Eur Respir J, 1994, 7, 907–913
DOI: 10.1183/09031936.94.07050907
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

Increased urinary LTE_4 excretion following inhalation of LTC_4 and LTE_4 in asthmatic subjects

P.E. Christie,* P. Tagari**, A.W. Ford-Hutchinson**, C. Black**, A. Markendorf+, M. Schmitz-Schumann+, T.H. Lee++.

Increased urinary LTE_4 excretion following inhalation of LTC_4 and LTE_4 in asthmatic subjects. P.E. Christie, P. Tagari, A.W. Ford-Hutchinson, C. Black, A. Markendorf, M. Schmitz-Schumann, T.H. Lee. ©ERS Journals Ltd 1994.

ABSTRACT: Urinary leukotriene E_4 (LTE_4) increases during exacerbations of asthma and following antigen challenge. We determined whether urinary LTE_4 excretion reflects sulphidopeptide leukotrienes in the airways of asthmatic patients.

Urinary LTE_4 concentration was measured prior to and 1.5 and 3.5 h following inhalation of bronchoconstrictive doses of leukotriene C_4 (LTC_4) or LTE_4 in eight asthmatic subjects. Increasing doses of agonist were inhaled until a 35% fall in specific airways conductance (sGaw) was achieved.

There was no significant difference between the 53±3% (mean±SEM) fall in sGaw following inhalation of LTC_4 (63.1 ng geometric mean, GM, range 5.8–527.5 ng) and the 43±4% fall in sGaw following inhalation of LTE_4 7.94 ng/GM (range 132–3701 ng). The LTE_4 excretion rate increased significantly from 2.95 (range 0.6–17.5) ng·h^{-1} to 4.67 (range 0.8–20) ng·h^{-1} at 1.5 h following LTC_4 inhalation; and from 1.8 (range 0.07–6.7) ng·h^{-1} to 6.9 (range 2.9–27.3) ng·h^{-1} at 1.5 h following LTE_4 inhalation; and had returned from baseline by 3.5 h. There was a correlation between the dose of LTC_4 inhaled and LTE_4 excreted in the urine (r= 0.82 and r=0.72, respectively). The % recovery of LTE_4 in the urine, of the total dose of inhaled LTC_4 or LTE_4 administered, was 6.9±4.1% and 0.8±0.3%, respectively.

Thus, inhalation of bronchoconstricting doses of LTC_4 or LTE_4 alter urinary LTE_4 excretion in a dose-dependent fashion. This indicates that urinary LTE_4 can be used as a marker of sulphidopeptide leukotriene synthesis in the lungs of patients with asthma.

Eur Respir J., 1994, 7, 907–913.

*Swiss Institute for Allergy and Asthma Research, Davos, Switzerland. +Hochgebirgsklinik, Wolfgang-Davos, Switzerland. ++Dept of Allergy and Allied Respiratory Diseases, U.M.D.S., Guys' Hospital, London, UK. **Merck-Frosst, Quebec, Canada.

Correspondence: T.H. Lee
Department of Allergy and Allied Respiratory Disorders
4th Floor
Hunts House
Guys Hospital
London SE1 9RT
UK

Keywords: Asthma
leukotriene E_4
mediators
urine

Received: September 13 1993
Accepted after revision December 22 1993

The work was supported in part by the National Asthma Campaign, UK, and Sandoz Pharmaceuticals, Basel, Switzerland.

The sulphidopeptide leukotrienes (LTC_4, LTD_4 and LTE_4), previously recognized as slow-reacting substance of anaphylaxis (SRS-A) [1, 2], they are derived from arachidonic acid by the action of 5-lipoxygenase which generates 5-hydroperoxy-eicosatetranoic acid and then leukotriene A_4 (LTA_4). LTA_4 is metabolized by the addition of glutathione to LTC_4. LTC_4 may be converted by γ-glutamyl transpeptidase to generate LTD_4, which is converted by a dipeptidase to generate LTE_4 [3, 4].

In man, there is rapid metabolism of LTC_4 to LTD_4 and then to LTE_4. LTE_4 may be further metabolized to oxidation products which are excreted into bile and urine [5–7]. *In vitro* the sulphidopeptide leukotrienes LTC_4, LTD_4 and LTE_4, contract smooth muscle and enhance microvascular permeability [8–10]. In humans, they are potent bronchoconstrictor agents when inhaled and increase nonspecific bronchial hyperresponsiveness [10–12]. Combined reversed phase-high performance liquid chromatography (RP-HPLC) and radioimmunoassay (RIA) has enabled urinary LTE_4 concentration to be measured as an estimate of the production of sulphidopeptide leukotrienes *in vivo* [13]. Previous work has demonstrated an increase in LTE_4 excretion during an acute exacerbation of asthma compared to the recovery stage [14], and an increase in LTE_4 excretion following antigen challenge in asthmatic subjects [14, 15]. In a group of asthmatic subjects who were aspirin sensitive, baseline urinary LTE_4 concentration was raised with further release of LTE_4 following aspirin challenge [16]. There is no association between LTE_4 concentration and baseline lung function or degree of bronchial hyperresponsiveness to histamine [17, 18].

