

Urinary leukotriene E₄ and 9 α , 11 β -prostaglandin F₂ concentrations in mild, moderate and severe asthma, and in healthy subjects

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Summary

Background Airway inflammation in asthma is associated with cysteinyl leukotriene and prostaglandin D₂ production. Measurement of urinary metabolites of these eicosanoids may be useful for monitoring asthma patients. However, the influence of asthma phenotype and severity on basal urinary excretion of these metabolites is unknown.

Objective To compare urinary leukotriene (LT)E₄ and 9 α , 11 β -prostaglandin (PG)F₂ concentrations in large groups of mild, moderate and severe asthmatic patients and healthy control subjects.

Methods Asthma severity, treatment and aspirin sensitivity were assessed by questionnaire in 168 asthmatic patients. Basal LTE₄ and 9 α , 11 β -PGF₂ concentrations were measured in urine samples from these patients and from 175 control subjects using enzyme immunoassays.

Results Urinary LTE₄ was correlated with 9 α , 11 β -PGF₂ in both control subjects and asthmatic patients ($P < 0.002$). Median LTE₄ and 9 α , 11 β -PGF₂ concentrations in patients with severe asthma were significantly reduced compared with mild asthmatic patients ($P < 0.05$ and < 0.001 , respectively). Urinary 9 α , 11 β -PGF₂, but not LTE₄ was lower in asthmatic patients using inhaled corticosteroids ($P < 0.02$). Multiple regression analysis indicated that urinary 9 α , 11 β -PGF₂ concentration was negatively correlated with asthma severity ($P = 0.003$) and also with % predicted FEV₁ (forced expiratory volume in 1 s) ($P = 0.005$).

Conclusions Baseline urinary LTE₄ and 9 α , 11 β -PGF₂ concentrations are of limited value in discriminating between patients with different severities of asthma. Reduced urinary LTE₄ and 9 α , 11 β -PGF₂ in patients with severe asthma suggest that direct or indirect effects of high-dose corticosteroid therapy combined with other factors associated with severe asthma may influence eicosanoid production. However, the negative association of urinary 9 α , 11 β -PGF₂ with lung function suggests an adverse effect of chronic PGD₂ production on lung function in asthma, irrespective of severity.

Keywords aspirin-intolerant asthma, asthma severity, corticosteroids, cysteinyl leukotrienes, inflammation, leukotriene E₄, prostaglandin D₂, 9 α , 11 β -prostaglandin F₂, urine

Submitted 16 June 2003; revised 18 August 2003; accepted 15 November 2003

Introduction

Chronic inflammation associated with asthma is characterized by infiltration and activation of eosinophils in the lung [1]. The cysteinyl leukotrienes (cysLT), which are major eicosanoid products of activated eosinophils, mast cells, basophils and monocytes [2, 3], have been implicated in the bronchoconstriction, increased mucus production, oedema, smooth muscle proliferation and airway remodelling that are characteristic of asthma [3–6]. A proportion of the leukotriene (LT) C₄ and D₄ produced in the lungs is rapidly catabolized to LTE₄, and approximately 7% of a total

inhaled dose of LTC₄ appears in the urine as LTE₄ [7], indicating that measurement of urinary LTE₄ provides a satisfactory indication of *in vivo* cysLT production in the lungs [8].

Activation of mast cells as a consequence of antigen–antibody-mediated cross-linking of IgE receptors on the surface of these cells, is thought to be an important feature of the allergic asthmatic response [9]. Prostaglandin (PG)D₂ is the major cyclooxygenase product released by activated mast cells and early studies demonstrated increased production of this bronchoconstricting and vasodilating eicosanoid in the airways of asthmatic patients following antigen challenge [10]. PGD₂ is unstable and is metabolized in the human lung by NADPH-dependent 11-ketoreductase to 9 α , 11 β -PGF₂, which is rapidly excreted in the urine. Although alveolar macrophages have also been reported to have the capacity to synthesize PGD₂ [11], mast cells are the major source of

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PGD₂ suggesting that its urinary metabolite, 9 α ,11 β -PGF₂ may be a useful marker of mast cell activation in the lungs [12].

