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A geranyl acetophenone targeting cysteinyl leukotriene synthesis prevents allergic airway inflammation in ovalbumin-sensitized mice

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ABSTRACT

Asthma is associated with increased pulmonary inflammation and airway hyperresponsiveness. The current use of corticosteroids in the management of asthma has recently raised issues regarding safety and lack of responsiveness in 5–10% of asthmatic individuals. The aim of the present study was to investigate the therapeutic effect of a non-steroidal small molecule that has cysteinyl leukotriene (cysLT) inhibitory activity, upon attenuation of allergic lung inflammation in an acute murine model. Mice were sensitized with ovalbumin (OVA) and treated with several intraperitoneal doses (100, 20, 2 and 0.2 mg/kg) of 2,4,6-trihydroxy-3-geranylacetophenone (tHGA). Bronchoalveolar lavage was performed, blood and lung samples were obtained and respiratory function was measured. OVA sensitization increased pulmonary inflammation and pulmonary allergic inflammation was significantly reduced at doses of 100, 20 and 2 mg/kg with no effect at the lowest dose of 0.2 mg/kg. The beneficial effects in the lung were associated with reduced eosinophilic infiltration and reduced secretion of Th2 cytokines and cysLTs. Peripheral blood reduction of total IgE was also a prominent feature. Treatment with tHGA significantly attenuated altered airway hyperresponsiveness as measured by the enhanced pause (Penh) response to incremental doses of methacholine. These data demonstrate that tHGA, a synthetic non-steroidal small molecule, can prevent acute allergic inflammation. This proof of concept opens further avenues of research and development of tHGA as an additional option to the current armamentarium of anti-asthma therapeutics.

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Introduction

Asthma affects over 300 million people and is currently one of the commonest chronic diseases in the world, with a rising prevalence, particularly in developing countries (Barnes, 2010). Allergic asthma is mainly characterized by airway inflammation, mucus hypersecretion and airway hyperresponsiveness (Barnes, 2008). Activation and increased synthesis of Th2 cytokines such as interleukin (IL)-4, IL-5 and IL-13 lead to release of numerous proinflammatory molecules such as lipid-derived mediators, various chemokines and growth factors from inflammatory and structural cells, followed by inflammatory cell infiltration into the airways with ensuing pulmonary inflammation and bronchiol constriction (Hamid and Tulic, 2009). The activation of cells bearing allergen-specific IgE, especially mast cells and macrophages in the airways leads to the secretion of bronchoconstrictors such as histamine and cysteinyl leukotrienes (cysLT) that augment the development of allergic asthma (Barnes, 2008; Hamid and Tulic, 2009).

CysLTs are synthesized from arachidonic acid via the 5-lipoxygenase (5-LO) pathway (Dahlén, 2006). These lipid mediators exhibit proinflammatory characteristics whereby they have been demonstrated to promote airway eosinophilia, increased mucus hypersecretion, potent bronchoconstriction and tissue remodeling in asthmatic individuals (Busse and Kraft, 2005). Although combinatorial inhalatory treatment with corticosteroids and β_2 agonists remains the first choice in the treatment of asthma, the use of cysLT inhibitors such as montelukast, pranlukast and zileuton has received considerable attention (Montuschi and Peters-Golden, 2010). cysLT inhibition can be achieved by both receptor antagonists (montelukast, pranlukast, zafirlukast) and the 5-LO inhibitor (zileuton), with receptor antagonists being more preferable due to better bioavailability and safety. Zileuton remains to date the only 5-LO inhibitor used clinically in the management of elevated levels of cysLT in asthma, however its use has been limited by a modest but distinct incidence of hepatic enzyme elevation and moderate bioavailability, which initially demanded four times daily administration (Hamid and Tulic, 2009). However at least two properties of selective 5-LO inhibitors that distinguish them from cysLT receptor antagonists deserve emphasis and further investigation: (1) their greater effects on AHR in asthmatic patients than those of cysLT receptor

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antagonists (Dahlén, 2006); and (2) their greater efficacy in reducing nasal symptoms in patients with aspirin-sensitive asthma (ASA) (Dahlen et al., 1998).

Acetophenones are simple aromatic ketones. Their usage as drugs is limited to raw material in the synthesis of several pharmaceuticals (Gadamasetti and Braish, 2007; Sittig, 1988). To date there are few if any reports of acetophenones being used for the treatment of asthma. Dorsch et al. had demonstrated reductions in bronchial obstruction in guinea pigs sensitized with ovalbumin (OVA) or platelet-activating factor (PAF) following treatment with several acetophenones (Dorsch et al., 1992). They concluded that acetosyringenin provided superior attenuation of bronchial obstruction, followed by less active acetophenones such as apocynine, methoxy-androsin, picein and androsin. However their studies focused on effects upon bronchoconstriction alone and there was no subsequent publications extending their findings. We had previously isolated a geranyl acetophenone namely 2,4,6-trihydroxy-3-geranyl acetophenone (tHGA) (Fig. 1) from *Melicope ptelefolia* (Abas et al., 2006; Shaari et al., 2006). This compound differs from those described by Dorsch et al. in terms of the acylphloroglucinol group. We have recently shown that tHGA inhibits the synthesis of cysLTs in activated macrophages via inhibition of 5-lipoxygenase (5-LO) enzymatic activity (Shaari et al., 2011).

