In all subjects, both aspirin and methacholine produced a significant pulmonary reaction, as evidenced by fall in FEV_1 >20%. Maximal fall in FEV_1 after aspirin occurred between 2.5–3.5 h ($25.3\pm5.0\%$; $p<0.001$ paired t-test) (fig. 1). Following methacholine, bronchoconstriction developed within 30 min ($35.9\pm3.2\%$; $p<0.0001$ paired t-test) (fig.2). The dose of aspirin ranged from 20–160 mg (98.0 ± 17.4). Our patients had large individual differences

Table 2. – Baseline FEV_1, PC_{20}Mch before and 24 h after oral aspirin challenge

		Baseline FEV_1 % pred		PC_{20}Mch mg·ml^{-1}	
No	Patients Init	ASA day	Mch day	Before ASA	24 h after ASA
1	K.G.	72	71	1.6	1.2
2	K.B.	98	99	5.5	5.5
3	B.R.	122	111	10.5	9.0
4	S.Z.	114	114	34.0	32.0
5	K.J.	79	82	3.5	1.5
6	K.H.	103	104	3.5	0.6
7	G.H.	78	80	6.6	5.0
8	K.L.	79	73	3.6	3.2
9	G.J.	77	80	10.0	6.0
10	W.J.	74	75	13.0	8.8
Mean		89.5	88.8	9.1	7.2
SEM		5.7	5.2	3.0	2.4
Paired t-test		NS		p<0.003	

NS: nonsignificant; ASA: aspirin (acetylsalicylic acid); Mch: methacholine; FEV_1: forced expiratory volume in one second; PC_{20}Mch: provocation dose of methacholine producing a 20% fall in FEV_1.

Statistical methods

The repeated-measures analysis of variance (ANOVA) of logarithmic observation with dose of aspirin (or methacholine) as a covariable was used to analyse time-dependent changes in urine and serum concentration of examined compounds. The F stat-istic was employed to test the significance. Multiple-regression analysis was performed with FEV_1, tryptase, 11-dehydro-TXB_2 as the dependent variables and LTE_4 as the independent variable. Comparison of baseline and maximal values of examined compounds after aspirin provocation were analysed using paired Studen's t-test, or a nonparametric Wilcoxon's signed rank test. Correlations between PC_{20}Mch and threshold dose of aspirin, and likewise between the maximal fall of blood eosinophil count and PC_{20}Mch examined at 24 h after aspirin challenge, were determined by linear regression analysis. Data are given as mean±SEM.

in bronchial responsiveness to methacholine; PC_{20}Mch ranged from 1.6–34.0 mg·ml^{-1} (9.1 ± 3.0). There was no evident correlation between PC_{20}Mch and aspirin threshold dose ($r=0.46$, $p=0.09$). Twenty four hours after aspirin ingestion, the airway hyperresponsiveness increased significantly in all subjects (PC_{20}Mch range 1.2–32.0 mg·ml^{-1}, 7.2 ± 2.4; $p<0.003$ paired t-test) (table 2).

