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The effect of a novel, dual function histamine H_1 receptor antagonist/5-lipoxygenase enzyme inhibitor on in vivo dermal inflammation and extravasation

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Abstract

Leukotrienes and histamine are thought to play important roles in the development of dermatitis. This study evaluated the in vivo efficacy of 5-{4-[(aminocarbonyl)(hydroxy)amino]but-1-ynyl}-2-(2-{4-[(*R*)-(4-chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)benzamide (ucb 35440), a dual function histamine H_1 receptor antagonist/5-lipoxygenase enzyme inhibitor, in mouse skin. A single application of phorbol 12-myristate 13-acetate (PMA) was used to induce an acute inflammatory response over a 6-h period. PMA was applied on days 0, 2, 4, 7 and 9 to generate a chronic inflammatory response measured on day 10. ucb 35440 was applied topically at 1 h pre-PMA challenge and 3 h post-PMA challenge in the acute model. In the chronic PMA model, ucb 35440 was applied topically twice a day (AM and PM) on days 7, 8 and 9. Dose–response studies revealed that ucb 35440 inhibited PMA-induced ear weight gain with a 57% inhibition measured using a 3% w/v topical solution in the acute model. The compound appeared less potent in the chronic model with 43% inhibition measured using a 3% w/v topical solution of ucb 35440. Qualitative histologic assessment in PMA challenged ears showed that ucb 35440 produced a moderate reduction of polymorphonuclear cell infiltration in the acute model whereas, a more substantial reduction in polymorphonuclear infiltration was noted in the chronic model. In addition, the oral efficacy of ucb 35440 was evaluated in vivo against histamine-induced extravasation in guinea pig skin. Single oral doses of ucb 35440 (10 mg/kg in 0.5% methylcellulose suspension) at 1, 2, 6 or 24 h pre-histamine challenge produced minimal inhibition of histamine-induced extravasation in the dermis. However, when ucb 35440 (10 mg/kg in a 0.5% methylcellulose suspension) was orally administered 24 and 2 h prior to dermal histamine challenge, significant inhibition of extravasation was observed. Similar inhibition of histamine-induced extravasation was observed when animals were orally dosed twice a day (AM and PM 10 mg/kg in a 0.5% methylcellulose suspension) for 5.5 days prior to dermal histamine challenge. Collectively, these results suggest that ucb 35440 may represent an important therapeutic class for the treatment of dermatologic inflammatory conditions.

Keywords: 5-Lipoxygenase; Histamine; Phorbol ester; Inflammation; Extravasation

1. Introduction

Atopic dermatitis is a chronic inflammatory skin disease frequently observed in allergic individuals. Epidemiological studies indicate that the prevalence of atopic dermatitis has been increasing since the 1940s. Current evidence suggests an immunologic basis for atopic dermatitis with the hallmarks of increased immunoglobulin E and eosinophilia detected in peripheral blood in the majority of patients. Histologic findings also support an immunologic basis with dermal CLA+T cells and mast cell degranulation in the acute lesion and macrophages and eosinophils in the chronic lesion (Leung, 1999; Leung and Soter, 2001).

Current therapy focuses on treating symptoms of atopic dermatitis with a combination of moisturizers, antihistamines, antibiotics and topical corticosteroids with the

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aims of repairing barrier function, reducing itch, secondary infections and inflammation. Given the multiple anti-inflammatory actions of corticosteroids, it is not surprising that atopic dermatitis is often successfully controlled with steroid therapy. Importantly, however, steroids disrupt a number of cytokine networks involved in lymphocyte function resulting in immunosuppression. In addition, long-term topical use decreases collagen synthesis leading to skin atrophy (Oishi et al., 2002; Oikarinen et al., 1998). Because of these risks, new therapeutic approaches are being intensively investigated. Modulators of the immune and inflammatory responses such as interferon-γ (Ellis et al., 1999; Hanifin et al., 1993), phosphodiesterase inhibitors (Hanifin et al., 1996) and an immunomodulator, tacrolimus, have shown some efficacy in atopic dermatitis (Reitamo et al., 2002a,b). Tacrolimus inhibits calcineurin phosphatase activity thereby preventing transcription of a variety of cytokines. Thus, as with corticosteroids, tacrolimus has broad immunosuppressive actions but does not cause skin atrophy (Reitamo et al., 1998).

