

Urinary concentrations of 15-epimer of lipoxin A₄ are lower in patients with aspirin-intolerant compared with aspirin-tolerant asthma

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Summary

Background Although an abnormality in arachidonic acid metabolism may be responsible for aspirin-intolerant asthma (AIA), there is little knowledge about the concentrations of urinary lipoxin A₄ (LXA₄) and the 15-epimer of LXA₄ (15-epi-LXA₄) in relation to asthma severity in AIA subjects.

Objective The purpose of this study is to estimate urinary LXA₄ and the 15-epimer concentrations to investigate lipoxins in AIA.

Methods In this study, we examined AIA, aspirin-tolerant asthma (ATA) and healthy control groups. The AIA and ATA groups were subdivided into the severe asthma and non-severe asthma subgroups. Urinary LXA₄, 15-epi-LXA₄ and leukotriene E₄ (LTE₄) were quantified using enzyme immunoassay after separating these compounds using high-performance liquid chromatography.

Results The urinary LXA₄ concentration was significantly lower than the 15-epi-LXA₄ concentration in the asthmatic subjects. The AIA group showed significantly lower urinary 15-epi-LXA₄ ($P < 0.01$) and higher urinary LTE₄ concentrations ($P < 0.05$) than the ATA group. Comparison of 15-epi-LXA₄ concentrations between the severe asthmatic and non-severe asthmatic subjects in the AIA and ATA groups revealed that the decreased 15-epi-LXA₄ concentration may be related to aspirin intolerance, but not asthma severity. Receiver operator characteristic curves demonstrated that the concentration ratio of LTE₄ to 15-epi-LXA₄ was superior to 15-epi-LXA₄ concentration and LTE₄ concentration as a predictive factor for aspirin intolerance.

Conclusions and Clinical Relevance We have demonstrated for the first time that urinary 15-epi-LXA₄ concentration is significantly higher than LXA₄ concentration in both the AIA and ATA groups. 15-Epi-LXA₄ concentration was significantly lower in the AIA group with an increased urinary LTE₄ concentration than in the ATA group. An imbalance between proinflammatory cysteinyl-leukotrienes and anti-inflammatory 15-epi-LXA₄ may be involved in AIA pathogenesis.

Keywords 15-epimer of lipoxin A₄, aspirin-intolerant asthma, asthma severity, leukotriene E₄, lipoxin A₄

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Introduction

The clinical syndrome aspirin-intolerant asthma (AIA) is characterized by aspirin/non-steroidal anti-inflamma-

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tory drug (NSAID) intolerance, bronchial asthma and chronic rhinosinusitis with nasal polyposis [1]. Several clinicoepidemiologic studies [2–4] have demonstrated that AIA is one of the common risk factors for the development of severe asthma. An imbalanced eicosanoid production is presumably associated with the degree of airflow obstruction and asthma severity in AIA subjects, because eicosanoids have both beneficial and detrimental effects on the lung.

Measurement of urinary concentrations of mediators demonstrated significant differences in eicosanoid concentrations between AIA and aspirin-tolerant asthma (ATA) subjects. The basal urinary concentration of leukotriene (LT) E_4 is significantly higher in AIA subjects [5], and aspirin provocation increases the urinary concentrations of LTE $_4$ and LTB $_4$ glucuronide in AIA subjects [6]. We have recently reported that the urinary concentration of prostaglandin E_2 (PGE $_2$), an indicator of PGE $_2$ biosynthesis in the kidney, is significantly lower in AIA subjects [7], although Mastalerz *et al.* have not observed the difference in the basal concentration of urinary PGE $_2$ metabolites between AIA and ATA subjects [8]. The concentrations of urinary PGE $_2$ metabolites differently decreased following the administration of aspirin or celecoxib between AIA and ATA subjects [8].

