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Anti-bacterial and anti-inflammatory properties of capric acid against *Propionibacterium acnes*: A comparative study with lauric acid

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ABSTRACT

Background: *Propionibacterium acnes* (*P. acnes*) is a commensal bacterium which is possibly involved in acne inflammation. The saturated fatty acid, lauric acid (C12:0) has been shown to possess antibacterial and anti-inflammatory properties against *P. acnes*. Little is known concerning the potential effects of its decanoic counterpart, capric acid (C10:0).
Objective: To examine the antibacterial and anti-inflammatory activities of capric acid against *P. acnes* and to investigate the mechanism of the anti-inflammatory action.
Methods: The antimicrobial activity of fatty acids was detected using the broth dilution method. An evaluation of *P. acnes*-induced ear edema in mice was conducted to evaluate the *in vivo* anti-inflammatory effect. To elucidate the *in vitro* anti-inflammatory effect, human SZ95 sebocytes and monocytic THP-1 cells were treated with *P. acnes* alone or in the presence of a fatty acid. The mRNA levels and secretion of pro-inflammatory cytokines were measured by qRT-PCR and enzyme immunoassay, respectively. NF-κB activation and MAPK expression were analyzed by ELISA and Western blot, respectively.
Results: Lauric acid had stronger antimicrobial activity against *P. acnes* than capric acid *in vitro* and *in vivo*. However, both fatty acids attenuated *P. acnes*-induced ear swelling in mice along with microabscess and significantly reduced interleukin (IL)-6 and CXCL8 (also known as IL-8) production in *P. acnes*-stimulated SZ95 sebocytes. *P. acnes*-induced mRNA levels and secretion of IL-8 and TNF-α in THP-1 cells were suppressed by both fatty acids, which inhibited NF-κB activation and the phosphorylation of MAP kinases.
Conclusion: Our data demonstrate that both capric acid and lauric acid exert bactericidal and anti-inflammatory activities against *P. acnes*. The anti-inflammatory effect may partially occur through the inhibition of NF-κB activation and the phosphorylation of MAP kinases.

1. Introduction

Acne vulgaris is the most common disease of the pilosebaceous unit. Multiple factors are considered to be involved in acne pathogenesis, follicular hyperkeratinization, *Propionibacterium acnes* (*P. acnes*)-induced inflammation, and excessive sebum production, which may serve as a nutrient source for *P. acnes* [1]. The role of *P. acnes*, a Gram-positive anaerobic bacterium species, in the pathogenesis of acne is supported by the activation of the inflammatory pathway through Toll-like receptor (TLR) binding [2,3]. *P. acnes* has been implicated in the pathogenesis of inflammatory acne by stimulating keratinocytes and sebocytes and macrophages to produce pro-inflammatory cytokines [4,5]. The interaction between *P. acnes* and infiltrating monocytes and lymphocytes may also play an important role in the pathogenesis of inflammatory acne [6]. *P. acnes* stimulates the production of the pro-inflammatory cytokines, interleukin (IL)-1β, CXCL8 (IL-8) and tumor necrosis factor (TNF)-α by human peripheral blood mononuclear cells and monocytic THP-1 cells [7,8]. Subsequently, the cytokines bind their receptors within the epidermis, infundibulum and sebaceous glands to participate in the inflammatory response. Moreover, active lipid mediators derived from arachidonic acid (AA), such as leukotrienes (LT), prostaglandins (PG), are

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other pro-inflammatory mediators thought to be involved in acne inflammation [9,10]. Interestingly, AA has been demonstrated to further regulate the immune response by enhancing the expression of IL-6 from sebocytes [11].

The nuclear factor kappa B (NF-κB) pathway and the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) cascades have been proposed as the two major mechanisms for modulation of the production of pro-inflammatory molecules, which are prominent contributors to chronic inflammatory responses [12]. Both NF-κB and MAPK pathways have been proposed to be related with *P. acnes*-induced inflammatory cytokine synthesis. *P. acnes* binds to TLRs on keratinocytes, sebocytes and dendritic cells, activating signaling cascades that enlist transcription factors and phosphokinases such as NF-κB and MAPK [13]. Grange et al. [5] demonstrated that *P. acnes* leads to degradation of IκB, stimulation of the MAPK pathway and to increased IL-8 production in keratinocytes.

Both capric acid (decanoic acid, C10:0) and lauric acid (dodecanoic acid, C12:0) have been shown to be powerful bactericidal agents *in vitro* [14]. Capric acid exhibits antibacterial activity against several Gram-positive and Gram-negative bacteria, anti-fungal and antiviral activity [15]. Nakatsuji et al. [16] reported that lauric acid exhibited significant antimicrobial and anti-inflammatory activities against *P. acnes.* Although the anti-*P. acnes* properties of lauric acid are well-documented, the mechanism of action has not been completely elucidated. In the preliminary studies, we investigated whether capric acid could suppress *P. acnes*-induced IL-8 production by THP-1 cells. The results showed that capric acid, at a concentration of 100 μM, significantly reduced IL-8 release by *P. acnes*-stimulated THP-1 cell. Therefore, the purpose of this study was to evaluate the anti-bacterial and anti-inflammatory activity of capric acid and lauric acid, and then investigate their mechanism of anti-inflammatory action in a cellular model, in order to better understand the possible anti-acne potential of capric acid.

