Prostaglandin E_2 systemic production in patients with asthma with and without aspirin hypersensitivity

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ABSTRACT

Background: A special regulatory role for prostaglandin E_2 has been postulated in aspirin-induced asthma. A study was undertaken to investigate the effects of aspirin on the systemic production of prostaglandin E_2 and cysteinyl leucotrienes in patients with asthma.

Methods: The urinary concentrations were determined of two main prostaglandin E_2 metabolites (13,14-dihydro-15keto-PGE_2 using a commercial enzyme immunoassay and 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid by gas chromatography/mass spectrometry) and leucotriene E_4 using an immunoassay. Determinations were performed at baseline and following oral aspirin and celecoxib challenges in two well-defined asthma phenotypes: aspirin-sensitive and aspirin-tolerant patients.

Results: Aspirin precipitated bronchial reactions in all aspirin-sensitive patients but in none of the aspirin-tolerant patients. Celecoxib 400 mg was well tolerated by all patients except for one with aspirin-induced asthma. At baseline, the mean levels of prostaglandin E_2 metabolites did not differ between the groups. Following different aspirin provocation doses, the mean levels of the two main prostaglandin E_2 metabolites were decreased in the aspirin-tolerant group but remained unchanged in the aspirin-sensitive group. The dose of aspirin had no effect on the magnitude of the response on the prostaglandin E_2 metabolites and its duration. In both groups, urinary prostaglandin E_2 metabolites decreased following celecoxib challenge. No correlation was found between prostaglandin E_2 metabolites and leucotriene E_4.

Conclusions: Aspirin-precipitated asthmatic attacks are not associated with changes in the systemic production of prostaglandin E_2. In contrast, the systemic production of prostaglandin E_2 becomes depressed by aspirin in non-sensitive patients. This different response might indicate COX-1-dependent prostaglandin E_2 control of inflammatory cells in aspirin-induced asthma. Thus, PGE_2 is released during the clinical reactions to aspirin through an alternative COX-2 pathway. The clinical implications of this finding are in line with current observations of good tolerance of the selective COX-2 inhibitors in aspirin-sensitive patients.

Prostaglandin E_2 (PGE_2) is a bioactive compound formed by actions of cyclooxygenase (COX) and specific PGE synthases.[1] In human airways PGE_2 is produced by many cells including epithelium, smooth muscle, alveolar cells, macrophages, phagocytes and lymphocytes.[1 2] In vitro, PGE_2 relaxes smooth muscle and displays a number of inhibitory effects on mast cell degranulation, synthesis of leucotriene B_4, activation of granulocytes and T cells.[3–5] PGE_2 elicits a large number of biological effects acting through four receptors: EP1, EP2, EP3 and EP4.[6–8] The response of target cells to PGE_2 varies according to the spectrum of receptors they express.

PGE_2 might be of special importance in aspirin-induced asthma. This is a distinct clinical syndrome affecting 5–10% of adults with asthma.[9 10] Asthma attacks triggered by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) are associated with inhibition of COX[11 12] (specifically COX-1 but not COX-2) and are characterised by overproduction of cysteinyl-leucotrienes (cys-LTs).[13–15] Inhaled PGE_2 protects against both aspirin-precipitated attacks of asthma and the massive release of urinary LTE_4.[16 17] The protection does not seem to be achieved through relaxant effects on bronchial smooth muscle, but rather by suppression of the mediators released from the proinflammatory cells. The incriminated cells could be mast cells or eosinophils. PGE_2 greatly inhibits oxygen bursts in granulocytes, including eosinophils,[4] and slows down the biosynthesis of cys-LTs in peripheral blood mononuclear cells.[18]

Because of the rapid metabolism of PGE during sampling and isolation of plasma,[1] measurement of stable metabolites in urine rather than the parent compound is the most efficacious method of assessing their endogenous production.[19] Moreover, human kidneys are abundant source of prostanoids, including PGE_2. Local biosynthesis of PGE_2, dependent on both constitutive COX-1 and inducible COX-2 in the kidneys, varies with age and salt intake. It is also affected by several drugs including angiotensin converting enzyme inhibitors.[20] PGE_2 produced in the kidneys is excreted as the parent compound and does not affect the pool of metabolites produced by systemic inactivation of PGE_2.[21] The main urinary metabolites of PGE_2 levels are believed to reflect global PGE_2 production. The predominant pathway of PGE_2 metabolism has been shown to involve transformation of PGE_2 by the 15-OH PGDH enzyme to 15-keto-PGE_2. Subsequently, 15-keto-PGE_2 is rapidly converted to its main metabolite of PGE_2 (13,14-dihydro-15keto-PGE_2; PGE_2-M) by the enzyme 15-keto-prostaglandin-Δ^{13} reductase.[22] Oxidation of 13,14-dihydro-15keto-PGE_2 leads to formation of a major metabolite of PGE_2 (9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid; tetranor-PGE-M). Tetranor-PGE-M is a stable urinary metabolite of PGE_1 and PGE_2 and is used as a urinary marker of PGE_2 biosynthesis.[23 24]

We studied the urinary excretion of two major PGE_2 metabolites, reflecting the systemic

Table 1 Clinical characteristics of study patients

	AIA (n = 19)	ATA (n = 21)	p value
Age (years)	42.4 (13.3)	43.6 (12.5)	0.613 (NS)
	41 (31–53)	43 (36–53)	
Sex (F/M)	11/8	13/8	0.967 (NS)
Duration of asthma (years)	7.8 (7.5)	10.6 (9.4)	0.328 (NS)
	5 (1–11)	7 (3–18)	
Inhaled steroids (yes/no)	11/8	17/4	0.170 (NS)
Inhaled steroids (μg/day)	1690 (712)	1424 (821)	0.204 (NS)
	1600 (1000–2000)	1000 (800–1600)	
FEV_1 baseline (% predicted) placebo day	90.4 (10.7)	90.5 (12.1)	0.683 (NS)
	94.8 (82.5–98.1)	91.4 (81.5–93.6)	
FEV_1 baseline (% predicted) aspirin day	91.6 (10.8)	94.1 (12.0)	0.708 (NS)
	93.0 (87.7–98.4)	94.0 (81.9–100.2)	
FEV_1 baseline (% predicted) celecoxib day	92.4 (12.4)	90.0 (13.8)	0.631 (NS)
	90.9 (85.7–98.6)	86.6 (78.0–95.7)	
Total IgE (IU/ml)	182.3 (229.8)	206.0 (241.6)	0.844 (NS)
	121 (45.4–226)	91.9 (39.9–345)	
Skin prick test (n) positive/negative	8/7	9/9	0.849 (NS)
Blood eosinophil count	425.4 (328.4)	356.7 (246.7)	0.667 (NS)
	371 (159–669)	296 (1164–502)	

Values are expressed as mean (SD) or median (25–75% percentiles).
AIA, aspirin-induced asthma; ATA, aspirin-tolerant asthma; FEV_1, forced expiratory volume in 1 s; NS, not significant.

production of this prostaglandin. The studies were carried out in patients with aspirin-sensitive and aspirin-tolerant asthma, both at baseline and after challenge with aspirin and celecoxib. We expected to be able to identify aspirin intolerance by a decrease in systemic PGE_2 production following aspirin challenge. We also looked at the possible relationship between release of PGE_2 metabolites and that of cys-LTs. To our knowledge, we are the first to perform such investigations.

