

Effects of a 5-lipoxygenase inhibitor, ABT-761, on exercise-induced bronchoconstriction and urinary LTE₄ in asthmatic patients

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ABSTRACT: The novel 5-lipoxygenase (5-LO) inhibitor, ABT-761, was investigated for its effect on exercise-induced bronchoconstriction in asthmatic subjects. The relationship between 5-LO inhibition and effects on the response of the airways to exercise was examined.

In a double-blind, randomized, crossover clinical trial, 10 patients with mild to moderate persistent asthma (who exhibited a fall in forced expiratory volume in one second (FEV₁) \geq 20% following standardized exercise challenge) received 200 mg ABT-761 or matched placebo, orally, 5 h prior to exercise on two study days, 7–10 days apart. Lung function, urinary leukotriene E₄ (LTE₄) and *ex vivo* calcium ionophore-stimulated LTB₄ release in whole blood were measured prior to dosing, prior to exercise and at various time points up to 4 h post-exercise.

The mean (SD) maximal percentage fall in FEV₁ after exercise was 27.1 (12)% on placebo and 19.9 (10)% on ABT-761 days, respectively ($p < 0.05$). Post-exercise fall in FEV₁ was significantly attenuated at 5, 10, 15 and 30 min after exercise and the mean area under curve, representing the overall effect of exercise from 0–45 min post-challenge, was also significantly attenuated by ABT-761 ($p < 0.001$). *Ex vivo* LTB₄ release was inhibited by more than 80% throughout the 4 h post-exercise period, indicating that 5-LO was extensively inhibited at all time points. Urinary LTE₄ in the post-exercise period was significantly lower after ABT-761 day than after placebo (40.1 (17.6) versus 89.8 (58.2) pg·mg creatinine⁻¹; $p < 0.05$). Inhibition of LTB₄ release in ABT-761-treated patients correlated positively with the attenuation of post-exercise FEV₁ decline ($r = 0.711$; $p < 0.05$).

We conclude that ABT-761 is effective in suppressing exercise-induced bronchoconstriction and that this protection is related quantitatively to the degree of 5-lipoxygenase inhibition.

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Bronchoconstriction following exercise is frequently observed in patients with bronchial asthma. This reaction, known as exercise-induced asthma (EIA), is an incompletely understood expression of airway hyperresponsiveness that appears to be related to cooling and drying of the intrapulmonary airways [1, 2]. It has consequent direct effects on airway smooth muscle or indirect effects through the release of inflammatory mediators from cells in the airway mucosa or submucosa, including mast cells and eosinophils [3–5]. The response of airways to exercise consists of an immediate bronchoconstriction that subsides within an hour. A late response is observed occasionally, but is highly variable in incidence [6, 7], indicating a probable difference in aetiology to allergen responses.

Recently, the possible involvement of the bronchoconstrictor sulphidopeptide leukotrienes C₄, D₄, and E₄ (LTC₄, LTD₄ and LTE₄, respectively) in EIA has been suggested by the demonstration of elevated urinary concentrations of LTE₄ in asthmatic children after exercise [8]. The involvement of 5-lipoxygenase (5-LO) metabolites of arachidonic

acid in exercise-induced bronchoconstriction has been demonstrated by the ability of 5-LO inhibitors or leukotriene receptor antagonists [9–12] to attenuate (to a variable extent) the bronchoconstriction induced in asthmatic patients by exercise [13–16] or by inhalation of cold, dry air [17]. The degree of attenuation by 5-LO inhibitors of the bronchoconstriction induced by these challenges has not, to date, been compared with the magnitude of the compounds' pharmacological action, as determined by the suppression of LTE₄ accumulation in the urine or the inhibition of *ex vivo* LTB₄ production by leukocytes.

