An Aspirin-Triggered Lipoxin A4 Stable Analog Displays a Unique Topical Anti-Inflammatory Profile

Arndt J. Schottelius, Claudia Giesen, Khusru Asadullah, Iolanda M. Fierro, Sean P. Colgan, John Bauman, William Guilford, Hector D. Perez and John F. Parkinson

_J Immunol_ 2002; 169:7063-7070; doi: 10.4049/jimmunol.169.12.7063

http://www.jimmunol.org/content/suppl/2002/12/03/169.12.7063.DC1

References
This article cites 43 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/169/12/7063.full#ref-list-1

Subscription
Information about subscribing to _The Journal of Immunology_ is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
An Aspirin-Triggered Lipoxin A₄ Stable Analog Displays a Unique Topical Anti-Inflammatory Profile

Arndt J. Schottelius,* Claudia Giesen,* Khusru Asadullah,* Iolanda M. Fierro,† Sean P. Colgan,‡ John Bauman,‡ William Guilford,‡ Hector D. Perez,§ and John F. Parkinson²

Lipoxins and 15-epi-lipoxins are counter-regulatory lipid mediators that modulate leukocyte trafficking and promote the resolution of inflammation. To assess the potential of lipoxins as novel anti-inflammatory agents, a stable 15-epi-lipoxin A₄ analog, 15-epi-16-p-fluorophenoxy-lipoxin A₄ methyl ester (ATLa), was synthesized by total organic synthesis and examined for efficacy relative to a potent leukotriene B₄ (LTB₄) receptor antagonist (LTB₄-R-Ant) and the clinically used topical glucocorticoid methylprednisolone aceponate. In vitro, ATLa was 100-fold more potent than LTB₄-R-Ant for inhibiting neutrophil chemotaxis and trans-epithelial cell migration induced by FMLP, but was ~10-fold less potent than the LTB₄-R-Ant in blocking responses to LTB₄. A broad panel of cutaneous inflammation models that display pathological aspects of psoriasis, atopic dermatitis, and allergic contact dermatitis was used to directly compare the topical efficacy of ATLa with that of LTB₄-R-Ant and methylprednisolone aceponate. ATLa was efficacious in all models tested: LTB₄/floprost-, calcium ionophore-, croton oil-, and mezerein-induced inflammation and trimellitic anhydride-induced allergic delayed-type hypersensitivity. ATLa was efficacious in mouse and guinea pig skin inflammation models, exhibiting dose-dependent effects on edema, neutrophil or eosinophil infiltration, and epidermal hyperproliferation. We conclude that the LXA₄ and aspirin-triggered LXA₄ pathways play key anti-inflammatory roles in vivo. Moreover, these results suggest that ATLa and related LXA₄ analogs may have broad therapeutic potential in inflammatory disorders and could provide an alternative to corticosteroids in certain clinical settings. The Journal of Immunology, 2002, 169: 7063–7070.

Lipoxin A₄ (LXA₄) is a short-lived, tetaene eicosanoid with potent anti-inflammatory activities (Ref. 1 and references therein). LXA₄ is produced locally at sites of inflammation by transcellular biosynthesis via interaction of neutrophils with platelets or other leukocytes with epithelial cells. Endogenous LXA₄ synthesis in neutrophils is primed by cytokines (2) and elevated in neutrophils from asthmatic subjects (3–5). LXA₄ may exert its anti-inflammatory effects through signals generated by binding to a high affinity, G protein-coupled LXA₄ receptor, ALX-R (1). ALX-R is conserved in mammals; it is constitutively expressed on neutrophils, eosinophils, and monocytes and is induced on epithelium and vascular endothelium, thus being ideally localized to effect a counter-regulatory role in promoting the resolution of cell-mediated inflammatory responses. ALX-R expression on neutrophils and eosinophils correlates with the ability of lipoxins to potently inhibit chemotaxis and transcellular migration of these cells. Aspirin triggers 15-epi-LXA₄ formation both in vitro and in vivo via a mechanism involving cyclooxygenase-2 inhibition (6). Aspirin-triggered 15-epi-lipoxins (ATLs) retain the anti-inflammatory properties of LXA₄ and may mediate in part aspirin’s therapeutic effects (7).