There were no differences between the baseline urinary levels of either LTE_4 or 11-dehydro-TXB_2 on aspirin and methacholine days (151.4 ± 48 8 pg·mg^{-1} creatinine (Cr) *versus* 161.6 ± 46.7 pg·mg^{-1}Cr for LTE_4; and 597.9 ± 80.0 pg·mg^{-1}Cr *versus* 557.7 ± 47.0 pg·mg^{-1}Cr for 11-dehydro-TXB_2). The excretion of LTE_4 increased after aspirin provocation, reaching a maximum between 4–6 h (1053.3 ± 417.8; $p<0.04$ paired t-test) (fig. 1). The elevation of LTE_4 was accompanied by a significant reduction of 11-dehydro-TXB_2 in urine (from 597.9 ± 80.0 pg·mg^{-1}Cr at baseline to 279.9 ± 43.0 pg·mg^{-1}Cr at 24 h of study; $p<0.005$ paired t-test) (fig. 1).
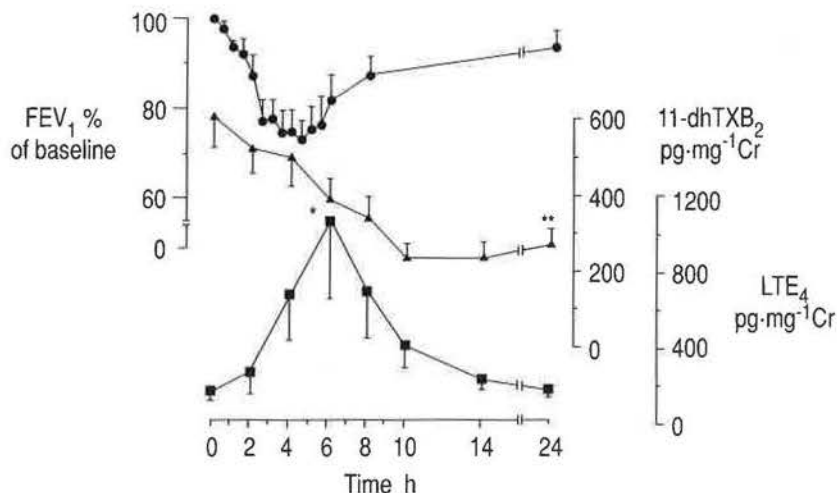
Fig. 1. – Oral aspirin challenge produces a fall in FEV₁ (solid circles), depression of urinary 11-dehydro-TXB₂ (solid triangles); pg·mg⁻¹ creatinine (Cr) p<0.001 ANOVA) and increase in urinary LTE₄ (solid squares; pg·mg⁻¹Cr; p<0.001 ANOVA) in 10 AIA patients. *: p<0.04; and **: p<0.005 (paired t-test) as compared with baseline. Each point represents mean±SEM. FEV₁: forced expiratory volume in one second; 11-dhTXB₂: 11-dehydro-thromboxane B₂; LTE₄: leukotriene E₄; Cr: creatinine; ANOVA: analysis of variance; AIA: aspirin-induced asthma.
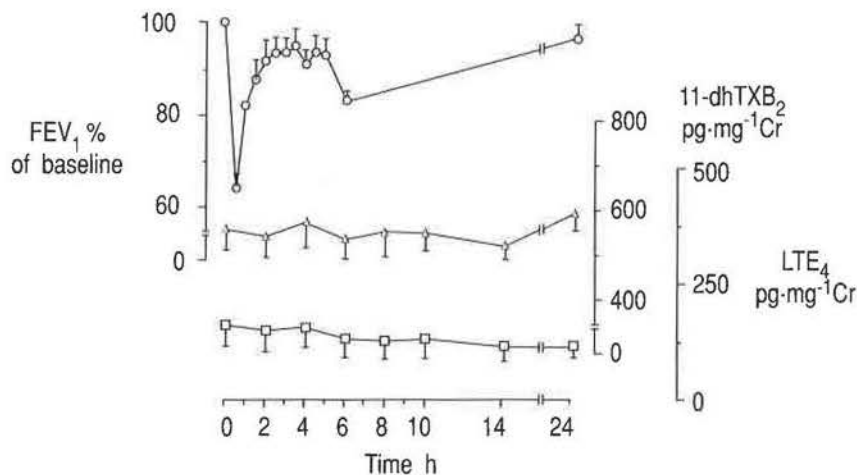

Fig. 2. – Methacholine inhalation results in a significant fall in FEV₁ (open circles), but does not effect urinary levels of 11-dehydro-TXB₂ (open triangles), or of LTE₄ (open squares) in 10 AIA patients. Each point represents mean±SEM. For abbreviations see legend to table I.

The intensity of both LTE₄ and TXB₂ responses depended on the dose of aspirin used (p<0.001 ANOVA). Peak LTE₄ levels were not correlated to maximum fall in FEV₁ (r=0.154; p=0.33). Methacholine challenge did not cause any changes in LTE₄ or 11-dehydro-TXB₂ urinary levels (fig. 2).

Following aspirin challenge, mean serum tryptase rose, reached a maximum at 4 h and then gradually declined, returning to baseline levels after 24 h (fig. 3). Mean levels at baseline were 0.77±0.11 ng·ml⁻¹, range 0.46–1.24 ng·ml⁻¹; and at 4 h 2.21±0.75 ng·ml⁻¹, range 0.62–7.10 ng·ml⁻¹ (p<0.04 Wilcoxon's signed rank test). In our patients we observed no evident relationship between aspirin-related clinical symptoms, such as scarlet flushing of the skin, rhinitis or blockage of the nose, and serum tryptase or urinary LTE₄ levels. Following methacholine challenge, serum tryptase levels showed no change; in all subjects the value remained between 0.44–0.66 ng·ml⁻¹ throughout the study period.

