Anti-Inflammatory Activity of a Potent, Selective Leukotriene A₄ Hydrolase Inhibitor in Comparison with the 5-Lipoxygenase Inhibitor Zileuton

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ABSTRACT

Leukotriene A₄ hydrolase (LTA₄H) catalyzes production of the proinflammatory lipid mediator, leukotriene (LT) B₄, which is implicated in a number of inflammatory diseases. We have identified a potent and selective inhibitor of both the epoxide hydrolase and aminopeptidase activities of recombinant human LTA₄H (IC₅₀, approximately 10 nM). In a murine model of arachidonic acid-induced ear inflammation, the LTA₄H inhibitor, JNJ-26993135 (1-[4-(benzothiazol-2-yloxy)-benzyl]-piperidine-4-carboxylic acid), dose-dependently inhibited ex vivo LTB₄ production in blood, in parallel with dose-dependent inhibition of neutrophil influx (ED₅₀, 1–3 mg/kg) and ear edema. In murine whole blood and in zymosan-induced peritonitis, JNJ-26993135 selectively inhibited LTB₄ production, without affecting cysteinyl leukotriene production, while maintaining or increasing production of the anti-inflammatory mediator, lipoxin (LX) A₄. The 5-lipoxygenase (5-LO) inhibitor zileuton showed inhibition of LTB₄, LTC₄, and LXA₄ production. Although zileuton inhibited LTB₄ production in the peritonitis model more effectively than the LTA₄H inhibitor, the influx of neutrophils into the peritoneum after 1 and 2 h was significantly higher in zileuton- versus JNJ-26993135-treated animals. This difference may have been mediated by the increased LXA₄ levels in the presence of the LTA₄H inhibitor. The selective inhibition of LTB₄ production by JNJ-26993135, while increasing levels of the anti-inflammatory mediator, LXA₄, may translate to superior therapeutic efficacy versus 5-LO or 5-LO-activating protein inhibitors in LTB₄-mediated inflammatory diseases.

Leukotrienes are biologically active metabolites of arachidonic acid that are implicated in a range of inflammatory diseases. Leukotriene (LT) B₄ production is initiated by the conversion of free arachidonic acid to an unstable epoxide intermediate, LTA₄, by 5-lipoxygenase (5-LO) in the presence of the accessory 5-LO-activating protein (FLAP). Hydrolysis of LTA₄ by the enzyme LTA₄ hydrolase (LTA₄H) produces the proinflammatory mediator LTB₄ (Haeggström, 2000). In addition to this epoxide hydrolase activity, LTA₄H has aminopeptidase activity toward a variety of substrates, although the in vivo function of this activity is not known. LTA₄ and arachidonic acid are also precursors for biosynthesis of lipoxins, which are endogenous anti-inflammatory agents believed to promote resolution of inflammatory responses (Sershan, 2005). LTB₄ is able to recruit and activate inflammatory cells, which in turn can cause tissue damage and disease. Elevated levels of LTB₄ have been implicated in the pathological etiology of inflammatory diseases including psoriasis, inflammatory bowel disease, chronic obstructive pulmonary disease, rheumatoid arthritis, asthma, and cystic fibrosis (Tager and Luster, 2003). Recently, a number of studies have focused on a role for LTB₄ in cardiovascular diseases, including atherosclerosis, myocardial infarction, and stroke (Aiello et al., 2002; Friedrich et al., 2003; Helgdottir et al., 2004; Huang et al., 2004; Subbarao et al., 2004). More recent data (Helgdottir et al., 2006) has shown that a particular LTA₄ gene haplotype, HapK, confers modest risk of myocardial infarction in an Icelandic cohort and in European Americans but confers a 3-fold higher risk in African Americans. Furthermore, LTA₄ and 5-LO show abundant expression in...
human atherosclerotic lesions, and their expression correlates with symptoms of plaque instability (Qiu et al., 2006). An LTA4H inhibitor would decrease LTB4 production and may thus have therapeutic potential in all of the above-mentioned diseases.

