Profile of eicosanoid generation in aspirin-intolerant asthma and anaphylaxis assessed by new biomarkers

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Background: It has recently demonstrated that a free radical–mediated pathway generates prostaglandins (PGs) and the corresponding prostaglandin enantiomers (*ent*-PGs). Aspirin-intolerant asthma and anaphylaxis accompany PGD_2 overproduction, possibly associated with mast cell activation via the COX pathway. However, free radical-mediated PG generation in the pathophysiology of these diseases, which can be demonstrated by measuring urinary *ent*-$PGF_2\alpha$, has not been reported.

Objectives: To evaluate the characteristic profile of eicosanoid generation via the COX and/or free radical–mediated pathway underlying aspirin-intolerant asthma and anaphylaxis.

Methods: A comparative group analysis consisted of asthma (n = 17) and anaphylaxis (n = 8, none with aspirin-induced anaphylaxis) cases. Urinary eicosanoid concentrations were quantified as follows: 2,3-dinor-9α,11β-PGF_2 by gas chromatography-mass spectrometry; leukotriene E_4, 9α,11β-PGF_2, and PGs by enzyme immunoassay.

Results: 2,3-Dinor-9α,11β-PGF_2 is a more predominant PGD_2 metabolite in urine than 9α,11β-PGF_2. At baseline, the aspirin-intolerant asthma group (n = 10) had significantly higher leukotriene E_4 and lower PGE_2 concentrations in urine than the aspirin-tolerant asthma group. During the reaction, the urinary concentrations of leukotriene E_4 and PGD_2 metabolites correlatively increased, but with markedly different patterns of the mediator release, in the aspirin-intolerant asthma group and the anaphylaxis group, respectively. The urinary PGD_2 metabolites and primary PGs were significantly decreased in the aspirin-tolerant asthma group. Urinary *ent*-$PGF_2\alpha$ concentrations were significantly increased in the anaphylaxis group but not the aspirin-intolerant asthma group.

Conclusions: When assessed by urinary 2,3-dinor-9α,11β-PGF_2, PGD_2 overproduction during aspirin-intolerant bronchoconstriction was clearly identified, regardless of COX inhibition. It is evident that free radical–mediated PG generation is involved in the pathophysiology of anaphylaxis. (J Allergy Clin Immunol 2010;125:1084-91.)

Key words: *Aspirin, asthma, anaphylaxis, cysteinyl-leukotriene, 2,3-dinor-9α,11β-PGF_2, ent-$PGF_2\alpha$, urine*

Arachidonic acid metabolites such as cysteinyl leukotrienes (CysLTs)[1] and prostaglandins (PGs)[2] play important roles in the pathophysiology of various inflammatory diseases. Because these compounds are rapidly metabolized *in vivo*, the concentration of urinary metabolites provides a time-integrated estimate of the production of the parent compounds, allowing us to detect their generation *in vivo*.[3] Urinary leukotriene (LT) E_4 is the most reliable analytical biomarker for monitoring the endogenous synthesis of CysLTs.[4] Similarly, urinary 9α,11β-PGF_2 and the later appearing 2,3-dinor-9α,11β-PGF_2 are relatively major metabolites of PGD_2[5,6] (see this article's Fig E1 in the Online Repository at www.jacionline.org). Among the LTC_4-producing cells such as eosinophils, basophils, and mast cells, it is only the mast cell that produces significant quantities of PGD_2.[7] Urinary PGD_2 metabolites are markedly increased in patients with mastocytosis,[8,9] so the urinary PGD_2 metabolites are evidently useful biomarkers of mast cell activation.[5,6] Furthermore, the measurement of PGD_2 metabolites represents a more sensitive strategy than measurement of serum tryptase.[10,11] However, recently, some problems have arisen about whether quantification of urinary 9α,11β-PGF_2 concentrations provides a valid assessment of systemic PGD_2 production, as follows:

