Prostaglandin D_2: A dominant mediator of aspirin-exacerbated respiratory disease

Katherine N. Cahill, MD,[a,b,d] Jillian C. Bensko,[a,c] Joshua A. Boyce, MD,[a,b,d] and Tanya M. Laidlaw, MD[a,b,d] *Boston, Mass*

Background: Aspirin desensitization followed by high-dose aspirin therapy is routinely performed for patients with aspirin-exacerbated respiratory disease (AERD). Little is known about the contributions of mediators other than cysteinyl leukotrienes to aspirin reactions and to the therapeutic benefit of high-dose aspirin therapy.

Objective: We investigated differences in urinary eicosanoid metabolite levels and blood eosinophil counts in patients with AERD who tolerate and those who fail aspirin desensitization and also in patients with AERD who were successfully treated with high-dose aspirin therapy.

Methods: Twenty-nine patients with AERD were stratified into those who tolerated aspirin desensitization (group I) and those who did not (group II). Urine was analyzed for eicosanoid metabolites at baseline, during aspirin reactions, and during high-dose aspirin therapy. Blood was analyzed for cell differentials at baseline and during aspirin therapy.

Results: Basal prostaglandin D_2 metabolite (PGD-M; 13.6 ± 2.7 vs 7.0 ± 0.8 pmol/mg creatinine [Cr], $P < .05$) and thromboxane metabolite (TX-M; 1.4 ± 0.3 vs 0.9 ± 0.1 pmol/mg Cr, $P < .01$) levels were higher in group II than in group I. During aspirin reactions, PGD-M levels remained unchanged, whereas TX-M levels (0.7 ± 0.1 pmol/mg Cr, $P = .07$) tended to decrease in group I. In contrast, PGD-M levels increased dramatically in group II (61.3 ± 19.9 pmol/mg Cr, $P < .05$), whereas TX-M levels did not change. The decrease in FEV_1 inversely correlated with basal urinary levels of both leukotriene E_4 and PGD-M. Blood eosinophil and basophil levels increased and urinary PGD-M levels (2.2 ± 0.8 pmol/mg Cr, $P < .001$) decreased on 2 months of high-dose aspirin therapy in group I.

Conclusion: Failure to tolerate aspirin desensitization in a subset of patients with AERD is associated with prostaglandin D_2 overproduction. The increase in blood eosinophil and basophil counts during high-dose aspirin therapy might reflect the functional consequences of decreased prostaglandin D_2 release and the therapeutic benefit of aspirin. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Aspirin-exacerbated respiratory disease, Samter triad, nasal polyps, asthma, prostaglandin D_2, thromboxane, aspirin desensitization, cysteinyl leukotrienes, urinary eicosanoids, eosinophils*

Aspirin-exacerbated respiratory disease (AERD) is characterized by asthma, eosinophilic nasal polyposis, and respiratory reactions upon ingestion of COX-1 inhibitors. The pathogenesis of AERD involves dysregulation of arachidonic acid (AA) metabolism, as indicated by excessive basal generation of the cysteinyl leukotrienes (cysLTs) leukotriene (LT) C_4, LTD_4, and LTE_4, which is further increased by COX-1 inhibitors. A long-standing hypothesis for the cause of reactions to COX-1 inhibitors in patients with AERD has been that COX-1 inhibition dysregulates 5-lipoxygenase (5-LO), resulting in overproduction of cysLTs. Reactions typically occur at a threshold aspirin dose of 40 to 160 mg (sufficient to block COX-1 but not COX-2), with symptoms generally confined to the respiratory tract. During reactions, both eosinophils and basophils are rapidly recruited to the respiratory tissue, accompanied by a decrease in blood eosinophil counts.[1,2] The reaction is usually followed by a desensitized state, during which patients can benefit from high-dose daily aspirin, which improves disease control.[3,4] Neither the mechanism responsible for the recruitment of effector cells nor the basis of the therapeutic benefit of high-dose aspirin are known.

AA is metabolized intracellularly to LTC_4 through the actions of 5-LO and LTC_4 synthase.[5-7] LTC_4 is released and converted to LTD_4, which in turn is rapidly metabolized to the stable end-metabolite LTE_4 and excreted in the urine.[8] At least 3 receptors (type 1 [$CysLT_1R$] and type 2 cysteinyl leukotriene receptors and GPR99)[9-12] mediate the actions of cysLTs *in vivo*. Urinary LTE_4 measurements reflect systemic cysLT production, and basal levels of LTE_4 in the urine of patients with AERD are higher than those in control subjects with aspirin-tolerant asthma (ATA).[11] Patients with AERD who experience large (>30%) decreases in FEV_1 during aspirin reactions have both higher basal urinary LTE_4 levels and greater aspirin-induced increases in LTE_4 levels than patients who experience lesser reductions in airflow.[13] Drugs that inhibit $CysLT_1R$ or 5-LO attenuate the severity of respiratory symptoms and blunt the reductions in FEV_1 that occur during aspirin reactions, validating the role of cysLTs.[14] Although the efficacy of these drugs justifies their use as prophylaxis for desensitization to aspirin, therapeutic responses to them are not uniform among patients with AERD, and some experience severe aspirin-induced reactions despite prophylaxis. Therefore it is likely that mediators other than cysLTs contribute to the disease in general and to the reactions to COX-1 inhibitors in particular. However, little is known about these other mediators.

From [a]the Department of Medicine, Harvard Medical School, and the Divisions of [b]Rheumatology, Immunology, and Allergy and [c]Pulmonary and Critical Care Medicine, and [d]the Jeff and Penny Vinik Center for Allergic Disease Research, Brigham and Women's Hospital, Boston.

Supported by National Institutes of Health grants (National Institute of Allergy and Infectious Diseases: AI007306-27, U19 AI095219-01, and 5U19AI070412-06 with Opportunity Fund Subaward No. 153556/153044; and National Heart, Lung, Blood Institute: K23HL111113-02) and by generous contributions from the Vinik Family and the Kaye Family.

Disclosure of potential conflict of interest: The authors have received research support from the National Institutes of Health/National Institute of Allergy and Infectious Diseases.

Received for publication May 27, 2014; revised July 1, 2014; accepted for publication July 15, 2014.

Corresponding author: Tanya M. Laidlaw, MD, Brigham and Women's Hospital, 1 Jimmy Fund Way, Smith Building, Room 626B, Boston, MA 02115. E-mail: tlaidlaw@partners.org.

