ABT-761 Attenuates Bronchoconstriction and Pulmonary Inflammation in Rodents

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ABSTRACT

Our primary goal has been to discover leukotriene biosynthesis inhibitors with characteristics that are appropriate for use as clinical agents. The success of the use of zileuton in the treatment of asthma led us to explore further the use of the N-hydroxyurea class of 5-lipoxygenase inhibitors as longer-acting compounds with good lung penetration. A variety of in vitro and in vivo methods were used to evaluate a large number of compounds, from which ABT-761 \((\text{R})-N-(3-(5-(4-fluorophenylmethyl)thien-2-yl)-1-methyl-2-propynyl)-N-hydroxyurea\) was selected for study. ABT-761 exhibited potent and selective inhibition of leukotriene formation both in vitro and in vivo. More importantly, the compound potently inhibited antigen-induced bronchospasm in guinea pigs when given either prophylactically or therapeutically. In addition, ABT-761 was a potent inhibitor of eosinophil influx into the lungs of Brown Norway rats. These data provide added support for the role of leukotrienes in both bronchospasm and eosinophilic inflammation and characterize ABT-761 as a particularly potent inhibitor of leukotrienes formed in pulmonary tissues. These data combined with the excellent pharmacokinetic characteristics of the compound indicate its potential use in the treatment of leukotriene-dependent human disease.

Considerable evidence suggests that leukotrienes contribute to human asthma (Henderson, 1994; Israel, 1994; Wenzel et al., 1995). Although much of this evidence was initially derived from research with isolated cells and tissues and with animal models, more recent clinical results with specific LTD₄ antagonists (Spector et al., 1994) and the specific 5-lipoxygenase inhibitor zileuton (Israel et al., 1993) have substantiated the role of leukotrienes in this common disease. Clinical findings with zileuton indicate that effective modulation of leukotriene formation in asthmatics requires not only that an agent be a potent inhibitor but also that tissue concentrations of the agent be maintained throughout the treatment period. Thus, optimal therapeutics will require long-acting agents with excellent tissue distribution. We previously reported a testing strategy that successfully predicted potency and duration of action for N-hydroxyurea containing 5-lipoxygenase inhibitors (Bell et al., 1995). This approach was used in the discovery of ABT-761 \((\text{R})-N-(3-(5-(4-fluorophenylmethyl)thien-2-yl)-1-methyl-2-propynyl)-N-hydroxyurea\) (Brooks et al., 1995). In the present study, we describe the characteristics of that compound; its biochemical profile, duration of action and pharmacokinetics in rodents; and its activity in reducing bronchoconstriction and pulmonary inflammation in rodent models of airway disease.

Materials and Methods

Bicinchoninic acid protein assay reagents were purchased from Pierce Chemical (Rockford, IL). HPLC columns were purchased from Regis Chemical Co. (Morton Grove, IL). Ficoll-Hypaque Mono-Poly Resolving medium was from Flow Laboratories (McLean, VA). EIA reagents were purchased from Cayman Chemical Co. (Ann Arbor, MI) or from PerSeptive Diagnostics (Cambridge, MA). Radioimmunoassay kits were from PerSeptive. ABT-761, zileuton, Bay X 1005, MK-476 and ZD-2138 were synthesized in our laboratories. RBL cell lysate 5-lipoxygenase inhibitor potency. The activity of agents in inhibiting the 5-lipoxygenase RBL-1 was performed according to the method of Jakschik et al. (1980) as modified in our laboratories (Carter et al., 1991).

Reversibility of human PMNL 5-lipoxygenase inhibition. The inhibition of isophorone A23187-induced leukotriene formation in human neutrophils and the reversibility of inhibition by ABT-761 was performed as described by Bell et al. (1995).

Human whole blood eicosanoid formation. Whole blood eicosanoid formation was measured as described by Bell et al. (1995).