In this communication we demonstrate that parenteral administration of tHGA, a new 5-LO inhibitor, has therapeutic effects in an acute allergic model of airway inflammation with doses as low as 2 mg/kg being as effective as a standard experimental dosing regimen of zileuton at 30 mg/kg. These findings highlight the potential character of tHGA as a non-steroidal drug lead for allergic asthma and enable further assessment of this compound with respect to preclinical studies on dose and route optimization, and pharmacokinetics.

Materials and methods

Synthesis of tHGA. A well stirred mixture of phloracetophenone (1.000 g, 6 mmol), geranyl bromide (0.876 g, 4.80 mmol), and anhydrous potassium carbonate (0.415 g, 3.00 mmol) in dry acetone (3.5 mL) was refluxed for 6 h. The reaction mixture was filtered and evaporated under reduced pressure to give an oily orange residue that was purified by flash column chromatography on Si gel (petroleum ether-EtOAc, 10:1) to afford 2,4,6-Trihydroxy-3-geranylacetophenone (tHGA) as a light yellow powder; mp 128–130 °C. ¹H NMR (CD₃OD) δ_H 1.58 (3 H, s, Me), 1.63 (3 H, s, Me), 1.76 (3 H, s, Me), 2.64, (3H, s, COMe), 1.96 (2H, q, *J* = 7.5 Hz), 2.06 (2H, m), 3.21 (2H, d, *J* = 6.5 Hz), 5.08 (1H, t, *J* = 7 Hz), 5.20 (1H, t, *J* = 6.5 Hz), 5.92 (1H, s, ArH); IR (KBr) ν_{max} 3405, 1627 cm⁻¹; EIMS *m/z* (%) [M]⁺ 304 (38), 289 (3), 261 (9), 235 (25), 181 (100).

Animals. Male Balb/c mice, aged 8–10 weeks were obtained from the Institute of Medical Research, Kuala Lumpur, Malaysia. The mice were housed at the Physiology Lab Animal Experimentation Room, which was maintained at 22–24 °C with a 12 h dark/light cycle, fed a commercial lab animal pellet and provided water ad libitum under specific pathogen-free conditions according to standard guidelines for the care and use of laboratory animals (National Research, 1996). All experiments were conducted under protocols approved by the Animal Experimentation Ethics Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

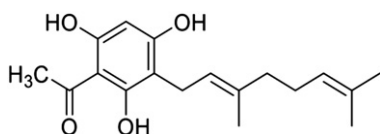


Fig. 1. Chemical structure of 2, 4, 6-trihydroxy-3-geranyl acetophenone (tHGA).

Allergen sensitization and exposure. The mice were sensitized by initial intraperitoneal injection of 0.1 mL phosphate-buffered saline (PBS) containing ovalbumin (OVA; 500 µg/mL, grade 3; Sigma-Aldrich, St. Louis, MO, USA) and alum (10% w/v). The mice were further sensitized with the same reagent at weekly intervals over the next two weeks (total immunizations = 3). One week later (day 21), the mice were exposed to aerosols of allergen (1% OVA in PBS, pH 7.4) for 30 min on three consecutive days (days 21–23). The aerosol exposure was performed in a chamber using an ultrasonic nebulizer (Omron, Japan). Control mice were sensitized with OVA and exposed to aerosols of PBS.

Administration of tHGA. Doses of tHGA (100, 20, 2.0 and 0.2 mg/kg) were prepared in a vehicle consisting of 1% w/v DMSO and 1% w/v Tween 80 in PBS pH 7.2. tHGA was administered intraperitoneally on 3 consecutive days from day 21 to day 23, 1 h prior to OVA aerosolization. Mice administered with tHGA appeared healthy, showed regular weight gain and activity levels similar to control mice, and had no ulceration at the injection sites. Zileuton (30 mg/kg) was used as a drug control, prepared in the same vehicle and administered through the same route. Our preliminary experiments had demonstrated that the vehicle used had no significant effect upon airway inflammation and mediator secretion and therefore mice sensitized and exposed to OVA were treated with the vehicle alone.

Bronchoalveolar lavage and cell counts. Mice were sacrificed on day 26 (three days after the final challenge dose of OVA). Bronchoalveolar lavage fluid (BALF) was obtained by flushing both lung lobes with 0.9 mL cold PBS pH 7.2. Briefly, the trachea of the animals was cannulated with a 22G feeding needle and tied with a surgical thread. The lungs were repeatedly flushed with cold PBS four times and the BALF was centrifuged at 400 g for 10 min at 4 °C. The supernatant was kept at –80 °C prior to assay. The cell pellet was resuspended in PBS and cytospins were prepared on a Hettich centrifuge with cytospin adaptors. Smears were air-dried overnight and stained with Wright's stain for differential cell counts. Total cell counts were performed on the remaining cell suspension whereby an equal volume was mixed with trypan blue and counted in a hemocytometer.