1. Concentrations of 9α,11β-PGF_2 were below the detection limit whereas the concentrations of 2,3-dinor-9α,11β-PGF_2 were markedly higher in human urine when estimated by liquid chromatography-mass spectrometry/mass spectrometry.[12]
2. 2,3-Dinor-9α,11β-PGF_2 showed a marked cross-reactivity (about 10%) to the antibody against 9α,11β-PGF_2 in commercially available enzyme immunoassay (EIA).[5]

These findings lead us to speculate that urinary 2,3-dinor-9α,11β-PGF_2 concentration might be a more reliable indicator in determining PGD_2 production *in vivo*. In contrast, quantification of primary PGs in urine has been shown to reflect predominantly renal production but not systemic PG production.[13]

There is another pathway that arachidonic acid is metabolized *in vivo* by a free radical–mediated mechanism to yield a series of PG-like compounds termed isoprostanes independent of the catalytic activity of the COX enzyme.[14] In contrast with COX-derived PGs, which is an optically pure, free radical–mediated peroxidation of arachidonic acid generates a racemic mixture of PGs. Thus, the presence of the enantiomer to COX-derived PG demonstrates that the PG is generated via a free radical-mediated mechanism[15] (see this article's Table E1 in this article's Online Repository at www.jacionline.org). The concentration of PG

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Abbreviations used

AIA: Aspirin-intolerant asthma
ATA: Aspirin-tolerant asthma
CysLT: Cysteinyl leukotriene
EIA: Enzyme immunoassay
ent-$PGF_2\alpha$: Prostaglandin $F_2\alpha$ enantiomer
GC-MS: Gas chromatography–mass spectrometry
GC-MS-NCI: Gas chromatography–mass spectrometry–negative-ion chemical ionization
L-ASA: Lysine-aspirin
LT: Leukotriene
NSAID: Nonsteroidal anti-inflammatory drug
PG: Prostaglandin
PGEM: 13,14-Dihydro-15keto-prostaglandin E_2
Tetranor-PGDM: 11,15-Dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid
Tetranor-PGEM: 9,15-Dioxo-11α-hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid

enantiomer in urine is a reliable index of systemic isoprostane and lipid peroxidation.[15,16] Quantification of urinary $PGF_2\alpha$ enantiomer (*ent*-$PGF_2\alpha$) actually provides a valuable tool to assess oxidant stress in cigarette smokers and in subjects with hypercholesterolemia.[16]

The clinical syndrome aspirin-intolerant asthma (AIA) is characterized by aspirin/nonsteroidal anti-inflammatory drug (NSAID) intolerance, bronchial asthma, and chronic rhinosinusitis with nasal polyposis.[17,18] Aspirin/NSAID-induced asthma reactions are developed through the pharmacological effect of COX-1 inhibitors,[19,20] which are accompanied by CysLTs and PGD_2 release.[21,22] Inhaled PGE_2 protects against both aspirin-induced bronchoconstriction and the massive release of urinary LTE_4,[23,24] so a critical deficiency in PGE_2 "braking" has been postulated as 1 possible mechanism for the AIA reaction.[25] The plasma aspirin concentration is adequate to inhibit COX[26]; however, the reasons for the discrepancy between COX inhibition by aspirin and the PGD_2 overproduction in AIA are unknown. The pathophysiologic response of anaphylaxis is also produced by inflammatory mediators such as CysLTs and PGD_2 released possibly from mast cells.[27] Considering the finding that human mast cells generate intracellular reactive oxygen species that are functionally linked to mast cell activation,[28] we hypothesized that a free radical–mediated mechanism might be also responsible for PGD_2 production during acute exacerbation of anaphylaxis and AIA. There has been no comparative study of urinary arachidonic acid metabolites including the free radical–mediated PG generation comprehensively evaluated by validated methods between AIA and anaphylaxis.