Aspirin provocation induced a gradual fall in blood eosinophil count (282.8±62.7 cells·mm⁻³ at baseline, and 127.0±43.2 cells·mm⁻³ at 4 h) and serum ECP levels (10.6±1.4 ng·ml⁻¹ at baseline, and 6.8±1.4 ng·ml⁻¹ at 4 h) (fig. 4). Eosinophil count reached a minimum at 6 h (101.9±35.6 cells·mm⁻³; p<0.01 paired t-test), while ECP serum levels increased nonsignificantly to 14.1± 6.8 ng·ml⁻¹ at the same time, and returned to 10.7±1.9 ng·ml⁻¹ at 8 h (fig. 4). Eosinophil count returned to initial values at 24 h after aspirin challenge (fig. 4). The fall in blood eosinophils at 6 h correlated with PC₂₀Mch examined 24 h after oral aspirin challenge (r=0.66, p<0.04). Following methacholine inhalation, the serum ECP levels and eosinophil count showed no changes.

Following lysine-aspirin inhalation, all subjects studied developed significant bronchoconstriction, expressed as a fall in FEV₁ exceeding 20% of baseline (28.3±3.9%; p<0.02 paired t-test). The reaction developed within about 40 min (range 15–60 min).
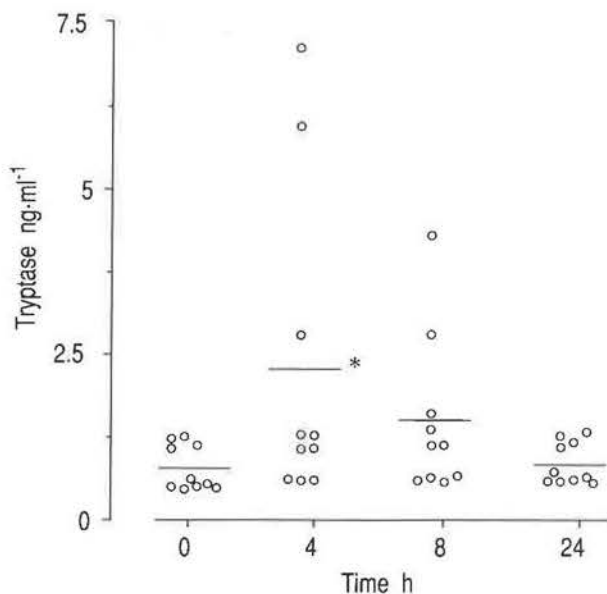
Fig. 3. – Effect of oral aspirin challenge on serum tryptase levels (ng·ml^{-1}) in AIA patients. Solid lines show arithmetic mean. *: indicates p<0.04 (Wilcoxon's test) at 4 h after aspirin challenge in comparison with baseline.
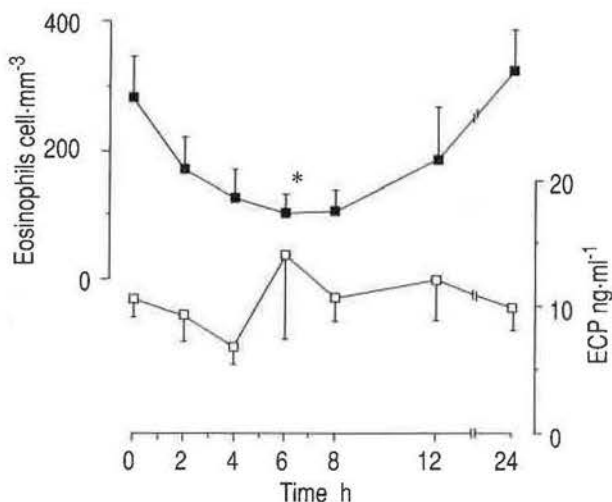

Fig. 4. – Oral aspirin challenge induces fall in blood eosinophil count (solid squares) and nonsignificant increase in serum ECP levels (open squares) in 10 AIA patients. *: indicates p<0.005 (paired t-test) at 6 h after aspirin challenge in comparison with baseline. Each point represents mean±SEM. ECP: eosinophil cationic protein; AIA: aspirin-induced asthma.

