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Psidium guajava extract inhibits thymus and activation-regulated chemokine (TARC/CCL17) production in human keratinocytes by inducing heme oxygenase-1 and blocking NF-κB and STAT1 activation

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ABSTRACT

Psidium guajava (*P. guajava*) is a food and medicinal plant with antioxidant, anti-inflammatory, and anti-allergic activities that support its traditional uses. The aim of this study was to determine the effects of *P. guajava* ethyl acetate extract (PGEA) on atopic dermatitis and to investigate the possible mechanisms by which PGEA inhibits cytokine-induced Th2 chemokine expression in HaCaT human keratinocyte cells. We found that PGEA suppressed the IFN-γ/TNF-α-co-induced production of thymus and activation-regulated chemokine (TARC) protein and mRNA in HaCaT cells. Additionally, PGEA inhibited the TNF-α/IFN-γ-co-induced activation of NF-κB and STAT1 and increased the expression of heme oxygenase-1 (HO-1) protein and mRNA. HO-1 inhibitor enhanced the suppressive effects of PGEA on TNF-α/IFN-γ-co-induced TARC production and gene expression. Collectively, these data demonstrate that PGEA inhibits chemokine expression in keratinocytes by inducing HO-1 expression and it suggests a possible therapeutic application in atopic dermatitis and other inflammatory skin diseases.

1. Introduction

Atopic dermatitis is a complex eczematous skin disease, accompanied by severe itching, that is affected by both genetic and environmental factors (Lee et al., 2007). Atopic dermatitis is estimated to affect approximately 8–25% of the human population worldwide, and its incidence is increasing (Weston and Howe, 2008). The inflammatory infiltrates in skin lesions consist not only of lymphocytes, but also of macrophages, eosinophils, mast cells, and Langerhans cells (Rudikoff and Lebwohl, 1998; Breuer et al., 2006).

Skin inflammatory processes are highly dependent on Th2 chemokine family (Pivarcsi and Homey, 2005). The chemokines are a superfamily of small cytokines that regulate trafficking of various types of leukocytes (Qi et al., 2009). Thymus and activation-regulated chemokine (TARC/CCL17), a Th2-type CC chemokine, is constitutively expressed in the thymus and is also produced by keratinocytes (Vestergaard et al., 1999), dendritic cells (Imai et al., 1996; Sallusto et al., 1998), endothelial cells (Campbell et al., 1999), bronchial epithelial cells (Sekiya et al., 2000), and fibroblasts (Yu et al., 2002). TARC is a ligand for CCR4, which is predominantly expressed on Th2 lymphocytes, basophils, and natural killer cells (Sallusto

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et al., 1998; Nickel et al., 1999; Godisk et al., 1997). Macrophage-derived chemokine (MDC/CCL22) is closely related to TARC and is constitutively produced by dendritic cells, B cells, macrophages, keratinocytes, and epithelial cells (Hino et al., 2007). Previous studies have demonstrated that serum levels of TARC and MDC are significantly elevated in atopic dermatitis patients and that increased levels of these chemokines correlate with increased severity of disease (Shimada et al., 2004; Hashimoto et al., 2006). Cutaneous T cell-attracting chemokine (CTACK/CCL27) is selectively expressed in skin (Reiss et al., 2001), particularly in epidermal keratinocytes, in which it is constitutively expressed (Kunkel and Butcher, 2002), and may also be involved in various inflammatory skin diseases (Kakinuma et al., 2003). This CC chemokine is a ligand for CCR10 (Kunkel and Butcher, 2002; Ishikawa-Mochizuki et al., 1999).

The exposure of keratinocytes to interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) leads to the abnormal expression of cytokines and chemokines, including TARC; these factors are believed to increase infiltration of monocytes/T cells into the site of inflammation in the skin (Sebastiani et al., 2002). NF-κB is activated when cells are stimulated with TNF-α and IFN-γ, which are both involved in the transcriptional activation of responsive genes (Adcock, 1997). The signal transducers and activators of transcription 1 (STAT1) proteins are also pivotal regulators of the IFN-γ-induced immune response in keratinocytes (Ju et al., 2009).

