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Original Article

Urinary leukotriene E_4 and 11-dehydro-thromboxane B_2 excretion in children with bronchial asthma

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Abstract

Background: Cysteinyl leukotrienes (CysLTs) and thromboxane (TX) A_2 have been implicated in the pathogenesis of bronchial asthma. Urinary leukotriene E_4 (LTE_4) and 11-dehydro-TXB_2 ($11DTXB_2$) levels are often used to assess the production of CysLTs and TXA_2. However, few studies have examined the products of these two mediators in the same asthmatic patients. To define the potential roles of CysLTs and TXA_2 in the pathogenesis of bronchial asthma in children, their urinary levels were measured in the present study.

Methods: Urinary LTE_4 and $11DTXB_2$ 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 LTE_4 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 103–389 pg/mg creatinine). Urinary $11DTXB_2$ 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 (median 252 pg/mg creatinine; range 41–716 pg/mg creatinine). Comparing different stages of asthma, LTE_4 levels during an acute attack were significantly higher ($P < 0.05$) than during the stable condition; however, there was no difference in urinary TXB_2 levels.

Conclusions: The present findings suggest that high levels of CysLTs and TXA_2 are associated with the pathogenesis of bronchial asthma. The measurement of urinary LTE_4 and $11DTXB_2$ would be useful in understanding the individual pathogenesis of asthmatic children.

Key words: bronchial asthma, cysteinyl leukotrienes, 11-dehydro-thromboxane B_2, leukotriene E_4, thromboxane A_2.

Introduction

Cysteinyl leukotrienes (CysLTs), namely leukotrienes C_4, D_4 and E_4, and thromboxane (TX) A_2 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.[1–5,11,12] A potent bronchoconstrictor, TXA_2 is generated from arachidonic acid by cyclooxygenase. Enhanced TXA_2 release has also been reported in asthmatic patients after allergen challenge.[7] Owing to the significant roles of CysLTs and TXA_2, their inhibitors or receptor antagonists have been developed extensively and recently some drugs have become available.[13]

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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 TXA_2 products of asthmatic patients with those of healthy control subjects. Leukotriene E_4 is a stable product of CysLTs and is considered an index of the systemic production of CysLTs production in humans.[14] 11-Dehydro-thromboxane B_2 ($11DTXB_2$) is the most abundant degradation product of TXB_2 and is also considered an index of systemic TXA_2 production.[15–17] The measurement of urinary LTE_4 and $11DTXB_2$ is a non-invasive method for assessing the production of CysLTs and TXA_2 *in vivo*, respectively. Oosaki *et al*.[18] previously established sensitive and selective methods of determination of urinary LTE_4 by enzyme immunoassay (EIA) and Ruitta *et al*.[16] established the method of determining urinary $11DTXB_2$ by radioimmunoassay (RIA). In the present study, using these methods, the products of CysLTs and TXA_2 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 using inhaled disodium cromoglycate (DSCG) and β_2-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 Glolabal 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 LTE_4 or $11DTXB_2$ 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 β_2-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 β_2-adrenergic receptor agonists and/or corticosteroid drip infusion. Urine samples from nine asthmatic children were collected 2 days after treatment.

Measurement of LTE_4

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. [^{3}H]-LTE_4 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; LTE_4 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 [^{3}H]-LTE_4 radioactivity and also corresponded to the

Table 1 Characteristics of patients and urinary levels of leukotriene E_4 and 11-dehydro-thromboxane B_2