A number of LTA4H inhibitors have previously been described, some of which have shown anti-inflammatory efficacy in vivo (for review, see Penning, 2001). In this study, we describe the pharmacology of a new LTA4H inhibitor, JNJ-26993135 (Fig. 1). This compound was shown to be a potent, selective inhibitor of recombinant LTA4H and ionophore-stimulated LTB4 production in whole blood and was orally efficacious in reducing neutrophil influx and edema in an arachidonic acid-induced ear inflammation model. In whole-blood assays in vitro and in a zymosan-induced peritonitis model in vivo, JNJ-26993135 inhibited LTb4 production and tended to increase anti-inflammatory lipoxin A4 production. In contrast, the 5-lipoxygenase inhibitor zileuton inhibited not only LTb4 (and LTC4) production but also lipoxin production. The data presented here reveal a potential therapeutic advantage of LTA4H inhibitors versus 5-lipoxygenase or FLAP inhibitors, namely selective inhibition of the potent proinflammatory mediator, LTb4, while leaving intact or increasing the endogenous anti-inflammatory, proresolution mediator, LXA4.

Materials and Methods

Preparation of Recombinant Human LTA4H Hydrolase. LTA4H-encoding DNA was amplified by polymerase chain reaction and cloned into pFastBac1 (Invitrogen, Carlsbad, CA) for expression in Spodoptera frugiperda cells. Recombinant LTA4H enzyme was purified from the infected S. frugiperda cells as described previously (Gierse et al., 1993) and adjusted to 0.29 mg/ml protein in 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, and 5% glycerol, and EDTA-free Complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). The specific activity of the enzyme was approximately 3.8 mmol/min/mg.

Preparation of Substrate. LTA4 substrate was prepared from the methyl ester of LTA4 (Cayman Chemical, Ann Arbor, MI) by treatment under nitrogen with 67 M equivalents of NaOH, at room temperature, for 40 min. The LTA4 substrate in its free acid form was kept frozen at −80°C until needed.

Epoxide Hydrolase Assay. LTA4 hydrolase inhibitor, JNJ-26993135, was prepared as a 10 mM stock solution in DMSO and diluted in the assays so that the final DMSO concentration did not exceed 0.1%. Recombinant human LTA4H (36 ng) was incubated with various concentrations of test compound for 10 min at room temperature in assay buffer (0.1 M potassium phosphate, pH 7.4, 5 mg/ml fatty acid-free bovine serum albumin) in a volume of 50 μl. The solution was then adjusted to 200 μl with assay buffer, and 25 μl of the substrate, LTA4, was added (final concentration, 40 mM; 0.13 μM; final volume, 225 μl). After 10 to 30 min at room temperature, the assay was terminated by diluting 20-fold in assay buffer. The amount of LTB4 produced was assayed by enzyme immunoassay (EIA) (catalog no. 901-068; Assay Designs, Inc., Ann Arbor, MI). The concentration of compound that was required for half-maximal inhibition of recombinant enzyme activity (IC50) was calculated by nonlinear regression using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA), one-site binding competition.

Aminopeptidase Assay. Aminopeptidase activity was determined by a modification of the procedure of Rudberg et al. (2004). Recombinant human LTA4H (375 ng) was preincubated for 15 min at room temperature in assay buffer (50 mM Tris-HCl, pH 8.0, 100 mM KCl) in the presence of different concentrations of JNJ-26993135 (0.2% final DMSO concentration). An equal volume of 2-fold concentrated substrate (L-α-lanine-4-nitro-anilide hydrochloride; A9325; Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 1 mM in a volume of 110 μl. Aminopeptidase activity was determined by monitoring absorbance at 405 nm. IC50 values were determined as described above for epoxide hydrolase activity.

