Original Article

Urinary leukotriene E₄ and 11-dehydro-thromboxane B₂ excretion in children with bronchial asthma

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ABSTRACT

Background: Cysteinyl leukotrienes (CysLTs) and thromboxane (TX) A₂ have been implicated in the pathogenesis of bronchial asthma. Urinary leukotriene E₄ (LTE₄) and 11-dehydro-TXB₂ (11DTXB₂) levels are often used to assess the production of CysLTs and TXA₂. However, few studies have examined the products of these two mediators in the same asthmatic patients. To define the potential roles of CysLTs and TXA₂ in the pathogenesis of bronchial asthma in children, their urinary levels were measured in the present study.

Methods: Urinary LTE₄ and 11DTXB₂ levels were measured by enzyme immunoassay (EIA) and radioimmunoassay (RIA), respectively. Urine samples from asthmatic children were measured during the stable condition and during an acute attack.

Results: Urinary LTE₄ levels during an acute attack (median 476 pg/mg creatinine; range 191–1100 pg/mg creatinine) and during the stable condition (median 332 pg/mg creatinine; range 128–965 pg/mg creatinine) were significantly higher (P < 0.05) than those of controls (median 233 pg/mg creatinine; range 128–965 pg/mg creatinine). Urinary 11DTXB₂ levels during an acute attack and during the stable condition (median 1666 (range 110–5105) and 1009 (range 46–6070) pg/mg creatinine, respectively) were significantly higher (P < 0.05) than those of controls.

Conclusions: The present findings suggest that high levels of CysLTs and TXA₂ are associated with the pathogenesis of bronchial asthma. The measurement of urinary LTE₄ and 11DTXB₂ would be useful in understanding the individual pathogenesis of asthmatic children.

Key words: bronchial asthma, cysteinyl leukotrienes, 11-dehydro-thromboxane B₂, leukotriene E₄, thromboxane A₂.

INTRODUCTION

Cysteinyl leukotrienes (CysLTs), namely leukotrienes C₄, D₄ and E₄, and thromboxane (TX) A₂ are considered to play important roles in bronchial asthma.¹–¹⁰ Cysteinyl leukotrienes are derived from arachidonic acid by the action of 5-lipoxygenase and increase vascular permeability, stimulate mucus secretion and induce bronchial hyperreactiveness and bronchoconstriction. Moreover, increased production of CysLTs in asthmatic patients in vivo has been observed in several studies.¹–¹⁰ A potent bronchoconstrictor, TXA₂ is generated from arachidonic acid by cyclooxygenase. Enhanced TXA₂ release has also been reported in asthmatic patients after allergen challenge.⁷ Owing to the significant roles of CysLTs and TXA₂, their inhibitors or receptor antagonists have been developed extensively and recently some drugs have become available.¹³
Although these mediators of asthma have been discussed previously, few studies have examined the products of these two mediators in the same asthmatic patients. In addition, few studies have compared the TXA₂ products of asthmatic patients with those of healthy control subjects. Leukotriene E₄ is a stable product of CysLTs and is considered an index of the systemic production of CysLTs production in humans.¹⁴ 11-Dehydro-thromboxane B₂ (11DTXB₂) is the most abundant degradation product of TXB₂ and is also considered an index of systemic TXA₂ production.¹⁵⁻¹⁷ The measurement of urinary LTE₄ and 11DTXB₂ is a non-invasive method for assessing the production of CysLTs and TXA₂ in vivo, respectively. Oosaki et al.¹⁸ previously established sensitive and selective methods of determination of urinary LTE₄ by enzyme immunoassay (EIA) and Ruitta et al.¹⁹ established the method of determining urinary 11DTXB₂ by radioimmunoassay (RIA). In the present study, using these methods, the products of CysLTs and TXA₂ were assessed in vivo in control subjects and in children with bronchial asthma during the stable condition and during an acute attack.

