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The relative responsiveness to inhaled leukotriene E_4, methacholine and histamine in normal and asthmatic subjects

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ABSTRACT: The relative bronchoconstricting potencies of leukotriene E_4 (LTE_4), methacholine and histamine have been compared in asthmatic and normal subjects. LTE_4 responsiveness in asthmatic subjects, as measured by the dose which produced a 35% fall in specific airways conductance (PD_{35}), ranged from 0.06–24.4 nmol (geom mean 4.1 nmol, n=20). This was significantly less than the PD_{35} in normal subjects (range 39.0–370 nmol, geom mean 105 nmol, n=6; $p<0.001$). There was a correlation between LTE_4 and methacholine responsiveness ($r=0.84$, $p<0.001$) and between LTE_4 and histamine responsiveness ($r=0.79$, $p<0.001$). LTE_4 was 73 times more potent than methacholine and 112 times more potent than histamine in asthmatic subjects. LTE_4 was 20 times more potent than methacholine and 58 times more potent than histamine in normal subjects. LTE_4 is a potent bronchoconstrictor agent, and LTE_4 responsiveness correlates with both histamine and methacholine responsiveness.

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Enhanced airway responsiveness to a wide range of stimuli is the hallmark of bronchial asthma [1]. Bronchoconstrictor agonists which include histamine [2], methacholine [3], prostaglandin (PG) $F_2\alpha$ [4] and PGD_2 [5] and physical stimuli such as exercise [6], and cold air hyperventilation [7] are more potent in constricting the airways of asthmatic subjects than those of normal individuals.

The sulphidopeptide leukotrienes (LT) C_4, LTD_4 and LTE_4 comprise the activity previously recognized as slow-reacting substance of anaphylaxis (SRS-A) [8]. In humans the leukotrienes are potent bronchoconstrictors when inhaled and airways responsiveness to LTC_4 and LTD_4 correlates with that to histamine and methacholine [9, 10]. There has only been one report in humans on the *in vivo* effects of LTE_4, the most stable of the sulphidopeptide leukotrienes. Davidson *et al.* [11] compared the airways response of six asthmatic and five normal subjects to inhaled LTE_4 and histamine, by measuring airways response in terms of both forced expiratory volume in one second (FEV_1) and flow at 30% of vital capacity above the residual volume ($\dot{V}30$).

There is very limited data on the relationship of LTE_4 responsiveness with other indices of non-specific airways responsiveness. We have therefore measured the airways responsiveness to LTE_4 in normal and asthmatic subjects and compared this with histamine and methacholine responsiveness.

Patients and methods

Twenty asthmatic subjects (8 males and 12 females) aged between 15 and 43 yrs (mean 27 yrs) were studied together with six normal subjects (4 males and 2 females) aged 23 to 36 yrs (mean 30 yrs). Asthmatic subjects were selected at random from patients attending the asthma clinic and normal subjects from volunteers working in the laboratory. Sixteen of the asthmatic subjects and two normal subjects were atopic. Baseline specific airways conductance (sGaw) of the asthmatic subjects ranged from 0.9–2.75 $s^{-1} \cdot kPa^{-1}$ and from 1.8–5.7 $s^{-1} \cdot kPa^{-1}$ for normal subjects. Fourteen asthmatic subjects were taking regular inhaled beclomethasone dipropionate and all asthmatic individuals were using inhaled $beta_2$-agonists as required. No subject was using theophylline or had taken oral corticosteroids or inhaled cromolyn for at least one month prior to the study. Medication was withheld for at least 8 h before each challenge and challenges were performed for each individual at the same time each day. The study was approved by the Guy's Hospital Ethical Committee and each subject gave informed consent.

Study design

All subjects attended the laboratory on two occasions at least two weeks apart. Subjects were challenged with

LTE_4 or with methacholine in random order. Nine asthmatic subjects and all normal subjects attended the laboratory on one more occasion and were challenged with histamine. The remaining eleven subjects declined a further bronchial challenge.

