Original Article

Urinary excretion of 9α,11β-prostaglandin F_2 and leukotriene E_4 in patients with exercise-induced bronchoconstriction

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Contributions: (I) TR Shin contributed to enroll of study subjects and wrote the manuscript; (II) JH Kim contributed to enroll of study subjects and carried out experiments; (III) CH Kim contributed to enroll of study subjects and analyzed the data; (IV) IG Hyun contributed to enroll of study subjects and analyzed the data; (V) JH Choi designed the overall study, contributed to enroll of the study subjects, analyzed the data and supervised this study.

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Background: Increased levels of mast cell-derived eicosanoids, such as prostaglandin (PG) D_2 and cysteinyl leukotrienes (CysLTs), have been reported in patients with exercise-induced bronchoconstriction (EIB), suggesting that mast cell activation is involved in the mechanism of EIB. However, it is still controversial since these results have not been reproduced in other studies. The aim of this study was to evaluate the role of PGD_2 and LTE_4 in adult asthma with EIB, as measuring urinary levels of their metabolites—9α,11β-PGF_2 and LTE_4 before and after an exercise challenge test.

Methods: Eight patients with asthma and EIB and five normal controls without EIB were enrolled. Exercise challenge tests comprised of 6 min of treadmill exercise or free running were performed in all study subjects, and urine samples before and 1 h after the challenge were collected. Urinary levels of 9α,11β-PGF_2 and LTE_4 were measured by enzyme immunoassay (EIA).

Results: No significant differences were observed in 9α,11β-PGF_2 and LTE_4 levels before/after the exercise challenge between patients with EIB and normal controls. No significant increases in urinary levels of 9α,11β-PGF_2 or LTE_4 were detected during the exercise challenge in patients with EIB and normal controls. No significant correlations were observed between the percent decrease in forced expiratory volume in 1 s (FEV_1) or percent changes in 9α,11β-PGF_2 and LTE_4 levels after the exercise challenge.

Conclusions: Urinary 9α,11β-PGF_2 and LTE_4 levels did not increase after an exercise challenge in patients with EIB, suggesting that urinary excretion of 9α,11β-PGF_2 and LTE_4 may not be a good marker of mast cell activation in patients with EIB.

Keywords: Asthma; exercise-induced; 9α,11β-prostaglandin (PG) F_2; leukotriene (LT) E_4

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Introduction

Exercise-induced bronchoconstriction (EIB) occurs predominantly among patients with asthma. Up to 90% of patients with asthma are thought to experience EIB (1). EIB occurs in some individuals without other features of asthma, with a prevalence of up to 20% (2).

Although the exact mechanism of EIB is not fully understood, hyperosmolarity and cooling of the airway surfaces resulting from hyperpnea is thought to trigger contraction of airway smooth muscle by releasing inflammatory mediators from activated inflammatory cells (3). Several studies have indicated that inflammatory mediators, including histamine, tryptase, leukotrienes (LTs), and prostaglandin (PG) D_2 are released into the airway from activated inflammatory airway cells, including eosinophils

and mast cells (4-7).

Activation of mast cells has been frequently implicated in the pathogenesis of EIB, as mast cell mediators, such as histamine, tryptase, PGD_2, cysteinyl leukotrienes (CysLTs), increase in induced sputum, exhaled breath condensate (EBC), and urine samples of patients with EIB after an exercise challenge test (4,5,8-14). However, the results are inconsistent with other studies, as no increases in histamine, tryptase, PGD_2, or CysLTs were detected in bronchial alveolar lavage fluid (BALF) or urine samples (15-18). The preventive effect of disodium cromoglycate or leukotriene receptor antagonists (LTRA) in patients with EIB further supports the importance of these mediators in EIB (4,10,11,19,20).