**METHODS**

**Subjects**

Twenty-nine children with bronchial asthma (19 males, 10 females) and nine control subjects (six males, three females) were enrolled in the study (Table 1). The mean age of asthmatic children and control subjects was 7 years (range 1–15 years) and 8 years (range 1–15 years), respectively. None of the patients had a history of aspirin sensitivity. Twenty-six of the asthmatic patients were receiving theophylline, all were receiving inhaled β₂-adrenergic receptor agonists, 10 were receiving inhaled corticosteroids and 17 were using a leukotriene receptor antagonist (LTRA). All patients were classified into one of four categories (intermittent, mild persistent, moderate persistent and severe persistent) according to Globalal Initiative for Asthma (GINA) guidelines (http://www.ginasthma.com/ Table 1). None of the patients had been treated previously with oral prednisolone (PSL) prior to their enrollment in the study. In the present study, the urinary excretion of LTE₄ or 11DTXB₂ was not influenced by the usage of LTRA or corticosteroids, because the patients kept the treatments unmodified throughout the duration of the study. Informed consent to participate in the study was obtained from all subjects or their parents.

Urine samples from children with bronchial asthma were measured during the stable condition and during an acute attack. Urine samples from nine asthmatic children selected at random (Table 1, patients 1–9) were also measured 2 days after treatment. The ‘stable condition’ refers to the condition in which the patients did not complain of any symptoms with or without receiving their usual medications. The ‘acute attack’ refers to a condition in which the patients complained of some active symptoms, cough and/or wheezing and/or chest tightness, which were occurring repeatedly on waking and/or disturbing sleep at night; therefore, they needed additional treatment to their usual treatments.¹⁹ Patients were treated with steroid and/or theophylline by injection and/or inhaled β₂-adrenergic receptor agonists and, 2 days after treatment, they felt better but were still complaining slightly of some asthmatic symptoms, such as cough and/or wheezing and/or chest tightness.

Urine samples were collected when the asthmatic children visited our hospitals during the stable condition maintaining their usual treatments. Urine samples from asthmatic children were also collected on arrival at hospital when they had acute asthma attacks. Patients were treated with theophylline and/or β₂-adrenergic receptor agonists and/or corticosteroid drip infusion. Urine samples from nine asthmatic children were collected 2 days after treatment.

**Measurement of LTE₄**

Urine samples were stored at −80°C and analyzed within 1 month of collection. An aliquot of urine was removed to determine creatinine concentration. The urinary creatinine level was determined using a Creatinine test kit (Pure Auto S CRE-L; Daiichi-kagaku, Tokyo, Japan).

Approximately 3000 d.p.m. [³H]-LTE₄ was added to each urine sample as an internal standard and the urine was applied to a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) that had been preconditioned by the serial addition of methanol and distilled water. Then, the cartridge was washed with distilled water, followed by 40% methanol; LTE₄ was eluted with 80% methanol. This elution was dried with nitrogen gas and was dissolved in the elution buffer used in high-performance liquid chromatography (HPLC; 486 Tunable Absorbance Detector; Waters) and the solution was injected onto a C18 reverse-phase column (CAPPCELL PAC UG 120; Shiseido, Tokyo, Japan). The fractions that contained peak [³H]-LTE₄ radioactivity and also corresponded to the
Table 1  Characteristics of patients and urinary levels of leukotriene E\textsubscript{4} and 11-dehydro-thromboxane B\textsubscript{2}

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<th>Mite score</th>
<th>Severity (GINA)</th>
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<th>Theophylline</th>
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Median: 577.5
Average: 7 684.5 4 4

GINA, Global Initiative for Asthma (GINA) guideline (http://www.ginasthma.com/).
HD, house dust; LTRA, leukotriene receptor antagonist; DSCG, disodium cromoglycate; LTE\textsubscript{4}, leukotriene E\textsubscript{4}; 11DTXB\textsubscript{2}, 11-dehydro-thromboxane B\textsubscript{2}.
retention time of authentic LTE₄ were dried and resuspended in assay buffer, which was supplied in the Leukotriene C₄,D₄/E₄ enzyme-immunoassay system (Amersham, Buckinghamshire, UK). Urinary LTE₄ concentrations determined by EIA were corrected for recovery of [³H]-LTE₄. The urinary LTE₄ level was expressed as pg/mg creatinine.