Production of lipoxin (LX), which is an anti-inflammatory compound produced by cell-to-cell interaction (Fig. 1), has been examined in AIA and ATA subjects using whole blood after stimulation with calcium ionophore in some studies. AIA is characterized by a lower capacity for biosynthesis of lipoxin A $_4$ (LXA $_4$) [9] and a significantly lower LXA $_4$ concentration in activated whole blood [10, 11], sputum [12] and bronchoalveolar lavage fluid (BALF) [13] from subjects with severe

asthma than from those with milder asthma. On the other hand, the 15-epimer of LXA $_4$ (15-epi-LXA $_4$), which exerts about a twofold stronger inhibitory effect on granulocyte adhesion than LXA $_4$ [14], is also produced by cell-to-cell interaction involving acetylated cyclooxygenase (COX)-2 and 5-lipoxygenase [15] in the presence of aspirin or by potential endogenous acetylating agents [16] (Fig. 1). AIA subjects show a decreased expression level of the COX-2 transcript in nasal polyps [17] or bronchial epithelial cells [18], which may result in a suppressed generation of 15-epi-LXA $_4$. There has been only one study demonstrating the presence of LXA $_4$ in urine using enzyme immunoassay (EIA) without separation; however, it was found that the antibody for LXA $_4$ cross-reacts with 15-epi-LXA $_4$ [19].

In this study, we quantified urinary LXA $_4$ and 15-epi-LXA $_4$ after separating these compounds using high-performance liquid chromatography (HPLC). This method enabled us to precisely determine whether LXA $_4$ is present in urine samples or whether the observed concentrations resulted from the cross-reactivity of the antibody with 15-epi-LXA $_4$. Furthermore, we examined the relationship among LXA $_4$ or 15-epi-LXA $_4$ concentration, asthma severity and intolerance to aspirin/NSAIDs.

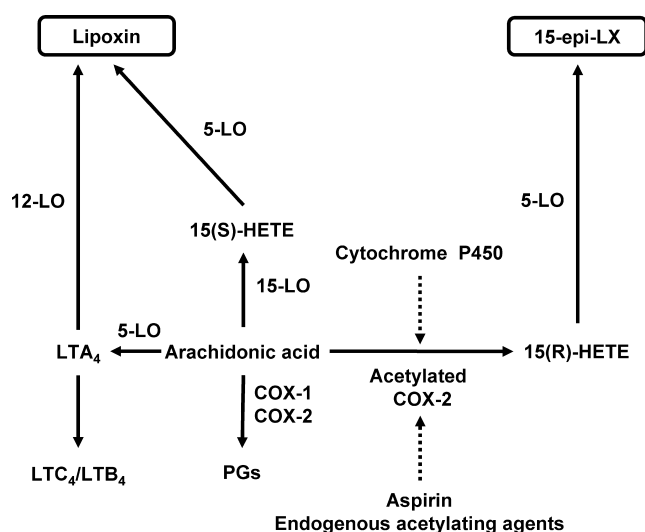


Fig. 1. Pathway for production of lipoxin (LX), 15-epimer of LX (15-epi-LX) and other eicosanoids. Leukotriene (LT) A $_4$, which is a product of arachidonic acid oxygenation by 5-lipoxygenase (LO), is a precursor of LTC $_4$ and LTB $_4$. Arachidonic acid is converted to prostaglandins (PGs) and thromboxane by cyclooxygenase 1 (COX-1) or COX-2. 15 (S)-hydroxyeicosatetraenoic acid (HETE) is produced from arachidonic acid by 15-LO, and the (R) enantiomer of 15-HETE is formed by cytochrome P-450 or COX-2 acetylated either by exogenous aspirin or by endogenous acetylating agents. LX is produced from either 15 (S)-HETE by 5-LO or LTA $_4$ by 12-LO. 15-epi-LX is produced from 15 (R)-HETE by 5-LO. The racemic mixture of 15-HETE is also non-enzymatically formed by oxidation of arachidonic acid.

Materials and methods

Subjects and study design

Subjects with asthma, who were classified into the AIA and ATA groups, were enrolled in this study. Adult asthmatic subjects were recruited from among the outpatients of Sagami National Hospital (Table 1). The diagnosis of asthma was based on the American Thoracic Society criteria [20]. AIA patients were subjected to the single-blind provocation test to confirm aspirin sensitivity during the 2 years before the study by a previously described method [5]. They were intravenously administered doubling doses of lysine aspirin (12.5–200 mg equivalent to aspirin). Asthmatic subjects showing a decrease in forced expiratory volume in 1 s (FEV $_{1.0}$) by 20% or greater as compared with the baseline were assigned to the AIA group. Subjects with respiratory tract infection within 6 weeks, ischaemic cardiovascular diseases or renal or liver dysfunction were excluded from the study. None of the subjects of the groups received aspirin, NSAIDs or COX-2-selective inhibitors for at least 6 months prior to urine collection. Healthy control subjects were non-atopic healthy hospital staff members; none of them were taking any medication. Permission to conduct this study was obtained from the Ethics Committee of Sagami National Hospital, St. Marianna University School of