METHODS

Subjects

The study population consisted of 19 patients with aspirin-induced asthma (AIA) and 21 patients with aspirin-tolerant asthma (ATA). The characteristics of the study patients are shown in table 1.

The diagnosis of aspirin intolerance was confirmed by oral aspirin provocation tests performed during the 24 months preceding the study. All patients with ATA occasionally used aspirin without any adverse reactions. The patients had stable asthma and their baseline forced expiratory volume in 1 s (FEV_1) was >70% of the predicted value on the study day. None had experienced an exacerbation or a respiratory tract infection in the 6 weeks preceding the study. The subjects were instructed to withhold medications that decrease bronchial responsiveness prior to aspirin/celecoxib challenge. Short-acting β_2 agonists were not used 8 h before the challenge. Long-acting β_2 agonists and theophylline were withdrawn for 24 h. Short-acting antihistamines and cromones were stopped 5 days before the challenge. Inhaled steroids were allowed at a dose of $\leqslant$2000 μg budesonide per day. None of the patients was treated with systemic corticosteroids or leucotriene modifying drugs.

Basal urinary levels of PGE_2-M, tetranor-PGE-M and LTE_4 were measured in 30 healthy subjects. The controls tolerated NSAIDs well and had no history of adverse reactions to aspirin and other aspirin-like drugs.

The patients gave informed consent and the study was approved by the university ethics committee.

Study design

The study consisted of two phases. In the first phase, at 1-week intervals, the patients underwent aspirin and celecoxib testing. The single-blind placebo-controlled oral challenge test with aspirin was carried out on two consecutive days. On the first day four capsules of placebo were administered every 1.5 h. On the second day the patients were challenged with increasing doses of 27, 44, 117, 312 mg aspirin at 1.5 h intervals up to the cumulative dose of 500 mg.[25] On the eighth day a single dose of 400 mg celecoxib was administered.

In the second phase of the study, following a 2-week run-in period, the same patients with ATA again underwent provocation tests with aspirin at a cumulative dose of 188 mg (ie, the dose which precipitated asthma attacks in nine patients with AIA). The purpose of the second phase was to exclude a dose dependence of the results obtained in the first phase in the ATA group. In the first phase, all patients with ATA were given aspirin in a dose of 500 mg while provocation doses of aspirin among patients with AIA differed. A single-blind placebo-controlled oral challenge test with aspirin was carried out on two consecutive days. On the first day three capsules of placebo were administered every 1.5 h. On the second day the patients were challenged with increasing doses of 27, 44 and 117 mg aspirin at 1.5 h intervals up to the cumulative dose of 188 mg.

Placebo, aspirin and celecoxib had an identical appearance. The challenge procedure with aspirin and/or celecoxib was interrupted if a bronchospastic reaction occurred (FEV_1 fell $\geqslant$20%) or if the maximum cumulative dose of aspirin and a single dose of celecoxib was reached. The cumulative dose of aspirin causing a 20% fall in FEV_1 was calculated and recorded as the provocation dose of aspirin (PD_{20}).

FEV_1 and extrabronchial symptoms were recorded at baseline, before the challenge tests and then every 30 min until 6 h after the last dose of aspirin and celecoxib.

In patients with a positive aspirin challenge (AIA), urine samples were collected for measurement of PGE_2-M, tetranor-PGE-M and LTE_4 estimations performed at baseline, at the time of appearance of the bronchial symptoms (time 0) and 2 and 4 h later. In patients with ATA in whom the aspirin challenge was negative, urine samples were collected at baseline, 1.5 h after the last aspirin dose (ie, when the cumulative doses of 500 mg (first phase) and 188 mg (second phase) were reached (time 0)), and then 2 and 4 h later.

In case of a single dose of celecoxib, urine samples were collected in the same manner as for the aspirin provocation challenge in patients with ATA and AIA.

Lung function tests

Pulmonary function tests were performed on a flow-integrating computerised pneumotachograph (Pneumoscreen; E Jaeger, Germany).

Urinary levels of PGE_2-M, tetranor-PGE-M and LTE_4

Urinary levels of PGE_2-M (Cayman Chemical, Prostaglandin E metabolite EIA Kit) and LTE_4 (Cayman Chemical, Ann Arbor, Michigan, USA) were measured in unpurified urine samples by direct enzyme immunoassay.[26] Measurements were made at the same time, in duplicates, using the same batch of the reagents. The results were expressed as pg/mg creatinine. The urinary concentration of tetranor-PGE-M was measured by gas chromatography/mass spectrometry[27 28] (see methods in the online data supplement).

Statistical analysis

Summary statistics were expressed as mean (M), standard deviation (SD), median (Me) and 25% and 75% percentiles. General Linear Model (GLM) including repeated measures analysis of variance, which takes into account the fact that the outcome measurements are repeated over time within subjects, was used for multiple comparisons. Logarithmic transformation was used when needed as variance stabilising transformation. To describe better the changes in time for log-transformed data, 95% confidence intervals (CI) in fold difference units[29] were constructed.

Correlation between variables was estimated with the Spearman rank order correlations. A p value of $\leqslant 0.05$ was considered statistically significant.

RESULTS

Clinical reactions

There was no statistical difference in clinical characteristics between the patients with AIA (positive aspirin challenge test) and those with ATA (negative aspirin challenge test), table 1. None of the patients developed symptoms after administration of placebo. In the AIA group, bronchial reactions developed after 27 mg in one subject, after 44 mg in one subject, after 117 mg in nine subjects and after 500 mg in six. The mean cumulative dose of aspirin was 188 mg. One patient developed asthma and anaphylactic shock after receiving aspirin at a dose of 177 mg. In another patient, abdominal pain accompanied by a transient increase in urinary and serum amylase (6718 U/l (normal range 32–640 U/l) and 2031 U/ml (normal range 30–110 U/ml), respectively) and serum lipase (615 U/l (normal range 23–300 U/l)) was recorded.