Histamine and methacholine challenges

Inhalation challenges were performed as previously described using a Hudson nebulizer linked to a breath activated dosimeter [12]. Delivery of air to the nebulizer was regulated to a pressure of 138 kPa (20 pounds per square inch), for a duration of 0.6 s from the start of inspiration of each breath. Under these conditions the Hudson nebulizer delivers droplets with a mass median aerodynamic diameter of 1.6 μm and the output of the nebulizer is 2.6 μl per breath. Following baseline measurements of sGaw the subjects inhaled 5 breaths of phosphate buffered saline (PBS) as a control. Each inhalation started at functional residual capacity and terminated at approximately 70% of baseline vital capacity; a 5 s breath hold was maintained at the end of each inhalation. If the decrease in sGaw was <10% the patients were subjected to histamine or methacholine challenge. Two-fold increasing concentrations of histamine acid phosphate or methacholine chloride (Sigma, Poole, Dorset) diluted in PBS were inhaled from a concentration of 0.5 $mg \cdot ml^{-1}$ (1.6 mM and 2.6 mM, respectively) to 1.6 $mg \cdot ml^{-1}$ (54.2 mM and 83.2 mM, respectively) for asthmatic subjects and 2.0 $mg \cdot ml^{-1}$ (6.5 mM and 10.2 mM, respectively) to 6.4 $mg \cdot ml^{-1}$ (208 mM and 324 mM, respectively) for normal subjects. Specific airways conductance (sGaw) was measured 2 min after each inhalation and increasing concentrations were administered until sGaw had fallen by more than 35%. The coefficient of variation for histamine and methacholine challenges has previously been shown to be 15% in this laboratory [13]. Asthmatic subjects bronchoconstrict at concentrations of less than 0.8 $mg \cdot ml^{-1}$.

LTE_4 inhalation challenges

LTE_4 was prepared by total chemical synthesis as described previously [14] and frozen under argon at -70°C. LTE_4 was analysed before inhalation challenge by reverse phase high performance liquid chromatography (RP-HPLC) on a 10 micron C_{18} ultrasil-ODS column (4.6 250 mm; Beckman Instruments Inc, Berkeley, CA)

Table 1. – The doses of LTE_4 and methacholine and histamine which produced a 35% fall in sGaw (PD_{35}) in normal and asthmatic subjects

Subjects	$PD_{35}LTE_4$	PD_{35} Methacholine	PD_{35} Histamine	Relative Potency LTE_4/Methacholine	Relative Potency LTE_4/Histamine
asthmatic	nmol	μmol	μmol		
1	2.90	0.76	0.9	261	309
2	16.00	0.70	0.7	44	45
3	4.00	0.51	2.2	127	550
4	7.00	0.26	2.6	36	371
5	5.20	0.23	0.3	44	51
6	9.00	0.32	0.4	36	45
7	19.00	0.90	3.0	47	158
8	4.40	0.26		58	
9	12.20	0.12		9	
10	6.10	0.94		154	
11	1.20	0.11		92	
12	17.80	0.25		14	
13	2.90	0.28		97	
14	6.20	0.90		145	
15	6.00	0.47	0.5	78	90
16	24.40	0.80	0.8	33	33
17	0.06	0.01		241	
18	7.20	1.90		263	
19	0.75	0.07		93	
20	0.28	0.05		178	
geometric mean	4.10	0.30	0.09	73	112
normal					
1	172.0	0.8	8.0	4.4	46
2	370.0	4.1	39.0	11.0	105
3	90.0	1.1	3.2	12.2	36
4	56.6	3.3	4.4	59.2	78
5	39.0	1.4	4.2	35.1	107
6	115.0	6.2	3.2	53.9	28
geometric mean	105.9	2.1	6.2	20.0	58

at a flow rate of 1 ml·min^{-1} with 65% methanol (BDH), 34.9% water, 0.1% acetic acid, pH 5.6, as solvent. Absorbance was monitored with an on-line spectrophotometer linked to an integrator (Spectraphysics, model SP 4270). The purity of LTE_4 before challenge was confirmed by its elution as a single peak at its unique retention time of 25±0.4 min (mean±SEM, n=10) in this solvent system. The concentration of the stock solution was assessed by UV scanning at 280 nM assuming an extinction coefficient of 40,000 cm^{-1}·M^{-1} and dilutions of LTE_4 were prepared in PBS. On four occasions samples of the solution remaining in the nebulizer after the highest concentration of LTE_4 was administered were analysed by RP-HPLC and by measurement of ultraviolet absorbance at 280 nm to determine if the purity or concentration of LTE_4 had changed during nebulization. LTE_4 after nebulization eluted as a single peak when subjected to RP-HPLC and the concentration differed by less than 10% from pre-nebulization values.

