Published in final edited form as:
J Invest Dermatol. 2009 October ; 129(10): 2480–2488. doi:10.1038/jid.2009.93.

Antimicrobial Property of Lauric Acid Against *Propionibacterium acnes*: Its Therapeutic Potential for Inflammatory Acne Vulgaris

Teruaki Nakatsuji[1,2], **Mandy C. Kao**[1,2], **Jia-You Fang**[3], **Christos C. Zouboulis**[4], **Liangfang Zhang**[5,6], **Richard L. Gallo**[1,2], and **Chun-Ming Huang**[1,2,6]

[1]Division of Dermatology, Department of Medicine, University of California, San Diego, California, USA

[2]VA San Diego Healthcare Center, San Diego, California, USA

[3]Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan

[4]Department of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany

[5]Department of Nanoengineering, University of California, San Diego, California, USA

[6]Moores Cancer Center, University of California, San Diego, California, USA

Abstract

The strong bactericidal properties of lauric acid (C12:0), a middle chain-free fatty acid commonly found in natural products, have been shown in a number of studies. However, it has not been demonstrated whether lauric acid can be used for acne treatment as a natural antibiotic against *Propionibacterium acnes* (*P. acnes*), which promotes follicular inflammation (inflammatory acne). This study evaluated the antimicrobial property of lauric acid against *P. acnes* both *in vitro* and *in vivo*. Incubation of the skin bacteria *P. acnes*, *Staphylococcus aureus* (*S. aureus*), and *Staphylococcus epidermidis* (*S. epidermidis*) with lauric acid yielded minimal inhibitory concentration (MIC) values against the bacterial growth over 15 times lower than those of benzoyl peroxide (BPO). The lower MIC values of lauric acid indicate stronger antimicrobial properties than that of BPO. The detected values of half maximal effective concentration (EC_{50}) of lauric acid on *P. acnes*, *S. aureus*, and *S. epidermidis* growth indicate that *P. acnes* is the most sensitive to lauric acid among these bacteria. In addition, lauric acid did not induce cytotoxicity to human sebocytes. Notably, both intradermal injection and epicutaneous application of lauric acid effectively decreased the number of *P. acnes* colonized with mouse ears, thereby relieving *P. acnes*-induced ear swelling and granulomatous inflammation. The obtained data highlight the potential of using lauric acid as an alternative treatment for antibiotic therapy of acne vulgaris.

INTRODUCTION

Acne vulgaris is the most common disorder of human skin that affects up to 80% of individuals in their lives (Dreno and Poli, 2003). Acne has many different symptoms including comedones,

Correspondence: Dr Chun-Ming Huang, Department of Medicine, University of California, Rm2317A, 3350 La Jolla Village, San Diego, California 92161, USA. chunming@ucsd.edu.

CONFLICT OF INTEREST
The authors state no conflict of interest.

papules, pustules, nodules, cysts and pilosebaceous inflammation. Among these, inflammatory lesions of acne are of the greatest concern to patients because they may lead to acne scarring, thereby inducing adverse psychological effects. *propionibacterium acnes* (*P. acnes*) is a Gram-positive anaerobic bacterium that mostly resides in the pilosebaceous follicles of the skin. Although *P. acnes* is a member of the normal skin commensal bacterial flora, it plays a critical role in the development of inflammatory acne when it overgrows and colonizes the pilosebaceous unit (Leeming *et al.*, 1988; Cunliffe and Gollnick, 2001; Leyden, 2001; Bojar and Holland, 2004). Genomic analysis of *P. acnes* has shown a variety of *P. acnes* pathogenicity (Bruggemann *et al.*, 2004). Further studies on the entire genome of *P. acnes* has revealed numerous genes that regulate products involved in degrading host molecules and triggering inflammation (Bruggemann, 2005; Rosen, 2007). It has also been widely accepted that inflammatory acne is induced by host immune reactions to *P. acnes. P. acnes* releases chemoactive factors that attract the immune system cells such as neutrophils, monocytes, and lymphocytes (Webster and Leyden, 1980; Burkhart *et al.*, 1999). Previous studies have found that *P. acnes* stimulates the production of proinflammatory cytokines such as interleukins −1β, −8, −12, and tumor necrosis factor-α. *P. acnes*-induced cytokines are mediated by toll-like receptor 2 (Kim *et al.*, 2002; Kim, 2005; Nagy *et al.*, 2006).

As reduction in *P. acnes* numbers in the hair follicle by antimicrobial agents correlates with clinical improvement of acne in patients, antibiotics have been used to treat acne for several decades and are still widely prescribed for acne patients. The oxidizing agent benzoyl peroxide (BPO) has been one of the most frequently used topical medications for acne treatment. It is often used as the first line treatment for patients suffering from mild to moderate acne. BPO is also used to treat severe acne in combination with a systemic medication such as an antibiotic agent and isotretinoin. The principal action mechanism of BPO is thought to be antibacterial activity, though slight anti-inflammatory effects and very mild anti-comedogenic effects may also exist (Gollnick and Schramm, 1998).