0091-6749/$36.00

© 2014 American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2014.07.031

Abbreviations used

AA:	Arachidonic acid
AERD:	Aspirin-exacerbated respiratory disease
ATA:	Aspirin-tolerant asthma
Cr:	Creatinine
cysLT:	Cysteinyl leukotriene
$CysLT_1R$:	Type 1 cysteinyl leukotriene receptor
DP:	D prostanoid
DP_2/CRTH2:	D prostanoid receptor 2/chemokine receptor homologous molecule expressed on T_H2 lymphocytes
ICS:	Inhaled corticosteroid
5-LO:	5-Lipoxygenase
LT:	Leukotriene
PG:	Prostaglandin
PGI_2:	Prostacyclin
PGD-M:	Prostaglandin D metabolite
PGE-M:	Prostaglandin E metabolite
PGI-M:	Prostacyclin metabolite
TX-M:	Thromboxane metabolite

COX-1 and COX-2 convert AA to prostaglandin (PG) H_2, which is converted to PGE_2, PGD_2, PGF_2, thromboxane, and prostacyclin (PGI_2) by cell type–restricted synthases. PGE_2 can induce bronchodilation,[15] suppress activation of mast cells and eosinophils, and block 5-LO activation.[16,17] Although PGE_2 is thought to play a protective role in patients with AERD, less is known about the roles of other COX products. PGD_2, the major COX product of mast cells, and its metabolite, 9α,11β-PGF_2, induce bronchoconstriction in asthmatic patients,[18] and asthmatic patients are hyperresponsive to PGD_2 compared with healthy control subjects.[19] PGD_2 is also a vasodilator[20] and is chemotactic for eosinophils, basophils, T_H2 cells, and type 2 innate lymphoid cells by acting at chemokine receptor homologous molecule expressed on T_H2 lymphocytes (CRTH2), also known as the D prostanoid (DP) 2 receptor.[21,22] Plasma levels of 9α,11β-PGF_2[23,24] in patients with AERD exceed those in patients with ATA and healthy control subjects and increase modestly within minutes of aspirin challenge.[24] Bronchoalveolar lavage fluid levels of PGD_2 remain unaffected by endobronchial challenge of patients with AERD using lysine aspirin, whereas metabolites of other PGs decrease.[25] These studies suggest that COX function and production of PGD_2 in patients with AERD might be at least partly resistant to aspirin. Although the bronchoconstriction, vasodilation, and inflammation-promoting actions of PGD_2 fit with a role in patients with AERD, its functions in patients with the disease remain largely unexplored.

We identified a subset of patients with AERD who did not tolerate desensitization to oral aspirin. Despite prophylaxis with the $CysLT_1R$ antagonist montelukast, these patients did not advance beyond a threshold dose of aspirin or had difficulty tolerating escalating doses of aspirin caused by ongoing cutaneous symptoms, gastrointestinal symptoms, or both. We hypothesized that differences in eicosanoid generation might account for differences in reaction severity and the development of systemic extrapulmonary symptoms in this subgroup. Here we demonstrate that patients with AERD who are unable to tolerate desensitization display markedly dysregulated production of PGs, particularly PGD_2, the levels of which increase dramatically during their reactions. The production of PGD_2 correlates with the severity of airflow obstruction during clinical reactions. We also show that patients who are successfully desensitized and then treated with high-dose aspirin exhibit sharply reduced systemic production of PGD_2, but not cysLTs, and demonstrate that reduced tissue flux of classical effector cells (eosinophils and basophils) is potentially a beneficial consequence of reduced PGD_2 generation.

METHODS

Patient selection and stratification

Patients with AERD who underwent aspirin desensitization at Brigham and Women's Hospital (Boston, Massachusetts) between 2009 and 2014 and signed informed consent forms to have urine samples, blood samples, or both collected were included in the study. All subjects had a history of asthma, nasal polyposis, and characteristic respiratory reactions upon ingestion of COX-1 inhibitors. All were offered desensitization because of refractory rhinosinusitis, nasal polyposis, or both, as specified in the 2007 practice parameters.[26] Aspirin desensitizations were performed while patients were not receiving the 5-LO inhibitor zileuton so that the production of cysLTs could be monitored. Patients took their regularly prescribed inhaled corticosteroids (ICSs) with or without long-acting β-agonists the morning of desensitization, as applicable. ICS use at the time of desensitization was recorded as low, medium, and high dose.[27] All but 2 subjects received montelukast (10 mg) the evening before and the morning of aspirin desensitization to attenuate the severity of respiratory symptoms during the reaction.[14] Patients were assigned to either group I (those who tolerated desensitization, n = 23) or group II (those who did not tolerate the procedure because of intractable abdominal pain, rash, or unresolved lower respiratory tract symptoms; n = 6). Demographic and clinical data were extracted from medical records at the time of desensitization.

Control subjects with ATA had a history of physician-diagnosed asthma and had tolerated a COX-1 inhibitor in the past 6 months. All subjects were nonsmokers.

Aspirin desensitization protocol

Two subjects from group I had a history of a previous reaction to nonsteroidal anti-inflammatory drug ingestion that required epinephrine administration and underwent aspirin desensitization in our medical intensive care unit. All other subjects underwent desensitization in our outpatient clinic. Oral aspirin desensitizations started with 40 mg of aspirin, followed by dose increases (81, 162, and 325 mg) every 90 minutes.[28] Patients were observed for respiratory symptoms, ocular injection, flushing, rash, and abdominal pain. The aspirin dose that caused upper and/or lower respiratory symptoms was recorded as the provocative dose. FEV_1 for each patient in the outpatient clinic was recorded at baseline, before each dose, and at the time of reaction.

Urinary eicosanoid measurements

Basal (before aspirin administration) urine samples were collected from all subjects. Urine was also collected for patients with AERD 180 minutes after the onset of aspirin-induced reactions. Fourteen patients with AERD also provided urine samples after at least 8 weeks of 1300 mg/d aspirin. Patients with ATA provided basal urine samples that were collected off of all nonsteroidal anti-inflammatory drugs for more than 1 week. A subset of patients with ATA (n = 5) also had urine collected 3 hours after ingestion of 325 mg of aspirin. All urine samples were stored at −80°C and analyzed by using gas chromatography–mass spectrometry at Vanderbilt University. As described previously, concentrations of LTE_4,[29] the major urinary thromboxane metabolite 11-dehydrothromboxane B_2 (TXB-M),[30] the major PGD_2 metabolite 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid (PGD-M),[31] the PGE metabolite 9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid (PGE-M), and the prostacyclin metabolite 2,3-dinor-6-keto-PGF1α (PGI-M), were measured and reported as picomoles per milligram of creatinine (Cr).