In normal healthy volunteers, the measurement of urinary LTE_4 following infusion of radiolabelled LTC_4 as either a bolus or infusion [19, 20], or inhalation of LTD_4 [21], suggests that urinary LTE_4 may act as a marker for whole body production of sulphidopeptide leukotrienes. No similar studies have been performed in asthmatic subjects and it is unknown how the local deposition and release of these mediators in asthmatic

airways alters urinary LTE_4 excretion. We have, therefore, determined the urinary LTE_4 excretion before and following inhalation of LTC_4 or LTE_4 in eight asthmatic subjects.

Methods

Subjects

Eight asthmatic subjects were studied (4 males, 4 females) aged 18–48 yrs, of whom three were atopic (table 1). Asthma was defined by a history of episodic wheezing and a greater than 20% reversibility of resting forced expiratory volume in one second (FEV_1) following 400 μg inhaled albuterol. Extrinsic asthmatic subjects demonstrated a greater than 3 mm wheal, as compared to the diluent control, in response to skin-prick tests to at least two common aeroallergens: grass pollen, tree pollen, cat dander, dog hair, *Dermatophagoides pteronyssinus* and *D. farinae*. Subject medication included inhaled albuterol and inhaled beclomethasone. Medication was withheld 8 h prior to provocation on each study day. Subjects had not taken antihistamines or cromolyn in the month prior to the study, and no subject had experienced an upper respiratory tract infection in the preceding month or during the study. The study protocol was approved by the Hochgebirgsklinik, Davos-Wolfgang Ethics Committee and each subject gave informed consent.

Study protocol

Subjects attended the laboratory on two occasions, separated by at least 10 days, when inhalation challenge with inhaled LTC_4 or LTE_4 was performed in a single-blind, randomized fashion. On attending the laboratory on the second occasion, subject No. 6 refused LTC_4 inhalation challenge. Urine samples were collected at baseline, 1.5 and 3.5 h following inhalation of LTC_4 or LTE_4.

Measurements of airway calibre

Measurements of specific airways conductance (sGaw) were made in a total body plethysmograph linked to a digital computer (Bodytest, Jaeger Ltd).

Inhalation challenge

Inhalation challenges were performed using the Asthma Provocation System (APS) Jaegar dosimeter which delivers compressed air at a pressure of 1.6 bar (22.8 psi) for a duration of 0.6 s from the start of each breath. Under these conditions, the nebulizer delivers droplets with a mass median aerodynamic diameter of 1.9 μ, and the output of the nebulizer is 5.8 $\mu l \cdot breath^{-1}$. The mean of five measurements of sGaw were recorded at baseline. Provided baseline sGaw was >0.7 $s \cdot kPa^{-1}$, inhalation challenge with agonist proceeded. Subjects inhaled control solution (10 breaths of phosphate buffered saline) (PBS). Each inhalation started at functional residual capacity and terminated at approximately 70% baseline vital capacity. A 5 s breathhold was maintained at the end of each inhalation. If the decrease in sGaw was <10% from baseline value, subjects underwent inhalation challenge with LTC_4 or LTE_4.

Inhalation challenge with LTC_4 and LTE_4

LTC_4 and LTE_4 were prepared by total chemical synthesis, as described previously, and frozen under argon at -70°C [1]. Each leukotriene was analysed before inhalation challenge by RP-HPLC on a 10 μ C_{18} ultrasil-ODS column (4.6×250 mm; Beckman Instruments Inc., Berkley, CA, USA), at a flow rate of 1 $ml \cdot min^{-1}$

Table 1. – Characteristics of patients studied

Subject No.	Age yrs	Sex	Atopy	Treatment	FEV_1 % pred	PD_{35}sGaw LTC_4 nmol	PD_{35}sGaw LTE_4 nmol
1	22	F	–	A	81	0.005	0.85
2	18	M	–	AB	98.5	0.40	0.20
3	45	M	–	AB	123	0.46	8.13
4	41	F	–	AB	105	0.018	0.13
5	24	F	–	A	99	0.04	3.2
6	48	M	+	AB	89	ND	0.20
7	48	F	+	A	89	0.42	7.5
8	46	M	+	AB	112	0.004	8.0
Mean	36				99.5	0.05	1.27
SEM	4.5				4.8		

The mean is the arithmetic mean for age and % predicted FEV_1; and the geometric mean for $PD_{35}sGawLTC_4$ and $PD_{35}sGawLTE_4$. LTC_4: leukotriene C_4; LTE_4: leukotriene E_4; sGaw: specific airway conductance; A: inhaled albuterol 200 μg *b.d. p.r.n.*; B: inhaled beclomethasone dipropionate 200 μg *b.d*; ND: LTC_4 inhalation challenge not performed in this subject; FEV_1: forced expiratory volume in one second; PD_{35}: provocative dose producing a 35% fall in sGaw.

with 65% methanol (BDH), 34.9% water, 0.1% acetic acid, pH 5.6, as solvent. Absorbance was monitored with an on-line spectrophotometer at 280 nm, linked to an integrator (Spectraphysics, Mountain View, CA, USA model SP 4270). The purity of each leukotriene was confirmed before challenge by its co-elution as a single peak at the identical retention times of the respective synthetic standards. The concentration of each leukotriene solution was assessed by ultraviolet scanning at 280 nm, assuming an extinction coefficient of 40,000 $cm^{-1} \cdot M^{-1}$, and dilutions of each leukotriene were prepared in PBS.