Biosynthesis of leukotrienes by chopped lung. Cynomolgus monkey lung was obtained from these laboratories. The lung tissue was cut into 1- to 2-mm cubes and continuously oxygenated at 4°C in

ABBREVIATIONS: LTB₄, leukotriene B₄; LTD₄, leukotriene D₄; 5-HETE, 5-hydroxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; RBL-1, rat basophilic leukemia cells; DMSO, dimethylsulfoxide; PBS, phosphate buffered saline; EIA, enzyme immunoassay; HPMC, hydroxypropyl methyl cellulose; AA, arachidonic acid; TXB₂, thromboxane B₂; Cdyn, dynamic compliance; PMNL, polymorphonuclear leukocytes; FLAP, 5-lipoxygenase activating protein.
modified Krebe-Henseleit buffer composed of 118 mM NaCl, 1.1 mM MgSO_{4}•7H_{2}O, 1.8 mM CaCl_{2}, 24.9 mM NaHCO_{3}, 1.0 mM KH_{2}PO_{4} and 11.1 mM glucose; washed; and resuspended in Krebs' buffer at a concentration of 100 mg/ml. Tissues were preincubated with vehicle (0.5% v/v DMSO) or test compound for 10 min. Calcium ionophore A23187 (20 μM) in DMSO or DMSO alone was added, and tissues were further incubated for 30 min. The tissue samples were transferred to an ice bath and centrifuged at 500 × g for 15 min at 4°C, and supernatants were removed and analyzed for LTB_{4} by EIA (Cayman Chemical Co.).

Isolated guinea pig trachea studies. Tracheal spiral strips were prepared and contractions were performed as described by Malo et al. (1994). For most agonist-induced contractions, each tissue was precontracted with 100 μM ABT-761 for 15 min before the addition of a single concentration of LTD_{4}, acetylcholine (10 μM), histamine (10 μM), PGD_{2} (1 μM) or U-44069 (0.1 μM).

Determination of drug plasma concentrations. Animals were dosed and plasma concentrations of ABT-761 were determined as described previously for A-79175 (Bell et al., 1994), with UV detection at 275 nm.

Rat peritoneal anaphylaxis model. Inhibition of leukotriene formation in the rat was performed as previously described (Young et al., 1991). Briefly, rats were passively sensitized to bovine serum albumin, and 3 hr later they were challenged in the peritoneal cavity with antigen. The peritoneal cavity was lavaged 15 min later, and the fluids were analyzed for leukotriene content by EIA.

Rat pleural inflammation model. Pleural inflammation was induced in male rats according to the method of Rao et al. (1983). Animals were dosed with experimental compounds in 0.2% HPMC 1 hr before the intrapleural injection of the calcium ionophore A23187. The rats were lightly anesthetized with Penthrane and injected intrapleurally with 0.5 ml of 2% ethanol in injectable saline (Abbott Laboratories) containing 20 μg of A23187. Thirty minutes later, the animals were killed, and the pleural cavities were lavaged with ice-cold saline. The lavage fluid was then added to ice-cold methanol (final methanol concentration, 30%) to lyse cells and precipitate protein. Eicosanoids were determined by EIA as described above.

AA-induced bronchospasm in the anesthetized guinea pig. Adult male albino Hartley-strain guinea pigs were anesthetized with an intraperitoneal injection of pentobarbital (20–24 mg/kg) and urethane (1.0 g/kg). After the induction of anesthesia, the trachea was surgically exposed and intubated with an endotracheal tube. All animals were permitted to spontaneously breathe room air.

Air-flow rate (ml/sec), tidal volume (ml/breath) and transpulmonary pressure (cm H_{2}O) were recorded simultaneously according to previously published methods (Malo et al., 1994), and C \text{P}_{\text{L}}\text{O}_{2}, a measurement of the status of peripheral airways, was automatically calculated by an on-line computer. The jugular vein was cannulated for the delivery of drugs and solutions to the animal. The animal was kept warm during the experiment by being placed on a heated table; the core temperature was monitored rectally.

Animals were orally dosed before anesthesia at the times noted. The animals were orally pretreated with intravenous meclofenamic acid (10 mg/kg) and propranolol (1 mg/kg) at 10 min before AA challenge. After the pretreatments, the animals were challenged with intravenous arachidonic acid (10 mg/kg). Measurements for compliance and conductance calculations were made every 20 sec for a period of 15 min after AA challenge.

Antigen-induced bronchospasm in the anesthetized guinea pig. Bronchoconstriction in sensitized guinea pigs was induced and measured as described in detail by Malo et al. (1994). For studies in which the compound was delivered after antigen challenge, a slightly different protocol was used. Immediately after ovalbumin (10 mg/ml, 30 sec) aerosol administration, the decrease in C \text{P}_{\text{L}}\text{O}_{2} was observed. When a 20%, 40% or 60% decrease in C \text{P}_{\text{L}}\text{O}_{2} was attained, an intravenous injection of ABT-761 or albuterol was given in 1 min. Measurements for compliance and conductance calculations were made every minute for a period of 40 min after intravenous administration of ABT-761. Control responses were determined after the same injection with only PEG-400.