Patient no.	Gender	Age (years)	Serum IgE (U/mL)	HD score	Mite score	Severity (GINA)	Steroid treatment during attack	Theophylline	LTRA	DSCG	β_2-Adrenergic receptor agonist	Steroid inhalant	Stable condition LTE_4	Stable condition $11DTXB_2$	Acute attack LTE_4	Acute attack $11DTXB_2$	After treatment LTE_4	After treatment $11DTXB_2$
1	M	7	1784	5	6	Moderate	+	+	–	+	+	–	183	861	671	1285	156	2130
2	M	5	200	4	5	Mild	+	+	–	+	+	–	212	2166	611	1188	295	820
3	M	14	900	6	6	Moderate	+	–	–	+	+	+	482	745	1100	563	443	785
4	M	1	21	0	0	Severe	+	+	+	+	+	–	373	282	280	3122	524	2708
5	M	9	70	0	0	Moderate	+	+	+	+	+	–	480	1059	546	2096	771	2137
6	F	10	199	2	2	Moderate	+	+	+	+	+	–	253	1515	437	2247	231	842
7	F	5	82	6	6	Mild	+	+	–	+	+	–	188	2150	409	589	340	492
8	F	9	294	4	4	Severe	+	+	–	+	+	+	484	131	797	166	473	533
9	F	7	1281	4	4	Moderate	+	+	+	+	+	–	556	1009	483	2087	872	1515
10	M	10	653	2	3	Moderate	+	+	+	+	+	+	378	904	615	1847		
11	M	4	208	4	5	Moderate	+	+	+	+	+	–	341	1842	468	3254		
12	M	5	2892	4	4	Moderate	+	+	+	+	+	+	274	163	553	2461		
13	M	3	401	5	5	Moderate	+	+	+	+	+	–	519	2462	567	2352		
14	M	9	265	5	5	Mild	+	+	+	+	+	–	254	1476	369	2499		
15	M	3	180	5	6	Moderate	+	+	+	+	+	+	332	1392	424	480		
16	M	3	1381	3	4	Moderate	+	+	–	+	+	+	606	167	191	379		
17	M	9	890	2	1	Mild	+	+	+	+	+	–	165	873	269	1259		
18	M	9	703	6	5	Severe	+	+	+	+	+	+	278	804	334	939		
19	M	2	1417	6	6	Moderate	+	+	+	+	+	–	755	3535	743	4075		
20	M	9	578	5	6	Mild	+	+	+	+	+	–	286	793	284	1700		
21	M	8	62	2	0	Intermittent	+	–	–	+	+	–	365	1992	371	258		
22	M	4	334	3	3	Moderate	+	+	+	+	+	+	328	3483	394	2548		
23	M	2	394	5	6	Mild	+	+	+	+	+	–	610	6070	980	5104		
24	F	15	301	0	0	Moderate	+	+	–	+	+	+	284	46	1100	311		
25	F	5	866	6	6	Intermittent	–	–	–	+	+	–	727	196	245	110		
26	F	7	1300	5	6	Mild	+	+	–	+	+	–	259	1837	476	2413		
27	F	7	1000	2	1	Mild	–	+	–	+	+	–	128	903	289	1318		
28	F	13	597	5	5	Mild	–	+	–	+	+	–	332	1026	504	1666		
29	F	3	600	6	6	Moderate	+	+	+	+	+	+	965	261	528	429		
Median			577.5										332	1009	476	1666	443	842
Average		7	684.5	4	4								393.0	1384.2	518.6	1680.9	456.1	1329.2

GINA, Global Initiative for Asthma (GINA) guideline (http://www.ginasthma.com/).

HD, house dust; LTRA, leukotriene receptor antagonist; DSCG, disodium cromoglycate; LTE_4, leukotriene E_4; $11DTBX_2$, 11-dehydro-thromboxane B_2.

retention time of authentic LTE_4 were dried and resuspended in assay buffer, which was supplied in the Leukotriene C4/D4/E4 enzyme-immunoassay system (Amersham, Buckinghamshire, UK). Urinary LTE_4 concentrations determined by EIA were corrected for recovery of [^{3}H]-LTE_4. The urinary LTE_4 level was expressed as pg/mg creatinine.

Measurement of 11DTXB$_2$

The 11DTXB$_2$ 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 11DTXB$_2$ was eluted by ethyl acetate. The pooled ethyl acetate was evaporated to dryness with nitrogen gas. The dried residue, containing 11DTXB$_2$, 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 11DTXB$_2$, was dried with nitrogen gas and the amount of 11DTXB$_2$ was quantitated by RIA (11-Dehydrothromboxane B2 [^{125}I] RIA kit; Perkin Elmer Life and Analytical Sciences, Boston, MA, USA). The urinary 11DTXB$_2$ 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 LTE_4 and 11DTXB$_2$ levels

Urinary levels of LTE_4 and 11DTXB$_2$ were measured to define the potential roles of CysLTs and TXA_2 in children with bronchial asthma.