2. Materials and methods

2.1. Materials

The strain of *P. acnes* (BCRC10723) was obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). *P. acnes* was cultured in brain heart infusion (BHI) broth (Difco, Detroit, MI, USA) with 1% glucose. The bacteria were cultured in an anaerobic atmosphere using BBL GasPak systems (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). The human monocytic THP-1 cell line (BCRC 60430) was also obtained from the Bioresource Collection and Research Center. THP-1 cells were maintained in RPMI 1640 (Gibco, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere with 5% CO_2. Human SZ95 sebocytes [17] were maintained in Sebomed basal medium (Biochrom, Berlin, Germany), supplemented with 5 ng/ml human recombinant epidermal growth factor (Sigma–Aldrich, St. Louis, MO, USA), 50 μg/mL gentamicin (Sigma), and 10% (v/v) FBS, at 37 °C in a humidified atmosphere with 5% CO_2. The assay kits for IL-8, IL-6, and TNF-α were purchased from Invitrogen (Carlsbad, CA, USA). Arachidonic, caporic, caprylic, capric and lauric acid were purchased from Sigma–Aldrich.

2.2. In vitro *antimicrobial activity assay*

The antimicrobial susceptibility of capric acid was compared with that of lauric acid as previously described [16,18]. Briefly, *P. acnes* was incubated in BHI broth with 1% glucose for 72 h under anaerobic conditions and adjusted to yield approximately 1×10^6 colony-forming units (CFU)/mL. Fatty acids were dissolved in 0.05% (v/v) DMSO. In sterile 96-well microtiter plates, 100 μL of fatty acid was diluted with BHI broth and added to wells containing 100 μL of the bacterial suspension in BHI broth. Two-fold serial dilutions were made in broth over a range to give concentrations of fatty acid. The control received 0.05% (v/v) DMSO alone. Triplicate samples were performed for each test concentration. After incubation for 72 h at 37 °C under anaerobic conditions, the plates were mixed well and then absorbance at 600 nm was measured by a microplate reader to estimate bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of a tested compound which inhibited the visible growth of *P. acnes*.

The minimum bactericidal concentrations (MBCs) of capric acid and lauric acid against *P. acnes* were determined according to the method described previously [16], with some modification. *P. acnes* (1×10^7 CFU/mL) was incubated with fatty acids at various concentrations in PBS on a 96-well plate (100 μL/well) under anaerobic conditions. The vehicle control received only 0.05% (v/v) of DMSO. *P. acnes* was incubated with different concentrations of fatty acids for 5 h. After incubation, the reaction mixture was diluted 1:10 to $1:10^4$ with PBS and 10 μL of the dilutions was spotted on BHI agar plates. After the liquid of the *P. acnes* suspension was absorbed into the agar, the plates were incubated at 37 °C under anaerobic conditions for 2 days, and the CFU of *P. acnes* was counted. The MBC was defined as the lowest concentration of a test compound which prevented the growth of *P. acnes* after subculture on a BHI agar plate which is free of test compound.

2.3. P. acnes-*induced inflammation* in vivo

Eight-week-old male ICR mice were purchased from the BioLASCO Taiwan Co., Ltd., Yilan, Taiwan. All animal experiments were approved by the Animal Care Committee of the National Taiwan Normal University. *In vivo* anti-inflammatory activity of capric acid and lauric acid was then evaluated using the following procedure which has been described previously [8]. In the preliminary testing, intra-dermal sole injection of capric acid or lauric acid (up to 4 μg/10 μL) did not cause any visible adverse reaction. Therefore, an administered dosage of 4 μg/10 μL was used for the following experiments. *P. acnes* (6×10^7 CFU per 10 μL in PBS) was intradermally injected into the right ear of ICR mice. Left ears received an equal amount (10 μL) of PBS ($n = 5$). Ten microliters of capric acid (2 and 4 μg/site) in 5% DMSO in PBS was injected into the same location of both ears right after *P. acnes* or PBS injection ($n = 5$). Twenty-four hours after bacterial injection, the increase in ear thickness was measured using a micro-caliper (Mitutoyo, Kanagawa, Japan). Mice were then sacrificed with carbon dioxide asphyxiation and ear disks of 4.0 mm diameter were punched out and weighed. The extent of edema was evaluated by the weight difference between the left and the right ear disk. The increase in ear thickness and weight of the *P. acnes*-injected ear was calculated and expressed as percentage of the PBS-injected control. For histological examination, paraffin embedded ears were vertically cut into cross-sections. The cross-sections were stained with hematoxylin and eosin (H&E) and then viewed under a microscope for the evaluation of inflammatory response.

To determine *P. acnes* number in the ear after 24-h bacterial injection, the ear was cut off and sterilized using povidone–iodine solution followed by 75% (v/v) ethanol. The disinfection procedure was repeated once. The inflamed nodule of mice ear was punched with a 5.0 mm biopsy. The punch biopsy was homogenized in