Pharmacological Characterization of SC-57461A (3-[Methyl][3-[4-(phenylmethyl)phenoxy]propyl]amino]propanoic Acid HCl), a Potent and Selective Inhibitor of Leukotriene A₄ Hydrolase II: In Vivo Studies

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ABSTRACT
Leukotriene (LT) A₄ hydrolase is a dual function enzyme that is essential for the conversion of LTA₄ to LTB₄ and also possesses an aminopeptidase activity. SC-57461A (3-[methyl][3-[4-(phenylmethyl)phenoxy]propyl]amino]propanoic acid HCl) is a potent inhibitor of human recombinant LTA₄ hydrolase (epoxide hydrolase and aminopeptidase activities, Kᵢ values = 23 and 27 nM, respectively) as well as calcium ionophore-induced LTB₄ production in human whole blood (IC₅₀ = 49 nM). In the present study, we investigated its action in several animal models. Oral activity was evident from the ability of the compound to inhibit mouse ex vivo calcium ionophore-stimulated blood LTB₄ production with ED₅₀ values at 1.0 and 3.0 h of 0.2 and 0.8 mg/kg, respectively. A single oral dose of 10 mg/kg SC-57461A blocked mouse ex vivo LTB₄ production 67% at 18 h and 44% at 24 h, suggesting a long pharmacodynamic half-life. In a rat model of ionophore-induced peritoneal eicosanoid production, SC-57461 inhibited LTB₄ production in a dose-dependent manner (ED₅₀ = 0.3–1 mg/kg) without affecting LTC₄ or 6-keto-prostaglandin F₁₅₂₀ production. Oral pretreatment with SC-57461 in a rat reversed passive dermal Arthus model blocked LTB₄ production with an ED₉₀ value of 3 to 10 mg/kg, demonstrating good penetration of drug into skin. Plasma level of intact SC-57461 (3 h after oral gavage dosing with 3 mg/kg) was 0.4 g/ml, which corresponds to 80% inhibition of dermal LTB₄ production. Oral or topical pretreatment with SC-57461A 1 h before challenge with arachidonic acid blocked ear edema in the mouse. SC-57461A is a competitive, selective, and orally active inhibitor of LTA₄ hydrolase in vivo, making it useful to explore the contribution of LTB₄ to a number of inflammatory diseases.
DMSO and 1% Tween 80 to fasted (18 h) adult male outbred mice (CD-1, 20–30 g; Charles River Breeding Laboratories, Portage, MI) by gavage. One or 3 h after dosing, mice were anesthetized with methoxyflurane and blood was collected on heparin (20 U/tube) from the retro-orbital sinus and added to microtiter plates (100 μl) along with an equal volume of RPMI-1640. Calcium ionophore A-23187 (20 μg/ml final concentration) was added and the mixture incubated 20 min at 37°C in a humidified incubator. The reaction was terminated by centrifugation (600g, 10 min, 4°C). Supernatants were analyzed for LTB4 by ELISA (Cayman Chemical, Ann Arbor, MI).

Rat Peritoneal Eicosanoid Production. Fasted CD rats (Charles River Laboratories, Inc., Wilmington, MA) for 18 h were given test compounds by gavage (10% DMSO, 90% saline/1% Tween 80) 1 h before i.p. injection of 20 μg of calcium ionophore A23187. Five minutes later, rats were euthanized with CO2 and the peritoneal cavity lavaged with ice-cold Dulbecco’s phosphate-buffered saline. Lavage fluid eicosanoids (LTB4, LTC4, PGE1) were analyzed by ELISA (Cayman Chemical). Reversed Passive Dermal Arthus (RPA) Reaction in Rat. Test compounds or vehicle (10% DMSO, 90% isotonic saline/1% Tween 80) were administered by gavage to fasted male (150–300 g) CD rats (Charles River Laboratories, Inc.) 1 h before initiating the Arthus reaction. At 1 h after dosing, animals were lightly anesthetized with ether and immune complexes were generated by intradermal injection of 200 μg of rabbit anti-chicken egg albumin IgG (Cappel, West Chester, PA) in 0.2 ml of water/site, followed immediately by i.v. injection via the tail vein of 10 mg of egg albumin (ICN, Costa Mesa, CA) in 0.5 ml of phosphate-buffered saline (PBS), pH 7.4. The control animals were given PBS alone. The inflammatory reaction was allowed to develop for 2 h or as indicated in the figure legends. Blood was collected for ex vivo LTB4 production by cardiac puncture before sacrifice by CO2 inhalation. Dermal injection sites were excised, placed on dry ice, and stored at −70°C. SC-57461 (free acid) was measured in plasma samples (Yuan et al., 1996) by reversed phase high-performance liquid chromatography with UV detection (LC Resources, Inc., McMinnville, OR).