A number of studies have demonstrated increased urinary LTE₄ concentrations following challenge of asthmatic patients with allergen or aspirin, and also during spontaneous acute asthma episodes (reviewed by Kumlin [13]). Similarly, increased urinary 9 α ,11 β -PGF₂ has been measured after aspirin challenge [12], during allergen-induced early- and late-phase asthmatic reactions [14], and in acute asthma and exercise-induced asthma in children [15]. Recently, a method based on immunoaffinity purification followed by enzyme immunoassay (EIA) to determine urinary LTE₄ concentrations has been carefully validated [16]. In addition, the measurement of 9 α ,11 β -PGF₂ in unextracted urine by EIA has been validated as a simple, rapid and specific method for evaluating PGD₂ production and hence mast cell activation in the lungs [17]. However, previous studies on basal urinary excretion of LTE₄ and 9 α ,11 β -PGF₂ in healthy subjects and asthmatic patients have used small numbers of subjects, with different sampling and assay methods, and have shown wide variation in results (Table 1). Furthermore, the question whether the degree of chronic asthma severity and airway inflammation influence basal urinary LTE₄ and 9 α ,11 β -PGF₂ concentrations has not been previously addressed.

Therefore, the aims of this study were to assess basal urinary LTE₄ and 9 α ,11 β -PGF₂ concentrations in a large group of healthy control subjects, and in asthmatic patients, who were carefully characterized with respect to the severity of their asthma. It was hypothesized that categorization of asthma patients according to the chronic severity of their disease, based both on recent history and on current parameters such as lung function and medication use, would

reflect the degree of ongoing airway inflammation, and that this in turn, would result in altered urinary excretion of LTE₄ and 9 α ,11 β -PGF₂ in the different patient groups. We therefore aimed to assess the influence of patient phenotype characteristics, including asthma severity and treatment, on urinary LTE₄ and 9 α ,11 β -PGF₂ concentrations.

Materials and methods

Subjects

Asthmatic patients were randomly selected from those attending the Asthma and Allergy Research Institute and Sir Charles Gairdner Hospital, Perth, Australia. These institutions treat patients drawn from the entire Perth metropolitan area and whose asthma ranges from mild to severe. In order to recruit control subjects from a comparable population and to supplement our mild patient group, a random mail out was used. A total of 168 patients with physician-diagnosed asthma were recruited and completed a detailed questionnaire, which was used to assess the severity of their asthma. This assessment was based on the National Asthma Education and Prevention Program, Expert Panel Report II guidelines [26], and uses the following combination of current symptoms and maintenance treatment to assess asthma severity: (i) lung function (% predicted FEV₁ (forced expiratory volume in 1 s)), (ii) daily inhaled corticosteroid (beclomethasone equivalent) dose over the previous 3 months, (iii) frequency of rescue medication use in the previous 2 weeks, (iv) frequency of daytime symptoms in the previous 2 weeks and (v) frequency of night-time awakening due to asthma in the previous 2 weeks. Three additional criteria were used to assess the recent asthma history of each patient: (i) use of oral corticosteroids in the preceding 12 months; (ii) unplanned visits to a general practitioner due to asthma in the preceding 12 months and (iii) hospital admissions for asthma in the preceding 12 months. Patients were classified as being mild, moderate or severe with respect to their score for each criterion and were placed in the overall category in which they were classified for the majority of criteria. On this basis patients were categorized as having mild asthma (severity = 1, n = 66), moderate asthma (severity = 2, n = 52) or severe asthma (severity = 3, n = 50). The control group comprised 175 healthy, non-asthmatic subjects who completed a brief questionnaire relating to their general health status. The characteristics of the control subjects and asthmatic patients are presented in Table 2.

If patients indicated aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) as possible triggers for their asthma, they completed a second questionnaire, which assessed the nature of these adverse reactions in detail. We have previously used a similar validated questionnaire to assess the prevalence of aspirin intolerance in asthmatic patients [27]. Information was obtained on the history and age of onset of nasal polyps, the specific drug(s) causing adverse reactions (from a list of named drugs including aspirin, aspirin derivatives and NSAIDs), the number of occasions on which adverse reactions had been experienced, the time to onset of symptoms, incidence of hospitalization following ingestion of these drugs, and specific symptoms (from a

Table 1. Previously reported baseline urinary LTE₄ and 9 α ,11 β -PGF₂ concentrations in asthmatic and control subjects