Immunoassay of BALF. The concentrations of interleukin (IL)-4, IL-5 and IL-13 were determined by immunoassay kits according to the manufacturer's instructions. The kits used were Mouse IL-4 and IL-5 ELISA Sets (BD Pharmingen, San Diego, CA, USA, and Cat. No. 555232 & 555236) and Mouse DuoSet IL-13 (R&D Systems, Cat. No. DY413). Concentrations of cysLTs were assayed with an enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI, USA; Cat. No. 520501) according to the manufacturer's instructions.

Lung histopathology. Lungs were instilled with 10% buffered formalin, removed and fixed in the same solution. After paraffin embedding, sections for microscopy were stained with hematoxylin and eosin (H&E) and periodic acid schiff (PAS). Airway inflammation was quantified in the peribronchiol and perivascular region of the airways. Average numbers of inflammatory cells were determined by dividing the total number of inflammatory cells in peribronchiol and perivascular regions with the total number of airways of each section. Goblet cell hyperplasia was determined by enumerating the number of PAS-positive cells in the airways. Average number of goblet cells was determined by dividing the total number of goblet cells in the airways with total number of airways per slide. Six sections were counted for each animal.

Assay of serum IgE. Immediately following the sacrifice blood was drawn through cardiac puncture using a 26G needle. Serum was subjected to an ELISA to determine concentrations of total serum IgE

(Mouse IgE ELISA Set; Cat. No. 555248; BD Pharmingen, San Diego, CA, USA).

Measurement of airway hyperresponsiveness (AHR). The responsiveness of mice to increasing concentrations of aerosolized methacholine was measured for 24 h (day 24) after the last challenge using whole body plethysmography (Buxco Electronics, Inc. Troy, NY). The main indicator of airflow obstruction, enhanced pause (Penh), which shows strong correlation with the airway resistance examined by standard evaluation methods, was calculated from the box waveform (Hamelmann et al., 1997). Aerosolized saline or methacholine was nebulized in increasing concentrations (6, 12, 25, 50 mg/mL) for 1 min, and Penh measurements were taken for 5 min after each dose. Penh values for the 5 min intervals were averaged and used to compare results across treatment groups and individual mice.

Statistical analysis. Data were analyzed using one-way ANOVA. Significant differences between experimental treatments were tested with Dunnett's post hoc test. All data is expressed as the mean $\pm$ S.E.M. of ten mice per group. Differences are considered significant when $P < 0.05$.

Results

Effects of tHGA on BALF cell counts

OVA-sensitized and -exposed mice showed increased total cells counts in BALF compared with control mice sensitized with OVA and exposed to PBS. These increases were due to significant increases in eosinophil numbers only (Fig. 2). Lymphocytes, macrophages and neutrophils remained at normal levels despite induction of airway inflammation (data not shown). tHGA (100, 20 and 2 mg/kg) treatment significantly decreased the number of eosinophils and consequently total cell counts.

tHGA reduces Th2 cytokine and cysLT concentrations in BALF

To investigate the effects of tHGA upon secretion of Th2 cytokines and cysLT, we measured the concentrations of IL-4, IL-5, IL-13 and cysLT in BALF by immunoassay (Fig. 3). OVA sensitization and exposure induced significant increases in the concentration of all cytokines and cysLT in BALF. tHGA treatment (100, 20 and 2 mg/kg) significantly reduced concentrations back to baseline values. tHGA at 0.2 mg/kg failed to exert any effects.

tHGA reduces total serum IgE concentrations

Concentrations of serum total IgE were increased in OVA-sensitized and -exposed mice. tHGA treatment (100, 20 and 2 mg/kg) caused significant reductions back to baseline levels (Fig. 4).

Effect of tHGA on lung histopathology

Fig. 5 shows representative micrographs of both H&E- and PAS-stained sections of both PBS and OVA exposed mice. While control mice showed robust pathological changes in allergic pulmonary inflammation (eosinophil and mononuclear cell infiltration around airways and vessels and goblet cell hyperplasia), mice treated with tHGA at 100, 20 and 2 mg/kg demonstrated significant attenuation of pathological changes, whereas the lowest dose had no significant effect.

