Effects of single-dose zileuton on bronchial hyperresponsiveness in asthmatic patients treated with inhaled corticosteroids


ABSTRACT: The aim of the study was to determine whether zileuton, an inhibitor of 5-lipoxygenase, attenuated bronchial hyperresponsiveness (BHR) in asthmatic subjects who had marked BHR during maintenance treatment with inhaled corticosteroids (ICS).

In a randomized, double-blind, placebo-controlled, cross-over study, a challenge test with histamine (provocative concentration of histamine producing a 20% fall in forced expiratory volume in one second (FEVi) (PC_{20, hist})) and with ultrasonically nebulized distilled water (UNDW) (provocative dose of UNDW producing a 20% fall in FEVi (PD_{20, UNDW})) was performed in seven patients with asthma after intake of either 400 mg zileuton or placebo. All patients (mean age 33 yrs, mean FEV1 111% of predicted) had marked BHR, as indicated by a mean PD_{20, UNDW} of 4.74 mL under treatment for at least 6 months with up to 800 μg ICS (mean 536 μg daily). On four different occasions, separated by at least 5 days, two UNDW and two histamine challenge tests were performed in random order 3 h after a morning dose of either zileuton or placebo.

Neither zileuton nor placebo changed baseline airway calibre prior to provocation. Zileuton increased PC_{20, hist} from 0.99 to 5.64 mg·mL\(^{-1}\) (2.1 doubling doses; p<0.03 compared to placebo), and increased PD_{20, UNDW} from 3.10 to 9.31 mL (1.3 doubling doses; p<0.05 compared to placebo).

In conclusion, a single dose of 400 mg zileuton attenuates bronchial hyperresponsiveness to histamine and ultrasonically nebulized distilled water in asthmatic patients with marked bronchial hyperresponsiveness during treatment with inhaled corticosteroids.


Current therapy for asthma is aimed at suppressing the inflammatory process in the wall of the airways, thus reducing symptoms of bronchial hyperresponsiveness (BHR) and airways obstruction. Inhaled corticosteroids (ICS) have been shown to be very effective in this respect. This may result in a tendency towards prescribing high doses for prolonged periods of time. In this respect, concerns about the safety of ICS have recently been addressed [1, 2]. ICS may reduce serum cortisol levels, alter biochemical markers of bone metabolism, and, in children, suppress growth velocity. The long-term clinical significance of these changes is not yet clear.

Other classes of anti-inflammatory agents for the treatment of asthma are currently under investigation. One of these drugs, zileuton (N-(1-(benzo(θ)thien-2-ylethyl))-N-hydroxyurea), an inhibitor of the 5-lipoxygenase pathway, has been shown to be efficacious in patients with asthma [3–6]. Single-dose studies have shown an attenuation of the asthmatic response to cold dry air [7], but not after allergen challenge [8]. In aspirin-sensitive asthmatics, 2.4 mg zileuton daily for 1 week prevented the fall in forced expiratory volume in one second (FEV1) in response to ingestion of aspirin [9]. A similar dose, administered for 2 days, attenuated exercise-induced bronchoconstriction [10]. A 13 week treatment period with zileuton, 1.6 or 2.4 mg daily, resulted in a 58% increase in the cold air minute ventilation required to cause a 15% decrease in FEV1 [11]. Asthma symptoms and FEV1 improved after 4 [12] to 13 weeks [13] of treatment with a similar dose of zileuton, without clear side-effects [12].

Whether zileuton has an additional effect on BHR in patients already treated with ICS is unknown. Such an action would be of potential clinical relevance, since it would allow tapering of the dosage of ICS. In the present study, this question was addressed by measuring the effect of a single dose of 400 mg zileuton during challenge with histamine (which, in the main, acts directly on airway smooth muscle) and ultrasonically-nebulized distilled water (UNDW) (which induces bronchoconstriction by the release of several mediators) in patients with asthma who were on maintenance treatment with ICS.

