Respiratory Medicine (2003) **97**, 1225–1229

respiratoryMEDICINE

High levels of urinary leukotriene E_4 excretion in steroid treated patients with severe asthma

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KEYWORDS
Urinary LTE_4;
Severe asthma;
Glucocorticoid-dependent

Summary Urinary LTE_4 reflects the whole body production of the cysteinyl-leukotrienes (LTC_4, LTD_4 and LTE_4) that are established mediators in asthma. The influence of chronic inhaled and oral glucocorticoid treatment on urinary excretion of leukotriene (LT) E_4 was investigated in subjects with asthma. Enzyme immunoassay analysis of LTE_4 was performed in spot urine samples collected from 40 patients with severe asthma, 25 patients with mild–moderate asthma and 20 non-asthmatic control subjects. Urinary LTE_4 was significantly higher in patients with severe asthma (69.7 ± 5.5) as compared to mild–moderate asthma (45.7 ± 3.3 with $P < 0.0004$) and control (42.5 ± 2.5 with $P < 0.0001$). Despite chronic systemic treatment with glucocorticoids, chronically severe asthma had presented with higher levels of LTE_4 compared to mild–moderate asthma and healthy controls. The findings support previous indications that one important component in asthmatic airway inflammation, the cysteinyl-leukotriene pathway remains relatively unopposed by oral glucocorticoids.

Introduction

Asthma is a chronic inflammatory disorder of the airways in which cysteinyl leukotrienes (CysLTs; LTC_4, LTD_4, and LTE_4) play a central role.[1] Leukotrienes are inflammatory mediators derived from arachidonic acid by the action of 5-lipoxygenase leading to the formation of LTA_4.[2] The action of LTC_4 synthase subsequently leads to the formation of LTC_4, which is transformed into LTD_4 and LTE_4. The potent biological action of the CysLTs includes bronchoconstriction,[3] induction of plasma exudation [4] as well as promotion of mucus secretion in the airways.[5] Several inflammatory cells possess the biosynthetic capacity to produce CysLTs, and LTE_4 is the end-product of metabolism CysLTs in the human lung.[6] It is established that measurement of the excretion of LTE_4 in the urine provides an index of CysLT production, and can be used in patients with asthma.[7–9]

Glucocorticoids are the most potent and effective anti-inflammatory agents used in the treatment of asthma.[10] There are severe asthmatics who require long-term treatment with systemic glucocorticoids to control their symptoms and these patients are thus usually called glucocorticoid-dependent severe asthmatics.[11] We have previously demonstrated that long-term corticotherapy in vivo attenuated the formation of 5-lipoxygenase products in human polymorphonuclear leukocytes (PMN) stimulated ex vivo but no effect was seen after short-term in vivo or in vitro treatment.[12]

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doi:10.1016/S0954-6111(03)00253-1

The aim of the present study was to determine if baseline urinary excretion of LTE_4 was different in severe asthma with patients treated chronically with moderate to high doses of oral steroids. To test this hypothesis we compared a group of patients with severe asthma to a group of patients with mild–moderate asthma and a group of non-asthmatic control subjects.

Patients and methods

Patients

Asthma was defined according to the American Thoracic Society criteria.[13] None of the patients suffered from afflictions that could affect urinary LTE4 (e.g. kidney or liver function abnormalities or other inflammatory diseases).[14–16] Clinical characteristic of all the subjects are presented in Table 1.

Forty non-smoking patients with severe asthma ranging in age from 22 to 74 years were included in the study according to methods previously described.[17] Severe asthma was determined according to 2 years follow-up of these patients when despite a clinical optimal management, we felt to wean these patients from oral steroids. All these patients were treated on a regular basis with prednisone (mean dose $\pm$ SD: 31.3 ± 23.1 mg/day) and 2000 μg of inhaled fluticasone propionate, 100 μg of salmeterol and 10 mg/kg of long acting theophylline. Four of the patients were aspirin-intolerant as documented by history. The compliance with prednisone therapy was assessed by measuring 8 AM plasma and saliva cortisol levels (Table 1).

Twenty-five non-smoking patients with mild–moderate asthma ranging in age from 23 to 69 years were also included. They were all symptomatic and their asthma was classified as mild–moderate according to the GINA guidelines.[18] Half of them were treated with low doses of inhaled steroids (less than 1000 μg) (Table 1). All these patients had no symptoms at the time of investigation.

Twenty non-smoking healthy volunteers ranging in age from 25 to 63 years were also included in the study. None of the healthy volunteers had ever suffered from asthma and had no respiratory tract infection.

Spot urine samples were collected in the morning (between 8.30 and 10.30 pm) and a venipuncture was performed in severe asthmatic patients for evaluation of plasma cortisol, which was evaluated using a competitive radioimmunoassay. Moreover, at the same time of venipuncture, saliva was collected and salivary cortisol was measured using a commercial enzyme immunoassay kit. This study was performed after informed consent and fulfilled the criteria of the ethic committee of the University of Montpellier.