Effects of tHGA on airway hyperresponsiveness to methacholine

Respiratory dysfunction was assessed as an increased Penh in response to increasing doses of methacholine. OVA-sensitized and -

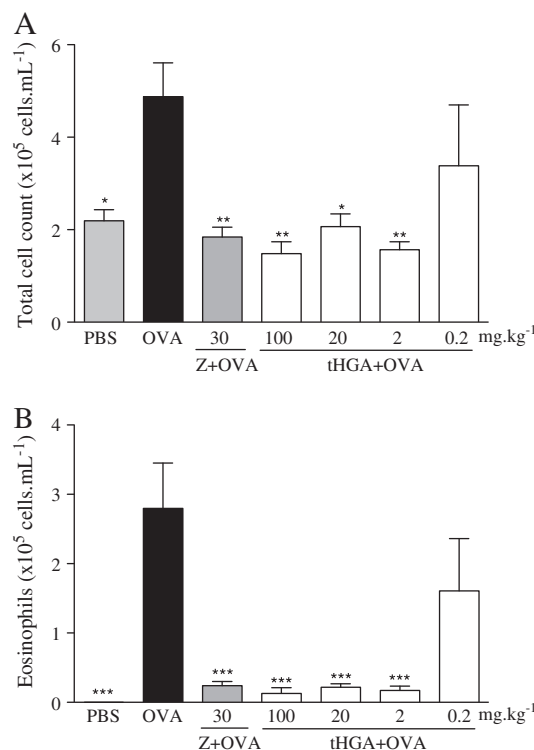


Fig. 2. Effect of tHGA on total cell (A) and eosinophil counts (B) in BALF. Trypan blue stained cell pellets were counted with a hemacytometer and differential cell counts (only eosinophil counts were significantly altered) were obtained by examination of Wright's stained cytosmears. Data are the mean $\pm$ SEM. Significant difference from OVA-sensitized and challenged mice, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Z + OVA = zileuton-treated group.

exposed mice showed altered respiratory function (Fig. 6), which manifested as increased Penh values compared with those seen in control mice sensitized with OVA and challenged with aerosols of PBS. In contrast, OVA-sensitized and -exposed mice treated with tHGA showed a range of dose-dependent changes in respiratory function; doses of 20 and 100 mg/kg as well as zileuton showed significant reduction in respiratory changes at the highest methacholine challenge dose. The lower tHGA doses of 0.2 and 2 mg/kg did not cause significant reduction of Penh but showed a diminishing trend.

Discussion

Leukotrienes are products of the 5-LO pathway and have been demonstrated to cause significant infiltration of lung tissue with inflammatory cells and bronchoconstriction in asthma (Evans et al., 2008; Peters-Golden and Henderson, 2007). Compounds that inhibit the synthesis of LTs such as zileuton, MK-886 and MK-591 have demonstrated efficacy in patients with asthma. Herein, we demonstrate that tHGA, a 5-LO/cysLT inhibitor, attenuates both pulmonary inflammation and hyperresponsiveness in a well established mouse model of allergic airway inflammation. Since zileuton is the only approved 5-LO inhibitor for clinical use we used it as a control in assessment of the various parameters measured. Zileuton is a N-hydroxyurea derivative, and its mechanism of action is likely to involve chelation of the iron at the active site of 5-LO, thereby blocking the enzyme's redox potential (Dahlén, 2006). In contrast to zileuton we have shown that tHGA is not a metal-chelator, indicating a possible competitive (non-redox) mechanism for its interaction with 5-LO (Shaari et al., 2011). Non-redox type LO inhibitors have been shown to have fewer side effects than redox or iron-chelating agents (McMillan and Walker, 1992). In contrast to drugs that target more ubiquitous inflammation pathways, such as the prostaglandin or tumor necrosis factor

