Effect of a 5-lipoxygenase inhibitor on leukotriene generation and airway responses after allergen challenge in asthmatic patients

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Abstract

The effect of a single oral dose (800 mg) of zileuton (A-64077), a specific 5-lipoxygenase inhibitor, on the early and late airway responses to inhaled allergen was studied in a randomised, double blind, placebo controlled, and crossover trial in nine subjects with atopic asthma. Leukotriene generation was also assessed in vivo by measuring urinary leukotriene (LT) E4 excretion, and ex vivo by measuring calcium ionophore stimulated whole blood LTB4 production. Zileuton almost completely inhibited ex vivo LTB4 production but reduced urinary excretion of LTE4 by only about half. There was a trend for the early asthmatic response to be less on the day of zileuton treatment, but this did not reach statistical significance (p = 0.08). The zileuton induced reduction in maximum fall in FEV1, in the early asthmatic response was, however, significantly related to the reduction in urinary LTE4 excretion (r = 0.8), but not to the reduction in LTB4 generation ex vivo. There was no significant change in the allergen induced late asthmatic response, or in the increase in airway responsiveness to methacholine following antigen. The results provide some support for the hypothesis that the cysteinyl leukotrienes have a role in the allergen induced early asthmatic response. More complete in vivo inhibition of 5-lipoxygenase may be needed to produce a significant reduction in airway response to allergen challenge.

The leukotrienes, products of the 5-lipoxygenase pathway of arachidonic acid metabolism, are thought to be important mediators in the pathogenesis of asthma because their biological activities produce changes that are similar to those seen in asthma.13 The cysteinyl leukotrienes, LTA4, LTD4, and LTE4, are potent bronchoconstrictor agents in normal and asthmatic subjects,14 and LTE4 has been reported to increase airway hyperresponsiveness.1 LTD4 is a potent chemotactic agent for leucocytes,4 and may be important in mediating the inflammatory process in asthmatic airways. LTD4 can increase mucus production in human airway preparations5 and airway microvascular leakage in animals.6 Several clinical studies with cysteinyl leukotriene antagonists in asthmatic subjects have shown a slight reduction in the early asthmatic response to inhaled allergen11 12 and in cold air induced bronchoconstriction.13 The compounds studied were relatively weak cysteinyl leukotriene antagonists, however, and they would in addition leave the effects of the dihydroxy acid LTB4 unopposed. An alternative approach is to inhibit the 5-lipoxygenase enzyme to reduce synthesis of all the leukotriens. Several different classes of 5-lipoxygenase inhibitors have been found to be effective in animal models of asthma.14-16 No significant reduction of allergen induced bronchoconstriction was, however, proposed by piriprost17 or nafazatrom18 in two clinical studies in man—but nafazatrom was found not to prevent ex vivo leukotriene production in man after oral dosing despite having 5-lipoxygenase inhibitory activity in vitro (the activity of piriprost in man was not studied).

After exposure to inhaled allergen there is an early and late bronchoconstrictor response in asthmatic subjects and an increase in airway responsiveness. Several mediators, including the leukotrienes, are generated during antigen challenge and may be important in mediating the airway responses. Zileuton, a hydroxamic acid 5-lipoxygenase inhibitor,19 has been shown to inhibit calcium ionophore stimulated human neutrophil production of LTB4 in man ex vivo after oral dosing,20 and is effective in inhibiting allergen induced contraction of tracheal smooth muscle in the guinea pig.21 We have investigated the efficacy of zileuton (800 mg) on the generation of cysteinyl leukotriene in vivo (assessed by urine LTE4 concentration) induced by inhaled allergen and on the generation of LBT4 ex vivo (by calcium ionophore stimulated whole blood) in asthmatic subjects. We assessed the inhaled allergen induced airway responses of the early and late asthmatic response and changes in airway responsiveness.

Methods

Subjects
Eleven non-smoking men with atopic asthma (mean age 28, range 19–44 years) with an FEV1 above 70% predicted were recruited (table). Subjects were having inhaled salbutamol only, apart from one who was also inhaling steroids and taking oral theophyllines. All subjects had been shown to have a dual asthmatic response to allergen on screening. None had had an upper respiratory tract infection or exacerbation of asthma.
within six weeks of starting the study. Non-steroidal anti-inflammatory drugs were not allowed during the study period. The study was approved by the ethics committee of the National Heart and Lung Hospitals, and fully informed written consent was obtained from each subject.

Subjects had to be healthy at the physical examination and to have normal results in biochemical and haematological tests and a normal electrocardiogram before entry into the study. These were repeated during the study and at the end to assess the safety and tolerability of zileuton.