ABT-761 (R(+)-N-(3-(5-(4-fluorophenylmethyl)-2-thienyl)-1-methyl-2-propynyl)-N-hydroxy-urea) is a novel, second generation 5-LO inhibitor. In previous studies, a single dose of 200 mg ABT-761 has been shown to inhibit LTB₄ release from human neutrophils *ex vivo* 4 h post-dosing and to maintain this inhibition for a further 5 h [18]. Maximal plasma concentrations occur approximately 5 h after oral dosing, with 50% elimination occurring at approximately 15 h (data on file at Abbott Laboratories).

Since 5-LO products are of possible importance in the pathophysiology of EIA, drugs that inhibit the synthetic enzyme may be of therapeutic benefit in this condition. We report here the results of a placebo-controlled, double-blind crossover study of the effects of a single dose of 200 mg ABT-761 in 10 patients with EIA on exercise-induced bronchoconstriction during room air breathing and on urinary LTE₄ excretion. Inhibition of *ex vivo* whole blood LTB₄ release was determined as an indicator of the degree of 5-LO inhibition and compared with the drug's effects on the response of the airways to exercise.

Materials and methods

Patients

Ten nonsmoking patients (five male, five female; mean age 31 yrs, range 24–45 yrs) with mild to moderate persistent asthma (table 1) were included in the study. All subjects demonstrated at least a 20% fall in FEV₁ following treadmill exercise on a screening day. They were able to abstain from inhaled short- or long-acting β_2 -adrenoceptor agonists for 8 h or 24 h, respectively, and from theophylline for 48 h. None received oral or inhaled steroids or inhaled nedocromil sodium for at least 4 weeks prior to the study. No caffeine was ingested for 12 h and no alcohol for 24 h prior to the study visits. Patients whose baseline lung function parameters (forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC)) were at least 60% of predicted values [19], with <15% variability in absolute values between the study days, were included. Patients were otherwise in good health and had no history of significant diseases other than bronchial asthma. They had not experienced an acute exacerbation of asthma or an upper respiratory tract infection within 6 weeks prior to the study. Physical examination, routine laboratory tests, chest radiograph and electrocardiogram (ECG) were normal.

Study design

The study was designed as a double-blind, randomized, placebo-controlled, two-period crossover trial. On a screen-

ing day, within 14 days prior to the beginning of the study, medical history, asthma history and vital signs (blood pressure, cardiac frequency (*f*C), respiratory frequency (*f*R), temperature) were taken and patients underwent a physical examination. ECG and laboratory tests including drug and alcohol analysis were carried out. Baseline lung function was measured and the FEV₁ decline after treadmill exercise was determined.

The two study periods consisted of 3 days each: the study day, a control visit 24–48 h post-dosing and a control visit 7–10 days post-dosing. The control visit for period 1 was the study day in period 2, allowing a 7–10 day washout between study days.

On each study day, prior to receiving medication, vital signs, ECG and pulmonary function were recorded and blood and urine were taken for measurements of plasma ABT-761, whole blood LTB₄ release and urinary LTE₄. Thirty minutes after the beginning of breakfast, patients received randomized ABT-761 or placebo, supplied as four capsules of 50 mg ABT-761 (total dose 200 mg), or matching placebo. Vital signs and ECG were recorded again 90 min after dosing. Five minutes before the end of the 5 h post-dosing period, pulmonary function was measured and blood and urine were collected for plasma drug concentration, whole blood LTB₄ release and urinary LTE₄ determinations.

Exercise challenge was commenced 5 h after administration of drug or placebo. Pulmonary function tests were performed 5, 10, 15, 30, 45 and 60 min after the completion of the exercise challenge and again at 2 and 4 h post-exercise. Vital signs and ECG were recorded regularly and blood for plasma ABT-761 and whole blood LTB₄ release were taken at 15 min, 2 h and 4 h post-exercise. Urine was also collected at the end of the 4 h post-exercise period.

On the study day and at each control visit (24–48 h and 7–10 days post-dosing), physical examination and reports of the patients' medical condition, asthma status, concomitant medication and any adverse events were made. Blood was taken at the 24–48 h post-dosing visit for plasma ABT-761 determination. Each patient was crossed over after the 7–10 day washout period.

The study was approved by the Ethics Commission of the Chamber of Physicians of Schleswig-Holstein. Written consent was obtained from all volunteers.