LXA₄ and ATLs undergo rapid metabolic inactivation by PG dehydrogenase-mediated oxidation and reduction (8, 9). These metabolites have reduced affinity for ALX-R and decreased anti-inflammatory potency. Chemical modifications to the C15-C20 region of LXA₄ and ATLs prevent metabolic inactivation, thus providing stable analogs with superior pharmaceutical characteristics (10). The stable lipoxin analogs inhibit neutrophil transcellular migration, pathogen- and TNF-α-induced epithelial cell IL-8 release, and vascular permeability of mouse ear skin exposed to inflammatory stimuli (Ref. 1 and references therein). In TNF-α-induced dorsal air pouch inflammation, stable LXA₄ analogs have potent local and systemic anti-inflammatory efficacy via down-regulation of proinflammatory cytokine and chemokine networks (11, 12). More specifically, lipoxins inhibit cytokine-stimulated IL-1β, macrophage inflammatory protein-2, and superoxide production while stimulating the anti-inflammatory cytokine IL-4 in neutrophils (11). Eosinophil-driven allergic reactions are also inhibited by stable LXA₄ analogs (13, 14). Lipoxins inhibit eosinophil chemotaxis, and IL-5 and eotaxin secretion (13). Moreover, lipoxins inhibit mesangial cell proliferation (15). Finally, lipoxins have been shown to inhibit the transcription factor NF-κB, which is a central regulator of inflammatory molecules and also is pivotal for proliferation and anti-apoptosis (16). Thus, an anti-proliferative effect can add to the anti-inflammatory mechanisms of lipoxins that interfere with the activation and migration of inflammatory cells.
Results to date suggest that the LXA4/ALX-R pathway promotes counter-regulatory signals to diverse proinflammatory mediators and that stable LXA4 analogs may have therapeutic potential in inflammatory and autoimmune disease. Because of the limited amounts of synthetic LXA4 analogs available, a role for LXA4/ALX-R in regulating cutaneous inflammation has not been studied systematically, and there is limited information on the efficacy profile of LXA4 analogs in industry standard animal models that represent distinct mechanisms of clinically relevant cutaneous inflammation. Quantitative results comparing the potency and efficacy of a stable LXA4 analog to other anti-inflammatory agents, including clinically relevant standards, such as glucocorticoids, is also lacking. To address these points, the topical efficacy of a stable ATL analog synthesized in bulk, namely 15-epi-16-p-fluorophenoxylipoxin A4 (ATLa; Fig. 1), was tested in a variety of skin inflammation models. The models were chosen to evaluate potential utility in distinct dermatoses, since they exhibit pathological features found in irritant contact dermatitis, psoriasis, allergic contact dermatitis, urticaria, and atopic dermatitis. Anti-inflammatory potency and efficacy were compared with those of a leukotriene B4 (LTB4) receptor antagonist (LTB4-R-Ant) and methylprednisolone aceponate (MPA), a mid-potent glucocorticoid that is marketed in Europe and frequently used for topical treatment of atopic dermatitis in children. The present results indicate that ATLa displays broad topical efficacy in all models of skin inflammation examined and proved to be dose-dependent for inhibiting edema, leukocyte infiltration, and epidermal hyperproliferation. ATLa gives a unique anti-inflammatory profile, suggesting potential use in topical treatment of dermatoses.

Materials and Methods

The stable analog ATLa (Fig. 1) was synthesized using previously described methods (17). Material of >95% purity was qualified against a synthetic ATLa standard (provided by Dr. N. Petasis (University of Southern California, Los Angeles, CA) and Dr. C. N. Serhan (Brigham and Woman’s Hospital, Harvard University, Boston, MA)) using 1H nuclear magnetic resonance, HPLC coinjection with photodiode array UV-visible detection (two methods), and liquid chromatography-mass spectrometry. The LTB4 receptor antagonist ZK-158252 (18, 19) (LTB4-R-Ant), Iloprost, and LTB4 for in vivo studies were synthesized at Schering AG (Berlin, Germany, and were performed in accordance with the ethical guidelines of Schering. Female NMRI mice (26–28 g) or pigmented white guinea pigs (Charles River, Wilmington, MA; 200–250 g) were housed according to institutional guidelines of the Schering animal facility. NMRI mice are outbred Swiss mice from Lynch to Poiley (National Institutes of Health, Bethesda, MD; albino, AA, BB, cc, DD, histocompatibility H-2a). Eight to 11 animals were randomly allocated to the different treatment groups.