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Patients and methods

Study design

This study was carried out using a randomized, double-blind, placebo-controlled, cross-over design. Seven patients with asthma according to the American Thoracic Society (ATS) criteria [14], who had marked BHR during treatment for at least 6 months with up to 800 μg ICS, were studied. They were tested on four different occasions separated by at least 5 days within a period of 4 weeks. Two challenge tests with UNDW (provocative dose of UNDW causing a 20% fall in FEV1) (PD20.UNDW)) and two with histamine (provocative concentration of histamine causing a 20% fall in FEV1 (PC20.Hist)) were performed in random order 3 h after an 09:00 h dose of either zileuton or placebo.

Subjects

Nonsmoking patients with allergic asthma, who exhibited marked BHR under maintenance treatment with ICS for at least 6 months, were selected. Allergy was defined as an elevated specific immunoglobulin E (IgE) or a positive skin-prick test for house dust mite or at least two other common aeroallergens [15]. Patients were included if their FEV1 was above 70% of predicted [16] and PD20.UNDW was below 8 mL. The second criterion was set for two reasons: firstly, from previous studies in our laboratory it appeared that this level of PD20.UNDW was associated with a PC20.Hist of less than 4 mg·mL⁻¹ [17, 18]; and, secondly, these levels of BHR provide the opportunity for measurable improvement by the drug under investigation. The current use of ICS (beclomethasone dipropionate or budesonide, either by metered-dose inhaler (MDI) or by dry powder) was not allowed to be higher than 800 μg daily. All subjects used salbutamol as rescue medication. Patients on long-acting β₂-agonists, theophylline and oral anticoagulants, as well as patients who were pregnant or breast-feeding, were excluded from participation in the study.

A total number of 17 patients, fulfilling the requirements with regard to current medication, were screened to participate. Ten subjects were not enrolled into the study, since the screening UNDW challenge test appeared to be negative. By chance, the seven subjects participating in the study were all female. Laboratory testing (complete blood count, serum chemistry) was performed after each provocation to assess the safety and tolerability of zileuton.

Measurement of lung function and BHR

Subjects did not use ICS or rescue medication for at least 8 h before each visit and rested 30 min before the measurements were started. Baseline FEV1 on the four study days had to be within 10%, otherwise the test was postponed to a later day. Lung function and responses to provocation with histamine and UNDW were assessed by FEV1, measured with a flow-volume curve recorded on a heated pneumotachograph (Spiro analyzer ST 250®; Fukuda Sangyo Co., Tokyo, Japan). Baseline lung function was recorded as the best of three reproducible values of FEV1 (within 5%) before the provocation tests.

The same technician from the lung function department always checked the patients morning FEV1. If the patient fulfilled the entry criteria, the technician then delivered the correct tablet according to the randomization list. Randomization was performed in blocks of four prior to the start of the study. For each participant number a sequence of measurements with either tablet "A" or "B" was listed according to the randomization list. The four tests (i.e. A-Hist, A-UNDW, B-Hist, B-UNDW) were sequenced at random. The patients received oral zileuton (capsules, 400 mg) or placebo capsules of identical appearance (both supplied by Abbott Laboratories) according to a blinded and randomized code. This dose of zileuton was chosen since leukotriene B₄ (LTB₄) production by ex vivo calcium ionophore-stimulated whole blood from healthy volunteers was inhibited by up to 80% of baseline. This response was studied in dosages of 200, 400, 600 and 800 mg of zileuton, and this reduction appeared to plateau at a dose of 400 mg [19]. Thus, 400 mg appears to be a dose that might be expected to have some physiological effect.

Three hours after intake, FEV1 was measured again. Subsequently, the provocation tests were performed. The histamine provocation test was carried out according to the method of Cockcroft et al. [20]. For 2 min, patients inhaled doubling concentrations of histamine acid phosphate by tidal breathing, increasing from 0.03 to 16 mg·mL⁻¹. Histamine was nebulized with a DeVilbiss 646 nebulizer (DeVilbiss, Somerset, PA, USA) with a fixed output of 0.13 mg·mL⁻¹. PC20.Hist was determined in mg·mL⁻¹ by interpolating the last two points of the dose-response curve on a semilogarithmic scale. Responsiveness to UNDW was assessed according to the method of Groo et al. [17]. UNDW was generated with an ultrasonic nebulizer (Ultraneb 99; DeVilbiss, Somerset, PA, USA) at a fixed output of 2.00±0.05 mg·mL⁻¹. After inhalation of 20 L of ambient air through the system, patients inhaled doubling volumes of air with UNDW (3, 5, 10, up to 160 L), measured with a Wright respirometer (British Oxygen Co., London, UK). The respirometer was placed between the aerosol hose and the mouthpiece by means of a two-way valve. Before and after each test, the nebulizer chamber and aerosol hose were weighed to determine the exact amount of distilled water inhaled. The cumulative amount of inhaled water (mL) causing a 20% fall in FEV1 from post-air values (PD20.UNDW) was calculated by linear interpolation on a semilogarithmic curve.