Selectivity of JNJ-26993135 versus 5-LO Receptors, Transporters, and Ion Channels. Selectivity of JNJ-26993135 was evaluated by Cerep, Inc. (Paris, France) against 50 other targets. These targets represent major classes of biogenic amine receptors, neurotransmitter receptors, ion channel binding sites, and neurotransmitter transporters. At 10 μM, JNJ-26993135 showed minimal cross-reactivity in all the assays (see Table 1).

In Vivo Experiments. All animal experiments described in this study were performed after review of the protocols and approval by the Institutional Animal Care and Use Committee.

Murine Whole-Blood Leukotriene B4, Cysteinyl Leukotriene, Lipoxin A4, and PGE2 Assays. For in vitro assays of ionophore-stimulated lipid mediator production, CD-1 mice were euthanized, and blood was collected in heparin-containing syringes by cardiac puncture. The blood was diluted 1:2 (LTB4, LXA4) or 1:15 (LTC4/D/E, PGE2) in RPMI 1640 medium, and 200-μl aliquots of the diluted blood were added to wells of a 96-well tissue culture plate. JNJ-26993135 or the 5-lipoxygenase inhibitor zileuton were added at different concentrations to the diluted whole blood (final DMSO concentration of 0.1%) and preincubated for 15 min at 37°C in a humidified incubator. For murine ex vivo analysis of LTB4 production, blood was obtained from BALB/c mice 4 h after oral dosing of JNJ-26993135 and was diluted 1:1 in RPMI 1640 medium, after which 200-μl aliquots of the diluted blood were added to a microtiter plate. Calcium ionophore A23187 (Sigma Chemical Co.) was added to samples for both in vitro and ex vivo whole-blood assays (final concentration, 20 μg/ml). The incubation was continued under the same conditions for an additional 10 to 30 min to allow eicosanoid formation. The reaction was terminated by centrifugation (208 g; 10 min at 4°C) to form a cell pellet, and the amount of LTB4 produced was assayed in the supernatants (diluted 1:5 to 1:15) by enzyme immunoassay. For LXA4 measurement, supernatant samples were assayed without dilution using EIA kits from Neogen (catalog number 401070; Lansing, MI). According to the manufacturer, the LTB4, EIA assay is selective for LTB4, with 5% cross-reactivity for 6-trans-12-epi-LTB4 and 6-trans-LTB4. The LXA4 EIA assay from Neogen is selective for LXA4, with 5% cross-reactivity for 5(S,6(R)-hydroxyeicosatetraenoic acid and 1% for lipoxin B4 (Levy et al., 1993). PGE2 and cysteinyl leukotrienes were measured using Assay Designs EIA (catalog numbers 900-001 and 901-070, respectively). IC50 values were determined as described above for epoxide hydrolase activity.

Arachidonic Acid-Induced Ear Inflammation. Arachidonic acid (catalog number 181198; Calbiochem, San Diego, CA) was added to a stock solution of 1 g/ml was diluted to 100 mg/ml in 100% acetone for experimental use. JNJ-26993135 or vehicle [20% hydroxypropyl-b-cyclodextrin (HPβCD)] were administered orally at t = −1 h to female BALB/c mice. At t = 0, 2 mg of arachidonic acid in acetone was applied to one ear of each mouse (n = 8–10 per group), and acetone was applied to the other ear. At t = 3 h, the mice were sacrificed, and blood was drawn for ex vivo whole-blood LTB4 production assays and measurement of compound levels. At the same time, 8-mm ear biopsies were taken, weighed, and frozen at −80°C for future analysis of neutrophil influx, as measured by myeloperoxidase activity.
Myeloperoxidase Assay. Ear biopsies were thawed and roughly minced into FastPrep tubes with lysing matrix D (catalog number 6913-100; Qiogene Inc., Carlsbad, CA) before the addition of 0.5 ml of freshly made extraction buffer (0.3 M sucrose, 0.22% cetyltrimethylammoniumbromide, 2.5 mM citrate). Samples were run on the FastPrep homogenization instrument for 30 s at a speed of 5 m/s before storage on ice. Samples were then centrifuged for 10 min at 14,000 rpm in a microcentrifuge. In a 96-well plate, 10 μl of the resultant supernatant was added to 90 μl of freshly made dilution buffer (10 mM citrate, pH 5.0, 0.22% cetyltrimethylammoniumbromide). Tetramethylbromide (20 μl) was added, and the plate was mixed gently and incubated at room temperature for 1 h. The reaction was then stopped with 100 μl of 1 M H2SO4, and the plate was read in a spectrophotometric plate reader at 405 nm.