A novel strategy that may lack the immunosuppressive liability of corticosteroids and tacrolimus is an anti-leukotriene approach. Leukotrienes are bioactive lipids derived from the metabolism of arachidonic acid by the enzyme 5-lipoxygenase. Several studies have demonstrated elevated levels of leukotrienes in blister fluid collected from human eczema and psoriasis skin lesions (Duell et al., 1988; Fogh et al., 1989; Reilly et al., 2000; Ruzicka et al., 1986) and leukotrienes are known to induce many of the characteristics of atopic dermatitis. For example, leukotrienes induce vasodilation, edema and neutrophil infiltration at intracutaneous injection sites in human skin (Soter et al., 1983). In addition, intradermal injection of leukotriene B_4 elicits a scratching response in mice (Andoh and Kuraishi, 1998). Further evidence that leukotrienes may play a role in atopic dermatitis comes from clinical observations in which leukotriene receptor antagonists have been shown to be effective in treating atopic dermatitis (Capella, 2001; Carucci et al., 1998; Yanase and David-Bajar, 2001).

The current study was performed to evaluate the activity of a novel dual function (histamine H_1 receptor antagonist/5-lipoxygenase enzyme inhibitor) compound, 5-{4-[(aminocarbonyl)(hydroxy)amino]but-1-ynyl}-2-(2-{4-[(*R*)-(4-chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy) benzamide (ucb 35440) (Wypij et al., 2002), in a murine phorbol 12-myristate 13-acetate (PMA)-induced skin inflammation model and in a guinea pig histamine-induced dermal extravasation model.

2. Materials and methods

2.1. Animals

Female CD-1 mice (Crl:CD-1 (ICR) BR) were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA). Animals were utilized at 8–10 weeks of age. Male Hartley guinea pigs (350–400 g; ~4–5 weeks old) were obtained from Elm Hill Breeding Labs (Chelmsford, MA). All animals had free access to standard rodent (LabDiet 5001) or guinea pig chow (LabDiet 5025, Purina, St. Louis, MO, USA) and de-ionized water. All animals were acclimated for a minimum of 3 days prior to experimentation, and all protocols were approved by the UCB Institutional Animal Care and Use Committee.

2.2. PMA-induced inflammation model in the mouse

PMA was dissolved in acetone at a concentration of 0.01% w/v. All solutions were applied (10 μl) to the inner and outer surfaces of each ear using a micropipette.

2.2.1. Acute model

Animals were dosed topically with 10 μl of test compounds (0.3–3.0% w/v) or vehicle 1 h pre-challenge and 3 h post-challenge with PMA. At 6 h post-challenge, animals were euthanized, ear punch biopsies collected using a 5/16 in. leather punch and individually weighed on a Mettler-Toledo (AB-204-S) balance. Ear biopsies were then fixed in Glyo-fixx (Thermo-Shandon, Pittsburgh, PA, USA) and processed for routine paraffin sections. Five-micrometer sections were stained with hematoxylin and eosin.

2.2.2. Chronic model

PMA was applied on days 0, 2, 4, 7 and 9. Dosing of test compounds or vehicle (10 μl) occurred twice a day (AM and PM) on days 7, 8 and 9. On days when dosing of compound and PMA challenge occurred, compound was administered 3 h pre-challenge and 3 h post-challenge. On day 10, animals were euthanized, ear biopsies obtained, weighed and fixed for histologic analysis. Five-micrometer paraffin sections were stained with hematoxylin and eosin.

2.3. Histamine-induced wheal and flare model in the guinea pig

Animals were fasted 12 h prior to oral dosing with ucb 35440 (10 mg/kg in a 0.5% methylcellulose suspension). This dose of ucb 35440 has previously been shown to significantly reduce both histamine-induced bronchoconstriction and ex vivo LTB4 generation in the guinea pig (data not shown). At 2, 4, 6 or 24 h following oral dosing, all animals were anesthetized via intra-muscular injection of 80 mg/kg Ketamine and 12 mg/kg Xylazine. Evans blue, dissolved in sterile 0.9% NaCl at 10 mg/ml, was administered intravenously (i.v.) at 10 mg/kg. Ten minutes after systemic administration of Evans blue, the back of the animal was shaved and each animal was injected (50 μl) intradermally (i.d.) at four discrete sites with one of the following: saline, and three doses of histamine (0.1, 1.0, 10.0 μg). Fifteen minutes following these i.d. injections, the animals were sacrificed and the skin carefully removed.