Statistical analysis

PC20 and PD20 values were log transformed before analysis and compared by analysis of variance using the Statistical Package for the Social Sciences (SPSS) version 5.0 software [21]. Paired data were analysed using the Wilcoxon signed rank test. Carry-over effects were analysed according to Pocock [22] using the Mann-Whitney U-test. Results are presented as means and standard errors of the means (SEM). Significance was accepted at a p-value of less than 0.05.
Results

Patient characteristics are summarized in Table 1. Their baseline FEV₁ ranged 86–134% pred. The daily use of ICS ranged 200–800 μg daily (mean 536 μg). Under this medication all patients had marked BHR, as indicated by a mean PD₂₀,UNDW of 4.74 mL at entry.

Table 1. - Patients characteristics at baseline

<table>
<thead>
<tr>
<th>No.</th>
<th>Pt</th>
<th>Age (yrs)</th>
<th>ICS (mg·day⁻¹)</th>
<th>ß₂ use (mg·day⁻¹)</th>
<th>Prestudy FEV₁ (L)</th>
<th>PD₂₀,UNDW (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>BDP, 400</td>
<td>0.1 p.r.n.</td>
<td>3.53</td>
<td>115</td>
<td>3.01</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>BDP, 750</td>
<td>0.4 p.r.n.</td>
<td>2.70</td>
<td>134</td>
<td>7.98</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>BUD, 800</td>
<td>0.8</td>
<td>2.41</td>
<td>86</td>
<td>3.61</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>BDP, 200</td>
<td>0.4 p.r.n.</td>
<td>3.17</td>
<td>88</td>
<td>3.97</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>BUD, 400</td>
<td>0.8</td>
<td>4.01</td>
<td>119</td>
<td>5.48</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>BDP, 400</td>
<td>0.1 p.r.n.</td>
<td>3.42</td>
<td>111</td>
<td>3.18</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>BDP, 800</td>
<td>1.6</td>
<td>3.00</td>
<td>125</td>
<td>6.00</td>
</tr>
</tbody>
</table>

Mean 33 536 3.18 111 4.74
SEM 5 92 0.20 7 0.69

Pt: patient; ICS: inhaled corticosteroids; ß₂ use: mean daily use of ß₂-agonist in the previous 6 months; FEV₁: forced expiratory volume in one second; PD₂₀,UNDW: provocative dose of ultrasonically nebulized distilled water causing a 20% fall in FEV₁ from baseline; BDP: beclomethasone dipropionate; BUD: budesonide.

Fig. 1. - Individual data for the provocative concentration of histamine causing a 20% fall in forced expiratory volume in one second (PC₂₀,Hist) after placebo (open symbols) and zileuton (closed symbols). Values are presented as mean±SEM, increasing from 0.99 (0.29) mg·mL⁻¹ after placebo to 5.64 (2.27) mg·mL⁻¹ after zileuton (2.1 doubling doses; p<0.03). The same symbols for individual patients are used in figures 1 and 2.

Fig. 2. - Individual data for the provocative dose of ultrasonically nebulized distilled water causing a 20% fall in forced expiratory volume in one second (PD₂₀,UNDW) after placebo (open symbols) and zileuton (closed symbols). Values are presented as mean±SEM, increasing from 3.10 (0.56) mL after placebo to 9.31 (3.38) mL after zileuton (1.3 doubling doses; p<0.05). The same symbols for individual patients are used in figures 1 and 2.