In this study, a new biomarker of the urinary *ent*-$PGF_2\alpha$ was used to confirm the involvement of another pathway for PG production via a free radical-mediated mechanism, leading to the measurement of its counterparts $PGF_2\alpha$ and PGE_2 in urine as indicators of renal production. In addition, we used gas chromatography–mass spectrometry–negative-ion chemical ionization (GC-MS-NCI) for the quantification of 2,3-dinor-9α,11β-PGF_2 in urine from subjects with anaphylaxis. Song et al[12] have recently recommended the measurement of the 11,15-dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor-PGDM) concentration by liquid chromatography-mass spectrometry/mass spectrometry to be a sensitive biomarker of PGD_2 production. However, tetranor-PGDM could not be chosen for gas chromatography–mass spectrometry (GC-MS) analysis because the compound has 2 keto groups. Subsequently, we evaluated the characteristic profile of eicosanoid generation through the COX pathway and/or free radical–mediated isoprostane pathway between anaphylaxis and AIA.

METHODS

An extended version of this article's Methods section is available in the Online Repository at www.jacionline.org.

Subjects and study design

We conducted a hospital-based prospective study from January 2007 to July 2008. As shown in Table I, anaphylactic subjects (n = 8) were enrolled in study I (urinary biomarkers in anaphylaxis). The diagnosis of anaphylaxis was based on the international criteria.[29,30] We excluded subjects with vasodepressor syncope, systemic mastocytosis, hereditary angioedema, psychiatric disorders, and cardiovascular or respiratory events. None with AIA or aspirin-intolerant urticaria/angioedema type were included in study I. Adult subjects with asthma (n = 17) clinically requiring a lysine-aspirin (L-ASA) provocation test because of a suspicion of intolerance to aspirin/NSAIDs were included in study II (comparison study of AIA and aspirin-tolerant asthma [ATA] groups). The diagnosis of asthma and asthma severity were based on the American Thoracic Society criteria[31] and the Global Initiative for Asthma guidelines,[32] respectively. Subjects with an upper respiratory tract infection in the previous 6 weeks, renal or liver dysfunction, hypertension, or autoimmune disease were excluded from this study. All patients' characteristics are shown in Table I. Permission to conduct the study was obtained from the Ethics Committee of the Sagamihara National Hospital, and all of the subjects who participated in this study gave written informed consent.

Provocation test

We performed a single-blind provocation test for the diagnosis of a specific cause of anaphylaxis[27,29] or aspirin intolerance[22,33] by a previously described method. Although all medications were withheld for at least 24 hours before the day of provocation to the furthest extent possible, certain medications were frequently needed to stabilize bronchial airways in the patients with severe AIA.[34,35] As shown in Table I, subjects with asthma producing a fall in FEV_1 of 20% or greater compared with the baseline were assigned as AIA (n = 10; mean cumulative dose of L-ASA, 60 mg). Subjects receiving even the maximum provocative dose of aspirin (cumulative dose of L-ASA, 375 mg) without significant bronchoconstriction or other adverse symptom were assigned as having ATA (n = 7). The subjects with anaphylaxis did not receive L-ASA or NSAID agents in the 4 weeks preceding the study or after the provocation test.

Assay of LTE_4, 9α,11β-PGF_2, PGE_2, $PGF_2\alpha$, and ent-$PGF_2\alpha$

Urine samples were collected at baseline (between 8:00 and 10:00 AM) and 0 to 3, 3 to 6, 6 to 9, and 9 to 24 hours after the provocation test.[36,37] As shown in Tables E1 and E2 in this article's Online Repository at www.jacionline.org, the samples were analyzed by EIA (Cayman Chemical, Ann Arbor, Mich) for urinary concentrations of LTE_4,[38,39] 9α,11β-PGF_2,[40] PGE_2, $PGF_2\alpha$, and *ent*-$PGF_2\alpha$ after purification with HPLC.[22,39] Recovery of the eicosanoids throughout the purification procedure was more than 75%. The urinary eicosanoid concentrations were expressed as picograms per milligram of creatinine.

Quantification of 2,3-dinor-9α,11β-PGF_2 by GC-MS-NCI

After purification by HPLC under the conditions shown in Table E2, 2,3-dinor-9α,11β-PGF_2 was quantified with GC-MS-NCI by using ^{18}O-labeled