Zymosan-Induced Peritonitis. Female CD-1 mice were pretreated orally, with vehicle, JNJ-26993135, or zileuton (both at 30 mg/kg), 30 min before i.p. challenge with 0.5 ml of zymosan solution (Sigma catalog number Z4250, 2 mg/ml in sterile phosphate-buffered saline). At the indicated times, mice were euthanized by CO2 overdose. The peritoneal cavity was flushed with 3 ml of lavage buffer (phosphate-buffered saline with 0.1% bovine serum albumin and 5 mM EDTA). The lavage fluid was transferred into 5-ml polypropylene tubes and kept on ice. The total number of leukocytes in lavage fluid was determined using a Z2 Coulter Counter. Differential leukocyte counts were made on Cytospin preparations that had been stained with a modified Wright's stain (Diff-Quik) by light microscopy. The peritoneal lavage samples were thawed on ice and diluted in assay buffer (1:5 for LTB4 and 1:100 for LTC4) and assayed by commercially available EIA kits (catalog numbers 901-068, and 901-070; Assay Designs, Inc.), following the manufacturer's instructions. For LXA4 measurement, supernatant samples were assayed without dilution using commercially available EIA kits (catalog number 401070; Neogen), following the manufacturer's instructions. Data were analyzed to compare drug-treated groups with vehicle-treated animals. A Student's t test was performed to detect significant differences below p = 0.05 using Prism (GraphPad software version 4.02).

results

Inhibition of LTC4 Hydrolase Activity in Vitro. The epoxide hydrolase activity of recombinant human LTC4 hydrolase for LTB4 formation was potently and dose-dependently inhibited by JNJ-26993135, with a mean IC50 value of 12 ± 10 (Table 1). Similar dose-dependent inhibition was observed for the peptidase activity, yielding an IC50 of approximately 6 nM. Thus, JNJ-26993135 is similarly potent at inhibiting both hydrolase and peptidase activities of recombinant human LTC4H (Fig. 2). Dose-dependent inhibition of ionophore-stimulated LTB4 production in murine blood diluted 1:2 was also observed for JNJ-26993135, with an IC50 of 339 ± 101 nM (Table 1), approximately 30-fold less potent than for the isolated enzyme. This relative decrease in potency could partially be ascribed to protein binding because the IC50 value obtained when the plasma proteins were diluted further (1:1.5 dilution of blood) was 89 ± 28 nM (n = 4).

Selectivity of JNJ-26993135. As shown in Table 1 and Fig. 2, although JNJ-26993135 dose-dependently inhibited LTB4 production in ionophore-stimulated blood, it had no significant effects on LTC4, lipoxin A4, or PGE2 production, indicating that the compound had no inhibitory effect on lipoygenase or cyclooxygenase activities, respectively. JNJ-26993135 was also tested for inhibitory activity on agonist binding to 50 different receptors, transporters and ion channels by Cerep, Inc. (Table 2). JNJ-26993135 showed less than 30% inhibition of any of the targets at a concentration of 10 μM.

