Thorax 1984;**39**:500–504

Comparative effects of inhaled leukotriene C_4, leukotriene D_4, and histamine in normal human subjects

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ABSTRACT The comparative actions of inhaled leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), and histamine were studied in six normal subjects. LTC_4 and LTD_4 were shown to be more potent bronchoconstrictors than histamine, with a more sustained action. LTC_4 and LTD_4 caused wheezing without cough or throat irritation and were shown to act on large and small airways.

For many years it has been suggested that the allergic mediator slow reacting substance of anaphylaxis (SRS-A) might have an important bronchoconstrictor role in asthma. Recently the structure of SRS-A from various species has been elucidated and its biological activity shown to be due to leukotrienes (LTs), the newly discovered 5-lipoxygenase metabolites of arachidonic acid. LTD_4 accounts for the activity of guinea pig SRS-A on intestinal and respiratory smooth muscle[1]; human SRS-A is made up of LTC_4 and LTD_4,[2] and rat SRS-A contains LTC_4, LTD_4, and LTE_4.[3]

In experimental animals leukotrienes have been shown to cause bronchoconstriction when given either intravenously[4] or by aerosol.[5] In guinea pigs intravenous administration of SRS-A caused greater changes in dynamic compliance than resistance, suggesting that their major action is on small airways.[6]

Leukotrienes have also been given by inhalation to human volunteers[7–9] and shown to be more active than histamine in causing bronchoconstriction. The principal measurement of lung function has been the flow at 30% of vital capacity above residual volume ($\dot{V}_{max30}$); this is thought to be mainly dependent on the function of small airways. We have studied the bronchoconstrictor action of inhaled LTC_4 and LTD_4 relative to that of histamine in a group of six normal volunteers. We measured specific airways conductance (sGaw) and V_{max30}. As leukotrienes are unstable compounds we have attempted to evaluate the loss of activity of LTC_4 and LTD_4 during nebulisation.

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Accepted 17 February 1984

Methods

We studied six normal, non-atopic subjects (five men and one woman) with a mean age of 28·5 years (range 21–35 years). All were lifelong non-smokers. Ethical permission was obtained and all participants gave informed consent.

All subjects were trained to perform panting manoeuvres in a constant volume whole body plethysmograph (Collins) for the measurement of sGaw.[10] They were taught to produce reproducible partial expiratory flow volume curves for measurement of maximum flow at $\dot{V}_{max30}$[11] (PK Morgan transfer factor spirometer with a PK Morgan differentiator recorded on a Hewlett Packard X-Y recorder).

Aerosol for inhalation was generated from a Wright nebuliser containing 2 ml of test solution driven by compressed air at a flow rate of 7 litres a minute; the solution was nebulised for two minutes during normal tidal breathing through the open mouth. The output of the nebuliser was 0·165 ml/min.

To construct the dose-response curves for histamine, sGaw was measured at 60, 80, 100, 120, and 140 seconds and the mean value calculated; $\dot{V}_{max30}$ was then measured in triplicate and the mean calculated. First the diluent (0·9% saline and chlorbutol

BP 0·5%) was inhaled; the response to this served as a baseline for comparison with other values. Histamine acid phosphate was then inhaled at a starting concentration of 0·1 mmol/l. Doses were repeated at fivefold or 10 fold higher concentrations every 10 minutes until a fall in sGaw of 35% or more was achieved.

On another day the time course of recovery from histamine induced bronchoconstriction was studied. Sufficient histamine to produce a fall in sGaw of about 35% was given. sGaw was measured after one minute and then every 15 seconds until two minutes. Measurements were repeated at three, five, seven minutes, etc, until sGaw had returned to near its baseline value.

Dose-response curves for LTC_4 and LTD_4 were obtained by the method used for the histamine dose-response curve. Since leukotrienes have a more prolonged action than histamine in human isolated bronchi[12] and in normal human volunteers,[8 9] further measurements of sGaw and $\dot{V}_{max30}$ were made after six, 11, and 16 minutes. Normal saline, which was used as the diluent, was inhaled first and the values obtained were used as the baseline in further analysis. Leukotrienes were synthesised by Dr J Rokach, Merck Frosst Laboratories.[13]

Solutions for inhalation were made up in normal saline immediately before use from stock solutions of LTC_4, 1·4 mmol/l, and LTD_4, 2 mmol/l, to give 10 fold increasing concentrations. The starting dose of LTC_4 was 14 nmol/l and of LTD_4 20 nmol/l.