For LTC_4 and LTE_4 challenges, each subject inhaled 10 breaths of geometrically increasing concentrations starting at 4×10^{-8} M and 4×10^{-7} M up to a maximum concentration of 1×10^{-5} M and 1×10^{-4} M for LTC_4 and LTE_4, respectively. The initial concentration of each inhaled leukotriene was determined from previous studies [22, 23]. sGaw was measured at 2 and 5 min, and then at 5 min intervals for 15 min. If a 35% decrease in sGaw was not achieved, the concentration of leukotriene in the nebulizer was increased by three fold and the protocol was repeated.

Measurement of urinary LTE_4

Urine was collected prior to and at 1.5 and 3.5 h following inhalation of LTC_4 and LTE_4. The volume of urine was recorded and a 50 ml aliquot saved. The free radical scavenger 4 hydroxy-2,2,6,6-tetramethylpiperidino-oxy free radical (4-Hydroxy TEMPO; Aldrich Chemical Co., Milwaukee, WI) was added at a final concentration of 1 mM, and the samples adjusted to pH 9.0 with NaOH to stabilize endogenous leukotriene metabolites. The samples were coded and stored at -70°C until measurement of LTE_4 as described previously [13]. [^{3}H] LTC_4 1.14 nCi (38.4 $kCi \cdot mol^{-1}$; NEN, Lachine, Quebec, Canada) was added per millilitre of thawed urine samples, and the pH of the samples was adjusted to pH 5.4 with acetic acid. [^{3}H] LTC_4 and endogenous LTE_4 were then extracted from 10 ml aliquots of urine using an "in-line" reversed-phase precolumn (C_{18} Adsorbosphere, 5 μm diameter packing material; Alltech, Mandel Scientific, Lachine, Quebec, Canada). Leukotrienes were retrogradely eluted, *via* twin switching valves, onto a reversed-phase 10 mm diameter, 15 cm long, analytical column (C_{18} HS, 3 μm diameter packing material; Alltech, Deerfield, III, USA) with a mobile phase consisting of MeOH:ammonium buffer (0.1% containing 1 mM disodium ethylenediaminetetra-acetic acid (EDTA); pH 5.4) in the proportions 70:30 (v:v) at a flow rate of 1 $ml \cdot min^{-1}$. The column was calibrated for the retention times of synthetic LTC_4, (5.33±0.05 min, n=18) and LTE_4 (14.01±0.15 min, n=18). The radioactivity of the fractions eluting with retention time of synthetic LTC_4 was assessed by liquid scintillation spectrometry to determine leukotriene recovery. Fractions eluting with retention time of synthetic LTE_4 were evaporated to dryness under vacuum, and the LTE_4 concentration was measured by specific radioimmunoassay as described previously [13]. The sensitivity of the assay is 8 $pg \cdot ml^{-1}$. The intra- and interassay coefficients of variation were 12 and 16%, respectively.

Statistical analysis

At baseline and at 1.5 and 3.5 h following inhalation of LTC_4 or LTE_4, urinary volume, creatinine and leukotriene concentrations were measured, and urinary LTE_4 was expressed as $pg \cdot mg^{-1}$ creatinine. The LTE_4 excretion rate in $ng \cdot h^{-1}$ was calculated. The baseline LTE_4 excretion rate was determined from the LTE_4 concentration in the urine at baseline and the mean rate of creatinine excretion over the 3.5 h period, since we have previously shown that creatinine excretion rate does not vary significantly over this period of time [24]. The increase in LTE_4 concentration was determined as the difference in LTE_4 concentration between the value at baseline and 1.5 h, and 1.5 and 3.5 h. The percentage nanogram recovery of LTE_4 in the urine was determined as the nanogram increase in urinary LTE_4 concentration, compared to baseline urinary LTE_4 divided by total nanogram amount of LTC_4 or LTE_4 inhaled at the mouth. Values of LTE_4 concentration were log transformed prior to analysis. The "t-test" for paired observations was used to compare baseline sGaw on the separate study days and percentage change in sGaw following bronchoconstriction with LTC_4 or LTE_4. Comparison of urinary LTE_4 excretion rate and increase in urinary LTE_4 at baseline, 1.5 and 3.5 h following inhalation of LTC_4 or LTE_4 was performed using the Wilcoxon matched pairs test. The correlation between the increase in urinary LTE_4 excretion and the total inhaled dose of LTC_4 or LTE_4 at the mouth was calculated by least squares linear regression.

Results

LTC_4 study day

The cumulative dose of LTC_4 inhaled was 63.1 ng (GM), (range 5.8–527.5 ng) and was accompanied by a 53±3% (mean±SEM) fall in sGaw (table 2).