Self-disinfection of the human skin surface through various modes of action has long been suggested (Burtenshaw, 1945). Free fatty acids (FFAs) and antimicrobial peptides have been known to be responsible for at least part of the self-antimicrobial disinfecting activity of the skin surface against microbial colonization (Gallo and Huttner, 1998; Drake *et al.*, 2008). FFAs are secreted from sebaceous glands as sebum triacylglycerides and subsequently released through hydrolyzation by lipases of the commensal bacterial flora, including *P. acnes* (Holland *et al.*, 1981). Various FFAs have shown antibacterial activity against a range of Gram-positive bacteria, but not Gram-negative bacteria (Wille and Kydonieus, 2003; Georgel *et al.*, 2005; Skrivanova *et al.*, 2005; Drake *et al.*, 2008). Lauric acid (C12: 0), a minor component in the sebum, is the most potent antimicrobial saturated fatty acid (Wille and Kydonieus, 2003). It is commonly found in natural products like coconut palm and milk. Although lauric acid exerts strong antimicrobial activity against many Grampositive bacteria (Kabara *et al.*, 1972; Kitahara *et al.*, 2004; Rouse *et al.*, 2005; Skrivanova *et al.*, 2005), it is unknown whether it has a similar effect on *P. acnes in vivo*; therefore, the potential of lauric acid to be used as a natural antibacterial agent in acne therapy is also unknown. In this study, we have demonstrated the potential of lauric acid as an alternative option for antibacterial therapy in acne treatment. Interestingly, lauric acid showed stronger antimicrobial activity as compared with BPO against skin bacteria, including *P. acnes, in vitro*. It also showed therapeutic potential against *P. acnes*-induced inflammation *in vivo*.

RESULTS

Antimicrobial effects of lauric acid and BPO against skin bacteria

To compare dose-response effects of lauric acid and BPO on the growth of bacteria that are present in the skin flora, *P. acnes*, *Staphylococcus aureus* (*S. aureus*), and *Staphylococcus*

epidermidis (*S. epidermidis*) were co-cultured with either agent at various concentrations for 72, 24, and 48 hours, respectively. Bacterial growth was evaluated by measuring absorbance at 600 nm (Figure 1). The length of cultivation period was determined based on the growth speed of the bacteria. Both lauric acid and BPO were tested at concentrations of twofold serial dilutions from 0.24 to 500 μg ml^{-1}. Higher concentrations of both components were not evaluated because they precipitated during incubation (Figure S1) and interfered with the measurement of optical density at 600 nm (OD_{600}). The minimal inhibitory concentration (MIC), the lowest concentration to prevent bacterial growth of lauric acid on the bacterial growth, is over 15 times lower than that of BPO. This suggests that lauric acid has much stronger antimicrobial activity than BPO (Table 1). The half maximal effective concentration of lauric acid on *P. acnes* growth was the lowest among the bacteria tested, suggesting that *P. acnes* is more sensitive than *S. aureus* and *S. epidermidis* (Table 1). The values of MIC (1.95 μg ml^{-1}) and the half maximal effective concentration (1.5 μg ml^{-1}) of lauric acid were also determined using a different strain of *P. acnes* (ATCC 11827) (Figure S2).

To determine minimal bactericidal concentration (MBC) of lauric acid, *P. acnes* was anaerobically incubated in PBS with several concentrations of lauric acid for 5 hours at 37 °C. After incubation, the bacteria were diluted with PBS and spotted on an agar plate to count a colony-forming unit (CFU) (Figure 2). We found that when lauric acid concentration is higher than 60 μg ml^{-1}, it started killing *P. acnes*. In contrast, no killing was observed when lauric acid is at 50 μg ml^{-1}. Therefore, we estimated the MBC of lauric acid against *P. acnes* (ATCC 6919) was 60 μg ml^{-1}. The same experiments were carried out to another strain of *P. acnes* (ATCC 11827) leading to an MBC of 80 μg ml^{-1} (Figure S3).

Cytotoxicity of lauric acid

Human sebocyte is one of the major target cells of *P. acnes* in acne patients. So cytotoxicity of lauric acid was examined using the human immortalized sebaceous gland cell line, SZ95 (Zouboulis *et al.*, 1999) (Figure 3). In the study, SZ95 sebocytes were incubated with lauric acid at various concentrations from 0.24 to 500 μg ml^{-1} for 18 hours at 37 °C and cell viability was determined subsequently. It was found that lauric acid did not affect sebocyte viability at the concentrations tested. Notably, lauric acid did not influence the viability of SZ95 sebocytes even at the high concentration of 125 μg ml^{-1}, at which *P. acnes* was completely killed as shown in Figure 2.

Inflammatory acne model using mouse ear

To induce inflammation *in vivo* using *P. acnes*, 1×10^7 CFU of living *P. acnes* were intradermally injected into the ear of Institute of Cancer Research (ICR) mice. Significant ear swelling (Figure 4b) was observed in *P. acnes*-injected ear 24 hours after the injection. In contrast, no swelling was observed in the PBS-injected ear (Figures 4a). Histological observation revealed that injection with *P. acnes* induced a considerable increase in the number of infiltrated inflammatory cells (Figure 4b). Transmission electron microscopy showed colonized and/or phagocytized *P. acnes* in the macrophage-like cells 24 hours after bacterial injection (Figure 4c and d), but no bacteria were observed in the PBS-injected ear (Figure 4e). In addition, fluorescent immunohistochemistry was employed to visualize *P. acnes* injected ear and PBS-injected ear, respectively, using antimouse CD11b IgG, a conventional macrophage marker. The infiltration of $CD11b^+$ macrophages was observed at the site where *P. acnes* was administrated (Figure 4f), whereas no $CD11b^+$ macrophages were observed in PBS-injected controls (Figure 4g).