Skin inflammation models

Because of the acute character of the models, ATLa, LTB4-R-Ant, or MPA was applied topically at the same time as the elicitation of the inflammatory reaction. Tissue weight (ears or dorsal skin punch biopsies) served as a criterion for edema formation. Peroxidase activity in skin homogenates served as a measure of total granulocyte (neutrophil and eosinophil) infiltration, and elastase activity served as a specific measure of neutrophil infiltration.

LTB4/Iloprost-induced inflammation

The stable PGI2 analog Iloprost enhances LTB4-induced ear inflammation, leading to edema and a neutrophilic infiltrate with a maximum reaction 24 h after elicitation (20). Ten microliters of 0.003% (w/v) LTB4 and 0.003% (w/v) Iloprost in ethanol/isopropylmyristate (95/5), with or without the respective anti-inflammatory agent, were applied dorsally to mouse ears. Animals were euthanized with CO2 24 h after application. Ears were cut off, weighed as an indicator for edema formation, and snap-frozen. Ears (area, ~1 cm2) were homogenized in 2 ml of buffer containing 0.5% HTAB and 10 mM MOPS (pH 7.0) in a Poltron(R) PT 3000 homogenizer (KINETICA, Lucerne, Switzerland) set at maximum speed (30,000 rpm). Samples were centrifuged, and 75 μl of supernatants were transferred to 96-deep-well plates (Beckman, Palo Alto, CA). The supernatants were then used to determine peroxidase and elastase activity as a measure for infiltrating granulocytes (peroxidase) and neutrophils (elastase), as described below. Anti-inflammatory effects of a given compound were defined as the percent inhibition of edema formation and peroxidase and elastase activities.

Calcium ionophore-induced inflammation

The calcium ionophore A-23187, applied topically, induces acute inflammation with edema and granulocyte infiltration that peaks at ~24 h (21). Ten microliters of a 0.1% (w/v) solution of A-23187 in ethanol/isopropylmyristate (95/5), with or without the respective anti-inflammatory agent, were applied dorsally to mouse ears. Animals were euthanized 24 h later, and the ears were processed as described for the LTB4/Iloprost inflammation model.

Croton oil-induced inflammation

The nonspecific contact irritant croton oil leads to acute inflammation and is characterized by edema formation and a mainly granulocytic cell infiltration into the skin (22). Ten microliters of 1% (v/v) croton oil in ethanol/isopropylmyristate (95/5), with or without the respective anti-inflammatory agent, were applied dorsally to mouse ears. Animals were euthanized at 24 h, and the ears were processed as described for the LTB4/Iloprost inflammation model.

Mezerein-induced inflammation

Mezerein causes acute inflammation, with edema formation and granulocyte infiltration within 24 h and epidermal hyperproliferation within ~72 h (23). Ten microliters of 0.05% (w/v) mezerein in ethanol/isopropylmyristate (95/5) with or without the respective anti-inflammatory agent were applied dorsally to mouse ears. For inflammation end points, animals were euthanized at 24 h, and ears were processed as described for the LTB4/
Iloprost inflammation model. As an indication for hyperproliferation, epidermal thickness was determined morphometrically in Formalin-fixed, plastic-embedded, sectioned, and toluidine blue-stained specimens obtained from the ears of mice euthanized at 72 h.

