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Plasma Levels of Leukotriene E_4 and 9α, 11 β-PGF_2 in Asthmatic Young Children during Exacerbation and Convalescence

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Background: Asthma is increasingly recognized as a mediator-driven inflammatory process in the lungs. Leukotrienes (LTs) and prostaglandins (PGs), which are two families of proinflammatory mediators generated via the arachidonic acid metabolism, have been shown to play an important role in the inflammatory cascade of the airways. The aim of this study was to examine whether plasma leukotriene E_4 (LTE_4) and 9α, 11 β-PGF_2 were biomarkers of asthma exacerbations in 2- to 5-year-old children. **Methods:** Sixty 2- to 5-year-old children who were hospitalized for acute asthma were assigned to receive budesonide (Pulmicort) (n = 32) or oral prednisolone (n = 28). The budesonide group received nebulized fenoterol (Berotec) together with the nebulized budesonide, and the prednisolone group received nebulized fenoterol and oral prednisolone. Pulmonary index scores (PIS), including respiratory rate, wheezing, inspiratory/expiratory ratios, and the use of accessory muscles during respiration, were measured as indices of clinical progression. Blood samples were collected at admission and discharge and plasma concentrations of 9α, 11 β-PGF_2 and LTE_4 were determined. **Results:** Fifty-two children completed the study; both budesonide (n = 28) and prednisolone (n = 24) treatment significantly improved acute asthma symptoms and PIS; however, there was no significant difference in plasma levels of LTE_4 and PGF_2 between asthma exacerbation and convalescence in both groups. **Conclusion:** Plasma levels of LTE_4 and 9α, 11β-PGF_2 were not good indicators of exacerbation in asthmatic children.

Key words: asthma, child, LTE_4, 9α, 11 β-PGF_2, PIS

INTRODUCTION

Chronic inflammation associated with asthma is characterized by infiltration and activation of eosinophils in the lungs[1]. Cysteinyl leukotrienes (cysLTs), which are major eicosanoid products of activated eosinophils, mast cells, basophils, and monocytes[2], play an important role in asthma via the 5-lipoxygenase pathway. The plasma leukotriene C_4 (LTC_4) is the dominant arachidonic acid metabolite released in lung tissue. This molecule is very unstable and rapidly converts to LTD_4, and finally to a less potent metabolite, LTE_4. The latter is the most stable of the three metabolites and is excreted in the urine[3]. It has been shown that approximately 7% of an inhaled dose of LTC_4 appears in the urine as LTE_4[4], which suggests that measurement of urinary LTE_4 provides a useful indication of the levels of cysLT production in the lungs[5].

Activation of mast cells is thought to be an important feature of the allergic asthmatic response[6]. Prostaglandin D_2 (PGD_2) is the major cyclooxygenase product released by activated mast cells; it is unstable and is metabolized in the human lung by NADPH-dependent 11-ketoreductase to 9α, 11 β-PGF_2 and is rapidly excreted in the urine. Although alveolar macrophages are also reported to have the capacity to synthesize PGD_2[7], mast cells are the major source of this molecule, which suggests that its urinary metabolite, 9α, 11 β-PGF_2 may be a useful marker of mast cell activation in the lungs[8]. For these reasons, the blood levels of LTE4 and PGF_2 reflect mast cell activity in the human lungs as well as the status of airflow limitation.

It has been shown that urinary LTE_4 ($uLTE_4$) and urinary PGF_2 ($uPGF_2$) are significantly elevated during acute attacks of atopic asthma, and that their levels fall in

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the convalescent phase[9]. Previous studies have shown that inhaled corticosteroids have a greater impact on $uPGF_2$ than on $uLTE_4$[10]. The current diagnosis of asthma in young children 2-5 years of age relies on clinical symptoms and signs and on the therapeutic response to β-2 agonists. As there is no reliable pulmonary test available for asthmatic children younger than 5 years of age, measurement of the blood levels of LTE_4 and PGF_2 may serve as a useful marker of the degree of airway obstruction in these children and can be helpful in guiding their management.

The aim of this study was to investigate changes in plasma levels of LTE4 and 9α, 11 β-PGF_2 during acute exacerbation and during the convalescent phase in 2- to 5-year-old asthmatic children who were treated by either oral prednisolone or inhaled budesonide. We also analyzed the discrepancies between blood levels of the end metabolites of eicosanoids between the two groups.

