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

P.N.R. Dekhuijzen, G.P. Bootsma, P.L.M.L. Wielders, L.R.M. van den Berg, J. Festen, C.L.A. van Herwaarden

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**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 ($FEV_1$) ($PC_{20,Hist}$)) and with ultrasonically nebulized distilled water (UNDW) (provocative dose of UNDW producing a 20% fall in $FEV_1$ ($PD_{20,UNDW}$)) was performed in seven patients with asthma after intake of either 400 mg zileuton or placebo. All patients (mean age 33 yrs, mean $FEV_1$ 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.**
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Dept of Pulmonary Diseases, Academic Hospital Nijmegen, Nijmegen, The Netherlands

Correspondence: P.N.R. Dekhuijzen
Dept of Pulmonary Diseases
Academic Hospital Nijmegen
PO Box 9101
6500 HB Nijmegen
The Netherlands

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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(*b*)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 ($FEV_1$) 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 $FEV_1$ [11]. Asthma symptoms and $FEV_1$ 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.

*For editorial comment see page 2701*

## 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 $FEV_1$) ($PD_{20,UNDW}$)) and two with histamine (provocative concentration of histamine causing a 20% fall in $FEV_1$ ($PC_{20,Hist}$)) 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 $FEV_1$ was above 70% of predicted [16] and $PD_{20,UNDW}$ 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 $PD_{20,UNDW}$ was associated with a $PC_{20,Hist}$ 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 $\beta_2$-agonists, theophylline and oral anticoagulative drugs, as well as patients who were pregnant or breast-feeding, were excluded from participation in the study.

A total number of 17 patients, fulfiling 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 $FEV_1$ 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 $FEV_1$, 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 $FEV_1$ (within 5%) before the provocation tests.

The same technician from the lung function department always checked the patients morning $FEV_1$. 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" of "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 $B_4$ ($LTB_4$) production by *ex vivo* calcium ionophone-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, $FEV_1$ 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$^{-1}$. $PC_{20,Hist}$ 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 Groot *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 $FEV_1$ from post-air values ($PD_{20,UNDW}$) was calculated by linear interpolation on a semilogarithmic curve.

### *Statistical analysis*

$PC_{20}$ and $PD_{20}$ 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 $PD_{20,UNDW}$ of 4.74 mL at entry into the study and a mean $PC_{20,Hist}$ of 0.99 mg·mL$^{-1}$ after placebo (fig. 1). Zileuton was well tolerated. No haematological or biochemical changes were observed after treatment.

Table 1. – Patients characteristics at baseline

| Pt No. | Age yrs | ICS mg·day$^{-1}$ | $\beta_2$ use mg·day$^{-1}$ | Prestudy FEV1 L | Prestudy FEV1 % pred | PD20,UNDW mL |
|---|---|---|---|---|---|---|
| 1 | 19 | BDP, 400 | 0.1 *p.r.n.* | 3.53 | 115 | 3.01 |
| 2 | 57 | BDP, 750 | 0.4 *p.r.n.* | 2.70 | 134 | 7.98 |
| 3 | 36 | BUD, 800 | 0.8 | 2.41 | 86 | 3.61 |
| 4 | 20 | BDP, 200 | 0.4 *p.r.n.* | 3.17 | 88 | 3.97 |
| 5 | 27 | BUD, 400 | 0.8 | 4.01 | 119 | 5.48 |
| 6 | 27 | BDP, 400 | 0.1 *p.r.n.* | 3.42 | 111 | 3.18 |
| 7 | 44 | BDP, 800 | 1.6 | 3.00 | 125 | 6.00 |
| Mean | 33 | 536 | | 3.18 | 111 | 4.74 |
| SEM | 5 | 92 | | 0.20 | 7 | 0.69 |

Pt: patient; ICS: inhaled corticosteroids; $\beta_2$ use: mean daily use of $\beta_2$-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.