Leukotriene E_4 was measured by EIA. Urinary LTE_4 levels are plotted in Fig. 1. Urinary LTE_4 levels in asthmatic children during the stable condition (332 (128–965) pg/mg creatinine) was significantly higher ($P < 0.05$) than that of control subjects (233 (103–389) pg/mg creatinine; Fig. 1a). Comparing the different conditions of asthma, LTE_4 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 $B_2$was measured by RIA and was detectable in all urine samples. Urinary 11DTXB$_2$ levels are shown in Fig. 2. Urinary 11DTXB$_2$ 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 11DTXB$_2$ levels during an acute attack (1666 (110–5105) pg/mg creatinine) and during the stable condition (Fig. 2b).

Urinary levels of LTE_4 and 11DTXB$_2$ were observed during the stable condition, an acute attack and 2 days after treatment in nine asthmatic children (Fig. 3). Urinary LTE_4 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 11DTXB$_2$ levels exhibited different patterns after an attack. Urinary 11DTXB$_2$ levels increased from 1009 pg/mg creatinine (range 131–2106 pg/mg creatinine) during the stable condition to 1285 pg/mg creatinine (range 166–3122 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 11DTXB$_2$ 2 days after treatment (Fig. 3b).

Correlations between urinary LTE_4 and 11DTXB$_2$

We assessed the relationship between LTE_4 and 11DTXB$_2$ 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

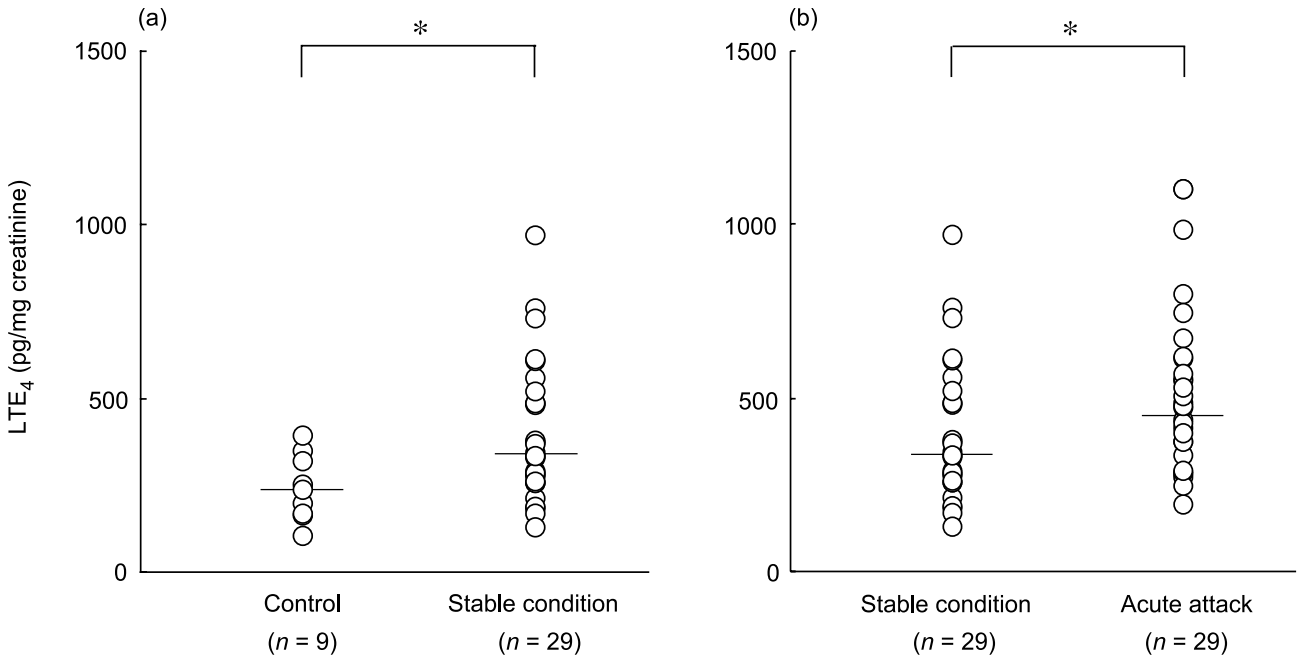

Fig. 1 (a) Urinary leukotriene E_4 (LTE_4) levels in asthmatic children during the stable condition (median 332 pg/mg creatinine; range 128–965 pg/mg creatinine) and in controls (median 233 pg/mg creatinine; range 103–389 pg/mg creatinine). (b) Urinary LTE_4 levels in asthmatic children during an acute 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. *$P < 0.05$.