METHODS

Patients and Study Design

Patients who had fever at admission were assigned to the budesonide group and those who exhibited a normal temperature at admission were assigned to the prednisolone group. Informed consent was obtained from the parents of all subjects. The children recruited were of preschool age (between 2 and 5 years old). A diagnosis of childhood asthma was made according to the criteria defined by the National Asthma Education and Prevention Program, Expert Panel Report II, Guidelines for the Diagnosis and Management of Asthma. These included recurrent viral-associated wheezing (> 3 episodes during the 12 months preceding the study) that was responsive to β-2 adrenergic agonist treatment and repeated coughing at night and on exertion[11]. Subjects who did not complete blood collection and those who did not respond to β2 adrenergic agonists were excluded.

From June 1, 2007, to March 31, 2008, 60 children who were eligible for the study were assigned into one of two treatment groups. The budesonide group patients received nebulized fenoterol solution (1.25 mg/2 mL; fenoterol hydrobromide, Boehringer Ingelheim) at 0.125 mg/kg every 6 h or as required, to a maximum of 1.25 mg, and nebulized budesonide inhalation suspension (1 mg/2 mL; Pulmicort Respules, AstraZeneca, Sodertalje, Sweden) at 0.05 mg/kg every 12 hours[12]. Patients in the prednisolone group received the same fenoterol regimen, together with oral prednisolone administration at a dose of 1 mg/kg per day.

Demographic data, including age, gender, FAS (Family Allergy Scores), serum IgE, total eosinophil count, serum SGOT, SGPT, BUN, and Phadiatop CAP, were obtained at admission. Blood samples were drawn from each patient at admission and discharge and serum total IgE, allergen-specific IgE, total eosinophil counts, and blood biochemistry were determined. The allergen-specific IgE was measured using a UniCAP-100 autoanalyzer (Phadiatop, Pharmacia, Sweden) and the values obtained were divided into six analytical classes: class 0, < 0.35 kU/L; class 1, 0.35-0.70 kU/L; class 2, 0.70-3.50 kU/L; class 3, 3.50-17.5 kU/L; class 4, 17.5-50.0 kU/L; class 5, 50.0-100.0 kU/L; and class 6, > 100 kU/L. The PIS values were calculated based on respiratory rate, wheezing, inspiration/expiration ratio, and the need to use accessory muscles[13]. In the scoring system, a score of 0 to 3 was assigned to each variable. The final scores ranged from 0 to 12, with higher scores indicating higher severity (Table 1). The plasma concentration levels of LTE_4 and 9α, 11 β-PGF_2 were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (Cayman Chemical system). Assays were performed using the protocols recommended by the manufacturer.

Table 1 Pulmonary Index Score

Scores	Respiratory Rate	Wheezing sound*	I:E Ration	Accessory Muscle Usage**
0	< 30 bpm	None	5/2	0
1	31-45	Terminal Expiration with stethoscope	5/3-5/4	±
2	46-60	Entire Expiration with stethoscope	1/1	++
3	> 60 bpm	Inspiration and Expiration without stethoscope	< 1/1	+++

* If no wheezing due to minimal air exchange, score3.
**: Accessory muscle use was scored by assessment of sternocleidomastoid activity;
0: no apparent activity; ± sign, questionable increase.;
++signs, increase apparent.;
+++: signs, maximal activity.
This table was from Becker et al's original paper[13].

Statistical Analyses

Demographic data, including age, gender, FAS, serum IgE, total eosinophil count, SGOT, SGPT, BUN, Phadiatop CAP, and urine analysis, were analyzed using the χ^2 test and one-way analysis of variance. Changes in plasma levels of LTE_4 and 9α, 11 β-PGF_2 between the two groups before and after treatment were analyzed using the Wilcoxon signed rank test. Differences between the budesonide and prednisolone groups were analyzed using the Mann—Whitney test. A two-tailed P value < 0.05 was considered significant.

Table 2 Demographic Data on admission

	Pulmicort group	Prednisolone group	P value
Age (yrs)	3.59±1.10	3.78±0.93	0.45
Gender (M/F)	1.53±0.51	1.48±0.51	0.85
Phadiotop CAP kU/L	1.13±1.52	2.07±2.27	0.68
Total eosinophil count, ul	129.81±185.37	307.03±305.56	0.38
Serum IgE IU/L	138.74±177.86	231.93±257.95	0.72
SGOT IU/L	28.68±7.70	29.52±6.08 0.68	0.68
SGPT IU/L	16.71±5.54	17.96±4.27	0.84
BUN Mg/dL	8.41±4.08	9.38±3.74 0.026	0.026
Creatinine Mg/dL	0.46±7.752E-02	0.48±8.953E-02	0.85
FAS*	0.84±0.92	1.41±0.93	0.44
CRP** Mg/dL	2.17±2.81	0.84±1.23	0.74