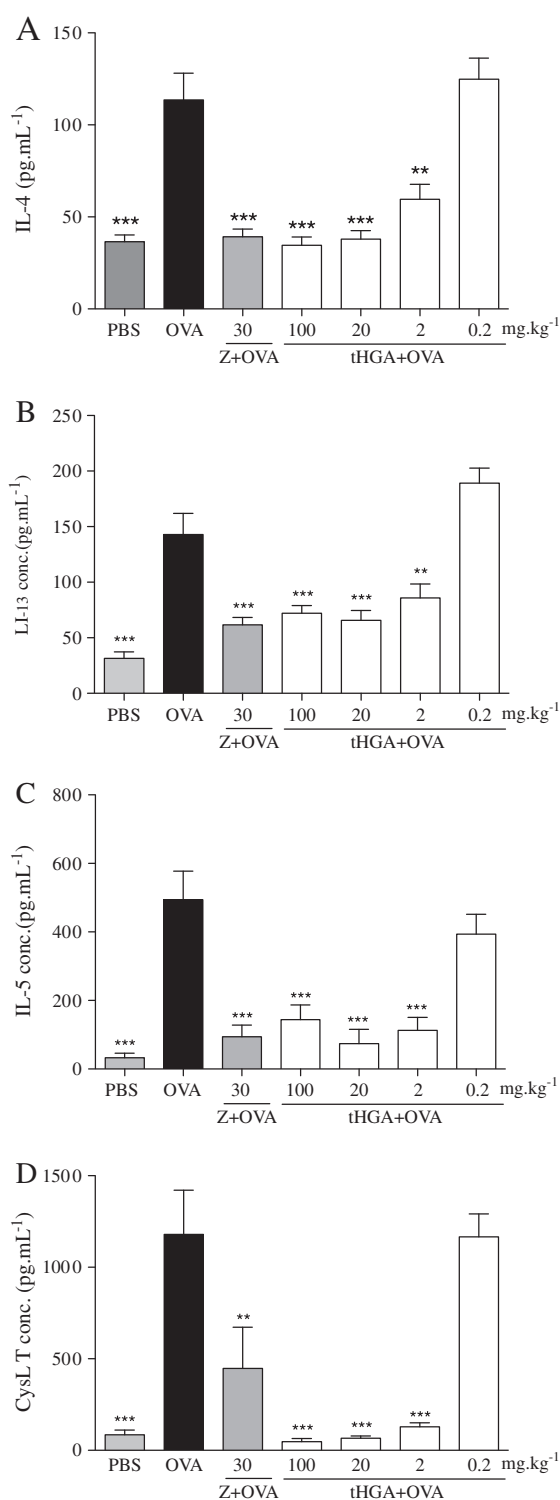


Fig. 3. Effect of tHGA on cytokine and cysLT concentrations in BALF. Concentrations of (A) IL-4, (B) IL-13, (C) IL-5 and (D) cysLT were assayed with ELISA and EIA kits. Data are expressed as means $\pm$ SEM. Significant difference from OVA-sensitized and challenged mice, * P <0.05, ** P <0.01 and *** P <0.001. Z + OVA = zileuton-treated group.

(TNF) pathways, no major side effects have been observed with chronic complete inhibition of leukotriene synthesis, as achieved in MK-591 clinical trials or as in FLAP-deficient or 5-LO-deficient mice (Byrum et al., 1997; Chapman et al., 1994; Chen et al., 1994). Given that leukotrienes are deleterious in both the acute and chronic aspects of respiratory and cardiovascular disease, the therapeutic potential of new anti-inflammatory leukotriene inhibitors is enormous.

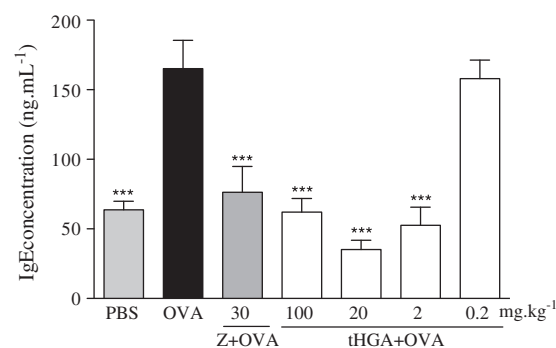


Fig. 4. Concentrations of total serum IgE. Values shown are the mean $\pm$ SEM. Significant difference from OVA-sensitized and challenged mice, *** P <0.001. Z + OVA = zileuton-treated group.

The significant reduction in Th2 cytokines in BALF can be related to the 5-LO/cysLT inhibitory effects of tHGA. Previous studies have demonstrated that cysLT activates the synthesis and secretion of these cytokines. In turn these particular cytokines play an important regulatory role in the development of asthma. One of the important mechanisms by which cysLTs promote an allergic asthmatic phenotype is by amplifying the generation of Th2 cytokines. This has been shown in vitro to occur via stimulation of release of preformed IL-4 (Bandeira-Melo et al., 2002), as well as via transcriptional upregulation of various Th2 cytokines (Tohda et al., 1999). IL-4 plays an important role in up-regulating the expression of endothelial vascular cell adhesion molecule-1 (VCAM-1) that interacts with very late antigen-4 (VLA-4) to promote eosinophil recruitment (Nials and Uddin, 2008; Wills-Karp et al., 1998). IL-4 and IL-13 are reported to contribute to B cell IgE isotype switching (Palmqvist et al., 2007). Furthermore these cytokines were shown to contribute to AHR and allergic inflammation in IL-4- and IL-13-deficient mice (Brusselle et al., 1994; Walter et al., 2001). IL-5 on the other hand regulates eosinophils (Yamaguchi et al., 1988) and the onset of airways hyper-reactivity and lung damage (Kips, 2001). IL-5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model (Foster et al., 1996).

Further evidence that cysLTs regulate Th2 expression is found in animal disease models (Underwood et al., 1996). In a study involving chronic administration of montelukast in a murine model of chronic antigen-induced asthma it was shown that montelukast attenuated the increased levels of IL-4 and IL-13 messenger RNA and protein observed in the lungs and BALF (Henderson et al., 2002). Likewise, montelukast has been shown to attenuate IL-4 messenger RNA synthesis and augment IFN-gamma mRNA expression in an OVA sensitization and challenge model in Brown Norway rats (Nag et al., 2002). These data corroborate findings in which macrophages and T lymphocytes isolated from 5-LO knockout mice demonstrated increased IFN-gamma synthesis (Peters-Golden et al., 2002). Taken together, these findings indicate that cysLTs contribute to the development of an allergic asthmatic phenotype by increasing the ratio of Th2:Th1 cytokines and it is obvious that tHGA can alter this balance through attenuation of Th2 cytokine synthesis at the least.