To analyze skin for eicosanoids, dermal samples were homogenized in 100% methanol and subsequently allowed to sit on ice (MeOH adjusted to 16.7% in H2O) for 30 min before centrifugation for 25 min at 2000 g (4°C). The supernatants were passed through C18 Sep-Pak cartridges (20515; Waters, Milford, MA); and the eicosanoids were eluted with methyl formate, evaporated, and reconstituted in ELISA buffer for TxB2 and LTB4 determination (Cayman Chemical). The percentage of recovery for a reference LTB4 sample was 55%.

AA-Induced Ear Edema in Mouse. AA was solubilized in acetone and a 20-μl aliquot was applied to the right ear (10 μl on inner ear and 10 μl on outer ear) of male CD-1 mice (25–30 g). The right ear of control mice received identical treatment with the acetone vehicle. Drugs were administered orally (drug dissolved in saline vehicle with 2% DMSO and 1% Tween 80) or topically (drug dissolved in acetone vehicle) 1 h before AA administration. The animals were sacrificed by CO2 inhalation 1 h after application of AA. The ears were immediately excised and weighed (in grams). Edema was calculated as the increase in right ear weight of mice treated with AA compared with increase in right ear weight of control mice treated with acetone vehicle.

Materials. SC-57461 and SC-57461A (>95% purity), N-(1-benzo[b]thien-2-ylthyl)-N-hydroxy urea (zileuton), and N-[5-[5-(4-fluorophenyoxy)-2-furanyl]-1-methyl-2-propynyl]-N-hydroxy-urea (A-78773) were synthesized by the Chemistry Department at Pharmac Research and Development, Skokie, IL.

Statistical Analysis. Points and bars in each figure denote mean ± standard error. All statistical analyses were done with either Dunnett’s t test or Student’s t test. Significance levels are indicated in legends.

Results

Inhibition of Mouse ex Vivo LTB4 Production. To assess the ability of a compound to inhibit LTB4 production in vivo, blood samples were collected after oral administration and the blood stimulated with calcium ionophore ex vivo. Dose-dependent inhibition of LTB4 production by SC-57461 was observed at 1 and 3 h after oral dosing (Fig. 1). The ED50 values for SC-57461A were 0.2 mg/kg at 1.0 h and 0.8 mg/kg at 3.0 h. It was similar in potency to A-78773 (ED50 values of 0.2 at 1.0 h and 1.0 at 3.0 h), but considerably more potent than zileuton (ED50 values of 1.0 at 1.0 h and 4.5 at 3.0 h).

To determine the duration of pharmacodynamic activity in the mouse, compounds were administered orally at 10 mg/kg and blood obtained at 6, 18, and 24 h later for ex vivo LTB4 production. A single dose of 10 mg/kg SC-57461A blocked LTB4 production (n = 2 studies; five mice per study) 79% at 6 h, 67% at 18 h, and 44% at 24 h.