TABLE I. Baseline clinical characteristics according to group stratification

Clinical characteristics	Patients with ATA (n = 10), mean ± SEM	Group I, patients with AERD (n = 23), mean ± SEM	Group II, patients with AERD (n = 6), mean ± SEM	*P* value (group I vs group II)
Age (y)	36.3 ± 3.3	47.3 ± 2.3	47.3 ± 1.7	.99
Male sex	40%	39%	50%	.67
Baseline FEV_1 (% predicted)	91% ± 6%	84% ± 3%	86% ± 4%	.63
FEV_1/FVC ratio	80% ± 3%	73% ± 2%	71% ± 2%	.87
Total IgE (IU/mL)		168.0 ± 35	344.4 ± 85	.22
Peripheral blood eosinophils (K/μL)		0.66 ± 0.11	0.40 ± 0.20	.16
ICS + LABA	90%	91%	100%	.33
High	0%	9%	17%	
Medium	10%	57%	50%	
Oral prednisone	0%	13%	17%	1.0
Montelukast use	40%	91%	100%	1.0

Data are shown as means ± SEMs.
FVC, Forced vital capacity; *LABA*, long-acting β-agonist.

TABLE II. Clinical characteristics of patients with AERD stratified by group after the provocative dose of aspirin

Clinical characteristics during aspirin desensitization			
	Group I, patients with AERD (n = 23), mean ± SEM	Group II, patients with AERD (n = 6), mean ± SEM	*P* value
Maximum change in FEV_1	−15% ± 3%	−30% ± 10%	<.05
Abdominal symptoms	26%	83%	<.05
Rash	0%	100%	<.001
Provocative aspirin dose (mg)	113 ± 16	68 ± 30	.18

Data are shown as means ± SEMs.

Peripheral blood leukocyte counts

Blood from subjects at baseline and during high-dose aspirin therapy was collected for complete blood counts (LabCorp, Burlington, NC) in a subset of group I subjects (n = 11).

Statistics

All data presented are means ± SEMs and were normally distributed. Baseline clinical characteristics and signs of the reaction to aspirin were compared between group I and group II patients with AERD by using a 2-tailed *t* test or by using a Fisher test for categorical data. Basal and post-aspirin ingestion eicosanoid levels from group I and group II patients with ATA were compared by using a 1-way ANOVA, followed by unpaired, 2-tailed *t* tests if the ANOVA *P* value was less than .05. *P* values reported represent *t* test results. Basal and reaction eicosanoid levels for each subject were compared by using a paired 2-tailed *t* test. The Pearson correlation coefficient was used for comparisons between urinary eicosanoid levels and decrease in FEV_1 or change in blood eosinophil counts. For all analyses, a *P* value of less than .05 was considered statistically significant. All analyses were performed with GraphPad Prism version 6.03 for Windows (GraphPad Software, La Jolla, Calif; www.graphpad.com).

The Brigham and Women's Hospital Human Subjects Institutional Review Board approved the study, and all subjects provided written consent.

RESULTS

Clinical characteristics of patients and reactions

Between 2009 and 2014, 111 patients with AERD underwent aspirin desensitizations at our institution and agreed to participate in clinical research. Of these, 29 patients provided urine for eicosanoid analysis, and 11 provided blood for complete blood counts before desensitization and after 2 months of aspirin treatment. Twenty-three (group I) completed the desensitization and successfully initiated treatment with high-dose aspirin, while 6 (group II) were unable to tolerate the desensitization because of marked extrapulmonary symptoms (n = 5) or failure of lower respiratory tract symptoms to resolve (n = 1). Age, race, baseline FEV_1 (percent predicted), FEV_1/forced vital capacity ratio, ICS use, total IgE levels, and peripheral blood eosinophil counts were not statistically different between the groups (Table I). Use of ICSs or oral steroids remained the same throughout the study.

Patients with AERD in both groups I and II experienced reductions in their FEV_1 (15% ± 3% for group I and 30% ± 10% for group II, $P < .05$, Table II) during reactions to aspirin. The provocative aspirin dose (log_2) that triggered the reaction was not significantly different between the 2 groups (Table II) and did not correlate with PG metabolite levels. Abdominal pain during reactions was more common ($P < .05$) and more severe in group II, with 4 subjects reporting sharp stabbing pain, 3 reporting nausea, and 1 experiencing watery diarrhea. A rash appeared during the reactions in all subjects in group II, whereas none of the subjects in group I had a rash ($P < .001$). The rash consisted of an erythematous and pruritic macular eruption that began on the distal upper and lower extremities bilaterally. It often involved the palmar and plantar surfaces (Fig 1), spread proximally, and was not associated with urticaria or angioedema. Five of the 6 subjects in group II were unable to tolerate aspirin therapy with dose escalation because of the persistence of gastrointestinal symptoms, rash, or both. The 1 subject in group II who eventually was able to tolerate aspirin therapy required a slower desensitization spaced over 2 days, as she continued to have a rash and lower respiratory reactions with each successive dose of aspirin.

Urinary eicosanoid measurements and relationship to clinical outcomes

Basal urinary LTE_4 levels were higher in group II patients with AERD (2.9 ± 1.0 pmol/mg Cr) than in the ATA control group (0.2 ± 0.09 pmol/mg Cr, $P < .01$, Fig 2). Compared with their respective prereaction basal levels, the urinary LTE_4 levels in both groups of patients with AERD increased during the reaction

FIG 1. Rash observed after the provocative dose of aspirin in 3 group II patients with AERD.

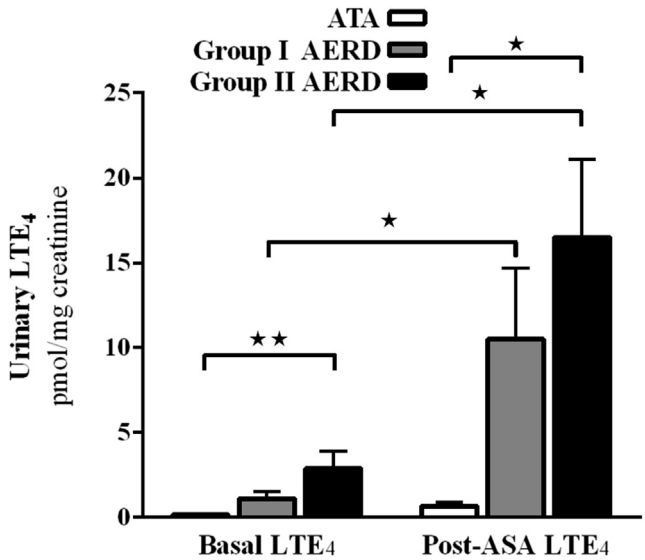

FIG 2. Basal and post-aspirin *(ASA)* urinary LTE_4 levels. Basal and aspirin-induced urinary LTE_4 levels analyzed by using gas chromatography–mass spectrometry from control subjects with ATA (basal, n = 10; after aspirin, n = 5), group I patients with AERD (n = 23), and group II patients with AERD (n = 6) are shown. Data are expressed as means ± SEMs. ★$P < .05$ and ★★$P < .01$.

to aspirin (group I, 10.5 ± 1.8 pmol/mg Cr; group II, 16.5 ± 4.6 pmol/mg Cr; $P < .05$ for both). Peak LTE_4 levels were not significantly different.