Inhibition of Whole-Blood Eicosanoid Production Compared with the 5-Lipoxygenase Inhibitor Zileuton. As discussed above and shown in Fig. 2, A and B, JNJ-26993135 significantly reduced LTB4 production in murine whole blood, with no effect on LTC4, lipoxin A4, or PGE2 production, indicating that the pathway shunting to cysteinyl leukotrienes. Likewise, no inhibition was observed of LXA4 or PGE2 synthesis (Fig. 2, C and D). A trend toward increased LXA4 synthesis was observed (Fig. 2C). When a similar experiment was performed in human whole blood, dose-dependent inhibition of LTB4 production by JNJ-26993135 was observed, with a trend toward increased LXA4 synthesis (results not shown). In contrast (as shown in Fig. 2, A–C), the 5-LO inhibitor zileuton blocked the production of the anti-inflammatory mediator, LXA4, in addition to inflammatory eicosanoids, LTB4 and LTC4. No inhibition of PGE2 synthesis was observed for zileuton up to 6 μM, but minimal, possibly nonspecific, inhibition was observed at 30 μM zileuton (Fig. 2D).

Pharmacokinetics of JNJ-26993135 in Mice and Dogs. Oral availability of JNJ-26993135 was determined from the pharmacokinetics of the compound in mice and dogs. Results are summarized in Table 3. The compound was rapidly absorbed after oral administration (tmax = 0.5 and 1 h, in mice and dogs, respectively) and eliminated with a half-life of approximately 3 h in mice and 4 h in dogs. The compound showed excellent oral availability in both species (>100 and 65%, in mice and dogs, respectively). The oral

<table>
<thead>
<tr>
<th>Assay</th>
<th>Activity</th>
<th>IC50</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant human LTC4H enzyme</td>
<td>Epoxide hydrolase</td>
<td>12 ± 10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Aminopeptidase</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>LTB4 production</td>
<td>339 ± 101</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>LTC4 production</td>
<td>&gt;30,000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>LXA4 production</td>
<td>&gt;30,000</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>PGE2 production</td>
<td>&gt;30,000</td>
<td>2</td>
</tr>
</tbody>
</table>
Cmax at 10 mg/kg was approximately 4-fold higher in dogs than the same dose in mice (23.5 versus 6.4 μM, respectively), whereas the AUC was approximately 6-fold higher.

**TABLE 2**

Selectivity of JNJ-26993135 versus a panel of 50 receptors, transporters, and ion channels

<table>
<thead>
<tr>
<th>Assay</th>
<th>Inhibition at 10 μM</th>
<th>Assay</th>
<th>Inhibition at 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (h)</td>
<td>10</td>
<td>NK3 (h)</td>
<td>21</td>
</tr>
<tr>
<td>A2A (h)</td>
<td>10</td>
<td>Y1 (h)</td>
<td></td>
</tr>
<tr>
<td>A3 (h)</td>
<td>13</td>
<td>Y2 (h)</td>
<td></td>
</tr>
<tr>
<td>α1 (Nonselective)</td>
<td>11</td>
<td>NT1 (h)</td>
<td></td>
</tr>
<tr>
<td>α2 (Nonselective)</td>
<td>12</td>
<td>Δ2 (h)</td>
<td></td>
</tr>
<tr>
<td>β1 (h)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT1 (h)</td>
<td>µ (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BZD (central)</td>
<td>19</td>
<td>ORL1 (h)</td>
<td></td>
</tr>
<tr>
<td>B2 (h)</td>
<td>11</td>
<td>5-HT1A (h)</td>
<td></td>
</tr>
<tr>
<td>CCKA (h) (CCK1)</td>
<td>12</td>
<td>5-HT1B</td>
<td></td>
</tr>
<tr>
<td>D1 (h)</td>
<td>12</td>
<td>5-HT2A (h)</td>
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</tr>
<tr>
<td>D2S (h)</td>
<td>14</td>
<td>5-HT3 (h)</td>
<td></td>
</tr>
<tr>
<td>ETA (h)</td>
<td>14</td>
<td>5-HT4a (h)</td>
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<tr>
<td>GABA (nonselective)</td>
<td>12</td>
<td>5-HT6 (h)</td>
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<td>GAL2 (h)</td>
<td>11</td>
<td>5-HT7 (h)</td>
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<td>IL-8B (h) (CXCR2)</td>
<td>12</td>
<td>sat (nonselective)</td>
<td>13</td>
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<tr>
<td>CCR1 (h)</td>
<td>15</td>
<td>VIP1 (h) (VPAC1)</td>
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<tr>
<td>H1 (central)</td>
<td>15</td>
<td>Ca²⁺ channel (L, verapamil site)</td>
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</tr>
<tr>
<td>H2</td>
<td>15</td>
<td>K + V channel</td>
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</tr>
<tr>
<td>MC4 (h)</td>
<td>11</td>
<td>SK + Ca channel</td>
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</tr>
<tr>
<td>ML1</td>
<td>12</td>
<td>Na⁺ channel (site 2)</td>
<td>23</td>
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<tr>
<td>M1 (h)</td>
<td>20</td>
<td>Cl⁻ channel</td>
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</tr>
<tr>
<td>M2 (h)</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3 (h)</td>
<td>20</td>
<td>NE transporter (h)</td>
<td>28</td>
</tr>
<tr>
<td>NK2 (h)</td>
<td>20</td>
<td>DA transporter</td>
<td></td>
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</table>