Eicosanoids, such as PGD_2 and CysLTs, act as potent bronchoconstrictors and are important proinflammatory mediators in asthma pathogenesis (21). PGD_2 is the major eicosanoid released from activated mast cells and is an established marker of mast cell activation (22). LTE_4 is an end metabolite of CysLTs mainly released from mast cells and eosinophils (23). Measuring their metabolites in urine, such as 9α,11β-PGF_2 and LTE_4, is validated as a marker of allergic inflammation in patients with asthma (22,23), and collecting urine is a simple, convenient and noninvasive sampling technique (23). A few studies have determined urinary excretion of both 9α,11β-PGF_2 and LTE_4 together in patients with EIB. One UK study group demonstrated increased urinary 9α,11β-PGF_2 and LTE_4 in elite athletes with EIB (12), whereas a Swedish group did not find an increase in urinary LTE_4 levels after exercise in adult patients with asthma, but found an increase in urinary 9α,11β-PGF_2 (9).

The aim of this study was to investigate the role of mast cell activation in patients with EIB by measuring urinary excretion of 9α,11β-PGF_2 and LTE_4 before and after an exercise challenge test in adult patients with asthma and EIB and in normal controls.

Materials and methods

Study subjects

Eight patients with a history of EIB and five normal controls were enrolled retrospectively. Each subject performed an exercise challenge test. Eight of the study subjects were positive responders, and five were non-responders. All patients with EIB had mild asthma. A diagnosis of asthma was established based on either a positive response to methacholine challenge test (PC_{20} <25 mg/mL) or a positive response to the mannitol bronchoprovocation test (PD_{15} <635 mg). One patient with EIB had physician-diagnosed asthma for 3 years. He refused to perform a test for asthma diagnosis. Subjects were excluded if baseline forced expiratory volume in 1 s (FEV_1) was ≤70% of the predicted value, if the patient was treated for asthma exacerbation within the previous month, or if the subject had parenchymal lung disease on chest radiography.

Seven subjects had atopy, as diagnosed by at least one positive reaction to allergy skin prick tests or multiple simultaneous allergen tests (AdvanSure AlloScreen®, LG, Seoul, Korea) which included common inhalant allergens, such as *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, tree pollen, grass pollen, ragweed, mugwort, *Alternaria*, and animal dander. This study was approved by the local Institutional Review Board, and written informed consent was obtained from all participants.

Spirometry, methacholine and mannitol bronchial provocation

Spirometry and methacholine challenges were conducted in accordance with the guidelines of the American Thoracic Society (24). Spirometry was performed with a Vmax™ (Sensormedics, Milan, Italy). Mannitol bronchial provocation was conducted according to the manufacturer's instructions (Aridol®, Pharmaxis Ltd. Frenchs Forest, NSW, Australia). Inhaled corticosteroids, leukotriene modifiers, or any bronchodilators (short- or long-acting inhaled β_2 agonists, methylxanthines, etc.) were stopped at least 1 week before the spirometry and bronchial provocation tests.

Exercise challenge

Exercise challenges were performed at least 1 week apart from the methacholine or mannitol bronchial provocation tests. Inhaled corticosteroids, leukotriene modifiers, or any bronchodilators (short- or long-acting inhaled β_2 agonists, methylxanthines, etc.) were stopped at least 1 week before the exercise challenge. The protocol mostly followed the standardized guidelines of the American Thoracic Society (24). Exercise challenges were performed by either free-running or treadmill exercise for 6 min at ≥85% of maximum heart rate. Each subject wore a nose clip during the exercise challenge. Spirometry was conducted before and 3, 6, 9, 12, 15, and 30 min after exercise. A positive response was defined ≥15% decrease in FEV_1.