PGE-M was the most abundant prostanoid metabolite detected at baseline in all 3 groups (Fig 3, *A*, left panel). The basal levels of PGD-M ($P < .05$) and thromboxane metabolite (TX-M; $P < .01$) were higher in group II (13.6 ± 2.7 and 1.4 ± 0.3 pmol/mg Cr, respectively) than in group I (7.0 ± 0.8 and 0.9 ± 0.1 pmol/mg Cr, respectively). Total basal urinary PG metabolite levels in group II subjects exceeded those in group I subjects by approximately 2-fold (43 ± 2 vs 23 ± 2 pmol/mg Cr, $P < .01$, data not shown).

After the provocative dose, urinary PGD-M levels increased in 8, decreased in 12, and remained unchanged in 3 of the 23 subjects in group I but increased in all 6 subjects in group II. As a result, the PGD-M level during the reaction was approximately 7-fold higher in the urine of group II subjects than that seen in group I subjects (61.3 ± 19.9 vs 9.6 ± 1.8 pmol/mg Cr, respectively; Fig 3, *A*, right panel; $P < .001$). Those 8 subjects in group I who showed increased PGD-M levels during reactions had a basal level of 5.6 ± 0.6 pmol/mg Cr, which increased to 18.2 ± 3.2 pmol/mg Cr. PGI-M (0.7 ± 0.2 vs 0.3 ± 0.05 pmol/mg Cr, $P < .001$) and TX-M (2.4 ± 0.7 vs 0.7 ± 0.1 pmol/mg Cr, $P < .001$) levels during the reaction were significantly higher in the post-aspirin urine of group II than group I (Fig 3, *A*). Total PG metabolite levels in the urine of group II subjects were significantly greater than total metabolites in the urine of group I subjects during reactions (85 ± 22 vs 21 ± 3 pmol/mg Cr, $P < .001$, data not shown). All PG metabolite levels decreased in the urine of the control subjects with ATA (n = 5, data not shown) after ingestion of 325 mg of aspirin, with the exception of 1 subject who had an increase in PGD_2 levels. Fig 3, *B*, shows the $\log_2$ of the fold change (post-aspirin compared with basal levels) of each metabolite in all 3 subject groups.

Basal and reaction eicosanoid levels were assessed for correlation with a decrease in FEV_1 during aspirin reactions. Both basal urinary LTE_4 ($r = -0.461$, $P < .05$) and PGD-M ($r = -0.438$, $P < .02$) levels inversely correlated with maximum decrease in FEV_1 during the reaction (Fig 4). Reaction PGD-M ($\log_2$) levels also correlated with decrease in FEV_1 during reactions ($r = -0.41$, $P < .05$, data not shown).

To determine the effect of high-dose aspirin therapy on the production of eicosanoids in patients with AERD, we compared basal levels of urinary PG metabolites and LTE_4 with levels after 8 weeks of high-dose aspirin therapy in group I subjects (n = 14). Aspirin therapy resulted in significant decreases in PGE-M (8.7 ± 2.1 pmol/mg Cr, $P < .01$), PGI-M (0.14 ± 0.03 pmol/mg Cr, $P < .01$), TX-M (0.2 ± .0.1 pmol/mg Cr, $P < .001$), and PGD-M (2.2 ± 0.8 pmol/mg Cr, $P < .001$) levels from basal levels, but there was no change in LTE_4 levels (2.7 ± 1.2 pmol/mg Cr, $P = .24$, Fig 5). The 1 subject in group II who went on to tolerate high-dose aspirin also demonstrated a decrease in PGD-M levels from baseline after 8 weeks.

Peripheral blood leukocyte counts and relationship to urinary PGD-M levels

Absolute peripheral blood eosinophil, basophil, and neutrophil counts were assessed in group I patients with AERD (n = 11) at baseline and again during high-dose aspirin therapy. While receiving high-dose aspirin therapy, eosinophil counts increased in all subjects (0.31 ± 0.06 to 0.93 ± 0.19 $10^3/\mu L$, $P < .01$), and basophil counts increased or stayed the same (0.06 ± 0.02 to 0.09 ± 0.02 $10^3/\mu L$, $P = .08$). Neutrophil counts did not change (Fig 6). The percentage increase in eosinophil counts during high-dose aspirin therapy tended to inversely correlate to the percentage decrease in urinary PGD-M levels from baseline during high-dose aspirin therapy ($r = -0.51$, $P = .16$, data not shown). The 1 subject in group II who went on to tolerate high-dose