The effects of tHGA upon tissue eosinophilia and peripheral IgE concentrations are also explained through inhibition of cysLT synthesis. Apart from their indirect actions on eosinophils mediated by enhanced Th2 cytokine generation, cysLTs directly promote recruitment (Spada et al., 1994) and inhibit apoptosis of eosinophils (Lee et al., 2000). Furthermore LTD4 has been shown to enhance the adhesive properties of eosinophils through increased expression of the adhesion molecules CD11b and CD18, thus causing greater adhesion to intercellular adhesion molecule-1 (ICAM-1) (Nagata et al., 2002). Furthermore LTD4 was also shown to regulate the growth of eosinophilic precursors from bone marrow or peripheral blood following activation

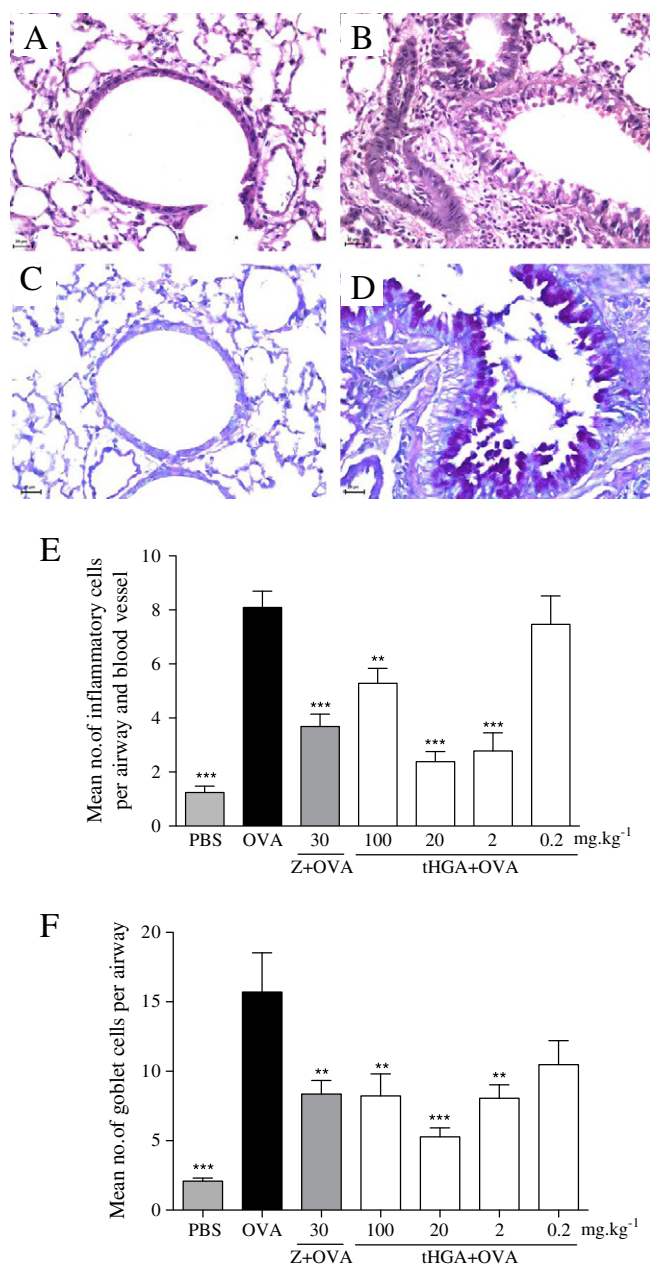


Fig. 5. Airway inflammation in OVA-treated mice. Lung tissue (upper and lower lobes of left lung) of PBS-treated control mice (A, C) and OVA-sensitized/challenged mice (B, D) was fixed in 10% buffered formalin and stained with hematoxylin and eosin (A, B) or Periodic Acid Schiff (C, D) and examined by light microscopy. Bar = 100 μ m. Quantitative analysis of inflammatory cell infiltration (E) and goblet cell metaplasia (F). Data are expressed as mean $\pm$ SEM. Significance difference from OVA-sensitized and challenged mice, ** $P < 0.01$, *** $P < 0.001$. Z + OVA = zileuton-treated group.

with either IL-5 or granulocyte macrophage colony-stimulating factor (GM-CSF) (Braccioni et al., 2002). These latter results may explain the consistent finding that anti-leukotriene drugs attenuate eosinophil numbers not only in the lungs, but also in the peripheral blood (Pizzichini et al., 1999).