**Trimellitic anhydride-induced delayed-type hypersensitivity (DTH)**

Sensitization with the occupational contact allergen trimellitic anhydride (TMA) induces a DTH reaction, with prominent eosinophil infiltration 24 h after challenge (24), which, in contrast to other types of acute contact dermatitis, is characterized by a mixed Th1/Th2 reaction. Mice were sensitized on days 0 and 1 by a single application of 50 μl of 3% (w/v) TMA in acetone/isopropylmyristate (80/20) onto a shaven area of 2 × 2 cm on the right flank. The DTH reaction was induced on day 5 by challenging the animals with a single application of 10 μl of 3% (w/v) TMA in acetone/isopropylmyristate (80/20) with or without the respective anti-inflammatory substance onto the dorsal sides of both ears. Animals were euthanized 24 h after challenge, and ears were processed as described for the LTB4/Iloprost inflammation model.

**Peroxidase activity assay**

Peroxidase activity as a measure of total granulocyte infiltration was measured as previously described (25). Briefly, tetramethylbenzidine (TMB) dihydrochloride was used as a sensitive chromogen substrate for peroxidase. To convert TMB into TMB dihydrochloride, 34 μl of 3.7% hydrochloric acid (equimolar) was added to 5 mg of TMB. Then 1 ml of DMSO was added. This stock solution was slowly added to sodium acetate-citric acid buffer (0.1 mol/L, pH 6.0) in a ratio of 1:100. Two hundred microliters of the AMC standard 7-amino-4-methylcoumarin (5 mM in ethanol). Menlo Park, CA) at 380 nm and compared against a standard curve with the AMC standard. Absolute extinction numbers were used to express peroxidase activity.

**Elastase activity assay**

Elastase activity was measured by fluorescence of 7-amino-4-methyl-coumarin (AMC) that is released from the substrate MeO-Succ-Ala-Ala-Pro-Val-AMC (Bachem, Torrance, CA). Homogenized samples in HTAB were diluted 1/10 in cetrimide buffer (0.3% cetridme, 0.1 M Tris, and 1 M NaCl, pH 8.5). The substrate MeO-Succ-Ala-Ala-Pro-Val-AMC (300 mM in DMSO) was diluted 1/100 in cetrimide buffer to a working concentration of 3 mM. In cetrimide buffer, diluted samples were pipetted in multwell plates, and the reaction was started by addition of the AMC substrate at 37°C. The reaction was stopped after 1 h with ice-cold 100 mM Na2CO3, and samples were measured in a Spectra Max Gemini (Molecular Devices, Menlo Park, CA) at 380 nm and compared against a standard curve with the AMC standard 7-amino-4-methylcoumarin (5 mM in ethanol).

**Statistical analysis**

For all animal models statistical analysis was performed with the so-called modified Hemm (inhibition) test, which was developed by Schering’s Department of Biometrics based on the program SAS System for Windows 6.12 (SAS Institute, Cary, NC). To determine the inhibitory effect of anti-inflammatory compounds, the difference between the respective mean value of the positive controls and the mean value of the vehicle controls was set at 100%, and the percentile change by the test substance was estimated: % change = [(mean value positive group – mean value negative group)/ (mean value negative group – mean value control group)] × 100. To test whether the change caused by the treatment is different from zero, a 95% confidence interval was calculated under consideration of the variance of observations within the entire experiment. If the interval did not include zero, the hypothesis that there is no change was rejected at the level of α = 0.05. For each experiment IC50 values were determined graphically.