Sephadex-induced eosinophilia in Brown Norway rats. Eosinophilia was induced in Brown Norway rats by the injection of Sephadex (Walsh, 1993; Walsh et al., 1994). Brown Norway rats weighing 130 to 150 g were orally dosed with ABT-761 in 0.2% HPMC (Abbott Laboratories). The animals were then injected in the central tail vein with 1 ml of 0.5 mg/ml Sephadex G-200. Control animals received equal volumes of saline. For the next 3 days, the rats were dosed either once or twice a day with either drug or HPMC. On day 3, rats were anesthetized by a nonlethal intraperitoneal injection of 25% urethane (1 ml, 0.006 × body weight). Tracheal intubation was performed, and the airways were lavaged with 2 × 5 ml of phosphate-buffered saline without Ca^{2+} containing 10 U/ml of heparin. The fluid was recovered manually through gentle aspiration. The fluid recovered from each lavage was pooled, and the volume was measured. Total cell counts were performed using a Coulter counter (model ZB1). A 1-ml aliquot of bronchoalveolar lavage fluid was added to 2 ml of ice-cold methanol to precipitate the protein. This mixture of methanol and bronchoalveolar lavage fluid was allowed to stand overnight at −20°C. Precipitated material was removed by centrifugation, and levels of eicosanoids were determined as above. The remaining bronchoalveolar lavage fluid was centrifuged (1500 × g); the cellular pellets were washed twice in a calcium-free phosphate-buffered saline, resuspended in 5 ml of phosphate-buffered saline and then centrifuged and stained with Wright-Giemsa for differential counts.

Statistical methods. Percentage inhibition was computed by comparing individual values in treatment groups with the mean value of the control group. Statistical significance was determined using one-way analysis of variance and Duncan’s multiple-range test. Linear regression was used to estimate IC_{50} and ED_{50} values.

Results

Characterization of ABT-761 as a selective 5-lipoxygenase inhibitor in vitro. ABT-761 (fig. 1) was tested in a number of biochemical assays to validate its use in the pulmonary models. Studies using cell lysates from RBL cells, a rich source of 5-lipoxygenase activity, indicated that the compound is a direct inhibitor of 5-lipoxygenase. In three studies, ABT-761 inhibited the formation of 5-HETE with an average IC_{50} value of 23 nM in incubations using 6 μM AA as substrate (table 1). This potency was greater than that observed for the standard inhibitors zileuton (Carter et al., 1991) and ZD-2138 (McMillan et al., 1992). The inhibitory potency of ABT-761 varied as a function of substrate concentration, yielding IC_{50} values of 23 to 151 nM for substrate concentrations of 6 to 65 μM, respectively. These data suggest that ABT-761 is a direct competitive inhibitor of 5-lipoxygenase.

ABT-761 was also found to inhibit human 5-lipoxygenase activity in a concentration-dependent manner in purified PMNL stimulated with the calcium ionophore A23187. Data of ABT-761. Control responses were determined after the same injection with only PEG-400.

Fig. 1. Chemical structure of ABT-761 [(F)-N-(3-(5-(4-fluorophenylmethyl)-2-thienyl)-1-methyl-2-propynyl)-N-hydroxyurea].

Activity of ABT-761

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1995) gave IC50 values of 28 and 280 nM, respectively. ABT-761 was found to inhibit the formation of LTB4 in two experiments, from cynomolgus monkeys. ABT-761 was more potent than the IC50 value for 5-lipoxygenase inhibition, as indicated by inhibition of LTB4 in the same donors. 15-HETE was also inhibited, but the inhibition was only 50% at the highest concentration tested (100 μM). Taken together, these results support the conclusion that ABT-761 is a selective inhibitor of 5-lipoxygenase.

Inhibition of ionophore-induced leukotriene formation from cynomolgus monkey lung fragments. Calcium ionophore A23187 (20 μM) induced the formation of significant amounts of LTB4 (45 ± 4.4 ng/g of tissue) from lung fragments taken from cynomolgus monkey, albeit very slowly. This slow rate of metabolism in vitro translated to a long duration in vivo. ABT-761 had an estimated oral half-life of 16 hours in monkeys and 15 hours in humans (Brooks et al., 1995).