The final concentrations of acetylsalicylic acid inhaled varied from 1–2 mg (table 1). In none of the subjects did we observe any additional symptoms usually accompanying oral challenges, *i.e.* skin flushing, rhinitis, conjunctivitis *etc*. Throughout the study period, serum tryptase showed no changes and remained within the range 0.07–0.46 in all patients. Thus, patients reacting to oral aspirin with increased serum tryptase levels, reacted to inhaled lysine-aspirin with asthma, but had normal serum tryptase levels. Similarly, no changes in ECP were observed (data not shown).

Discussion

It has recently been reported that asthmatic attacks precipitated by oral aspirin [5], or inhaled lysine-aspirin [17], are associated with increase in the excretion of urinary LTE$_4$. We observed a similar association and at the same time noticed inhibition of cyclooxygenase, as evidenced by a fall in urinary 11-dehydro-TXB$_2$. In contrast, methacholine challenge, producing comparable bronchial obstruction, did not alter eicosanoid production. Eicosanoid response, therefore, is not evoked by a nonspecific bronchoconstriction. Similarly, WESTCOTT *et al.* [18] recently showed that methacholine challenge did not alter excretion of urinary LTE$_4$ in patients with allergic asthma.

We have not measured urinary LTE$_4$ following placebo administration at the time when aspirin threshold dose was predetermined. Placebo, however, produced neither clinical symptoms nor pulmonary function alteration in our patients. Others [5], reported lack of LTE$_4$ excretion after placebo administration in AIA.

In our patients, bronchoconstriction began within 30 min after aspirin ingestion. The dose of aspirin varied from 20–160 mg. Dosage seemed to determine the magnitude of the urinary eicosanoid response; changes in both LTE$_4$ and 11-dehydro-TXB$_2$ were correlated to the amount of aspirin received. The lowest values of FEV$_1$ occurred at 2.5–3.0 h, while the maximal raise of LTE$_4$ was recorded between 4–6 h. Such a course of LTE$_4$ excretion following aspirin provocation was similar to that reported by CHRISTIE and co-workers [5]. It is consistent with urinary LTE$_4$ response to LTD$_4$ inhalation [19], and intravenous infusion of LTC$_4$ in healthy subjects [20]. Since quantitation of LTE$_4$ in urine is related to systemic synthesis of peptidoleukotrienes [20], increased excretion of LTE$_4$ may reflect changes in leukotriene synthesis in the lungs of an aspirin-intolerant patient with asthma.

We observed no relationship between urinary excretion of LTE$_4$ and initial airway nonspecific hyperresponsiveness. Furthermore, airways response to methacholine was not correlated to threshold doses of aspirin, reported by other authors [12, 21]. However, 24 h after oral aspirin provocation the bronchial reactivity to methacholine increased significantly. This is consistent with recent observations [22] indicating that sulphidopeptide leukotrienes enhance nonspecific airway responsiveness in asthmatic patients.

We noticed no differences in urinary LTE$_4$ response between patients on chronic systemic corticotherapy as compared to those who took no steroids. Systemic administration of steroids does not alter eicosanoid levels in asthmatic airways [23]. In aspirin-sensitive asthmatics, steroids can attenuate clinical reaction to aspirin [24], but only when given at a dose distinctly higher than that used by our patients.

The mechanism of leukotrienes overproduction in AIA is unknown. A popular concept states that under utilization of arachidonic acid, *via* blocked cyclooxygenase, increases its availability for 5-lipoxygenase. As a result, arachidonic acid is shunted from one pathway to the other, and leukotrienes are formed in increased

quantities. For reasons explained elsewhere [10, 25] we do not favour this concept. Lack of inverse relationship between fall in urinary 11-dehydro-TXB$_2$ and rise in urinary LTE$_4$, observed in this study, also speaks against the "shunting" idea. A more plausible explanation, proposed by KUEHL et al. [26], suggests that prostaglandin E$_2$ (PGE$_2$) exerts direct inhibitory action on initiation of leukotriene biosynthesis. A local deficiency of PGE$_2$, as a consequence of cyclooxygenase inhibition, might remove a damping mechanism controlling leukotriene production, and released leukotrienes would result in asthma attacks. Depressed PGE$_2$ levels [27] and rise in leukotrienes [27–29] were also observed in nasal lavage fluid obtained from aspirin-intolerant asthmatics following local or oral aspirin challenges. If AIA is caused by viral infection, as suggested by a recent hypothesis [30], then temporary removal of PGE$_2$ would set free specific cytotoxic T lymphocytes, which in turn would attack the infected cells of the respiratory tract, precipitating asthma.