**Results**

**Human neutrophil chemotaxis and transcellular migration**

A hallmark of inflammation is the movement of circulating leukocytes into tissues via chemotaxis and transcellular migration across endothelial and epithelial cell barriers (26). Classical chemotactic mediators include the bacterial derived N-formylpeptide, fMLP, and the proinflammatory eicosanoid, LTB4. Human neutrophil chemotaxis to fMLP (10 nM) was inhibited in a dose-dependent manner by ATLa with an IC50 of ~0.1 nM and a maximal effect at ~10 nM (data not shown). ATLa was ~100-fold more potent than the specific LTB4 receptor antagonist (LTB4-R-Ant; IC50 = ~10 nM). In contrast, LTB4-R-Ant inhibited neutrophil chemotaxis induced by LTB4 (10 nM) more potently (IC50 = ~1 nM) than ATLa (IC50 = ~10 nM). In addition to chemotaxis, ATLa potently inhibited neutrophil transepithelial migration to 10 nM fMLP or 10 nM LTB4 (Fig. 2). Inhibition was dose dependent, with an IC50 of ~1 nM against fMLP and ~10 nM against LTB4. As expected, LTB4-R-Ant inhibited LTB4-induced transepithelial migration to LTB4 (IC50 = ~1 nM), but was virtually ineffective on transepithelial migration to fMLP (~20% inhibition at 1 μM). Thus, in addition to potently inhibiting human neutrophil chemotaxis, ATLa potently inhibits neutrophil transmigration through epithelial cell monolayers, a finding consistent with previous in vitro studies (10). The present results correlate with the finding that the receptor ALX-R is expressed on both human neutrophils and human epithelial cells and may mediate LX4-dependent effects in both cell types (1). Our results also suggest that ATLa might display broader anti-inflammatory potential than LTB4-R-Ant, since neutrophil responses to more than one mediator are potently attenuated.

**Efficacy of ATLa in cutaneous inflammation models**

To characterize its anti-inflammatory potential for human skin diseases, ATLa was examined in diverse models of skin inflammation. The models are based on cutaneous reactions to exogenous stimuli that provoke a variety of symptoms, including edema, neutrophil or eosinophil infiltration, and epidermal hyperproliferation.
The models encompass features of irritant dermatitis, allergic contact dermatitis, and some aspects of psoriasis and atopic dermatitis. A limitation of the models is their acute character, which does not allow for the assessment of therapeutic administration. Therefore, compounds were tested in a preventive manner by coapplication with the proinflammatory reagents. ATLa was compared with two reference compounds: LTB4 R-Ant and the topically active glucocorticoid, MPA. Comparative data for ATLa and LTB4 R-Ant in all models are summarized in Table I. Data for MPA are provided in the text and figures.

**LTB4/prost-induced inflammation**

As a direct correlate to antagonism of LTB4 responses in vitro (see above), ATLa was tested in an LTB4-dependent model. Topical ATLa dose-dependently inhibited edema as well as neutrophil infiltration (Fig. 3). IC50 values were ~40 µg/cm2; and complete inhibition was achieved at 300 µg/cm2 for both efficacy end points (mean of three independent experiments). The anti-inflammatory effects of ATLa in this model were comparable to the selective LTB4-R-Ant, which exhibited IC50 in the range of 20–30 µg/cm2 for all parameters tested. The results in this model are consistent with the potent in vitro functional antagonism of LTB4-stimulated neutrophil responses by both ATLa and LTB4-R-Ant. Given their known anti-inflammatory mechanism of action (27), glucocorticoids such as MPA are not active in this direct model of LTB4-induced inflammation and were not tested.

**Calcium ionophore-induced inflammation**

To examine the influence of ATLa on inflammation mediated by endogenous LTB4 production, efficacy was tested in calcium ionophore-induced inflammation in mice (Table I). ATLa exerted dose-dependent efficacy on all end points in this model (IC50 = <100–200 µg/cm2), with complete inhibition of cell infiltration and ~70% inhibition of edema at 1000 µg/cm2. ATLa was equipotent to LTB4-R-Ant in this model, but was 100- to 1000-fold less potent than MPA (IC50 = 0.1–10 µg/cm2). To demonstrate efficacy across species, ATLa effects were also tested in calcium ionophore-induced inflammation in the ears of guinea pigs (Table I). Inhibitory effects were similar in extent to LTB4-R-Ant. The data confirm that ATLa has anti-inflammatory efficacy in two species.