Oxidative stress has been implicated in cutaneous damage in various inflammatory skin diseases, including atopic dermatitis (Tsukahara et al., 2003). By protecting cells from damage by free radicals, heme oxygenase 1 (HO-1) plays an anti-inflammatory role during oxidative stress (Lee et al., 2007) and is a critical factor in the response to oxidative injury. Accordingly, the pharmacologic induction of HO-1 expression has been suggested as a novel potential strategy for the treatment of various inflammatory diseases.

The guava *Psidium guajava* (*P. guajava*) is grown in tropical and subtropical countries as a food and is also widely used in folk medicines (Han et al., in press). It is commonly used to treat gastrointestinal and respiratory disturbances and as an anti-inflammatory agent (Gutiérrez et al., 2008). The ethyl acetate extract of *P. guajava* leaves contains polyphenols, which are typical free radical scavengers (Hsieh et al., 2007). In addition to their antioxidant properties, some polyphenols have other medicinal properties, including anti-cancer, anti-inflammatory and anti-allergic activities (Hsieh et al., 2007). Our previous studies demonstrated that *P. guajava* extract suppresses the IgE-mediated allergic response transduced by FcεRI-signaling in mast cells (Han et al., in press). In the present study, we investigated the effects of a *P. guajava* ethyl acetate extract (PGEA) on the development of atopic dermatitis and on regulatory mechanisms in human keratinocytes.

2. Materials and methods

2.1. Materials

Chemicals and cell culture materials were obtained from the following sources: Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS), Life Technologies, Inc. (Carlsbad, CA, USA); WST-1 (2-[4-iodophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium, monosodium salt) assay kit, Roche Co. (London, UK); TNF-α and IFN-γ, from Sigma Aldrich Co. (St. Louis, MO, USA); zinc protoporphyrin (ZnPP), Calbiochem (La Jolla, CA, USA); luciferase assay system, Promega (Sunnyvale, CA USA); pCMV-β-gal, Clontech (Palo Alto, CA, USA); NF-κB luciferase reporter construct, Stratagene (Grand Island, NY, USA); LipofectAmine 2000 and SYBR Safe DNA Gel Stain kit, Invitrogen, Inc. (Carlsbad, CA, USA); TARC immunoassay reagents for cytokine assays, R&D Systems (Minneapolis, MN, USA); anti-β-actin, anti-lamin B, anti-IκB-α, anti-Nrf2, and anti-p65 antibodies, Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); protein assay kit, Bio-Rad Laboratories, Inc. (Hercules, CA, USA); primary antibodies (against HO-1, phospho-IκB-α, phospho-p65, and STAT1/phospho-STAT1) and horseradish peroxidase-linked secondary antibodies (against rabbit and mouse IgG), Cell Signaling Technologies (Danvers, MA, USA); ECL chemiluminescence system and polyvinylidene difluoride (PVDF) membranes, Amersham Pharmacia Biotech (Uppsala, Sweden). Polymerase chain reaction (PCR) oligonucleotide primers were custom-synthesized by Bioneer Co. (Korea). All chemicals were of the highest grade commercially available.

2.2. Plant material

PGEA was prepared as described by Seo et al. (2005). Briefly, the air-dried leaves of *P. guajava* (130 g) were extracted with ethyl acetate, and the extract powder resulting after drying (PGEA yield, 0.15%) was dissolved in dimethyl sulfoxide (DMSO).

2.3. Cell culture

The human keratinocyte cell line HaCaT was grown in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (10% FBS-DMEM) at 37 °C in a humidified incubator under a 5%-CO_2 atmosphere. Stock solutions of PGEA dissolved in DMSO were directly applied to the culture medium for 30 min before the addition of TNF-α/IFN-γ. Control cells were treated with DMSO alone.

2.4. Cytotoxicity assays

PGEA cytotoxicity was assessed using a WST-1 (2-[4-iodophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium, monosodium salt) assay kit according to the manufacturer's instructions. Briefly, HaCaT cells were seeded into 96-well plates and grown in 10% FBS-DMEM at 37 °C for 24 h. Then, PGEA was added to the culture medium at various concentrations (0.1–100 μg/mL). Additionally, TNF-α and IFN-γ (each 20 ng/ml) were incubated with or without PGEA treatment. Relative cytotoxicity was quantified by measuring the absorbance at 550 nm (a wavelength at which PGEA does not absorb) on a Varioskan spectrofluorimeter (Thermo Electron Co., Waltham, MA, USA).