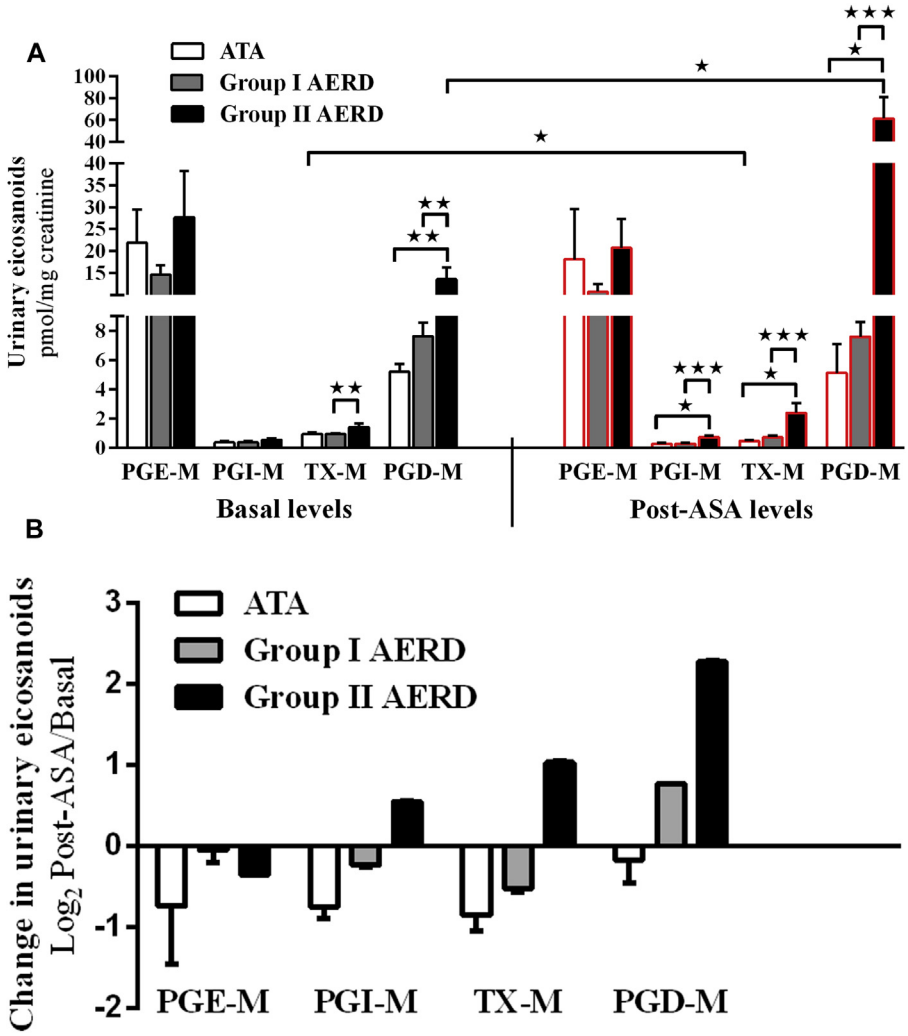

FIG 3. PG levels at baseline and after aspirin *(ASA)*. **A,** Basal *(left panel)* and post-aspirin *(right panel)* urinary PG levels in patients with ATA (basal, n = 10; after aspirin, n = 5), group I patients (n = 23), and group II patients (n = 6) are shown. **B,** Log_2 of change from basal urinary PG levels induced by aspirin administration is shown for the 3 patient groups. Data are expressed as means ± SEMs. ★P < .05, ★★P < .01, and ★★★P < .001.

aspirin also demonstrated an increase in absolute peripheral blood eosinophil counts at 8 weeks.

DISCUSSION

AERD involves complex dysregulation of proinflammatory (cysLTs and PGD_2) and anti-inflammatory (PGE_2) eicosanoid mediators. Clinical reactions to drugs that block COX-1 are characterized by increases in urinary LTE_4 levels and have been thought to be largely driven by the effector functions of cysLTs.[32] To our knowledge, ours is the first comprehensive examination of PG generation in patients with AERD undergoing a therapeutic desensitization procedure. Unexpectedly, subjects unable to tolerate the procedure because of cutaneous and gastrointestinal symptoms (despite the use of a $CysLT_1R$ antagonist) not only generate higher levels of cysLTs than those who tolerate the procedure but also do not suppress several prostanoids at a threshold dose of aspirin. In particular, subjects unable to tolerate the procedure generate markedly more PGD_2 during reactions than do those who tolerate desensitization. These findings imply a potential role for "aspirin-resistant" PGD_2 as a mediator of AERD. Additionally, the suppression of PGD_2 that results from high-dose aspirin therapy might prevent the recruitment of PGD_2-responsive effector cells to the target tissue. This could account for why therapy with high-dose aspirin improves disease control, while leaving the high baseline urinary LTE_4 levels typical of patients with this disease unaltered,[24,33,34] and increasing blood eosinophil and basophil counts.

The demographic and physiologic characteristics of the 23 subjects who tolerated the desensitization procedure (group I) were comparable with the characteristics of those who did not tolerate the procedure (group II, Table I). Every subject in group I had respiratory symptoms during the procedure, but these resolved, and all subjects successfully continued on to higher aspirin doses without additional symptoms. The subjects in group II had greater bronchoconstriction (Table II) and were unable to complete the procedure because of ongoing abdominal pain, an atypical rash, or both (Fig 1). The development of a rash during

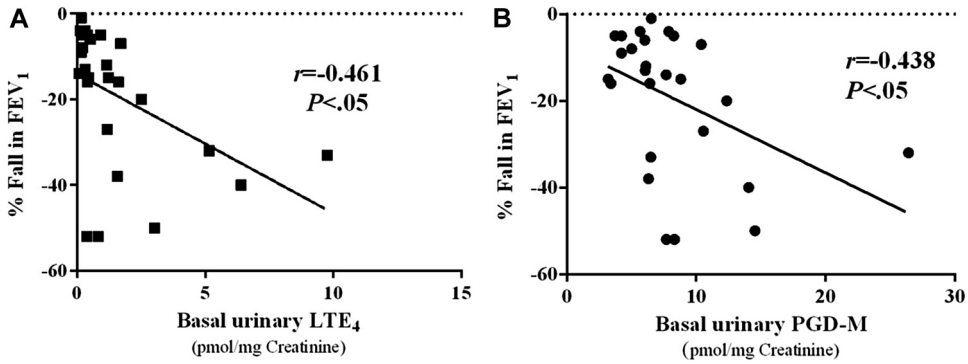

FIG 4. Basal urinary eicosanoid levels correlate with change in FEV_1 during aspirin desensitization. Basal urinary LTE_4 **(A)** and PGD-M **(B)** levels plotted against the corresponding percentage change in FEV_1 during aspirin reaction for each patient with AERD are shown. The effect size, as determined by using the Pearson correlation coefficient, is denoted as an *r* value.

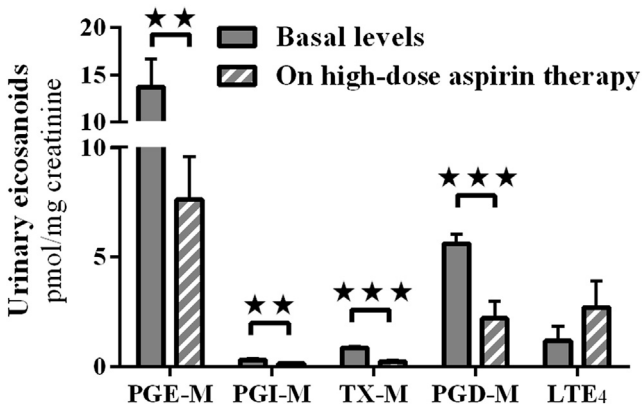

FIG 5. Effect of high-dose aspirin therapy on urinary eicosanoid levels in patients with AERD. Urinary eicosanoid levels of group I patients with AERD (n = 14) are shown at baseline before aspirin and after 650 mg of twice-daily aspirin therapy for 8 weeks. Data are expressed as means ± SEMs. ★★P < .01 and ★★★P < .001.

desensitization signaled a more challenging desensitization for subjects. The gastrointestinal manifestations could well reflect the actions of mediators released from gut mast cells during the reaction to aspirin.[35] The dose of aspirin that provoked symptoms was not statistically different between the groups (Table II) and did not correlate with reaction PGD-M levels, which is consistent with previous studies reporting that neither the severity of reactions nor the changes in urinary LTE_4 levels depend on the dose of aspirin ingested.[13,36] We cannot exclude the possibility that the subjects in group II would achieve desensitization using an alternative protocol with intranasal ketorolac, which might be better tolerated than the standard oral aspirin desensitization protocol used in our study.[37]

The correlation between basal LTE_4 levels and the decrease in FEV_1 (Fig 4, *A*) is consistent with the observations of Daffern et al,[13] who reported that basal urinary LTE_4 levels predict the severity of airflow obstruction during aspirin challenge. Unlike the study by Daffern et al, however, all but 2 subjects in our cohort were pretreated with a $CysLT_1R$ antagonist. Thus the fact that basal urinary LTE_4 levels correlated with the decrease in FEV_1 in our subjects suggests that the cysLT-dependent component of airflow obstruction involves receptors other than $CysLT_1Rs$. Because we found that PGD-M levels at baseline and during reactions also correlated with the decrease in FEV_1 (Fig 4, *B*), the bronchoconstricting effects of PGD_2 could account for a portion of the bronchial response to aspirin that resists $CysLT_1$ blockade.