Effects of lauric acid on *P. acnes in vivo* and *P. acnes*-induced inflammation

To examine the antimicrobial effect of lauric acid *in vivo*, mouse ears were intradermally injected with *P. acnes* (1×10^7 CFU). The *P. acnes*-injected site was then injected with lauric

acid (2 μg) (Figure 5) for 1 day. Injection of lauric acid significantly reduced *P. acnes*-induced ear swelling (Figure 5a) and the number of *P. acnes* colonized within the ear (Figure 5b). Histological images showed that injection of lauric acid reduced granulomatous response to *P. acnes* (Figure 5d) compared with injection of an equal amount of vehicle (Figure 5e), whereas sole injection of lauric acid (without pre-injection of *P. acnes*) did not induce inflammatory reaction (Figure 5c). Furthermore, a TUNEL assay illustrated that the injection of lauric acid did not trigger skin cells to undergo apoptosis (Figure S4). To examine a therapeutic potential of lauric acid by epicutaneous application for acne therapy, lauric acid (150 μg) in Vaseline was applied epicutaneously on the ear injected with *P. acnes* (1 ± 10^7 CFU). Epicutaneous application of lauric acid for 1 day noticeably reduced *P. acnes*-induced ear swelling (Figure 6a) and the number of *P. acnes* colonized within the ear (Figure 6b). Furthermore, data from TUNEL assays indicated that epicutaneous application of lauric acid did not result in the apoptosis of differentiated keratinocytes (Figure 6c). These data suggest that epicutaneous application of lauric acid can effectively relieve *P. acnes*-induced inflammation without detrimental effects on skin cells.

DISCUSSION

P. acnes produces a number of extracellular enzymes and metabolites that can directly damage host tissues (Hoeffler, 1977; Holland *et al.*, 1981; Cove *et al.*, 1983; Hoffler *et al.*, 1985). One of the well-known enzymes is extracellular triacylglycerol lipase that produces FFAs by hydrolyzing triglycerides in sebum (Miskin *et al.*, 1997). Sebum FFAs, if overproduced, induce very mild inflammation and assist bacterial adherence and colonization in sebaceous follicles (Puhvel and Sakamoto, 1977; Gribbon *et al.*, 1993). On the other hand, FFAs of various chain lengths (C^8- C^{18}) have antibacterial activity against a range of Gram-positive bacteria, but not against a number of Gram-negative bacteria (Wille and Kydonieus, 2003; Georgel *et al.*, 2005; Skrivanova *et al.*, 2005; Drake *et al.*, 2008). Lauric acid is a minor sebum component (1-2% of total sebum FFA) (Bach and Babayan, 1982), but it is the most active antimicrobial FFA (Kabara *et al.*, 1972; Kitahara *et al.*, 2004; Rouse *et al.*, 2005; Skrivanova *et al.*, 2005). Palmitoleic acid isoforms (C16:1Δ6 or C16:1Δ9), a major FFA in the sebum, causes a reduction in the growth of the Gram-positive bacteria *Staphylococcus*, *Streptococcus*, and *Fusobacterium*, but is ineffective against *P. acnes* (Wille and Kydonieus, 2003). Previous studies have demonstrated antimicrobial activities of middle chain saturated FFAs, including lauric acid, against *P. acnes in vitro* (Puhvel and Reisner, 1970; Higaki, 2003). Despite a number of studies on antimicrobial activity of FFAs *in vitro* have been made, little effort has been conducted to demonstrate the antimicrobial activity of FFAs against *P. acnes in vivo* to evaluate its potential for acne therapy.

Our results indicate that lauric acid exerts the inhibitory effect on the growth of skin bacteria such as *P. acnes*, *S. aureus*, and *S. epidermidis* at a concentration 15 times lower than that of BPO, a frequently used oxidizing agent in acne treatment (Figure 1, Table 1). In addition, ED_{50} values from dose-response curves showed that *P. acnes* is the most sensitive bacteria to lauric acid among the skin bacteria tested (Figure 1 and Figure S2, Table 1). Lauric acid exerts antimicrobial activity on two *P. acnes* strains (ATCC 6919 and ATCC 11827) (Figure 1 and Figure S2) and other strains isolated from human sebaceous material (Puhvel and Reisner, 1970). Because acne patients may harbor different skin microbes and *P. acnes* strains, it is worthwhile to test the potency of lauric acid on a variety of clinical bacteria isolates in the future. In addition, although it has been known that FFAs have antibacterial activities against many Gram-positive bacteria (Drake *et al.*, 2008), it is worthwhile to investigate if lauric acid exhibits antibacterial activity against Gramnegative bacteria in skins. It has been demonstrated that FFAs can be integrated into liposomes (Araseki *et al.*, 2002). Engineering a lauric acid-integrated liposome may circumvent the precipitation problem of water-insoluble lauric acid at high concentrations. The MBC assay indicated that 5 hours incubation of 100 μg ml^{-1} of

lauric acid completely killed two *P. acnes* strains at (1×10^7 CFU per ml) (Figures 2 and Figure S3). Determination of the bactericidal effects of lauric acid as a function of bacterial numbers may provide valuable information for treatments of various stages of acne vulgaris. On the other hand, when lauric acid was incubated with human sebocytes for 18 hours, whose activity is highly involved in the development of acne, lauric acid did not show cytotoxicity at the concentrations exerting antimicrobial activity (Figure 3). In incubations of the Gram-positive bacteria *Clostridium perfringens* or *S. aureus* with lauric acid or capric acid (C10:0), respectively, separation in inner and outer membranes and complete cytoplasmic disorganization were observed in the treated cells despite the lack of alteration in cell wall structure and cell size (Bergsson *et al.*, 2001;Skrivanova *et al.*, 2005). Taken together, lauric acid may specifically disrupt bacterial membranes but not mammalian cell membranes.