**Measurement of 11DHTXB₂**

The 11DHTXB₂ was extracted from an acidified sample by adding an equal volume of octadecylsilyl silica powder (ODS) suspension (80 mg/mL in 40% ethanol) followed by mixing, centrifuging (at 2000 g for 3 min at room temperature) and either decanting or aspirating. The pellet was washed with an acidic alcohol solution and then with petroleum ether for deproteinizing and defatting. The 11DHTXB₂ was eluted by ethyl acetate. The pooled ethyl acetate was evaporated to dryness with nitrogen gas. The dried residue, containing 11DHTXB₂, was dissolved in the eluent (acetonitril : chloroform : acetic acid, 10 : 90 : 0.5, v/v/v) and applied to the open silica mini column (Bond Elute Si; VARIAN, Palo Alto, CA, USA). The column was washed with the eluent (acetonitril : chloroform : acetic acid, 20 : 80 : 0.5, v/v/v). The elution buffer, containing the 11DHTXB₂, was dried with nitrogen gas and the amount of 11DHTXB₂ was quantitated by RIA (11-Dehydrothromboxane B₂ [¹²⁵I] RIA kit; Perkin Elmer Life and Analytical Sciences, Boston, MA, USA). The urinary 11DHTXB₂ level was also expressed as pg/mg creatinine.

**Statistical analyses**

The Mann–Whitney unpaired U-test was used to compare controls and asthmatic children during the stable condition and the Wilcoxon paired test was used to compare asthmatic children during the stable condition and during an acute attack. Correlation was analyzed by Pearson correlation analysis. The percentage of changes was calculated using the following equation: % change = (level during stable condition – level during attack) × 100/level during attack. Data are expressed as the median (range) and P < 0.05 was considered significant.

**RESULTS**

Urinary LTE₄ and 11DHTXB₂ levels

Urinary levels of LTE₄ and 11DHTXB₂ were measured to define the potential roles of CysLTs and TXA₂ in children with bronchial asthma.

Leukotriene E₄ was measured by EIA. Urinary LTE₄ levels are plotted in Fig. 1. Urinary LTE₄ levels in asthmatic children during the stable condition (332 (128–965) pg/mg creatinine) were significantly higher (P < 0.05) than that of control subjects (233 (103–389) pg/mg creatinine; Fig. 1a). Comparing the different conditions of asthma, LTE₄ levels during an acute attack (476 (191–1100) pg/mg creatinine) were significantly higher (P < 0.05) than those during the stable condition (Fig. 1b).

11-Dehydro-thromboxane B₂ was measured by RIA and was detectable in all urine samples. Urinary 11DHTXB₂ levels in asthmatic children during the stable condition (1009 (46–6070) pg/mg creatinine) were significantly higher (P < 0.05) than those of control subjects (252 (41–716) pg/mg creatinine; Fig. 2a). However, there was no significant difference in 11DHTXB₂ levels during an acute attack (1666 (110–5105) pg/mg creatinine) and during the stable condition (Fig. 2b).

Urinary levels of LTE₄ and 11DHTXB₂ were observed during the stable condition, an acute attack and 2 days after treatment in nine asthmatic children (Fig. 3). Urinary LTE₄ increased from 373 pg/mg creatinine (range 183–556 pg/mg creatinine) during the stable condition to 546 pg/mg creatinine (range 280–1100 pg/mg creatinine) during an acute asthma attack and then decreased to 443 pg/mg creatinine (range 156–872 pg/mg creatinine) 2 days after treatment (Fig. 3a). In contrast, urinary 11DHTXB₂ levels exhibited different patterns after an attack. Urinary 11DHTXB₂ levels increased from 1009 pg/mg creatinine (range 131–2106 pg/mg creatinine) during the stable condition to 546 pg/mg creatinine (range 280–1100 pg/mg creatinine) during an acute asthma attack and gradually decreased to 842 pg/mg creatinine (range 492–2708 pg/mg creatinine) 2 days after treatment. However, each patient showed variable levels of urinary 11DHTXB₂ 2 days after treatment (Fig. 3b).