The baseline urinary LTE_4 concentration on the LTC_4 inhalation study day was 30.1 GM (range 5.8–150) $pg \cdot mg^{-1}$ creatinine. Following inhalation of LTC_4 there was a significant increase in urinary LTE_4 concentration at 1.5 h to 65.1, (range 13.9–154) $pg \cdot mg^{-1}$ creatinine ($p=<0.05$). At 3.5 h the urinary LTE_4 concentration was 45.1 GM, (range 0–93) $pg \cdot mg^{-1}$ creatinine which was similar to that of baseline urinary LTE_4 concentration ($p=>0.05$) (fig. 1). There was no correlation between baseline urinary LTE_4 concentration and the provocative dose of LTC_4 producing a 35% fall in sGaw (PD_{35} sGaw LTC_4), which was 0.05 GM, (range 0.004–0.46 nmol ($p=>0.05$).

Table 2. – The doses of LTC_4 or LTE_4, inhaled increases in LTE_4 and the percentage recovery of LTE_4 in the urine at 1.5 and 3.5 h after leukotriene inhalation

Subject No.	Inhaled dose ng	% fall in sGaw	Increase in LTE_4 ng		% recovery LTE_4	
			1.5 h	3.5 h	1.5 h	3.5 h
LTC_4 inhalation						
1	5.8	42	1.76	0	30.3	0
2	527.5	63	5.58	0	1.1	0
3	527.5	49	3.7	0	0.7	0
4	18.8	54	1.9	5.4	10.1	29
5	58	58	0.27	0.06	0.46	0.1
7	527.5	48	4.49	0	0.85	0
8	5.8	56	0.3	4.4	5.2	76
Mean	63.1	53	1.5	0.05	6.9	15.0
SEM		3.0			4.1	10.9
LTE_4 inhalation						
1	407.5	35	3.2	0	0.78	0
2	132.6	39	1.22	1.72	0.92	1.3
3	3701	36	40.8	19.2	1.1	0.5
4	132.6	58	4	0	3.0	0
5	3701	30	8	2.26	0.2	0.06
6	132.6	65	0.2	0	0.15	0
7	3701	40	3.3	1.1	0.09	0.0
8	3701	39	12.8	0	0.34	0
Mean	794	43	3.7	2.95	0.82	0.44
SEM		4.0			0.33	0.24

Means are the geometric means except for the % recovery of LTE_4 in the urine and % fall in sGaw which is the arithmetic mean±SEM. For further abbreviations see legend to table 1.

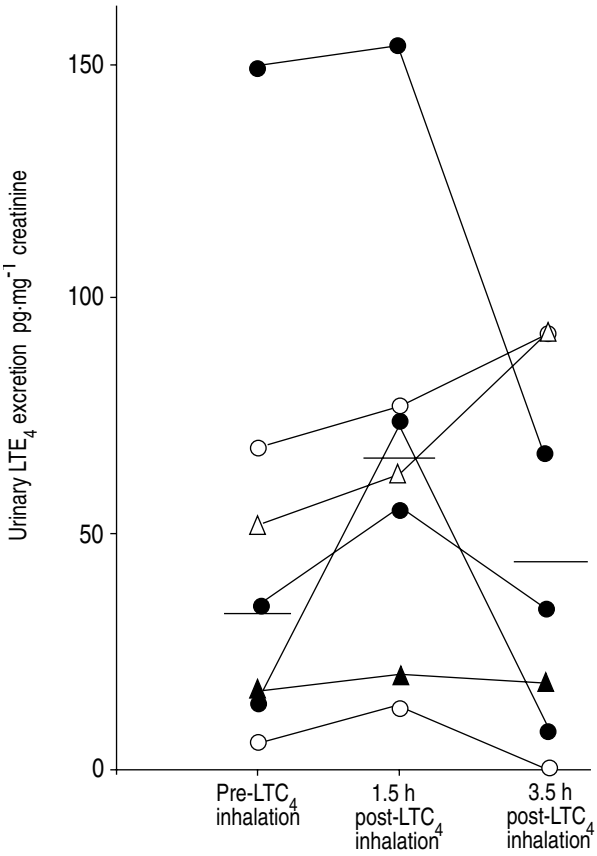

Fig. 1. – The increase in urinary leukotriene E_4 (LTE_4) excretion in asthmatic subjects prior to and at 1.5 and 3.5 h post-inhalation of leukotriene C_4 (LTC_4). Symbols denote inhaled dose of LTC_4:—●—: 520 ng; —▲— : 58 ng; —△— : 18 ng;—○—: 5 ng. Bars represent geometric means.