Subject group	<i>n</i>	LTE ₄ (pg/mg creatinine)	9 α ,11 β -PGF ₂ (pg/mg creatinine)	Reference
Non-asthmatic	29	210	–	[18]
Asthmatic	8	208	–	
Non-asthmatic	17	34	–	[19]
Asthmatic	31	43	–	
Non-asthmatic	5	84	–	[20]
Asthmatic	8	110	–	
Non-asthmatic	9	132	–	[8]
Asthmatic	26	156	–	
Non-asthmatic	6	50	–	[21]
Asthmatic	12	95	–	
Non-asthmatic	7	184	–	[22]
Asthmatic	9	163	–	
Non-asthmatic	16	68	–	[23]
Asthmatic	19	74	–	
Non-asthmatic	9	–	477	[12]
Asthmatic	14	–	548	
Asthmatic	10	68	181	[24]
Non-asthmatic	50	337	1060	[25]
Asthmatic	66	483	910	
Non-asthmatic	175	27	219	This study
Asthmatic	168	24	208	

LTE₄, leukotriene E₄; 9 α ,11 β -PGF₂, 9 α ,11 β -prostaglandin F₂.

Table 2. Characteristics of asthmatic patients and control subjects

	Control	Mild asthma	Moderate asthma	Severe asthma
Number	175	66	52	50
Female (%)	52.0	66.7	75.0	70.0
Age (mean $\pm$ SD)	49.5 $\pm$ 15.1	45.4 $\pm$ 15.2	49.4 $\pm$ 13.0	53.9 $\pm$ 13.1
BMI (mean $\pm$ SD)	26.1 $\pm$ 3.9	26.9 $\pm$ 6.1	28.0 $\pm$ 5.9	28.7 $\pm$ 5.9
Atopic (%)	48.6	87.9	82.7	78.0
% predicted FEV ₁ (mean $\pm$ SEM)	99.4 $\pm$ 1.3	93.5 $\pm$ 1.8	74.1 $\pm$ 2.6	59.8 $\pm$ 3.3
Current smokers (%)	6.9	0	3.8	6.0
Past smokers (%)	45.1	31.8	42.3	40.0
Medication use				
Oral corticosteroids (%)*	—	13.6	61.5	78.0
Frequency of use (mean times/year)	—	1.3	1.5	2.9
Inhaled corticosteroids (%)†	—	57.6	96.2	96.0
Mean inhaled steroid dose (μ g/day $\pm$ SEM)‡	—	821 $\pm$ 91	1654 $\pm$ 141	2923 $\pm$ 194
β_2 -adrenoceptor agonists (%)§	—	69.7	92.3	98.0

*Percentage of patients who had used oral corticosteroids in last 12 months. †Percentage of patients using inhaled corticosteroids. ‡Beclomethasone equivalent dose over the previous 3 months. §Percentage of patients using β_2 -adrenoceptor agonists. BMI, body mass index; FEV₁, forced expiratory volume in 1 s.

comprehensive list of possible symptoms). Individuals reporting any adverse reaction to these drugs were further categorized into (1) individuals reporting minimal respiratory response or undefined responses, (2) individuals who had experienced more than one episode with characteristic symptoms of aspirin intolerance or (3) individuals who had undergone a formal aspirin challenge or who reported multiple characteristic episodes, often resulting in hospitalization. For this study, data for a group of 33 patients from categories 2 or 3 only (aspirin-intolerant asthma (AIA) patients, Table 3) was compared with the data for the 135 patients who reported no history of aspirin intolerance (aspirin-tolerant asthma (ATA) patients).

In order to assess whether urinary LTE₄ and 9 α , 11 β -PGF₂ concentrations varied with time of sampling, urine samples were grouped according to time of collection. The collection periods were 08:00–11:00 (control, n = 65; asthma, n = 57), 11:00–14:00 (control, n = 67; asthma, n = 67) and 14:00–17:00 (control, n = 43; asthma, n = 39). To assess day-to-day variation within subjects, urine samples were collected from 11 control subjects and nine asthmatic patients on three occasions approximately 1 week apart. Urinary LTE₄ and 9 α , 11 β -PGF₂ concentrations in these samples were measured and the mean values at weeks 1–3 were compared.