Table 1. Clinical characteristics of subjects

	Control	ATA		AIA		P value
		Non-severe	Severe	Non-severe	Severe	
No. of subjects (female), <i>n</i>	10 (2)	16 (6) 9 (3)	7 (3)	15 (11) 5 (4)	10 (7)	
Age, years*	46.3 (17.3)	59.2 (20.3) 54.8 (23.0)	64.9 (16.1)	53.9 (16.0) 51.8 (26.7)	55.0 (8.8)	NS
Duration of asthma, years*	NA	12.3 (13.2) 8.3 (12.1)	17.3 (13.8)	13.1 (10.9) 13.4 (14.6)	12.9 (9.5)	NS
Peripheral eosinophils (cells/mm ³)*	NM	415.0 (332.7) 362.9 (325.1)	488.0 (366.6)	630.2 (404.4) 685.5 (417.7)	608.1 (419.7)	NS
FEV ₁ (% predicted), %*	NM	88.0 (20.1) 96.4 (15.6)	77.1 (21.0)	81.7 (16.9) 92.9 (15.4)	78.9 (17.0)	NS
Treatments						
Inhaled corticosteroids (ICS), <i>n</i>	NA	11 4	7	14 4	10	
Daily ICS dose among treated [†] (mcg, budesonide equivalent)	NA	400 (100–1300)	1600 (600–1800)	1600 (800–1800)	1600 (1600–2000)	NS
Oral corticosteroids, <i>n</i>	NA	2 0	2	3 0	3	
LTRAs, <i>n</i>	NA	8 3	5	7 2	5	NS

*Values are means (SD).

[†]Values are medians (interquartile range).AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NS, not significant; FEV_{1,0}, forced expiratory volume in 1 s; LTRA, leukotriene receptor antagonist; NA, not applicable; NM, not measured.

Medicine, and all the subjects gave their written informed consent.

Asthma severity

Asthma severity was determined on the basis of the criteria developed by the Severe Asthma Research Program (SARP) [4, 10]. The subjects were followed up for at least 6–12 months with dose titration of drugs in accordance with the level of control of asthma. The asthmatic subjects were further classified into the severe asthma and non-severe asthma subgroups, as defined on the basis of the SARP criteria.

Quantification of LTE₄, LXA₄ and 15-epi-LXA₄ in urine

Urine samples were collected between 9:00 and 11:00 AM, and aliquots were stored at –40°C until analysis. Urinary LTE₄ concentration was quantified using EIA after purification using HPLC, as reported previously [21]. Before urine collection, peripheral blood was collected from the subjects to count the number of eosinophils.

Urinary LXA₄ and 15-epi-LXA₄ concentrations were measured using EIA (Neogen Corp. Lexington, KY, USA) after separation using HPLC. Briefly, urine (2 mL) was passed through an Empore C18 disk cartridge (SD type), and both LXA₄ and 15-epi-LXA₄ were eluted from the

cartridge using 0.5 mL of methanol. HPLC was performed on a Discovery RP Amide C16 column (Supelco, Park Bellefonte, PA, USA) using a solvent mixture of methanol : distilled water : acetic acid (55 : 45 : 0.1, v/v/v) at a flow rate of 1.0 mL/min at 37°C. The fractions corresponding to the retention times of LXA₄ (approximately 18.3 min) and 15-epi-LXA₄ (approximately 19.6 min) were collected (Fig. 2). After the fractions were diluted with distilled water, the fraction was loaded onto an Empore C18 cartridge. LXA₄ and 15-epi-LXA₄ were recovered from the cartridge using 0.5 mL of methanol. The methanol extracts were stored at –40°C, and LXA₄ and 15-epi-LXA₄ concentrations were determined using the corresponding EIA kits within 1 or 2 weeks. The concentrations were expressed as pg/mg of creatinine.