Table 1 Clinical characteristics of the study subjects

Subject	Sex	Age	BMI	%dFEV$_1$[a]	BA	PC$_{20}$ (mg/mL)	FEV$_1$ (%)[a]	FEV$_1$/FVC (%)[b]	Atopy
Patients									
1	M	17	19.7	20	Y	23.00	115	77	N
2	M	21	28.1	23	Y[c]		100	85	Y
3	M	18	28.7	16	Y[d]		93	63	N
4	M	19	22.2	19	Y	12.30	116	83	Y
5	M	19	17.6	40	Y	0.93	81	69	N
6	M	20	29.0	28	Y	0.84	75	63	Y
7	M	19	23.9	23	Y	3.76	76	66	Y
8	M	20	23.3	15	Y	3.48	73	58	Y
Mean ± SD		19.1±1.2	24.1±4.3	23.0±8.0		7.4±8.7	91.1±17.7	70.5±10.0	
Controls									
9	M	19	34.6	5	N		91	76	Y
10	M	22	26.5	8	Y[c]		84	82	NA
11	M	1	20.5	8	N		122	94	Y
12	M	21	25.4	3	N		81	79	NA
13	M	22	23.0	4	N		105	85	NA
Mean ± SD		20.6±1.5	26.0±0.3	5.6±2.3			96.6±17.0	83.2±6.9	

[a], %dFEV$_1$, percent decrease in FEV$_1$ on the exercise challenge test; [b], P<0.05 between patients and controls; [c], physician-diagnosed asthma; [d], patient diagnosed with asthma by positive reaction to mannitol bronchoprovocation test. FEV$_1$, forced expiratory volume in 1 s; SD, standard deviation; Y, yes; N, no; NA, not applicable; BMI, body mass index; FVC, forced vital capacity.

Measurement of urinary 9α,11β-PGF_2 and LTE_4 concentrations

Urine was collected before and 1 h after the end of the exercise challenge test. The urine samples were stored at −20 ℃ until analysis. The 9α,11β-PGF_2 and LTE_4 were measured by enzyme immunoassay (EIA) using an EIA kit according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, USA). Urinary excretion of 9α,11β-PGF_2 and LTE_4 is expressed as ng/mmol creatinine. Creatinine was measured in all urine samples using a commercially available colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

All data are expressed as means ± standard deviation (SD). Mean values of the mediators between the two groups were compared using the Mann-Whitney *U*-test. Differences in the mediators before and after exercise were compared using the Wilcoxon signed-rank test. The correlation between percent decrease in FEV$_1$ and percent change in the mediators during the exercise challenge test was analyzed using Spearman's correlation analysis. A P value <0.05 was considered to indicate significance. All statistical analyses were performed using SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics of the study subjects are summarized in *Table 1*. Mean age was 19.7±1.5 years (range, 17-22 years), and all subjects were male. Two of the subjects were smokers (<2 pack-year). No significant differences were observed in age, sex, or body mass index between the patients with EIB and controls. Five of the eight patients with EIB had atopy. Baseline FEV$_1$ (% predicted) was significantly lower in patients with EIB than that in controls (91.1±17.7 *vs.* 96.6±17.0, P=0.02), and baseline FEV$_1$/forced vital capacity (%) was also lower in patients with EIB than that in the control group (70.5±10.0 *vs.* 83.2±6.9, P<0.05). The maximum percent decrease in FEV$_1$ after exercise