None of the patients with ATA developed any clinical symptoms following aspirin or celecoxib challenges. In one of 17 patients with AIA the celecoxib challenge produced dyspnoea and FEV_1 fell by 21%. This was the same patient who developed shock after aspirin administration. The case has been described elsewhere.[30] The remaining 16 patients in the AIA group tolerated celecoxib very well.

All the symptoms were relieved by short-acting β_2 agonists. Systemic corticosteroids were required in three cases only. Subcutaneous adrenaline was administered to the patient who developed shock.

Urinary PGE_2-M levels

Phase I

At baseline, urinary levels of PGE_2-M did not differ significantly between the study groups and healthy control subjects (table 2, $p = 0.52$). After placebo administration, no significant differences in urinary PGE_2-M levels were found between the study groups ($p = 0.58$, ANOVA). In patients with AIA, urinary PGE_2-M levels increased 2 h following placebo administration compared with baseline values ($p = 0.01$; 95% CI 1.030 to 1.722 baseline; fig 1A).

PGE_2-M values on the day of aspirin challenge differed significantly between the AIA and ATA groups ($p<0.001$, ANOVA). The levels did not change at any time during the observation period in patients with AIA but, in the ATA group, urinary PGE_2-M concentrations were decreased 2 h ($p<0.001$; 95% CI 0.417 to 0.688 baseline) and 4 h ($p<0.001$; 95% CI 0.339 to 0.559 baseline) following aspirin challenge tests compared with baseline values. The lowest level was reached 4 h after aspirin administration (fig 1B).

Following celecoxib challenge, urinary concentrations of PGE_2-M were significantly higher in patients with AIA ($p = 0.04$, ANOVA). In the AIA and ATA study groups urinary PGE_2-M levels decreased at 2 h ($p = 0.08$ and $p = 0.05$; 95% CI 0.557 to 0.918 baseline, respectively) and 4 h ($p = 0.04$; 95% CI 0.532 to 0.889 baseline and $p = 0.007$; 95% CI 0.521 to 0.857 baseline, respectively) following celecoxib challenge tests compared with baseline values (fig 1C). The dose of aspirin had no effect on the magnitude of the response of PGE_2-M and its duration.

Table 2 Baseline values of eicosanoids in patients with AIA or ATA and healthy controls (values represent means of three estimations performed in each patient 1 week apart)

				p Value		
	AIA (n = 19)	ATA (n = 21)	Healthy (n = 30)	AIA vs ATA	AIA vs HC	ATA vs HC
Urinary PGE_2-M (pg/mg creatinine)	477.1 (277.69)	480.7 (222.1)	879.47 (210.78)	0.95	0.88	0.72
	464 (281–600)	451 (342–538)	403.5 (303–763)			
Urinary tetranor-PGE-M (ng/mg creatinine)	11.05 (9.64)	10.27 (7.83)	10.06 (8.43)	0.79	0.96	0.87
	7.2 (5.4–11.1)	7.9 (5.0–12.4)	7.6 (4.9–10.5)			
Urinary LTE_4 (pg/mg creatinine)	1846.6 (2747.4)	342.0 (277.7)	257.0 (180.2)	<0.001	<0.001	0.75
	1347 (355–2036)	220 (160–361)	222 (119–315)			

Values are expressed as mean (SD) and median (25–75% percentiles).
AIA, aspirin-induced asthma; ATA, aspirin-tolerant asthma; PGE_2-M, 13,14-dihydro-15keto-PGE_2; tetranor-PGE-M, 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid; LTE_4, leucotriene E_4.

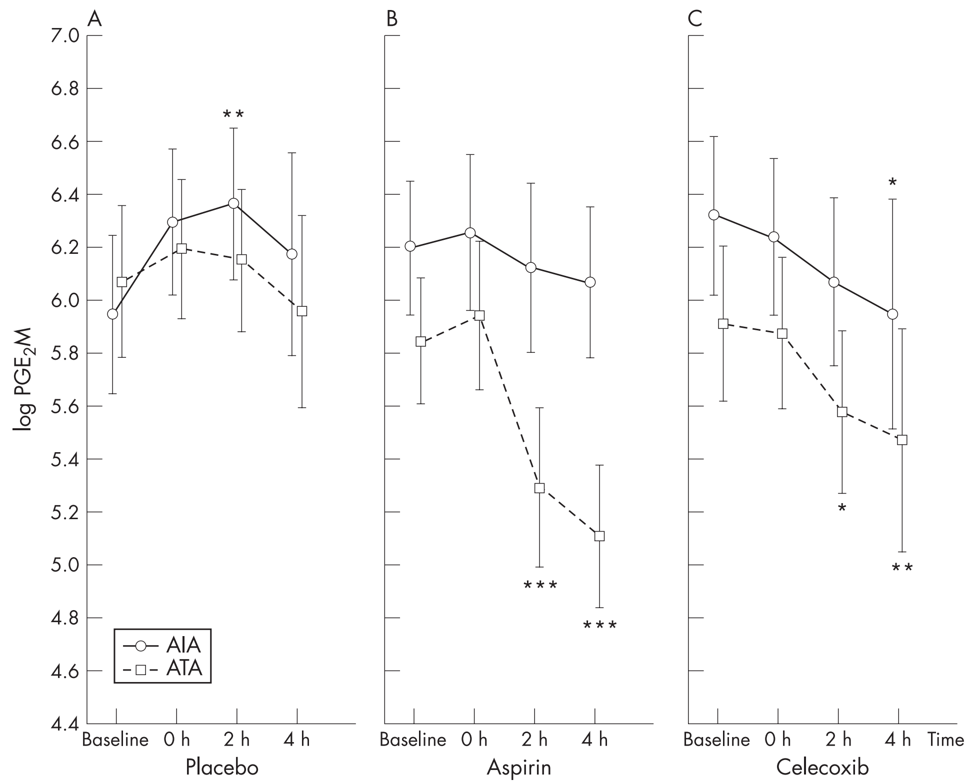

Figure1 Urinary levels of the prostaglandin E_2 (PGE_2) metabolite 13,14-dihydro-15keto-PGE_2 (PGE_2-M) in pg/mg creatinine before and after (A) placebo, (B) aspirin and (C) celecoxib challenges in patients with aspirin-induced asthma (AIA) and aspirin-tolerant asthma (ATA) (phase I). ***$p \leqslant 0.001$; **$p \leqslant 0.01$; *$p \leqslant 0.05$.