Although significant effects of tHGA upon AHR were noted, we do acknowledge the limitations of whole body plethysmography in the assessment of AHR. The use of this technique as a sole indicator of AHR still remains under intense debate and in many instances confirmatory tests that employ invasive techniques to measure dynamic compliance and resistance have been advocated. Nevertheless there have also been arguments as to the validity of invasive methods since they may alter baseline breathing patterns. Further investigations

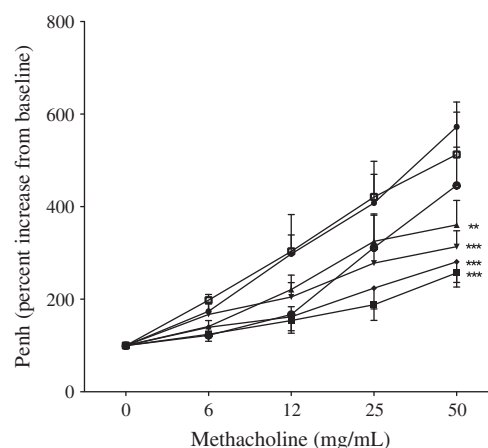


Fig. 6. Effect of tHGA treatment on airway hyperresponsiveness to aerosolized methacholine. —●— OVA-sensitized and challenged mice, —■— PBS controls, —▲— Zileuton 30 mg/kg, —▼— tHGA 100 mg/kg, —◆— tHGA 20 mg/kg, —●— tHGA 2 mg/kg, and —■— tHGA 0.2 mg/kg. Values are expressed as mean $\pm$ SEM. Significant differences compared to OVA-sensitized group, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

upon the pharmacology of tHGA will employ both non-invasive and invasive methods to gain a more definitive insight of the effect upon AHR. It is also important to note that the present findings, in which all markers associated with inflammation were reduced following treatment, do not explain the exact mechanism of action and our discussion is speculative based on existing literature. It is also possible that lower cysLTs in the tHGA groups are not measures of inhibitor activity, but general markers of inflammation that track with the others. We are currently investigating the mechanism of action of tHGA through dissection of major proinflammatory pathways in both cellular and animal models.

In conclusion, we have demonstrated that our previous findings regarding tHGA's inhibitory effects upon cysLT synthesis *in vitro* can be extended and confirmed in a standard acute model of murine allergic airway inflammation. Doses of 2 mg/kg and above are effective in alleviating all common parameters of pulmonary dysfunction in the above model and it is possible that these effects are due solely to the inhibition of 5-LO enzyme activity and subsequent synthesis of cysLTs. Further evaluation of tHGA in terms of its effects upon other proinflammatory mediators and their signaling pathways including evaluation of tHGA in both inhalatory and oral dosage forms is envisioned. Current experiments are evaluating effects of tHGA upon tissue remodeling in chronic models that better mimic human asthma.

Conflict of interest

The authors do not have any conflict of interest.

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References

- Abas, F., Lajis, N.H., Israf, D.A., et al., 2006. Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. *Food Chem.* 95, 566–573.
- Bandeira-Melo, C., Woods, L., Phofofo, M., et al., 2002. Intracrine cysteinyl leukotriene receptor-mediated signaling of eosinophil vesicular transport-mediated interleukin-4 secretion. *J. Exp. Med.* 196, 841–850.
- Barnes, P.J., 2008. Immunology of asthma and chronic obstructive pulmonary disease. *Nat. Rev. Immunol.* 8, 183–192.