Table 1. – Patient characteristics

Patient No.	Sex M/F	Age yrs	Height cm	Weight kg	FEV ₁ % pred	FEV ₁ % FVC	PC20 mg·mL ⁻¹	Therapy
1	M	26	185	67	87.9	80.3	2.03	β
2	M	33	187	84	99.1	71.5	1.12	β , CS
3	M	26	184	98	108.0	79.2	0.81	β
4	M	24	180	92	80.9	69.4	1.21	β
5	F	27	172	56	69.9	66.4	0.17	β , CS
6	F	30	171	58	96.2	74.5	2.56	β
7	F	35	176	65	96.8	71.9	0.05	β , CS
8	F	45	178	63	84.3	72.8	0.30	β
9	M	24	181	83	71.3	61.0	0.28	β
10	F	42	166	58	78.0	62.9	0.45	β
Mean		31	178	72	87.2	71.0	0.53*	
SD		-	-	-	12.6	6.3	0.16–1.80*	

β_2 -agonists were withheld for 8 h (short-acting) or 24 h (long-acting) prior to study. Corticosteroids had not been used within 4 weeks of study. *: Geometric mean with geometric range of SD. M: male; F: female; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; PC20: provocative concentration of methacholine causing a 20% fall in FEV₁; β : β_2 -agonist; CS: inhaled corticosteroids.

Pulmonary function measurements

Lung function parameters (FEV₁ and FVC) were measured with a triple V transducer connected to spirometry equipment (Oxycon Champion; Jaeger Co., Würzburg, Germany). Subjects wore a nose clip during lung function measurement procedures. The best values of three FEV₁ and FVC manoeuvres were taken [19].

Exercise challenge

Patients underwent a treadmill exercise on the screening day to establish the appropriate level of exercise for the treatment periods. The exercise challenge was set at a level that increased *f*C to 80–90% of predicted maximum [20]. Exercise was carried out on a treadmill (EL 2000; Jaeger); speed and inclination were based on the height and age of the patients. The treadmill exercise consisted of three steps: a period of 2 min with a target *f*C of 50% maximum, 2 min with a target *f*C of 70% maximum and 8 min with 80–90% of predicted maximum *f*C [20]. Patients inspired normal room air; ambient temperature and humidity were maintained at 20–25°C, and 50–60%, respectively. Ventilation, *f*R and *f*C during exercise were determined every minute and ECG was monitored continuously.

Whole blood LTB₄ release assay

Five millilitre blood samples were collected in heparinized tubes at all time points except pre-dose, for which 20 mL was collected (four 5 mL samples, three stimulated and one unstimulated). Pre-dose stimulated and all post-dose samples were stimulated by the addition of 10 µL Ca²⁺ ionophore A23187 (24 µM in dimethyl sulphoxide (DMSO); final concentration 47.7 nM); pre-dose unstimulated samples were sham-stimulated with 10 µL DMSO. Samples were incubated for 30 min at 37°C, after which reactions were terminated by incubation in an ice-water bath for at least 15 min. Cell-free plasma was separated by centrifugation (1,000 × *g* for 10 min at 4°C) and stored at -20°C until required for measurement of LTB₄ by high pressure liquid chromatography (HPLC). Estimates of percent inhibition of LTB₄ release were calculated according to the following equation:

$$\text{Percentage inhibition} = \left(1 - \frac{S_t - U_0}{S_0 - U_0}\right) \cdot 100$$

where *S*_{*t*} = plasma concentration of LTB₄ at time *t* post-dosing after stimulation of LTB₄ production; *S*₀ = mean plasma LTB₄ concentration from triplicate determinations at pre-dosing, after stimulation of LTB₄ production; and *U*₀ = plasma concentration of LTB₄ at pre-dosing without stimulation of LTB₄ production (*i.e.* unstimulated).

Urinary LTE₄ assay

Urine samples were collected in separate containers without preservatives. The urine volume of each sample period was measured and 50 mL aliquots were taken and stored at -20°C until required for measurement of LTE₄ by HPLC.