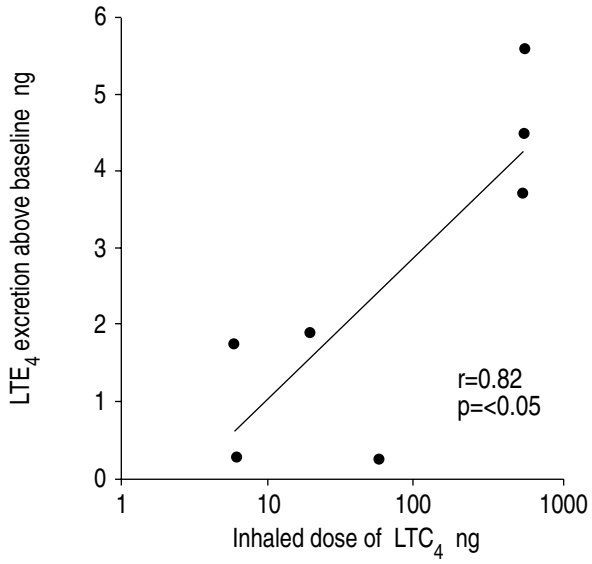

Fig. 2. – The correlation between the cumulative dose of leukotriene C_4 (LTC_4) inhaled and the increase in urinary leukotriene E_4 (LTE_4) above baseline LTE_4 at 1.5 h post-inhalation in asthmatic subjects.

Following inhalation of LTC_4, the increase in LTE_4 excretion at 1.5 h was 1.54 GM (range 0.27–5.5) ng ($p=<0.05$) (table 2). There was a positive correlation between the cumulative dose of LTC_4 inhaled at the mouth and the increase in LTE_4 over baseline values at 1.5 h following inhalation of LTC_4 ($r=0.82$; $p=<0.05$) (fig. 2).

The recovery of LTE_4 in the urine at 1.5 h following inhalation of LTC_4 was 6.9±4.1% (mean±SEM) (table 2). There was no significant recovery of LTE_4 in the urine at 3.5 h following inhalation of LTC_4.

Table 3. – The rate of urinary LTE_4 excretion ($ng \cdot h^{-1}$) after LTC_4 or LTE_4 inhalation

Subject	Post LTC_4 inhalation h			Post LTE_4 inhalation h		
No.	0	1.5	3.5	0	1.5	3.5
1	0.81	1.98	0	1.16	3.3	0.6
2	5.5	9.2	5.1	6.0	6.8	0.6
3	17.5	2.0	7	0.07	27.3	19.3
4	5.2	6.6	8.8	6.7	9.4	5.6
5	0.6	0.8	0.6	1.8	7.2	3.3
6	ND	ND	ND	2.7	2.9	1.8
7	1.5	4.4	1.1	1.6	3.8	2.3
8	6.2	6.4	9.2	4.7	13.3	0.1
GM	2.95	4.67	3.5	1.8	6.9	2.2

Time 0 denotes leukotriene excretion rate prior to LTC_4 or LTE_4 inhalation. GM: geometric mean; ND: challenge not performed in this subjects. For further abbreviations see legend to table 1.

The basal LTE_4 excretion rate on the LTC_4 inhalation day was 2.95 GM (range 0.6–17.5) $ng \cdot h^{-1}$ (table 3). Following inhalation of LTC_4 there was a significant increase in LTE_4 excretion rate to 4.67 GM, (range 0.8–20) $ng \cdot h^{-1}$ at 1.5 h (p=0.02), which had returned toward baseline by 3.5 h.

LTE_4 study day

The dose of LTE_4 inhaled was 794 GM (range 132.6–3701) ng, which was accompanied by a 43±4% (mean ±SEM) fall in sGaw (table 2).

The baseline LTE_4 concentration on the LTE_4 inhalation study day was 18.3 GM, (range 0.6–68) $pg \cdot mg^{-1}$ creatinine, which did not differ from the baseline LTE_4 concentration on the LTC_4 inhalation study day (p=0.4). Following inhalation of LTE_4, there was a significant increase in urinary LTE_4 concentration at 1.5 h to 84.8 GM, (range 18.9–209) $pg \cdot mg^{-1}$ creatinine (p=<0.05) (fig. 3). At 3.5 h the urinary LTE_4 concentration was 55 GM, (range 6.4–232) $pg \cdot mg^{-1}$ creatinine, which was similar to that of baseline urinary LTE_4 concentration (p=>0.05). There was no correlation between baseline urinary LTE_4 concentration and the PD_{35} sGaw LTE_4, which was 1.27 GM, (range 0.20–8.13) nmol.

Following inhalation of LTE_4, the increase in LTE_4 excretion over baseline at 1.5 h was 3.7 GM, (range 0.2–40.8) ng (p=<0.05), which had returned toward baseline value by 3.5 h (table 2). There was a positive correlation between the cumulative dose of LTE_4 inhaled at the mouth and the increase in LTE_4 over baseline LTE_4 at 1.5 h (r=0.72; p=<0.05) (fig. 4).

The basal LTE_4 excretion rate on the LTE_4 inhalation day was 1.8 GM, (range 0.07–6.7) $ng \cdot h^{-1}$ which did not differ significantly from that of the LTC_4 inhalation day (p=>0.05). Following inhalation of LTE_4 there was a significant increase in LTE_4 excretion rate to 6.9 $ng \cdot h^{-1}$ GM, (range 2.9–27.3) $ng \cdot h^{-1}$ at 1.5 h (p=<0.05), which had returned toward basline by 3.5 h (table 3).