Zileuton increased PC₂₀,Hist from 0.99 (0.29) to 5.64 (2.27) mg·mL⁻¹ after placebo (0.99 (0.29) mg·mL⁻¹ after zileuton (2.1 doubling doses; p<0.03). The same symbols for individual patients are used in figures 1 and 2.

Baseline FEV₁ before administration of zileuton or placebo was similar on the four occasions (Table 2). Neither zileuton nor placebo changed FEV₁ 3 h after intake. Thus, zileuton did not cause acute bronchodilation in these patients.

Zileuton increased PC₂₀,Hist from 0.99 (0.29) to 5.64 (2.27) mg·mL⁻¹ after placebo (0.99 (0.29) mg·mL⁻¹ after zileuton (2.1 doubling doses; p<0.03). The same symbols for individual patients are used in figures 1 and 2.

To exclude some unexpected order effect, Table 3 lists the individual data set out by order of challenge and drug with the gap between the challenges applied. Apparently, no order effect was present; e.g. patients who received zileuton at the last challenge (Nos. 1, 3, 6 and 7) and patients who received placebo at the last challenge (Nos. 2, 4 and 5) both showed improvements in the level of hyperresponsiveness.

Table 2. - FEV₁ at baseline and at the start of each provocation test

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Zileuton</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FEV₁ before histamine</td>
<td>FEV₁ before UNDW</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>After 3 h</td>
</tr>
<tr>
<td>1</td>
<td>3.58</td>
<td>3.53</td>
</tr>
<tr>
<td>2</td>
<td>2.65</td>
<td>2.71</td>
</tr>
<tr>
<td>3</td>
<td>2.42</td>
<td>2.35</td>
</tr>
<tr>
<td>4</td>
<td>3.22</td>
<td>3.30</td>
</tr>
<tr>
<td>5</td>
<td>4.03</td>
<td>4.01</td>
</tr>
<tr>
<td>6</td>
<td>3.51</td>
<td>3.71</td>
</tr>
<tr>
<td>7</td>
<td>3.03</td>
<td>3.13</td>
</tr>
<tr>
<td>Mean</td>
<td>3.21</td>
<td>3.25</td>
</tr>
<tr>
<td>SEM</td>
<td>0.21</td>
<td>0.22</td>
</tr>
</tbody>
</table>

FEV₁: forced expiratory volume in one second; Pt: patient; UNDW: ultrasonically nebulized distilled water.
Table 3. FEV1 at baseline and at the start of each provocation test in the sequence of the tests performed.

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Visit 1 FEV1 baseline and after 3 h</th>
<th>Challenge</th>
<th>Visit 2 FEV1 baseline and after 3 h</th>
<th>Challenge</th>
<th>Visit 3 FEV1 baseline and after 3 h</th>
<th>Challenge</th>
<th>Visit 4 FEV1 baseline and after 3 h</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Day 0 3.58±3.53 L</td>
<td>Z-Hist</td>
<td>Day 7 3.78±3.59 L</td>
<td>P-UNDW</td>
<td>Day 14 3.68±3.52 L</td>
<td>P-Hist</td>
<td>Day 21 3.76±3.54 L</td>
<td>Z-UNDW</td>
</tr>
<tr>
<td>2</td>
<td>Day 0 2.80±2.83 L</td>
<td>P-UNDW</td>
<td>Day 6 3.04±2.98 L</td>
<td>Z-UNDW</td>
<td>Day 13 2.65±2.71 L</td>
<td>Z-Hist</td>
<td>Day 20 2.73±2.40 L</td>
<td>P-Hist</td>
</tr>
<tr>
<td>3</td>
<td>Day 0 2.31±2.47 L</td>
<td>P-UNDW</td>
<td>Day 7 2.32±2.30 L</td>
<td>P-Hist</td>
<td>Day 13 2.42±2.35 L</td>
<td>Z-Hist</td>
<td>Day 19 2.50±2.39 L</td>
<td>Z-UNDW</td>
</tr>
<tr>
<td>5</td>
<td>Day 0 4.03±4.01 L</td>
<td>Z-Hist</td>
<td>Day 8 3.79±3.67 L</td>
<td>3.17</td>
<td>Day 15 4.04±3.69 L</td>
<td>P-Hist</td>
<td>Day 22 3.95±3.56 L</td>
<td>0.93</td>
</tr>
<tr>
<td>6</td>
<td>Day 0 3.71±3.76 L</td>
<td>P-UNDW</td>
<td>Day 5 3.57±3.77 L</td>
<td>0.438</td>
<td>Day 12 3.75±3.86 L</td>
<td>Z-UNDW</td>
<td>Day 19 3.51±3.71 L</td>
<td>2.273</td>
</tr>
</tbody>
</table>

Z: zileuton; P: placebo. For further definitions see legend to table 2.