- Barnes, P.J., 2010. New therapies for asthma: is there any progress? *Trends Pharmacol. Sci.* 31, 335–343.
- Braccioni, F., Dorman, S., O'Byrne, P., et al., 2002. The effect of cysteinyl leukotrienes on growth of eosinophil progenitors from peripheral blood and bone marrow of atopic subjects. *J. Allergy Clin. Immunol.* 110, 96–101.
- Brusselle, G.G., Kips, J.C., Tavernier, J.H., et al., 1994. Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin. Exp. Allergy* 24, 73–80.
- Busse, W., Kraft, M., 2005. Cysteinyl leukotrienes in allergic inflammation: strategic target for therapy. *Chest* 127, 1312–1326.
- Byrum, R.S., Goulet, J.L., Griffiths, R.J., Koller, B.H., 1997. Role of the 5-lipoxygenase-activating protein (FLAP) in murine acute inflammatory responses. *J. Exp. Med.* 185, 1065–1075.
- Chapman, K.R., Friedman, B.S., Shingo, S., et al., 1994. The efficacy of an oral inhibitor of leukotriene synthesis (MK-0591) in asthmatics treated with inhaled steroids. *Am. J. Respir. Crit. Care Med.* 149, A215.
- Chen, X.S., Sheltzer, J.R., Johnson, E.N., Funk, C.D., 1994. Role of leukotrienes revealed by targeted disruption of the 5-lipoxygenase gene. *Nature* 372, 179–182.
- Dahlén, S.E., 2006. Treatment of asthma with antileukotrienes: first line or last resort therapy? *Eur. J. Pharmacol.* 533, 40–56.
- Dahlen, B., Nizankowska, E., Szczeklik, A., et al., 1998. Benefits from adding the 5-lipoxygenase inhibitor zileuton to conventional therapy in aspirin-intolerant asthmatics. *Am. J. Respir. Crit. Care Med.* 157, 1187–1194.
- Dorsch, W., Wagner, H., Neszmelyi, A., Stuppner, H., 1992. Acetophenones: highly active antiasthmatic compounds of plant origin. *Int. Arch. Allergy Immunol.* 99, 493–495.
- Evans, J.F., Ferguson, A.D., Mosley, R.T., Hutchinson, J.H., 2008. What's all the FLAP about?: 5-lipoxygenase-activating protein inhibitors for inflammatory diseases. *Trends Pharmacol. Sci.* 29, 72–78.
- Foster, P.S., Hogan, S.P., Ramsay, A.J., et al., 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183, 195–201.
- Gadamasetti, K., Braish, T., 2007. *Process Chemistry in the Pharmaceutical Industry*, Volume 2. CRC Press, pp. 142–145.
- Hamelmann, E., Schwarze, J., Takeda, K., et al., 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* 156, 766–775.
- Hamid, Q., Tulic, M., 2009. Immunobiology of asthma. *Annu. Rev. Physiol.* 71, 489–507.
- Henderson, W.J., Tang, L., Chu, S., et al., 2002. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am. J. Respir. Crit. Care Med.* 165, 108–116.
- Kips, J.C., 2001. Cytokines in asthma. *Eur. Respir. J. Suppl.* 34, 24–33.
- Lee, E., Robertson, T., Smith, J., et al., 2000. Leukotriene receptor antagonists and synthesis inhibitors reverse survival in eosinophils of asthmatic individuals. *Am. J. Respir. Crit. Care Med.* 161, 1881–1886.
- McMillan, R.M., Walker, E.R.H., 1992. Designing therapeutically effective 5-lipoxygenase inhibitors. *Trends Pharmacol. Sci.* 13, 323.
- Montuschi, P., Peters-Golden, M.L., 2010. Leukotriene modifiers for asthma treatment. *Clin. Exp. Allergy* 40, 1732–1741.
- Nag, S., Lamkhioed, B., Renzi, P., 2002. Interleukin-2-induced increased airway responsiveness and lung Th2 cytokine expression occur after antigen challenge through the leukotriene pathway. *Am. J. Respir. Crit. Care Med.* 165, 1540–1545.
- Nagata, M., Saito, K., Tsuchiya, K., et al., 2002. Leukotriene D4 upregulates eosinophil adhesion via the cysteinyl leukotriene 1 receptor. *J. Allergy Clin. Immunol.* 109, 676–680.
- National Research Council, 1996. *Institute of Laboratory Animal Research, Commission on Life Science. Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington DC.
- Nials, A.T., Uddin, S., 2008. Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis. Model. Mech.* 1, 213–220.
- Palmqvist, C., Wardlaw, A.J., Bradding, P., 2007. Chemokines and their receptors as potential targets for the treatment of asthma. *Br. J. Pharmacol.* 151, 725–736.
- Peters-Golden, M., Henderson, W.R., 2007. Leukotrienes. *N. Engl. J. Med.* 357, 1841–1854.
- Peters-Golden, M., Bailie, M., Marshall, T., et al., 2002. Protection from pulmonary fibrosis in leukotriene-deficient mice. *Am. J. Respir. Crit. Care Med.* 165, 229–235.
- Pizzichini, E., Leff, J., Reiss, T., et al., 1999. Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. *Eur. Respir. J.* 14, 12–18.
- Shaari, K., Safri, S., Abas, F., Lajis, N.H., Israf, D.A., 2006. A geranylacetophenone from the leaves of *Melicope ptelefolia*. *Nat. Prod. Res.* 20, 415–419.
- Shaari, K., Suppaiah, V., Lam, K.W., et al., 2011. Bioassay-guided identification of an anti-inflammatory prenylated acylphloroglucinol from *Melicope ptelefolia* and molecular insights into its interaction with 5-lipoxygenase. *Bioorg. Med. Chem.* 19 (21), 6340–6347.
- Sittig, M., 1988. *Pharmaceutical Manufacturing Encyclopedia*. William Andrew Publishing/Noyes, p. 39,177.
- Spada, C., Nieves, A., Krauss, A., et al., 1994. Comparison of leukotriene B4 and D4 effects on human eosinophil and neutrophil motility in vitro. *J. Leukoc. Biol.* 55, 183–191.
- Tohda, Y., Nakahara, H., Kubo, H., et al., 1999. Effects of ONO-1078 (pranlukast) on cytokine production in peripheral blood mononuclear cells of patients with bronchial asthma. *Clin. Exp. Allergy* 29, 1532–1536.
- Underwood, D., Osborn, R., Newsholme, S., et al., 1996. Persistent airway eosinophilia after leukotriene (LT) D4 administration in the guinea pig: modulation by the LTD4 receptor antagonist, pranlukast, or an interleukin-5 monoclonal antibody. *Am. J. Respir. Crit. Care Med.* 154, 850–857.
- Walter, D.M., McIntire, J.J., Berry, G., et al., 2001. Critical Role for IL-13 in the development of allergen-induced airway hyperreactivity. *J. Immunol.* 167, 4668–4675.
- Wills-Karp, M., Luyimbazi, J., Xu, X., et al., 1998. Interleukin 13: central mediator of allergic asthma. *Science* 282, 2258–2261.
- Yamaguchi, Y., Suda, T., Suda, J., et al., 1988. Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J. Exp. Med.* 167, 43–56.