Inhibition of Rat Peritoneal Eicosanoid Production. To assess selectivity of leukotriene inhibition in vivo, a rat model of calcium ionophore A23187-induced eicosanoid production was used (Rao et al., 1993a; Raychaudhuri et al., 1993). Injection of ionophore into the pleural cavity elicited an increase in both lipoxygenase (LTC4, LTB4) and cyclooxygenase products (6-keto-PGF1α). Potent and dose-dependent inhibition (ED50 = 0.3–1.0 mg/kg p.o.) of ionophore-stimulated peritoneal LTB4 production was observed with SC-57461 given by gavage 1 h before ionophore (Fig. 2). However, no significant effects on LTC4 or 6-keto-PGF1α were detected.

In contrast to SC-57461, A-78773 (10 mg/kg) blocked production of LTC4 by greater than 50%, which is predicted on the basis of its ability to inhibit the 5-LO pathway (data not shown). However, neither A-78773 nor SC-57461 affected production of the cyclooxygenase metabolite 6-keto-PGF1α.

Rat Reversed Passive Dermal Arthus Reaction. The anti-inflammatory activity of SC-57461 in skin was evaluated in a rat RPA reaction. Test compounds were administered orally 1 h before immune complex formation and the reaction was allowed to develop for 2 h. SC-57461 significantly inhibited LTB4 formation in a dose-responsive manner (Fig. 3). The maximum inhibition of LTB4 formation was...
Fig. 2. Effect of SC-57461 on rat ionophore-induced peritoneal eicosanoid production. Oral administration of inhibitor at different doses (mg/kg) 1 h before ionophore. Eicosanoids were measured 5 min after challenge. n = 5 to 6/dose group. *, p < 0.05 compared with control ionophore-stimulated levels.

Fig. 3. Inhibition of dermal eicosanoids in rat RPA by SC-57461. Rats were orally dosed 1 h before immune-complex formation and sacrificed 2 h later. SC-57461 ED₅₀ and ED₉₀ values were approximately 0.3 and 10 mg/kg, respectively. n = 5 rats/dose group. *, p < 0.05 compared with antigen-antibody-stimulated levels.

>90% at 10 mg/kg. A-78773 was slightly more potent with an ED₉₀ of 1 to 3 mg/kg (data not shown). Neither SC-57461 nor A-78773 significantly altered TXB₂ formation.

This model was also used to determine plasma levels of drug at efficacious doses. SC-57461 was administered 1 h before initiation of RPA reaction. Two hours after intradermal injection (3 h after SC-57461 administration), blood was obtained and plasma levels of SC-57461 measured. A dose of 1 mg/kg SC-57461 resulted in an 80% inhibition of dermal LTB₄ with 0.06 μg/ml SC-57461 detected in the plasma (Table 1). At a higher dose of 3 mg/kg, a plasma value of 0.4 μg/ml was detected with greater (87%) inhibition of dermal LTB₄. The plasma concentration of SC-57461 was dose-dependent with the highest concentration at 30 mg/kg. The approximate ED₉₀ dose of SC-57461 in this experiment was 3 mg/kg. The corresponding mean SC-57461 plasma concentration, taken to be the target plasma concentration for efficacy, was 0.4 μg/ml.

**Inhibition of AA-Induced Ear Edema in Mouse.** SC-57461A was tested for its ability to inhibit AA-induced edema on the mouse ear. AA can elicit an acute edematous reaction to the ear associated with increases in both lipoxygenase and cyclooxygenase products (Opas et al., 1985; Rao et al., 1993b; Chen et al., 1994). Application of AA (2 mg/ear) consistently elicited a 2- to 2.5-fold increase in weight after 1 h. After oral administration, doses of 0.8, 4, and 20 mg/kg resulted in significant inhibition of edema (Fig. 4A). SC-57461A was also active (Fig. 4B) after topical application (ED₅₀ = 110 μg/ear), being nearly equipotent with A-78773 (ED₉₀ = 100 μg/ear; data not shown).

**Discussion**

SC-57461A is a potent, selective, and competitive inhibitor of LTA₄ hydrolase with excellent activity in whole animals. It is the first LTA₄ hydrolase inhibitor to be extensively characterized in vivo. This drug shows good oral bioavailability across species, and its activity in the skin demonstrates good tissue penetration after oral or topical administration, making it potentially useful in the clinical management of inflammatory dermal diseases.