STUDY PROTOCOL

Allergen challenge

On a screening day the atopy of each subject was confirmed by obtaining a positive skin-prick test response to either Dermatophagoides pteronyssinus or mixed grass pollen allergen extracts (Pharmacia, Milton Keynes) dissolved in normal saline. Subjects first inhaled the diluent, aerosolised with a Wright nebuliser (volume 2 ml, flow rate 7 l/min, output 0–2 ml/min), through a face mask with open mouthed tidal breathing for two minutes, and FEV₁ was then measured for 15 minutes to exclude a bronchoconstrictor response to diluent. Cumulative doses of allergen were then inhaled in the same way to identify subjects with a dual asthmatic response. The initial allergen concentration used for inhalation was that which caused a 2–3 mm diameter weal in the skinprick test. Doubling concentrations of allergen were inhaled until an early asthmatic response (fall in FEV₁ of at least 20% of the post-diluent value) was seen. No further allergen was then given, FEV₁ was measured up to eight hours to detect any late asthmatic response, which was defined as a fall in FEV₁ of at least 15% of post-diluent value. If a dual response was seen, the final allergen dose reached was used on the two allergen challenge study days. Of the 17 subjects screened, six subjects with an isolated early asthmatic response were not enrolled into the main study.

Two to three weeks later subjects returned for the first allergen challenge study. Zileuton (800 mg) or matching placebo was ingested in the morning, and allergen challenge was performed three hours later. FEV₁ was measured every 10 minutes for the first hour after challenge, and then hourly for the next seven hours. At least two weeks later subjects returned for the second study to continue with an identical protocol provided that the baseline FEV₁ was within 15% of that on the first study day. If not, they were asked to return for another study day.

FEV₁ was measured with a dry bell spirometer (Vitalograph, Buckingham) with the subject sitting upright. The mean of three good expiratory efforts was taken. Before each test subjects rested in a warm room for at least 15 minutes.

Non-specific airway responsiveness was assessed according to the method of Cockcroft et al.²² on the day before and after each allergen test day, at the same time of the day for each subject. The provocative dose of methacholine needed to reduce FEV₁ by 20% of the post-diluent value (PC₂₀ FEV₁) was determined. Methacholine was inhaled in the same way as allergen.

Assessment of 5-lipoxygenase inhibition

5-Lipoxygenase activity after allergen challenge was assessed by measuring urinary LTE₄ excretion.²¹ Immediately before challenge subjects emptied their bladder and urine was then collected for four hours. Urine volume and pH were measured and aliquots of 30 ml were stored at −70°C. LTE₄ was extracted from the urine by reverse phase high performance liquid chromatography, tritiated LTE₄ being used as an internal standard,²⁴ and measured by radioimmunoassay (Amersham International, Amersham). The efficacy of zileuton was also assessed by calcium ionophore stimulated whole blood LTB₄ production ex vivo. Three and a half, four, five, seven, nine, and 10 hours after it was given 5 ml of whole blood was incubated with 5 µl of 50 mM calcium ionophore for 15 minutes and centrifuged, and the supernatant was stored at −70°C. LTB₄ was extracted by high performance liquid chromatography, and assayed by radioimmunoassay.

Drug level

Plasma level of drug was measured at 3, 5, 4 and 5 hours post-dose on both placebo and active days. Zileuton was extracted by high performance liquid chromatography and measured by ultraviolet absorbance as developed by Abbott Laboratories.

ANALYSIS

PC₂₀ values were log transformed before analysis. FEV₁ and PC₂₀ values were compared by analysis of variance. Paired data were analysed by the Wilcoxon sign rank test and the relation between two variables by regression analysis. Results are presented as means with the standard errors of the mean in parentheses unless otherwise stated. p < 0.05 was accepted as significant.

Results

SUBJECTS

Of the 11 subjects who showed a late response
Effect of zileuton (800 mg orally) on early and late asthmatic responses after allergen challenge. ● Zileuton; ○ placebo. Values are means with 1 SEM.

Effect of zileuton on calcium ionophore stimulated whole blood leukotriene (LT) B₄ production. Values are means with 1 SEM. *p < 0.005.

AIRWAY RESPONSES
Baseline values before allergen challenge on placebo and treatment days were closely matched (fig 1). No period or treatment effect was found by two way analysis according the the method of Hills and Armitage. There was a trend for the fall in FEV₁ during the early asthmatic response to be less with zileuton, but this did not reach statistical significance at any point (maximum fall in FEV₁: placebo 1·08 (0·25) l, zileuton 0·83 (0·21) l; p = 0·18). The maximum difference between placebo and zileuton was 40 minutes after allergen challenge, which is after the nadir of the fall in FEV₁ (fig 1; difference in FEV₁: −0·33 (0·16) l; p = 0·08). There was no difference between placebo and zileuton in the late asthmatic response (maximum fall in FEV₁: placebo 1·16 (0·24) l, zileuton 0·92 (0·27) l; p = 0·22). There were no significant differences in the areas under the curve during the early or late phases.

Figure 2  Effect of zileuton on airway responsiveness to inhaled methacholine before and after allergen challenge. PC₂₀ FEV₁, is the provocative dose (mg/ml) of inhaled methacholine needed to cause a fall in FEV₁ of 20% of the post-diluent value, and individual values for each subject before and after allergen challenge are shown. The geometric mean and 95% confidence interval for each day are also shown.