Pharmacokinetic studies with ABT-761 were performed in several other species. A summary of the data obtained in rats, guinea pigs and mice is shown in table 2. In all three species, the compound gave excellent pharmacokinetic results. The compound was particularly long-lived in guinea pig, for which a plasma half-life of 7.6 hours was determined from intravenous studies. In general, the time to the maximal plasma concentration of ABT-761 was somewhat long, 5 to 6.5 hours, but it gave classic absorption curves, as demon-

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### TABLE 1

**In vitro potency and selectivity of ABT-761 vs. selected inhibitors**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Broken cell IC50&lt;sup&gt;a&lt;/sup&gt; (μM)</th>
<th>PMNL IC50&lt;sup&gt;a&lt;/sup&gt; (μM)</th>
<th>Whole blood eicosanoid IC50&lt;sup&gt;a&lt;/sup&gt; (μM)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LTB&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>ABT-761</td>
<td>23 (19–29)</td>
<td>23</td>
<td>0.150</td>
</tr>
<tr>
<td>Zileuton</td>
<td>92 (50–140)</td>
<td>700</td>
<td>(0.1–0.2)</td>
</tr>
<tr>
<td>ZD-2138</td>
<td>1400</td>
<td>9</td>
<td>(0.64–0.84)</td>
</tr>
<tr>
<td>Bay X 1005</td>
<td>I.A.</td>
<td>60</td>
<td>6.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> 5-HETE formation from arachidonic acid in RBL lysates. Data are calculated for mean value of duplicate determinations.

<sup>b</sup> Calcium ionophore A23187 induced LTB<sub>4</sub> biosynthesis in human PMNL. Data are from five donors for ABT-761 and two for the other compounds. Determinations are mean values of triplicate determinations for the Abbott compounds.

<sup>c</sup> Ionophore stimulated eicosanoid release in human whole blood. Data are from six and three donors for ABT-761 and zileuton, respectively.

<sup>d</sup> Values within parentheses are 95% confidence limits.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species (route&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Bioavailability&lt;sup&gt;b&lt;/sup&gt;</th>
<th>t&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (hr)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt; (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABT-761</td>
<td>Mouse (p.o.) (5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>1.5</td>
<td>3.6&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Mouse (i.v.) (5)</td>
<td></td>
<td></td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>Rat (p.o.) (4)</td>
<td>60</td>
<td>1.5</td>
<td>3.0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rat (i.v.) (4)</td>
<td></td>
<td></td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>Guinea pig (p.o.) (4)</td>
<td>90</td>
<td>5.0</td>
<td>10.4&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Guinea pig (i.v.) (4)</td>
<td></td>
<td></td>
<td>10.4&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Compound dosed by oral gavage in 0.2% HPMC at 4 mg/kg (mouse, rat and guinea pig) or intravenously in 20% DMSO, 40% ethanol and 40% saline or in 9% mucin.

<sup>b</sup> Bioavailability from comparison of oral and intravenous studies.

<sup>c</sup> Intravenous half-life determined from the slope of the elimination curves.

<sup>d</sup> Maximum plasma concentration achieved.

<sup>e</sup> Number of animals is given within parentheses.

<sup>f</sup> Estimated oral half-life.
strated by the similarity of estimated oral half-lives with those calculated from intravenous data. The half-life in the rat was somewhat shorter than that in the other species. However, the bioavailability of the compound was quite good, even in that species (60%), and it was excellent in the other two (90–100%).

**Leukotriene inhibition studies in rodents.** An anaphylactic reaction in the rat peritoneal cavity produces large amounts of LTE₄ and lesser amounts of LTB₄ and TXB₂ (Young et al., 1991). ABT-761 was found to be a potent inhibitor of leukotriene biosynthesis but not of TXB₂ in this model. When the compound was orally dosed as a 3-hr pre-treatment, the compound gave dose-related inhibition of LTE₄ formation. An ED₅₀ value of 1.4 mg/kg was calculated from these data for LTE₄ inhibition. In the same experiment, the ED₅₀ value for the inhibition of LTB₄ biosynthesis was 0.6 mg/kg (fig. 2). There was no significant difference in the inhibition seen for the two classes of leukotrienes. The potency of ABT-761 was ~3-fold greater than that previously obtained for zileuton (Carter et al., 1991). It was also more potent than ZD-2138 or Bay X 1005. In contrast to the leukotriene inhibition seen with the compound, no inhibition of TXB₂ formation was seen at the highest dose tested (10 mg/kg).

