Hyperleukotrieneuria in Patients with Allergic and Inflammatory Disease

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ABSTRACT
Cysteinyl leukotrienes (CysLTs: leukotrienes C₄, D₄, and E₄) have long been implicated in the pathogenesis of asthma and several allergic diseases. LTE₄ has been identified as a major metabolite of LTC₄, and urinary LTE₄ (U-LTE₄) is considered as the most reliable analytic parameter for monitoring the endogenous synthesis of CysLTs. From recent studies on the U-LTE₄ associated with adult stable asthma we identified four factors for hyperleukotrieneuria, namely, aspirin intolerance, eosinophilic nasal polyposis (ENP), vasculitis, and severe asthma. In ENP, there is prominent infiltration of eosinophils in the sinus and polyp tissues, which is linked to adult asthma and aspirin sensitivity, and ENP is the most important factor for the overproduction of CysLTs in asthmatics. We also demonstrated that anaphylaxis and eosinophilic pneumonia (EP) are associated with a marked increase in the U-LTE₄ concentration. Under these disease conditions, U-LTE₄ may be one of the candidate biomarkers. Moreover, the changes in U-LTE₄ concentrations may provide valuable information concerning therapeutic targets.

KEY WORDS
anaphylaxis, aspirin-intolerant asthma (AIA), Churg-Strauss syndrome (CSS), cysteinyl leukotrienes (CysLTs), nasal polyp

STABLE ASThma WITH HYPERLEukOTRIENURIA
Leukotrienes (LTs) are downstream products of the metabolism of cell or nuclear membrane phospholipids. In certain inflammatory cells such as eosinophils, mast cells, and other inflammatory cells, degradation to arachidonic acid (AA) by phospholipase A₂ occurs at the nuclear membrane, which indicates the biologic functions of the LTs once they are formed.¹⁻⁴ ⁵-lipoxygenase activation at the cytoplasmic or nuclear membrane then leads to the production of an unstable intermediate known as leukotriene A₄ (LTA₄), which can be further metabolized, depending on cell type, to LTB₄ or the cysLTs (LTC₄, LTD₄, and LTE₄). LTC₄ synthase metabolizes LTA₄ to LTC₄ via glutathione transferase. LTC₄ is then rapidly metabolized to LTD₄ and LTE₄ through the enzymes gamma-glutamyl transpeptidase and a dipeptidase. LTC₄ and LTD₄ both have very short half-lives, whereas LTE₄ appears to be the most stable of the three, with the longest half-life.³

LTE₄ has been identified as a major metabolite of LTC₄; urinary LTE₄ (U-LTE₄) has been considered as the most reliable analytic parameter for monitoring the endogenous synthesis of CysLTs.⁶ We previously described an efficient procedure for the precise quantification of LTE₄ in a small volume of urine, which was achieved mainly by the use of an Empore extraction disk cartridge.⁷ Asano et al. reported that no systematic variation in urinary LTE₄ excretion rates over the course of a day was observed in either normal subjects or patients with stable asthma.⁸ Even under a clinically stable condition, the U-LTE₄ concentration in patients with aspirin-intolerant asthma (AIA) is significantly higher than that in patients with aspirin-tolerant asthma (ATA) (Fig. 1).⁹⁻¹² We evaluated the clinicopathological factors associated with the increase in U-LTE₄ concentration in asthmatics and have confirmed that the U-LTE₄ concentration in AIA patients is significantly higher than those in ATA patients. Depending on asthma severity, ATA patients with severe asthma and AIA patients with poor pulmonary function showed a significant increase in U-LTE₄ concentration, but the extent of increase was not large (Fig. 2).¹¹ Moreover, we have demonstrated
for the first time that nasal polyposis is one of the most important factors that indicate hyperleukotrieniuria. Recently, we have determined another clinicopathological factor for hyperleukotrieniuria in patients with adult asthma. Churg-Strauss syndrome (CSS) is characterized by the presence of asthma, eosinophilia, and small-vessel vasculitis with granuloma. The natural history of CSS is, first, the appearance of eosinophilic rhinosinusitis, followed several years later by the development of difficult asthma with marked peripheral blood eosinophilia, and finally the development of systemic vasculitis. We have demonstrated that the U-LTE\textsubscript{4} concentration is elevated in the acute phase of CSS. However, the U-LTE\textsubscript{4} concentration significantly increases in patients with not only eosinophilic vasculitides, including CSS, but also noneosinophilic vasculitides (Fig. 3).