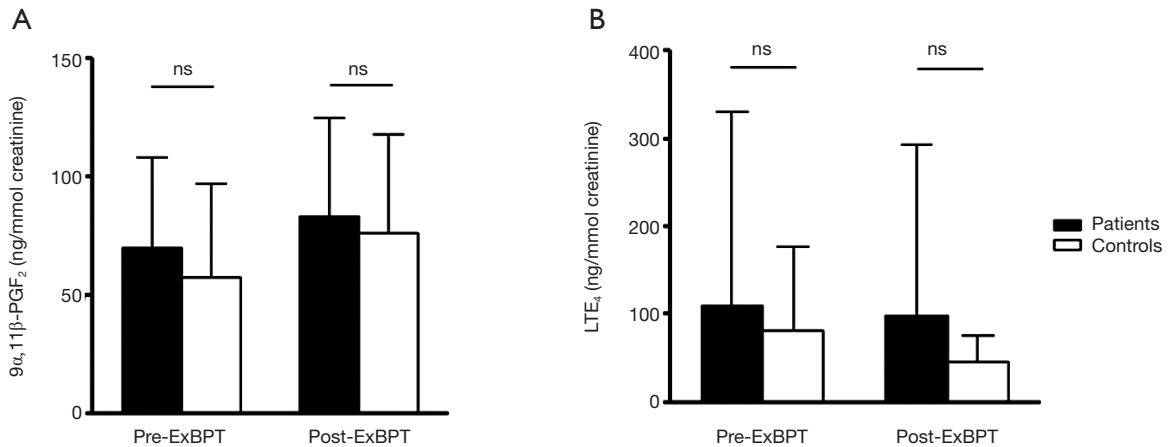

Figure 1 Urinary excretion of 9α,11β-PGF_2 (A) and LTE_4 (B) before and after the ExBPT in the study subjects. ns, not significant; PG, prostaglandin; LT, leukotriene; ExBPT, exercise bronchoprovocation test.

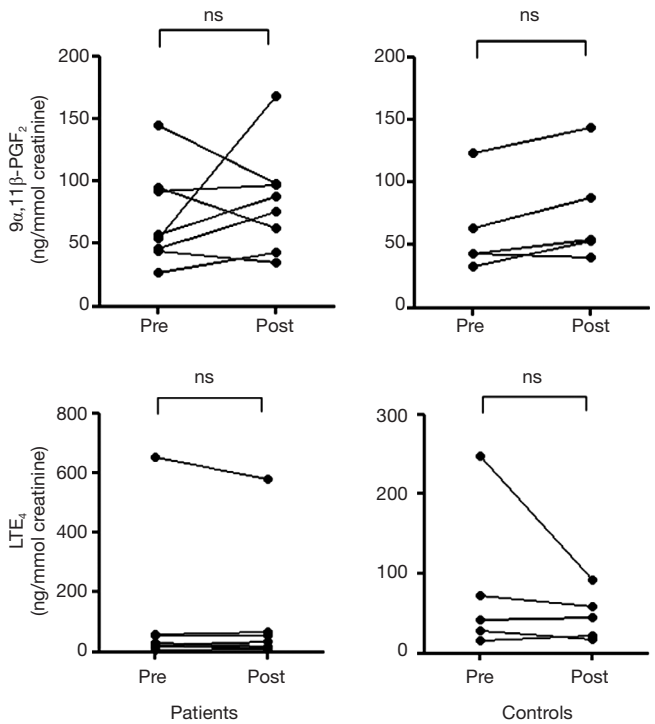

Figure 2 Changes in urinary excretion of 9α,11β-PGF_2 and LTE_4 before and after the exercise bronchoprovocation test in patients with EIB and controls. ns, not significant; PG, prostaglandin; LT, leukotriene; EIB, exercise-induced bronchoconstriction.

was 23.0%±8.0% in patients with EIB, whereas it was 5.6%±2.3% in normal controls (P<0.05).

No significant difference was observed in the baseline 9α,11β-PGF_2 level between patients with EIB and controls (70.2±38.0 *vs.* 61.4±36.3 ng/mmol creatinine), and no significant difference was observed in the 9α,11β-PGF_2 level after the exercise challenge between patients with EIB and controls (83.5±41.4 *vs.* 76.1±41.7 ng/mmol creatinine) (*Figure 1*). No difference was observed in the urinary LTE_4 level before (109.7±220.5 *vs.* 81.7±95.5 ng/mmol creatinine) or after the exercise challenge (99.5±195.2 *vs.* 47.3±30.3 ng/mmol creatinine) between patients with EIB and controls. Urinary excretion of 9α,11β-PGF_2 in patients with EIB did not increase significantly after the exercise challenge (*Figure 2*). Urinary excretion of LTE_4 also did not significantly change after the exercise challenge. The maximum percent decrease in FEV_1 during the exercise challenge test in patients with EIB tended to be positively correlated with the percent change in 9α,11β-PGF_2 level (r=0.67, P=0.07) (*Figure 3*). The percent change in LTE_4 in patients with EIB was not correlated with the maximum percent decrease in FEV_1 on the exercise challenge test (r=0.47, P>0.05).