The recovery of LTE_4 in the urine 1.5 h following inhalation of LTE_4 was 0.82±0.33% (mean±SEM)

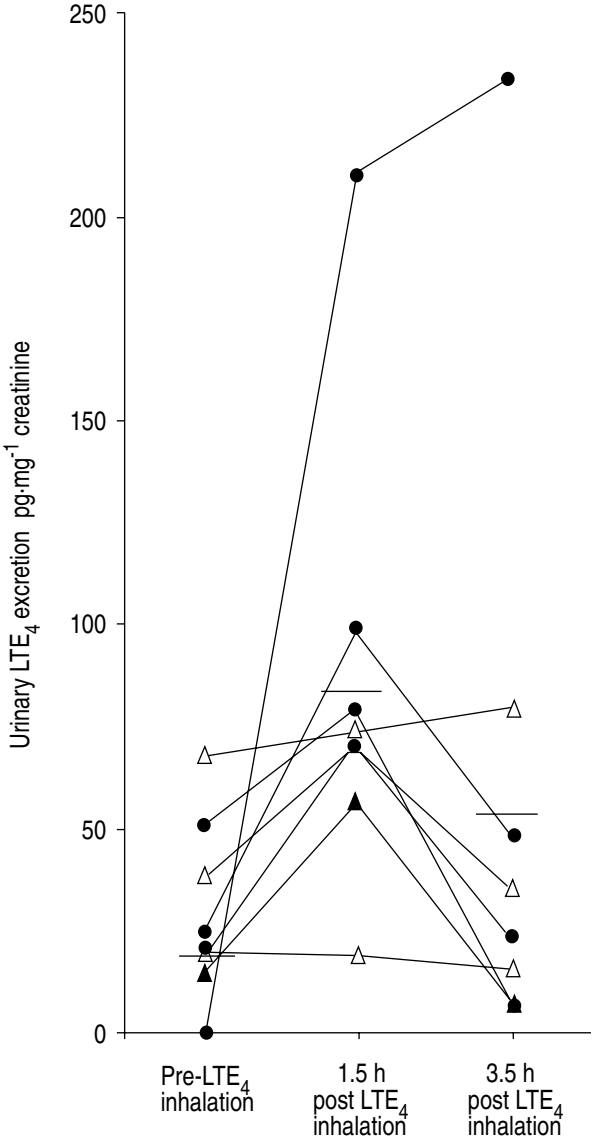

Fig. 3. – The increase in urinary leukotriene (LTE_4) excretion in asthmatic subjects prior to and at 1.5 and 3.5 h post-inhalation of LTE_4. Symbols denote inhalation of LTE_4: —●— : 3700 ng; —▲— : 400 ng; —△—: 130 ng. Bars represent geometric means.

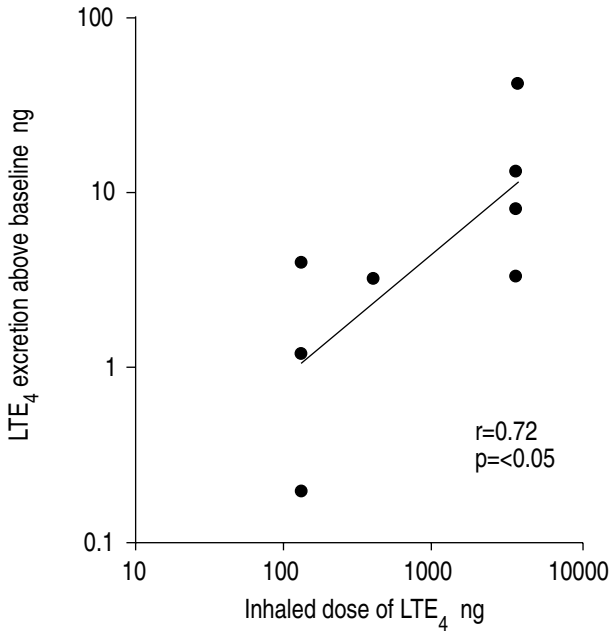

Fig. 4. – The correlation between the cumulative dose of leukotriene E_4 (LTE_4) inhaled and the increase in urinary LTE_4 above baseline LTE_4 at 1.5 h post-inhalation in asthmatic subjects.

(table 2). There was no significant recovery of LTE_4 in the urine at 3.5 h following inhalation of LTE_4.

Discussion

This study has demonstrated that inhalation of LTC_4 or LTE_4 in asthmatic subjects resulted in an increase in the LTE_4 excreted into the urine, and the increase correlated with the total dose of LTC_4 or LTE_4 inhaled. This suggests that urinary LTE_4 excretion can reflect change in sulphidopeptide leukotrienes in the airways of asthmatic subjects. This was supported in prior studies, where endogenous release of sulphidopeptide leukotriene after antigen-induced bronchoconstriction or acute asthma [14] resulted in increased urinary LTE_4 excretion. Although the dose of LTE_4 or LTC_4 inhaled at the mouth may differ from that administered to the bronchi, the observation that increases in LTE_4 excretion correlated with the dose of LTC_4 or LTE_4 administered at the mouth suggests that even small changes in pulmonary levels of leukotriene are reflected by changes in LTE_4 excretion. Although subject medication including inhaled albuterol and inhaled beclomethasone were withheld 8 h prior to inhalation challenge, one cannot exclude the possibility that these drugs may have an effect on leukotriene release, metabolism or excretion.

