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

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ARTICLE INFO

Article history:
Received 30 August 2013
Received in revised form 19 October 2013
Accepted 31 October 2013

Keywords:
Capric acid
Lauric acid
Propionibacterium acnes
Antibacterial
Anti-inflammation

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.

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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 monocyctic 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 IKK, 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 humified atmosphere with 5% CO₂. Human S295 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 humified atmosphere with 5% CO₂. 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 × 10⁶ 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 × 10⁶ 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 6 h. After incubation, the reaction mixture was diluted 1:10 to 1:10⁴ 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 × 10⁷ 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 weighted. 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
300 µL of sterile PBS with a hand tissue grinder. CFUs of *P. acnes* in the mice ear were counted by plating serial dilutions (1:10² to 1:10⁵) of the homogenate on a BHI agar plate. These agar plates then were anaerobically incubated for 72 h at 37 °C.

2.4. Determination of the viability of cells

THP-1 cells (2 × 10⁶ cells/mL) were maintained in 96-well culture plates with various concentrations of fatty acids. After 24 h of incubation, 20 µL of Alamar blue reagent (Invitrogen, Carlsbad, CA, USA) was added to each well. Two hours later, the optical density (OD) of the resulting medium was measured, and the difference in the absorbance values at 570 and 600 nm was calculated using a Synergy HT multidetection microplate reader (BioTek). S295 sebocytes (1 × 10⁶ cells/mL) were cultured in 96-well culture plates in the presence of various concentrations of fatty acids. Cell viability was determined 24 h later using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

2.5. Measurement of cytokine production in human S295 sebocytes and monocyctic THP-1 cells

The anti-inflammatory activity of capric acid and lauric acid was examined in *P. acnes*-stimulated S295 sebocytes and monocyctic THP-1 cells in vitro. To prepare the *P. acnes* suspension for the sequential stimulation of cells, the log-phase bacterial *P. acnes* culture was harvested, washed with PBS, and then centrifuged at 10,000 × g for 5 min. After two additional washes in PBS, the *P. acnes* pellet was re-suspended in RPMI medium or Sebomed basal medium without antibiotics. Human monocyctic THP-1 cells and S295 sebocytes were respectively seeded at 2 × 10⁶ cells/mL and 1 × 10⁶ cells/mL in 96-well plates with 10% FBS/RPMI medium or 10% FBS/Sebomed basal medium, and were treated with fatty acid alone or stimulated with live *P. acnes* (wet weight 200 µg/mL) alone or in combination with different concentrations of fatty acid for a 24-h incubation period. Cell-free supernatants were collected, and concentrations of IL-6, and IL-8 were analyzed with respective enzyme immunoassay kits.

2.6. RNA isolation and quantitative real-time polymerase chain reaction (PCR)

Total RNA was isolated with the TRizol reagent (Invitrogen), according to the manufacturer’s instructions. Complementary DNA was generated from 2 µg of total RNA, with the oligo (dT) primer and 1 µL of reverse transcriptase (Promega, Madison, WI, USA). We used IL-8 5'-TGCCAAGGAGTGCTAAAG-3' and 5'-CTCCA-CAACCCTCTGCA-3' primers, TNF-α 5'-TCTTCTGCTGACTCTTG-3' and 5'-ATCTCTACGTCCAGCATTG-3' primers, and GADPH 5'-GTGAGGTGAGTCAGG-3' and 5'-TGAGGCTCAATTGAGGTC-3' primers. The primers amplified a 157 bp fragment of the IL-8 cDNA, a 224 bp fragment of the TNF-α cDNA, and a 113 bp fragment of the GADPH cDNA. Real-time PCRs were conducted in an iCycler iQ Real-Time detection system (Bio-Rad, Hercules, CA, USA), using iQ™ SYBR Green Supermix (Bio-Rad). Thermal cycling conditions for all assays were initial denaturation at 95 °C for 3 min and 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Melting analysis was performed by denaturing at 95 °C for 1 min and cooling to 55 °C for 1 min followed by heating at the rate of 0.5 °C cycle with holding 10 s from 55 °C to 95 °C. The relative amounts of the PCR products were analyzed by iQ™ optical system software, vers. 2.1.