LTA₄ is a suicide substrate for both LTA₄ hydrolase and 5-LO. Therefore, treatment with an LTA₄ hydrolase inhibitor may lead to an accumulation of LTA₄ that would inactivate both LTA₄ hydrolase and 5-LO, resulting in total leukotriene inhibition. In contrast, LTA₄ could be metabolized via leukotriene C₄ synthase to LTC₄/LTD₄, thus increasing the total amount of cysteinyl leukotrienes. Our data from the rat peritoneal assay suggest little shunting to the LTC₄/LTD₄ pathway. Moreover, it was previously reported that partial inhibition of LTA₄ hydrolase by bestatin did not alter the production of cysteinyl leukotrienes in a rat lung perfusion model challenged with ionophore (Muskardin et al., 1994). Thus, there is no evidence to date to support that inhibition of LTA₄ hydrolase results in significant shunting to other leukotrienes.

Alternatively, LTA₄ can spontaneously hydrolyze into its biologically inactive 6-trans LTB₄ isomers as was observed after blockade by LTA₄ (Evans et al., 1986). Nonenzymatic metabolism of this type may help explain the lack of shunting via other pathways. Interestingly, we observe about a 2-fold increase in 6-trans LTB₄ in mouse plasma after inhibition of ionophore-induced LTB₄ production ex vivo (data not shown).

SC-57461A demonstrated good oral activity in both the mouse and the rat. It was particularly potent in the mouse ex vivo assay with half-maximal inhibitory effects on ionophore-
We used the RPA model to characterize LTB₄ inhibition and correlate plasma level with efficacy after oral administration. RPA is an immune complex-induced model that is complement-, cytokine-, mast cell-, and neutrophil-dependent (Baily and Strum, 1983; Mulligan and Ward, 1992; Ramos et al., 1992). Lesions, characterized by edema, erythema, and increased eicosanoid biosynthesis (LTB₄, TxB₂), were elicited by intradermal injection of rabbit anti-chicken egg albumin IgG followed immediately by intravenous injection of the respective antigen. RPA has a more pathophysiological relevant agonist (immune complexes) than the calcium ionophore-, arachidonic acid-, or phorbol ester-mediated models; and a relevant target tissue (skin) that requires a compound to distribute out of blood and into tissue, an advantage over ex vivo blood assays. In this model, SC-57461A dose dependently inhibited RPA-induced dermal LTB₄ production with no significant effect on TxB₂ production, again supporting a selective action. SC-57461A also reduced neutrophil infiltration as evidenced by decreased accumulation of myeloperoxidase (data not shown).

The efficacious dose in the RPA model may be representative of the dose that will be efficacious in diseases such as psoriasis. Therefore, the plasma level of parent compound was determined to define the efficacious plasma concentration after oral dosing. A dose of 3 mg/kg SC-57461 resulted in 87% inhibition of LTB₄ with 0.4 μg/ml of parent compound detected in the plasma 3 h after administration.

SC-57461A was efficacious both orally and topically against AA-induced edema in the mouse ear. This strongly suggests that LTB₄ contributes to the AA-edema response. However, maximal inhibition of edema after SC-57461A did not exceed 80%, suggesting LTB₄ is not the sole mediator. Similarly, maximal doses of A-78773 in our study (data not shown) and in data reported previously (Bell et al., 1995) did not completely inhibit edema, thus suggesting a partial role for lipoxygenase products in mediating the AA-induced edema.

The efficacy of SC-57461A in several models of inflammation further support a role for LTB₄ as an important mediator of arachidonic-induced inflammation. This is also supported by recent studies in LTA₄ hydrolase-deficient mice, which show blunted responses to zymosan-induced peritonitis and arachidonic-induced mouse ear inflammation (Byrum et al., 1999). It remains to be seen whether a selective inhibitor of LTA₄ hydrolase is more efficaciously clinically than leukotriene biosynthesis inhibitors, which operate via different mechanisms (e.g., 5-LO inhibitors, phospholipase A₂ inhibitors).

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References


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