At baseline and 4 h following aspirin challenge, urinary PGE_2-M levels were increased only in patients with AIA with the highest levels of total serum IgE (Spearman $r = 0.6$; $p = 0.03$). Baseline concentrations of PGE_2-M in the urine of healthy controls were 1.59-fold (59%) greater in men than in women ($p = 0.01$). In contrast, there was no relationship between gender and urinary PGE_2-M levels at baseline or following any of the challenges in the patient groups studied ($p = 0.18$, ANOVA).

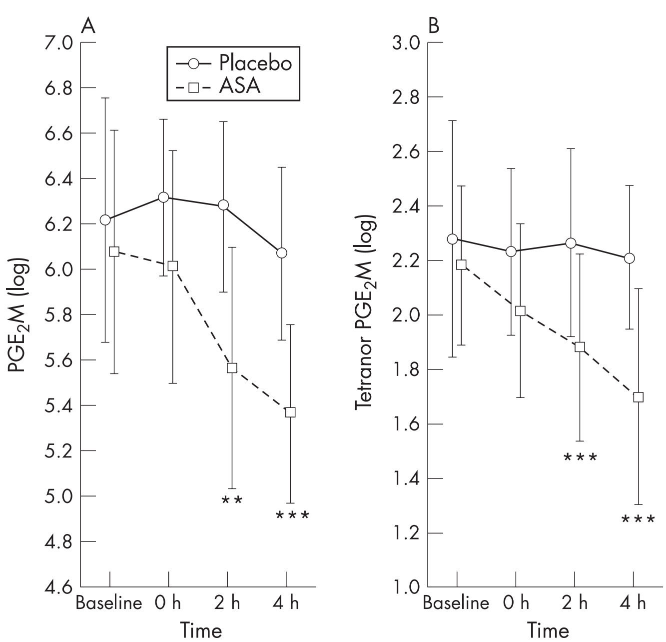

Figure 2 Urinary levels of prostaglandin E_2 (PGE_2) metabolites before and after placebo and a cumulative dose of 188 mg aspirin in patients with aspirin-tolerant asthma (phase II). (A) 13,14-dihydro-15keto-PGE_2 (PGE_2-M (PGE_2-M), pg/mg creatinine; (B) 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor-PGE-M), ng/mg creatinine. ASA, aspirin. ***$p \leqslant 0.001$; **$p \leqslant 0.01$; *$p \leqslant 0.05$.

Phase II

At baseline, urinary levels of PGE_2-M did not differ significantly between placebo and aspirin days in patients with ATA ($p = 0.38$). After placebo administration, no significant differences in urinary PGE_2-M levels were found. Urinary PGE_2-M levels decreased 2 h ($p = 0.005$; 95% CI 0.488 to 0.737 baseline) and 4 h ($p<0.001$; 95% CI 0.399 to 0.603 baseline) following a cumulative dose of 188 mg aspirin compared with baseline values in patients with ATA (fig 2A).

Urinary 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid (tetranor-PGE-M)

Phase I

At baseline, urinary levels of tetranor-PGE-M did not differ significantly between both study groups and healthy control subjects (table 2, $p = 0.94$, ANOVA). Following placebo administration, no significant differences in urinary tetranor-PGE-M levels were found between patients with AIA and those with ATA ($p = 0.55$, ANOVA). The levels did not change at any time during the observation period ($p = 0.99$, ANOVA, fig 3A).

Tetranor-PGE-M levels following aspirin challenge did not change at any time of the observation period in patients with AIA ($p = 0.23$, ANOVA) but, in patients with ATA, the levels decreased significantly ($p<0.001$, ANOVA). In the ATA group, urinary concentrations of tetranor-PGE-M decreased at 2 h ($p = 0.001$; 95% CI 0.489 to 0.748 baseline) and 4 h ($p<0.001$; 95% CI 0.391 to 0.615 baseline) following aspirin challenge tests compared with baseline values (fig 3B).

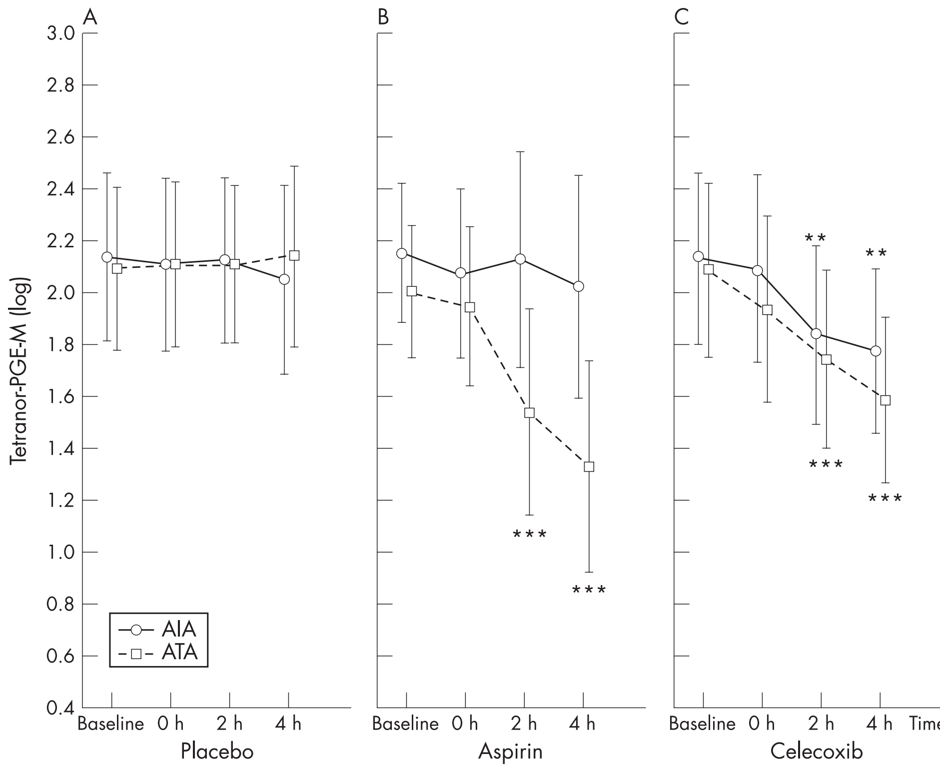

Figure 3 Urinary levels of the prostaglandin E_2 (PGE_2) metabolite 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor-PGE-M) in ng/mg creatinine before and after (A) placebo, (B) aspirin and (C) celecoxib challenges in patients with aspirin-induced asthma (AIA) and those with aspirin-tolerant asthma (ATA) (phase I). ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$.