Although previous studies have monitored the production of PGs during aspirin challenges in patients with AERD, none had simultaneously monitored all PG metabolites in subjects with vastly different clinical responses to aspirin. PGE_2 suppresses 5-LO activity.[38] PGD_2 and thromboxane A_2 are potent bronchoconstrictors,[39] and PGI_2 is a vasodilator that decreases airflow when inhaled.[40] Thus all PGs measured in this study are potentially relevant to AERD. The increase in total PG metabolites observed in group II during reactions to aspirin (from 43.2 ± 9.4 to 85.2 ± 22.1 pmol/mg Cr) contrasts with the aspirin-induced changes in PG levels observed in group I and in the control subjects with ATA. Although much of the increase in total PG levels in group II reflects PGD-M levels, TX-M and PGI-M levels tended to increase in response to aspirin challenge in this group, whereas both tended to decrease in group I (Fig 3). Although PGE_2 is bronchoprotective in patients with AERD because of inhibition of mast cell activation and 5-LO, PGE-M levels did not change significantly in either group during aspirin reactions. It is possible that the high PGE_2 levels generated by the kidney mask changes that reflect aspirin-induced suppression of its production in respiratory tissue[41,42] at the low doses that elicit reactions. The modest reduction in PGE-M levels observed in group I after 8 weeks of treatment might reflect the ability of high-dose aspirin to interfere with COX-2 (from which most renal PGE_2 is derived), as well as COX-1. The fact that both LTE_4 and PG levels increased simultaneously suggests that aspirin provokes the release of large quantities of AA in group II subjects by a to-be-determined mechanism. Additionally, the comparative resistance of their PG metabolites to suppression by aspirin at doses that block COX-1 (but not COX-2) suggests that COX-2 activity might mediate PG production in group II subjects.[43] COX-2 protein expression by mast cells is increased in bronchial biopsy specimens from patients with AERD compared with that seen in control subjects with ATA.[44] COX-2 selective antagonists are almost universally well tolerated by patients with AERD,[45] but whether COX-2 antagonists might actually be therapeutic in group II patients with AERD remains to be determined.

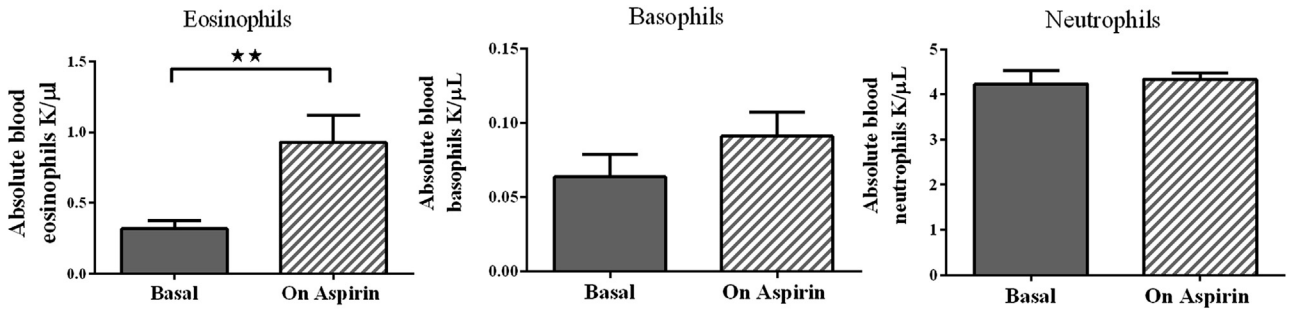

FIG 6. Peripheral blood eosinophil counts increase on high-dose aspirin therapy. Blood eosinophil, basophil, and neutrophil counts (K/μL) for group I patients with AERD (n = 11) at baseline and during high-dose aspirin therapy are shown. Data are expressed as means ± SEMs. ★★$P < .01$.

Because the effects of high-dose aspirin therapy on PG metabolites had not previously been studied comprehensively in patients with AERD, we sought to determine the effect of high dose-aspirin therapy on urinary eicosanoid levels in group I subjects. The sharply reduced levels of PGI-M, TX-M, and PGD-M in the urine of group I subjects during aspirin therapy (Fig 5) suggest that suppression of these bronchoconstrictive PGs might contribute to the clinical benefit of high-dose aspirin therapy. PGD_2 is potently chemotactic for eosinophils and basophils, both of which express D prostanoid receptor 2/chemokine receptor homologous molecule expressed on T_H2 lymphocytes (DP_2/CRTH2).[21] Aspirin challenges induce a reduction in blood eosinophil counts in patients with AERD,[2] potentially reflecting (in retrospect) their recruitment to the tissue. Both eosinophil and basophil counts increase in nasal lavage fluid after aspirin challenge,[1] without an accompanying increase in the concentrations of eosinophil-active chemokines. Remarkably, eosinophil counts, but not neutrophil counts (which lack DP2/CRTH2), increased (Fig 6) in all successfully desensitized subjects in our study and tended to correlate with the reduction in PGD-M levels. We suspect that PGD_2, through DP_2/CRTH2, might facilitate the persistent eosinophilic inflammation of the respiratory tract that characterizes AERD. The suppression of PGD_2 generation by high-dose aspirin therapy might increase the circulating pool of $DP_2/CRTH2^+$ effector cells by removing the chemotactic gradient that supports their recruitment to the tissue. Suppression of effector cell recruitment could contribute to the therapeutic benefit of high-dose aspirin in patients with AERD, which is clearly not due to changes in cysLT production.[46]