Significant antibiotic resistance and multiple drug resistance have been identified for *P. acnes* strains from acne patients with long-term antibiotic treatments (Eady *et al.*, 2003; Nord and Oprica, 2006). Moreover, biofilm formation by *P. acnes* increases its resistance against antimicrobial agents (Coenye *et al.*, 2007). These problems of resistance may cause the failure of antibiotic treatment against acne. Lauric acid, however, has been shown to exhibit antimicrobial activity against methicillin-resistant *S. aureus* (Kitahara *et al.*, 2004). A previous study showed that antimicrobial FFA treatment led to less frequent development of spontaneous resistant bacteria strains compared with treatments with the frequently used antibiotics, tetracycline and metronidazole (Petschow *et al.*, 1996). In addition, bactericidal effects of lauric acid was equally observed on both planktonic and biofilm-formed bacteria (Chavant *et al.*, 2004). Thus, lauric acid may have the potential to be used as an effective antibacterial treatment for antibiotic-refractory acne.

The study of immune responses to *P. acnes* required an acne animal model, which would be an important tool in the evaluation of the antimicrobial activity of lauric acid against *P. acnes in vivo*. Proliferation of *P. acnes* starts in the microcomedone, which is the precursor acne lesion characterized by hyperkeratinization, formation of a keratin plug, and increase in sebum secretion by the sebaceous gland (Leeming *et al.*, 1988; Leyden *et al.*, 1998; Cunliffe and Gollnick, 2001; Leyden, 2001). Overgrowth of *P. acnes* in the microcomedones results in the rupture of the epithelium of the sebaceous follicle, allowing bacteria to enter the dermis. Consequently, bacteria contact with the host immune system, causing granulomatous inflammation (typical inflammatory acne) (Kligman, 1974; Toyoda and Morohashi, 2001; Degitz *et al.*, 2007). To create an animal model for *P. acnes*-induced inflammation, the ear of ICR mouse was intradermally injected with *P. acnes*. Ear swelling was then measured according to a rat ear model as described earlier (De Young *et al.*, 1984, 1985). Our data show that intradermal challenge of mouse ear with *P. acnes* attracts numerous macrophages to the site of *P. acnes* (Figure 4). The profile of the granulomatous inflammation in the mouse ear is similar to that of the inflammatory acne in the human sebaceous follicle because numerous *P. acnes* have been observed inside phagosomes of an infiltrating macrophage in an inflammatory acne lesion (Toyoda and Morohashi, 2001). *P. acnes* is resistant to phagocytes and is able to survive in macrophages (Webster *et al.*, 1985). In our previous data obtained using a tissue chamber model integrated with a dermis-based cell-trapped system to mimic the *in vivo* microenvironment of acne lesions, injection of living *P. acnes* into the intradermally implanted tissue chamber attracted Gr-1^+ neutrophils and CD11b^+ macrophages into the chamber (Nakatsuji *et al.*, 2008d) and increased the level of proinflammatory cytokine and macrophage inflammatory protein-2 in the chamber fluid (Nakatsuji *et al.*, 2008a, b). We have recently developed effective vaccines for *P. acnes* associated inflammation as an alternative treatment for acne. The vaccines consist of killed-whole organism of *P. acnes* and a cell wall-anchored *P. acnes* sialidase (Nakatsuji *et al.*, 2008a, b, c). The vaccine may be effective against *P. acnes* outside of a cell but not against phagocytized and/ or invading bacteria. However, *P. acnes* inside cells may be susceptible to the antimicrobial activities of lauric acid, which is cell

membrane permeable. Our data demonstrate that either intradermal or epicutaneous administration of lauric acid with *P. acnes* decreased the number of detected bacteria in the ear, thereby relieving *P. acnes*-induced ear swelling and granulomatous inflammation (Figures 5 and 6). In addition, application of lauric acid did not trigger the skin cells to undergo apoptosis (Figures 6 and Figure S4). These data suggest that lauric acid can suppress pathogenicity of *P. acnes in vivo*, but is harmless to host cells. As human skin is in general thicker than mouse ear skin, a higher dosage with repeated applications of lauric acid may be required for acne treatments in clinics.

Epicutaneous application of lauric acid enhances the activity of antibiotic agents, skin permeability, and transdermal drug delivery of antibiotic chemicals (Lee *et al.*, 1994; Kravchenko *et al.*, 2003). Conjugation of lauric acid with synthetic antimicrobial peptide results in an increased ability of the compound to permeabilize bacteria membranes (Chu-Kung *et al.*, 2004). Thus, a combination of topical antimicrobial therapies for acne with lauric acid may induce synergistically enhanced effectiveness. In conclusion, we demonstrated the antimicrobial property of lauric acid against *P. acnes in vitro* and its therapeutic effects on *P. acnes*-induced inflammation *in vivo* using the ICR mouse ear model. The obtained data highlight the potential of using lauric acid as an alternative treatment option to the antibiotic therapy of acne vulgaris.

