European Journal of Pharmacology 797 (2017) 53–64

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

An orally active geranyl acetophenone attenuates airway remodeling in a murine model of chronic asthma

Yu Zhao Lee[a], Khozirah Shaari[b], Manraj Singh Cheema[a], Chau Ling Tham[a], Mohd Roslan Sulaiman[a], Daud Ahmad Israf[a,*]

[a] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ARTICLE INFO

Keywords:
Geranyl acetophenone
Airway remodeling
Subepithelial fibrosis
Airway inflammation
Cytokine
Chronic asthma

ABSTRACT

2,4,6-Trihydroxy-3-geranyl acetophenone (tHGA) is a synthetic compound that is naturally found in *Melicope ptelefolia*. We had previously demonstrated that parenteral administration of tHGA reduces pulmonary inflammation in OVA-sensitized mice. In this study, we evaluated the effect of orally administered tHGA upon airway remodeling in a murine model of chronic asthma. Female BALB/C mice were sensitized intraperitoneally with ovalbumin (OVA) on day 0, 7 and 14, followed by aerosolized 1% OVA 3 times per week for 6 weeks. Control groups were sensitized with saline. OVA sensitized animals were either treated orally with vehicle (saline with 1% DMSO and Tween 80), tHGA (80, 40, 20 mg/kg) or zileuton (30 mg/kg) 1 h prior to each aerosolized OVA sensitization. On day 61, mice underwent methacholine challenge to determine airway hyperresponsiveness prior to collection of bronchoalveolar lavage (BAL) fluid and lung samples. BAL fluid inflammatory cell counts and cytokine concentrations were evaluated while histological analysis and extracellular matrix protein concentrations were determined on collected lung samples. Oral tHGA treatment attenuated airway hyperresponsiveness and inhibited airway remodeling in a dose-dependent fashion. tHGA's effect on airway remodeling could be attributed to the reduction of inflammatory cell infiltration and decreased expression of cytokines associated with airway remodeling. Oral administration of tHGA attenuates airway hyperresponsiveness and remodeling in OVA-induced BALB/c mice. tHGA is an interesting compound that should be evaluated further for its possible role as an alternative non-steroidal pharmacological approach in the management of asthma.

1. Introduction

Asthma is characterized as a chronic respiratory disease involving airway inflammation and hyperresponsiveness (AHR) (Barnes, 2008). It is estimated that 300 million people worldwide suffer from asthma (Barnes, 2010) with high prevalence amongst children (Pedersen et al., 2011). Increased concentrations of pulmonary inflammatory mediators as a consequence of persistent inflammation are associated with airway structural changes, termed airway remodeling (Manso et al., 2012). Airway remodeling includes the disruption of epithelial integrity, subepithelial fibrosis and collagen deposition, smooth muscle cell hyperplasia and hypertrophy, increased mucus production and submucosal glands and airway wall thickening (Bergeron et al., 2010). In particular, abnormally high amounts of collagen, fibronectin and tenascin-C have been reported to be deposited in the lamina reticularis beneath the airway epithelium (Takayama et al., 2006). The pathophysiology of airway remodeling involves interactions between a vast array of cells namely eosinophils, T-lymphocytes, mast cells, smooth muscle cells, epithelial cells and fibroblasts (Girodet et al., 2011).

Recent studies have suggested corticosteroids, the gold standard in asthma treatment, as being ineffective in reversing airway structural changes (Doerner and Zuraw, 2009; Royce and Tang, 2009). Moreover, in severe asthma patients, AHR is not abrogated subsequent to corticosteroid treatment (Baraket et al., 2012) as supported by several earlier pioneering studies (Lundgren et al., 1988; Juniper et al., 1990). Currently, there are no targeted therapies for reversing airway structural changes in asthma (Pascual and Peters, 2005).

The geranyl acetophenone, 2,4,6-trihydroxy-3-geranyl acetophenone (tHGA), contains a bioactive principle of the phloroglucinol structure-core (Shaari et al., 2006). The acylphloroglucinol group naturally found in many natural products exhibits many interesting biological properties (Chung, 1995). Our earlier studies revealed that

* Corresponding author.
E-mail address: daudaia@upm.edu.my (D.A. Israf).

http://dx.doi.org/10.1016/j.ejphar.2017.01.011
Received 30 August 2016; Received in revised form 5 January 2017; Accepted 11 January 2017
Available online 12 January 2017

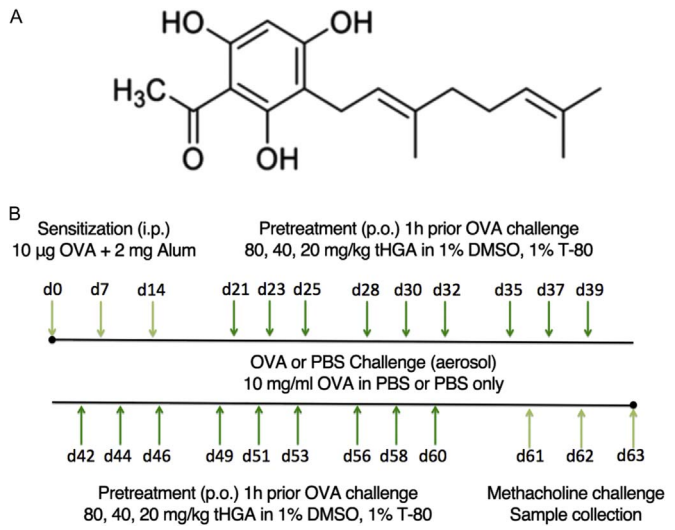

Fig. 1. (A) Chemical structure of 2,4,6-trihydroxy-3-geranyl acetophenone (tHGA). (B) Experimental design: Mice were sensitized with 10 µg OVA absorbed in 2 mg Alum at day 0, 7 and 14. After day 14, mice were sensitized with aerosolized OVA or PBS thrice per week up to 6 weeks. Treated mice were administered vehicle or different doses of tHGA or zileuton (drug control) orally 1 h prior every OVA sensitization. Mice were challenged with methacholine 72 h after the last OVA sensitization to analyze airway hyperresponsiveness and killed for BAL fluid and lung sample collection.