The timing of urine samples at 1.5 and 3.5 h was chosen from studies in asthmatic subjects where maximal LTE_4 excretion occurs within 4 h following antigen challenge [14]. The increase in urinary LTE_4 excretion following inhalation of LTC_4 or LTE_4 occurred within 1.5 h and returned towards baseline by 3.5 h. These results are consistent with rapid plasma clearance and local action of the sulphidopeptide leukotrienes.

There was no significant difference in the degree of bronchoconstriction following inhalation of LTC_4 or LTE_4 in the asthmatic subjects studied. There was a correlation between the dose of LTC_4 or LTE_4 inhaled and the increase in leukotriene excretion, such that a small increase in LTE_4 excretion occurred in subjects who had the greatest airway responsiveness to inhaled LTC_4 or LTE_4. Thus, release of endogenous pulmonary leukotrienes, which could play a role in bronchoconstriction, may be reflected by alterations in urinary LTE_4 concentration.

Approximately 7% of the total dose of LTC_4 inhaled in asthmatic subjects was recovered in the urine at 1.5 h. This finding is similar to that in a study by VERHAGEN *et al.* [21], where the fractional conversion of inhaled LTD_4 to urinary LTE_4 in normal subjects was 3% [21]. Following infusion of radiolabelled LTC_4 into healthy volunteers, 4–6% appeared in the urine as LTE_4 [19, 20]. Subsequent metabolism of LTE_4 to oxidative analogues, and biliary excretion of the sulphidopetide leukotrienes, which were not assessed in this study, probably accounts for the remaining excretion. In two subjects (Nos. 4 and 8), there was an increased percentage recovery of LTE_4 following LTC_4 inhalation at 3.5 h. There were no clinical features distinguishing these two subjects and, whilst one cannot exclude the possibility that there was altered leukotriene metabolism in these subjects, it is more likely that an overestimation of the degree of excretion occurred due to the smaller dose of LTC_4 inhaled in these subjects.

The more rapid cellular uptake of LTE_4, compared to LTC_4 and subsequent metabolism of LTE_4 to metabolites not measured in this study, may account for the decreased recovery of LTE_4 in the urine at 1.5 h following LTE_4 inhalation. UEHARA *et al.* [25] suggested that there are energy-dependent uptake processes for the transport of sulphidopeptide leukotrienes into cells, with a rank order of $LTE_4 > LTD_4 > LTC_4$.

The baseline urinary LTE_4 level in the asthmatic subjects studied was similar to that reported previously [14–16], and the 2–3 fold increase in LTE_4 excretion following inhalation of LTC_4 or LTE_4 was similar to that observed following antigen challenge [14, 15]. We were unable to demonstrate a correlation between urinary LTE_4 excretion and the degree of LTC_4- or LTE_4-induced bronchoconstriction consistent with prior studies [16, 17]. This is probably explained by the large variation in sensitivity of the airways to inhaled leukotrienes. Cumulative inhalations of 132–3701 ng LTE_4 (an approximate 40 fold range) produced similar changes in sGaw (table 2).

In summary, we have demonstrated that a wide range of bronchoconstrictive doses of cysteinyl leukotrienes can be quantitatively recovered in the urine of asthmatic subjects. These results do not differ from previous studies in normal subjects [19–21]. The data suggest that the leukotriene-driven "tone" of asthmatic airways, as demonstrated by the bronchodilatory effects of LTD_4 receptor antagonists [26, 27], is not caused by a defect in the metabolism or transfer of LTC_4 from

the lung. The ability of urinary LTE_4 determinations to detect sub-nanogram changes in pulmonary leukotriene levels suggests that such measurements may accurately reflect alterations in the sulphidopeptide leukotriene status of the asthmatic lung [14–17].