MATERIALS AND METHODS

Preparation of bacteria

P. acnes (ATCC 6919 and ATCC 11827) (American Type Culture Collection, Manassas, VA) was cultured on Brucella broth agar (BD, Sparks, MD), supplemented with 5% (v v^{-1}) defibrinated sheep blood (LAMPIRE Biological Laboratories, Pipersville, PA), vitamin K (5 μg ml^{-1}, Remel, Lenexa, KS), and hemin (50 μg ml^{-1}, Remel), under anaerobic conditions using Gas-Pak (BD) at 37 °C. Single colonies were inoculated in Reinforced Clostridium Medium (Oxford, Hampshire, England) and cultured at 37 °C until reaching around OD_{600}=1.0 (logarithmic growth phase) under anaerobic conditions. *S. aureus* (ATCC 35556) or *S. epidermidis* (ATCC 12228) was cultured on tryptic soy broth (Sigma, St. Louis, MO) agar overnight at 37 °C. The bacteria from single colonies were cultured in tryptic soy broth overnight at 37 °C. The overnight culture was diluted 1:20 and cultured until reaching around OD_{600}=1.0. These bacteria were harvested by centrifugation at 5,000 *g* for 10 minutes, washed with PBS, and suspended to an appropriate amount of PBS for the experiments.

In vitro antimicrobial assays

MIC of lauric acid was compared with that of BPO, which has long been used clinically in acne treatment. Lauric acid (Sigma) or BPO (Fisher scientific, Pittsburgh, PA) were dissolved in DMSO and then added to bacteria suspension to obtain the final concentration of 5% (v v^{-1}) DMSO. *P. acnes* (1×10^6 CFU per ml) was incubated with lauric acid or BPO at the concentrations of twofold serial dilution (0.24-500 μg ml^{-1}) in Reinforced Clostridium Medium on a 96-well microplate (100 μl per well) under anaerobic conditions for 72 hours. *S. aureus* or *S. epidermidis* (1×10^6 CFU per ml) were incubated with the same concentrations of lauric acid and BPO in tryptic soy broth under aerobic conditions for 24 or 48 hours, respectively. The control received only 5% (v v^{-1}) DMSO. After incubation, the plates were mixed well and then absorbance at 600 nm was measured by a microplate reader to estimate bacterial growth.

To determine MBC of lauric acid against *P. acnes*, *P. acnes* (1×10^7 CFU per ml) was incubated with lauric acid at various concentrations (12.5-100 μg ml^{-1}) in PBS on a 96-well microplate (100 μl per well) under anaerobic conditions. The control received only 5% (v v^{-1}) of DMSO.

The growth of *P. acnes* was decreased 1 hour after incubation and completely eliminated 3 and 5 hours after incubation with lauric acid (Figure S5). The reaction mixture was diluted 1:10-1:10^6 with PBS. MBC was determined by spotting the dilution (5 μl) on a Brucella broth agar plate for the counting of CFUs.

Cytotoxicity of lauric acid on human sebocytes

The immortalized human sebaceous gland cell line SZ95 (Zouboulis *et al.*, 1999), was cultured on 96-well plates in Sebomed basal medium (Biochrom, Berlin, Germany) supplemented with 5 ng ml^{-1} human recombinant epidermal growth factor (Sigma), 10% (v v^{-1}) heat-inactivated fetal bovine serum (Mediatech Inc., Herndon, VA), at 37 °C under an atmosphere of 5% (v v^{-1}) CO_2 in air. Lauric acid was prepared in DMSO and added to the culture medium at various concentrations for the final concentration of 5% DMSO. SZ95 sebocytes (1×10^5 cells per well) were incubated with lauric acid for 18 hours at 37 °C. As a negative control, an equal amount of DMSO was added to the culture medium. Triton X-100 (0.01%) was used to achieve 100% of cell cytotoxicity. After incubation, cell viability of sebocytes was determined with acid phosphatase assays (Martin and Clynes, 1991). Cells were washed with PBS three times and incubated with 100 μl of 10m$_M$ *p*-nitrophenyl phosphate in acid phosphatase assay buffer [1 $_M$ sodium acetate buffer, pH 5.5, containing 0.1% (w v^{-1}) Triton X-100] for 1 hour at 37 °C. After that, 10 μl of 1N NaOH was added to stop the reaction and absorbance at 405 nm was measured. Cytotoxicity of lauric acid was calculated as the percentage of cytotoxicity of Triton X-100 ((the OD_{405} difference without and with lauric acid treatment) ÷ (the OD_{405} difference without lauric acid and with Triton X-100 treatment) × 100 (%)).

Transmission electron microscopy

Ears of ICR mice were intradermally injected with 1×10^7 CFU per 20 μl of live *P. acnes* (ATCC 6919) suspension in PBS or PBS alone. The ear was excised 24 hours after bacterial challenge, fixed in Karnovsky's fixative (4% paraformaldehyde, 2.5% glutaraldehyde, 5m$_M$ $CaCl_2$ in 0.1 $_M$ Na Cacodylate buffer, pH 7.4) overnight at 4 °C followed by 1% OsO_4 in 0.1 $_M$ Na Cacodylate buffer, pH 7.4, *en bloc* staining with 4% uranyl acetate in 50% ethanol, and subsequently dehydrated using a graded series of ethanol solutions followed by propylene oxide and infiltration with epoxy resin (Scipoxy 812, Energy Beam Sciences, Agawam, MA). After polymerization at 65 °C overnight, thin sections were cut and stained with uranyl acetate (4% uranyl acetate in 50% ethanol) followed by bismuth subnitrate. Sections were examined at an accelerating voltage of 60 kV using a Zeiss EM10C electron microscope (Carl Zeiss, Thornwood, NY).