For LTE_4 challenges, each subject inhaled geometrically increasing concentrations of LTE_4 starting at a concentration of 0.18 µg·ml^{-1} (0.4 µM) in asthmatic subjects and 4.6 µg·ml^{-1} (10 µM) in normal subjects as determined from previous studies [11]. sGaw was measured every 5 min for 15 minutes. If a 35% decrease in sGaw was not achieved within 15 minutes the concentration of LTE_4 in the nebulizer was increased by 3-fold and the protocol repeated. The timings of observations were based on the results of previous studies [11]. Bronchoconstriction commences at 5 minutes after inhalation of LTE_4 with maximal bronchoconstriction occurring at between 10–15 minutes after inhalation.

Measurements of sGaw were made in a total body plethysmograph linked to a digital computer as previously described [15]. Four to six measurements of sGaw were recorded at each time point and the mean value was calculated. Baseline sGaw was >0.9 s^{-1}·kPa^{-1} prior to each challenge in asthmatic subjects. Mean baseline sGaw measurements did not differ significantly in the asthmatic subjects on the three study days, being 1.49±0.09 s^{-1}·kPa^{-1}, 1.52±0.09 s^{-1}·kPa^{-1}, and 1.44±0.09 s^{-1}·kPa^{-1} prior to methacholine, LTE_4 and histamine challenges, respectively.

Analysis of data

The cumulative dose of histamine or methacholine required to produce a 35% fall in sGaw (PD_{35}) was determined by linear interpolation from the log dose-response curve. Log transformed data was analysed by Student's t-test and correlations were assessed by the linear regression analysis.

Results

Relative potencies of LTE_4, methacholine and histamine asthmatic subjects. The LTE_4 PD_{35} sGaw in asthmatic subjects ranged from 0.06–24.4 nmol (geom mean 4.1 nmol, n=20), the methacholine PD_{35} sGaw ranged from 0.01–1.9 µmol (geom mean 0.30 µmol, n=20), and the histamine PD_{35} sGaw ranged from 0.3–3.0 µmol (mean 0.9 µmol, n=9) (table 1). The relative potency of LTE_4 to methacholine (PD_{35} Meth/PD_{35} LTE_4) ranged from 9–263 (geom mean 73, n=20). The relative potency of LTE_4 to histamine (PD_{35} Hist/PD_{35} LTE_4) ranged from 45–550 (geom mean 112, n=9).

Normal subjects. The LTE_4 PD_{35} sGaw in normal subjects ranged from 39–370 nmol (geom mean 105.9 nmol, n=6) (table 1), the methacholine PD_{35} sGaw ranged from 0.8–6.2 µmol (geom mean 2.1 µmol, n=6), and the histamine PD_{35} sGaw in ranged from 3.2–39 µmol (geom mean 6.2 µmol, n=6). The airways responsiveness to LTE_4, methacholine and histamine was significantly greater in asthmatic than in normal subjects ($p<0.001$) (tables 1 and 2). The relative potency of LTE_4 to methacholine in normal subjects ranged from 4.4–59.2 (geom mean 20, n=6). The relative potency of histamine to LTE_4 in normal subjects ranged from 28 to 107 (geom mean 58, n=6). Asthmatic subjects were 26 times more sensitive to LTE_4 than normal subjects whereas they were 7 times more sensitive to methacholine and histamine.

Correlation of airways responsiveness to LTE_4 with that to methacholine and histamine

There was a positive correlation between LTE_4 PD_{35} and methacholine PD_{35} ($r=0.84$, $p<0.001$, n=26) (fig. 1) and between LTE_4 PD_{35} and histamine PD_{35} ($r=0.79$, $p<0.001$, n=15) when linear regression was calculated for both asthmatic and normal subjects (fig. 2).