When using authentic LXA₄ and 15-epi-LXA₄ (0.4 ng), which were purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and Merck KGaA (Darmstadt, Germany), respectively, the overall recovery of LXA₄ and 15-epi-LXA₄ by our method including extraction with an Empore C18 cartridge and purification by HPLC was about 70%. The detection limit of the LXA₄ and 15-epi-LXA₄ EIA kits was 20 pg/mL. The 15-epi-LXA₄ EIA kit showed very little cross-reactivity with LXA₄ (3%). The LXA₄ EIA kit showed 24% cross-reactivity with 15-epi-LXA₄.

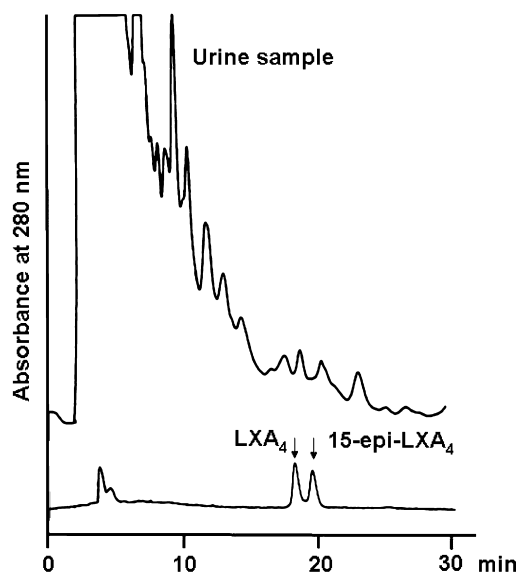


Fig. 2. Separation of extract of urine (upper) and authentic lipoxin A_4 (LXA_4) and 15-epimer of LXA_4 (15-epi- LXA_4) (lower 5.0 ng each) using high-performance liquid chromatography.

Statistical analyses

For samples with LXA_4 and 15-epi- LXA_4 concentrations below the lowest detection limits, the data were expressed as half the detection limit in statistical analyses. Data are expressed as median and inter-quartile range or mean and SD. The Kruskal–Wallis test was performed for comparison among the three groups in addition to post hoc tests. Between-group comparisons were performed using non-parametric Mann–Whitney U -test. Correlation analysis was performed using Spearman's rank correlation coefficient. Comparison of categorical variables was performed using Fisher's exact probability test. Differences were considered significant when the P value was less than 0.05. Receiver-operating characteristic (ROC) curves were constructed using JMP 8.0 software (SAS Institute, Cary, NC, USA).

Results

Clinical characteristics of subjects

Table 1 shows the clinical characteristics of the subjects enrolled in this study. There were no significant differences in age, duration of asthma, eosinophil count and spirometry baseline between the ATA and AIA groups.

After the provocation test, broncho-constriction was induced in AIA subjects at cumulative doses of 87.5 mg in 11 subjects, 187.5 mg in two subjects and 387.5 mg in two subjects. Several subjects experienced symptoms such as abdominal pain, flushing, cough, nasal obstruction, tearing, nausea and chest tightness.

No significant difference was observed in $FEV_{1.0}$ between the AIA and ATA groups. Statistical evaluation using Fisher's exact probability test revealed that there was no significant difference in the number of severe asthmatic subjects between the ATA and AIA groups, suggesting that there was no significant difference in the prevalence of severe asthma between these groups. The dose of inhaled corticosteroids tended to be slightly higher in the AIA group than in the ATA group, but the difference was not statistically significant ($P = 0.06$).

Urinary mediator concentrations in AIA, ATA and healthy control groups

Table 2 shows a summary of urinary LXA_4 , 15-epi- LXA_4 and LTE_4 concentrations. The urinary LTE_4 concentration was significantly higher in the AIA group than in the ATA ($P < 0.05$) and healthy control ($P < 0.01$) groups. The urinary 15-epi- LXA_4 concentrations were significantly higher than urinary LXA_4 concentration in the ATA ($P < 0.01$) and AIA subjects ($P < 0.05$). The urinary 15-epi- LXA_4 concentration was significantly higher in the ATA subjects than in the AIA ($P < 0.01$) and healthy control ($P < 0.01$) subjects. There was a significant correlation between urinary LXA_4 and 15-epi- LXA_4 concentrations in the ATA subjects ($r_s = 0.48$, $P = 0.05$), but not in the AIA or healthy control subjects. There was no significant correlation of urinary LTE_4 concentration with urinary LXA_4 or 15-epi- LXA_4 concentration in the AIA, ATA or healthy control subjects. A significantly negative correlation was found between urinary 15-epi- LXA_4 concentration and the number of peripheral blood eosinophils in the ATA subjects ($r_s = -0.71$, $P < 0.05$), but not in the AIA subjects. No significant correlation was observed between cumulative dose of aspirin during the provocation test and LTE_4 concentration or 15-epi- LXA_4 concentration in AIA subjects. There was no correlation between urinary LXA_4 or 15-epi- LXA_4 concentration and any of the other clinical parameters such as $FEV_{1.0}$.