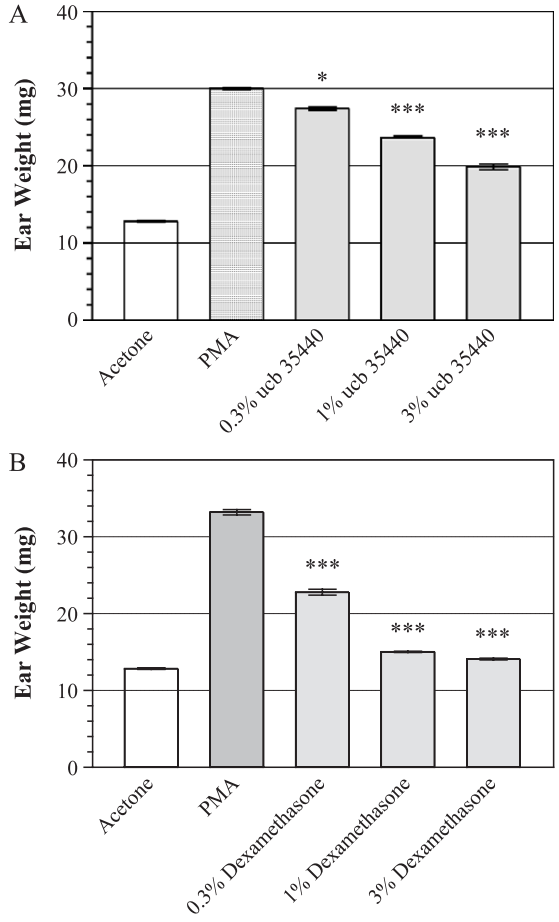

Fig. 1. Effect of ucb 35440 on PMA-induced ear weight in the acute mouse model. Ears were treated with 10 μl of 0.3–3.0% w/v of ucb 35440 (A) or 0.3–3.0% w/v of dexamethasone (B) 1 h pre-challenge and 3 h post-challenge with PMA (10 μl of a 0.01% w/v solution). Vehicle ears were treated with acetone. Ear weights were measured 6 h post-challenge. Weights are reported as mean±S.E.M. ($N \geq 6$ per group; *$P<0.05$, ***$P<0.001$ compared to PMA alone).

Measurement of the Evans blue wheals deposited in the skin was taken from the subcutaneous surface. A digital caliper (Control, Model 62379-531) was used to measure the wheal diameters in the *x* (rostral–caudal) and *y* (lateral–medial) planes. In separate sets of experiments, ucb 35440 (10 mg/kg in a 0.5% methylcellulose suspension) was orally administered 24 and 2 h prior to histamine challenge or orally dosed twice a day (AM and PM; 10 mg/kg in 0.5% methylcellulose suspension) for 5.5 days prior to dermal histamine challenge as outlined above.

2.4. Data analysis

All ear weights were measured and recorded. Sample masses were analyzed versus the maximal control for inhibition of edematous response (% inhibition) according to the following formula: % inhibition=100×[1.0−((P−((PMA+ucb 35440 treated)−(acetone treated ear)/(PMA+ethanol treated ear)−(acetone treated ear))]. Wheal diameters were measured and recorded. Sample diameters were analyzed versus the maximal control for wheal and flare (% inhibition) according to the following formula: % inhibition=100×[1.0−((ucb 35440 treated+histamine ID)−(ucb 35440 treated+saline ID)/(methylcellulose+histamine ID)−(methylcellulose+saline ID))]. Statistical analysis of data was accomplished using one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Tests. All statistical analysis and graphing of data were performed using GraphPad Prism (version 3.00, 1999, GraphPad Software).