Fluorescence immunohistochemistry

To induce inflammation, mouse ear was intradermally injected with live *P. acnes* (ATCC 6919) as described above. The ear was excised 24 hours after bacterial injection, fixed in Optimal Cutting Temperature (OCT) compound (Sakura Finetek, Torrance, CA), and frozen at −80 °C. The tissue block was cross-sectioned with Cryostat and fixed in 10% formamide in PBS. The sections were blocked with PBS containing 5% bovine serum albumin (BSA) and anti-mouse CD16/CD32 IgG (5 μg ml^{-1}) (BD Biosciences Pharmingen, Sparks, MD) for 30 minutes, and incubated with biotinylated anti-mouse CD11b IgG (5 μg ml^{-1}) (BD Biosciences), a conventional macrophage marker. Tetramethylrhodamine isothiocyanate (TRITC)-streptavidin conjugate (5 μg ml^{-1}) (ZYMED, Carlsbad, CA) was applied to the section, which was incubated for 20 minutes at room temperature, followed by 4′-6-Diamidino-2-phenylindole (Sigma). Images were obtained using an Olympus BX41 fluorescent microscope (Olympus, Center Valley, PA).

To examine the toxicity of lauric acid *in vivo*, the ear was excised for cross-sections 24 hours after intradermal injection or epicutaneous application of lauric acid. To detect the apoptotic

cells, the tissue sections were stained with a DeadEnd Fluorometric Terminal Deoxynucleotidyl Transferase TUNEL System (Promega, Madison, WI) followed by immuno-reactions to rabbit anti-mouse keratin 10 (K10) IgG (1:1,000 dilution) (Covance, Berkeley, CA) and goat anti-rabbit IgG-TRITC conjugate (5 μg ml^{-1}).

P. acnes-induced inflammation

P. acnes (ATCC 6919) (1×10^7 CFU per 20 μl in PBS) was intradermally injected into left ears of ICR mice. Right ears received with an equal amount (20 μl) of PBS. Lauric acid (2 μg20μl^{-1} in 5% DMSO in PBS) was injected into the same location of both ears right after *P. acnes* or PBS injection. As a control, an equal volume (20 μl) of 5% DMSO in PBS was intradermally injected into both the ears. For epicutaneous application, lauric acid (150 μg in 5% acetone mixed with 15 mg of Vaseline (Sigma)) was applied on the surface of the ear skin right after intradermal injection with *P. acnes* (1×10^7 CFU per 20 μl) or PBS (20 μl). Epicutaneous application of 5% acetone mixed with 15 mg of Vaseline served as a control. For histological observation, the ear was cross-sectioned, stained with hematoxylin and eosin (Sigma), and viewed on a Zeiss Axioskop2 plus microscope (Carl Zeiss). The increase in ear thickness was measured using a micro caliper (Mitutoyo, Kanagawa, Japan) 24 hours after the bacterial injection. The increase in ear thickness of the *P. acnes*-injected ear was calculated as percentage of a PBS-injected control.

To determine *P. acnes* number in the ear, the ear was cut off and punched with an 8 mm biopsy punch 24 hours after *P. acnes* injection. Wet kimwipes were used to remove the Vaseline remaining on the ear surfaces. The punch biopsy was homogenized in 200 μl of sterile PBS with a hand tissue grinder. CFUs of *P. acnes* in the ear were enumerated by plating serial dilutions ($1:10^2$-$1:10^8$) of the homogenate on a Brucella agar plate. To count colonies, the plate was anaerobically incubated for 72 hours at 37 °C. All experiments using mice were conducted according to institutional guidelines for animal experiments.

Statistical analysis

Data are presented as mean±SE. The Student's *t*-test was used to assess the significance of independent experiments. The criterion $P<0.05$ was used to determine the statistical significance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health Grants (R01-AI067395-01, R21-R022754-01 and R21-I58002-01). We thank Dr Y.-T. Liu for providing valuable comments and Corbin Clawson for proofreading.

Abbreviations

BPO, benzoyl peroxide; CFU, colony forming unit; FFA, free fatty acids; ICR, Institute of Cancer Research; MBC, minimal bactericidal concentration; P. acnes, Propionibacterium acnes.

REFERENCES

Araseki M, Yamamoto K, Miyashita K. Oxidative stability of polyunsaturated fatty acid in phosphatidylcholine liposomes. Biosci Biotechnol Biochem 2002;66:2573–7. [PubMed: 12596850]

Bach AC, Babayan VK. Medium-chain triglycerides: an update. Am J Clin Nutr 1982;36:950–62. [PubMed: 6814231]

Bergsson G, Arnfinnsson J, Steingrimsson O, Thormar H. Killing of Gram-positive cocci by fatty acids and monoglycerides. Apmis 2001;109:670–8. [PubMed: 11890570]

Bojar RA, Holland KT. Acne and *Propionibacterium acnes*. Clin Dermatol 2004;22:375–9. [PubMed: 15556721]

Bruggemann H. Insights in the pathogenic potential of *Propionibacterium acnes* from its complete genome. Semin Cutan Med Surg 2005;24:67–72. [PubMed: 16092793]

Bruggemann H, Henne A, Hoster F, Liesegang H, Wiezer A, Strittmatter A, et al. The complete genome sequence of *Propionibacterium acnes*, a commensal of human skin. Science 2004;305:671–3. [PubMed: 15286373]

Burkhart CG, Burkhart CN, Lehmann PF. Acne: a review of immunologic and microbiologic factors. Postgrad Med J 1999;75:328–31. [PubMed: 10435165]

Burtenshaw JML. Self-disinfection of the skin: a short review and some original observations. Brit Med Bull 1945;3:161–9.