LTE_4 measurements

Enzyme immunoassay (EIA) analysis of urinary LTE_4 was performed with rabbit polyclonal antiserum and acetylcholine esterase-linked tracer (Cayman Chemical, Ann Arbor, MI, USA) as previously described.[19] Microtitre plates were coated with a mouse monoclonal anti-rabbit IgG antibody. Solutions of synthetic LTE_4 standard, LTE_4 acetylcholine esterase tracer and LTE_4 antiserum prepared in EIA buffer were used. Several dilutions of unextracted urine samples were added in duplicates. After incubation overnight in darkness at room temperature, the enzyme substrate (Ellman's reagent) was added. The working detection limit was 8 pg/ml.

Table 1 Clinical characteristic of subjects.

	Asthmatics		
	Controls	Mild–moderate	Severe
Number	20	25	40
Age (years)*	46.0 ± 12.9	47.8 ± 13.2	54.6 ± 15.3
Sex	12F/8M	9F/16M	15F/25M
FEV1 (% of predicted)	N.A.	82.7 ± 15.9	62.3 ± 22.9**
Oral steroids dose (mg/day)	No	No	31.3 ± 23.1
Inhaled steroids dose (μg/day)	No	456 ± 488	2000
Plasma cortisol (μg/100 ml)[a]	N.A.	N.A.	4.9 ± 6.9
Salivary cortisol (μg/100 ml)[b]	N.A.	N.A.	0.2 ± 0.4

*Significant difference using Kruskal–Wallis test $P = 0.02$.
**Significant difference using Mann–Whitney test $P = 0.0005$.
[a]Normal value : 10–22 μg/100 ml.
[b]Normal value : >0.2 μg/100 ml.

Creatinine was measured by a colorimetrical alkaline picrate method (Sigma, St. Louis, MO, USA) and results are expressed as ng LTE_4 per mmol creatinine.

Statistical analysis

All the data are expressed as median and interquartile or mean $\pm$ SD. For comparison of LTE_4 excretion among the three groups, a non-parametric Kruskal–Wallis and a post hoc Bonferroni–Dunn test were performed. All the correlations were analysed using the Spearman rank test. The significance level was set at $P<0.05$.

Results

Clinical features

Subject characteristics are summarized in Table 1. In severe asthma patients FEV_1 was lower than in patients with mild–moderate asthma (62.3 $\pm$ 22.9% predicted versus 82.7 $\pm$ 15.9, with $P<0.0005$ using the Mann–Whitney U test).

The plasma and saliva levels of cortisol in the severe asthmatics were decreased as compared to normal levels (Table 1). This supports compliance with the prednisone therapy.

LTE_4 excretion

The urinary excretion of LTE_4 in the three groups is presented in Fig. 1. Urinary LTE_4 was significantly higher in patients with severe asthma (69.7 $\pm$ 5.5) as compared to mild–moderate asthmatics (45.7 $\pm$ 3.3, $P = 0.0004$ using Kruskal–Wallis test) and control subjects (42.5 $\pm$ 2.5, $P<0.0001$ using Kruskal–Wallis test). There was no significant difference in LTE_4 levels between patients with mild–moderate asthma and healthy controls (45.7 $\pm$ 3.3 vs. 42.5 $\pm$ 2.5, NS).

Data correlations

There was an inverse correlation between the FEV_1 value and the dose of oral steroids in severe asthma (Rho $= -0.593$, $P = 0.0002$), and a direct correlation between the plasma and saliva cortisol values (Rho $= 0.714$, $P = 0.0001$).

No correlation was found between the urinary LTE_4 and any of the clinical parameters (FEV_1, steroid doses, plasma or saliva cortisol).

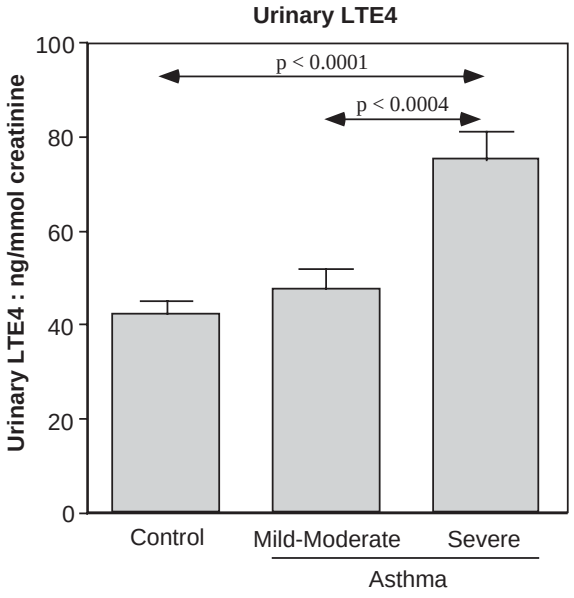

Figure 1 Urinary leukotriene E_4 (LTE_4) in mild–moderate asthmatics ($n = 25$), severe asthmatics ($n = 40$) and control subjects ($n = 20$). The Kruskall–Wallis test gave an overall significance difference ($P < 0.0001$). * $P < 0.0001$ and ** $P = 0.0004$ using a post hoc Bonferroni–Dunn test are the significant difference with the severe asthma group.

