

Clinical aspects of allergic disease

Urinary leukotriene E₄ in the assessment of nocturnal asthma

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Background: Urinary leukotriene E₄ (LTE₄) is a marker of the body's production of cysteinyl LTs, important mediators of airway inflammation. The role of the latter in nocturnal asthma is a topic of increasing interest.

Objective: This investigation was aimed at determining whether nighttime attacks are associated with increased release of LTs, expressed by urinary LTE₄, and the relationship between the two phenomena.

Methods: Three groups were studied: group A, seven control subjects; group B, nine asthmatic patients without nocturnal attacks; and group C, nine asthmatic patients with a comparable daytime FEV₁ but who were experiencing nocturnal exacerbations (morning dips in peak expiratory flow >20%). Urine was collected over 24 hours in three samples (9:00 AM to 3:00 PM; 3:00 PM to 9:00 PM; and 9:00 PM to 9:00 AM). LTE₄ was measured by high-performance liquid chromatography and radioimmunoassay and expressed as nanograms per millimole of creatinine.

Results: No significant differences between urinary LTE₄ were noticed within groups A and B. Conversely, in group C urinary LTE₄ at night (geometric mean with 95% confidence interval: 35.16 with 28.77-42.85) was significantly higher than that of the other samples (respectively 23.12 with 17.78-30.06, $p < 0.05$; and 25.18 with 21.03-30.13, $p < 0.02$); it was also significantly higher than in all the samples of other groups. A significant ($p < 0.02$) linear correlation was observed between morning dip in peak expiratory flow and the log urinary LTE₄ in the nocturnal sample.

Conclusion: These results indicate the role of LTs in nocturnal asthma and suggest that urinary LTE₄ may be a useful marker of this condition. (*J ALLERGY CLIN IMMUNOL* 1996;97:735-41.)

Key words: Asthma, nocturnal asthma, leukotrienes, LTE₄, airway inflammation

Cysteinyl leukotrienes (LTC₄, LTD₄, LTE₄) are important spasmogenic and proinflammatory mediators in bronchial asthma.¹ LTE₄ is a stable product of their metabolism, excreted in urine²⁻⁴,

Abbreviations used

LT: Leukotriene
PEF: Peak expiratory flow

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therefore, with normal renal function, its urinary concentration may be assumed to be a reliable marker of the overall LT production in the body. A significant increase in this concentration was first reported by Taylor et al.⁵ after antigen inhalation challenge, as well as after an acute asthmatic attack.

Thereafter, similar results have been reported both in spontaneous exacerbations of asthma⁶ and in models of bronchoconstriction induced by expo-

sure to allergens,⁷⁻⁹ aspirin,^{10,11} and platelet-activating factor¹²; whereas measurements carried out in exercise-induced bronchoconstriction have led to conflicting results.^{13,14}

Nocturnal exacerbations are very common events among patients with asthma: airway inflammation is likely to be a major contributor to the pathogenesis of such exacerbations¹⁵ with the intervention of many different cell types,^{16,17} some of which (mast cells, eosinophils, and mononuclear phagocytes) are known to be capable of generating cysteinyl LTs.¹⁸

This study was aimed at assessing the role of the latter mediators in the pathogenesis of nocturnal asthma: the assessment was based on the evaluation of the pattern of LTE₄ excretion over 24 hours in patients with asthma who were having recurrent nocturnal exacerbations, in comparison with patients with asthma not having nocturnal exacerbations and with control subjects.

METHODS

Subjects

Three groups of volunteers were studied, after the investigation had been approved by the appropriate ethical committee. In group A there were seven control subjects (men, aged 26 to 37 years), selected because of the lack of family and personal history of asthma or other respiratory diseases; all of them showed spirometric and peak expiratory flow (PEF) values within normal limits. In group B there were nine patients (men, aged 26 to 60 years) affected by bronchial asthma, defined according to the American Thoracic Society criteria,¹⁹ selected because of stable conditions (no symptom exacerbation or change in therapeutic regimen in the 2 months preceding the study), no recent history of nocturnal asthma, and absence of morning dips in PEF (i.e., a ratio of early morning value to maximal daily value higher than 80%) in the 2 weeks preceding the study. In group C there were nine patients with nonseasonal bronchial asthma (men, aged 35 to 70 years), with a degree of daytime FEV₁ not statistically different from that of group B, but who were having nocturnal exacerbations, as expressed by recurrence of morning dips in PEF (i.e., a ratio of early morning value to maximal daily value lower than 80%) over 5 days or more in the week preceding the study.