Previous studies reported that levels of PGD_2 metabolites in urine, plasma, or bronchoalveolar lavage fluid either remain unchanged[4,24,33,47] or increase modestly with aspirin challenge in patients with AERD.[48] The differences between these and our data likely reflect differences in sampling, the heterogeneous nature of AERD,[49] and the variable proportions of subjects with the phenotype defined by group II. The pharmacologic properties of PGD_2 and its metabolites make it likely that they play a causal role in the severe extrapulmonary manifestations of reactions to aspirin in group II. The significant correlation between basal urinary PGD-M levels and the decrease in FEV_1 during aspirin reaction suggests that they also contribute to the respiratory response to aspirin (Fig 4). PGD_2-mediated bronchoconstriction is sensitive to blockade with antagonists of the thromboxane receptor.[48,50] PGD_2 also causes cutaneous vasodilation by acting at DP_1 receptors,[21] and it seems plausible that the rash experienced by subjects in group II (Fig 1) could reflect the direct effects of PGD_2 in the skin microvasculature. Finally, the selective changes in blood eosinophil and basophil counts resulting from high-dose aspirin therapy in our study (Fig 6) might well reflect a prominent function for PGD_2 in recruiting $DP_2/CRTH2^+$ effector cells to the respiratory tissues. Drugs are under development that block thromboxane, DP_1, and DP_2/CRTH2 receptors.[50,51] We speculate that these drugs, alone or in combination, could permit safe desensitization to aspirin in the PGD_2-overproducing subgroup of patients with AERD and could replicate some of the therapeutic benefits of high-dose aspirin by preventing the chemotaxis of $DP_2/CRTH2^+$ cells into the tissues. Our study is a necessary prerequisite to such trials.

We thank Jing Cui, MD, PhD, for her statistical assistance during manuscript preparation.

Key messages

- **Cutaneous and gastrointestinal symptoms during aspirin desensitization and failure to tolerate desensitization in patients with AERD are associated with marked increases in PGD_2 generation.**
- **High-dose aspirin therapy in patients with AERD suppresses PGD_2 production and increases peripheral blood eosinophil counts.**

REFERENCES

1. Kupczyk M, Kurmanowska Z, Kuprys-Lipinska I, Bochenska-Marciniak M, Kuna P. Mediators of inflammation in nasal lavage from aspirin intolerant patients after aspirin challenge. Respir Med 2010;104:1404-9.
2. Sladek K, Szczeklik A. Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma. Eur Respir J 1993;6:391-9.
3. Stevenson DD, Simon RA, Mathison DA. Aspirin-sensitive asthma: tolerance to aspirin after positive oral aspirin challenges. J Allergy Clin Immunol 1980;66:82-8.
4. Swierczynska-Krepa M, Sanak M, Bochenek Grazyna, Strek P, Cmiel A, Gielicz A, et al. Aspirin desensitization in patients with aspirin-induced and aspirin-tolerant asthma: a double-blind study. J Allergy Clin Immunol 2014 [Epub ahead of print].
5. Nicholson DW, Ali A, Vaillancourt JP, Calaycay JR, Mumford RA, Zamboni RJ, et al. Purification to homogeneity and the N-terminal sequence of human leukotriene C_4 synthase: a homodimeric glutathione S-transferase composed of 18-kDa subunits. Proc Natl Acad Sci U S A 1993;90:2015-9.