Correlations between urinary LTE₄ and 11DHTXB₂

We assessed the relationship between LTE₄ and 11DHTXB₂ in children with bronchial asthma (Fig. 4). No relationship was noted between these prostanoids in children with bronchial asthma or in the controls. In plots of changes from levels observed during an attack to
Fig. 1  (a) Urinary leukotriene E₄ (LTE₄) levels in asthmatic children during the stable condition (median 332 pg/mg creatinine; range 128–965 pg/mg creatinine) and in controls (median 233 pg/mg creatinine; range 103–389 pg/mg creatinine). (b) Urinary LTE₄ levels in asthmatic children during an acute asthma attack (median 476 pg/mg creatinine; range 191–1100 pg/mg creatinine) and during the stable condition (median 332 pg/mg creatinine; range 128–965 pg/mg creatinine). Horizontal bars indicate median values. *P < 0.05.

Fig. 2  (a) Urinary 11-dehydro-thromboxane B₂ (11DTXB₂) levels in asthmatic children during the stable condition (median 1009 pg/mg creatinine; range 46–6070 pg/mg creatinine) and in controls (median 252 pg/mg creatinine; range 41–716 pg/mg creatinine). (b) Urinary 11DTXB₂ levels in asthmatic children during an acute asthma attack (median 1666 pg/mg creatinine; range 110–5105 pg/mg creatinine) and during the stable condition (median 1009 pg/mg creatinine; range 46–6070 pg/mg creatinine). Horizontal bars indicate median values. *P < 0.05.
levels during the stable condition, the changes in LTE4 were not related to changes in 11DTXB2 in children with bronchial asthma (Fig. 5). Neither gender, age, serum IgE nor eosinophil count had any relationship with urinary levels of LTE4 or 11DTXB2 (data not shown). One patient (no. 5) had high eosinophil counts (1344/µL during the stable condition; 871/µL during an acute attack; and 2567/µL when he felt better 2 days after treatment). However, the eosinophil count did not correlate with urinary levels of LTE4 or 11DTXB2. There was no significant correlation between urinary levels of LTE4 and the severity of asthma; however, the severity of asthma in patients with high levels of urinary LTE4 were classified as ‘moderate persistent’ or ‘severe persistent’.

**DISCUSSION**

Cysteinyl leukotrienes and TXA2 are considered to play important roles in the pathogenesis of bronchial asthma. The relationship between urinary LTE4 and 11DTXB2 in the pathogenesis of asthma has been reported by several investigators;2–5,8,11,13,20 because of the instability of CysLTs and TXA2, the end-products of the cascade were determined. However, most studies have been

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**Fig. 3** Urinary leukotriene E4 (LTE4) and 11-dehydro-thromboxane B2 (11DTXB2) levels in nine children with bronchial asthma during the stable condition, an acute asthma attack and 2 days after treatment. (a) Urinary LTE4 levels increased from a median of 373 pg/mg creatinine (range 183–556 pg/mg creatinine) during the stable condition to 546 pg/mg creatinine (range 280–1100 pg/mg creatinine) during an acute asthma attack, decreasing again to 443 pg/mg creatinine (range 156–872 pg/mg creatinine) 2 days after treatment. (b) Urinary 11DTXB2 levels were apt to increase from a median of 1009 pg/mg creatinine (range 131–2166 pg/mg creatinine) during the stable condition to 1285 pg/mg creatinine (range 166–3122 pg/mg creatinine) during an acute asthma attack and then decrease slowly to 842 pg/mg creatinine (range 492–2708 pg/mg creatinine) 2 days after treatment. *P < 0.05.

**Fig. 4** Relationship between urinary leukotriene E4 (LTE4) and 11-dehydro-thromboxane B2 (11DTXB2) levels. No relationship was noted between these prostanoids in (a) control subjects, (b) asthmatic children while in the stable condition and (c) asthmatic children during an acute asthma attack.
performed in adults. In the present study, we have demonstrated the relationship between urinary LTE4 and 11DTXB2 in children with bronchial asthma.