All of the subjects belonging to groups B and C were being treated with inhaled steroids (beclomethasone dipropionate on a regular basis) and inhaled β_2 -agonists (as needed) before selection; the treatment was kept unmodified throughout the study.

All subjects gave written informed consent to participation in the study.

Study design

On the study day all urine voided over 24 hours was collected from each subject in three samples, relevant,

respectively, to the periods 9:00 AM to 3:00 PM, 3:00 PM to 9:00 PM, and 9:00 PM to 9:00 AM. On the same day subjects had FEV₁ measured in the afternoon, between 3:30 and 5:30 PM. PEF monitoring was continued through the study day.

Measurement of urinary LTE₄

Urine samples were stored at -80°C until analysis. Samples were thawed and to each 5 ml aliquot, 4000 cpm of tritiated-LTE₄ was added (Du Pont de Nemours Italiana-NEN Products, Cologno M., Italy). The sample was loaded into an LC18 cartridge (Supelco, Bellefonte, Pa.), preconditioned with methanol 100% (10 ml), distilled water (10 ml), and formic acid at pH 3. Cysteinyl LTs were eluted with methanol 80% and water at pH 6; this fraction was then evaporated to dryness and reconstituted with 500 μl of aqueous methanol (40%). Separation of LTE₄ was performed by high-performance liquid chromatography with a System Gold apparatus (Beckman Instruments Inc., Fullerton, Calif.), equipped with a UV168 detector, an RP-18 precolumn (Waters Associates, Milford, Mass.), and an Ultrasphere 25 cm octadecylsilyl 5 μm column (Beckman). Gradient system included two solvents (a, 50/50/0.02 methanol, water and acetic acid at pH 5.5 with ammonium hydroxide; b, methanol 100%). A flow rate of $1\text{ ml} \cdot \text{min}^{-1}$ was used: under these conditions LTE₄ was eluted at between 25 and 30 minutes. Thirty-five 1 ml fractions were collected and dried; 200 μl of each fraction was used for the assessment of recovery and LTE₄ elution by measuring radioactivity (LS1801 β -counter, Beckman). Eight hundred microliters of fraction containing tritiated LTE₄ and the three fractions eluted before and after the peak were evaporated and redissolved in 250 μl of radioimmunoassay buffer; then LTE₄ was measured by a radioimmunoassay commercial kit (Amersham Italia, Milan, Italy). LTE₄ was the radioligand, and samples were quantified against an LTE₄ standard curve with a monoclonal antibody for LTC₄ that had good cross-reactivity with LTE₄. All measurements were made in duplicate, corrected for recovery (45% to 65%), and expressed in nanograms per millimole of creatinine; the latter was evaluated by a spectrophotometric method (Sera Pak Ames, Miles Italiana S.p.A., Milan, Italy). Previously established inter- and intra-assay coefficients of variability were 12% and 7%, respectively; sensitivity was 8 pg.

Statistical analysis

Because of the skewness of distribution, data were expressed in terms of geometric means and 95% confidence intervals of the mean.²⁰ Correlations were examined by parametric (least-square method) regression analysis; comparisons were made by analysis of variance, testing each pair of results, taken separately, by Fisher's protected least significant difference.²¹