Are cysteinyl leukotrienes specific mediators for AIA? A note of caution seems warranted. In our study, the intensity of aspirin-precipitated bronchial obstruction showed no correlation to release of leukotrienes in urine. Rise in urinary LTE$_4$ was observed in clinical reactions, precipitated not only by aspirin, but also by allergens [7, 17]. Interestingly, preliminary studies have demonstrated that both allergen- and aspirin-provoked bronchoconstriction can be attenuated or prevented by leukotriene inhibitors. In these studies, most but not all of the subjects were partially protected [31, 32, 33]. It seems that cysteinyl leukotrienes could act as one of the final mediators, their release being triggered by specific stimuli, different in various types of asthma.

Mast cells could be a source of leukotrienes. In AIA, their activation has been a matter of controversy. Some authors have reported a rise in plasma histamine levels [34, 35], and mast-cell associated neutrophil chemotactic activity [36], following challenge in patients with AIA. Others have provided data [37, 38] pointing to a lack of mast cell activation. Pretreatment with terfenadine, an H$_1$ histamine receptor antagonist, offered protection against bronchospasm provoked by inhaled lysine-aspirin in only one of six patients with AIA [12]. In another study [39], pretreatment with clemastine, a receptor blocking antihistamine, gave protection against flushing, rhinorrhea and headache in 10 AIA patients challenged with oral aspirin; reduction in bronchospasm intensity was, however, observed in only 5 of the 10 patients. Ketotifen, equipped with potent antihistamine activity, reduced or prevented aspirin reactions in the majority of patients studied [40, 41]. Pretreatment with disodium cromoglycate, a mast-cell stabilizer, gave conflicting results [42, 43].

In the present study, we measured serum tryptase, a specific marker of mast cell activation [44]. In the group of 10 patients studied, mean serum tryptase level increased significantly within 4 h of aspirin ingestion, preceding by 2 h the peak excretion of urinary LTE$_4$. In contrast, inhalation challenge with lysine-aspirin, resulting in a comparable bronchoconstriction, did not produce any alternation in serum tryptase levels. Four of the patients who underwent both challenges, and responded with serum tryptase rise to oral aspirin, showed no such response to aspirin inhalation. Oral, but not inhalation challenges, were associated with non-respiratory symptoms, such as flushing, rhinorrhea, headache and conjunctivial irritation. Tryptase, therefore, could have been released from degranulating mast cells of skin, nose or cerebral vessels. Our previous results [39], demonstrating that clemastine protects against non-respiratory symptoms provoked by aspirin, are consistent with such a hypothesis. In a recent study, BOSSO et al. [45] observed a serum tryptase rise only in those aspirin-intolerant asthmatics who responded to oral aspirin challenge with severe respiratory reactions extending to skin and/or gastrointestinal tract.

Searching for a cellular origin of leukotrienes we also looked at eosinophils. We observed a significant fall in blood eosinophil count, reaching a minimum at 6–8 h after aspirin provocation. The fall correlated with airway hyperresponsiveness to methacholine, examined at 24 h of the study. This might reflect eosinophil recruitment into the respiratory tract after aspirin provocation. Bronchoalveolar eosinophilia [46], and a parallel fall in blood eosinophil count [47] was reported during the late response to allergen inhalation in asthmatic subjects. Depression of the blood eosinophil count following aspirin ingestion was accompanied by a rise in ECP which, however, did not reach levels of statistical significance. ECP is a protein, cytotoxic for human bronchial epithelium, released from eosinophil upon activation [48]. Interestingly, the highest ECP values following aspirin were recorded in one of our AIA patients who was atopic. These observations suggest involvement of eosinophil in pathogenesis of aspirin-provoked bronchoconstriction in some patients with AIA.

Our study confirms two recent reports [5, 18] on increased excretion of urinary LTE$_4$ in aspirin-provoked asthma. Furthermore, it demonstrates that leukotriene overproduction is accompanied by cyclooxygenase inhibition, and that the magnitude of leukotriene response is related to the dose of aspirin used. In aspirin-sensitive asthmatics, following oral aspirin challenge, blood eosinophil count falls, and eosinophils and mast cells often show signs of activation. These cells may be the source of bronchoconstrictor leukotrienes, as well as of other mediators, responsible for additional skin, eye and nasal symptoms in aspirin-precipitated attacks of asthma.

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