Inhalation Challenge with Sulfidopeptide Leukotrienes in Human Subjects*

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The close relationship between asthma and other allergic disorders has been a basis for the continued suspicion that the pathogenesis of asthma is related in part to the synthesis and release of mediators of immediate hypersensitivity. Prominent among the mediators known to be released in such a response are two bronchoactive substances: histamine, and a slow-reacting substance of anaphylaxis (SRS-A). Since, in human subjects, antihistamine treatment is not of general benefit in the therapy of asthma, a substantial effort was made in the 1960s and 1970s to identify the structure of the aforementioned slow-reacting substance of anaphylaxis, or SRS-A. In June, 1979, Murphy and co-workers identified leukotriene (LT) C4, (5(S)-hydroxy 6(R)-glutathionyl-7,9 trans-11,14 cis eicosatetraeonic acid) as a major constituent of SRS-A. Shortly thereafter, the 6-cysteinyl-glycl and 6-cysteineyln analogs of LTC4 were identified as LTD4, and LTE4, respectively. These three compounds together have been shown to encompass the activity formerly attributed to SRS-A. In the six years since the chemical identification of these mediators, their subsequent availability in pure form by total chemical synthesis has led to a virtual explosion of knowledge in this field.

Of substantial importance to the question of the mechanism of asthma and asthmatic responses has been the confirmation that these compounds (cumulatively known as sulfidopeptide leukotrienes because of a peptide linked to the lipid backbone at C-6) are potent airway contractile agonists in the lungs of animals and man. Effects of sulfidopeptide leukotrienes in animals have been reviewed in depth recently. In this review, we will concentrate on the effects of these compounds on human airways. We will first address the effects of inhalation of sulfidopeptide leukotrienes on normal subjects, and then on subjects with asthma. We will compare and contrast the effects of inhalation of these agents as determined by a number of investigative groups among normal and asthmatic subjects and address the issue of airway hyperresponsiveness to inhalation of these compounds.

In Vitro Studies

Shortly after the identification of the leukotrienes, evidence appeared that the leukotrienes were potent constrictors of isolated human airway contractile tissues. Sven Dahlen and co-workers demonstrated that LTC4 and LTD4, constrict isolated bronchial tissues obtained from the apparently normal lung tissue surrounding surgical specimens removed for cancer of the lung. They demonstrated that the administration of 1 nM concentrations of LTC4 and LTD4 elicited an overall contractile effect similar to that with 1,000 nM histamine, establishing a potency ratio in this preparation of 1/1,000. Shortly thereafter, Hanna and co-workers presented similar information. Their report not only confirmed the relative potency of the sulfidopeptide leukotrienes compared to histamine in isolated human tissues, but also demonstrated that the responses of bronchioles, pulmonary parenchymal strips, and isolated pulmonary arteries to these compounds were similar. Relative potency of these compounds as compared to histamine has also been confirmed by Siros and co-workers. In summary, the available in vitro human data demonstrate that the sulfidopeptide leukotrienes are about 1,000 times more potent airway constrictors than histamine.

Bronchoconstriction Indices

If leukotrienes are important in asthma, they must be able to mediate airway constriction in intact human subjects. The capacity of these compounds to elicit airway constriction is now well-established, as outlined below. Of particular importance in evaluating the effects of these compounds is the observation that the amount of substance required to achieve a given effect varies substantially with the pulmonary mechanical index used to assess response. Although a number of different investigative groups have used different response indices, most groups have reported the effects of inhalation of these compounds (in aerosol form) on the flow rate measured low in the vital capacity (approximately 30 percent of vital capacity above residual volume) obtained from partial expiratory flow volume relationships, the V₃₋₅-P. A partial expiratory flow volume maneuver (PEFV) is a forced expiratory maneuver initiated from about 60 percent of vital...
capacity above residual volume, rather than from total lung capacity. A maneuver initiated at total lung capacity (TLC) is referred to as a maximal-expiratory flow-volume maneuver, or MEFV. This test was originally developed to avoid the bronchodilatory effects of inspiration to total lung capacity. Although the precise mechanism of bronchodilation during large inspirations has not been determined in man, data from animal studies suggest that maximal inspiratory maneuvers selectively reverse constriction of peripheral airways, rather than central airways or parenchymal contractile tissues. Thus, it is reasonable to assume that during a forced expiratory maneuver initiated from TLC, rather than 60 percent of TLC, there may be transient (2 to 3 min) selective peripheral airway bronchodilation attendant to this maneuver. Further, when compared to the flow rate measured at a single volume low in the vital capacity, the FEV₁ (the integral of flow over the upper vital capacity) is likely more sensitive to alterations in the state of large airways. Thus, by comparing the effects of leukotriene inhalation assessed by using the FEV₁ with the effects on the V₂₅-P, both the full inspiration and the airway properties limiting flow contribute to the relatively greater small airway sensitivity of the V₂₅-P.