TABLE I. Characteristics of subjects

Subject No.	Age (yr)	Sex	Atopy	Smoker	FEV ₁ (% pred)	Urinary LTE ₄ concentrations (ng/mmol of creatinine)		
						9 AM-3 PM	3 PM-9 PM	9 PM-9 AM
Group A								
1	34	M	No	No	102	44.9	39.5	36.3
2	26	M	No	No	102	20.2	13.8	16.9
3	33	M	No	No	96	17.9	21.9	16.7
4	34	M	No	No	94	32.5	12.0	25.6
5	27	M	No	No	104	12.5	14.7	12.3
6	37	M	No	No	97	15.0	22.6	20.8
7	37	M	No	No	92	17.0	13.4	18.1
Mean	32.6*				98.1*	20.79†	18.11†	19.86†
	4.4				4.6	13.71-31.55	12.25-26.70	14.39-27.35
Group B								
1	50	M	No	No	119	14.6	12.1	11.2
2	44	M	Yes	Yes	82	12.5	12.0	13.1
3	53	M	No	No	101	32.0	19.1	22.8
4	30	M	Yes	Yes	88	10.8	13.7	30.6
5	60	M	No	Ex	56	11.5	9.30	14.8
6	26	F	Yes	No	93	11.9	15.7	17.4
7	42	M	No	Ex	75	30.7	39.4	40.2
8	27	M	Yes	Ex	107	22.9	32.5	24.5
9	37	F	No	Ex	53	42.0	47.8	47.5
Mean	41.0*				86.0*	18.49†	19.10†	22.03†
	12.0				22.2	12.39-27.67	12.19-27.99	14.99-32.43
Group C								
1	37	F	No	Ex	100	22.5	20.4	24.6
2	70	F	No	No	63	27.0	34.5	37.0
3	35	M	Yes	No	53	32.9	19.3	26.9
4	40	F	No	No	95	30.2	25.9	52.9
5	40	M	No	No	73	28.8	26.7	27.6
6	39	F	No	No	88	23.2	24.9	35.2
7	40	F	Yes	No	79	10.8	36.4	42.1
8	43	F	No	Ex	100	17.5	19.0	46.5
9	52	M	Yes	No	83	24.9	25.1	33.0
Mean	44.0*				81.6*	23.12†	25.18†	35.16†
	10.9				16.4	17.78-30.06	21.03-30.13	28.77-42.85

*Arithmetic mean and standard deviation.

†Geometric mean and confidence interval.

RESULTS

Main characteristics of investigated subjects and individual values of urinary LTE₄ concentrations in different samples are shown in Table I.

No correlation between the log concentrations of the 3:00 PM to 9:00 PM period (assumed as baseline) and FEV₁ (expressed as percent of predicted value) was found, either in the overall series (Fig. 1) or within each group.

As concerns comparisons within groups, no significant differences between urinary LTE₄ concentrations in samples relevant to different

periods of the day were observed in control subjects; similar results were found in patients with asthma not experiencing nocturnal attacks. Conversely, in subjects with nocturnal asthma the excretion of LTE₄ was significantly higher during the night than in the other two periods under investigation ($p < 0.05$ vs the 9:00 AM to 3:00 PM sample; $p < 0.02$ vs the 3:00 PM to 9:00 PM sample) (Fig. 2).

As concerns comparisons between groups, although mean values relevant to group C were always higher, the difference in LTE₄ concentra-

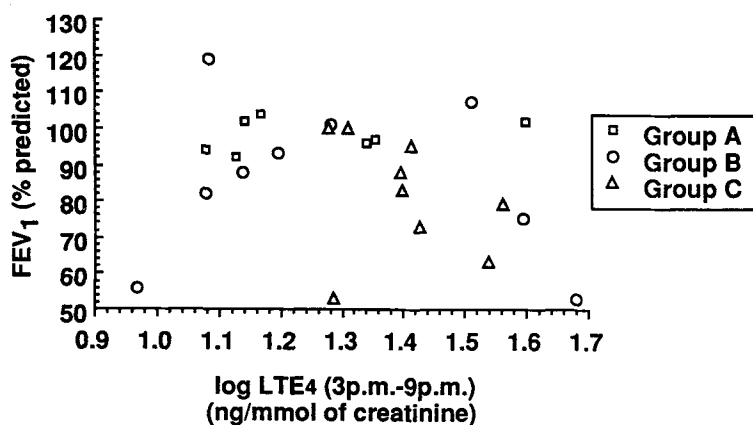


FIG. 1. Relationship between log concentrations of LTE_4 in urine sample relevant to the period 3:00 PM to 9:00 PM and FEV_1 (expressed as percent of predicted normal value).