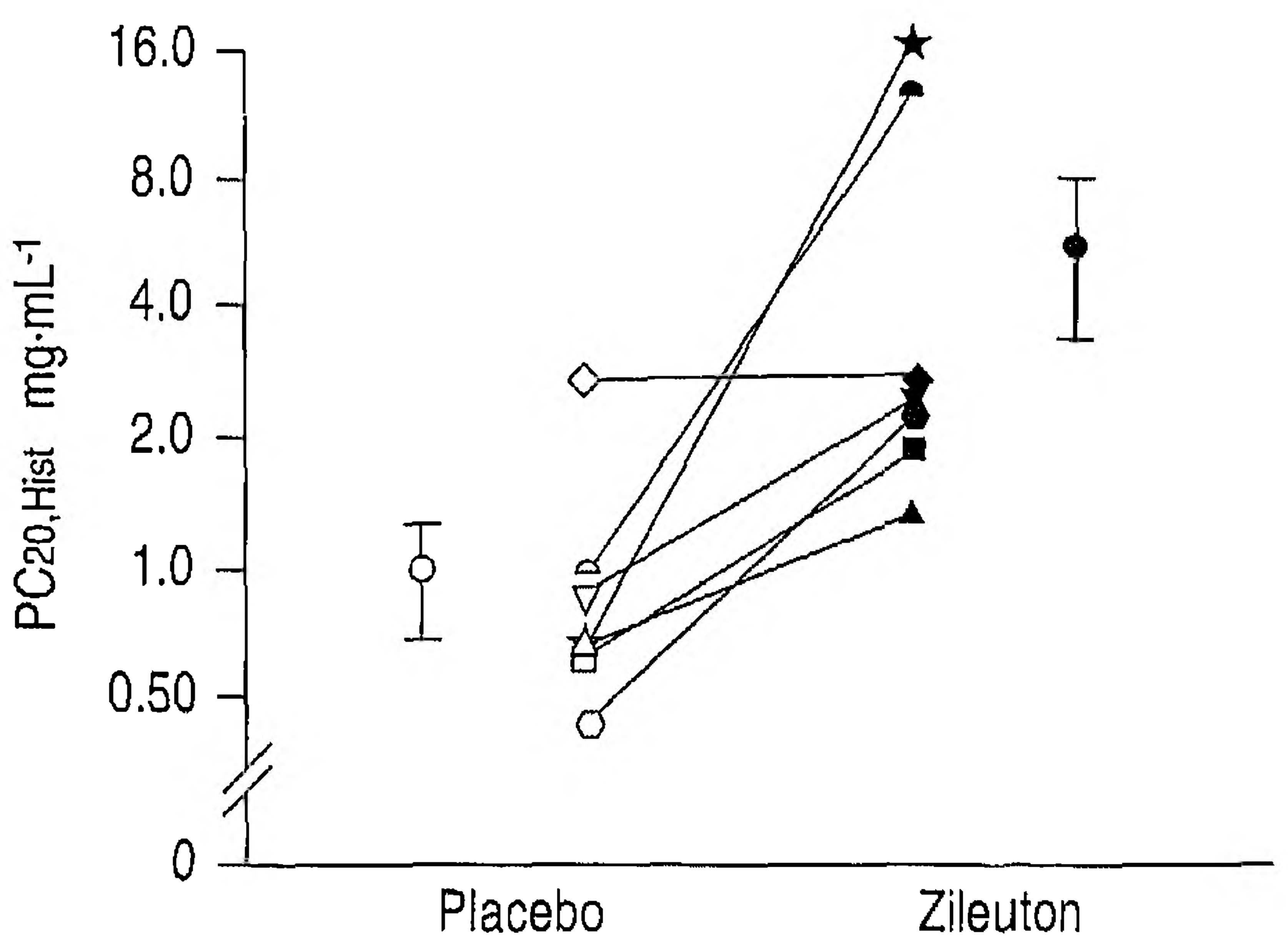


Fig. 1. – Individual data for the provocative concentration of histamine causing a 20% fall in forced expiratory volume in one second (PC20,Hist) after placebo (open symbols) and zileuton (closed symbols). Values are presented as mean±SEM, increasing from 0.99 (0.29) after placebo to 5.64 (2.27) mg·mL$^{-1}$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