**CysLTs OVERPRODUCTION IN ACUTE ASTHMA**

1) **SPONTANEOUS ATTACK**

In acute asthmatics, the urinary excretion of LTE\textsubscript{4} was significantly higher on admission with an asthma attack, and returned to control levels when the patient’s conditions improved. However, the increased level of U-LTE\textsubscript{4} was only 2-fold higher than that of healthy controls (Fig. 4). Oosaki also reported that there is a significant correlation between changes (%) in urinary eosinophil protein X (EPX), which may originate from eosinophil activation, and those in urinary LTE\textsubscript{4} during the spontaneous attack state. Moreover, Green et al. reported that the decreases in FEV\textsubscript{1} are significantly correlated to U-LTE\textsubscript{4} concentrations in patients with acute exacerbation of asthma. Tanaka and his colleagues found that the U-LTE\textsubscript{4} concentration at night significantly increased in
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Fig. 3 Urinary LTE4 (A) and EDN (B) concentration in each group. Urinary concentrations are expressed by using the log scale. Horizontal bars indicate medians. *p < 0.05; †p < 0.01. HC: healthy control, RA: rheumatoid arthritis, VD: non-eosinophilic vasculitis, CSS: Churg-Strauss syndrome. (Adapted from Higashi et al.14)

patients with nocturnal asthma.18 These findings suggest that systemic CysLTs overproduction during a spontaneous attack is mainly caused by eosinophils, and that it is closely related to human airflow limitation.

2) ALLERGEN-INDUCED ASTHMATIC REACTION

The urinary LTE4 concentration increases during the first 3h after allergen inhalation in atopic patients.19,20 However, no significant increase is observed in the late phase during allergen-induced bronchoconstriction (Fig. 5).19 Moreover overproduction of CysLTs in allergen-induced asthma was found to be not associated with an increased concentration of LTB4 glucuronide in urine.19 Very recently, we have quantified lipid mediators in exhaled breath condensate (EBC) and their corresponding urinary metabolites before and after allergen inhalation. In the patients with allergen-induced early asthmatic responses (EARs), the decreased percentage in FEV1 significantly correlated with the increase in CysLTs concentration in EBC but not with the increase in U-LTE4 concentration after allergen inhalation (Fig. 6).21 There was a significant correlation between increased concentrations of CysLTs and PGD2 in EBC collected in patients with EARs after allergen inhalation. However, the increase in urinary 9α, 11β-PGF2 (metabolite presumably related to mast cell activation), concentrations did not correlate with either the increase in PGD2 concentration in EBC or that in LTE4 concentration in urine.21 These results suggest that (1) human EARs may be mainly induced by CysLTs generated by pulmonary mast cells and (2) urinary LTE4

Fig. 4 Change in urinary LTE4 concentration before and after treatment in patients with the spontaneous asthma attack. *p < 0.05; **p < 0.01; ***p < 0.01 compared with improved state. (Adapted from Oosaki et al.19)
and PGD₂ metabolite may not be direct indicators of eicosanoid production in the airway.