When 15-epi- LXA_4 concentration in the same urine samples was measured twice, 2 months apart, there was no significant difference between the measured concentrations (mean $\pm$ SD, 14.73 ± 5.63 vs. 15.88 ± 8.68 , $n = 8$), and the ratio of 15-epi- LXA_4 concentration in the first measurement to that in the second measurement was 1.02 ± 0.37 , suggesting that 15-epi- LXA_4 remained stable during storage at -40°C for at least 2 months, and 15-epi- LXA_4 concentration can be measured with a good reproducibility.

Urinary LTE_4 and 15-epi- LXA_4 concentrations and their ratio depending on asthma severity

In the severe asthma subgroup, the AIA subjects showed significantly lower urinary 15-epi- LXA_4 and

Table 2. Urinary mediator concentrations and concentration ratios of urinary LTE₄ to 15-epi-LXA₄ in healthy control, ATA and AIA subjects

	Control	ATA		AIA	
		Non-severe	Severe	Non-severe	Severe
Urinary LTE ₄ concentration, pg/mg-creatinine	53.4 (51.5–135.5)	144.3 (73.6–251.5) 143.9 (74.2–184.6)	155.4 (74.1–310.0)	345.1* (188.9–1231.0) 345.1 (162.3–431.7)	346.4† (222.9–1541.8)
Urinary LXA ₄ concentration, pg/mg-creatinine	9.8 (8.0–15.1)	18.6 (11.4–25.1) 13.6 (11.3–19.2)	22.5 (18.8–34.2)	11.3 (6.0–16.3) 14.4 (11.3–16.6)	7.8 (3.1–15.7)
Urinary 15-epi-LXA ₄ concentration, pg/mg-creatinine	22.5 (10.0–58.4)	95.3‡ (64.6–142.0) 88.8 (65.3–128.7)	139.2 (60.1–142.3)	21.5 (13.7–52.4) 21.5§ (13.5–51.7)	21.2¶ (14.3–47.3)
Concentration ratio of urinary LTE ₄ to 15-epi-LXA ₄	4.3 (1.3–9.4)	1.9 (1.0–2.9) 1.7 (0.8–3.2)	2.2 (1.4–2.8)	14.5 (6.2–37.0) 6.7** (4.2–15.6)	21.7†† (9.7–38.1)

Values are medians (interquartile range). The results were analysed using non-parametric Mann–Whitney *U*-test.

*Significantly higher in AIA subjects than in ATA ($P < 0.05$) and control subjects ($P < 0.01$).

† $P < 0.01$ when compared with severe ATA subjects.

‡ $P < 0.01$ when compared with control subjects and AIA subjects.

§ $P < 0.05$ when compared with non-severe ATA subjects.

¶ $P < 0.01$ when compared with severe ATA subjects.

||Significantly higher in AIA subjects than in ATA subjects ($P < 0.01$) and control subjects ($P < 0.05$).

** $P < 0.01$ when compared with non-severe ATA subjects.

†† $P < 0.01$ when compared with severe ATA subjects.