In general, these bronchoprovocation challenges are performed such that incrementally greater concentrations of aerosolized bronchoconstrictor substances are inhaled until a specific response (ie, 20 percent decrease in FEV₁) is achieved. Degree of sensitivity is quantified by reporting the interpolated drug concentration that would have been required to achieve a given response, termed the effective concentration (EC) for a given response. The lower the EC, the more sensitive the subject.

**SUMMARY OF PUBLISHED DATA**

**Normal Subjects**

Holroyde and co-workers were the first to report the effects of inspired aerosols generated from solutions of leukotrienes on airway tone in normal man. They demonstrated that LTC₄ and LTD₄ induced bronchoconstriction in two normal subjects within 5 min of aerosol exposure, with the effect lasting from 15 to 20 min. As with all aerosol exposure studies, the amount of bronchoactive material that actually reached the airways is difficult to precisely quantify. However, it is common practice in bronchoprovocation studies to quantitate the concentration of the solutions from which the bronchoactive agent was nebulized and relate this concentration to bronchoconstrictor effects. In this study, the nebulizer concentration of LTD₄ that would have been required to result in a 30 percent decrease in the V₂₅-P was 4 μM in one subject and 16 μM in the other (Fig 1). Further, the investigators reported that leukotriene inhalation (over this concentration range) had only minimal effects on the FEV₁, but induced coughing near RV. Inhalation of a leukotriene receptor antagonist, FPL55712 or FPL59257, inhibited the bronchoconstrictor response to inhaled

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21509/ on 04/02/2017)

**Figure 1.** Concentration of LTD₄ required in a nebulizer reservoir to achieve a specific effect in a number of subjects. The effects used were a 30 percent decrease in flow rate measured from partial flow volume curves (V₂₅-P), or a 15 to 20 percent decrease in the FEV₁. Each symbol represents data from a different group. Note the substantial overlap in results between normal and asthmatic subjects when V₂₅-P is used as an index of responsiveness. There is less overlap when FEV₁ is used as the responsiveness index. Asterisk indicates that mean data from six subjects is plotted as a single point. Concentrations for the Bisgaard study were calculated from data in the published report.
LTD₄ thus establishing the role of the LTD₄ receptor in the bronchoconstrictor response.¹⁶

Weiss and co-workers, in a series of manuscripts,¹⁶⁻¹⁸ reported that inhalation of LTC₄ and LTD₄ decreased the Vₑ₋ₑ⁻⁻ in five normal subjects. They used inhalation of aerosols generated from solutions of LTC₄ or LTD₄ of varying concentration in these studies. Solution concentrations were increased in approximately three-fold increments until a specific effect was achieved. They reported that nebulizer concentrations of LTC₄ and LTD₄ of about 1 to 10 µM were required to achieve a 30 percent decrease in Vₑ₋ₑ⁻⁻ (Fig 1). They also measured histamine responses in these individuals and thereby found that LTC₄ and LTD₄ were about 3,500 times more potent than with histamine in reducing the Vₑ₋ₑ⁻⁻.