**Croton oil- and mezerein-induced inflammation**

Croton oil and mezerein are phorbol ester compounds that elicit an inflammatory reaction triggered by protein kinase C activation. This leads to the release of various proinflammatory mediators, which induce edema, cellular infiltration, and epidermal hyperproliferation. In the model of croton oil-induced inflammation, ATLa and LTB4-R-Ant inhibited edema formation and cell infiltration dose-dependently, with IC50 values in the range of 200–600 µg/cm2 (Table I). MPA inhibited these parameters with higher potency than both ATLa and LTB4-R-Ant (IC50 = <0.2 µg/cm2). In the mezerein-induced inflammation model, IC50 values for edema and cell infiltration measured at 24 h were ~100–150 µg/cm2 for ATLa (Table I and Fig. 4). LTB4-R-Ant showed similar potency to ATLa, with IC50 values from 100–260 µg/cm2 for these end points. MPA was significantly more potent for all inflammatory parameters (IC50 = 0.1–0.75 µg/cm2). Edema formation was inhibited by 70–80%, and cellular infiltration was completely inhibited at a dose of 1000 µg/cm2 for the eicosanoids and at 10 µg/cm2 for the glucocorticoid.

Mezerein induces a marked epidermal hyperproliferation: epidermal thickness at 72 h increased ~5-fold (see Fig. 5A, i, 11 µm; ii, 52 µm; and iv, 10 µm). ATLa caused a potent, dose-dependent inhibition of epidermal hyperproliferation (IC50 = ~120 µg/cm2). Complete inhibition of hyperproliferation was achieved at the highest dose (1000 µg/cm2; Fig. 5A, iii, 10 µm; and v; and Fig. 5B), with near-complete inhibition observed at 300 µg/cm2. ATLa was 3-fold more potent than LTB4-R-Ant (IC50 = ~400 µg/cm2; Fig. 5B) on this end point. The potency of MPA on epidermal hyperproliferation was not directly compared with ATLa in these experiments, but is in the same same range as its anti-inflammatory effects (data not shown).

<table>
<thead>
<tr>
<th>Table I. Summary of efficacy data for ATLa, LTB4-R-Ant, and MPA in skin inflammation models*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model and Species</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>LTB4/prost, mouse</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Calcium ionophore, mouse</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Calcium ionophore, guinea pig</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mezerein, mouse</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Croton oil, mouse</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Trimellitic anhydride (DTH), mouse</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

* The table summarizes the IC50 for ATLa and LTB4-R-Ant for each efficacy end point tested in the cutaneous inflammation models.

a Mean ± SEM from three independent experiments.

b Estimated IC50 outside of dose range tested.

c Dose response not established.
Trimellitic anhydride-induced DTH

TMA stimulates a T cell-mediated inflammatory reaction with a characteristic of DTH. Application of TMA to sensitized animals evokes a mixed cellular infiltrate composed primarily of eosinophils, but with some neutrophils. In this model MPA exerts very potent effects on edema formation and neutrophil infiltration (IC_{50} < 0.2 µg/cm²), but weaker effects on eosinophil infiltration, as measured with the
Derma and dermal hyperproliferation induced by mezerein (II), that was with marked epidermal hyperproliferation at 72 h was determined as described in Materials and Methods. Histological sections (×25 magnification) of mouse ear skin show the normal morphology of the vehicle control (i), with marked epidermal and dermal hyperproliferation induced by mezerein (ii), that was completely inhibited by 1000 μg/cm² ATLa treatment (iii). The higher magnifications (×100) for mezerein (iv) and mezerein plus ATLa (v) treatments demonstrate a decrease in leukocyte infiltration. Big round structures in the dermis represent cross-sections of hair follicles.

FIGURE 5. ATLa inhibits mezerein-induced epidermal hyperproliferation. (i) Mezerein was applied, as described in Fig. 4, and epidermal hyperproliferation at 72 h was determined as described in Materials and Methods. Histological sections (×25 magnification) of mouse ear skin show the normal morphology of the vehicle control (i), with marked epidermal and dermal hyperproliferation induced by mezerein (ii), that was completely inhibited by 1000 μg/cm² ATLa treatment (iii). The higher magnifications (×100) for mezerein (iv) and mezerein plus ATLa (v) treatments demonstrate a decrease in leukocyte infiltration. Big round structures in the dermis represent cross-sections of hair follicles. B. The bar graph shows the percent inhibition of mezerein-induced epidermal hyperproliferation by ATLa (■) compared with that by LTB₄ R-Ant LTB₄ R-Ant (□). ANOVA Compounds were co-applied with mezerein. Results are expressed as the mean ± SEM of animals per group experiments. * p < 0.05 vs vehicle control.