5-HT, serotonin; DA, dopamine.

**TABLE 3**

Pharmacokinetic parameters for JNJ-26993135 in mice and dogs

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex (Route)</th>
<th>Dose</th>
<th>n</th>
<th>Cmax</th>
<th>tmax</th>
<th>AUC(0-∞)</th>
<th>t</th>
<th>CL/F</th>
<th>Vdss</th>
<th>F</th>
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<tbody>
<tr>
<td>Balb/c mouse</td>
<td>Female (i.v.)</td>
<td>2</td>
<td>3</td>
<td>5.69</td>
<td>0</td>
<td>7.13</td>
<td>2.74</td>
<td>1</td>
<td>2.5</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Female (p.o.)</td>
<td>10</td>
<td>3</td>
<td>6.37</td>
<td>0.5</td>
<td>40.73</td>
<td>3.18</td>
<td>0.88</td>
<td>NA</td>
<td>114</td>
</tr>
<tr>
<td>Beagle dogs</td>
<td>Female (i.v.)</td>
<td>2</td>
<td>2</td>
<td>14.9</td>
<td>0</td>
<td>76.3</td>
<td>4</td>
<td>0.07</td>
<td>0.42</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Female (p.o.)</td>
<td>10</td>
<td>2</td>
<td>23.5</td>
<td>1</td>
<td>248.7</td>
<td>6.1</td>
<td>0.12</td>
<td>NA</td>
<td>65</td>
</tr>
</tbody>
</table>

Cmax at 10 mg/kg was approximately 4-fold higher in dogs than the same dose in mice (23.5 versus 6.4 μM, respectively), whereas the AUC was approximately 6-fold higher.
amounts of the compound orally to mice, 1 h before arachidonic acid challenge to the ear. Three hours later (4 h after the compound was dosed orally), JNJ-26993135 significantly inhibited \( (p < 0.0001) \) the ex vivo ionophore-stimulated LTB\(_4\) production at 3, 10, and 30 mg/kg (Fig. 3). The concentration of JNJ-26993135 in the plasma increased proportionally with increasing doses (Table 4). At 3 mg/kg, approximately 50\% inhibition was observed, at a plasma concentration of 1 \( \mu M \), diluted to 0.5 \( \mu M \) in the assay. The topical arachidonic acid-induced neutrophil influx was inhibited in a dose-dependent manner, with an estimated ED\(_{50}\) of between 1 and 3 mg/kg p.o., at a plasma concentration between 0.3 and 1 \( \mu M \). The arachidonic acid-induced ear edema was also dose-dependently inhibited by JNJ-26993135, although the extent of inhibition was lower than for the neutrophil influx. This result is consistent with the fact that edema is mediated not only by LTB\(_4\) but also by the cysteinyl leukotrienes, which are not affected by inhibition of LTA\(_4\)H. The dose dependence of measured plasma concentrations of JNJ-26993135 (Table 4), ex vivo inhibition of ionophore-induced LTB\(_4\) production in blood, and inhibition of neutrophil influx and edema thus demonstrate a direct relationship between the in vivo pharmacokinetics and pharmacodynamic activity of this compound.