If no fall in sGaw occurred, inhalation was repeated after 20 minutes at a concentration 10 times higher. If a fall in sGaw occurred the next concentration was given 45 minutes later, after we had checked that sGaw and $\dot{V}_{max30}$ had returned to their baseline values. When a 35% or greater fall in sGaw had occurred no further doses were given and further measurements of sGaw and $\dot{V}_{max30}$ were made at 30, 45, and 60 minutes.

To investigate the stability of leukotrienes during nebulisation, samples of the solutions of LTC_4 and LTD_4 that had been put into the nebuliser jar and those remaining at the end of inhalation were collected and assayed against standard LTC_4 and LTD_4 on strips of guinea pigs ileum smooth muscle superfused with Tyrode solution and blocked with mepyramine and hyoscine.[14]

Results

Inhalation of histamine, LTC_4, and LTD_4 caused bronchoconstriction in all subjects. The bronchoconstriction caused by all three agents was reflected in a fall in both sGaw and $\dot{V}_{max30}$ (fig. 1).

The concentration of solutions required to cause a 35% drop in sGaw (PD_{35}) for each subject is shown in table 1. For the group as a whole the geometric mean PD_{35} for histamine was 55 mmol/l, for LTC_4 73 μmol/l, and for LTD_4 89 μmol/l. For individuals the potency of LTC_4 in relation to that of histamine varied from 125:1 to 1800:1, with a mean of 975:1. For LTD_4 it varied from 285:1 to 1175:1 (mean 720:1).

The time course of bronchoconstriction for the group of six subjects is shown in figure 2. The onset of bronchoconstriction was within 90 seconds of the end of inhalation.

As a measure of the rate of recovery from bronchoconstriction the time taken for sGaw to return to 90% of the baseline value (TR_{90}) was determined; for histamine the mean (1 SD) was 9·9 (3·8) min, for LTC_4 32·3 (8·0) min, and for LTD_4 25·3 (5·4) min. The difference between the TR_{90} for LTC_4 and histamine was signficant (paired Student's t test) ($p < 0·005$) and the difference between LTD_4 and histamine was significant ($p < 0·001$). Subjects recovered from the bronchoconstriction induced by LTD_4 faster than from that induced by LTC_4 but the difference failed to reach significance.

All subjects found the dose of histamine that induced bronchoconstriction caused coughing, wheezing, and an unpleasant sensation in the throat. Inhalation of LTC_4 and LTD_4 caused a mild but not unpleasant sensation in the throat and no coughing but appreciable wheezing.

Assays of solutions of LTC_4 and LTD_4 showed that the leukotrienes lost 50·3% (4·6%) and 51·2% (5·1%) respectively of their biological activity during the two minute period in which compressed air passed through the solution.

Discussion

These results show that when LTC_4 or LTD_4 are inhaled by normal human subjects they cause bronchoconstriction and are much more active than histamine. Previous observations[7–9] have also shown leukotrienes to be bronchoconstrictor agents in man and the indices measured have suggested that they act selectively on the small airways. In these investigations $\dot{V}_{max30}$ was used as the measurement of airway function. This is thought to be a reflection of changes in small airways[11 15–17] Holroyde *et al*[7] found a significant change in flow at 1·5 litres above residual volume (approximately equivalent to $\dot{V}_{max30}$) but only a 3–6% and a 6–10% change in forced expiratory volume in one second (FEV_1). Because of the appreciable changes in $\dot{V}_{max30}$, the apparent lack of change in FEV_1, and the absence of upper airways irritation causing coughing and in view of animal data,[6] it has been postulated that