References

1. Lewis RA, Drazen JM, Austen KF, Clarke DA, Corey EJ. Identification of the C(6)-S-conjugate of leukotriene A with cysteine as a naturally occurring slow-reacting substance of anaphylaxis (SRS-A). Importance of the 11-cis-geometry for biological activity. *Biochem Biophys Res Commun* 1980; 285: 104–105.
2. Morris H, Taylor GW, Piper PJ, Tippins JR. Structure of slow-reacting substance of anaphylaxis from guinea-pig lung. *Nature* 1980; 285: 104–105.
3. Bach MK, Brashler J, Morton D. Solubilization and characterization of the leukotriene C_4 synthetase of rat basophil leukaemia cells. A novel particulate glutathione-S-transferase. *Arch Biochem* 1984; 230: 455–465.
4. Samuelsson B. Leukotrienes: mediators of hypersensitivity reactions and inflammation. *Science* 1983; 220: 568–575.
5. Huber M, Muller J, Leier I, *et al.* Metabolism of cysteinyl leukotrienes in monkey and man. *Eur J Biochem* 1990; 194: 309–315.
6. Zakrzewski JT, Sampson AP, Evans JM, Barnes NC, Piper PJ, Costello JF. The biotransformation *in vitro* of cysteinyl leukotrienes in blood of normal and asthmatic subjects. *Prostaglandins* 1989; 37(4): 425–444.
7. Sala A, Voelkel N, Maclouf J, Murphy RC. Leukotriene E_4 elimination and metabolism in normal human subjects. *J Biol Chem* 1990; 265(35): 21771–21778.
8. Dahlen SE, Bjork J, Hedquist P, *et al.* Leukotrienes promote plasma leakage and leucocyte adhesion in postcapillary venules: *in vivo* effects with relevance to the acute inflammatory response. *Proc Natl Acad Sci USA* 1988; 78: 3887–3891.
9. Soter NA, Lewis RA, Corey EJ, Austen KF. Local effects of synthetic leukotrienes (LTC_4, LTD_4, LTE_4 and LTB_4) in human skin. *J Invest Dermatol* 1983; 80: 115–119.
10. Drazen JM, Austen KF, Lewis RA, *et al.* Comparative airway and vascular activities of leukotrienes C_1 and D *in vivo* and *in vitro*. *Proc Natl Acad Sci USA* 1980; 77: 4354–4358.
11. Lee TH, Austen KF, Corey EJ, Drazen JM. LTE_4-airway hyperresponsiveness of guinea-pig trachea smooth muscle to histamine and evidence for 3 separate sulfidopeptide receptors. *Proc Natl Acad Sci USA* 1984; 81: 4922–4925.
12. Arm JP, Spur BW, Lee TH. The effect of inhaled leukotriene E_4 on the airway responsiveness to histamine in subjects with asthma and normal subjects. *J Allergy Clin Immunol* 1988; 82: 654–660.
13. Tagari P, Ethier D, Carry M, *et al.* Measurement of urinary leukotrienes by reversed-phase liquid chromatography and radioimmunoassay. *Clin Chem* 1989; 35: 388–391.
14. Taylor GW, Black P, Turner N, *et al.* Urinary LTE_4 after antigen challenge and in acute asthma and allergic rhinitis. *Lancet* 1989; 1: 584–588.
15. Smith CM, Christie PE, Hawksworth RJ, Thien F, Lee TH. Urinary leukotriene E_4 levels after allergen and exercise challenge in bronchial asthma. *Am Rev Respir Dis* 1991; 144: 1411–1413.
16. Christie PE, Tagari P, Ford-Hutchinson AW, *et al.* Urinary leukotriene E_4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991; 143: 1025–1029.
17. Westcott JY, Smith H, Wenzel SE, *et al.* Urinary leukotriene E_4 in patients with asthma. *Am Rev Respir Dis* 1991; 143: 1322–1328.
18. Smith CM, Hawksworth RJ, Thien FCK, Christie PE, Lee TH. Urinary leukotriene E_4 in bronchial asthma. *Eur Respir J* 1992; 5: 693–699.
19. Maltby NH, Taylor GW, Ritter JM, Moore K, Fuller RW, Dollery CT. Leukotriene C_4 elimination and metbolism in man. *J Allergy Clin Immunol* 1990; 85: 3–9.
20. Maclouf J, Antoine C, De Caterina R, *et al.* Entry rate and metabolism of leukotriene C_4 into vascular compartment in healthy subjects. *Am J Physiol* 1992; 263: 244–249.
21. Verhagen J, Bel EH, Kijne GM, *et al.* The excretion of leukotriene E_4 into urine following the inhalation of leukotriene D_4 by human individuals. *Biochem Biophys Res Commun* 1987; 148: 864–868.
22. Adelroth E, Morris MM, Hargreave FE, O'Byrne PM. Airway responsiveness to leukotrienes C_4 and D_4 and to methacholine in patients with asthma, and normal controls. *N Engl J Med* 1986; 315: 480–484.
23. O'Hickey SP, Arm JP, Rees PJ, Spur BW, Lee TH. The relative responsiveness to inhaled leukotrienes E_4, methacholine and histamine in normal and asthmatic subjects. *Eur Respir J* 1988; 1: 913–917.
24. Rasmussen JB, Eriksson LO, Tagari P, Margolskee D, Girard Y, Andersson K-E. Urinary LTE_4 excretion in antigen-provoked asthmatic patients treated with the inhaled LTD_4 antagonist L-648,051. *Allergy* 1992; 47: 599–603.
25. Uehara NK, Ormstad L, Orning L, Hammarstrom S. Characteristics of the uptake of cysteine-containing leukotrienes by isolated hepatocytes. *Biochem Biophys Acta* 1983; 732: 69–74.
26. Gaddy JN, Margolskee DJ, Bush RK, Williams VC, Busse WW. Bronchodilation with a potent and selective leukotriene D_4 (LTD_4) receptor antagonist (MK-571) in patients with asthma. *Am Rev Respir Dis* 1992; 148: 358–363.
27. Hui KP, Barnes NC. Lung function improvement in asthma with a cysteinyl-leukotriene receptor antagonist. *Lancet* 1991; 337: 1062–1063.