Plasma ABT-761

Serum levels of ABT-761 were determined from frozen plasma by HPLC.

Statistical analysis

Unless otherwise specified, all means presented are least-squares means from the particular model used. Within-group means are quoted with standard deviation (*SD*). Between-group differences are given with standard errors (*SEM*) of least-square means. All parametric analyses were based on F-tests derived from a two-period crossover analysis of variance (ANOVA) model with sequence, patients-within-sequence, period and treatment as factors. All efficacy variables were analysed for differences between treatments and for sequence effects at multiple time points. Sequence effects (as surrogates for carryover effects) were tested using patient-within-sequence as the error term.

Additional nonparametric analyses were based on Koch's nonparametric approach to the analysis of two-period crossover design. All sample sizes presented are appropriate for the summary statistics for pre-dosing, for a particular post-dosing interval, and for change from pre-dosing. Parametric tests and nonparametric tests resulting in *p*-values less than or equal to 0.050, when rounded to three digits, are reported as "significant" in the text. All *p*-values are based on two-tailed tests and were determined using Statistical Analysis System (SAS) procedures (version 6.09; SAS Institute, Inc., Cary, NC, USA).

Analyses of linear correlations between lung function results and parameters of leukotriene metabolism and ABT-761 plasma levels were carried out using SyStat (SyStat Inc., Evanston, IL, USA).

Results

Ambient conditions and baseline lung function

There were no differences in baseline lung function, *f*C or ambient conditions on the two study periods. The ambient temperature and humidity (mean ± *SEM*) were 23 ± 0.3 versus 23 ± 0.4°C and 57.2 ± 0.9 versus 56.2 ± 0.9 on placebo and ABT-761 days, respectively. The mean (*SD*) *f*C and pre-dose FEV₁ values of the patients were 67.2 (8.66) beats·min⁻¹ and 3.35 (1.02) L, respectively, on placebo day versus 68.3 (7.59) beats·min⁻¹ and 3.36 (0.88) L, respectively, on ABT-761 day. During exercise, *f*C and minute ventilation rose to maxima of 180 (9.95) beats·min⁻¹ and 86.4 (31.6) L on placebo day versus 182 (11.5) beats·min⁻¹ and 89.5 (32.3) L on ABT-761 treatment day.

Exercise challenge

Pre-exercise FEV₁ did not differ between study days (placebo: 3.39 (0.94) L; ABT-761: 3.46 (1.02) L; *NS*). The maximal percentage fall in FEV₁ post exercise was 27.1 (12)% after placebo versus 19.9 (10)% after ABT-761 treatment (fig. 1). The mean ± *SEM* difference of -7.20 ± 2.01% was statistically significant (*p* < 0.05), yield-

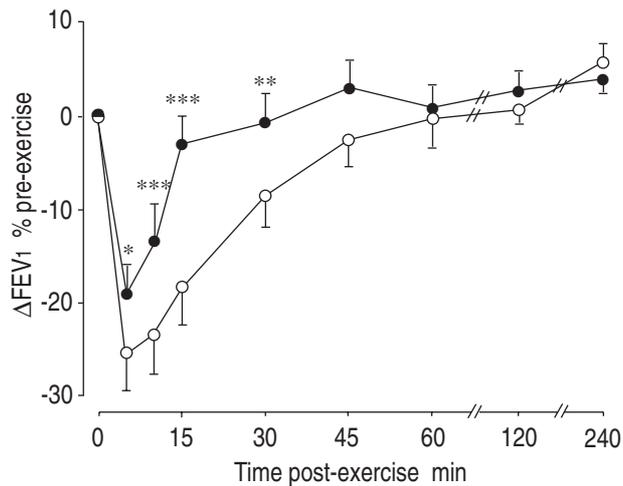


Fig. 1. – Mean \pm SEM post-exercise change in forced expiratory volume in one second (Δ FEV₁) in 10 patients treated with placebo (○) or ABT-761 (●) in a two-period, double-blind crossover manner. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, compared to placebo.

ing a mean inhibition of $22.0 \pm 12.8\%$. The inhibitory effect of ABT-761 on post-exercise maximal FEV₁ decline was seen in nine of the 10 patients (fig. 2a).