The messenger (m)RNA level of each sample for each gene was normalized to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

2.7. Detection of MAPK expression by Western blot analysis

Human monocyctic THP-1 cells were seeded at 2 × 10⁶ cells/mL in 6-cm dishes and were stimulated with viable *P. acnes* (wet weight 200 µg/mL) alone or co-incubated with various concentrations of tested samples. After 2 h of treatment, cells were harvested and washed with PBS. Whole cell lysates were prepared in a lysis buffer (Cell Signaling, Beverly, MA, USA) containing 10 mM phenylmethylsulfonyl fluoride (PMSF). The cell lysates were sonicated and cleared by centrifugation at 4 °C, 12,000 × g for 10 min. The protein concentration was measured by DC protein assay (Bio Rad). Aliquots of the lysates (each containing 30 µg of protein) were boiled for 5 min and electrophoresed on a 10% SDS–polyacrylamide gel. The resolved proteins were then transferred to PVDF membranes. Membranes were blocked by incubation in gelatin-NET buffer at room temperature, and then incubated with 1:1000 dilution of primary antibodies to MAPK, phosphor–MAPK (Cell Signaling Technology, Danvers, MA, USA) and anti-β-actin (Sigma), followed by horseradish peroxidase-conjugated secondary antibody according to the manufacturer’s instructions. Immuno-reactive proteins were detected with the enhanced ECL chemiluminescence Western blotting detection

![Fig. 1](image-url) Effect of various concentrations of capric acid and lauric acid on *P. acnes* growth under anaerobic conditions at 37 °C for 72 h (A). Increases in the concentration of both fatty acids reduce the bacterial growth. Arrows indicated that complete inhibitions were observed at 1 mM of capric acid and 0.25 mM of lauric acid. The bactericidal activity of capric acid and lauric acid was determined as MBC (B). The MBCs of capric acid and lauric acid were 20 and 10 mM, respectively. Data represent the mean ± SD. ND, non-detectable.
system (ChemeDoc XRS, Bio-Rad). Signal strengths were quantified using densitometric program (Image Lab, Bio-Rad).

2.8. NF-κB activation assay

NF-κB activation was analyzed using an NF-κB/p65 ActivELISA kit (Imgenex; San Diego, CA, USA). The kit can detect and quantify the nuclear-translocated p65 subunit. To determine the effects of fatty acids on P. acnes-induced activation of NF-κB in THP-1 cells, human monocytic THP-1 cells (3 × 10⁶ cells/mL) cultured in medium were stimulated with P. acnes (200 μg/mL) alone or in combination with the indicated concentrations of fatty acids for 16 h. Cytoplasmic and nuclear extracts were then prepared according to the manufacturer’s instructions. Briefly, the cytoplasmic fraction was collected in the supernatant of whole-cell lysates after centrifugation at 12,000 × g for 30 s at 4 °C. The nuclear pellet was re-suspended in 100 μL nuclear lysis buffer at 4 °C for 30 min, and the suspension was centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant containing the nuclear fraction was subjected to an enzyme-linked immunosorbent assay (ELISA) using specific anti-NF-κB antibodies, according to the manufacturer’s instructions. The absorbance was read at 405 nm using a Synergy HT multiplate detector.

2.9. Statistical analysis

All data are presented as means ± SD. Statistical analyses were performed using the SPSS 19.0 statistical package (Chicago, IL, USA). The Mann–Whitney U-test was used to compare differences between the vehicle and treatments. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Anti-bacterial activity of capric acid and lauric acid against P. acnes

In our preliminary study, we investigated the effect of saturated medium chain fatty acids, such as capric acid (C10:0), caprylic acid (C8:0), and caproic acid (C6:0) on the growth of P. acnes. Among these three fatty acids, caprylic acid exhibited the most potent antibacterial activity. In contrast, no inhibitory effect on P. acnes growth was observed when caprylic acid and lauric acid were administered at concentrations of 6.9 mM (1 mg/mL) and 8.6 mM (1 mg/mL), respectively.

To compare the effect of caprylic acid and lauric acid on the growth of P. acnes, bacteria were co-cultured with various concentrations of fatty acids for 72 h. The MIC values of caprylic acid and lauric acid were determined as 1 and 0.25 mM, respectively (Fig. 1A). We further evaluated the MBCs of caprylic acid and lauric acid (Fig. 1B). The MBC values of caprylic acid and lauric acid were 20 and 10 mM, respectively. The results indicated that lauric acid had superior anti-microbial activity against P. acnes in vitro than caprylic acid.