Discussion

In this study, we did not detect an increase in urinary 9α,11β-PGF_2 or LTE_4 levels after an exercise challenge test in patients with EIB, and urinary levels of 9α,11β-PGF_2 and LTE_4 after the exercise challenge in patients with EIB were not significantly higher than those in a control group. In addition, the percent decrease in FEV_1 during the exercise challenge test was not correlated with the percent changes in 9α,11β-PGF_2 and LTE_4 levels.

Activation of mast cells has long been implicated in the

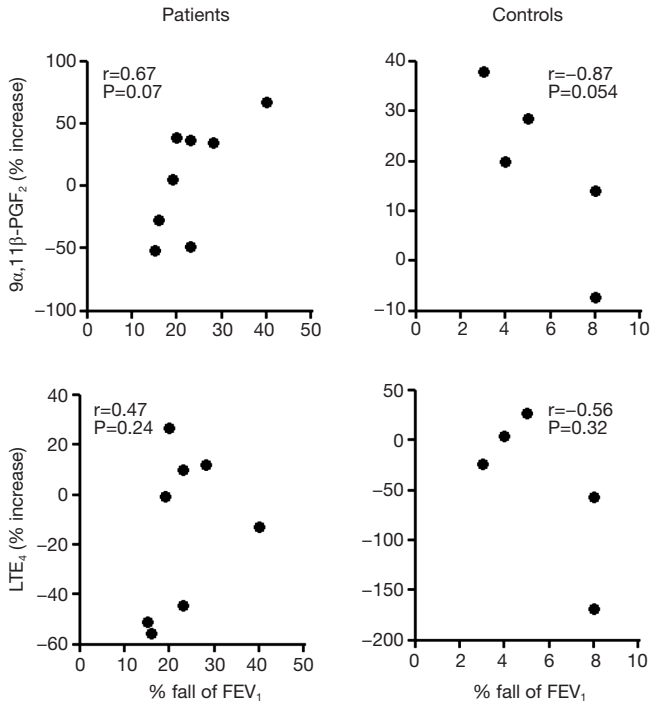

Figure 3 Correlation between percent decrease in FEV_1 with percent changes in urinary levels of 9α,11β-PGF_2 and LTE_4 before and after an exercise challenge in patients with EIB and controls. FEV_1, forced expiratory volume in 1 s; PG, prostaglandin; EIB, exercise-induced bronchoconstriction.

pathogenesis of EIB, as plasma histamine increases after exercise in patients with EIB, and the mast cell stabilizer disodium cromoglycate attenuates EIB (19). Histamine and tryptase release in induced sputum from EIB patients was also noted following an exercise challenge (4). Crimi *et al.* showed that the number of degranulated mast cells increases in bronchial biopsies from patients with EIB after an exercise challenge (7). However, other studies have not demonstrated an increase in histamine in plasma, BALF, bronchial lavage, or urine after exercise in patients with EIB, suggesting that mast cells may not contribute to the mechanism of EIB (9,15,16,25). Eosinophilic airway inflammation is also detected in patients with EIB, as eosinophils and eosinophil cationic proteins increase in induced sputum or BALF after exercise (5-7). However, Gauvreau *et al.* did not demonstrate eosinophilic airway inflammation after exercise in patients with EIB (26).

Eicosanoids, such as PGD_2 and CysLTs, are released from activated mast cells or eosinophils and are frequently isolated from induced sputum, BALF, and urine to investigate their roles in EIB. However, previous results have been inconsistent. Interestingly, significant increases in PGD_2 and/or CysLTs have been consistently observed in induced sputum and EBC (4-6,13), but not in BALF (15). Because EIB is initiated by drying and cooling of the airway, which causes bronchoconstriction in the segmental airways (27), BALF collected from the distal airway may not be appropriate to identify relevant airway inflammation.