tHGA was able to exert a dose-dependent inhibition of 5-lipoxygenase (5-LOX), cyclooxygenase (COX) activity and cysteinyl leukotriene (CysLT) secretion in LPS-induced macrophage cell lines (Shaari et al., 2011). We also demonstrated that synthetic tHGA exerted a dose-dependent inhibitory effect upon allergic airway inflammation in OVA-induced BALB/c mice following intraperitoneal administration (Ismail et al., 2012).

In this study, we demonstrate synthetic tHGA to be orally active in attenuating airway remodeling in a chronic murine model of asthma. Our findings provide further questions as to the molecular pathophysiological events altered by tHGA and the possibility of its development as a new non-steroidal oral lead for the management of allergic asthma.

2. Materials and methods

2.1. tHGA synthesis

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. ^{1}H NMR (CD_3OD) δ_H 1.58 (3H, s, Me), 1.63 (3H, s, Me), 1.76 (3H, 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) (Fig. 1A). tHGA purity was more than 99%.

2.2. Animals

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia. All studies involving animals are reported in accordance with the ARRIVE guidelines (Kilkenny et al., 2010; McGrath et al., 2010). One hundred and five female BALB/c mice purchased from the Institute for Medical Research (IMR), Malaysia (6–8 weeks old; 20–25 g) were divided into 6 groups (n=15): The Normal (N) group was induced with phosphate buffered saline (PBS) while Control (C), Vehicle (VEH), 80 mg/kg tHGA treated (80), 40 mg/kg tHGA treated (40), 20 mg/kg tHGA treated (20) and 30 mg/kg Zileuton (ZIL) treated groups were induced with aerosolized ovalbumin (OVA). All animals were kept under 12 h light-dark cycle with access to standard chow and water *ad libitum* according to standard guidelines for the care and use of laboratory animals (National Research Council, 1996).

2.3. Chronic allergic asthma induction

The immunization, treatment and sampling schedule is shown in Fig. 1B. Mice were sensitized initially with 0.1 ml PBS containing 100 µg/ml OVA (Sigma Aldrich, USA) and 10% w/v alum (Sigma Aldrich, USA) via intraperitoneal injection on day 0, 7 and 14. Commencing on day 21, the mice were exposed to aerosols of 1% OVA in PBS for 30 min on three days per week for a total of 6 consecutive weeks. Aerosolization was conducted in a perspex chamber attached to an ultrasonic nebulizer (Omron, Japan). Control mice were immunized with PBS without OVA.

2.4. tHGA treatment

Three different doses of tHGA (80 mg/kg, 40 mg/kg and 20 mg/kg) were tested. Doses were prepared in a vehicle consisting of 1% dimethyl sulfoxide (DMSO) and 1% Tween-80 in PBS. tHGA was administered via oral gavage from day 21 to day 60 1 h prior to each OVA aerosolized sensitization. Zileuton at a dose of 30 mg/kg was prepared in the same vehicle and also administered orally. Vehicle controls were given the vehicle (1% DMSO and 1% Tween-80 in PBS) orally.

2.5. Airway hyperresponsiveness (AHR) analysis

Airway resistance and dynamic compliance was measured by the invasive method according to Buxco FinePointe series RC system protocol (Buxco Research Systems, USA). Mice were anaesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine. When appropriate depth of anaesthesia was achieved as monitored by the loss of righting and pinch toe reflex, tracheotomy was performed and mice were intubated endotracheally with an 18 G cannula. Mechanical ventilation (Buxco Research Systems, USA) was provided at a rate of 150 breaths per min with tidal volume of 10 ml/kg. Intubated mice were connected to the Buxco RC system to be delivered nebulized PBS and methacholine doses of 0.5–8 mg/ml. Airway resistance and lung dynamic compliance were measured at 60 s after methacholine administration. Results were expressed as % change from the baseline values [(baseline value − value at each methacholine dose)/baseline value x 100].

2.6. Bronchoalveolar lavage and inflammatory cell counts

Bronchoalveolar lavage (BAL) fluid was collected by cannulating the exposed trachea with a 22 G feeding needle and tied with surgical thread. The lungs were repeatedly flushed four times with 0.9 ml cold PBS and centrifuged at 400g for 10 min at 4 °C. The supernatants were kept at −80 °C for cytokine assay while cell pellets were resuspended in PBS. Total cell counts were performed with a haemacytometer on cell suspensions with trypan blue staining. Remaining cell suspensions were used to prepare cytosmears. Smears were air-dried overnight prior to staining with Wright's stain for differential cell counts. A total of 300 cells were enumerated on 3 different random locations of each smear under 1000x magnification to determine the percentage of neutrophils, lymphocytes, eosinophils and macrophages.

2.7. Lung tissue histology

The left lung lobe was removed and fixed in 10% buffered formalin,