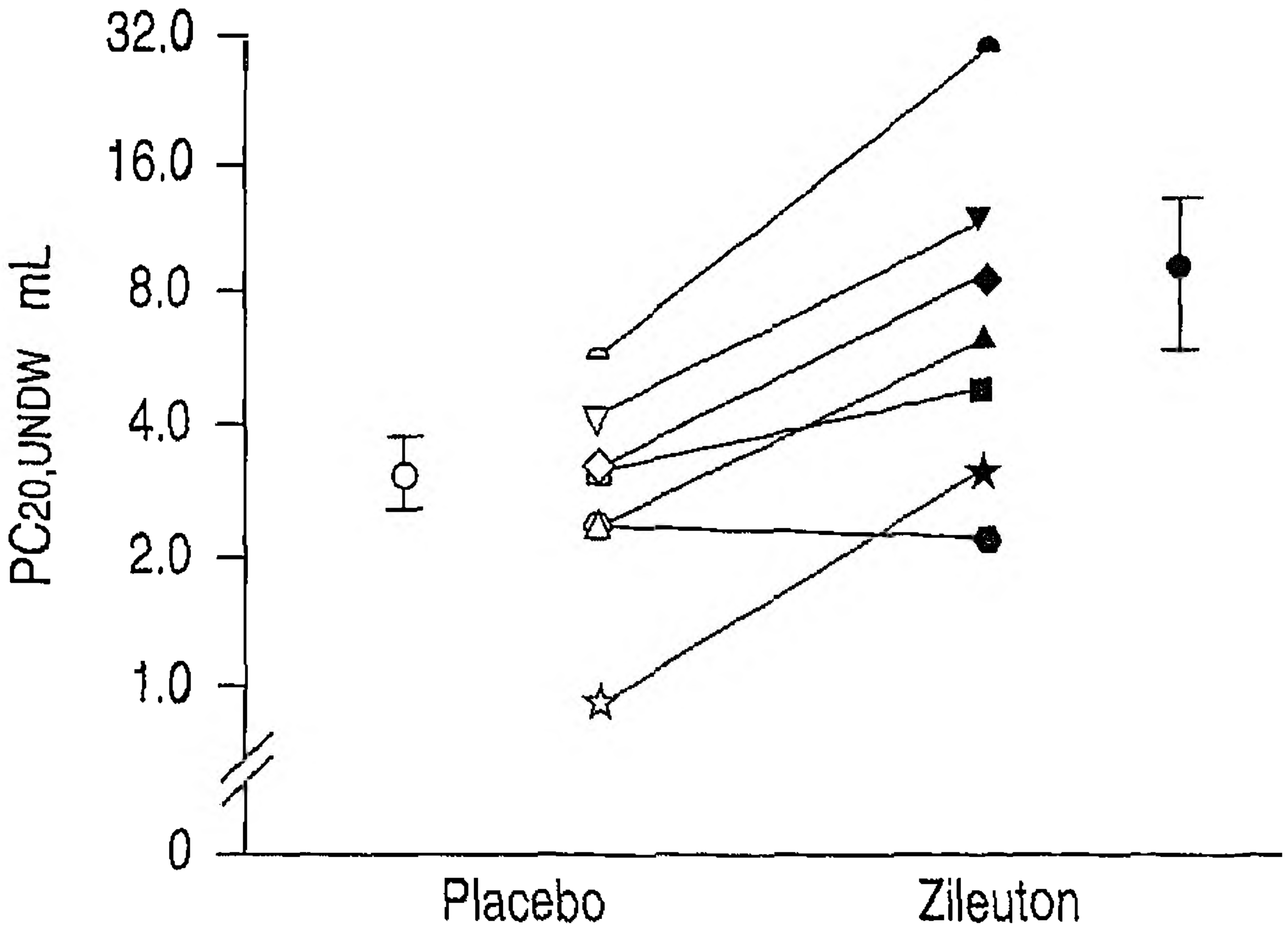


Fig. 2. – Individual data for the provocative dose of ultrasonically nebulized distilled water causing a 20% fall in forced expiratory volume in one second (PD20,UNDW) after placebo (open symbols) and zileuton (closed symbols). Values are presented as mean±SEM, increasing from 3.10 (0.56) after placebo 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.