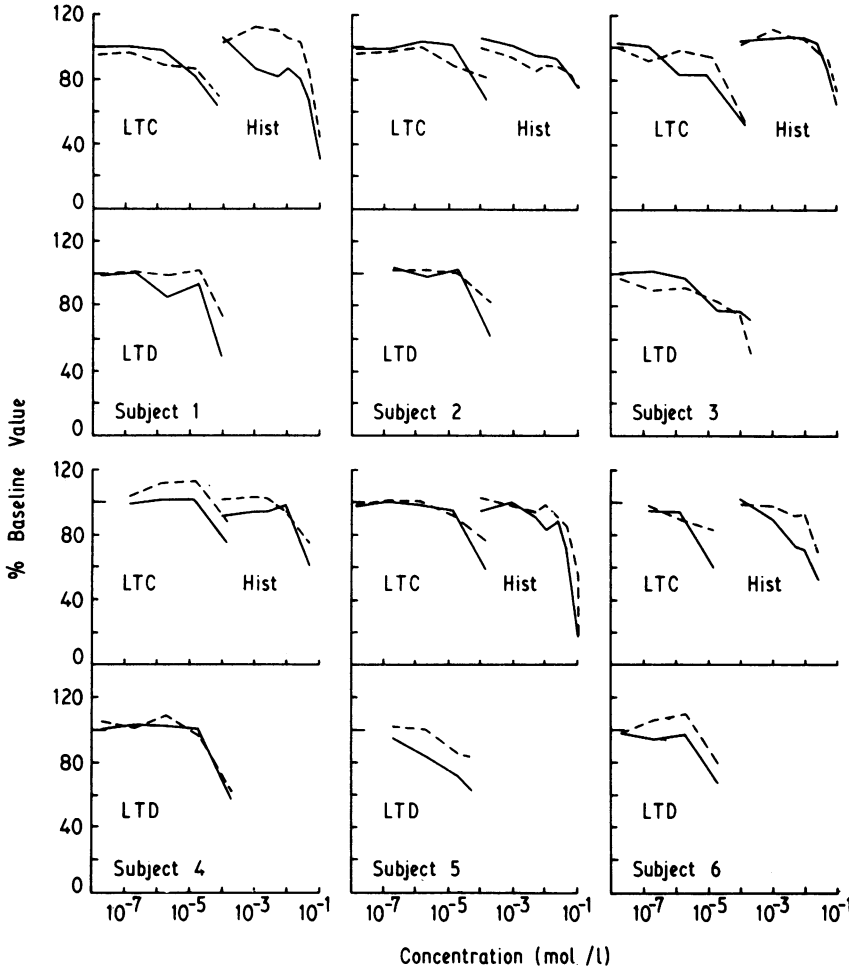

Fig 1 *Dose-response curves for inhaled histamine (Hist) and leukotrienes C_4 (LTC) and D_4 (LTD): percentages of the original baseline values of the flow at 30% of vital capacity above residual volume ($\dot{V}_{max30}$) and specific airways conductance (sGaw) against molar concentration (mol/l) for six normal subjects.*

leukotrienes act mainly on peripheral airways.[7 8] In addition to $\dot{V}_{max30}$, however, in the same subjects we measured sGaw, which in normal subjects predominantly represents large airways function.[15 18 19] The results suggest that leukotrienes contract both large and small airways. This is in agreement with the results of work in human lung preparations in vitro, where leukotrienes are at least as active in contracting human bronchus as in contracting parenchyma.[12 20] The changes in sGaw and $\dot{V}_{max30}$ observed in our study are broadly in line with changes in airways resistance and dynamic compliance observed by Ford-Hutchinson and his coworkers in squirrel monkeys (personal communication).

This study demonstrates that both LTC_4 and LTD_4 are more potent bronchoconstrictors than is histamine, but our values for the relative potencies

Original nebuliser concentration in mol/litre of histamine, LTC_4 and LTD_4 required to cause a 35% drop in sGaw (PD_{35})

Subject No.	*1*	*2*	*3*	*4*	*5*	*6*
Histamine	5×10^{-2}	$1{\cdot}8 \times 10^{-1}$*	10^{-1}	$4{\cdot}0 \times 10^{-2}$	$5{\cdot}3 \times 10^{-2}$	$1{\cdot}4 \times 10^{-2}$
LTC_4	$5{\cdot}6 \times 10^{-5}$	$1{\cdot}6 \times 10^{-4}$*	$5{\cdot}6 \times 10^{-5}$	$3{\cdot}2 \times 10^{-4}$*	10^{-4}	10^{-5}
LTD_4	$5{\cdot}6 \times 10^{-5}$	$1{\cdot}6 \times 10^{-4}$	$3{\cdot}1 \times 10^{-4}$*	$1{\cdot}4 \times 10^{-4}$	$4{\cdot}5 \times 10^{-5}$	$2{\cdot}8 \times 10^{-5}$*

*Value derived by extrapolation.