Measurement of urinary LTE₄ and 9 α , 11 β -PGF₂

Spot urine samples were collected from all subjects and stored at -80°C . Urine samples were purified using a cysLT immunoaffinity sorbent (Cayman Chemical, Ann Arbor, MI, USA) and then assayed for LTE₄ as previously described [16]. Briefly, after centrifugation (2500 g , 3 min) the urine sample (1 mL) was incubated with 20 μ L of cysLT immunoaffinity sorbent for 1 h at room temperature with gentle mixing. Samples were centrifuged (2500 g , 4 min), the supernatant discarded, and the resin was washed with phosphate-buffered saline (pH 7.4). Cold methanol (0.5 mL) was then added to the resin pellet, which was mixed and left at -20°C for 5 min. The supernatants were collected by centrifugation (6,500 g , 4 min) and the methanol evaporated in a SpeedVac

Table 3. Characteristics of patients and urinary LTE₄ and 9 α , 11 β -PGF₂ concentrations in aspirin intolerant and aspirin-tolerant asthmatic patients

	AIA	ATA
Number	33	135
Female (%)	72.7	69.6
Age (mean $\pm$ SD)	48.4 $\pm$ 12.4	49.4 $\pm$ 14.7
Asthma severity, n (%)		
Mild	9 (27.3)	57 (42.2)
Moderate	17 (51.5)	35 (25.9)
Severe	7 (21.2)	43 (31.9)
Atopic (%)	84.8	83.0
% predicted FEV ₁ (mean $\pm$ SEM)	79.0 $\pm$ 3.9	77.1 $\pm$ 2.1
Current smokers (%)	3.0	3.0
Past smokers (%)	33.3	38.5
Medication use		
Oral corticosteroids (%)*	54.5	45.9
Frequency of use (mean times/year)	2.1	2.2
Inhaled corticosteroids (%)†	90.9	76.3
Mean inhaled steroid dose (μ g/day $\pm$ SEM)‡	1617 $\pm$ 208	1941 $\pm$ 135
β_2 -adrenoceptor agonists (%)§	81.8	85.9
Urinary LTE ₄ (pg/mg creatinine), median (IQR)	30.3 (11.7–44.0)	23.5 (16.9–55.5)
Urinary 9 α , 11 β -PGF ₂ (pg/mg creatinine), median (IQR)	191.4 (93.4–358.4)	209.4 (136.3–427.5)

*Percentage of patients who had used oral corticosteroids in last 12 months. †Percentage of patients using inhaled corticosteroids. ‡Beclomethasone equivalent dose over the previous 3 months. §Percentage of patients using β_2 -adrenoceptor agonists. LTE₄, leukotriene E₄; 9 α , 11 β -PGF₂, 9 α , 11 β -prostaglandin F₂; AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; FEV₁, forced expiratory volume in 1 s; IQR, interquartile range.

A160 concentrator (Savant Instruments, Farmingdale, NY, USA). The dry residue was resuspended in 0.5 mL of assay buffer and LTE₄ concentrations were determined in duplicate using an EIA according to the manufacturer's instructions (Cayman Chemical). The recovery of LTE₄ from urine during immunoaffinity purification was 63–76% [16]. The detection

limit for the LTE_4 EIA is 36.1 pg/mL and all samples were sufficiently concentrated so that read-outs from the EIA were well above the detection limit. The intra-assay coefficient of variation, based on replicate analyses of 42 samples, was 9.8% with an inter-assay coefficient of variation of 19.4% as determined by three separate analyses of five randomly selected samples.

Concentrations of $9\alpha, 11\beta\text{-PGF}_2$ were assayed in unextracted, diluted (1:10) or undiluted urine samples using a specific EIA according to the manufacturer's instructions (Cayman Chemical). This assay has a detection limit of 15 pg/mL, and we measured an intra-assay coefficient of variation of 11.3%, based on replicate analyses of 30 samples, with an inter-assay coefficient of variation of 14.8%, determined from three separate analyses of five randomly selected samples. There was no cross-reactivity between the LTE_4 and $9\alpha, 11\beta\text{-PGF}_2$ EIAs ($< 0.01\%$). To correct for inter-subject variations in diuresis and concentration of urine, creatinine concentrations were measured for all urine samples using a spectrophotometric assay (Sigma Chemical, St Louis, MO, USA). Urinary LTE_4 and $9\alpha, 11\beta\text{-PGF}_2$ concentrations were expressed as picogram per milligram of creatinine.

Statistical analysis

Urinary LTE_4 and $9\alpha, 11\beta\text{-PGF}_2$ concentrations were not normally distributed, therefore median (interquartile range, IQR) values were calculated. Differences in median values between groups, and for urine samples collected at different times were assessed using the Mann-Whitney U -test, while correlations were determined using Spearman's rank correlation. Multiple linear regression was used to assess the relationships between urinary LTE_4 or $9\alpha, 11\beta\text{-PGF}_2$ concentrations and gender, age, body mass index (BMI), asthma severity, % predicted FEV_1 , atopy, aspirin intolerance, smoking history, oral and inhaled corticosteroid use and inhaled corticosteroid dose in asthmatic patients. In control subjects, the multiple regression model included gender, age, BMI, atopy and smoking history as independent variables.