The post-exercise fall in FEV₁ was significantly attenuated at four time points: at 5 min by $6.47 \pm 2.74\%$; at 10 min by $10.2 \pm 1.72\%$; at 15 min by $15.5 \pm 2.71\%$; and at 30 min by $8.07 \pm 2.56\%$ (fig. 1). Accordingly, the mean area under the curve (AUC), representing the overall drug effect AUC/min (percentage change) between 1 and 45 min was significantly reduced after ABT-761 treatment (mean AUC/min: -3.90 (8.20) versus -12.8 (10.7)% for placebo; mean difference: $8.93 \pm 1.76\%$, $p < 0.001$).

Return to 95% of baseline FEV₁ post exercise took 15.5 (16.0) min after ABT-761 versus 28.1 (17.4) min after placebo ($p < 0.05$).

Ca²⁺ ionophore-stimulated LTB₄ release

Mean pre-dose LTB₄ release during the two study periods was 151 (52.4) ng·mL⁻¹ (placebo) and 142 (39.6) ng·

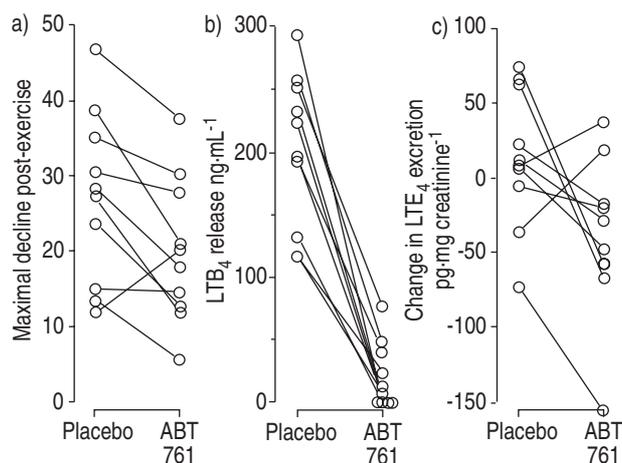


Fig. 2. – Pairwise representation of: a) maximum fall in forced expiratory volume in one second (FEV₁) after exercise; b) calcium ionophore-stimulated whole blood leukotriene B₄ (LTB₄) release 5 h after dosing; and c) change in urinary leukotriene E₄ (LTE₄) concentration between pre-dose and post-exercise periods during the placebo and ABT-761 study periods in 10 asthmatics.

mL⁻¹ (ABT-761). Following ABT-761 treatment, A23187-stimulated LTB₄ release into the plasma was significantly inhibited 5 h post-dosing (mean 85.8 (16.8)% inhibition) and up to 4 h post-exercise, by 86%, 87% and 82% at 15 min, 2 h and 4 h, respectively. The mean difference in percentage inhibition between the treatment periods was statistically significant ($p < 0.01$): $122 \pm 11.3\%$ at 5 h post-dose, and $114 \pm 10.0\%$, $157 \pm 27.3\%$ and $144 \pm 17.7\%$ at 15 min, 2 h and 4 h post-exercise, respectively. Individual data (fig. 2b) demonstrate that inhibition of LTB₄ release after ABT-761 treatment was observed in all 10 subjects (range: 49.7–100%).

Urinary LTE₄ excretion

The values of urinary LTE₄ excretion are shown in figure 3. After placebo, mean urinary LTE₄ level increased 4 h post exercise as compared to pre-dose by 13.3 (46.4) pg·mg creatinine⁻¹, from 76.5 (53.7) to 89.8 (58.2) pg·mg creatinine⁻¹. Following ABT-761 treatment there was a mean fall in urinary LTE₄ of 39.8 (53.0) pg·mg creatinine⁻¹, from 79.9 (62.2) to 40.1 (17.6) pg·mg creatinine⁻¹, corresponding to a 50.2% reduction in LTE₄ excretion and yielding a marked and statistically significant difference in post-exercise LTE₄ excretion between the two treatment periods (53.2 ± 21.8 pg·mg creatinine⁻¹, $p < 0.05$). This effect of ABT-761 treatment was observed in eight of the ten patients investigated (fig. 2c).