3. Results

3.1. ucb 35440 inhibits PMA-induced inflammation

Topical application of ucb-35440 resulted in a dose-dependent inhibition of PMA-induced mouse ear edema in

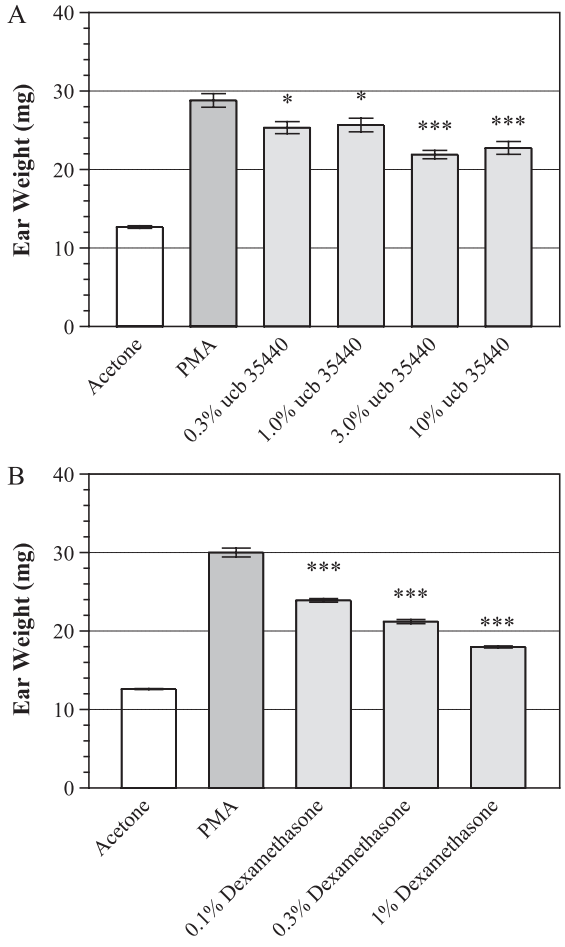

Fig. 2. Effect of ucb 35440 on PMA-induced ear weight in the chronic mouse model. Ears were treated with 10 μl of 0.3–10.0% w/v of ucb 35440 (A) or 0.1–1.0% w/v of dexamethasone (B) on days 7–9 as described in the Materials and Methods. Ear weights were measured on day 10. Weights are reported as mean±S.E.M. ($N \geq 6$ per group; *$P<0.05$, ***$P<0.001$ compared to PMA alone).

the acute model (Fig. 1A). Dose-dependent inhibition by ucb 35440 was not as apparent in the chronic model; however, a 3% w/v topical solution was more potent than either the 0.3% or the 1.0% topical solution (Fig. 2A). Dose–response studies demonstrated that ucb-35440 inhibited acute PMA-induced mouse ear edema with a 57% inhibition measured using a 3% w/v topical solution (Fig. 1A). The compound was less potent and less efficacious in the chronic model with a maximal inhibition of 43% measured using a 3% w/v topical solution (Fig. 2A). The positive control, dexamethasone, was more potent since a 1% w/v dose yielded a 94% inhibition in