Results

All asthmatic groups contained significantly more females compared with the control group ($\chi^2 = 12.8$, $P = 0.005$, Table 2) and there were also significantly more atopic subjects in the asthmatic groups ($\chi^2 = 47.1$, $P < 0.001$). Severe asthmatic patients were older than the mild asthmatic group ($P < 0.02$) and had a higher mean BMI than the control group ($P < 0.01$). Urinary LTE_4 concentrations were not significantly different between control subjects (median 26.6 pg/mg creatinine, IQR 15.4–47.0) and all asthmatic patients (24.2, 12.1–46.9). However, there was a small but statistically significant reduction in median urinary LTE_4 concentration in severe asthmatic patients (22.7, 9.7–33.2, $n = 50$) compared with control subjects ($P < 0.01$) and mild asthmatic patients (25.9, 18.7–56.2, $n = 66$, $P < 0.05$, Fig. 1). Urinary LTE_4 was not significantly different in patients using inhaled corticosteroids (23.7, 11.3–46.9, $n = 136$) compared with those who were not (25.6, 15.4–46.7, $n = 32$) and urinary LTE_4 did not correlate with inhaled corticosteroid dose. Similarly, urinary

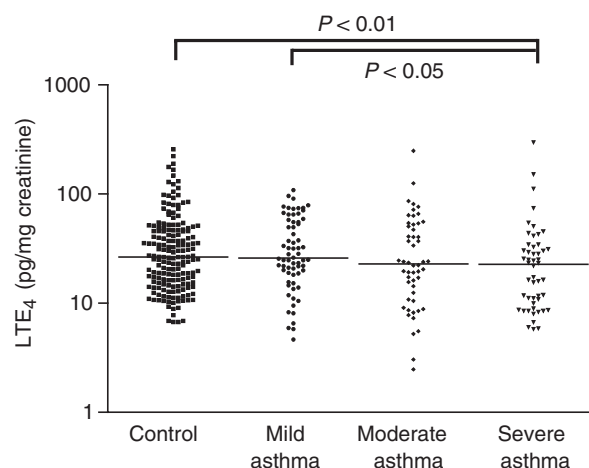


Fig. 1. Urinary leukotriene E_4 concentrations in control subjects ($n = 175$) and in mild ($n = 66$), moderate ($n = 52$) and severe asthmatic patients ($n = 50$). Horizontal bars indicate median values.

LTE_4 did not differ between patients who had ($n = 80$) or had not ($n = 88$) used oral corticosteroids in the previous 12 months.

Urinary $9\alpha, 11\beta\text{-PGF}_2$ in control subjects (median 218.7 pg/mg creatinine, IQR 136.7–390.2) did not differ significantly from that in all asthmatic patients (208.0, 129.1–406.1). However, the urinary $9\alpha, 11\beta\text{-PGF}_2$ concentration in severe asthmatic patients (160.6, 104.3–265.3) was substantially lower than that of control subjects ($P < 0.01$), mild (240.8, 161.4–494.9, $P < 0.001$) and moderate asthmatic patients (257.0, 122.9–415.5, $n = 52$, $P < 0.02$, Fig. 2). Median urinary $9\alpha, 11\beta\text{-PGF}_2$ concentration was significantly lower in patients using inhaled corticosteroids (191.2, 119.0–348.1, $n = 136$) compared with those who were not (330.4, 169.7–504.7, $n = 32$, $P < 0.02$) but there was no significant correlation between urinary $9\alpha, 11\beta\text{-PGF}_2$ and inhaled corticosteroid dose ($r_s = -0.12$, $P = 0.15$). Urinary $9\alpha, 11\beta\text{-PGF}_2$ concentrations were not significantly different in patients who had ($n = 80$) or had not ($n = 88$) used oral corticosteroids in the previous 12 months. Urinary $9\alpha, 11\beta\text{-PGF}_2$ concentrations