In the present study, urinary LTE4 levels in children with bronchial asthma during the stable condition were significantly higher than in control children. In addition, urinary LTE4 levels in children during an acute asthma attack were higher than during the stable condition. Asano et al.5 also demonstrated that patients with mild to moderate asthma excrete LTE4 in the urine at a greater rate than control subjects. Taylor et al.4 revealed that urinary LTE4 was significantly higher in asthma patients after antigen challenge than in control subjects. The results of the present study are consistent with previous findings in adult asthmatic patients.3,4,9,11,17,20

In the present study, urinary 11DTXB2 levels were higher in children with bronchial asthma than in controls. Unlike LTE4, urinary 11DTXB2 levels did not increase markedly during an acute attack.

Oosaki et al.3,20 reported on variations in urinary levels of these mediators in patients with spontaneous asthma attacks who were monitored for 3 days and whose state improved. The study of Oosaki et al.3,20 showed that urinary levels of LTE4 were significantly higher during the attack and returned to control levels once the patient’s state had improved. In the present study, the urinary levels of these prostanoids were measured in asthmatic children during the stable condition, during an acute attacks and 2 days after treatment. In eight children, urinary LTE4 levels increased during an acute attack and decreased 2 days after treatment. One patient (no. 8) exhibited a different pattern of urinary LTE4 excretion: levels decreased during an acute attack and then increased when she felt better 2 days after treatment. However, the urinary 11DTXB2 levels in this patient increased during an acute attack and then decreased 2 days after treatment. This patient had atopic-type bronchial asthma and was treated with theophylline, steroid inhalant, DSCG and a β2-adrenergic receptor agonist. Before she was enrolled in the study, she had been treated with an LTRA for 5 weeks. However, LTRA treatment had little effect on her asthma. Urinary 11DTXB2 levels tended to increase during an asthma attack and persisted 2 days after treatment. Similar to the findings of the present study, Oosaki et al. have shown that the median level of urinary 11DTXB2 was highest during the 3rd hospital day in atopic-type patients and during the 2nd hospital day in non-atopic-type patients.3

In the present study, urinary levels of LTE4 and 11DTXB2 were slightly higher than those reported previously.2–5,8,11,13,20 Osamura et al. had reported that urinary levels of 11DTXB2 were significantly high between 1 and 3 years after birth and that they tended to decrease gradually with age thereafter.21 Because all our subjects were children (1–15 years of age), this may explain why the urinary levels of 11DTXB2 were slightly higher in the present study than those reported previously.

Suzuki et al.2 reported that no significant relationship was observed between urinary LTE4 and 11DTXB2 in asthmatic patients. Oosaki et al.3 also examined the relationship in changes (%) between these two metabolites; however, they noted no significant difference. In the present study, consistent with results of previous studies, no relationship was observed between urinary LTE4 and 11DTXB2 in children with bronchial asthma. In addition, changes (%) in LTE4 levels were not associated with 11DTXB2 levels in children with bronchial asthma. This suggests that increases in the levels of these two metabolites are not correlated with one another.

Neither gender, age, serum IgE nor eosinophil count revealed any relationship with urinary levels of LTE4 or 11DTXB2. Eosinophils play an important role in the
pathogenesis of bronchial asthma and the eosinophil count is correlated with the clinical severity of the disease. However, there are few studies referring to the correlation between eosinophil count and urinary levels of LTE4 or 11DHTXB2. There was no significant correlation between urinary levels of LTE4 and the severity of asthma; however, the severity of the asthma in patients with high levels of urinary LTE4 tended to be classified as ‘moderate persistent’ or ‘severe persistent’.

In conclusion, we have shown significantly higher levels of urinary LTE4 and 11DHTXB2 in asthmatic children during the stable condition. These findings strongly suggest that the arachidonate cascade metabolites CysLTs and thromboxanes play certain roles in the pathogenesis of bronchial asthma in children. According to the differential changes in urinary levels of these metabolites during an acute attack, we suppose that an imbalance in the metabolism arises between the 5-lipoxygenase pathway and the cyclooxygenase pathway. The measurement of LTE4 and 11DHTXB2 in urine samples, which is a safe and easily available method of estimating the synthesis and release of the mediator in children, would be useful in understanding the pathogenesis of bronchial asthma.

REFERENCES