High concentrations of leukotrienes are also formed in rats administered an intrapleural injection of A23187 (Rao et al., 1993). ABT-761 administered orally inhibited the formation of LTE₄ in this model in a dose-related fashion, yielding an ED₅₀ value of 3 mg/kg. Thus, ABT-761 was found to inhibit leukotriene formation in both pleural and peritoneal cavities. Plasma level determinations of ABT-761 from blood collected at the termination of these studies were consistent with the pharmacokinetic studies (table 2) in that doses of 1 to 10 mg/kg p.o. resulted in plasma levels of 1 to 14 μM. In general, plasma levels needed to be >4 μM to achieve >50% inhibition of leukotrienes in either cavity. These data taken together with the biochemical and ex vivo results indicate that ABT-761 is a selective 5-lipoxygenase inhibitor with good oral potency.

![Fig. 2. Inhibition of the biosynthesis of LTB₄ and LTE₄ in the rat anaphylaxis model by ABT-761. Animals were passively sensitized to bovine serum albumin by intraperitoneal injection 3 hr before antigen challenge. ABT-761 or control vehicle was dosed orally 3 hr before the antigen challenge. Fifteen minutes after antigen challenge, the animals were killed, and the peritoneal cavities were lavaged. Products were determined by EIA; control values were 151 ± 23 ng/rat for LTE₄ and 10.6 ± 5.3 ng/rat for LTB₄. Each data point represents the mean value for seven or eight animals.](https://example.com/fig2.png)

**Bronchoconstriction models in guinea pigs.** Although there are no animal models of human asthma that completely mimic this disease, a number of models are available that appear to have similarities to the bronchoconstrictive component of the disease (Malo et al., 1994; Wegner et al., 1993). ABT-761 was used as an oral agent in two of these, both in guinea pigs: AA-induced bronchospasm and antigen-induced bronchospasm in actively sensitized animals.

 Intravenously administered AA to guinea pigs previously treated with meclofenamic acid and propranolol was found to induce a bronchoconstriction that usually reached a peak within 5 min and then subsided to a secondary plateau that was 40% to 50% of the maximum response (Malo et al., 1989). The initial phase of the bronchoconstriction from 0 to 5 min appears to be independent on leukotriene formation; selective inhibitors such as ABT-761 (fig. 3) and A-79175 do not block it. In addition, fatty acids such as oleic acid that are not 5-lipoxygenase substrates also induce the first portion of the bronchoconstrictive response when injected intravenously but return to base line quickly. In contrast, alternative substrates such as eicosapentaenoic acid gave the same response as AA. In addition, bronchoconstriction induced by intravenous injection of eicosapentaenoic acid was inhibited by the selective 5-lipoxygenase inhibitor zileuton.

Oral pretreatment with ABT-761 inhibited AA-induced bronchoconstriction at low oral doses (fig. 3). An oral ED₅₀ value of 3 mg/kg was calculated from dose-response data.

Actively sensitized guinea pigs respond to aerosolized ovalbumin challenge with a brisk, extensive bronchospasm (Malo et al., 1994). The pulmonary response in this model appears to be similar to the bronchospasm observed after antigen challenge of allergic asthmatics in the clinical laboratory or during an acute asthmatic attack. The bronchoconstrictive response in the guinea pig, however, is more extensive than that normally experienced by the chronic asthmatic because antigen challenge causes a 65% to 75% change in lung function measured as Cdyn. When ABT-761 was administered orally 5 hr before antigen challenge, the bronchospasm was inhibited. As shown in figure 4, ABT-761 at 3 mg/kg gave nearly complete inhibition of the bronchospasm in animals pretreated with mepyramine and meclofenamic acid. At a higher dose of 10 mg/kg, a maximum inhibition of ~85% was observed, whereas a dose of 1 mg/kg gave ~50% inhibition (data not shown). This activity is superior to that seen with zileuton previously (Malo et al., 1994) and, as shown in figure 4, is superior to the recently described cysLT₁ antagonist MK-476 (Jones et al., 1995). A time course of predosing was also performed, and the compound gave significant inhibition through 8 hr at a dose of 5 mg/kg (data not shown). As in the rat leukotriene inhibition studies, ABT-761 was linearly absorbed as determined by measuring plasma levels of compound at the termination of the bronchospasm studies. Plasma levels of ABT-761 were 4.5 to 5.5 μM at the 3 mg/kg dose and 12 to 15 μM at the 10 mg/kg dose.