Of interest in these studies were the observations that pre-treatment of subjects with aspirin did not alter the response to inhaled LTD₄. (The observation had been made, in animal studies, that pre-treatment with cyclo-oxygenase inhibitors enhanced the response to inhaled LTC₄ or LTD₄.¹⁶⁻¹⁸) They also reported the effects of inhaled LTD₄ on the FEV₁ in two normal subjects. In these two subjects, the concentrations of LTD₄ required to reduce the FEV₁ by 20 percent were 50 times greater than the concentrations required to reduce the Vₑ₋ₑ⁻⁻ by 30 percent. They interpret this observation to suggest that, in normal subjects, LTD₄ was a selective peripheral airway agonist.

Barnes and co-workers²⁰ reported data on the effect of inhaled LTC₄ and LTD₄ in six normal subjects. They used the technique of inhalation of graded concentrations of leukotrienes and, importantly, assessed the bioactivity of the solution remaining in the nebulizer reservoir after aerosolization of the leukotrienes. In their nebulizer system, about half the bioactivity was lost with aerosolization. They found that a mean initial nebulizer concentration of about 50 µM LTD₄ was required to reduce the Vₑ₋ₑ⁻⁻ by 30 percent; on the average, initial concentrations of about twice as great were required to reduce the specific airways conductance (SGaw). Compared to histamine, they found LTD₄ to be about 1,200 times more potent as a bronchoconstrictor agonist when the Vₑ₋ₑ⁻⁻ was used as the index of response. They interpreted these findings to suggest that, although the leukotrienes were more potent than histamine as airway constrictors, they did not have the selective peripheral effects suggested by Weiss et al.¹⁶⁻¹⁸ However, although SGaw may predominantly reflect central airway tone in the basal state, a decrement in SGaw may reflect alterations in central or peripheral airways. Thus, their findings are also consistent with a predominantly peripheral site of action of LTD₄, as compared to histamine.

Smith and co-workers²⁸ reported the effects of inhaled LTD₄ in six normal subjects. In this study, they measured specific conductance (SGaw), in addition to Vₑ₋ₑ⁻⁻ and FEV₁. They used the technique of exposure to aerosols generated from solutions of increasing leukotriene concentrations. In this study, methacholine (rather than histamine) was used as the reference agonist. The average concentration of LTD₄ required to decrease the SGaw or the Vₑ₋ₑ⁻⁻ by 30 percent was 180 µM (Fig 1). In contrast, the average effect of exposure to an aerosol generated from a solution of 900 µM LTD₄ was to decrease the FEV₁ by only 15 percent (Fig 1).

Thus, this study differs from those reported by Holroyde et al²⁰ and by us²⁶⁻²⁸ in that the amount of LTD₄ required to achieve a given response was about 45 times greater. There are a number of possible explanations for this difference. One is that Smith et al used the dipotassium salt of LTD₄, rather than the free acid; these may differ in potency. Another is that the protocol utilized by Smith et al incorporated intermittent inhalation to total lung capacity (TLC). This may have resulted in a diminished response to inhalation of leukotrienes. A third explanation is that the spectrum of responsiveness to inhaled leukotrienes, as judged by Vₑ₋ₑ⁻⁻, may not be unimodal; that is, within the normal population there may be two populations of individuals with bronchoresponsiveness to leukotrienes, varying by about 30- to 100-fold.

A final point made by Smith and co-workers²⁸ was that the response they measured—an equal effect of LTD₄ on Vₑ₋ₑ⁻⁻ and SGaw—was not indicative of the selective peripheral airway response to leukotrienes in normal subjects postulated by Weiss and co-workers.¹⁶⁻¹⁸ They reasoned that the SGaw should reflect predominantly large airway effects and, hence, if there was little large airway constriction, it should be only minimally altered. They explain the minimal change in the FEV₁ as due to the insensitivity of this index to large airway changes relative to the SGaw. However, an alternative explanation is that the SGaw was decreased due to marked peripheral airway constriction and that the minimal change in FEV₁ reflects the lack of a central airway effect. Resolution of this controversy will require more specific tests of central vs peripheral airway narrowing. This will likely require measurements of maximal expiratory flow rates while subjects inspire gases of varying physical properties.²⁶⁻²⁸ Of note are the findings of Weiss and co-workers³⁸ that the ratio of maximal expiratory flow while breathing a mixture of 80 percent helium and 20 percent oxygen to that achieved while breathing room air decreased after LTC₄ inhalation. This observation is more consistent with the theory of a selective peripheral airway effect of leukotrienes, rather than a widespread airway effect.