**Selective Inhibition of LTB\(_4\) Production in a Murine Model of Zymosan-Induced Peritonitis.** The effects of the LTA\(_4\) hydrolase inhibitor JNJ-26993135 on in vivo synthesis of LTB\(_4\), LTC\(_4\), and anti-inflammatory LXA\(_4\) were determined during acute inflammation in murine zymosan-induced peritonitis. Furthermore, the effects of JNJ-26993135 were also compared with the 5-LO inhibitor zileuton. Lipid mediator levels were analyzed at 1, 2, and 3 h after zymosan treatment. Eicosanoid levels were initially undetectable in peritoneal lavage (data not shown). Upon zymosan administration, LTB\(_4\) levels increased in vehicle-treated animals to peak at 2 h, whereas LTC\(_4\) and LXA\(_4\) were maximal between 1 and 2 h, all decreasing again at 3 h. When dosed orally, JNJ-26993135 (30 mg/kg) demonstrated a significant reduction, compared with vehicle controls, in peritoneal LTB\(_4\) levels at all time points (Fig. 4A), with no increase in proinflammatory LTC\(_4\) levels (Fig. 4B) and thus no pathway shunting to cysteinyl leukotrienes. Zileuton treatment blocked the production of LTB\(_4\) more effectively than JNJ-26993135 at 1 and 2 h and, in addition, totally inhibited synthesis of LXA\(_4\) and LTC\(_4\). In contrast, JNJ-26993135 showed a trend toward increased LXA\(_4\) levels in vivo (Fig. 4C). Differential cell counts from peritoneal lavage indicated that JNJ-26993135 significantly decreased total neutrophils at 1 and 2 h (40\% inhibition) compared with vehicle-treated animals (Fig. 4D), whereas zileuton-treated animals showed increased neutrophil influx \( (p < 0.05) \) at 1 h. At 2 h, significantly higher neutrophil levels were found in zileuton- versus JNJ-26993135-treated animals \( (p < 0.05) \) and marginally higher versus vehicle-treated mice. Thus, increased neutrophil levels were observed in zileuton-treated animals despite the fact that LTB\(_4\) levels were lower than in JNJ-26993135-treated mice at both 1- and 2-h time points.

To better define the effects of JNJ-26993135 treatment on LXA\(_4\) levels in this peritonitis model, the study was repeated with larger numbers of animals. In this experiment, treatment with JNJ-26993135 selectively inhibited LTB\(_4\) production (Fig. 5) and showed a clear, statistically significant increase \( (p < 0.05) \) in the LXA\(_4\) levels at 1 h (Fig. 5). Thus, in contrast to zileuton, JNJ-26993135 promotes the production of anti-inflammatory LXA\(_4\) in vivo.

**FIG. 3.** Dose-dependent inhibition by oral dosing of JNJ-26993135, of LTB\(_4\) production in blood, and neutrophil recruitment and ear edema in response to topical arachidonic acid. Vehicle (20% HP\(_{β}\)CD) or JNJ-26993135 (1–30 mg/kg) was administered orally to mice, 1 h before topical arachidonic acid challenge to the ear. Three hours later, blood was analyzed for ex vivo ionophore-stimulated LTB\(_4\) production (A), and ear biopsies were taken for measurement of neutrophil influx (from myeloperoxidase activity (B)) and edema (C), respectively. Results are expressed as percentage of vehicle control and represent the mean ± S.D. of 10 animals in each group.