ABT-761 was also examined in the antigen challenge model using a therapeutic protocol. Because no data were available from the literature with this type of protocol, a pilot study with albuterol was performed. As can be seen in figure 5, the control response to antigen challenge is a drop in Cdyn.
to \sim 65\% of base-line values. This occurs in the 3- to 5-min period after challenge. To examine the effect of dosing compound after bronchospasm had begun, albuterol was administered intravenously after a predetermined $C_{\text{dyn}}$ response had been achieved. When albuterol was administered after a reduction in $C_{\text{dyn}}$ of 40\% was observed, the bronchial tone was rapidly and almost completely normalized to base-line values (fig. 5). After a change of 60\%, however, the response to albuterol was considerably lessened (data not shown).

Eosinophilic pulmonary inflammation in the rat. Another important aspect of asthma is a chronic inflammation of the lung characterized by a dominant eosinophilic component. As with bronchoconstriction, a number of animal models exist that have characteristics similar to the human disease. The intravenous administration of Sephadex G-200 particles induces a lung eosinophilia in Brown Norway rats that is characterized by increases in the bronchoalveolar lavage fluid of cysteinyl leukotrienes on days 0 to 3 and in eosinophils on days 1 to 3 (Walsh et al., 1994; Namovic et al., 1996). At day 3, \sim 30\% of the cells lavaged from the lungs of rats receiving Sephadex were eosinophils compared with 0.15\% in the saline-treated controls. Monocytes are not increased in this model, but neutrophils (day 1) and lymphocytes (day 3) are modestly increased (Namovic et al., 1996).

When ABT-761 was administered twice daily for 3 days, a dose-dependent reduction in both the number of eosinophils found in the lavage fluid and the levels of cysteinyl leukotrienes in the lavage fluid was observed (fig. 6, a and b). In two other experiments, a similar activity of ABT-761 was observed with significant inhibition of cell influx and reduction of leukotrienes occurring at doses of 1 and 3 mg/kg (data not shown). In two of these experiments, no effect on PMNL number or lymphocyte number was observed at day 3. However, modest effects were seen on both cell types in a third experiment. As expected from the previous rat studies, plasma levels of ABT-761 were maintained throughout the 4-day study. Plasma levels of ABT-761 on day 4 before dosing (3 mg/kg) were 5 \mu M and peaked at \sim 12 \mu M at 2 to 3 hours after dosing.

Discussion

Human asthma is a complex disease with multiple initiating events and multifactorial causes and symptomatic sequelae. Current research aimed at understanding the underlying causes of the disease has focused on bronchoconstriction, pulmonary inflammation and airway hyperreactivity (Fischer et al., 1995; Israel, 1994; Kay, 1991) as important attributes of the disease. The recent availability of specific leukotriene modulators and success of some of those agents in the treatment of chronic asthma (Israel et al., 1993) have clearly shown that leukotrienes are important factors in the disease. However, the precise role of these potent mediators is not yet completely defined, and new, more potent, longer-lasting agents may be required to explore fully the role of
leukotrienes in the disease. ABT-761, as described in this study, appears to be such an agent. ABT-761 was found to be a direct reversible inhibitor of 5-lipoxygenase in broken cell preparations and against cellular leukotriene biosynthesis. The inhibition of 5-HETE formation in RBL lysates was dependent on the concentration of substrate (AA) consistent with the compound being a competitive inhibitor of 5-lipoxygenase. The compound was a much weaker inhibitor of other eicosanoid-metabolizing enzymes, such as cyclooxygenase and 12- and 15-lipoxygenase in calcium ionophore A23187-challenged human whole blood. These data indicate that the compound is similar in profile to zileuton (table 1) but ~4- to 5-fold more potent. The selectivity of ABT-761 was also somewhat greater because it inhibited whole blood LTB₄ formation at a 5-fold lower concentration and TXB₂ formation at approximately the same concentration. Comparisons of the molecule with ZD-2138 (McMillan et al., 1992) and Bay X 1005 (Hatzelman et al., 1994) were also interesting (table 1). As expected for a FLAP inhibitor (Hatzelman et al., 1994), Bay X 1005 did not inhibit the 5-lipoxygenase directly in RBL lysates but was effective, albeit 3-fold less potent than ABT-761, against neutrophil LTB₄ biosynthesis. ZD-2138 also had a inhibitory profile that was different than that of the two direct enzyme inhibitors, zileuton and ABT-761; it was much less potent in the lysate assay than in neutrophils or whole blood.