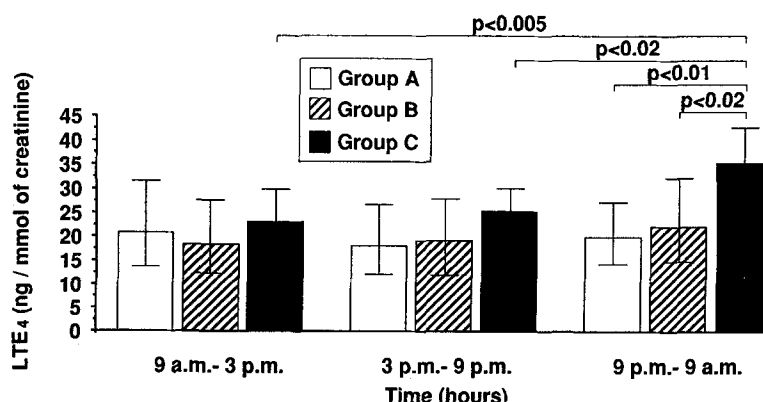


FIG. 2. Comparison between urinary concentrations of LTE_4 in different groups. Data are expressed as geometric means; 95% confidence intervals are also reported. Significance of statistical differences between nocturnal samples of group C and other samples is indicated.

tions was not statistically significant for samples relevant to the periods 9:00 AM to 3:00 PM and 3:00 PM to 9:00 PM. Conversely, in the nocturnal sample the concentration was significantly higher in group C than in the other two groups ($p < 0.01$ vs group A; $p < 0.02$ vs group B) (Fig. 2).

When the results of the three groups were examined together, a significant ($p < 0.02$) linear correlation was found between the magnitudes of morning dips in PEF and the log concentrations of LTE_4 in the nocturnal samples (Fig. 3).

DISCUSSION

Nocturnal exacerbations of asthma are very common²² and disturbing phenomena, with serious consequences for patients in terms of quality of life. In addition, the recurrence of attacks may have unfavorable prognostic implications: the mortality rate is increased at night,²³ and a worse

long-term clinical course has been observed in patients with recurrent attacks.²⁴

Both of these implications may be explained in the light of the airway inflammatory condition underlying this pattern of asthma. In fact, although the exact mechanisms leading to nocturnal attacks are still under debate, in recent years it has been repeatedly demonstrated by bronchoalveolar lavage that at least some of the cells and mediators of inflammation associated with the pathogenesis and clinical course of asthma in general are also involved in the nocturnal pattern of presentation. Practical monitoring of inflammation in asthma is a topic of increased interest: this unsolved problem is made even more difficult in the setting of nocturnal asthma, because invasive techniques (such as bronchoalveolar lavage) are not suitable because of the need to leave sleep as undisturbed as possible. Disruption would actually impair the

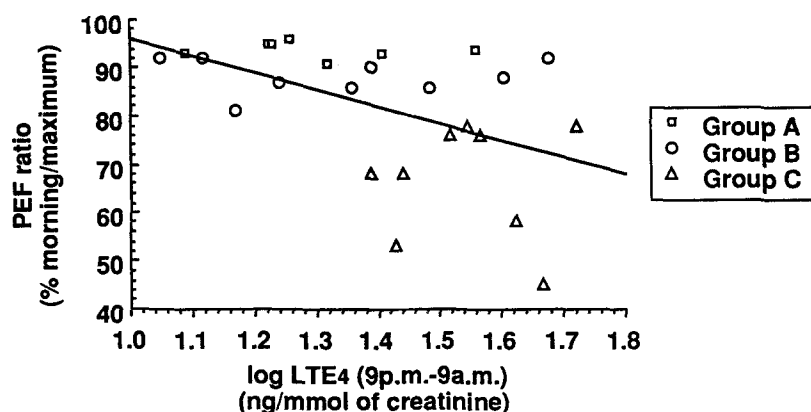


FIG. 3. Linear correlation between log concentration of LTE_4 in nocturnal urine sample and the morning dip in PEF (expressed as ratio of early morning value to maximum daily value).