Figure 3  Time course of effect of zileuton on calcium ionophore stimulated whole blood leukotriene (LT) B₄ production. Values are means with 1 SEM. *p < 0.005.

Figure 4  Effect of zileuton (■) and placebo (△) on (a) mean ex vivo leukotriene (LT) B₄ production and (b) urine LTE₄. Mean ex vivo LT₄ production refers to mean whole blood calcium ionophore stimulated LT₄ production for four hours after allergen challenge. Urine LTE₄ is the total urinary excretion of LTE₄ over four hours. Values are means with 1 SEM.
Baseline airway responses to inhaled metacholine before allergen challenge were very similar for placebo and zileuton (geometric mean PC_{20} FEV_{1} = 0.41 (95% confidence interval: 0.20–0.84) and 0.44 mg/ml (0.15–0.78). The increase in airway responsiveness after allergen challenge did not differ significantly after placebo and zileuton treatment (before/after challenge PC_{20} FEV_{1}, metacholine: placebo 2.13, zileuton 1.89; fig 2).

**EFFECTS ON IN Vivo AND EX Vivo LEUKOTRIENe GENERATION**

Zileuton substantially inhibited calcium ionophore stimulated whole blood LT_{B} production with maximum inhibition (93.1% of baseline; p < 0.005) four hours post-dose; after the dose had been given significant inhibition was still present at 10 hours after the dose (63.5% of baseline; p < 0.005; fig 3). Mean urinary LT_{E} excretion after allergen challenge was reduced by about half by zileuton treatment (placebo 111.5 (23.5), zileuton 58.2 (14.8) ng/mmol creatinine; p < 0.01; fig 4).

The zileuton induced change in maximum fall in FEV_{1}, for the early asthmatic response correlated with the reduction in urinary LT_{E} production (r = 0.8, p < 0.01; fig 5) but not with the inhibition of calcium ionophore stimulated whole blood LT_{B} production (r = 0.04, p > 0.5).

**PLASMA ZILEUTON CONCENTRATIONS**

Mean plasma zileuton concentrations three and a half, four, and five hours after ingestion were 3.37 (0.36), 3.03 (0.39) and 2.6 (0.27) µg/ml respectively on the days of zileuton treatment; no drug was detected on the placebo day. There was no relation between the peak drug concentrations and change in FEV_{1}, during the early and late asthmatic responses, reduction in urinary LT_{E} excretion, or calcium ionophore stimulated whole blood LT_{B} production.

**Discussion**

We found that zileuton at the dose used in the study partially reduced urinary LT_{E} excretion and substantially reduced calcium ionophore stimulated whole blood LT_{B} production. There was a trend towards a reduction in the fall in FEV_{1} during the early asthmatic response with zileuton, but neither this nor the late asthmatic response or increase in airway responsiveness differed significantly between zileuton and placebo. The change in the early asthmatic response induced by zileuton correlated with the change in urinary LT_{E} concentrations but not with the change in ex vivo LT_{B} production.

In vivo activation of the 5-lipoxygenase pathway in asthmatic patients during an asthmatic attack has been shown by the increased urinary excretion of LT_{E} and by increased concentrations of cysteinyl leukotrienes in bronchoalveolar lavage fluid recovered by fibreoptic bronchoscopy during the early and late responses to inhaled allergen challenge. In the present study the activation of 5-lipoxygenase in asthmatics was assessed by urinary excretion of LT_{E}, as this is simple and non-invasive and did not interfere with measurements of lung function. Urinary LT_{E} excretion after allergen challenge is likely to reflect airway leukotriene generation, as there was no evidence of systemic effects in any subjects. Zileuton reduced the urinary excretion of LT_{E} by about half, though not down to the range found in non-asthmatic subjects in this laboratory. This suggests that 5-lipoxygenase activity was only partially inhibited, and may explain the non-significant reduction in bronchoconstriction during the early asthmatic response. A significant reduction of airway response to allergen may be seen only if more complete inhibition of leukotrienes generation can be achieved, as the leukotrienes are very potent biological agents: the cysteinyl leukotrienes are thousands of times more potent than histamine in causing bronchoconstriction, and LT_{B} is one of the most potent chemotactic agents known.

The early bronchoconstrictor response after allergen challenge is thought to be due to release of mediators, such as histamine and leukotrienes, from inflammatory cells via IgE mediated mechanisms. Although not statis-
zileuton was more effective in inhibiting ex vivo production of the early asthmatic response. There was a trend towards a reduction in the late asthmatic constrictor response but this was not significant. Measurement of leukotriene production in vivo rather than ex vivo may be more useful in future studies of 5-lipoxygenase inhibitors in man. Our results would fit a role for the leukotrienes in the early asthmatic response to inhaled allergen, particularly if inhibition of leukotriene related airway responses requires near complete inhibition of the 5-lipoxygenase enzyme in vivo. Further studies with higher or repeated doses of zileuton or with more potent 5-lipoxygenase inhibitors in man are needed.

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