The whole blood eicosanoid data for ABT-761 data indicate a ~300-fold selectivity for the 5-lipoxygenase vs. thromboxane formation. Thus, when dosed in vivo, ABT-761 would be expected to completely block the formation of LTB₄ and the cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄. It would not be expected to inhibit the formation of other eicosanoids. This inhibition should then be readily reversible when plasma concentrations of ABT-761 decrease over time.

ABT-761 was also found to be an effective specific inhibitor of leukotriene formation in lung tissue from monkeys. In contrast, it did not have nonspecific effects on lung tissue, as measured in the guinea pig. The compound lacked general bronchodilator activity, as tested in guinea pig tracheal preparations. It had no effect on basal tone of the tissue and failed to block contractions elicited by histamine, acetylcholine, the thromboxane agonist LTD₄ or PGD₂. These studies support the use of ABT-761 to probe the role of leukotrienes in vivo in pulmonary tissue.

ABT-761 was designed to be resistant to glucuronidation (Bell et al., 1995; Brooks et al., 1995). This characteristic translated to excellent bioavailability and long plasma half-lives in several species. In addition, the compound was an orally bioavailable inhibitor in rodents, as demonstrated by potent inhibition of leukotriene production in the rat. Oral doses of 5 mg/kg inhibited leukotriene formation in the rat peritoneal cavity for several hours. Cysteinyl leukotrienes and LTD₄ were inhibited in parallel, consistent with the site of inhibition of ABT-761 being at 5-lipoxygenase. ABT-761 was more potent in the anaphylaxis assay than the other inhibitors discussed above. It was 3-fold more potent than zileuton and Bay X 1005 (table 3). Interestingly, ZD-2138, which was the most potent inhibitor against in vitro whole blood LTB₄ formation, was inactive in this model.

ABT-761 was examined in three protocols of bronchoconstriction in the guinea pig of increasing complexity. In an effort to simplify the bronchospastic response to a single-

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TABLE 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat anaphylaxis ED₅₀ mg/kg</th>
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<tbody>
<tr>
<td>ABT-761</td>
<td>1.4⁴</td>
</tr>
<tr>
<td>Zileuton</td>
<td>4.4⁴</td>
</tr>
<tr>
<td>ZD-2138</td>
<td>&gt;10⁶</td>
</tr>
<tr>
<td>Bay X 1005</td>
<td>4.0⁶</td>
</tr>
</tbody>
</table>

⁴ Cysteinyl leukotriene formation in rat peritoneal cavity induced by immune complexes.

⁵ Value calculated from a 4-point dose-response study. Each point n = 8.

⁶ Value is a mean of six separate studies.
mediator system, intravenous AA was administered as a challenge. This model was somewhat successful in that the bronchospasm observed was not histamine dependent as has been observed for a significant part of the response to antigen (Malo et al., 1994). The specific inhibitor ABT-761 was effective in inhibiting most (~75%) of the bronchospasm observed. Interestingly, FLAP antagonists were not effective in this model (Malo et al., 1993), but the cysLT1 antagonist ZD-198615 was effective (Malo et al., 1993). Moreover, an initial bronchospastic response was observed in the animals at early times (1–3 min) after challenge that was not leukotriene dependent on the basis of several criteria described in Results.

ABT-761 was also examined in an antigen-challenge model using actively sensitized animals. As described by us (Malo et al., 1994) and others (Kallos and Kallos, 1984; Piper, 1977), in the presence of an antihistamine and a cyclooxygenase inhibitor, the bronchoconstrictive response to aerosolized antigen in guinea pigs is blocked by leukotriene modulators, including 5-lipoxygenase inhibitors, cysLT1 receptor antagonists and FLAP antagonists (Malo et al., 1993) if administered before antigen challenge. ABT-761 was very effective in blocking antigen-induced bronchospasm giving nearly complete inhibition at oral doses of 3–10 mg/kg. Thus the molecule was more potent than zileuton (Malo et al., 1994), Bay X 1005 (Nagai et al., 1996) and the cysLT1 receptor antagonist MK-476 recently described by Jones et al. (1995) (fig. 5). Oral dosing of ABT-761 at 2 to 8 hr before antigen challenge resulted in a marked diminution of the response. Inhibition was seen at plasma levels similar (4–15 μM) to those required to inhibit leukotriene formation in the rat; this is consistent with the proposal that the inhibition of bronchospasm was derived from inhibition of leukotriene formation. In addition, these observations confirm the leukotriene dependence of this guinea pig model using the highly selective inhibitor ABT-761 and the selective cysLT1 receptor antagonist MK-476.