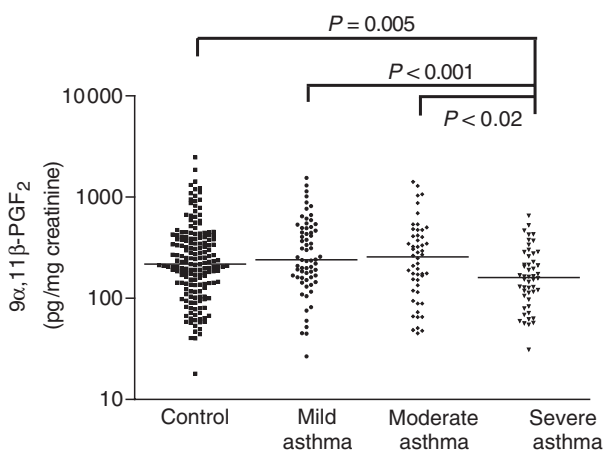


Fig. 2. Urinary $9\alpha, 11\beta$ -prostaglandin F_2 concentrations in control subjects ($n = 175$) and in mild ($n = 66$), moderate ($n = 52$) and severe asthmatic patients ($n = 50$). Horizontal bars indicate median values.

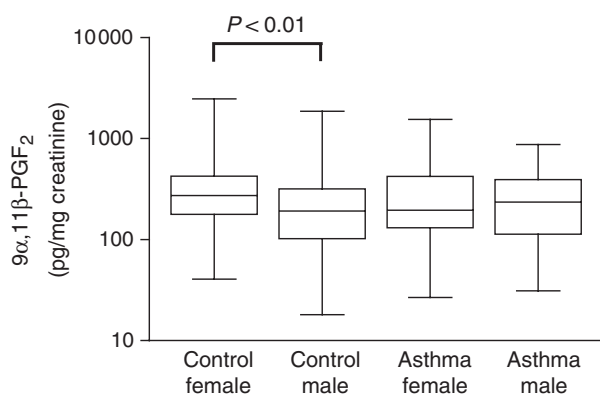


Fig. 3. Urinary $9\alpha, 11\beta$ -prostaglandin F_2 concentrations in female ($n = 91$) and male ($n = 84$) control subjects and in female ($n = 118$) and male ($n = 50$) asthmatic patients. Horizontal bars indicate median values and boxes the interquartile range.

were significantly correlated with LTE_4 concentrations in both control subjects ($r_s = 0.2515$, $P < 0.001$) and asthmatic patients ($r_s = 0.2412$, $P < 0.002$).

When compared with aspirin-tolerant patients, a significantly greater proportion of patients with a strong self-reported history of AIA were categorized as having moderate asthma ($\chi^2 = 8.1$, $P < 0.02$, Table 3), but other characteristics of the two groups did not differ significantly. Urinary LTE_4 concentrations in the AIA group (30.3, 16.9–55.5, $n = 33$) tended to be higher than those of the aspirin-tolerant group (23.5, 11.7–44.0, $n = 135$), although the difference did not reach statistical significance ($P = 0.19$). Urinary $9\alpha, 11\beta$ -PGF $_2$ concentrations did not differ significantly between the two groups.

Among the control subjects, males had significantly lower urinary $9\alpha, 11\beta$ -PGF $_2$ concentrations (192.1, 101.1–319.4) compared with females (272.8, 176.3–423.4, $P < 0.01$, Fig. 3). In multiple regression analyses of data for the control subjects, urinary $9\alpha, 11\beta$ -PGF $_2$ was positively correlated with female gender (standardized coefficient 0.16, $P = 0.029$) and urinary LTE_4 (standardized coefficient 0.27, $P < 0.001$), while urinary LTE_4 was positively correlated with age (standardized coefficient 0.17, $P = 0.023$). Multiple regression analysis of data for the asthmatic patients showed no significant correlation between urinary LTE_4 concentration and any of the independent variables. However, there were significant negative correlations between urinary $9\alpha, 11\beta$ -PGF $_2$ concentration and asthma severity, % predicted FEV $_1$, age and atopy (Table 4).

Among control subjects, urinary LTE_4 concentrations were higher in samples collected between 08:00 and 11:00 (34.0, 18.1–52.0, $P < 0.01$) or between 11:00 and 14:00 (27.3, 16.4–47.3, $P < 0.05$) compared with those collected between 14:00 and 17:00 (19.8, 13.3–36.3). There was a similar trend in the asthmatic patients although the differences were not statistically significant. Urinary $9\alpha, 11\beta$ -PGF $_2$ concentrations in control or asthmatic subjects did not vary with time of collection. In samples collected from the same subjects on three occasions approximately 1 week apart, median urinary LTE_4 and $9\alpha, 11\beta$ -PGF $_2$ concentrations at weeks 1–3 were not significantly different either in control subjects ($n = 11$) or asthmatic patients ($n = 9$).