*: FAS; Family Allergy Score
**: CRP; C-Reactive Protein

Table 3 PIS data

	Pulmicort group	Prednisolone group	P value
R.R. (in)	30.75±3.89	33.96±5.95	0.187
R.R. (o)	22.71±3.56	24.59±4.96	0.758
Wheezing (in)	1.18±0.90	1.54±0.78	0.330
Wheezing (o)	0	0	0
I : E ratio (in)	2.29±0.53	2.63±0.49	0.742
I : E ratio (o)	0.86±0.52	0.96±0.46	0.149
Muscle use (in)	1.57±0.50	1.67±0.48	0.582
Muscle use (o)	0.82±0.48	0.67±0.48	0.784
PIS total score (in)	5.79±1.55	6.67±1.34	0.524
PIS total score (o)	1.68±0.90	1.63±0.88	0.105
Hospital-stay	4.54±1.14	4.83±1.37	0.454

(in): on admission
(o): upon discharge

RESULTS

Demographic Data

Sixty patients were initially enrolled, of which 52 completed the study. Six patients were excluded because they did not complete blood collection at discharge. Two other patients were excluded because their clinical condition deteriorated and they were transferred to a medical center for further treatment. There were no significant differences in age, gender, FAS, serum IgE, total eosinophil count, SGOT, SGPT, or Phadiatop CAP between the two study groups ($P > 0.05$) (Table 2).

Changes in Pulmonary Function after Treatment

As shown in Table 3, in the budesonide group there was a significant improvement in total PIS (5.79±1.55 vs 1.68 ±0.90; $P < 0.001$), respiration rate (30.75±3.89 vs 22.71 ± 3.56; $P < 0.001$), wheezing scores (1.18±0.90 vs 0; P value not determined), inspiration/expiration ratio (2.29 ±0.53 vs 0.86±0.52; $P < 0.001$), muscle activity (1.57± 0.50 vs 0.82±0.48; $P < 0.001$). In the prednisolone group, there was also a significant improvement in PIS (6.67± 1.34 vs 1.63±0.88; $P < 0.001$), respiration rate (33.96± 5.95 vs 24.59±4.96; $P < 0.001$), wheezing scores (1.54± 0.78 vs 0; P value not determined), inspiration/expiration ratio (2.63±0.49 vs 0.96±0.46; $P < 0.001$), and muscle activity (1.67±0.48 vs 0.67±0.48; $P < 0.001$).

Comparison of Pulmonary Function between the Two Groups before and after Treatment

As shown in Table 3, there were no significant differences in total PIS between the two treatment groups at admission (6.67±1.34 vs 5.79±1.55; $P > 0.05$) and at discharge (1.63±0.88 vs 1.68±0.90; $P > 0.05$). The respiratory rate was not significantly different between the groups at admission (33.96±5.95 vs 30.75±3.89; $P > 0.05$) or at discharge (24.59±4.96 vs 22.71±0.56; $P > 0.05$). A significant overall improvement in wheezing scores was found after treatment in both groups, but there was no significant difference in these scores between groups at admission (1.54±0.78 vs 1.18±0.90; $P > 0.05$) or at discharge (0 vs 0; $P > 0.05$). The sharp downward slope indicated a remarkable clinical improvement. The inspiration/expiration ratio decreased after initiation of treatment in both groups, but there were no significant differences between groups at admission (2.63± 0.49 vs 2.29±0.53; $P > 0.05$) or at discharge (0.96±0.46 vs 0.86 ± 0.52; $P > 0.05$). An apparent overall improvement in sternocleidomastoid muscle activity was found in both groups of patients, with similar decreases in activity observed in both groups; activity scores were 1.67±0.48 vs 1.57± 0.50 ($P > 0.05$) at admission and 1.63± 0.88 vs 1.68 ±0.90 ($P > 0.05$) at discharge.

Changes in Plasma Concentration of LTE_4 and 9α, 11 β-PGF_2 after Treatment

The plasma concentration of LTE_4 was not significantly reduced by treatment with budesonide (236.91 ± 151.59 before vs 305.80±208.39 after treatment, $P > 0.05$) (Fig. 1A) nor with prednisolone (116.46±65.18 before vs 112.18 ±62.51 after treatment, $P > 0.05$). Similarly, plasma 9α, 11 β-PGF_2 levels did not change significantly after treatment with either budesonide (13.64±4.68 before vs 17.63 ±17.81 after treatment, $P > 0.05$) or prednisolone (6.95± 3.08 before vs 6.72.87±1.90 after treatment, $P > 0.05$) (Fig. 1B).