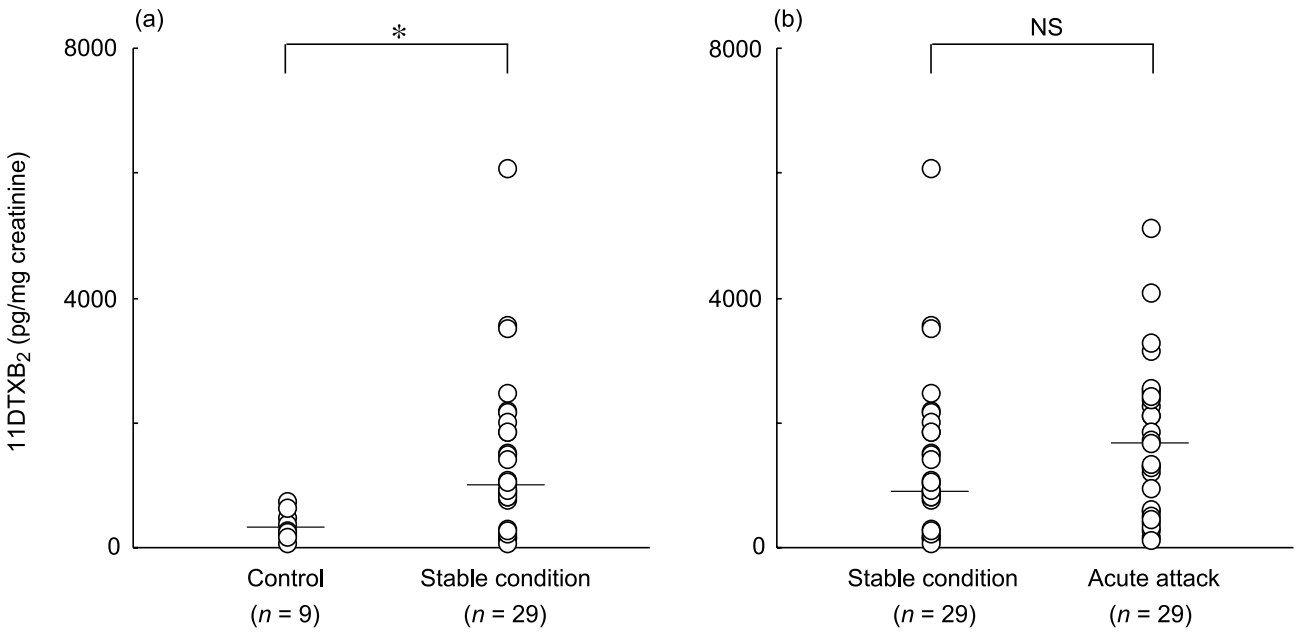

Fig. 2 (a) Urinary 11-dehydro-thromboxane B_2 ($11DTXB_2$) levels in asthmatic children during the stable condition (median 1009 pg/mg creatinine; range 46–6070 pg/mg creatinine) and in controls (median 252 pg/mg creatinine; range 41–716 pg/mg creatinine). (b) Urinary $11DTXB_2$ levels in asthmatic children 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. *$P < 0.05$.