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; LXA₄, lipoxin A₄; 15-epi-LXA₄, 15-epimer of LXA₄; LTE₄, leukotriene E₄.

higher urinary LTE₄ concentrations than the ATA subjects [median (interquartile range) AIA vs. ATA: 21.2 (14.3–47.3) vs. 139.2 (60.1–142.3) pg/mg-creatinine ($P < 0.01$) for 15-epi-LXA₄, 346.4 (222.9–1541.8) vs. 155.4 (74.1–310.0) pg/mg-creatinine ($P = 0.05$) for LTE₄]. In addition, even in the non-severe subgroup, the AIA subjects showed a significantly lower urinary 15-epi-LXA₄ concentration than the ATA subjects [21.5 (13.5–51.7) vs. 88.8 (65.3–128.7) pg/mg-creatinine ($P < 0.05$)] (Figs 3 and 4). The concentration ratio of urinary LTE₄ to 15-epi-LXA₄ was significantly higher in the AIA subjects than in the ATA subjects in both the severe and non-severe asthma subgroups [Fig. 5, 21.7 (9.7–38.1) vs. 2.2 (1.4–2.8) ($P < 0.01$) for severe asthma, 6.7 (4.2–15.6) vs. 1.7 (0.8–3.2) ($P < 0.01$) for non-severe asthma].

Receiver-operating characteristic curve analysis suggested that the concentration ratio of LTE₄ to 15-epi-LXA₄ was superior to 15-epi-LXA₄ concentration and LTE₄ concentration as a biomarker for diagnosis of aspirin sensitivity (Fig. 6). The area under curve values were as follows: 15-epi-LXA₄ concentration, 0.908 (95% CI, 0.743–0.971); LXA₄ concentration, 0.696 (0.477–0.852); LTE₄ concentration, 0.788 (0.576–0.910); the concentration ratio of LTE₄ to 15-epi-LXA₄, 0.977 (0.885–0.996); the concentration ratio of LTE₄ to LXA₄, 0.825 (0.620–0.932). As described above, the urinary LXA₄ concentration was significantly lower than the

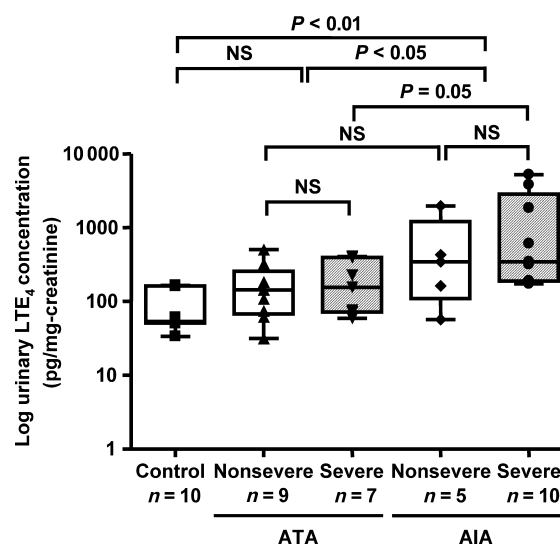


Fig. 3. Urinary leukotriene E₄ (LTE₄) concentrations in healthy control, aspirin-tolerant asthma (ATA) and aspirin-intolerant asthma (AIA) subjects. Data are presented as box plots showing medians and interquartile ranges. In the box plots, the lower boundary indicates the 25th percentile. The line within the box indicates the 50th percentile (median) and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the maximum and minimum values, respectively. The results were analysed using non-parametric Mann–Whitney *U*-test.

urinary 15-epi-LXA₄ concentrations in both ATA and AIA subjects, and the concentration ratio of LTE₄ to LXA₄ has a lower diagnostic value for AIA.

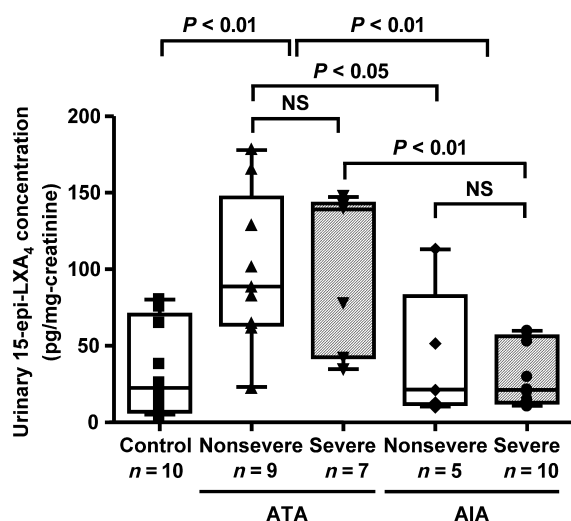


Fig. 4. Concentrations of urinary 15-epimer of LXA₄ (15-epi-LXA₄) in healthy control and aspirin-tolerant asthma (ATA) and aspirin-intolerant asthma (AIA) subjects. Data are presented similar to those in Fig. 3. The results were analysed using non-parametric Mann–Whitney *U*-test.