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 $PC_{20,Hist}$ from 0.99 (0.29) to 5.64 (2.27) mg·mL$^{-1}$ (2.1 doubling doses (DD); $p<0.03$ compared to placebo) (fig. 1). In six of the seven patients, a clear increase was observed. $PD_{20,UNDW}$ was increased from 3.10 (0.56) to 9.31 (3.38) mL (1.3 DD; $p<0.05$ compared to placebo) (fig. 2). Again, an improvement was observed in all but one patient (which was not the same patient as the one that showed no improvement of the $PC_{20,Hist}$).

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

| Pt | Zileuton | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|
| No. | FEV1 before histamine | | FEV1 before UNDW | | FEV1 before histamine | | FEV1 before UNDW | |
| | Baseline L | After 3 h L | Baseline L | After 3 h L | Baseline L | After 3 h L | Baseline L | After 3 h L |
| 1 | 3.58 | 3.53 | 3.76 | 3.54 | 3.68 | 3.52 | 3.78 | 3.59 |
| 2 | 2.65 | 2.71 | 3.04 | 2.98 | 2.73 | 2.40 | 2.80 | 2.83 |
| 3 | 2.42 | 2.35 | 2.50 | 2.39 | 2.32 | 2.30 | 2.31 | 2.47 |
| 4 | 3.22 | 3.30 | 3.12 | 3.32 | 3.19 | 3.24 | 3.13 | 3.05 |
| 5 | 4.03 | 4.01 | 3.79 | 3.67 | 4.04 | 3.69 | 3.99 | 3.56 |
| 6 | 3.51 | 3.71 | 3.75 | 3.86 | 3.57 | 3.77 | 3.71 | 3.76 |
| 7 | 3.03 | 3.13 | 3.14 | 3.36 | 3.15 | 3.29 | 3.17 | 3.11 |
| Mean | 3.21 | 3.25 | 3.30 | 3.30 | 3.24 | 3.17 | 3.27 | 3.20 |
| SEM | 0.21 | 0.22 | 0.19 | 0.19 | 0.22 | 0.22 | 0.23 | 0.17 |

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

| Pt No. | Visit 1 FEV1 baseline and after 3 h L | Visit 1 Challenge | Visit 2 FEV1 baseline and after 3 h L | Visit 2 Challenge | Visit 3 FEV1 baseline and after 3 h L | Visit 3 Challenge | Visit 4 FEV1 baseline and after 3 h L | Visit 4 Challenge |
|---|---|---|---|---|---|---|---|---|
| 1 | Day 0 | Z-Hist | Day 7 | P-UNDW | Day 14 | P-Hist | Day 21 | Z-UNDW |
| | 3.58→3.53 | 1.92 | 3.78→3.59 | 3.13 | 3.68→3.52 | 0.613 | 3.76→3.54 | 4.84 |
| 2 | Day 0 | P-UNDW | Day 6 | Z-UNDW | Day 13 | Z-Hist | Day 20 | P-Hist |
| | 2.80→2.83 | 2.32 | 3.04→2.98 | 6.32 | 2.65→2.71 | 1.348 | 2.73→2.40 | 0.658 |
| 3 | Day 0 | P-UNDW | Day 7 | P-Hist | Day 13 | Z-Hist | Day 19 | Z-UNDW |
| | 2.31→2.47 | 4.2 | 2.32→2.30 | 0.875 | 2.42→2.35 | 2.497 | 2.50→2.39 | 11.7 |
| 4 | Day 0 | P-Hist | Day 6 | Z-UNDW | Day 12 | Z-Hist | Day 19 | P-UNDW |
| | 3.19→3.24 | 2.739 | 3.12→3.32 | 8.78 | 3.22→3.30 | 2.806 | 3.13→3.05 | 3.20 |
| 5 | Day 0 | Z-Hist | Day 8 | Z-UNDW | Day 15 | P-Hist | Day 22 | P-UNDW |
| | 4.03→4.01 | >16 | 3.79→3.67 | 3.17 | 4.04→3.69 | 0.676 | 3.99→3.56 | 0.93 |
| 6 | Day 0 | P-UNDW | Day 5 | P-Hist | Day 12 | Z-UNDW | Day 19 | Z-Hist |
| | 3.71→3.76 | 2.35 | 3.57→3.77 | 0.438 | 3.75→3.86 | 2.20 | 3.51→3.71 | 2.273 |
| 7 | Day 0 | Z-UNDW | Day 7 | P-UNDW | Day 17 | P-Hist | Day 23 | Z-Hist |
| | 3.14→3.36 | >28.2 | 3.17→3.11 | 5.6 | 3.15→3.29 | 0.983 | 3.13→3.13 | 12.668 |