3.2. Effect of caprylic acid and lauric acid on P. acnes-induced inflammation in vivo

To induce inflammation in vivo, living P. acnes (6 × 10⁸ CFU per 10 μL in PBS) were intradermally injected into the mice ears. Histologically, microabscess was found in the dermis after 24-h P. acnes injection (Fig. 2A). The inflammatory cells in the mice ears predominantly consisted of neutrophils at H&E stained section (the insert of Fig. 2A). Prior to the determination of anti-inflammatory effect of caprylic acid and lauric acid in vivo, an intradermal injection test was performed to evaluate its skin irritation effect. Intradermal administration of caprylic acid (4 μg/site) or lauric acid (4 μg/site) alone produced no apparent irritation, such as ear swelling, redness, and cutaneous erythema (data not shown), and no increasing infiltrate of inflammatory cells (Fig. 2B). To examine the in vivo anti-inflammatory effect of caprylic acid and lauric acid (2 and 4 μg/site), mouse ears were intradermally injected with viable P. acnes for one day. Co-injection of caprylic acid or lauric acid significantly reduced P. acnes-induced ear swelling measured by ear thickness (Fig. 2C) and ear biopsy weight (Fig. 2D). Co-injection of 4 μg lauric acid or caprylic acid significantly reduced the number of microabscesses and polymorphonuclear leukocytes (PMNs) in the injection site (Fig. 2E, insert). The inhibitory effect of lauric acid was statistically significant (Fig. 2C, D).
of P. acnes colonized within the ear (Fig. 2E). In addition, lauric acid exhibited stronger inhibitory activity on colonized P. acnes than capric acid in vivo (p = 0.032), which was consistent with its antimicrobial effect in vitro.

3.3. Effects of capric acid and lauric acid on pro-inflammatory cytokine induction by P. acnes in vitro

Since capric acid and lauric acid exerted in vivo anti-inflammatory activity against P. acnes, we were interested in exploring further the action and mechanisms by which they suppress P. acnes-induced inflammatory responses. Prior to the comparative study, the cytotoxicity of fatty acids was examined. Capric acid and lauric acid had no significant cytotoxicity on SZ95 sebocytes (Fig. 3A) and THP-1 cells (Fig. 4A) up to concentrations of 100 μM and 125 μM, respectively. Treatment of both fatty acids (up to 100 μM) did not affect the basal levels of IL-6 and IL-8 of
significant test.

3.4. Fatty acids inhibited MAPK phosphorylation and NF-κB activation in P. acnes-stimulated THP-1 cells

To elucidate the underlying mechanism by which capric acid and lauric acid attenuate P. acnes-induced cytokine production, we evaluated the inflammation-related signaling cascades, such as NF-κB and MAPK, including extracellular signal-related kinase (ERK), p38-mitogen-activated kinase (p38), and c-Jun N-terminal kinase (JNK). Fig. 6 shows that the levels of phosphorylated p38, JNK, and ERK were significantly increased in response to P. acnes stimulation relative to the negative control in the absence of bacteria. Capric acid and lauric acid at a concentration of 100 µM significantly suppressed P. acnes-induced phosphorylated MAPK, such as p38, JNK, and ERK. As shown in Fig. 7, exposure of THP-1 cells to P. acnes for 16 h significantly increased NF-κB p65 translocation. Treatment with capric acid and lauric acid at a concentration of 25 µM significantly attenuated the increasing NF-κB p65 translocation in P. acnes-stimulated THP-1 cells after 16 h of incubation (Fig. 7).

3.5. Capric acid and lauric acid inhibited P. acnes-induced IL-8 production by arachidonic acid-pretreated THP-1 cells

IL-8 is a major chemotactic and activating peptide for neutrophils. As shown in Fig. 8A, treatment with arachidonic acid alone significantly increased IL-8 release by THP-1 cells. Co-treatment with arachidonic acid and P. acnes enhanced IL-8 production as compared with P. acnes alone (p = 0.001). Moreover, pre-treatment with arachidonic acid dramatically potentiated P. acnes-induced IL-8 production (Fig. 8A). In addition, co-treatment with capric acid and lauric acid significantly suppressed IL-8 induction by arachidonic acid alone in THP-1 cells (Fig. 8B). Notably, both capric acid and lauric acid effectively suppressed P. acnes-induced IL-8 production by arachidonic acid-pretreated THP-1 cells (Fig. 8C).

4. Discussion

The present study has been undertaken to demonstrate the in vitro and in vivo antibacterial and anti-inflammatory effect of capric acid and to compare its bioactivity with that of lauric acid. We provide here a preliminary description of the molecular basis of the anti-inflammatory action of capric acid and lauric acid in P. acnes-stimulated monocytic THP-1 cells. The down-regulation of pro-inflammatory cytokines by both fatty acids may partially be mediated by blocking the MAPK pathways and subsequent NF-κB activation.