Discussion

Urinary levels of LTE_4 were measured in patients with mild–moderate and severe asthma and in control subjects. We observed that the production of CysLTs, as reflected in urinary LTE_4 concentrations, was significantly higher in severe asthma as compared to mild–moderate asthma and control subjects. There was however no difference in urinary LTE_4 levels between the mild–moderate asthmatics and controls, which agrees well with previous findings.[20,21] The method used for the measurements of LTE_4 has been extensively validated [14] and there appears to be no diurnal variation of LTE4 excretion,[19] unless subjects have an asthma attack. In addition, for this study all samples were collected at the same time interval during the morning hours. Another potential bias, severe reduction in related function, could be excluded because all subjects had serum creatinine levels within the normal range. It is established that subjects with aspirin-intolerance have increased urinary LTE_4.[8,20] However, only four (10%) of the steroid-dependent asthmatic patients were aspirin-intolerant. The LTE_4 values for these subjects were not the highest ones and fell within the range of others. The higher levels remained even if these subjects were excluded. Finally, the small difference in age between the steroid-dependent

asthmatic patients and controlled asthmatics is unlikely to influence the results.

It is of note that the severe asthmatic patients had been treated for at least 2 years with oral and inhaled steroids, but despite this, they still presented recurrent threatening episodes of acute asthma, and excreted high levels of LTE_4 into the urine. In this context, there are observations suggesting that CysLTs indeed are important mediators of asthma exacerbations [9] and are well known to amplify inflammation and bronchoconstriction. The constant release of these mediators may therefore contribute to the persistence of symptoms leading to an uncontrolled asthma in this group of patients.

Concerning the action of glucocorticoids on inflammatory mediators, their effectiveness is different for different cellular functions and also relates to the duration of the treatment. In previous studies, it has been found that, there was no significant change in the level of eicosanoids, and more specifically urinary LTE_4, after short-term treatment for 6–9 days of prednisone in 14 atopic asthmatics.[22] Likewise, 2 weeks treatment with fluticasone propionate did not inhibit allergen-induced urinary LTE_4 excretion.[23] The higher urinary LTE_4 level in the steroid-dependent asthmatic patients in this study is therefore a further indication that steroids do not cause major inhibition of the biosynthesis of CysLTs in vivo. We cannot conclude from this cross-sectional study of spot samples whether or not steroids may have had some inhibitory effects on the leukotriene pathway, as the patients with severe asthma theoretically may have had even higher levels of LTE_4 before treatment. For example, in a recent study, it was found that long-term systemic glucocorticoid treatment did modify the biosynthesis of LTB_4 produced by stimulated blood PMNs ex vivo, whereas short-term treatment did not have any effect.[12] However, even if such an effect may be detectable also on the CysLT pathway, its importance appears marginal in view of the high levels of leukotriene excretion and hence production that was found in this investigation of patients that had been treated with oral steroids for two years or more. The patients' compliance with treatment was documented by measurements of plasma and salivary cortisol levels that in addition correlated well.

In summary, our study for the fist time demonstrates that patients with severe asthma treated with inhaled and oral steroids excrete higher levels of LTE_4 into the urine compared with non-asthmatics or patients with mild–moderate asthma. This generates the hypothesis that clinical control of asthma may be improved in severe asthma if the potent pro-inflammatory effects of the leukotrienes are opposed by treatment with anti-leukotriene drugs. Recent studies indeed support that anti-asthmatic effects over and above those produced by glucocorticoids may be obtained by addition of anti-leukotriene drugs.[24–27] Furthermore, the unopposed leukotriene production in these subjects may be one explanation of why they still had asthma exacerbations during the otherwise beneficial steroid treatment. CysLT are potent bronchoconstrictors and their inhalation produces changes in the lung perfusion-ventilation ratio that share many characteristics of an asthma attack.[28] Our findings warrant a study of the influence of long term addition of anti-leukotriene drugs to steroid therapy in severe asthmatic patients.

Acknowledgements

The technical assistance of Ms. Kerstin Ström is gratefully acknowledged. This study was supported by the Direction de la Recherche Clinique, CHU Montpellier (PHRC 1997 no. 7572 and AOI 1995 no. 7515), the Swedish Medical Research Council (99PU-12754, 14X-13047, 14X-9071), the Swedish Heart Lung Foundation, the Asthma and Allergy Foundation, Vårdalstiftelsen and Karolinska Institutet.

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