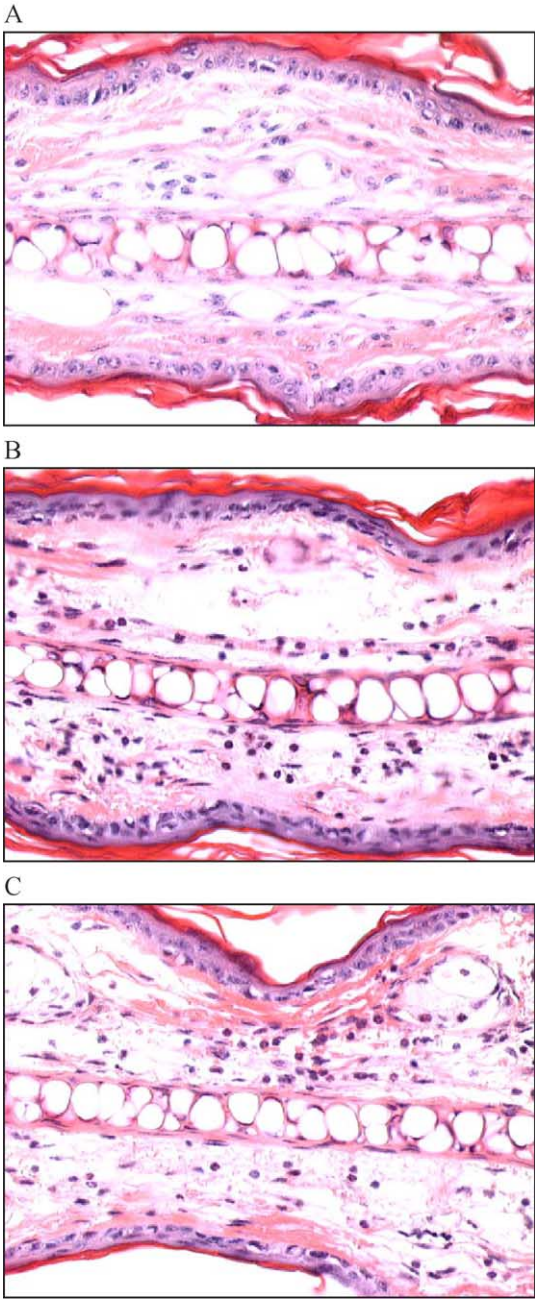

Fig. 3. Representative photomicrographs of H&E stained mouse ear cross sections in the acute PMA model. Ears were harvested 6 h post-treatment with acetone vehicle (A), 10 μl of a 0.01% w/v solution of PMA (B) or PMA plus 10 μl of a 3.0% w/v of ucb 35440 (C). Note the edema and polymorphonuclear cell influx in PMA-treated ears and the modest reduction in polymorphonuclear cell influx with ucb 35440 treatment (400× magnification).

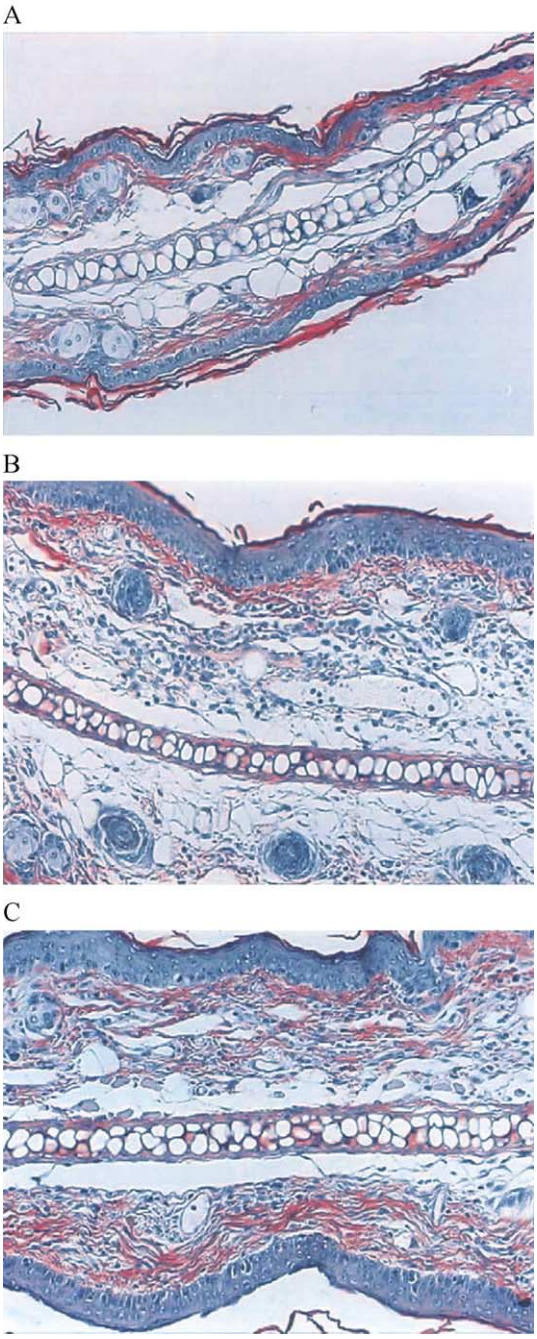

Fig. 4. Representative photomicrographs of hematoxylin and eosin stained mouse ear cross sections in the chronic PMA model. Ears treated with acetone vehicle (A), 10 μl of 0.01% w/v PMA (B) or PMA plus 3.0% w/v ucb 35440 (C) as described in the Materials and Methods. Ears were measured on day 10. Note the edema, vasodilatation, inflammatory cells and epidermal hyperplasia in PMA-treated ears and the reduction in inflammatory cells and edema with ucb 35440 treatment.

the acute model (Fig. 1B) and a 51% inhibition in the chronic model (Fig. 2B).