Many asthmatics have a decreased overall lung function compared with normal individuals, which can be partially reversed by the use of beta agonists. In most patients, the disease is chronic. Drugs used in asthma treatment thus must be able to alleviate ongoing bronchospasm as well as prevent pulmonary responses to a new insult. Recent clinical data with zileuton indicate that pulmonary function in asthmatics is improved acutely after a single dose of the compound (Israel, 1994). Because the compound does not cause bronchodilatation in normal subjects, the hypothesis has been proposed that ongoing leukotriene formation is responsible for a portion of the bronchospasm in these asthmatics and that inhibition of leukotriene formation would improve pulmonary function. In response to these concepts, we tested ABT-761 and albuterol for the effectiveness of these compounds against an ongoing bronchospasm in the antigen-challenged guinea pig model. These studies were done by inducing a compliance reduction of 40% followed by an intravenous bolus of either albuterol or ABT-761. Albuterol was able to significantly reverse the bronchoconstriction when administered at 40% compliance; however, the effect of the compound was substantially diminished when compliance had reached a 60% decrease before therapy. These data were not surprising given the known bronchodilatory mode of action of albuterol; however, the data did show that the bronchoconstrictive response to antigen is reversible in this model. More unexpected was the response to intravenous ABT-761 after bronchospasm had been initiated. The compound was able to reverse 50% of the bronchospasm in response to antigen. Whether this is because leukotrienes are responsible for 50% of the bronchospasm with the other half being initiated by another mediator or because insufficient ABT-761 was delivered could not be determined because the dose that was administered was the highest that was soluble in the intravenous vehicle. The data obtained for ABT-761 in the guinea pig, however, confirm the hypothesis that the bronchoconstrictive response to antigen requires the continual formation of leukotrienes. Thus, although the pulmonary response is more severe, the guinea pig model appears to mimic the human asthmatic response (Israel, 1994) to the extent that it also appears to require continual leukotriene formation for continued bronchoconstriction to occur.

Pulmonary inflammation is another important characteristic of human asthma (Kay, 1991). In contrast to other diseases, such as rheumatoid arthritis, the inflammation in asthma is predominantly eosinophilic (Martin et al., 1996). Several animal models of pulmonary inflammation have been reported. Recently, we modified one of these and found it useful for the characterization of compounds (Namovic et al., 1996). We have shown that the intravenous injection of Sephadex particles in Brown Norway rats results in a specific eosinophilic inflammation. The eosinophil influx is temporally associated with the appearance of cysteinyl leukotrienes in bronchoalveolar lavage fluid. ABT-761 administered orally twice daily for the 3-day course of the response gave potent and parallel inhibition of both the eosinophil influx as measured by bronchoalveolar lavage fluid cells as well as by bronchoalveolar lavage fluid leukotriene levels. Given the specificity of action of ABT-761 shown in this report, we propose that the effect on eosinophil influx observed in this model has a leukotriene component. At this point it is unclear whether the attraction of eosinophils to the lungs of treated rats is dependent predominantly on LTB4, cysteinyl leukotrienes or both. Both classes of leukotrienes have been shown to be chemotactic for human eosinophils, although data on rat eosinophils are unavailable. We are inclined to believe that LTB4 is the major eicosanoid chemottractant because MK-476 and ZD-204219, two selective cysLT1 receptor antagonists, are not as effective in this model as leukotriene inhibitors (Namovic et al., 1996). Whether these observations translate to other models of pulmonary eosinophilia or to asthma itself remains to be explored.

In conclusion, ABT-761 appears to have the specificity, duration of action and tissue penetration required for effective attenuation of both the bronchoconstrictive and inflammation components of pulmonary disease. Given the early clinical success of leukotriene modulation with agents such as zileuton and zafirlucast, the more potent, longer-lasting 5-lipoxygenase inhibitor ABT-761 should prove to be a useful pharmacological and clinical agent.

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References


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