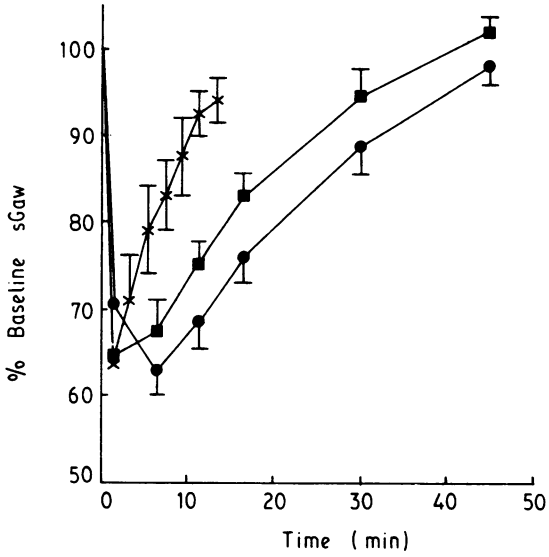

Fig 2 *Time course of recovery from bronchoconstriction induced by histamine (×——×) and leukotrienes C_4 (●——●) and D_4 (■——■): percentage of original baseline sGaw and standard error for the group of six normal subjects.*

differ from those of other authors. Weiss *et al*[8] found LTC_4 to be 600–9500 (geometric mean about 3900) times more potent than histamine in causing a 30% decrease in $\dot{V}_{max30}$. In a further study Weiss *et al*[9] found LTD_4 to be 5900 times more potent than histamine in the same group of subjects. There are several possible explanations for the difference in potency between the present study and those of Weiss and his colleagues. A different nebuliser was used in the two studies: whereas in our study a Wright nebuliser was used, Weiss and his colleagues used a De Vilbiss No 42 nebuliser with a dosimeter. There are two possible ways in which the choice of nebuliser may influence the result. Firstly, the difference in nebuliser may alter the site of deposition of the test solution. This is unlikely to be the explanation; Ryan *et al*[21] compared the results of a standard histamine challenge test in 10 asthmatic patients using a De Vilbiss nebuliser and a Wright nebuliser. They found that the nebulisers deposited the same dose in the lung, although the De Vilbiss nebuliser deposited more in the throat and central airways. The concentrations of histamine required to cause a 20% decrease in FEV_1 were similar. The second way in which the nebuliser may influence the result is by differences in the degree of oxidation of the leukotrienes. We have shown considerable changes in activity of LTC_4 and LTD_4 during nebulisation, and the potency of leukotrienes determined in this study may be significantly underestimated. The loss of potency when a De Vilbiss nebuliser is used in not known. Another possible reason for the observed difference is that with the small number of subjects studied—five by Weiss *et al* and six in this study—the apparent difference represents only the spectrum of sensitivity to leukotrienes within the normal population. The relative potencies of leukotrienes and histamine are assumed by comparing the PD_{35} for LTC_4 and LTD_4 to the PD_{35} for histamine; thus the ratio is determined by two values which themselves show large individual variations.

Sensitivity to histamine is not a good predictor of sensitivity to LTD_4. Griffin *et al*[22] found that a group of asthmatics had hypersensitivity to histamine, reacting to 1/100th the concentration to which normal subjects reacted; yet they were only three times as sensitive to LTD_4. The ratio of potency of histamine to that of leukotrienes, derived from two values which vary widely in individuals and have little direct relationship, would show even wider variation.

The time course of action of LTC_4 and LTD_4 in comparison with that of histamine is similar to the time course seen in human isolated bronchus[12 20] and in previous studies[8 9] and shows that leukotrienes induce prolonged bronchoconstriction.

The effects of inhalation of leukotrienes differ from those of histamine. Leukotrienes did not cause any cough or unpleasant irritation in the throat, which contrasts with the findings of Holroyde *et al*[7] but agrees with those of Weiss and his colleagues.[8 9]

In conclusion, therefore, our study has shown that in normal subjects inhaled LTC_4 and LTD_4 cause bronchoconstriction which is more potent and longer lasting than that induced by histamine, and that they exert this effect on large as well as small

airways.

We wish to thank the Asthma Research Council and King's College joint research committee for financial support. We thank Dr J Rokach, Merck Frosst Laboratories, for synthetic leukotrienes. We thank Mr S Galton and Mrs A Watson for skilled technical assistance, Miss M Rusbridge for illustrations, and Miss C Barton for typing the manuscript.

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