Histological evaluation of acute PMA challenged ears revealed prominent polymorphonuclear leukocyte recruitment in the dermis with accompanying connective tissue disruption and edema (Fig. 3B). Treatment with ucb 35440 resulted in a modest reduction in leukocyte recruitment and edema in the acute PMA challenged ears (Fig. 3C). Chronic PMA challenged ears displayed a marked epidermal hyperplasia, edema, connective tissue disruption and dilation of dermal vasculature as well as mononuclear and polymorphonuclear leukocyte recruitment (Fig. 4B). Treatment with ucb 35440 appeared to have a more substantial inhibitory effect on inflammatory/immune cell infiltrate and edema in the chronic model; however, increases in dermal connective tissue content were still observed in these ears. ucb 35440 had no apparent effect on the hyperplastic epidermis (Fig. 4C).

3.2. ucb 35440 inhibits histamine-induced wheal and flare

A single oral dose of ucb 35440 was not sufficient to produce a statistically significant inhibition of histamine-induced plasma extravasation in guinea pig skin (Fig. 5). While neither the 2 or 24 h pre-challenge doses of ucb 35440 alone produced significant inhibition, the combination of a 24 h pre-challenge dose followed by an additional 2 h pre-challenge dose produced a highly significant inhibition of extravasation induced by 0.1 μg and 1.0 μg histamine ($P<0.001$, Fig. 5). Evaluation of animals dosed twice a day with ucb 35440 for 5 days prior to histamine challenge also revealed substantial inhibition ($P<0.001$); however, the effect was not statistically greater than that observed with the 24 h plus 2 h pre-challenge group.

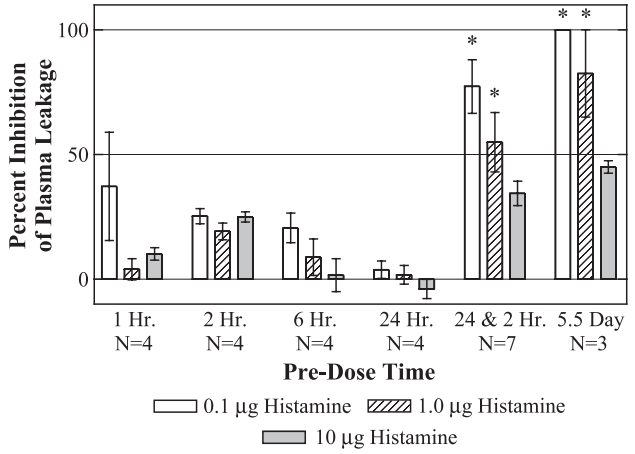

Fig. 5. Effect of ucb 35440 on histamine-induced extravasation in guinea pig skin. Guinea pigs received oral doses of ucb 35440 (10 mg/kg in 0.5% methylcellulose) at times indicated prior to intradermal histamine challenge injections in the skin as described in the Materials and Methods. Data are reported as mean percent inhibition±S.E.M. Note significant inhibition required multiple doses prior to histamine challenge (N=3–7/group;*$P<0.001$ compared to vehicle control).

4. Discussion

Atopic dermatitis is a chronic, pruritic inflammatory skin disease. While immune and inflammatory processes are central to the disease, itch is thought to be an important initiator and exacerbator of atopic dermatitis lesions. Given that degranulated mast cells are present and thus a source of histamine in acute atopic dermatitis lesions and histamine elicits itch, antihistamine therapy has been widely used in its treatment. However, small clinical trials have produced mixed results concerning the efficacy of antihistamines for reducing atopic dermatitis associated itch (Klein and Clark, 1999). Importantly, a large, well-controlled clinical trial recently demonstrated that addition of a nonsedating, histamine H_1 receptor antagonist to topical corticosteroid treatment resulted in a significant improvement in pruritus scores (Kawashima et al., 2003). This finding clearly supports a treatment strategy to block histamine-induced itch in conjunction with reducing inflammation associated with atopic dermatitis. Results from our study demonstrate that the histamine-1 receptor antagonist/5-lipoxygenase inhibitor, ucb 35440, inhibits PMA-induced increase in ear weight as well as histamine-induced wheal and flare reactions. Together, these findings suggest that histamine H_1 receptor antagonism coupled with 5-lipoxygenase inhibition may be an important therapeutic strategy for the treatment of inflammatory skin diseases.

