Journal of Ethnopharmacology 192 (2016) 248–255

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Barrier protective effects of 2,4,6-trihydroxy-3-geranyl acetophenone on lipopolysaccharides-stimulated inflammatory responses in human umbilical vein endothelial cells

Yi Joong Chong [a], Nazmi Firdaus Musa [a], Ng Chean Hui [b], Khozirah Shaari [b], Daud Ahmad Israf [a], Chau Ling Tham [a,*]

[a] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43300, Malaysia
[b] Faculty of Science, Universiti Putra Malaysia, Serdang 43300, Malaysia

ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form
6 June 2016
Accepted 9 July 2016
Available online 9 July 2016

Keywords:
2,4,6-trihydroxy-3-geranyl acetophenone (tHGA)
Melicope ptelefolia
Phloroglucinol
Barrier protective effect
Endothelial dysfunction
Lipopolysaccharides

ABSTRACT

Pharmocological relevance: 2,4,6-trihydroxy-3-geranyl acetophenone (tHGA), is a phloroglucinol compound found naturally in *Melicope ptelefolia. Melicope ptelefolia* has been used traditionally for centuries as natural remedy for wound infections and inflammatory diseases.
Aim of the study: Endothelial barrier dysfunction is a pathological hallmark of many diseases and can be caused by lipopolysaccharides (LPS) stimulation. Therefore, this study aims to investigate the possible barrier protective effects of tHGA upon LPS-stimulated inflammatory responses in human umbilical vein endothelial cells (HUVECs).
Materials and methods: HUVECs were pretreated with tHGA prior to LPS stimulation, where inflammatory parameters including permeability, monocyte adhesion and migration, and release of pro-inflammatory mediators were examined. Additionally, the effect of tHGA on F-actin rearrangement and adhesion protein expression of LPS-stimulated HUVECs was evaluated.
Results: It was found that pretreatment with tHGA inhibited monocyte adhesion and transendothelial migration, reduced endothelial hyperpermeability and secretion of prostaglandin E_2 (PGE_2). Additionally, tHGA inhibited cytoskeletal rearrangement and adhesion protein expression on LPS-stimulated HUVECs.
Conclusion: As the regulation of endothelial barrier dysfunction can be one of the therapeutic strategies to improve the outcome of inflammation, tHGA may be able to preserve vascular barrier integrity of endothelial cells following LPS-stimulated dysfunction, thereby endorsing its potential usefulness in vascular inflammatory diseases.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The endothelium is a functional barrier that lines the blood vessel, in which this barrier plays major roles in homeostasis including coagulation, fibrinolysis and immune responses. During inflammation, the endothelium is activated and releases a cascade of inflammatory mediators such as MCP-1 and IL-8 for recruitment of monocytes and neutrophils (Gavard, 2009; Zhang et al., 2011). Additionally, the endothelium also upregulates the expression of adhesion molecules and dissociates the tight junctional proteins to facilitate diapedesis and fluid permeation (Gavard, 2009; Harmey et al., 2002). In between the alteration of protein expression, the endothelium permeability is increased, and the recruitment of circulating leukocytes is heightened. Therefore this initiates the vascular barrier dysfunction cascade (Thiemermann et al., 1995). This is deleterious to the host as such hyperinflammatory activity may cause systemic shock and tissue damage, both of which may be fatal. Therefore, intervention on such severe inflammatory state is necessary.

LPS is found on the membrane of gram-negative bacteria, and it is frequently found in high concentration in patients who are suffering from septicemia and endotoxemia (Rivers et al., 2001). Activation of TLR4 by LPS will lead to downstream upregulation of various pro-inflammatory signaling molecules that play important role in propagating inflammatory responses such as NF-κB (Hoefen and Berk, 2002) and MAP kinases (Sawa et al., 2008). In high concentrations, LPS may lead to fatal conditions such as multiple organ dysfunction and septic shock (Dellinger et al., 2004), both of which are linked to vascular barrier disruption (Angus and Van der Poll, 2013; Balk, 2000). It has been suggested that therapeutic approaches aimed at maintaining vascular barrier integrity constitute a potential approach in the management of sepsis

* Corresponding author.
E-mail address: chauling@upm.edu.my (C.L. Tham).

http://dx.doi.org/10.1016/j.jep.2016.07.032
0378-8741/© 2016 Elsevier Ireland Ltd. All rights reserved.

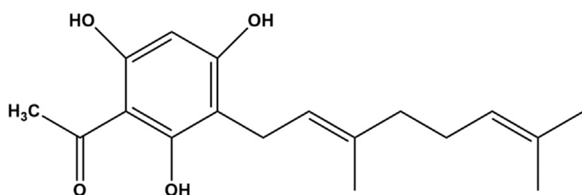

Fig. 1. Structure of 2,4,6-Trihydroxy-3-Geranyl Acetophenone.

(Dellinger et al., 2004; Goldenberg et al., 2011).

Melicope ptelefolia (Champ. ex Benth.) is a shrub growing in many areas of the Southeast Asia commonly used as a traditional Chinese medicine for the treatment of inflammatory reactions including abscesses, wound infection, eczema and dermatitis (Jiangsu New Medical School, 1986). In Malaysia, the shoots and young leaves of *Melicope ptelefolia* are believed to be high in nutritional and medicinal values and amongst the popular 'ulam' or green salad favored by the local Malay population. Our research group had successfully isolated a new natural product namely 2,4,6-trihydroxy-3-geranyl acetophenone (tHGA) from *Melicope ptelefolia* (Shaari et al., 2006). tHGA was identified as one of the three marker compounds that are found abundantly in young leaves but not in mature leaves of *Melicope ptelefolia* (Shuib et al., 2011). tHGA is a drug-like compound containing a phloroglucinol structural-core as the bioactive principle (Ng et al., 2006; Shaari et al., 2006) (Fig. 1).

