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

Kok P Hui, Ian K Taylor, Graham W Taylor, Paul Rubin, James Kesterson, Neil C Barnes, Peter J Barnes

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, LTC4, LTD4, and LTE4, are potent bronchoconstrictor agents in normal and asthmatic subjects,14 and LTD4 has been reported to increase airway hyperresponsiveness.1 LTD4 is a potent chemotactic agent for leucocytes,1 and may be important in mediating the inflammatory process in asthmatic airways. LTD4 can increase mucus production in human airway preparations6 and airway microvascular leakage in animals.10 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 LTB4 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) dis-
solved in normal saline. Subjects first inhaled
the diluent, aerosolised with a Wright nebu-
lariser (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 inhala-
tion was that which caused a 2-3 mm dia-
meter seal in the skin prick 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 per-
formed 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 re-
turned for the second study to continue with
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 on the day before and after each aller-
gen test day, at the same time of the day for
each subject. The provocative dose of metha-
choline needed to reduce FEV₁ by 20% of the
post-diluent value (PC₂₀ FEV₁) was deter-
mined. 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. 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 stan-
dard, and measured by radioimmunoassay
(Amersham International, Amersham). The
efficacy of zileuton was also assessed by cal-
cium 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 min-
utes 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 com-
pared 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

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**Characteristics of the asthmatic subjects**

<table>
<thead>
<tr>
<th>Subject No</th>
<th>Age (y)</th>
<th>FEV₁ l (% pred)</th>
<th>PC₂₀ methacholine* (mg/ml)</th>
<th>Drug†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>4.9 (98)</td>
<td>0.04</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>3.0 (81)</td>
<td>0.19</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>4.9 (117)</td>
<td>0.2</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>4.1 (98)</td>
<td>0.26</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>4.8 (117)</td>
<td>3.4</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>4.3 (100)</td>
<td>0.15</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>4.6 (105)</td>
<td>1.18</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>2.6 (84)</td>
<td>0.57</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>4.6 (112)</td>
<td>1.24</td>
<td>Salbutamol, beclometasone, theophylline</td>
</tr>
</tbody>
</table>

*The provocative dose of inhaled methacholine needed to cause a 20% fall in baseline FEV₁.
†Inhaled, apart from the theophylline (oral) taken by subject 9.
to inhaled allergen, two were withdrawn. One developed an exacerbation of asthma following a chest infection after the first study day (placebo). The other subject who had an FEV₁ of just over 70% predicted was withdrawn when his FEV₁ fell to below 70% predicted before allergen challenge. The remaining nine subjects completed the study, and all results were included in the analysis. Zileuton was well tolerated and there were no important biochemical, haematological, or electrocardiographic changes after treatment.

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.²⁵ 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.

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**Figure 1** Effect of zileuton (800 mg orally) on early and late asthmatic responses after allergen challenge. ● Zileuton; ○ placebo. Values are means with 1 SEM.

**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 LTB\(_4\) 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 LTE\(_4\) 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 LTE\(_4\) production (\(r = 0.8\), \(p < 0.01\); fig 5) but not with the inhibition of calcium ionophore stimulated whole blood LTB\(_4\) 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) \(\mu g/ml\) 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 LTE\(_4\) excretion, or calcium ionophore stimulated whole blood LTB\(_4\) production.

**Discussion**

We found that zileuton at the dose used in the study partially reduced urinary LTE\(_4\) excretion and substantially reduced calcium ionophore stimulated whole blood LTB\(_4\) production ex vivo. 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 LTE\(_4\) concentrations but not with the change in ex vivo LTB\(_4\) 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 LTE\(_4\), and by increased concentrations of cysteinyl leukotrienes in bronchoalveolar lavage fluid recovered by fiberoptic bronchoscopy during the early and late responses to inhaled allergen challenge. In the present study the activation of 5-lipoxygenase in airways was assessed by urinary excretion of LTE\(_4\), as this is simple and non-invasive and did not interfere with measurements of lung function. Urinary LTE\(_4\) 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 LTE\(_4\) 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 LTB\(_4\) 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 stas-
tically significant, the maximum reduction of the fall in FEV₁ produced by zileuton was after the nadir in the fall in FEV₁, following allergen challenge. This time course of action is consistent with the known action of leucotrienes and histamine, inhaled cysteinyl leukotrienes causing maximum bronchoconstriction at about 15 minutes, and histamine somewhat earlier. Leukotrienes are not stored by resting cells (unlike histamine), and are generated only after stimulation, so they would be expected to contribute more to the latter part of the early bronchoconstrictor response after allergen challenge. In a study by Britton and coworkers, in which a leukotriene antagonist, L649,923, reduced the early response to allergen, the maximum effect was seen after the nadir of the early asthmatic response. In another study an antihistamine was effective in the first 15 minutes.

In the fall in FEV₁-lipoxygenase adequately. Alternatively, this may be due to inadequate penetration of the nadir, in the fall in FEV₁.

The pattern of change in the early asthmatic response is similar to that in the present study. This may be because the activity of zileuton in the single dose of 800 mg used in this study may have been insufficient by that time, particularly when it was only partially effective during the early phase as measured by urinary LTE₄ levels. In two patients where serum drug concentrations seven hours after the dose were available little zileuton was detected.

Previous studies of 5-lipoxygenase inhibitors in man have depended on ex vivo methods of assessing its activity. In the present study zileuton was more effective in inhibiting ex vivo calcium ionophore stimulated whole blood LTB₄ production than in inhibiting leukotriene production in vivo as reflected by urinary LTE₄ excretion, and there was no correlation between these two variables. There are several possible explanations. The lesser effect in vivo may be due to inadequate penetration of zileuton into airway tissues, or a higher plasma concentration may be needed to suppress airways 5-lipoxygenase adequately. Alternatively, different inflammatory cells may have different susceptibilities to the effect of zileuton, such that the mast cells, macrophages, or eosinophils in the airways may not have been affected as much as neutrophils in the peripheral blood. In addition, the cellular generation of the cysteinyl leukotrienes may be less affected than that of LTB₄. The lack of relation between the changes in leukotriene generation ex vivo and the early asthmatic response suggest that this is not a useful method for assessing the clinical efficacy of 5-lipoxygenase inhibitors in man.

In this study a single oral dose (800 mg) of zileuton was only partially effective in inhibiting activation of 5-lipoxygenase in vivo by inhaled allergen despite near complete ex vivo inhibitory activity. There was a trend towards a reduction in the early asthmatic bronchoconstrictor 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.

17 Manns JS, Holgate ST. Effect of piriprost (U-60257), a novel lipoxygenase inhibitor, on allergen and exercise induced asthma. Thorax 1986;41:746-52.
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