Histologic evaluation of human lesions reveals T cells and degranulated mast cells in the acute lesion with macrophages and eosinophils in the chronic lesion (Leung, 1999; Leung and Soter, 2001). Given this complex pathology, it has been difficult to establish relatively short-term animal models for pre-clinical drug testing. Although not an allergen-driven model, the phorbol ester-induced mouse ear inflammation model produces edema and polymorphonuclear cell recruitment acutely (Carlson et al., 1985; Stanley et al., 1991) with macrophages and T-cells predominating by day 7 of the chronic model (Alford et al., 1992). Therefore, the PMA model mimics several aspects of human atopic dermatitis. In addition, this model provides opportunities to evaluate prophylactic treatment in the acute model as well as treatment of established lesions in the chronic model. New models including an epicutaneous sensitization model (Spergel et al., 1998), a food hypersensitivity model (Li et al., 2001), and mice strains which spontaneously develop dermatitis such as the cpdm and the NC/Nga mice (HogenEsch et al., 1993; Vestergaard et al., 2000) will likely serve as important models in the drug discovery setting. However, these models are yet to be completely characterized and require considerably more time and resources. Therefore, these models will likely be incorporated at a later stage in pre-clinical development.

The data provided in this report supports previous findings that blocking either the histamine type 1 receptor or the lipoxygenase pathway alone reduces acute phorbol-ester-induced edema (Carlson et al., 1985). Interestingly, however, our results in the chronic model indicate that the combination of histamine H_1 receptor antagonism plus 5-lipoxygenase inhibition is superior to either approach alone as ucb 35440 doubled the reduction in ear weight reported in a previous study for lipoxygenase inhibitors alone and was greater than 10-fold more efficacious than a histamine H_1 receptor antagonist alone (Stanley et al., 1991). What makes this finding more compelling is that in contrast to past studies, we did not apply a final dose of drug on the day of sacrifice as done by Stanley et al. (1991).

The increased efficacy of ucb 35440 in the chronic PMA model could be due to several factors. The first clue may be gleaned from the histamine-induced wheal and flare experiments in which multiple oral doses were required to inhibit the histamine response. The inhibition with multiple doses was clearly greater than an additive effect in the wheal and flare model leading us to hypothesize that ucb 35440 accumulates in the skin. Given that multiple doses of ucb 35440 were applied topically in the chronic PMA model, the compound may have accumulated in the skin thereby accounting for the enhanced efficacy. In addition, the combination of histamine H_1 receptor antagonism with 5-lipoxygenase inhibition may indeed be superior to either approach alone. While this has not been ruled out, previous reports indicate that histamine H_1 receptor antagonists alone do not block phorbol-ester-induced edema in the chronic model (Stanley et al., 1991), suggesting that 5-lipoxygenase inhibition accounts for the majority of the activity of ucb 35440 in the chronic PMA model.

Indeed, leukotrienes are known to induce a host of pro-inflammatory events including itch, vasodilation, edema as well as activation and migration of leukocytes (Andoh and Kuraishi, 1998; Fogh and Kragballe, 2000; Soter et al., 1983). In addition, elevated leukotriene levels have been detected in blister fluid collected from human eczema and psoriasis skin lesions (Duell et al., 1988; Fogh et al., 1989; Reilly et al., 2000; Ruzicka et al., 1986). Furthermore, leukocytes from humans with atopic dermatitis display enhanced release of leukotrienes (Ruzicka and Ring, 1987). Clinical observations with leukotriene receptor antagonists also strongly support a role for leukotrienes in dermatitis (Capella, 2001; Carucci et al., 1998; Yanase and David-Bajar, 2001). Interestingly, observations noted in the present study as well as previous reports suggest that 5-lipoxygenase inhibitors reduce inflammatory cell influx (Stanley et al., 1991).

In summary, the novel dual function histamine H_1 receptor antagonist/5-lipoxygenase inhibitor, ucb 35440, inhibits PMA-induced increase in ear weight as well as histamine-induced wheal and flare reactions. Importantly, this novel compound exhibited both topical and oral activity. Given the role of histamine and leukotrienes in allergic/inflammatory skin diseases, these results suggest that this unique class of molecules may be an important therapeutic advance in the treatment of these disorders.

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