---

**TABLE 4**

<table>
<thead>
<tr>
<th>Dose (mg/kg p.o.)</th>
<th>Plasma Concentration at 4 h (( \mu M ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
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In the present study, we describe the pharmacology of JNJ-26993135, a new potent and selective inhibitor of LTA4H. This compound dose-dependently inhibited the production of LTB4 by the human recombinant enzyme as well as in whole blood from mice (this study), rats, dogs, and humans (data not shown). JNJ-26993135 showed oral efficacy and a clear pharmacokinetic/pharmacodynamic relationship in murine arachidonic acid-induced ear inflammation. In zymosan-induced peritonitis, JNJ-26993135 selectively inhibited LTB4 synthesis and increased production of the anti-inflammatory mediator, LXA4.

LTB4 is a potent proinflammatory mediator, implicated in a number of diseases. Potential therapeutics for these diseases include 5-LO or FLAP inhibitors, which block the synthesis pathway upstream of LTA4, LTA4H inhibitors, and LTB4 receptor antagonists, which block the actions of LTB4 after synthesis. LTB4 antagonists generally are not equally effective at blocking both BLT1 and BLT2, whereas blocking LTB4 synthesis has the advantage of inhibiting downstream events through both receptors. The importance of targeting both receptors is indicated by recent data showing the role of BLT1 in a number of inflammatory models (Gelfand and Dakhama, 2006; Kim et al., 2006; Shao et al., 2006), as well as expression of BLT2 in human and murine mast cells (Lundeen et al., 2006), human dendritic cells (Shin et al., 2006), murine keratinocytes (Iizuka et al., 2005), and human synovial tissues (Hashimoto et al., 2003), all of which are relevant in inflammatory diseases.

LTA4H inhibitors specifically block the production of LTB4 from LTA4, without affecting the biosynthesis of lipoxins, which are also produced from LTA4 or from arachidonic acid through the activity of 5-lipoxygenase and 12- or 15-lipoxygenase. Lipoxins, such as LXA4, are known to play a role as endogenous anti-inflammatory agents and key mediators of the natural process of resolving an inflammatory response (Serhan, 2005). Furthermore, production of LXA4 has been described in a variety of inflammatory diseases, and decreased levels of LXA4 have been found in patients with severe versus moderate asthma (Levy et al., 2005) and in cystic fibrosis (Karp et al., 2004). Unlike LTA4 inhibitors, 5-LO or FLAP inhibitors block the leukotriene pathway upstream of LTA4 and LXA4, and we confirm this in the present study for the 5-LO inhibitor zileuton. In contrast, JNJ-26993135 selectively inhibited LTB4 synthesis and seemed to increase LXA4 synthesis. This may have resulted...
in the decreased number of neutrophils after 1 and 2 h in the LTA4H inhibitor- versus the zileuton-treated animals. After 24 h, analysis of the inflammation indicated that both vehicle- and zileuton-treated animals had higher total neutrophil counts compared with JNJ-26993135-treated animals, suggesting that the endogenous resolution process was somehow enhanced in the LTA4H inhibitor-treated animals.

In addition to demonstrating oral anti-inflammatory efficacy in the murine models described, JNJ-26993135 also demonstrated oral efficacy in trinitrobenzene sulfonic acid-induced colitis in rats (B. Whittle, J. Varga, A. Berko, K. Horvath, J. Riley, K. Lundeen, A. Fource, and P. Dunford, unpublished data) and favorable pharmacokinetic properties in mice and dogs. JNJ-26993135 or other LTA4H inhibitors may be candidates for further development in inflammatory diseases where LTβR is known to play a major role. In addition, our study with JNJ-26993135 reveals a potential therapeutic advantage of LTA4H inhibitors versus 5-lipoxygenase or FLAP inhibitors, namely selective inhibition of the potent proinflammatory mediator, LTβR, while leaving intact or increasing the endogenous anti-inflammatory, proresolusion mediator LXA4.

References


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