Following celecoxib challenge, no significant differences were found in urinary tetranor-PGE-M levels between patients with AIA and those with ATA (p = 0.58, ANOVA). Urinary tetranor-PGE-M levels in the AIA and ATA groups decreased at 2 h (p<0.001; 95% CI 0.586 to 0.933 baseline and p<0.001; 95% CI 0.565 to 0.888 baseline, respectively) and 4 h (p = 0.009; 95% CI 0.352 to 0.878 baseline and p<0.001; 95% CI 0.483 to 0.759 baseline, respectively) following celecoxib challenge compared with baseline values (fig 3C).

The dose of aspirin had no effect on the magnitude of the response of tetranor-PGE-M and its duration (p = 0.32, ANCOVA). The concentration of urinary tetranor-PGE-M in

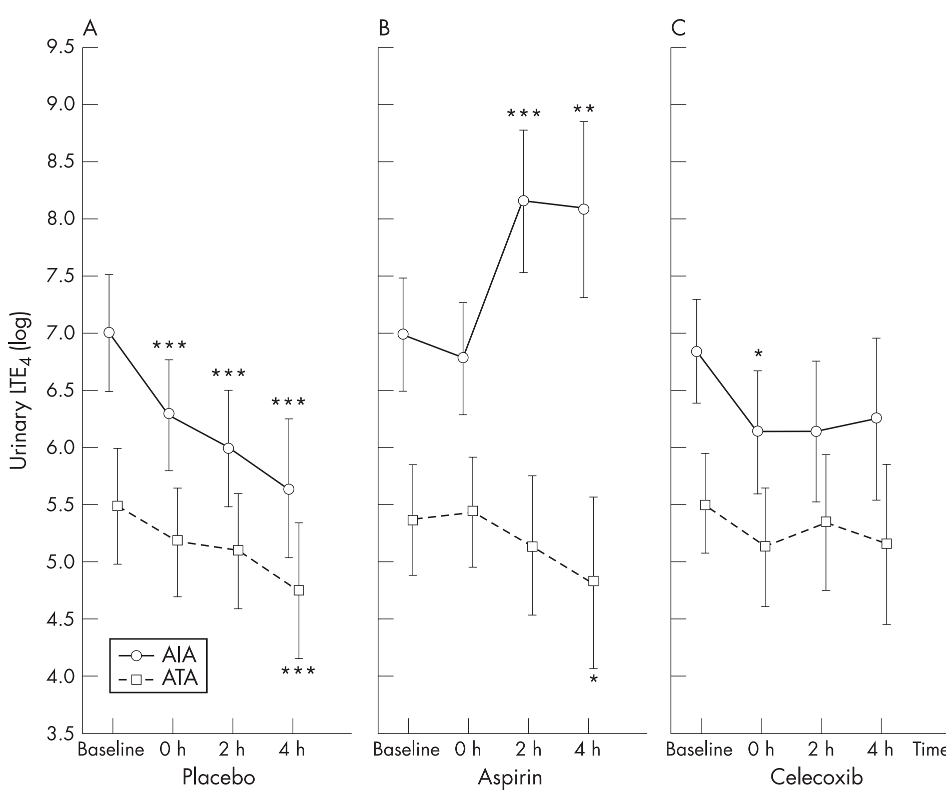

Figure 4 Urinary levels of leucotriene E_4 (LTE_4; pg/mg creatinine) before and after (A) placebo, (B) aspirin and (C) celecoxib challenges in patients with aspirin-induced asthma (AIA) and aspirin-tolerant asthma (ATA) (phase I). ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$.

individual patients with AIA did not depend on the cumulative dose of aspirin which caused bronchospasm.

Baseline urine concentrations of tetranor-PGE-M in healthy controls were 1.39-fold (39%) greater in men than in women ($p = 0.03$). In contrast, there was no relationship between gender and urinary tetranor-PGE-M levels at baseline or following any of the challenges in the patient groups studied ($p = 0.26$, ANOVA).

Phase II

At baseline, urinary levels of tetranor-PGE-M did not differ significantly between placebo and aspirin days in patients with ATA ($p = 0.51$). No significant differences in urinary tetranor-PGE-M levels were found after administration of placebo. Urinary tetranor-PGE-M levels decreased 2 h ($p<0.001$; 95% CI 0.558 to 0.962 baseline) and 4 h ($p<0.001$; 95% CI 0.466 to 0.887 baseline) following a cumulative dose of 188 mg aspirin compared with baseline values in patients with ATA (fig 2B).

Urinary LTE_4

Baseline urinary LTE_4 excretion (table 2) was higher in patients with AIA than in those with ATA ($p<0.001$, ANOVA) or healthy control subjects. In the AIA group a gradual decrease in the urinary LTE_4 level was seen following placebo administration reaching at time 0 ($p<0.001$; 95% CI 0.317 to 0.751 baseline) which continued to fall at 2 h ($p<0.001$; 95% CI 0.237 to 0.561 baseline) and 4 h ($p<0.001$; 95% CI 0.166 to 0.394 baseline).

Urinary LTE_4 levels increased at 2 h ($p = 0.001$; 95% CI 2.087 to 4.948 baseline) and 4 h ($p = 0.003$; 95% CI 1.931 to 4.579 baseline) following aspirin challenge tests compared with baseline values only in the patients with a positive aspirin challenge.

In patients with AIA, following celecoxib challenge urinary LTE_4 levels decreased at time 0 ($p = 0.02$; 95% CI 0.321 to 0.760 baseline) and remained stable 2 h (95% CI 0.320 to 0.762 baseline) and 4 h later (95% CI 0.359 to 0.851 baseline). There were no changes in urinary LTE_4 levels after celecoxib challenge in patients with ATA compared with baseline values (fig 4).

There was a positive correlation between the urinary LTE_4 levels (at baseline and following aspirin challenge) and the blood eosinophil count only in patients with AIA (Spearman $r = 0.78$; $p<0.001$).

Analysis of correlation between urinary LTE_4 and PGE_2-M levels

Following aspirin challenge, no correlation was found between urinary LTE_4 and PGE_2-M levels in patients with AIA. There was a positive correlation between urinary LTE_4 levels and the levels of PGE_2-M following aspirin challenge (at 4 h) only in patients with ATA (Spearman $r = 0.63$; $p = 0.006$). No correlation was found in either study group following placebo and celecoxib challenges.