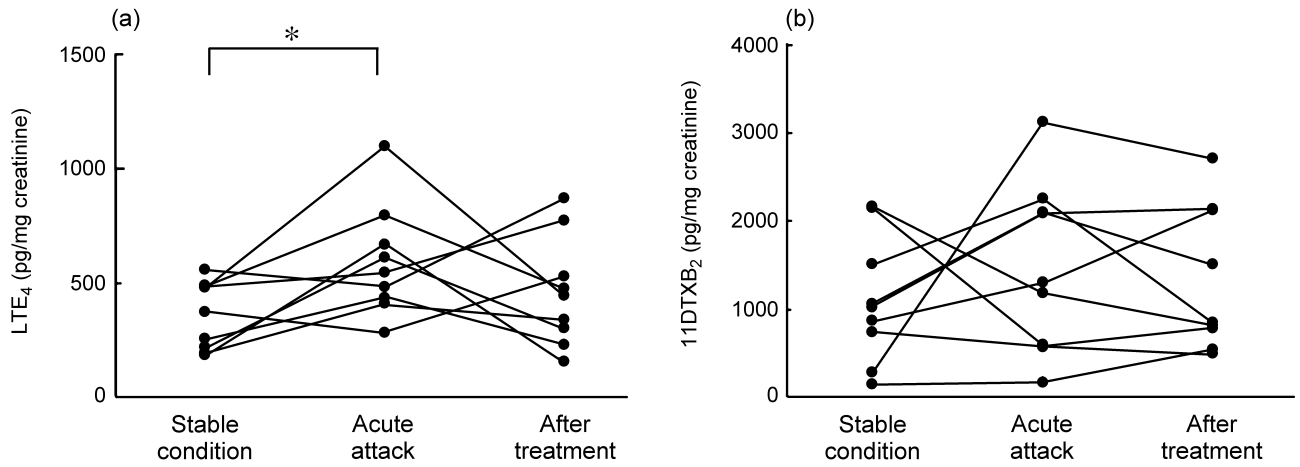

Fig. 3 Urinary leukotriene E_4 (LTE_4) and 11-dehydro-thromboxane B_2 ($11DTXB_2$) levels in nine children with bronchial asthma during the stable condition, an acute asthma attack and 2 days after treatment. (a) Urinary LTE_4 levels increased from a median of 373 pg/mg creatinine (range 183–556 pg/mg 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 $11DTXB_2$ 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. $^*P < 0.05$.

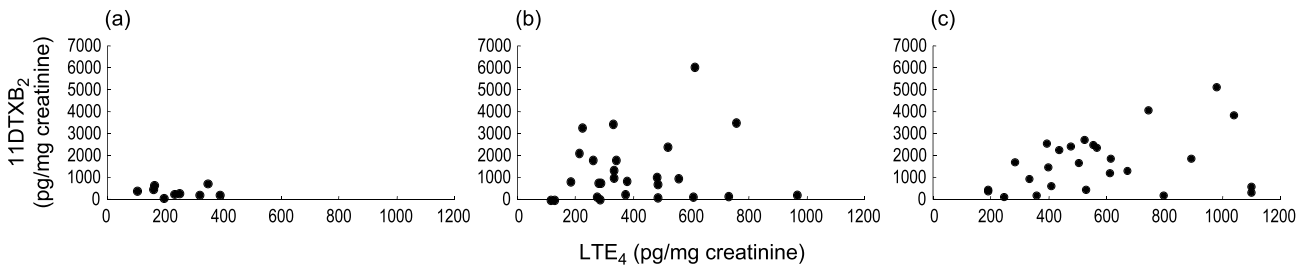

Fig. 4 Relationship between urinary leukotriene E_4 (LTE_4) and 11-dehydro-thromboxane B_2 ($11DTXB_2$) levels. No relationship was noted between these prostanoids in (a) control subjects, (b) asthmatic children while in the stable condition and (c) asthmatic children during an acute asthma attack.

levels during the stable condition, the changes in LTE_4 were not related to changes in $11DTXB_2$ in children with bronchial asthma (Fig. 5). Neither gender, age, serum IgE nor eosinophil count had any relationship with urinary levels of LTE_4 or $11DTXB_2$ (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 LTE_4 or $11DTXB_2$. There was no significant correlation between urinary levels of LTE_4 and the severity of asthma; however, the severity of asthma in patients with high levels of urinary LTE_4 were classified as 'moderate persistent' or 'severe persistent'.