DISCUSSION

Asthma is the most common chronic childhood disease

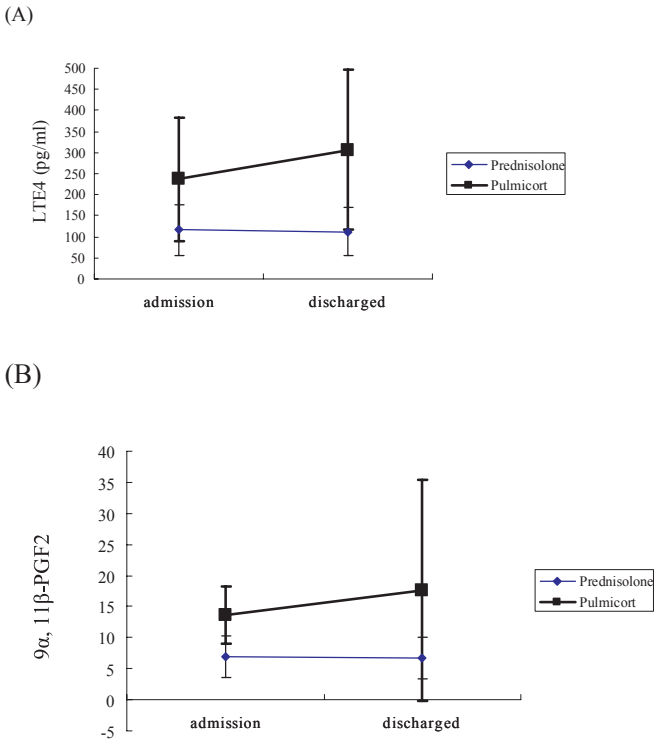

Fig. 1 Changes of plasma levels of LTE_4 (A) and 9α, 11 β-PGF_2 (B) after treatment.

in America. As many as 80% of asthmatic children exhibit clinical symptoms of asthma by 5 years of age[14]. Recent longitudinal studies show that many asthmatics experience symptoms during the first year of life[15]. The severity of asthma changes little with time; children with severe asthma in early childhood will have severe asthma up to 35 years of age[16]. Moreover, children who had wheezy lower respiratory tract illness during the first 3 years of life and whose wheezing episodes persisted up to the age of 6 have significantly lower levels of pulmonary function at age 6 when compared with children whose wheezing symptoms started after 3 years of age[17]; therefore, early clinical symptoms of asthma are likely to lead to significant lung function impairment in later life[18]. Asthma is a clinical syndrome that can be controlled but cannot be cured. Early diagnosis and early medical intervention may provide a better outcome for these patients.

CysLTs and PGD_2 are major eicosanoid products of activated eosinophils, mast cells, basophils, and monocytes[19] and are characteristic of asthma[20]. Activation of mast cells is thought to be an important feature of the allergic asthmatic response[21]. Measurement of urinary LTE_4 and 9α, 11β-PGF_2 is potentially useful for the assessment of chronic inflammation associated with eosinophil and mast cell activation in the airways of asthmatic patients[22]. This study showed that plasma LTE_4 and 9α, 11 β-PGF_2 levels were not different between the two groups during acute asthma exacerbation or convalescence. One possible explanation for these findings is that short-term administration of oral or inhaled corticosteroids to normal subjects or asthmatic patients did not alter urinary LTE_4 excretion[23]. A different study showed that increased urinary LTE4 was noted only in subjects with moderate to severe asthma[24]. The patients in our study had mild to moderate severity (PIS scores below 7) and, therefore, the levels of plasma LTE_4 and 9α, 11 β-PGF_2 were not significantly different between the exacerbation and convalescence stages of asthma. Moreover, there were no significant differences in the levels of these molecules between the budesonide and prednisolone groups.

Inhaled corticosteroids can be administered to young children using a nebulizer device and may have a lower risk for the development of side effects than systemic corticosteroids. For young children, who are growing and developing, the side effects of medications should be taken into serious consideration; however, jet nebulizers deliver approximately only 25% of the labeled budesonide dose to the patient; 25% of the remaining dose is delivered to the ambient air and 50% of the dose stays in the nebulizer[25]. In a study performed in 3-6-year-old children with asthma, the total systemic availability (pulmonary plus oral) of a budesonide inhalation suspension delivered by a jet nebulizer was found to be approximately 6% of the labeled dose[26]; therefore, the side effects of the nebulized corticosteroid may be lower when compared with systemic steroids. The therapeutic effects were not significantly different between the budesonide and prednisolone groups in the present study. Taking safety and effectiveness into consideration, nebulized corticosteroid treatment may be the best choice for asthmatic children.

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