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 $PC_{20,Hist}$ after zileuton in comparison to placebo, and, similarly, $PD_{20,UNDW}$ was improved in six of the seven patients. Neither zileuton nor placebo changed airway calibre, as indicated by no changes in $FEV_1$ during 3 h after intake.

To assess the efficacy of anti-inflammatory treatment, an indirect challenge (as with UNDW) may be of value since it mimics naturally occurring bronchoconstrictor stimuli and because it reflects the severity of asthma better than pharmacological stimuli [23, 24]. UNDW induces bronchoconstriction by cell-mediated events [24]. Challenge with UNDW may increase BHR and induce a late asthmatic response, just as occurs on exposure to allergens [25, 26]. The extent of the increase in $PD_{20,UNDW}$ (1.3 DD) in the present study was slightly less than in the study of Groot *et al.* [27], who found an increase of 1.8 DD after 4 weeks of beclomethasone dipropionate, 800 µg daily. This may be explained by the fact that at baseline the present patients were less responsive to UNDW, the $PD_{20,UNDW}$ being 4.74 mL (at intake) and 3.1 mL (after placebo), compared to 1.3 mL in the study by Groot *et al.* [27].

In contrast, inhaled histamine acts, in the main, directly on the airway smooth muscle [23]. The magnitude of increase in $PC_{20,Hist}$ after a single dose of 400 mg zileuton (2.1 DD) resembles the effects of maintenance therapy with ICS in previous studies. The average increase in $PC_{20,Hist}$ or methacholine after chronic treatment with beclomethasone dipropionate or budesonide, as recently reviewed by Barnes and Pedersen [28], is about 1–2 DD.

To the best of our knowledge, no single- or multiple-dose studies have been performed looking directly at histamine or methacholine reactivity after the administration of 5-lipoxygenase inhibitors. With leukotriene receptor antagonists, a small but significant reduction (from 0.30 to 0.48 $mg \cdot mL^{-1}$) in methacholine responsiveness was observed after a 1 week treatment of asthmatic patients with pranlukast [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 $FEV_1$ by ~0.35 L in patients with an $FEV_1$ of ~57–60% pred [12]. Such improvement was not observed in other studies [7, 8] or in the present patients, presumably because the $FEV_1$ 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 $PD_{20,UNDW}$ by an inhibitory action on leukotriene (LT)$C_4$, $LTD_4$ and $LTE_4$ production induced by osmolar changes. Indeed, UNDW appears to induce leukotriene production. Meslier *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 $LTB_4$ and $LTD_4$, 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 5-lipoxygenase activity should have been dissipated less than 16 h postingestion [11]. Looking at table 3, however, no order effect appeared to be present.

In conclusion, although the number of patients studied was small, the present data show that a single dose of 400 mg zileuton may attenuate bronchial hyperresponsiveness in steroid-dependent asthmatics. Additional studies are necessary to investigate whether similar effects are found after prolonged periods of treatment with zileuton.

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