Bisgaard and co-workers³⁸ studied the effects of LTD₄ in nine normal subjects. They measured the FEV₁ and the Vₑ₋ₑ⁻⁻ as well as peak expiratory flow rate.
and trapped gas volume. When the $V_{sc}$-P was used as the index of bronchoconstrictor responsiveness, the mean nebulizer concentration required to achieve a 30 percent decrease in this index was 12.5 $\mu$M (Fig 1). The concentration of LTD$_4$ required to decrease the FEV$_1$ by 12 percent, or to decrease the peak expiratory flow rate by 21 percent, was greater than 200 $\mu$M. In contrast, average nebulizer concentrations of 5.5 $\mu$M were required to increase the volume of trapped gas by 47 percent. Two points bear consideration. The average concentration of LTD$_4$ required to achieve a 30 percent decrease in the $V_{sc}$-P (12.5 $\mu$M) is more similar to that reported by Holroyde et al$^{24}$ and Weiss et al,$^{35,36}$ and substantially less than that reported by Smith et al.$^{24}$ Second, if LTD$_4$ had a profile of activity on both large and small airways as suggested by Smith et al, it seems unlikely that the peak expiratory flow rate (an index of large airway tone) would not have been altered in the majority of subjects when concentrations of 200 $\mu$M LTD$_4$ were placed in the nebulizer reservoir. Thus, this profile of activity also suggests that LTD$_4$ is a relatively selective peripheral airway agonist.

There is uniform agreement that the sulfidopeptide leukotrienes are potent bronchoconstrictor substances; however, there is less uniformity concerning their potency relative to histamine or methacholine. Four out of five groups$^{9,16,23-27}$ report LTD$_4$ to be approximately 1,000 to 6,000 times more potent than histamine when $V_{sc}$-P is used as the bronchoconstriction index. One group reported a mean potency ratio for LTD$_4$ to methacholine of 285:1,$^{24}$ mostly as a result of the insensitivity of their subjects to inhaled LTD$_4$, as assessed by $V_{sc}$-P (mean concentration to reduce $V_{sc}$-P by 30 percent of 180 $\mu$M). Of interest in this regard is the distribution of airway responsiveness to LTD$_4$ in the 26 normal subjects reported thus far (Fig 1). Note that the distribution appears bi-modal, with one cluster of data near EC$_{50}$ values of 10 $\mu$M and a second near 200 $\mu$M. If this small sample reflects the distribution of LTD$_4$ responsiveness in the normal population at large, then the leukotrienes would be clearly distinguished from other bronchoactive agents which have been shown to have a unimodal population distribution. Further, there is unresolved controversy about the site of action of leukotrienes in normal airways; some investigators believe that they are both central and peripheral airway constrictors of comparable potency,$^{23,24}$ while others believe that the evidence more cogently supports a predominantly peripheral site of response for these agonists.$^{14,35,36}$ Further studies will be required to clarify both of these issues.

**Asthmatic Subjects**

If leukotrienes are important bronchoconstrictors in asthmatic syndromes, then asthmatic subjects should manifest a bronchoconstrictor response after inhalation of these agonists. Based on experience with other agonists (including histamine, methacholine, and carbachol) there is good reason to believe that asthmatic subjects should be hyperresponsive to inhaled leukotrienes as well. It was clearly surprising to us, therefore, when $V_{sc}$-P was used as the bronchoconstrictor index, that a group of six mildly asthmatic subjects did not manifest the expected degree of hyperresponsiveness to inhaled LTD$_4$. In our study, we examined a group of six mildly asthmatic subjects who were hyperresponsive to inhaled histamine; the geometric mean of the histamine concentration required to reduce $V_{sc}$-P by 30 percent was $170$ $\mu$M. This was 1/140 of the mean concentration of histamine required for a similar effect in normal subjects. In this group of asthmatic subjects, the mean concentration of LTD$_4$ required to reduce $V_{sc}$-P by 30 percent was $1.2$ $\mu$M (range, 0.1 to 18 $\mu$M), while a mean concentration of 4 $\mu$M was required to produce a similar effect in normal subjects (Fig 1). Thus, the asthmatic subjects were more responsive than the normal subjects, but the degree of this hyperresponsiveness was substantially less than expected.