**HYPERLEUKOTRIENURIA IN SEASONAL ALLERGIC RHINITIS (SAR)**

CysLTs have been reported to play a primary role in the induction of nasal blockage associated with allergic rhinitis, with limited effect on other rhinitis symptoms. ³²⁻³⁵ We demonstrated for the first time that the basal U-LTE₄ concentration is significantly higher in SAR patients with severe nasal blockage than in those with mild or no nasal blockage and in healthy control subjects. ²⁶ More interestingly, there was no significant difference in the U-LTE₄ concentration between the patients with both SAR and asthma and SAR patients with severe nasal blockage (Fig. 7). ²⁶ There is a significant correlation between U-LTE₄ and 9α₁₁ βPGF₂ concentrations. On the other hand, there is no significant correlation between U-LTE₄ and eosinophil-derived neurotoxin (EDN) concentrations. ²⁶ These results indicate that nasal blockage may be induced by the overproduction of CysLTs generated by mast cells rather than by eosinophils in the nasal cavity of SAR patients.

**ROLE OF CysLTs IN THE PATHOGENESIS OF NASAL POLYPOSIS**

Nasal polyps are edematous semitranslucent masses in the nasal and paranasal cavities, mostly originating from the mucosal linings of the sinuses and prolaps-
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Fig. 8 Urinary LTE4 concentration in aspirin-tolerant asthma (ATA) and aspirin-intolerant asthma (AIA) patients with eosinophilic nasal polyposis (ENP) and those with normal sinuses. Urinary LTE4 concentration is expressed by using the log scale. The horizontal bars indicate medians. (Adapted from Higashi et al.11)

Fig. 9 A significant decrease in urinary LTE4 concentration between before and after the endoscopic sinus surgery without changing medication. The horizontal bars indicate medians. (Adapted from Higashi et al.11)

ing into the nasal cavities. Eosinophilic nasal polyposis (ENP), in which there is prominent infiltration of eosinophils and submucosal edema, is linked to comorbidities such as nonatopic asthma, adult-onset asthma, and aspirin intolerance, or may represent a part of a systemic disease such as CSS. Despite sinus surgery or intensive steroid therapy, the recurrence rate of ENP in patients with asthma is very high, and the pathogenesis of ENP has not been clarified.27

For the first time, we found that the U-LTE4 concentration is significantly high even in ENP patients without aspirin sensitivity (Fig. 8).11 We then confirmed the changes in U-LTE4 concentration after endoscopic sinus surgery without changing the medica-
CysLT OVERPRODUCTION IN HUMAN ANAPHYLAXIS

Anaphylaxis is a life-threatening generalized allergic reaction that occurs when antigens bind to immunoglobulin E on mast cells and basophiles causing the release of inflammatory mediators. We focused on the changes in inflammatory mediator concentrations during anaphylactic reactions in our recent study. We have demonstrated that anaphylaxis is associated with increased excretions of LTE4 and 9α, 11β-PGF2 (Fig. 10), and a significant correlation between the concentrations of these two mediators. The observations suggest that mast cells may participate in the generation of CysLTs during the anaphylactic reactions. The changes in mediator concentrations may provide valuable information on therapeuti-
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tic targets in anaphylaxis.

**EOSINOPHILIC PNEUMONIA (EP) AND CysLTs OVERPRODUCTION**

Eosinophilic pneumonia (EP) is a diffuse infiltrative lung disease characterized by alveolar and peripheral airway eosinophilia.\(^{38-40}\) Although eosinophils produce cysteiny1 leukotrienes (CysLTs) in large quantities, information on the relationship between CysLTs and eosinophilic pneumonia (EP) is lacking. We demonstrated that the urinary LTE4 concentration is significantly higher in EP patients during acute exacerbation than in asthma patients with acute exacerbation and healthy subjects (Fig. 11).\(^{41}\) and the concentration significantly decreases in EP patients during clinical remission. These findings suggest that CysLTs production is closely associated with the clinical conditions of EP patients. We also demonstrated that the progression of eosinophilic pneumonia is associated with elevated urinary leukotriene E4 and eosinophil-derived neurotoxin concentrations, which may originate from eosinophil activation.\(^{41}\) The leukotriene E4 concentration was correlated with the level of diffusing capacity of the lung for carbon monoxide during acute exacerbation.\(^{42}\) These findings suggest that the monitoring of the leukotriene E4 concentration may aid in the management of eosinophilic pneumonia patients.

**REFERENCES**