Discussion

Measurement of urinary LTE_4 and $9\alpha, 11\beta$ -PGF $_2$ is potentially useful in assessing chronic inflammation associated with eosinophil and mast cell activation in the airways of asthmatic patients [28]. However, it is not known whether phenotype characteristics such as severity of disease influence urinary concentrations in asthmatic patients. In this study, urinary LTE_4 and $9\alpha, 11\beta$ -PGF $_2$ concentrations varied widely both in control subjects and asthmatic patients, although the range of concentrations measured was similar to that previously reported for normal and asthmatic subjects (Table 1). Basal levels of cysLT and PGD $_2$ production probably maintain normal physiological processes such as smooth muscle tone, and these eicosanoids are presumably produced by the same cells (eosinophils, mast cells, basophils, monocyte-macrophages) in normal subjects and asthmatic patients [29]. However, it is not clear why basal urinary eicosanoid levels vary so widely between subjects. Possible contributing factors may be variation in the numbers and activation of eicosanoid producing cells or physiological variations in metabolism and urinary excretion.

This study showed that baseline urinary eicosanoid concentrations may not be useful in discriminating between asthmatic patients with differing severity of disease, possibly because small changes consequent to airway inflammation may be obscured by inter-subject variation and the dilution of eicosanoids produced in the lungs [30]. However, we did observe a small but statistically significant reduction in urinary LTE_4 in patients with severe compared with mild asthma, and a much more substantial reduction in urinary

Table 4. Multiple regression analysis for factors independently correlated with urinary $9\alpha, 11\beta$ -PGF $_2$ in asthmatic patients

	Regression co-efficient (95% CI)	Standardized regression co-efficient	Significance
Asthma severity (1 = mild, 2 = moderate, 3 = severe)	– 325.8 (– 532.9 to – 118.7)	– 0.96	$P = 0.002$
% predicted FEV $_1$	– 8.78 (– 14.89 to – 2.67)	– 0.79	$P = 0.005$
Atopy (1 = no, 2 = yes)	– 138.5 (– 253.3 to – 23.8)	– 0.2	$P = 0.018$
Age	– 4.08 (– 7.41 to – 0.74)	– 0.22	$P = 0.017$
Asthma severity* % predicted FEV $_1$	2.61 (0.14–5.09)	0.55	$P = 0.039$

The backward elimination multiple regression analysis with urinary $9\alpha, 11\beta$ -PGF $_2$ as the dependent variable included the following independent variables in the model: asthma severity, gender, age, BMI, % predicted FEV $_1$, aspirin intolerance, atopic status, smoking history, inhaled corticosteroid use and dose, oral corticosteroid use, urinary LTE_4 and appropriate interaction terms.

$9\alpha, 11\beta$ -PGF $_2$, $9\alpha, 11\beta$ -prostaglandin F_2 ; FEV $_1$, forced expiratory volume in 1 s; BMI, body mass index; LTE_4 , leukotriene E $_4$; *interaction term.

9 α ,11 β -PGF₂ in severe compared with mild and moderate asthmatic patients. Furthermore, there was an independent negative correlation between urinary 9 α ,11 β -PGF₂ and asthma severity.

One possible explanation for these findings is that increased oral and inhaled corticosteroid use by the severe asthmatic patients may have influenced urinary excretion of eicosanoid metabolites. In previous studies, short-term administration of oral or inhaled corticosteroids to normal subjects or asthmatic patients did not alter urinary LTE₄ excretion [31–34]. However, a reduction in BAL fluid LTC₄ concentrations was observed in asthmatic patients treated with beclomethasone for 2.5 years [35]. Furthermore, *ex vivo* studies have shown reduced Ca²⁺ ionophore-stimulated LTC₄ synthesis in leucocytes from asthmatics receiving oral corticosteroids [36, 37]. Contrasting effects of corticosteroids on the enzymes of the leukotriene biosynthetic pathway have also been reported. Thus, fluticasone inhibits LTC₄ secretion from activated eosinophils by down-regulating cytosolic phospholipase A₂ (cPLA₂) expression [38]. However, dexamethasone induces 5-lipoxygenase activating protein and 5-lipoxygenase mRNA and protein expression in monocytes [39]. The question of whether corticosteroids reduce cysLT generation is important in assessing the potential clinical benefit of adding leukotriene receptor antagonist therapy to corticosteroid therapy [35] and our results suggest that corticosteroids have a limited effect on cysLT production as assessed by urinary LTE₄ levels.