Urinary LTE_4 and 9α,11β-PGF_2 levels are widely used as a marker of allergic inflammation (22,23). However, urinary excretion results of LTE_4 and 9α,11β-PGF_2 in patients with EIB vary among studies. Several studies that have measured urinary LTE_4 failed to show a significant increase after exercise in patients with EIB, including the current study (9,17,18), whereas other studies demonstrated increase of urinary LTE_4 (10-12,28). In contrast, urinary 9α,11β-PGF_2 levels in previous studies increased significantly and consistently in patients with EIB after exercise (8,9,12). However, we could not demonstrate a significant increase in urinary 9α,11β-PGF_2 level in patients with EIB, although the percent change in 9α,11β-PGF_2 level tended to be positively correlated with the maximum percent decrease in FEV_1 during the exercise challenge test in patients with EIB (r=0.67, P=0.07) (*Figure 3*).

Several explanations are possible for these discrepancies among studies. First, individual variations in release of eicosanoids during exercise, such as race, age, sex, or body mass index may occur among patients with EIB. Second, the timing of urine collection may have influenced eicosanoid levels. Too early or too late a urine collection may have caused negative results. According to the kinetics of LTE_4 excretion, the majority of urinary LTE_4 is excreted within 2-4 h after allergen challenge or inhalation of LTD_4 (29,30). In this study, we collected urine 1 h after the end of the exercise challenge test, which may have been too early to detect urinary excretion of LTE_4 and/or 9α,11β-PGF_2 after exercise. However, two studies with positive results determined LTE_4 and/or 9α,11β-PGF_2 within 1-2 h after an exercise challenge (11). One study serially collected urine at 0-3, 3-6, 6-12, and 12-24 h after an exercise challenge, but did not show an increase in urinary LTE_4 at any time point (18). Furthermore, Park *et al.* did not demonstrate increased urinary excretion of LTE_4 6 h after exercise in Korean patients with EIB (28). Taken together, these results suggest that our timing of urine collection after the exercise challenge may not influence the negative results. Third, total release of LTS from the airway after an exercise challenge may be too low to detect in systemic

circulation and/or urine. It is also possible that locally produced mediators were trapped in areas of poor perfusion in response to airway cooling, resulting in poor absorption into systemic circulation (18). The release of urinary LTE_4 after an exercise challenge is about twofold higher than that at baseline (9,17,18), whereas a 4- or 12-fold increase in urinary LTE_4 has been reported after allergen challenge (18,30). In addition, when we measured urinary 9α,11β-PGF_2 in a patient with exercise-induced anaphylaxis as a positive control of mast cell activation, there was about 12-fold increase of urinary 9α,11β-PGF_2 after exercise challenge (80.1 *vs.* 998.9, data not shown). Thus, the role of mast cell activation in patients with EIB may be feeble compared to that during allergen provocation or systemic allergic reactions such as anaphylaxis. This explanation also agrees with the consistent increase in inflammatory mediators in induced sputum and EBC after an exercise challenge (4-6,13,14).

Mechanisms other than inflammatory mediator release from activated mast cells or eosinophils may be involved in EIB pathogenesis. Airway epithelial injury by thermal or osmotic stress decreases PGE_2 production, which has anti-inflammatory and bronchodilatory effects in patients with asthma (31). An increase in the CysLTs/PGE_2 ratio has been observed in induced sputum from patients with EIB after an exercise challenge (6). Sensory neuronal activation by electrical and chemical stimuli has been reported to be another mechanism of EIB (32).

Our study had several limitations. We had a relatively small number of subjects with heterogeneous clinical characteristics of atopy and smoking history; the exercise challenge protocols were inconsistent among the subjects, such as free-running or treadmill exercise; and we did not evaluate other mast-cell-specific inflammatory mediators such as histamine and tryptase.

Conclusions

Our findings do not support a role of inflammatory mediators such as PGD_2 and CysLTs, which are known as a marker of mast cell and eosinophil activation, in Korean patients with EIB. As activation of inflammatory cells in EIB was evidenced by an increase of inflammatory mediators in induced sputum or EBC in other studies (4-6,13,14), measuring urinary excretion of 9α,11β-PGF_2 and LTE_4 may not be a good marker for detection of airway inflammation in patients with EIB.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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