There was a mean 10.2 pg·mg creatinine⁻¹ fall in urinary LTE₄ concentration between the pre-dose and pre-exercise samples on the ABT-761, but this was not statistically significant in comparison to the small increase of 0.21 pg·mg creatinine⁻¹ on placebo day.

ABT-761 plasma levels

The mean \pm SEM (range) plasma level of ABT-761 5 min prior to exercise was 2.80 ± 0.31 (1.6–4.32) μ g·mL⁻¹ and remained constant up to 4 h post-exercise: 2.87 ± 0.25 (2.00–4.70) μ g·mL⁻¹.

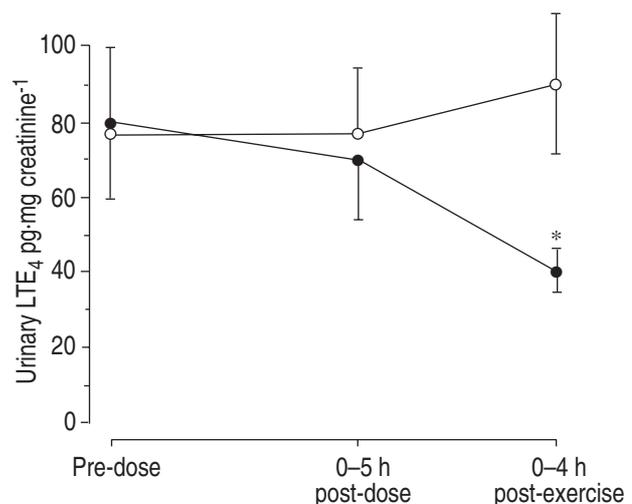


Fig. 3. – Mean \pm SEM leukotriene E₄ (LTE₄) concentrations in the urine of patients treated with placebo (○) or ABT-761 (●). There were no significant differences between LTE₄ levels for the three time periods within either group. *: $p < 0.05$, compared to placebo.

Relationships between post-exercise lung function and leukotriene metabolism

During the placebo period there was no significant relationship between post-exercise FEV₁ decline and stimulated LTB₄ biosynthesis estimated 5 min prior to exercise challenge ($r=0.230$, NS) nor between FEV₁ decline and urinary LTE₄ excretion 0–4 h post-exercise ($r=-0.468$, NS). After ABT-761 treatment, post-exercise FEV₁ decline was positively correlated ($r=0.654$, $p<0.05$) and the post-exercise overall effect negatively correlated ($r=-0.682$, $p<0.05$) with the stimulated LTB₄ level 5 min prior to exercise; *i.e.*, a smaller fall in FEV₁ was associated with a lower 5-LO activity in blood leukocytes. The overall effect on post-exercise FEV₁ reduction also showed a significant correlation to percentage inhibition of stimulated LTB₄ biosynthesis following ABT-761 treatment ($r=0.711$, $p<0.05$).

Neither post-exercise urinary LTE₄ excretion nor post-exercise FEV₁ decline correlated significantly with the ABT-761 plasma level.

Drug safety

ABT-761 was generally well tolerated. Two patients reported mild headache after dosing with ABT-761, possibly related to study medication. No other adverse events were reported.

Discussion

This study shows the protective effect of a single dose of 200 mg ABT-761 on exercise-induced bronchoconstriction, associated with inhibition of LTB₄ release from human neutrophils *ex vivo* and urinary LTE₄ excretion. Maximal FEV₁ decline was attenuated by 22%, the overall reduction of FEV₁ was reduced by 70% and the recovery time of FEV₁ to 95% of pre-exercise values was diminished by 55%. The maximal effect of ABT-761 on exercise-induced bronchoconstriction (fig. 1) was observed in the 15th minute and the immediate post-exercise bronchoconstriction was only marginally reduced.