Analysis of correlation between urinary LTE_4 and tetranor-PGE-M levels

Following placebo, aspirin and celecoxib challenges, no correlation was found between urinary LTE_4 and tetranor-PGE-M levels in patients with AIA ($p = 0.99$, $p = 0.27$, $p = 0.58$, respectively) or ATA ($p = 0.37$, $p = 97$, $p = 0.52$, respectively).

DISCUSSION

In this study we measured the two main metabolites of PGE_2 found in the urine: PGE_2-M by enzyme immunoassay and tetranor-PGE-M by gas chromatography/mass spectrometry. The latter method discriminated between genuine PGE_2 metabolites and the PGE_1 end products depending on diet. It has been reported that the mass spectrometry measurement of PGE_2 metabolites in urine is highly accurate and sensitive.[31]

Our results showed a similar baseline urinary level of PGE_2-M and tetranor-PGE-M in patients with AIA, those with ATA and healthy controls. In healthy subjects, as has been reported previously,[32] the level of both PGE_2 metabolites was significantly higher in men than in women. However, we did not observe these differences in patients with asthma. Aspirin challenge in patients with AIA had no effect on PGE_2-M and tetranor-PGE-M levels at any time during the observation period compared with baseline values. In contrast, in patients with ATA, urinary levels of both PGE_2 metabolites became significantly depressed following two aspirin challenges of either 500 mg or 188 mg. There is a tentative explanation for a paradoxical finding of urinary PGE_2 metabolites not changing during the positive aspirin challenge in patients with AIA. These metabolites decreased following comparable doses of aspirin in other subjects not sensitive to aspirin. Constitutively expressed COX-1, by its main product PGE_2, controls activation of inflammatory cells. Upon inhibition with aspirin, it is the second source of PGE_2 (ie, COX-2) which takes part and may contribute to urinary metabolites. This unmasking effect of aspirin was present only in patients with AIA and, as expected, celecoxib did not discriminate for aspirin sensitivity when urinary metabolites of PGE_2 were measured.

It has been postulated that COX-1 inhibition by aspirin, resulting in reduced PGE_2 production, provides the mechanism that triggers attacks of asthma.[33] This hypothesis has been supported by the following data:

- Inhalation of exogenous PGE_2 prevents bronchoconstriction provoked by aspirin and inhibits urinary excretion of cys-LTs.[16 17 33]
- Epithelial cells from surgically removed nasal polyps of patients with AIA produce less PGE_2 than the same cells from patients with ATA.[34]
- Peripheral blood cells from patients with AIA release less PGE_2 at baseline than those from healthy controls.[35]
- PGE_2 production by bronchial fibroblasts in patients with AIA is lower than in patients with ATA.[36]
- Lower expression of COX-2 in patients with AIA results in deficient production of PGE_2 in nasal polyps, thus contributing to an imbalance between eicosanoids.[37 38]
- Inflammatory cells infiltrating the nasal mucosa of patients with AIA are deficient in EP2 receptor.[39]
- Of 370 single nucleotide polymorphismss from 63 candidate genes, only a particular variant coding for EP2 was significantly associated with AIA.[40]

The evident discrepancy between the systemic biosynthesis of PGE_2, evaluated by its two main metabolites, and accumulating data for PGE_2 inhibition as the triggering mechanism of AIA could be explained by a cellular mechanism. Hypersensitivity to aspirin and other drugs of its class is a phenomenon linked to a specific inflammation[41] in which there is degranulation of mast cells in target organs such as the bronchial wall, nasal and paranasal mucosa and skin in patients with urticaria. This has been repeatedly documented by increased levels of PGD_2 in blood[41 42] and urine,[43 44] histamine in nasal secretions,[45–47] bronchoalveolar lavage fluid[48] and tryptase in blood.[49] It is plausible that activated mast cells can augment the biosynthesis of both PGE_2 and PGD_2. However, only PGD_2 was reported in relation to the challenge. It is also likely that inflammatory mediators released from the activated mast cells following a positive aspirin challenge could affect PGE_2 production through two mechanisms. First, they could upregulate PGE_2 biosynthesis in other cells through an intracellular calcium-dependent phospholipase A_2. This rise in systemic PGE_2 would depend on COX-2 activity and correlate with the number of inflammatory cells. In fact, a selective COX-2 inhibitor reduced urinary levels of both PGE_2 metabolites to the same extent in patients with AIA and those with ATA. Second, they could modulate PGE_2-related pathways. Indeed, in primary human airway epithelial cells, interleukin-13 depresses PGE_2 production but, at the same time, upregulates the activity of enzymes that metabolise PGE_2.[50] The activity of the enzymes involved in PGE_2 degradation could therefore be upregulated by some of the numerous proinflammatory substances that are released during an aspirin-induced asthma attack. Additional evidence is based on upregulation of the cysLT pathway in AIA. Together with eosinophils, mast cells are the main systemic source of cysLTs. The positive correlation between the post-challenge increase of LTE_4 excretion and the blood eosinophil count reflected mast cell- eosinophil functional unit, a hallmark of inflammation associated with cysLT overproduction.[51]

In this study the urinary LTE_4 levels at baseline and following aspirin challenge were in agreement with those shown previously.[16 17] However, it was surprising to find a lack of correlation between the urinary PGE_2 metabolites and LTE_4 levels in patients with AIA. Moreover, the dose of aspirin had no effect on the magnitude of the response of either of the PGE_2 metabolites and its duration in these patients.

In the ATA group, placebo, aspirin and celecoxib tended to reduce urinary excretion of LTE_4. A similar trend was observed following placebo and celecoxib in patients with AIA. These results suggest that, like placebo, aspirin and celecoxib in patients with ATA and celecoxib in patients with AIA do not affect cys-LT metabolism. The tendency of LTE_4 to decrease over time following placebo, aspirin and celecoxib administration in patients with ATA and after placebo and celecoxib in those with AIA may reflect the diurnal variation in cys-LT production in the body. However, this observation needs to be confirmed by further studies.

In one of 17 patients with AIA who developed shock following aspirin challenge, celecoxib triggered signs of bronchial obstruction and a rise in urinary LTE_4 levels while the remaining patients tolerated celecoxib very well. Special care is therefore needed if celecoxib is to be given to patients with severe aspirin intolerance. Following celecoxib challenge, urinary LTE_4 levels decreased in patients with AIA and remained stable throughout the observation period. This result differs from earlier observations.[52] Contrary to previous reports,[52] there were no changes in urinary LTE_4 levels after celecoxib administration in patients with ATA.