Discussion

The present study shows that a single dose of 400 mg zileuton improves BHR in asthmatic patients with marked BHR under maintenance treatment with ICS. Indeed, six out of seven patients showed an increase in PC20 Hist after zileuton in comparison to placebo, and similarly, PD20 UNDW was improved in six of the seven patients. Neither zileuton nor placebo changed airway calibre, as indicated by no changes in FEV1 during 3 h after intake.

To assess the efficacy of anti-inflammatory treatment, an indirect challenge (as with UNDW) may be of value after zileuton in comparison to placebo, and, similarly, six out of seven patients showed an increase in PC20, Hist after a single dose of 400 mg zileuton [29], Therefore, the magnitude of the acute effect of zileuton on histamine and UNDW reactivity observed in the present study is difficult to compare with previous data.

The mechanisms by which zileuton attenuates the response to histamine and UNDW in these asthmatics who are being treated with ICS are unknown. Previous studies showed that a single dose of 600 mg zileuton rapidly (i.e. within 1 h) increased FEV1 by ~0.35 L in patients with an FEV1 of ~57–60% pred [12]. Such improvement was not observed in other studies [7, 8] or in the present patients, presumably because the FEV1 was close to its predicted value in these studies. Thus, an increase in airway calibre is unlikely to be the explanation for the reduced responses to histamine and UNDW challenge after zileuton found in the present study.

Other mechanisms may be considered to explain the observed additive effect of zileuton on BHR, besides ICS, although we did not attempt to explore these possibilities. It might be postulated that zileuton has, besides ICS, an additional inhibitory effect on the activation of the 5-lipoxygenase pathway. Such action would explain the increase in PD20 UNDW by an inhibitory action on leukotriene (LT)C4, LTD4 and LTE4 production induced by osmolar changes. Indeed, UNDW appears to induce leukotriene production. MESSLER et al. [30] showed that intranasal installation of distilled water aerosol in responsive subjects with allergic rhinitis raised the humoral and cellular content of nasal lavage fluid, including LTB4 and LTD4, to a similar extent as after allergen challenge. In contrast, there is no evidence that post-histamine-induced responses might be mediated by leukotrienes. Histamine provocation did not provoke release of leukotrienes [31]. Nevertheless, it cannot be excluded that patients with still significant BHR despite ICS are those in whom bronchoconstriction is, in part, dependent on leukotrienes. Another potential explanation is that zileuton might act, in part, as an antihistamine. This could explain the effects on histamine reactivity as well as a smaller effect on UNDW. However, to our knowledge pharmacological data to support this concept are not available. Clearly, subsequent studies are required to explore the above-mentioned possibilities.
Finally, a potential order effect associated with tachyphylaxis should be considered. In this study, measurements were performed with an interval of at least 5 days. This study design was chosen since it is known that histamine provocation may induce a significant bronchoconstriction response to UNDW [17]. Tachyphylaxis for repeated histamine provocations may exist, as does refractoriness for UNDW, which may persist up to 4 h [17]. The half-life of zileuton is 2.5 h, and its effect on airway responses after allergen challenge in asthmatic patients is not asthma. Clin Allergy 1988; 18: 317–321.

A potential order effect associated with tachyphylaxis may induce a significant bronchoconstriction response to UNDW [17]. Tachyphylaxis for repeated histamine provocations may exist, as does refractoriness for UNDW, which may persist up to 4 h [17]. The half-life of zileuton is 2.5 h, and its effect on airway responses after allergen challenge in asthmatic patients is not asthma. Clin Allergy 1988; 18: 317–321.

References