Discussion

Cysteinyl leukotrienes and TXA_2 are considered to play important roles in the pathogenesis of bronchial asthma. The relationship between urinary LTE_4 and $11DTXB_2$ in the pathogenesis of asthma has been reported by several investigators;[2–5,8,11,13,20] because of the instability of CysLTs and TXA_2, the end-products of the cascade were determined. However, most studies have been

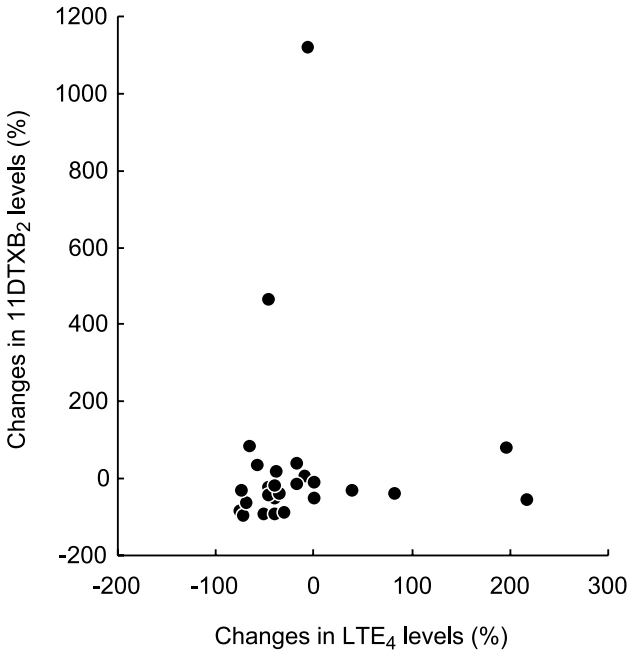

Fig. 5 Changes (%) in leukotriene E_4 (LTE_4) levels did not correlate with changes (%) in 11-dehydro-thromboxane B_2 ($11DTXB_2$) levels in children with bronchial asthma. The percentage change was calculated as follows: (level during stable condition – level during attack) × 100/level during attack.

performed in adults. In the present study, we have demonstrated the relationship between urinary LTE_4 and $11DTXB_2$ in children with bronchial asthma.

In the present study, urinary LTE_4 levels in children with bronchial asthma during the stable condition were significantly higher than in control children. In addition, urinary LTE_4 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 LTE_4 in the urine at a greater rate than control subjects. Taylor *et al*.[4] revealed that urinary LTE_4 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 $11DTXB_2$ levels were higher in children with bronchial asthma than in controls. Unlike LTE_4, urinary $11DTXB_2$ 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 LTE_4 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 LTE_4 levels increased during an acute attack and decreased 2 days after treatment. One patient (no. 8) exhibited a different pattern of urinary LTE_4 excretion: levels decreased during an acute attack and then increased when she felt better 2 days after treatment. However, the urinary $11DTXB_2$ 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 theophyline, steroid inhalant, DSCG and a β_2-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 $11DTXB_2$ 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 $11DTXB_2$ 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 LTE_4 and $11DTXB_2$ were slightly higher than those reported previously.[2–5,8,11,13,20] Osamura *et al*. had reported that urinary levels of $11DTXB_2$ 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 $11DTXB_2$ were slightly higher in the present study than those reported previously.

Suzuki *et al*.[2] reported that no significant relationship was observed between urinary LTE_4 and $11DTXB_2$ 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 LTE_4 and $11DTXB_2$ in children with bronchial asthma. In addition, changes (%) in LTE_4 levels were not associated with $11DTXB_2$ 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 LTE_4 or $11DTXB_2$. Eosinophils play an important role in the

pathogenesis of bronchial asthma and the eosinophil count is correlated with the clinical severity of the disease.[22] However, there are few studies referring to the correlation between eosinophil count and urinary levels of LTE_4 or $11DTXB_2$. There was no significant correlation between urinary levels of LTE_4 and the severity of asthma; however, the severity of the asthma in patients with high levels of urinary LTE_4 tended to be classified as 'moderate persistent' or 'severe persistent'.

In conclusion, we have shown significantly higher levels of urinary LTE_4 and $11DTXB_2$ 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-lipoxgenase pathway and the cyclooxygenase pathway. The measurement of LTE_4 and $11DTXB_2$ 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.

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