Smith and co-workers$^{24}$ studied a group of asthmatic subjects, also with mild disease. They found that inhalation of an aerosol generated from a solution containing an average of 8 $\mu$M LTD$_4$ resulted in a 30 percent decrement in $V_{sc}$-P (Fig 1). Compared to their group of normal subjects, who required concentrations of LTD$_4$ of 180 $\mu$M to achieve a 30 percent fall in $V_{sc}$-P, these asthmatic subjects were 22 times more sensitive to inhaled LTD$_4$. However, when compared to the data from other groups for normal subjects,$^{16,23,27}$ the group of asthmatic subjects studied by Smith et al were only minimally hyperresponsive to LTD$_4$. This group also reported sensitivity data when the SGaw or FEV$_1$ was used as a bronchoconstrictor index. A mean LTD$_4$ concentration of 2 $\mu$M was required to decrease the SGaw by 30 percent, and a mean concentration of 6 $\mu$M was required to reduce the FEV$_1$ by 15 percent. This group was the first to establish that, in asthmatic subjects, the potency of LTD$_4$ was not substantially related to the bronchoconstrictor index used to assess this potency, thus clearly distinguishing normal from asthmatic subjects.

Bisgaard and co-workers$^{27}$ studied eight mildly asthmatic subjects using protocol similar to that employed by others. They found that a mean nebulizer concentration of 0.75 $\mu$M was required to decrease $V_{sc}$-P by 30 percent, which was 3/4 of the concentration required to produce a similar effect in their normal subjects (Fig 1). They confirmed the finding of Smith et al$^{24}$ in their asthmatic subjects; that is, the relative amount of leukotriene required to produce a bronchoconstrictor effect was not substantially different when different bronchoconstrictor indices were utilized to assess
response. Thus, in contrast to the controversy in normal subjects, the responsiveness of asthmatic subjects reported by a number of investigative groups is similar. Furthermore, the observation that the bronchoconstrictor index used to assess responsiveness is not of great importance in determining the airway responsiveness in asthmatic groups suggests that, in this setting, these agonists do not have a selective peripheral airway effect.

**Summary**

What is the meaning of these findings to the practicing chest physician? First, leukotrienes are potent airway constrictors; they are capable of reproducing the type of airway constriction observed in asthma. The role of leukotrienes in this regard has yet to be established, but experiments to test the importance of these agents in this setting are likely to be performed soon. Specifically, several leukotriene receptor antagonists or synthesis inhibitors have been identified and may provide the tools needed to test this crucial hypothesis. Second, the leukotrienes are unique bronchoactive agents in that the degree of hyperresponsiveness between normal and asthmatic subjects varies markedly with the bronchoconstrictor index used to assess response. When one compares normal subjects to asthmatic subjects, there is substantial overlap in leukotriene sensitivity among groups when \( V_{\text{aw}} \cdot P \) is used as the bronchoconstrictor index. However, when the FEV, is used as the bronchoconstrictor index, there is little overlap in sensitivity between normal and asthmatic subjects, and the separation between the two groups is even more clearly made than it is with histamine or methacholine challenge. Thus, LTD, inhalation challenge may replace the histamine and methacholine challenges in the diagnosis of cryptic shortness of breath. Third, the differential sensitivity of various bronchoconstrictor indices in both normal and asthmatic subjects when leukotrienes are used may provide clues as to the locus of airway hyperresponsiveness in asthma. Thus, leukotrienes hold the promise of new ways to treat and diagnose asthma, as well as providing new insights into the pathobiology of the disease itself.

**References**


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