The present results strongly agree with another ABT-761 study in asthmatics with exercise-induced bronchoconstriction, which reported a mean inhibition of FEV₁ fall, expressed as reduction of AUC, of 61% [21]. Although the reduction in maximum post-exercise fall in FEV₁ by ABT-761 was greater in the other study (13%), this may be accounted for, at least in part, by the patients in the present study having a lower mean baseline FEV₁ (87% pred, compared to 91% in the study by VAN SCHOOR *et al.* [21]) and a larger maximum post-exercise fall in FEV₁ (27%, compared to 23% in the study of VAN SCHOOR *et al.* [21]), indicating that the population reported on here suffered asthma of slightly greater severity and exhibited greater responsiveness to exercise.

The presents results are also consistent with those obtained with zileuton (the first 5-LO inhibitor and predecessor of ABT-761) in exercise-induced asthma, where the maximum decrease in post-exercise challenge FEV₁ was 15% following 2 days of treatment with zileuton 600 mg *q.i.d.* and 28% following placebo treatment (mean inhibition ~43%) [22]. ISRAEL *et al.* [17], investigating the inhibitory effect of a single dose of 800 mg zileuton on

asthma induced by cold, dry air, reported an increase of the provocative dose causing a 20% fall in FEV₁ (PD₂₀) after zileuton treatment of about 26%.

ABT-761 treatment almost completely inhibited (~90%) the calcium ionophore-stimulated LTB₄ release from human neutrophils. This is in accordance with zileuton studies reported by HUI *et al.* [23] and ISRAEL *et al.* [17] which measured 93 and 74% inhibition, respectively, after ingestion of 800 mg doses. As compared with other drugs that inhibit 5-LO, the inhibitory effect of ABT-761 is relatively high. Following three doses of 250 mg of the 5-LO activating protein (FLAP) inhibitor, MK-0591, DIAMANT *et al.* [24] found an inhibition of ionophore-stimulated LTB₄ release of between 96 and 99%. Following ZD2138 (350 mg) NASSER and co-workers demonstrated an inhibition of 72 or 82% [25, 26]; a considerably smaller inhibitory effect was seen with MK-886 [27]. It is notable that in the studies with ZD2138 and MK-0591, almost total inhibition of *ex vivo* LTB₄ release was observed up to 24 h after dosing. In the case of MK-0591 this inhibition was even seen when plasma drug concentrations had diminished to less than 12% of peak levels, suggesting either that the drug was given in doses that were very much higher than required to bring about total inhibition of 5-LO activity or that an irreversible action on the activating protein was exerted.

It has not previously been possible to demonstrate correlations between inhibition of LTB₄ release, reflecting 5-LO inhibition, and attenuation of FEV₁ decline. While a direct correlation was not observed in the present study, there was a correlation between inhibition of LTB₄ release by ABT-761 and the drug's effect on overall exercise-induced reduction in FEV₁ over the full post-exercise period. This may be taken to indicate a relationship between systemic inhibition of 5-LO activity and inhibition of exercise-induced bronchoconstriction, although the degree to which 5-LO is inhibited in the lungs remains unclear [24].

The inhibitory potency of CysLT₁ receptor antagonists (*i.e.*, antagonists at receptors for LTC₄, LTD₄ and LTE₄) on exercise-induced bronchoconstriction seems to be slightly greater than that of 5-LO inhibitors [13, 15, 16]. The reported protective effects on maximal post-exercise FEV₁ decline range between 40% following ICI 204,219 treatment and 68% after MK-571 [13]. It is probably important, in this context, that the CysLT₁ antagonists were administered by inhalation; although systemic 5-LO activity is effectively abolished by inhibitors of FLAP and 5-LO, including ABT-761, there may be a residual local generation of leukotrienes in the airways sufficient to induce bronchoconstriction after exercise.