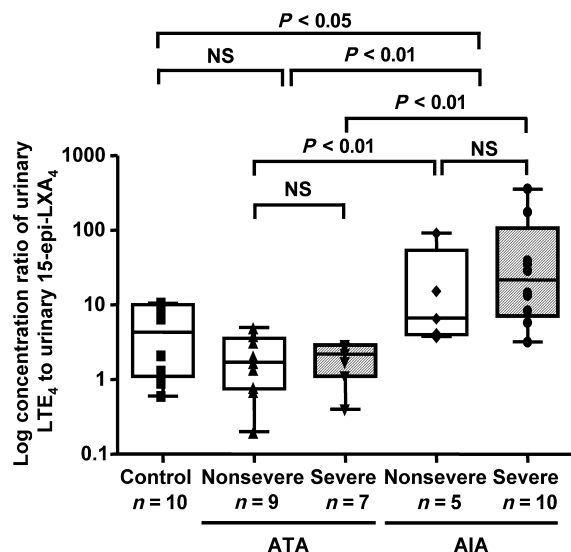


Fig. 5. Concentration ratio of urinary leukotriene E₄ (LTE₄) to 15-epimer of LXA₄ (15-epi-LXA₄) in healthy control, aspirin-tolerant asthma (ATA) and aspirin-intolerant asthma (AIA) subjects. Data are presented similar to those in Fig. 3. The results were analysed using non-parametric Mann–Whitney *U*-test.

Discussion

We have established a method for determining urinary LXA₄ and 15-epi-LXA₄ concentrations using the corresponding EIA kits after separating these compounds using HPLC to overcome the problem of significant cross-reactivity between the anti-LXA₄ antibody and 15-epi-LXA₄, which led us to an unexpected novel find-

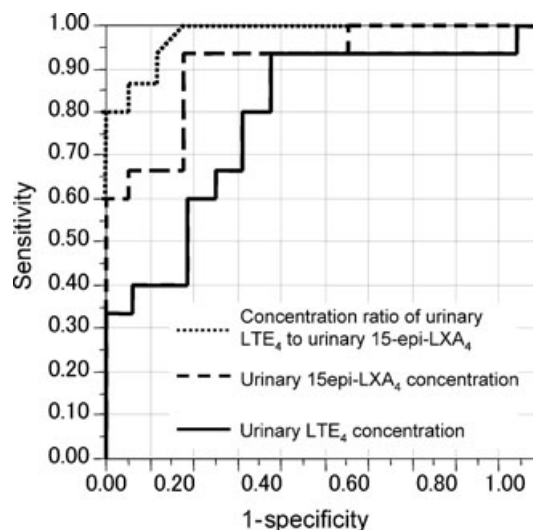


Fig. 6. Receiver operating characteristic curves for evaluation of urinary 15-epimer of LXA₄ (15-epi-LXA₄) concentration, urinary leukotriene E₄ (LTE₄) concentration and the concentration ratio of LTE₄ to 15-epi-LXA₄ for diagnosing aspirin sensitivity. The area under curve values for urinary 15-epi-LXA₄ concentration, urinary LTE₄ concentration and the concentration ratio of LTE₄ to 15-epi-LXA₄ were 0.908 (95% CI, 0.743–0.971), 0.788 (95% CI, 0.576–0.910) and 0.977 (95% CI, 0.885–0.996), respectively.

ing that the urinary 15-epi-LXA₄ concentration is significantly higher than the urinary LXA₄ concentration.