In conclusion, this study describes the systemic production of PGE_2 in asthma and points to differences between patients with AIA and ATA. The global production of PGE_2 remains unaffected by aspirin in patients with AIA compared with patients with ATA. The lack of depression of systemic PGE_2 biosynthesis following aspirin challenge in patients with AIA may be a consequence of mast cell activation with a secondary inflammatory response to the released mediators. However, this may not apply to local conditions in the bronchi where aspirin can suppress PGE_2 biosynthesis in patients with AIA.

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REFERENCES

1. **Funk CD.** Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001;**294**:1871–5.
2. **van Overveld FJ,** Jorens PG, De Backer WA, *et al*. Release of arachidonic acid metabolites from isolated human alveolar type II cells. *Prostaglandins* 1992;**44**:101–10.
3. **Peters SP,** Schulman ES, MacGlashan DW, *et al*. Pharmacological and biochemical studies of human lung mast cells. *J Allergy Clin Immunol* 1982;**69**:150.
4. **Gryglewski RJ,** Szczeklik A, Wandzilak M. The effect of six prostaglandins, prostacyclin and iloprost on generation of superoxide anions by zymosan of formyl-methionyl-leucyl-phenylanine. *Biochem Pharmacol* 1987;**36**:4209–13.
5. **Minakuchi R,** Wacholtz MC, Davis LS, *et al*. Delineation of the mechanism of inhibition of human T cell activation by PGE_2. *J Immunol* 1990;**145**:2616–25.
6. **Narumiya S,** Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 1999;**79**:1193–225.
7. **Narumiya S,** FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 2001;**108**:25–30.
8. **Balazy M,** McGiff J, Laniado-Schwartzman M, *et al*. The many faces of eicosanoids: prostaglandins, leukotrienes and other arachidonate metabolites. In: Frischman WH, Sonnenblick EH, Sica DA, eds. *Cardiovascular pharmacotherapeutics*: New York: McGraw-Hill, 2003:821–39.
9. **Wenzel SE.** Asthma: defining of the persistent adult phenotypes. *Lancet* 2006;**368**:804–13.
10. **Wenzel SE.** Phenotypes in asthma: useful guides for therapy, distinct biological processes, or both? *Am J Respir Crit Care Med* 2004;**170**:579–80.
11. **Szczeklik A.** The cyclooxygenase theory of aspirin-induced asthma. *Eur Respir J* 1990;**3**:588–93.
12. **Szczeklik A,** Sanak M. The broken balance in aspirin hypersensitivity. *Eur J Pharmacol* 2006;**533**:145–55.
13. **Christie PE,** Tagari P, Ford-Hutchinson AW, *et al*. Urinary leukotriene E_4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991;**143**:1025–9.
14. **Kumlin M,** Dahlén B, Björck T, *et al*. Urinary excretion of leukotriene E_4 and 11-dehydro-thromboxane B_2 in response to bronchial provocations with allergen, aspirin, leukotriene D_4 and histamine in asthmatics. *Am Rev Respir Dis* 1992;**146**:96–103.
15. **Wenzel SE.** The role of leukotrienes in asthma. *Prostaglandins Leukot Essent Fatty Acids* 2003;**69**:145–55.
16. **Szczeklik A,** Mastalerz L, Niżankowska E, *et al*. Protective and bronchodilator effects of prostaglandin E and salbutamol in aspirin-induced asthma. *Am J Respir Crit Care Med* 1996;**153**:567–71.
17. **Sestini P,** Armetti L, Gambaro G, *et al*. Inhaled PGE_2 prevents aspirin-induced bronchoconstriction and urinary LTE_4 excretion in aspirin-sensitive asthma. *Am J Respir Crit Care Med* 1996;**153**:572–5.
18. **Schafer D,** Schmid M, Gode UC, *et al*. Dynamics of eicosanoids in peripheral blood cells during bronchial provocation in aspirin-intolerant asthmatics. *Eur Respir J* 1999;13:638–46.
19. **Samuelsson B,** Granstrom E, Green K, *et al*. Prostaglandins. *Ann Rev Biochem* 1975;**44**:669–95.
20. **Miller MJ,** Westlin WF, McNeill H,*et al*. Renal prostaglandin efflux induced by vasopressin, dDAVP and arachidonic acid: contrasting profile and sites of release. *Clin Exp Pharmacol Physiol* 1986;**8**:577–84.
21. **Cagen LM,** McGiff JC. Measurement of prostaglandins and prostaglandin metabolites. In: Radzialowski FM, ed. *Hypertension research. Methods and models*. New York and Basel: Marcel Dekker, 1982:139–94.
22. **Hamberg M,** Samuelson B. On the metabolism of prostaglandin E_1 and E_2 in man. *J Biol Chem* 1971;**246**:6713–21.
23. **Hamberg M.** Inhibition of prostaglandin synthesis in man. *Biochem Biophys Res Commun* 1972;**49**:720–6.
24. **Honda H,** Fukawa K, Sawabe T. Influence of adjuvant arthritis on main urinary metabolites of prostaglandin F and E rats. *Prostaglandins* 1980;**19**:259–69.
25. **Niżankowska E,** Bestyńska-Krypel A, Ćmiel A, *et al*. Oral and bronchial provocation tests with aspirin for diagnosis of aspirin-induced asthma. *Eur Respir J* 2000;**15**:863–9.
26. **Kumlin M,** Stensvad L, Larsson LDB, *et al*. Validation and application of a new simple strategy for measurements of urinary leukotriene E_4 in humans. *Clin Exp Allergy* 1995;**25**:467–79.
27. **Schweer H,** Watzer B, Seyberth HW. Determination of seven prostanoids in 1 ml of urine by gas chromatography-negative ion chemical ionization triple-stage quadrupole mass spectrometry. *J Chromatogr* 1994;**652**:221–7.
28. **Schweer H,** Meese CO, Seyberth HW. Determination of 11α-hydroxy-9,15-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid and 9α,11α-dihydroxy-15-oxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid by gas chromatography/negative ion chemical ionization triple-stage quadrupole mass spectrometry. *Anal Biochem* 1990;**189**:54–8.
29. **Peat JK,** Unger WR, Combe D. Measuring changes in logarithmic data, with special reference to bronchial responsiveness. *J Clin Epidemiol* 1994;**47**:1099–108.
30. **Mastalerz L,** Sanak M, Gawlewicz-Mroczka A, *et al*. Different eicosanoid profile of the hypersensitivity reactions triggered by aspirin and celecoxib in a patient with sinusitis, asthma and urticaria. *J Allergy Clin Immunol* 2006;**118**:957–8.