Discussion

Interaction of LXA₄ and aspirin-triggered 15-epi-LXA₄ with ALX-R has recently emerged as an endogenous signaling pathway for anti-inflammation (1). The field has developed substantially in recent years with the availability of metabolically stable LXA₄ analogs, such as ATLa, that facilitate pharmacological experimentation in vitro and in vivo. Despite these advances, the lack of substantial quantities of synthetic lipoxin analogs for detailed evaluation of the potency and efficacy vs benchmark research and clinical standards has not yet been possible. To this end we have synthesized and qualified the lipoxin analog ATLa, which showed intriguing biological properties in earlier studies (1) (see Fig. 1). Our present results establish the following. 1) ATLa potently inhibited neutrophil chemotaxis and transcellular migration, confirming earlier reports (10), and was a broader inhibitor of neutrophil responses than our own potent LTB₄ receptor antagonist. 2) Topically applied ATLa dose-dependently inhibited cutaneous inflammation evoked by diverse inflammatory mediators, with complete inhibition of several efficacy end points. 3) ATLa was efficacious in at least two species: mouse and guinea pig. 4) ATLa potency in vivo was at least equivalent to a potent LTB₄ receptor antagonist, but considerably less potent than MPA, a clinically used topical glucocorticoid standard. 5) ATLa was shown for the first time to block inflammation-dependent epidermal hyperproliferation and to inhibit a T cell-dependent allergic cutaneous reaction. These findings confirm the actions of ATLa and firmly establish that lipoxin analogs, such as ATLa, are a novel therapeutic class that may be considered for topical treatment of human dermatoses of diverse etiology.

ATLa inhibition of neutrophil chemotaxis and transcellular migration to both LTB₄ and fMLP in vitro demonstrates a broader influence for ATLa than LTB₄ R-Ant. In addition to potent LTB₄ antagonism in vitro, ATLa exhibited dose-dependent topical efficacy in exogenous LTB₄/Iloprost-induced inflammation, with potency equivalent to LTB₄ R-Ant. Earlier studies with lipoxin analogs in this model did not establish dose-dependence or potency relative to a direct LTB₄ antagonist (6). ATLa potency in this model was quite remarkable given that ATLa is not a direct LTB₄ receptor antagonist (IC₅₀ = >10 μM) (28).
as the mean pounds were coapplied with TMA during challenge. Results are expressed.

Materials and Methods

in mice. Mice were sensitized and then challenged with TMA allergen as

ATLa prevents trimellitic anhydride allergen-induced DTH

FIGURE 6. ATLa prevents trimellitic anhydride allergen-induced DTH

in mice. Mice were sensitized and then challenged with TMA allergen as
described in Materials and Methods. The inhibitory effects of ATLa (■)

were compared with those of LTB₄ R-Ant (□) and MPA (▲). All com-

pounds were coapplied with TMA during challenge. Results are expressed

as the mean ± SEM of 10 animals per group. * ∕ p < 0.05 vs vehicle
control.

To explore the broader effects of ATLa, efficacy was investigat-
gated in cutaneous reactions triggered by stimuli that induce en-
dogenous release of various inflammatory mediators, including

LTB₄. Calcium ionophore-induced inflammation is more promi-
nent compared with the LTB₄/Illoprost model and is inhibited by

LTB₄ receptor antagonists (29). As expected, ATLa exhibited
dose-dependent topical efficacy with similar potency to LTB₄R-
Ant, but was less potent than MPA. Importantly, ATLa was shown
to inhibit ionophore-induced inflammation in two species: mouse
and guinea pig.