There is a discrepancy between the nearly complete inhibition of 5-LO-mediated arachidonic acid metabolism and the smaller extent of protection against bronchoconstriction following exercise, both in our study and an earlier study with zileuton [17]. This underlines the thesis that about half of the exercise response is mediated by mechanisms distinct from the leukotrienes [12]. The greatest inhibition of bronchoconstriction following treatment with 5-LO inhibitors is observed in aspirin-induced asthma, while the effects on bronchoconstriction following allergen challenges are very variable. The maximal protective effect was shown in aspirin-induced asthma following zileuton treatment (600 mg *q.i.d.* for 7 days)

resulting in a complete blockade of the reaction [28]. The maximal effect on early asthmatic reactions in allergen-induced asthma was an inhibition of about 80% following treatment with MK-0591 [24] or BAY-X-1005 [29]. In contrast there was no significant reduction of bronchoconstriction after allergen challenge following zileuton treatment [23, 25] despite inhibition of LTB₄ release.

Despite the relative lack of efficacy of zileuton on allergen-induced airway responses, ISRAEL *et al.* [30] showed a mean increase in FEV₁ of 13.4% in a 4 week placebo-controlled trial in patients with mild to moderate asthma, accompanied by a mean decrease in urinary LTE₄ excretion. In our study there was a slight (NS) but consistent (seven out of 10 patients) increase in urinary LTE₄ excretion post-exercise of 13.5%, but there was no significant correlation with lung function. It should be noted that the relatively small and variable inhibition of post-exercise urinary LTE₄ accumulation by ABT-761, despite the large and homogeneous inhibition of stimulated leucocyte LTB₄ production, is in agreement with findings in allergen-challenged asthmatic patients treated with zileuton [23]. The variability may be due to the variability of the method of urine sampling. An increase in urinary LTE₄ levels is well documented following antigen [13, 23–25, 31–33], aspirin [26, 34] and acute reversible airway obstruction [12], although the increase after allergen inhalation is also highly variable [24, 25] and can be very small [27]. Elevated baseline levels are known in aspirin-sensitive subjects [26, 31, 32, 34]. In children, KIKAWA *et al.* [8] demonstrated an increase in urinary LTE₄ excretion post-exercise, but this is not documented in adult subjects [32]. The relatively small degree of inhibition of LTE₄ excretion in the 4 h post-exercise period by ABT-761 and zileuton may reflect only a partial inhibition of 5-LO in the lungs, where a large proportion of the excreted LTE₄ is assumed to originate, although there may be other possible interpretations.

We are unable to explain the deviating lung function response following ABT-761 treatment of one subject (fig. 2a). This patient exhibited treatment effects, namely inhibition of LTB₄ release (by 75.1%) and LTE₄ excretion post-exercise (-131 pg·mg creatinine⁻¹), and ABT-761 plasma level was 2.18 µg·mL⁻¹. Another subject had only a marginal inhibitory effect on FEV₁ decline following ABT-761 and showed also no deviations in these parameters. This lack of responsiveness to 5-LO inhibition in individual patients is not uncommon, however: in a study of zileuton effects on responses to isocapnic hyperventilation, only nine of the 13 patients showed suppression of bronchoconstriction [17] and it is acknowledged that there is substantial heterogeneity among patients in their therapeutic response to 5-LO inhibitors [35].

The present results demonstrate that the 5-lipoxygenase inhibitor ABT-761, given orally as a single dose of 200 mg, has a significant protective effect against exercise-induced bronchoconstriction in patients with asthma, amounting to about 70% over 45 min. The drug also inhibits urinary leukotriene E₄ levels after exercise as compared to placebo and inhibits calcium-ionophore stimulated leukotriene B₄ release from human neutrophils by 90%, indicating extensive 5-lipoxygenase inhibition. Within the range of plasma ABT-761 concentrations achieved, the extent of protection against exercise-induced bronchoconstriction is related to the degree of inhibition of 5-lipoxygenase, indicating that 5-lipoxygenase activity is likely to be impor-

tant in the pathophysiology of exercise-induced asthma and that its inhibition may be of therapeutic benefit.

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