6. Lam BK, Penrose JF, Freedman GJ, Austen KF. Expression cloning of a cDNA for human leukotriene C_4 synthase, a novel integral membrane protein conjugating reduced glutathione to leukotriene A_4. Proc Natl Acad Sci U S A 1994;91:7663-7.
7. Welsch DJ, Creely DP, Hauser SD, Mathis KJ, Krivi GG, Isakson PC. Molecular cloning and expression of human leukotriene C_4 synthase. Proc Natl Acad Sci U S A 1994;91:9745-9.
8. Kumlin M, Stensvad F, Larsson L, Dahlén B, Dahlén SE. Validation and application of a new simple strategy for measurements of urinary leukotriene E4 in humans. Clin Exp Allergy 1995;25:467-79.
9. Kanaoka Y, Maekawa A, Austen KF. Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. J Biol Chem 2013;288:10967-72.
10. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, et al. Characterization of the human cysteinyl leukotriene cysLT1 receptor. Nature 1999;399:789-93.
11. Sarau HM, Ames RS, Chambers J, Ellis C, Elshourbagy N, Foley JJ, et al. Identification, molecular cloning, expression, and characterization of a cysteinyl leukotriene receptor. Mol Pharmacol 1999;56:657-63.
12. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, et al. Characterization of the human cysteinyl leukotriene 2 receptor. J Biol Chem 2000;275:30531-6.
13. Daffern PJ, Muilenburg D, Hugli TE, Stevenson DD. Association of urinary leukotriene E4 excretion during aspirin challenges with severity of respiratory responses. J Allergy Clin Immunol 1999;104:559-64.
14. White A, Ludington E, Mehra P, Stevenson DD, Simon RA. Effect of leukotriene modifier drugs on the safety of oral aspirin challenges. Ann Allergy Asthma Immunol 2006;97:688-93.
15. Benyahia C, Gomez I, Kanyinda L, Boukais K, Danel C, Leséche G, et al. PGE(2) receptor (EP(4)) agonists: potent dilators of human bronchi and future asthma therapy? Pulm Pharmacol Ther 2012;25:115-8.
16. Feng C, Beller EM, Bagga S, Boyce JA. Human mast cells express multiple EP receptors for prostaglandin E2 that differentially modulate activation responses. Blood 2006;107:3243-50.
17. Luo M, Jones SM, Phare SM, Coffey MJ, Peters-Golden M, Brock TG. Protein kinase A inhibits leukotriene synthesis by phosphorylation of 5-lipoxygenase on serine 523. J Biol Chem 2004;279:41512-20.
18. Beasley RC, Featherstone RL, Church MK, Faggerty P, Varley JG, Harris A, et al. Effect of a thromboxane receptor antagonist on PGD2- and allergen-induced bronchoconstriction. J Appl Physiol 1989;66:1685-93.
19. Hardy CC, Robinson C, Tattersfield AE, Holgate ST. The bronchoconstrictor effect of inhaled prostaglandin D2 in normal and asthmatic men. N Engl J Med 1984;311:209-13.
20. Cheng K, Wu TJ, Wu KK, Sturino C, Metters K, Gottesdiener K, et al. Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. Proc Natl Acad Sci U S A 2006;103:6682-7.
21. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. J Exp Med 2001;193:255-61.
22. Xue L, Salimi M, Panse I, Mjosberg JM, McKenzie AN, Spits H, et al. Prostaglandin D activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on T2 cells. J Allergy Clin Immunol 2014;133:1184-94.
23. Higashi N, Taniguchi M, Mita H, Yamaguchi H, Ono E, Akiyama K. Aspirin-intolerant asthma assessment using the urinary biomarkers, leukotriene E_4 and prostaglandin D_2 metabolites. Allergol Int 2012;61:393-403.
24. Bochenek G, Nagraba K, Nizankowska E, Szczeklik A. A controlled study of 9α,11β-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.
25. Szczeklik A, Sladek K, Dworski R, Bizankowska E, Soja J, Sheller J, et al. Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. Am J Respir Crit Care Med 1996;154:1608-14.
26. Macy E, Bernstein JA, Castells MC, Gawchik SM, Lee TH, Settipane RA, et al. Aspirin challenge and desensitization for aspirin-exacerbated respiratory disease: a practice paper. Ann Allergy Asthma Immunol 2007;98:172-4.
27. National Asthma Education and Prevention Program, third expert panel on the diagnosis and management of asthma. Expert panel report 3: guidelines for the diagnosis and management of asthma. Bethesda: National Heart, Lung, and Blood Institute; 2007. Available at: http://www.ncbi.nlm.nih.gov/books/NBK7232/.
28. Laidlaw TM, Kidder MS, Bhattacharyya N, Xing W, Shen S, Milne GL, et al. Cysteinyl leukotriene overproduction in aspirin-exacerbated respiratory disease is driven by platelet-adherent leukocytes. Blood 2012;119:3790-8.
29. Morrow JD, Minton TA. Improved assay for the quantification of 11-dehydrothromboxane B2 by gas chromatography-mass spectrometry. J Chromatogr 1993;612:179-85.
30. Awad JA, Morrow JD, Takahashi K, Roberts LJ 2nd. Identification of non-cyclooxygenase-derived prostanoid (F2-isoprostane) metabolites in human urine and plasma. J Biol Chem 1993;268:4161-9.
31. Morrow JD, Guzzo C, Lazarus G, Oates JA, Roberts LJ 2nd. Improved diagnosis of mastocytosis by measurement of the major urinary metabolite of prostaglandin D2. J Invest Dermatol 1995;104:937-40.
32. Christie PE, Tagari P, Ford-Hutchinson AW, Charlesson S, Chee P, Arm JP, et al. Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. Am Rev Respir Dis 1991;143:1025-9.
33. Szczeklik A, Sladek K, Dworski R, Nizankowska E, Soja J, Sheller J, et al. Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. Am J Respir Crit Care Med 1996;154:1608-14.
34. Yoshida S, Amayasu H, Sakamoto H, Onuma K, Shoji T, Nakagawa H, et al. Cromolyn sodium prevents bronchoconstriction and urinary LTE_4 excretion in aspirin-induced asthma. Ann Allergy Asthma Immunol 1998;80:171-6.
35. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. J Clin Invest 2003;112:1666-77.
36. Mastalerz L, Sanak M, Gawlewicz-Mroczka A, Gielicz A, Szczeklik A. Prostaglandin E2 systemic production in patients with asthma with and without aspirin hypersensitivity. Thorax 2008;63:27-34.
37. Lee RU, White AA, Ding D, Dursun AB, Woessner KM, Simon RA, et al. Use of intranasal ketorolac and modified oral aspirin challenge for desensitization of aspirin-exacerbated respiratory disease. Ann Allergy Asthma Immunol 2010;105:130-5.
38. Liu T, Laidlaw TM, Katz HR, Boyce JA. Prostaglandin E2 deficiency causes a phenotype of aspirin sensitivity that depends on platelets and cysteinyl leukotrienes. Proc Natl Acad Sci U S A 2013;110:16987-92.
39. Song WL, Stubbe J, Ricciotti E, Alamuddin N, Ibrahim S, Crichton I, et al. Niacin and biosynthesis of PGD(2)by platelet COX-1 in mice and humans. J Clin Invest 2012;122:1459-68.
40. Hardy CC, Bradding P, Robinson C, Holgate ST. Bronchoconstrictor and antibronchoconstrictor properties of inhaled prostacyclin in asthma. J Appl Physiol 1988;64:1567-74.
41. Yoshimura T, Yoshikawa M, Otori N, Haruna S, Moriyama H. Correlation between the prostaglandin D(2)/E(2) ratio in nasal polyps and the recalcitrant pathophysiology of chronic rhinosinusitis associated with bronchial asthma. Allergol Int 2008;57:429-36.
42. Szczeklik A, Gryglewski RJ, Olszewski E, Dembinska-Kiec A, Czerniawska-Mysik G. Aspirin-sensitive asthma: the effect of aspirin on the release of prostaglandins from nasal polyps. Pharamacol Res Commun 1977;9:415-25.
43. Smith WL, Meade EA, Dewitt DL. Interaction of PGH synthase isozymes-1 and -2 with nonsteroidal anti-inflammatory drugs. Adv Exp Med Biol 1997;400A:189-96.
44. Sousa A, Pfister R, Christie PE, Lane SJ, Nasser SM, Schmitz-Schumann M, et al. Enhanced expression of cyclo-oxygenase isoenzyme 2 (COX-2) in asthmatic airways and its cellular distribution in aspirin-sensitive asthma. Thorax 1997;52:940-5.
45. Woessner KM, Simon RA, Stevenson DD. The safety of celecoxib in patients with aspirin-sensitive asthma. Arthritis Rheum 2002;46:2201-6.
46. Nasser SM, Patel M, Bell GS, Lee TH. The effect of aspirin desensitization on urinary leukotriene E4 concentrations in aspirin-sensitive asthma. Am J Respir Crit Care Med 1995;151:1326-30.
47. Szczeklik A, Dworski R, Mastalerz L, Prokop A, Sheller J, Nizankowska E, et al. Salmeterol prevents aspirin-induced attacks of asthma and interferes with eicosanoid metabolism. Am J Respir Crit Care Med 1998;158:1168-72.
48. Larsson AK, Hagfjard A, Dahlen SE, Adner M. Prostaglandin D(2) induces contractions through activation of TP receptors in peripheral lung tissue from the guinea pig. Eur J Pharmacol 2011;669:136-42.
49. Bochenek G, Kuschill-Dziurda J, Szanfraniec K, Plutecka H, Szczeklik A, Nizankowska-Mogilnicka E. Certain subphenotypes of aspirin-exacerbated respiratory disease distinguished by latent class analysis. J Clin Allergy Immunol 2014;133:98-103.
50. Johnston SL, Bardin PG, Harrison J, Ritter W, Joubert JR, Holgate ST. The effects of an oral thromboxane TP receptor antagonist BAY u 3405, on prostaglandin D2- and histamine-induced bronchoconstriction in asthma, and relationship to plasma drug concentrations. Br J Clin Pharmacol 1992;34:402-8.
51. Busse WW, Wenzel SE, Meltzer EO, Kerwin EM, Liu MC, Zhang N, et al. Safety and efficacy of the prostaglandin D2 receptor antagonist AMG 853 in asthmatic patients. J Allergy Clin Immunol 2013;131:339-45.