quality of sleep and would potentially interfere with the phenomenon under investigation, because sleep itself may be a pathogenetic factor.²⁵

LTs are among the most important mediators of asthma: in particular, cysteinyl LTs in the airways are potent smooth muscle contraction agonists, and they affect microvascular permeability and stimulate mucus secretion.¹ LTE_4 is a stable product of metabolism of cysteinyl LTs, derived by cleavage of a glycine residue from LTD_4 , which, in turn, is the result of the removal of a glutamic acid residue from LTC_4 . In case of infusion^{2,4} or inhalation³ of cysteinyl LTs, LTE_4 is excreted in urine in a relatively constant proportion; therefore, its urinary concentration has been applied as an integrated measure of the overall LT production in the body in studies concerning both spontaneous^{5,6} and induced^{5,7-9,26,27} attacks of asthma.

The results of this study indicate that nocturnal exacerbations of asthma are accompanied by an increased release of cysteinyl LTs. Conversely, the results relevant to groups A and B confirm the absence of spontaneous significant day-to-night variations in the amount of LTE_4 excreted in urine, already reported in patients with asthma without nocturnal attacks.²⁷ In addition, urinary concentration of the mediator was found to be linearly correlated to the magnitude of morning dip in PEF: because the latter has been previously demonstrated to be a reliable marker of the degree of bronchoconstriction at night,²⁸ it is reasonable to assume that the release in LTs is directly involved in the pathogenesis of nocturnal bronchoconstriction.

Conversely, airway function during the day does not seem to be influenced by baseline release of LTs, as demonstrated by the lack of correlation

between FEV_1 and measurement of LTE_4 in the afternoon sample; however, the trend toward higher levels recorded in all urine samples of group C, though not statistically significant, is compatible with the belief that in subjects with nocturnal asthma some degree of airway inflammation is present throughout the day and causes increased bronchial reactivity, the effects of which are manifested at night because of the intervention of specific or nonspecific eliciting factors (e.g., allergens, sleep stages, posture, airway cooling, gastroesophageal reflux, etc.).

In these subjects the magnitude of nocturnal increases in LTE_4 , with respect to baseline levels, is lower than that reported for the early reaction to allergens: however, in the experimental setting of specific challenge tests the stimulus and the response are concentrated over a restricted period. Conversely, in spontaneously occurring nocturnal exacerbations of asthma, obstructive phenomena develop over several hours, often undergoing repeated changes of degree in both directions, which could correspond to the changing effect of the just cited eliciting factors, possibly resulting in oscillations in the magnitude of mediator release.

In addition, it must be emphasized that the actual figures relevant to LTE_4 concentrations underestimate to some extent the magnitude of 5-lipoxygenase mediators released in the airways during nocturnal attacks for two reasons. First, LTE_4 accounts for only a small proportion of the total metabolites from the lung. Second, urine was collected at the end of the night in order to avoid disturbing the spontaneous course of sleep; however, it has been repeatedly demonstrated that the nadir of respiratory function is reached around 4:00 AM.^{25,29} If so, sampling all urine spontaneously

voided up to 9:00 AM may actually result in a dilution effect caused by the contribution of the low concentration samples relevant to the earlier phase of the night. Conversely, it may be hypothesized that some inhibitory effect on the release of LTs may have been induced by treatment with inhaled corticosteroids; however, such an effect is not likely, because previous studies have shown that neither oral³⁰ nor inhaled steroids^{30,31} are able to affect urinary excretion of LTE₄ in different study models.

On the other hand, in spite of these possible limitations from the quantitative point of view, the degree of functional impairment at night (as reflected by the early morning residual obstruction) and the overall release of LTE₄ over the night showed a statistical correlation. This finding suggests some causative role of cysteinyl LTs in nocturnal asthma. Further clinical evaluations are warranted to evaluate the possibility of introducing this measurement as a noninvasive humoral marker of inflammation, possibly suitable for the follow-up of this common and potentially dangerous clinical condition.

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