Both LXA₄ and 15-epi-LXA₄ are metabolized by 15-hydroxyprostaglandin dehydrogenase, which converts 15-epi-LXA₄ into 15-oxo-LXA₄ [22]. The rate of conversion of LXA₄ is twofold higher than that of 15-epi-LXA₄, which may be attributed to the higher 15-epi-LXA₄ concentration than LXA₄ concentration in biological samples. If this explanation is plausible for the concentration of 15-epi-LXA₄ being higher than that of LXA₄, this method might be applied to other samples of biological fluid. When we preliminarily quantified LXA₄ and 15-epi-LXA₄ concentrations in BALF from subjects with interstitial lung disease (*n* = 6), it is surprising that the LXA₄ concentration in BALF was below the detection limit, whereas the median concentration of 15-epi-LXA₄ was 6.7 pg/mL in BALF (unpublished observations). This finding provides us further evidence that the 15-epi-LXA₄ concentration is significantly higher than the LXA₄ concentration in biological fluid. The results of our study indicated that LXA₄ concentration may be incorrectly determined in most previous studies in which 15-epi-LXA₄ and LXA₄ were quantified without separating them and have highlighted the significance of 15-epi-LXA₄ concentration as a biomarker of LX production.

Quantification of urinary LTE₄ provides a reliable means to assess the formation of total endogenous cysteinyl-leukotrienes (CysLTs) [23]. Urinary metabolites of

LXs have not been identified yet. Thus, it is still not clarified whether urinary LXs predominantly reflect local production in the kidney similar to urinary unmetabolized COX-derived prostaglandins [24, 25] or systemic production, which results from filtration of LXs from plasma. Although 15-epi-LXA₄ is also named aspirin-triggered 15-epi-LXA₄, urinary 15-epi-LXA₄ concentration was detectable even in the absence of aspirin administration.

Although no significant difference was observed in 15-epi-LXA₄ concentration between the severe asthmatic and non-severe asthmatic subjects in both the AIA and ATA groups, the 15-epi-LXA₄ concentration in the severe asthmatic subjects of the AIA group was significantly lower than that in severe asthmatic subjects of the ATA group. A similar difference was observed in the non-severe asthmatic subjects between the AIA and ATA groups, suggesting that the decreased concentration of 15-epi-LXA₄ may be related to aspirin intolerance. This finding is consistent with the findings of a lower LXA₄ concentration in the sino-nasal tissue in AIA subjects [26]. Aspirin/NSAID intolerance itself, but not severe asthma, may be associated with diminished 15-epi-LXA₄ production, and an imbalance of arachidonic acid metabolism including 15-epi-LXA₄ may be involved in AIA pathogenesis. 15(R)-hydroxyeicosatetraenoic acid, which is enzymatically formed by acetylated COX-2 or CYP2C9 in the cytochrome P-450 pathway [16, 27], is a precursor of 15-epi-LXA₄. Thus, the findings of this study suggest the possibility of a diminished expression of COX-2, acetylated COX-2 or cytochrome P-450 in AIA subjects. Further studies will be required to clarify the mechanism responsible for the decreased concentration of 15-epi-LXA₄ in AIA subjects.

It has been considered that baseline urinary LTE₄ concentration is a strong predictor of AIA [28]. When evaluated from ROC curves, the concentration ratio of urinary LTE₄ to urinary 15-epi-LXA₄ was a stronger predictive factor than the concentration of LTE₄ or 15-

epi-LXA₄ for the diagnosis of aspirin sensitivity in asthmatic subjects.

In contrast, the ATA subjects exhibited a significantly higher urinary 15-epi-LXA₄ concentration than the healthy control subjects ($P < 0.01$), and a significantly negative correlation between urinary 15-epi-LXA₄ concentration and the number of peripheral blood eosinophils. These findings support the idea that 15-epi-LXA₄ plays a protective role against allergic inflammation in animal models of asthma [29, 30]. In allergic airway inflammation, LXA₄ and 15-epi-LXA₄ block both bronchial hyperresponsiveness and pulmonary inflammation induced by eosinophils via the LXA₄ receptor, leading to decreases in the numbers of eosinophils and T lymphocytes and decreases in the concentrations of interleukin-5, interleukin-13, eotaxin, immunoglobulin E, prostanoids and CysLTs [31].

Inhibition of LXA₄ production by dexamethasone in alveolar macrophages has recently been reported [32]. As there was no significant difference between the AIA and ATA subjects in the dose of an inhaled corticosteroid, a significant difference in 15-epi-LXA₄ concentration between AIA and ATA subjects ($P < 0.01$) may not have resulted from the regulation of 15-epi-LXA₄ production by a corticosteroid.

In conclusion, our findings indicate that an imbalance between locally produced proinflammatory CysLTs and anti-inflammatory 15-epi-LXA₄ at baseline concentrations may play an important role in the pathogenesis of AIA.

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