31. **Murphey LJ,** Williams MK, Sanchez SC, *et al*. Quantification of the major urinary metabolite of PGE_2 by a liquid chromatographic/mass spectrometric assay: determination of cyclooxygenase-specific PGE_2 synthesis in healthy humans and those with lung cancer. *Anal Biochem* 2004;**334**:266–75.
32. **Szczeklik A.** Prostaglandin E_2 and aspirin-induced asthma. *Lancet* 1995;**345**:1056.
33. **Pavord ID,** Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E_2. *Lancet* 1994;**344**:436–8.
34. **Kowalski ML,** Pawliczak R, Woźniak 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.
35. **Kowalski ML,** Ptasińska A, Bienkiewicz B, *et al*. Differential effects of aspirin and misoprostol on 15-hydroxyeicosatetraenoic acid generation by leukocytes from aspirin-sensitive asthmatic patients. *J Allergy Clin Immunol* 2003;**112**:505–12.
36. **Pierzchalska M,** Szabo Z, Sanak M, *et al*. 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.
37. **Picado C,** Femandez-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. **Perez-Novo CA,** Watelet JB, Claeys C, *et al*. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. *J Allergy Clin Immunol* 2005;**115**:1189–96.
39. **Ying S,** Meng Q, Scadding G, *et al*. H. Aspirin sensitive rhinosinusitis is associated with reduce E-prostanoid 2 (EP2) receptor expression on nasal mucosal inflammatory cells. *J Allergy Clin Immunol* 2006;**117**:312–8.
40. **Jinnai N,** Sakagami T, Sekigawa T, *et al*. Polymorphisms in the prostaglandin E_2 receptor subtype 2 gene confer susceptibility to aspirin-intolerant asthma: a candidate gene approach. *Hum Mol Genet* 2004;**13**:3203–17.
41. **Lee SH,** Rhim T, Choi YS, Min JW, *et al*. Complement C3a and C4a increased in plasma of patients with aspirin-induced asthma. *Am J Respir Crit Care Med* 2006;**173**:370–8.
42. **Bochenek G,** Nagraba K, Nizankowska E,*et al*. A controlled study of 9alpha, 11beta-PGF2 (a prostaglandin D2 metabolite) in plasma and urine of patients with bronchial asthma and healthy controls after aspirin challenge. *J Allergy Clin Immunol* 2003;**111**:743–9.
43. **O'Sullivan S,** Dahlen B, Dahlen SE, *et al*. Increased urinary excretion of the prostaglandin D_2 metabolite 9 alpha, 11 beta-prostaglandin F_2 after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction. *J Allergy Clin Immunol* 1996;**98**:421–32.
44. **Szczeklik A,** Nizankowska E, Bochenek G, *et al*. Safety of a specific COX-2 inhibitor in aspirin-induced asthma. *Clin Exp Allergy* 2001;**31**:219–25.
45. **Ferreri NR,** Howland WC, Stevenson DD, *et al*. Release of leukotrienes, prostaglandins and histamine into nasal secretions of aspirin sensitive asthmatics during reactions to aspirin. *Am Rev Respir Dis* 1988;**137**:847–54.
46. **Fischer AR,** Rosenberg MA, Lilly CM, *et al*. Direct evidence for a role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. *J Allergy Clin Immunol* 1994;**94**:1046–56.
47. **Kowalski ML,** Sliwinska-Kowalska M, Igarashi Y, *et al*. Nasal secretions in response to acetylsalicylic acid. *J Allergy Clin Immunol* 1993;**91**:580–98.
48. **Szczeklik A,** Sladek K, Dworski R, *et al*. Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1996;**154**:1608–14.
49. **Sladek K,** Szczeklik A. Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma. *Eur Respir J* 1993;**6**:391–9.
50. **Trudeau J,** Hu H, Chibana K, *et al*. Selective downregulation of prostaglandin E2-related pathways by the Th2 cytokine IL-13. *J Allergy Clin Immunol* 2006;**117**:1446–54.
51. **Szczeklik A,** Sanak M. The broken balance in aspirin hypersensitivity. *Eur J Pharmacol* 2006;**533**:145–55.
52. **Gyllfors P,** Bochenek G, Overholt J, *et al*. Biochemical and clinical evidence that aspirin-intolerant asthmatic subjects tolerate the cyclooxygenase 2-selective analgetic drug celecoxib. *J Allergy Clin Immunol* 2003;**111**:1116–21.

Lung alert

Inhibition of NKCC1 may be beneficial in sepsis

Mortality related to bacteraemic pneumonia remains high, and previous studies have shown that the Na^+–K^+–Cl cotransporter (NKCC1) may have an important role in causing acute lung injury secondary to compromise of the alveolar-capillary barrier. Under normal physiological conditions, NKCC1 plays a central role in salt transport and volume regulation in epithelial and non-epithelial cells. This study investigated the host response to *Klebsiella pneumoniae* infection in an experimental model of bacteraemia in congenic mice lacking NKCC1 expression (NKCC1−/−) and control mice (NKCC1+/+).

Mice were infected with *K pneumoniae* and bronchalveolar lavage fluid (BALF) was analysed 48 h later. NKCC1−/− mice had significantly higher numbers of cells in BALF, in particular increased numbers of neutrophils and interleukin (IL)-10. There was also a 10-fold decrease in bacterial colony forming units (CFUs) in NKCC1−/− mice compared with controls. Hypothermia was also significantly less in NKCC1−/− mice 48 h after infection. Similar changes were noted in a model of acute inflammation after lipopolysaccharide stimulation, with significantly higher neutrophils, macrophages and IL-6 in NKCC1−/− mice. However, these effects were observed primarily in the pneumonic model and not in the peritonitic model.

This study shows that NKCC1 contributes to changes in pulmonary vascular permeability during inflammation, and loss of NKCC1 expression shows a protective effect against hypothermic sepsis and bacteraemia. Inhibitors specific for NKCC1 might provide a novel means of limiting sepsis in individuals with bacterial pneumonia.

► Nguyen M, Pace AJ, Koller BH. Mice lacking NKCC1 are protected from development of bacteremia and hypothermic sepsis secondary to bacterial pneumonia. *J Exp Med* 2007;**204**:1383–93

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