Croton oil is an irritant that stimulates keratinocytes in vitro and
in vivo to release the inflammatory mediators IL-1α, TNF-α, IL-8,
and GM-CSF via protein kinase C stimulation (30, 31). As ex-
pected, MPA showed anti-inflammatory effects at low doses,
whereas ATLa, although as efficacious as MPA, was ~1000-fold
less potent. ATLa efficacy in this model is consistent with lipoxins
inhibiting TNF-α-induced epithelial cell IL-8 release (32), and
TNF-α-induced neutrophil infiltration and cytokine/chemokine
networks in the mouse air pouch (11). Extensive ATLa inhibition
of both inflammatory responses (24 h) and epidermal hyperprol-
eration (72 h) in the mezerein-induced model is particularly note-
worthy. This is the first demonstration of an effect of lipoxins on
epidermal hyperproliferation. Epidermal hyperproliferation is a
frequent finding in some cutaneous disorders and is prominent in
psoriasis. The enhanced epidermal growth not only contributes sig-
ificantly to patient discomfort, but also complicates disease treat-
ment (33, 34). The inhibitory effect on epidermal hyperprolifera-
tion might be a direct anti-proliferative effect on keratinocytes,
since lipoxins have been shown to antagonize mitogenic effects
and to be anti-proliferative (15). Moreover, keratinocyte hyperpro-
liferation appears to be induced by IL-6 and IL-8, both of which
are inhibited by lipoxins (1, 32, 33, 35, 36). The strong inhibitory
effect of ATLa on epidermal hyperproliferation in combination with
its ability to locally inhibit chemokine networks suggest that
ATLa could be an effective topical treatment for psoriasis.

The occupational allergen TMA induces cutaneous and respira-
tory allergic reactions in man (37). TMA sensitizes and elicits a
DTH reaction in animals, with a mixed Th1 and Th2 character
(38). The pronounced efficacy of ATLa on edema formation as
well as on neutrophil and eosinophil cell infiltration in this model
is the first demonstration that lipoxin analogs can modulate a cu-
taneous, T cell-dependent allergic response. These results are con-
sistent with earlier studies showing that lipoxin analogs inhibit
eosinophil chemotaxis in vitro (39) and eosinophil-driven inflam-
mation in vivo (13, 14). Taken together these findings suggest that
ATLa could be explored for topical treatment of dermatoses with
a prominent eosinophil component, such as allergic contact der-
matitis or atopic dermatitis.

Lipoxins are produced in human asthmatics and potently atten-
uate human monocyte inflammatory responses (40). Moreover,
ATLa potently attenuates airway hyper-reactivity and eosinophilia
in murine allergic airway inflammation via multipronged suppres-
sion of inflammatory mediators (41). Such findings highlight the
anti-inflammatory profile of lipoxins as being distinct from LTB₄
receptor antagonists. The latter have shown poor efficacy in murine
allergic airway inflammation (42) and in human asthma clinical
trials (43).

In summary, the in vitro and in vivo characterization of ATLa
shows a unique anti-inflammatory profile and suggests its utility
for the topical treatment of inflammatory reactions in skin. While
less potent than the clinically used topical glucocorticoid MPA,
topical ATLa showed equivalent efficacy on most end points mea-
sured. To date there is no evidence to suggest that lipoxins would
be expected to cause skin atrophy or systemic endocrinological
side effects, which limit the long term use of several topical ste-
roids. ATLa and related lipoxins analogs might thus offer an al-
ternative approach to chronic treatment of dermatoses or treatment
of skin reactions that are steroid resistant. Based on the similarity
of cutaneous inflammatory reactions and their underlying mecha-
nisms to inflammation in other organs, the impressive therapeutic
effects of topically applied ATLa suggest potential utility in other inflammatory and autoimmune diseases. These include rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, acute respiratory distress syndrome, and ischemia/reperfusion injury. For these indications, further understanding of the systemic pharmacology, efficacy, and safety profile of ATLa and related lipoxin analogs is required.

Acknowledgments

We thank Eginhard Matzke (Research Business Area Dermatology, Schering AG) for excellent technical assistance. Drs. Nicolas A. Petasis and Giovani Bernasconi (Department of Chemistry, University of Southern California) provided synthetic samples of ATLa and general advice on the synthesis and handling of lipoxins.

References


10 nM fMLP

% inhibition of chemotaxis

Concentration (nM)

10 nM LTB4

% inhibition of chemotaxis

Concentration (nM)