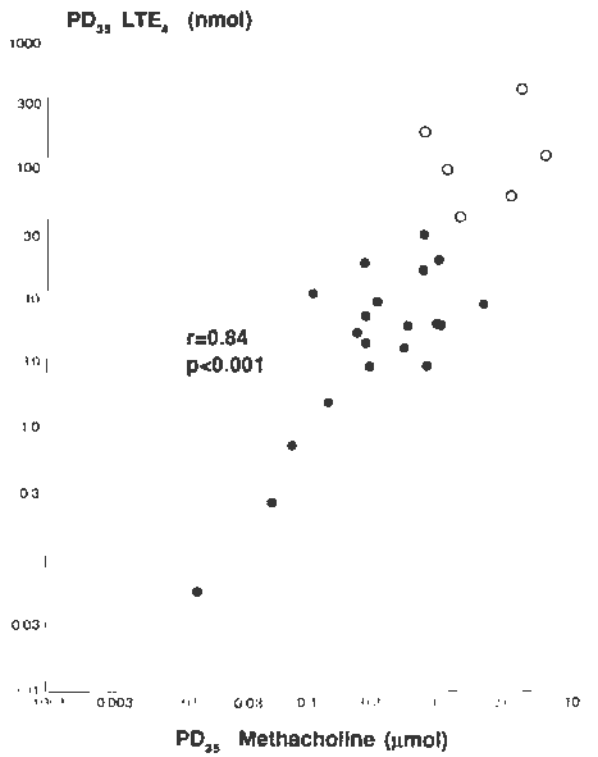

Fig. 1. – The correlation between LTE_4 PD_{35} and methacholine PD_{35}. ● asthmatic subjects; ○ normal subjects.

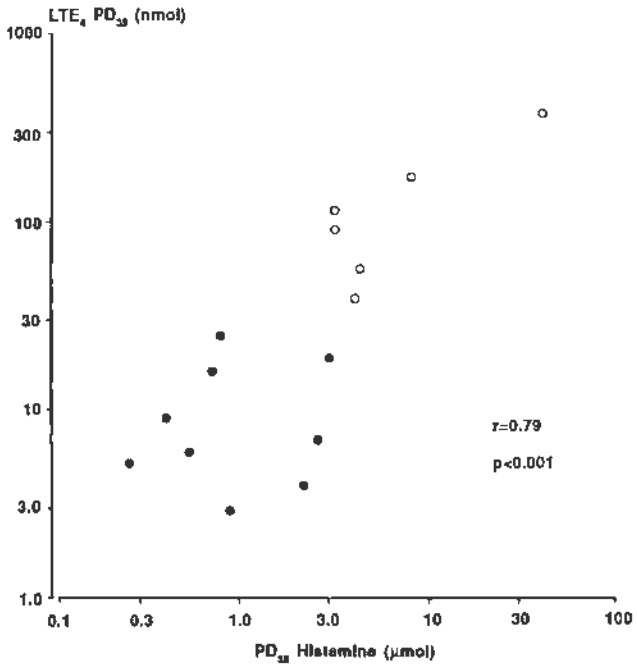

Fig. 2. – The correlation between LTE_4 PD_{35} and histamine PD_{35}. ● asthmatic subjects; ○ normal subjects.

Discussion

The airways responsiveness to LTE_4 has been compared to that of histamine and methacholine in normal and asthmatic subjects. Airways responsiveness to the bronchoconstrictor agents was assessed by constructing cumulative dose-response curves and the dose which produced a 35% decrease in specific airways conductance (PD_{35}) was obtained from the straight-line portion of the log dose-response curve by linear interpolation. The potencies of each agent were studied by comparison of the PD_{35} values.