Corticosteroids have a more marked inhibitory effect on prostanoid production by suppressing the expression of the key synthetic enzymes cPLA₂ and COX-2 [40, 41]. This may explain the reduced urinary 9 α ,11 β -PGF₂ concentrations in asthmatic patients using inhaled corticosteroids, the greater reduction in urinary 9 α ,11 β -PGF₂ compared with LTE₄ in patients with severe asthma and the independent negative correlation between 9 α ,11 β -PGF₂ levels and asthma severity. Furthermore, the significant positive correlation between LTE₄ and 9 α ,11 β -PGF₂ concentrations in both control subjects and asthmatic patients supports the possibility that cPLA₂ and its inhibition by corticosteroids may influence overall eicosanoid metabolism via both the 5-lipoxygenase and cyclooxygenase pathways.

Corticosteroids also reduce eosinophil and mast cell numbers in the lungs of asthmatic patients [42] and may thereby indirectly reduce cysLT and PGD₂ production. However, the severe asthmatic patients in this study were generally symptomatic on most days, despite using relatively high doses of inhaled corticosteroids. Although urinary 9 α ,11 β -PGF₂ was reduced in severe asthma, the independent negative correlation between 9 α ,11 β -PGF₂ and % predicted FEV₁ suggests that chronic PGD₂ production may be adversely affecting lung function in our total asthmatic population. Since asthma severity was likely to be strongly associated with corticosteroid use and dose, interaction terms for these variables were included in the multiple regression analysis. Corticosteroid use *per se* was not independently associated with reduced urinary eicosanoid concentrations, suggesting that a complex interaction of factors, such as airway remodelling, high-dose corticosteroid treatment and steroid resistance, that are closely associated with the severe asthma phenotype may be influencing urinary LTE₄ and 9 α ,11 β -PGF₂ concentrations.

Previous studies have shown significantly increased baseline urinary LTE₄ levels in AIA patients [8, 19, 24, 25, 43, 44], and while urinary LTE₄ levels were increased in AIA patients in the present study, the difference did not reach statistical significance. This may reflect an effect of corticosteroids in partially suppressing eosinophils and LTE₄ production in the AIA patients, more of whom were using oral and inhaled corticosteroids compared with aspirin-tolerant patients. Because we excluded from the analysis any individuals who were uncertain of their response to aspirin or NSAIDs, it is unlikely that any undiagnosed AIA patients were classified as being aspirin tolerant, potentially reducing the difference in urinary LTE₄ between AIA and ATA patients. However, our results are in keeping with previous reports that baseline urinary 9 α ,11 β -PGF₂ is not increased in AIA [12, 24, 25].

Increased urinary 9 α ,11 β -PGF₂ concentrations in control females may reflect the induction of cyclooxygenase-2 and increased synthesis of prostaglandins due to the fall in progesterone levels that precedes menstruation [45, 46]. The significant independent positive association between urinary LTE₄ concentration and age in control subjects suggests a general up-regulation of leukotriene metabolism with increasing age, whereas such an effect may be obscured in asthmatic patients. On the other hand, the negative correlation between age and urinary 9 α ,11 β -PGF₂ in asthmatic patients suggests other potential influences such as a reduction in prostaglandin production in post-menopausal women who are likely to have comprised approximately one-third of the asthmatic patients.

The significantly higher urinary LTE₄ levels in morning samples from control subjects is in keeping with a previous observation of a morning peak in urinary LTE₄ in healthy subjects [47]. Interestingly the latter study found that among asthma patients, a similar morning peak was confined to those with nocturnal asthma, possibly explaining the fact that LTE₄ was not significantly increased in morning samples in our whole asthmatic population.

This study suggests that measurement of baseline concentrations of urinary eicosanoids does not distinguish asthmatic patients from normal subjects, and there was a poor correlation between urinary eicosanoid concentrations and disease severity. Nevertheless, measurement of urinary eicosanoids may provide useful complementary information, as exemplified by our findings of significant negative correlations of urinary 9 α ,11 β -PGF₂ with lung function and severity of asthma. Serial measurements of urinary eicosanoids in individual patients may also be useful for monitoring disease activity, but this will require further studies to determine whether enhanced excretion of eicosanoids in certain individuals, both asthmatic and control, represents true phenotype differences and whether there are large within-subject variations over time.

Acknowledgement

This work was supported by the Australian Commonwealth Government through the Cooperative Research Centres Programme.

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