Chavant P, Gaillard-Martinie B, Hebraud M. Antimicrobial effects of sanitizers against planktonic and sessile Listeria monocytogenes cells according to the growth phase. FEMS Microbiol Lett 2004;236:241–8. [PubMed: 15251203]

Chu-Kung AF, Bozzelli KN, Lockwood NA, Haseman JR, Mayo KH, Tirrell MV. Promotion of peptide antimicrobial activity by fatty acid conjugation. Bioconjug Chem 2004;15:530–5. [PubMed: 15149180]

Coenye T, Peeters E, Nelis HJ. Biofilm formation by *Propionibacterium acnes* is associated with increased resistance to antimicrobial agents and increased production of putative virulence factors. Res Microbiol 2007;158:386–92. [PubMed: 17399956]

Cove JH, Holland KT, Cunliffe WJ. Effects of oxygen concentration on biomass production, maximum specific growth rate and extracellular enzyme production by three species of cutaneous propionibacteria grown in continuous culture. J Gen Microbiol 1983;85:3327–34. [PubMed: 6663280]

Cunliffe, WJ.; Gollnick, HP. Microbiology of acne. In: Cunliffe, WJ.; Gollnick, HP., editors. Acne. Martin Dunitz; Kent: 2001. p. 29-36.

De Young LM, Spires DA, Ballaron SJ, Cummins CS, Young JM, Allison AC. Acne-like chronic inflammatory activity of *Propionibacterium acnes* preparations in an animal model: correlation with ability to stimulate the reticuloendothelial system. J Invest Dermatol 1985;85:255–8. [PubMed: 3161957]

De Young LM, Young JM, Ballaron SJ, Spires DA, Puhvel SM. Intradermal injection of *Propionibacterium acnes*: a model of inflammation relevant to acne. J Invest Dermatol 1984;83:394–8. [PubMed: 6238104]

Degitz K, Placzek M, Borelli C, Plewig G. Pathophysiology of acne. J Dtsch Dermatol Ges 2007;5:316–23. [PubMed: 17376098]

Drake DR, Brogden KA, Dawson DV, Wertz PW. Thematic review series: skin lipids. Antimicrobial lipids at the skin surface. J Lipid Res 2008;49:4–11. [PubMed: 17906220]

Dreno B, Poli F. Epidemiology of acne. Dermatology 2003;206:7–10. [PubMed: 12566799]

Eady EA, Gloor M, Leyden JJ. *Propionibacterium acnes* resistance: a worldwide problem. Dermatology 2003;206:54–6. [PubMed: 12566805]

Gallo RL, Huttner KM. Antimicrobial peptides: an emerging concept in cutaneous biology. J Invest Dermatol 1998;111:739–43. [PubMed: 9804331]

Georgel P, Crozat K, Lauth X, Makrantonaki E, Seltmann H, Sovath S, et al. A toll-like receptor 2-responsive lipid effector pathway protects mammals against skin infections with gram-positive bacteria. Infect Immun 2005;73:4512–21. [PubMed: 16040962]

Gollnick H, Schramm M. Topical drug treatment in acne. Dermatology 1998;196:119–25. [PubMed: 9557245]

Gribbon EM, Cunliffe WJ, Holland KT. Interaction of *Propionibacterium acnes* with skin lipids *in vitro*. J Gen Microbiol 1993;139:1745–51. [PubMed: 8409917]

Higaki S. Lipase inhibitor for the treatment of acne. J Mol Cata B Enzym 2003;22:377–84.

Hoeffler U. Enzymatic and hemolytic properties of *Propionibacterium acnes* and related bacteria. J Clin Microbiol 1977;6:555–8. [PubMed: 201661]

Höffler U, Gehse M, Gloor M, Pulverer G. Enzyme production of propionibacteria from patients with acne vulgaris and healthy persons. Acta Derm Venereol 1985;65:428–32. [PubMed: 2416168]

Holland KT, Ingham E, Cunliffe WJ. A review, the microbiology of acne. J Appl Bacteriol 1981;51:195–215. [PubMed: 6457823]

Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP. Fatty acids and derivatives as antimicrobial agents. Antimicrob Agents Chemother 1972;2:23–8. [PubMed: 4670656]

Kim J. Review of the innate immune response in acne vulgaris: activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. Dermatology 2005;211:193–8. [PubMed: 16205063]

Kim J, Ochoa MT, Krutzik SR, Takeuchi O, Uematsu S, Legaspi AJ, et al. Activation of toll-like receptor 2 in acne triggers inflammatory cytokine responses. J Immunol 2002;169:1535–41. [PubMed: 12133981]