Our research group previously reported significant antioxidant and nitric oxide inhibitory activities of *Melicope ptelefolia,* suggesting its potential anti-inflammatory properties (Abas et al., 2006). Our recent work also demonstrated that tHGA prevents airway inflammation in an ovalbumin-induced murine model of acute allergic asthma. The airway hyperresponsiveness, pulmonary cellular infiltration, goblet cell metaplasia, cytokine and cysteinyl leukotriene secretion and systemic IgE concentrations were all reduced following systemic treatment of tHGA (Ismail et al., 2012). Bioassay studies have shown that tHGA exerts dose-dependent inhibition against 5-lipoxygenase (5-LOX) and both cyclooxygenase (COX) isoforms (Shaari et al., 2011), both of which play an important role in mediating LPS-stimulated inflammation. Virdis et al., revealed that inhibition of COX-2 improved the outcome of LPS-stimulated endothelial dysfunction due to reduction of pro-inflammatory signal transduction and reactive oxygen species (Virdis et al., 2005), therefore giving insights on the potential of tHGA on inhibiting LPS-stimulated vascular dysfunction. There have not been any attempts to evaluate the use of tHGA as an adjunct therapeutic in sepsis or LPS-associated endothelial dysfunction. Hence, this study was carried to evaluate the barrier protective effect of tHGA upon LPS-stimulated endothelial dysfunction.

2. Materials and methods

2.1. Reagents

Lipopolysaccharide (O111: B5), Evans Blue, Albumin and BCECF-AM were purchased from Sigma (St Louis, MO, USA). Sodium Fluorescein (Na-F), dimethyl sulfoxide and 3-(4,5-Dimethylthiazol-2-yl)−2-Diphenyltetrazolium Bromide (MTT) were purchased from Amresco (Solon, OH, USA). Water soluble tetrazolium-1 (WST-1) was purchased from Millipore (Billerica, MA, USA). Cell culture inserts and Collagen I was purchased from BD Biosciences (Franklin Lake, NJ, USA). Alexa Fluor-488 conjugated phalloidin was purchased from Invitrogen (Carlsbad, CA, USA). Mouse Anti-Human ICAM-1 (sc107), Mouse Anti-Human VCAM-1 (sc-13160) and goat anti-mouse IgG (sc-2005) was purchased fron Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Synthesis of tHGA

tHGA was synthesized according to previously described method (Ismail et al., 2012). 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_H1.58 (3 h, s, Me), 1.63 (3 h, s, Me), 1.76 (3 h, s, Me), 2.64, (3 h, s, COMe), 1.96 (2 h, q, J=7.5 Hz), 2.06 (2 h, m), 3.21 (2 h, d, J=6.5 Hz), 5.08 (1 h, t, J=7 Hz), 5.20 (1 h, t, J=6.5 Hz), 5.92 (1 h, s, ArH); IR (KBr) ν_{max} 3405, 1627 cm-1; EIMS m/z (%) [M]+304 (38), 289 (3), 261 (9), 235 (25), 181 (100).

2.3. Cell culture

Human Umbilical Vein Endothelial Cells (HUVECs) were purchased from Cascade Biologics. The cells were maintained in Endogro-LS Complete Media Kit (Merck Milipore) at 37 °C in a humidified incubator with 5% CO_2. U937, a human monocytic cell line, was purchased from ATCC. The cells were maintained at a density of 2×10^5–1×10^6 cells/mL in RPMI 1640 supplemented with 10% FBS. For this study, only HUVECs between passage 3 to passage 5 were used.

2.4. MTT cell viability assay

Effect of tHGA on viability of cells were examined using MTT reagent. HUVECs were grown at a concentration of 4×10^3 HUVECs/well. The seeding concentration is determined based on MTT optimization assay whereby the optimal seeding range of HUVECs for MTT is between 1.25×10^3 to 5×10^4 cells/well in 96 well plate (data not shown). On the next day, different concentrations of tHGA with the presence of 1 µg/mL LPS were added into each individual well and co-incubated in a humidified CO_2 incubator for 24 h at 37 °C. MTT (5 mg/mL) was then added followed by incubation in CO_2 incubator at 37 °C of 4 h. Finally, live cells containing formazon salt were then lysed with DMSO, and the amount of formazon salt was determined via measuring OD at 570 nm using microplate reader (Tecan Austria GmbH, Austria).

2.5. Adhesion assay

Monocyte adhesion to HUVECs was performed as described previously with some modification (Tham et al., 2015). Confluent HUVECs in 96 well plates were first pretreated with tHGA or Dexamethasone for 6 h, followed by LPS stimulation for 5 h. Meanwhile, U937 cells were prelabelled with 4 µg/mL BCECF-AM for 1 h immediately prior to co-culture. Following stimulation, HUVECs were washed with PBS, followed by co-cultured with 1×10^5 U937 in 100 µL media for 1 h to allow adhesion of the monocytes to the endothelial monolayer. Non-adherent cells were then washed thrice with PBS, and adhered U937 were lysed with 0.2% Triton-X 100. The fluorescence intensity was quantified at 485-nm excitation and 530-nm emission using a spectrofluorometer (Tecan M200 Infinite, Mannedorf, Switzerland).

Download English Version:

https://daneshyari.com/en/article/2544552

Download Persian Version:

https://daneshyari.com/article/2544552

Daneshyari.com