Free fatty acids (FFA) play an important role in the human innate immune system, particularly in the defense of skin and mucosal surfaces. There is 10–15 µg of FFA per square centimeter on human skin, among them lauric acid, myristic acid, palmitic acid, sapienic acid and cis-8-octadecenoic acid [15]. Lauric acid and its preparations of liposomes [19] and copolymers [20] have shown strong antimicrobial activity against P. acnes. The MIC values of both fatty acids obtained in this study were lower than their respective MBC value. However, lauric acid possesses stronger anti-P. acnes activity than capric acid. In addition, we observed that caprylic acid and caproic acid had no apparent inhibitory effect on growth of P. acnes. The antibacterial activity of each free fatty acid depends on its nature, e.g., chain length and the presence, number, and position of double bonds [15]. Ko et al. [21] reported that capric acid and lauric acid are nearly equally active against three Propionibacterium species including P. acnes, P. granulosum and P. avidum. However, comparisons of our study with other reports are complicated because a variety of methodological approaches were used to determine antibacterial activity.

Consistent with the previous finding of Nakatsuji and colleagues [16], lauric acid is effective against P. acnes-induced mouse ear inflammation in vivo (Fig. 2). Our results showed that injection of lauric acid or capric acid (4 µg/site) significantly reduced the number of P. acnes colonized within mice ear. Both fatty acids at dosage of 2 µg did not significantly reduce the P. acnes colonies (Fig. 2E). However, Nakatsuji et al. [16] demonstrated that injection of 2 µg lauric acid significantly reduced the number of P. acnes within the mice ear. This inconsistent observation perhaps resulted from our higher bacteria infection load (6 × 10³ CFU/site) than that of Nakatsuji’s study (1 × 10⁷ CFU/site).

Regarding anti-inflammatory activity of capric acid, Wu et al. [22] reported that capric acid suppressed PGE₂ production in

S295 sebocytes as well as IL-8 and TNF-α of THP-1 cells in the absence of P. acnes (data not shown).

Treatment with capric acid and lauric acid significantly suppressed P. acnes-induced IL-8 (Fig. 3B) and IL-6 (Fig. 3C) production by S295 sebocytes as well as IL-8 (Fig. 4B) and TNF-α (Fig. 4C) production by THP-1 cells. We further analyzed the mRNA levels of pro-inflammatory cytokines by quantitative real-time PCR (qRT-PCR). As shown in Fig. 5, capric acid and lauric acid suppressed the gene expressions of IL-8 and TNF-α in P. acnes-stimulated THP-1 cells.

Fig. 5. Capric acid and lauric acid suppressed P. acnes-induced pro-inflammatory cytokine mRNA expression in THP-1 cells. The expression level of mRNA was determined using a quantitative real-time PCR. The expression of cytokine mRNA was normalized to GAPDH mRNA and expressed as multiples of change with untreated THP-1 cells as the control. Each column shows the mean ± SD. *** denote significant difference from vehicle (DMSO) at p < 0.001 analyzed by Mann–Whitney U-test.
lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Capric acid has also been shown to inhibit nitric oxide (NO) production and inflammatory inducible NO synthase (iNOS) gene expression in LPS-stimulated RAW264.7 macrophages [23]. This study demonstrated capric acid relieved P. acnes-induced ear swelling of mice (Fig. 2). Therefore, we examined the action and mechanisms by which capric acid and lauric acid suppress P. acnes-induced inflammatory responses in vitro.

In the pathogenesis of acne inflammation, P. acnes plays an important initiating role by producing chemotactic factors, resulting in attracting of the immune system cells such as neutrophils, monocytes, and lymphocytes [3]. Previous studies have found that P. acnes stimulates the production of pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8, and TNF-α [24,25]. IL-8, a CXC-type chemokine, is a potent pro-inflammatory chemotactic factor that predominantly exerts its chemotactic effects on neutrophils [26]. Enhanced IL-8 levels were observed in P. acnes-stimulated peripheral blood mononuclear cells from patients with acne vulgaris [27]. TNF-α and IL-6 are also potent inflammatory molecules which have endocrine effects either in acute or chronic inflammation [28]. Elevated expression of IL-6 and IL-8 has been found in acne-affected skin [11]. Since these inflammatory mediators are thought to increase the inflammatory state of acne and to aggravate the initial acne lesion, we next investigated whether capric acid and lauric acid could inhibit pro-inflammatory cytokine production in P. acnes-stimulated SZ95 sebocytes and monocyctic THP-1 cells. Our findings provide evidence that both capric acid and lauric acid have a marked suppressive effect on P. acnes-induced IL-8 and IL-6 production by SZ95 sebocytes (Fig. 3) as well as IL-8 and TNF-α production by THP-1 cells (Fig. 4). Consequently, we evaluated whether capric acid and lauric acid affect mRNA expression of cytokines. Our result showed that capric acid and lauric acid attenuate the expression of P. acnes-induced IL-8 and TNF-α at the transcriptional level (Fig. 5).