LTE_4 enhances bronchial hyperresponsiveness in asthmatic subjects [13], there is bioconversion of LTC_4 and LTD_4 to LTE_4 during a contractile reaction [16] and LTE_4 may persist at the site of release for a prolonged period of time [17]. Since there is bioconversion of LTC_4 and LTD_4 to LTE_4, it is likely that LTE_4 will contribute significantly to airflow obstruction at the site of release of the sulphidopeptide leukotrienes. Our data demonstrates that LTE_4 is a potent bronchoconstrictor agent in normal and asthmatic subjects and confirms the work of Davidson *et al.* [11]. Using sGaw as the index of airway calibre, we found that LTE_4 was on average 20-fold and 73-fold more potent than methacholine and 58-fold and 112-fold more potent than histamine on a molar basis in normal and asthmatic subjects respectively. In addition, asthmatic subjects were approximately 25-fold more sensitive to LTE_4 than were normal subjects, but were only 7-fold more sensitive to histamine and methacholine.

We demonstrate for the first time that there is a positive correlation between LTE_4 PD_{35} and histamine and methacholine PD_{35} when both asthmatic and normal subjects are considered when the linear regression was calculated for both of these subjects. Comparisons of results in the literature are complicated by differences in the aerosol delivery technique and the details of the airway measurements made. One approach to compare data from different studies is to evaluate the responsiveness of groups of normal and asthmatic subjects studied by the same investigators in terms of their relative responsiveness to leukotrienes and to reference agonists [18] ([normals/asthmatics] responsiveness to reference agonist divided by [normals/asthmatics] responsiveness to a leukotriene). The relative responsiveness of normal individuals for the reference agonist, histamine or methacholine, is greater than the relative responsiveness to leukotrienes in three of the four published studies [9, 10, 19, 20]. That is, in three of the four studies, asthmatic subjects failed to exhibit the same degree of hyperresponsiveness to a leukotriene than to the reference agonist. In these studies airway response was determined by measurement of FEV_1, and flow rates at 30% and 40% of forced vital capacity. Using sGaw as a measurement of large airways calibre [21, 22], we have demonstrated airway hyperresponsiveness to LTE_4 in asthmatic subjects. Our results are in agreement with those of Smith *et al.* [9] in that the relative responsiveness to the leukotrienes in asthmatic subjects was 2- and 3-fold greater than that to histamine and methacholine, respectively, when airway calibre was determined by sGaw. These findings are consistent with the data of Davidson *et al.* [11] and suggest that in bronchial asthma there may be site selectivity for leukotriene hyperresponsiveness, as compared to histamine and methacholine responsiveness, and that asthmatic subjects may be more hyperresponsive to the leukotrienes in the large airways.

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Réactivité comparée à l'inhalation de leukotriène E_4, de la métacholine et d'histamine, chez des sujets normaux et asthmatiques. S. P. O'Hickey, J. P. Arm, P. J. Rees, B. W. Spur, T. H. Lee.

RÉSUMÉ: La puissance bronchoconstrictrice relative de LTE_4, de la métacholine et de l'histamine a été comparée chez des sujets asthmatiques et normaux. La réactivité des asthmatiques à l'égard de LTE_4, exprimée par la dose qui produit une chute de 35% de la conductance spécifique des voies aériennes (PD_{35}), varie entre 0.06 et 24.4 nmol (moyenne géométrique: 4.1 nmol, n=20). Cette valeur est significativement plus faible que la PD_{35} des sujets normaux (extrêmes: 39.0 à 370 nmol, moyenne géométrique: 105 nmol, n=6, p<0.001). L'on a observé une corrélation entre la réactivité à LTE_4 et la réactivité à la métacholine (r=0.84, p<0.001) ainsi qu'entre les réactivités à LTE_4 et à l'histamine (r=0.79, p<0.001). LTE_4 s'est avérée 73 fois plus puissante que la métacholine, et 112 fois plus puissante que l'histamine chez les sujets asthmatiques. Chez les sujets normaux, LTE_4 est 20 fois plus puissante que la métacholine et 58 fois plus puissante que l'histamine. LTE_4 est donc un agent bronchoconstricteur puissant, et la réactivité à LTE_4 est en corrélation à la fois avec celle à l'égard de l'histamine et à l'égard de la métacholine.
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