Both NF-κB and MAPK pathways have been proposed to be related with P. acnes-induced inflammatory cytokine production.

Fig. 6. Effect of capric and lauric acid on P. acnes-induced p38 (A), ERK (B), and JNK (C) activation in THP-1 cells. THP-1 cells were incubated 2 h without P. acnes (control), with P. acnes alone (DMSO vehicle), and with P. acnes in the presence of fatty acids. Data are presented as the mean ± SD. * and ** denote significant difference from vehicle (P. acnes alone) at p < 0.05 ( * ) and p < 0.01 (** ) and p < 0.001 ( *** ) analyzed by Mann–Whitney U-test.

Fig. 7. Suppressive effect of capric and lauric acid on P. acnes-induced NF-κB p65 activation in THP-1 cells. THP-1 cells were incubated 16 h without P. acnes (control), with P. acnes alone (DMSO vehicle), and with P. acnes in the presence of fatty acids. Data are presented as the mean ± SD. * and ** denote significant difference from vehicle (P. acnes alone) at p < 0.01 (** ) and p < 0.001 ( *** ) analyzed by Mann–Whitney U-test.
NF-κB has been demonstrated to be involved in the positive regulation of inflammatory and immune genes including those for IL-8, IL-2, TNF-α, monocyte chemoattractant protein-1, iNOS and cyclooxygenase (COX)-2 [29]. NF-κB and AP-1 have been reported to be activated in inflammatory acne lesions [29,30]. P. acnes is recognized by TLR2 and activates p38 and ERK MAPKs, thus contributing to IL-8 production [5]. These previous studies led us to examine the effect of capric acid and lauric acid on both signaling pathways. Further investigation of the molecular mechanisms revealed that treatment with capric acid and lauric acid suppressed MAPK phosphorylation (Fig. 6) and NF-κB activation (Fig. 7). Hence, our finding suggests that capric acid and lauric acid inactivate MAPK and NF-κB and this is likely to be important in the anti-inflammatory action of both fatty acids against P. acnes.

Both leukotrienes and prostaglandins are the eicosanoid metabolites originated from the arachidonic acid cascade. Enhanced 5-lipoxygenase and COX-2 was detected in acne-involved facial skin [11]. Moreover, arachidonic acid enhanced the level of IL-6 in SZ95 sebocytes, but those of TNFα and IL-1β were not affected [11]. LTB4 potentiates CGD-mediated intracellular signaling in peripheral blood mononuclear cells, resulting in enhanced secretion of pro-inflammatory cytokines [31]. Since metabolites of arachidonic acid may affect cytokine production, we investigated whether pre-treatment of arachidonic acid could potentiate P. acnes-induced IL-8 production by THP-1 cells. We found that treatment with arachidonic acid alone increased IL-8 level. P. acnes and its combination with pretreatment of arachidonic acid powerfully stimulated IL-8 release from THP-1 cells in comparison with untreated controls (Fig. 8A). Our finding suggests that metabolites of arachidonic acid may synergize P. acnes-induced IL-8 production and contribute to the worsening of acne inflammation, although its exact mechanism of action remains to be clarified. Interestingly, capric acid and lauric acid significantly inhibited IL-8 induction by arachidonic acid-stimulated THP-1 cells (Fig. 8B). Moreover, both fatty acids effectively inhibited P. acnes-induced IL-8 production by arachidonic acid-pretreated THP-1 cells (Fig. 8C). Nakatsuji et al. [32] reported that antibodies elicited by inactivated P. acnes in immunized mice decrease IL-8 production, thereby decreasing inflammation and improving acne. We therefore hypothesized that the anti-inflammatory effect of capric acid and lauric acid might be at least partly due to their suppressive effect on IL-8 production. As lauric acid is considered to be an effective agent for acne vulgaris therapy, capric acid may also have the potential to be a beneficial ingredient for acne inflammation.

In conclusion, P. acnes-induced inflammatory responses were inhibited by capric acid and lauric acid which suppressed the MAPK phosphorylation and NF-κB activation. Hence, our data suggested that capric acid may be a candidate for the anti-inflammatory treatment of acne.

Acknowledgement

This work was supported by the research grant, NSC 101-2320-B-003-002, from the National Science Council, Taipei, Taiwan.

References