Kitahara T, Koyama N, Matsuda J, Aoyama Y, Hirakata Y, Kamihira S, et al. Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant Staphylococcus aureus. Biol Pharm Bull 2004;27:1321–6. [PubMed: 15340213]

Kligman AM. An overview of acne. J Invest Dermatol 1974;62:268–87. [PubMed: 4274207]

Kravchenko IA, Golovenko NY, Larionov VB, Aleksandrova AI, Ovcharenko NV. Effect of lauric acid on transdermal penetration of phenazepam *in vivo*. Bull Exp Biol Med 2003;136:579–81. [PubMed: 15500077]

Lee CK, Uchida T, Kitagawa K, Yagi A, Kim NS, Goto S. Relationship between lipophilicity and skin permeability of various drugs from an ethanol/water/lauric acid system. Biol Pharm Bull 1994;17:1421–4. [PubMed: 7874070]

Leeming JP, Holland KT, Cuncliffe WJ. The microbial colonization of inflamed acne vulgaris lesions. Br J Dermatol 1988;118:203–8. [PubMed: 2964856]

Leyden JJ. The evolving role of *Propionibacterium acnes* in acne. Semin Cutan Med Surg 2001;20:139–43. [PubMed: 11594668]

Leyden JJ, McGinley KJ, Vowels B. *Propionibacterium acnes* colonization in acne and nonacne. Dermatology 1998;196:55–8. [PubMed: 9557227]

Miskin JE, Farrell AM, Cunliffe WJ, Holland KT. *Propionibacterium acnes*, a resident of lipid-rich human skin, produces a 33 kDa extracellular lipase encoded by gehA. Microbiology 1997;143(Part 5):1745–55. [PubMed: 9168624]

Nagy I, Pivarcsi A, Kis K, Koreck A, Bodai L, McDowell A, et al. *Propionibacterium acnes* and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. Microbes Infect 2006;8:2195–205. [PubMed: 16797202]

Nakatsuji T, Liu YT, Huang CP, Gallo RL, Huang CM. Antibodies Elicited by Inactivated *Propionibacterium acnes*-Based Vaccines Exert Protective Immunity and Attenuate the IL-8 Production in Human Sebocytes: Relevance to Therapy for Acne Vulgaris. J Invest Dermatol 2008a; 128:2451–7. [PubMed: 18463682]

Nakatsuji T, Liu YT, Huang CP, Gallo RL, Huang CM. Vaccination targeting a surface sialidase of *P. acnes*: implication for new Treatment of acne vulgaris. PLoS ONE 2008b;3:e1551. [PubMed: 18253498]

Nakatsuji T, Rasochova L, Huang CM. Vaccine therapy for *P. acnes*-associated diseases. Infect Disord Drug Targets 2008c;8:160–5. [PubMed: 18782033]

Nakatsuji T, Shi Y, Zhu W, Huang CP, Chen YR, Lee DY, et al. Bioengineering a humanized acne microenvironment model: proteomics analysis of host responses to *P. acnes* infection *in vivo*. Proteomics 2008d;8:3406–15. [PubMed: 18651708]

Nord CE, Oprica C. Antibiotic resistance in *Propionibacterium acnes*. Microbiological and clinical aspects. Anaerobe 2006;12:207–10. [PubMed: 17000123]

Petschow BW, Batema RP, Ford LL. Susceptibility of Helicobacter pylori to bactericidal properties of medium-chain monoglycerides and free fatty acids. Antimicrob Agents Chemother 1996;40:302–6. [PubMed: 8834870]

Puhvel SM, Reisner RM. Effect of fatty acids on the growth of *Corynebacterium acnes in vitro*. J Invest Dermatol 1970;54:48–52. [PubMed: 5416678]

Puhvel SM, Sakamoto M. A reevaluation of fatty acids as inflammatory agents in acne. J Invest Dermatol 1977;68:93–7. [PubMed: 137937]

Rosen T. The *Propionibacterium acnes* genome: from the laboratory to the clinic. J Drugs Dermatol 2007;6:582–6. [PubMed: 17668523]

Rouse MS, Rotger M, Piper KE, Steckelberg JM, Scholz M, Andrews J, et al. *In vitro* and *in vivo* evaluations of the activities of lauric acid monoester formulations against *Staphylococcus aureus*. Antimicrob Agents Chemother 2005;49:3187–91. [PubMed: 16048923]

Skrivanova E, Marounek M, Dlouha G, Kanka J. Susceptibility of *Clostridium perfringens* to C-C fatty acids. Lett Appl Microbiol 2005;41:77–81. [PubMed: 15960756]

Toyoda M, Morohashi M. Pathogenesis of acne. Med Electron Microsc 2001;34:29–40. [PubMed: 11479771]

Webster GF, Leyden JJ. Characterization of serum-independent polymorphonuclear leukocyte chemotactic factors produced by *Propionibacterium acnes*. Inflammation 1980;4:261–9. [PubMed: 7429606]

Webster GF, Leyden JJ, Musson RA, Douglas SD. Susceptibility of *Propionibacterium acnes* to killing and degradation by human neutrophils and monocytes *in vitro*. Infect Immun 1985;49:116–21. [PubMed: 2989178]

Wille JJ, Kydonieus A. Palmitoleic acid isomer (C16:1delta6) in human skin sebum is effective against gram-positive bacteria. Skin Pharmacol Appl Skin Physiol 2003;16:176–87. [PubMed: 12677098]

Zouboulis CC, Seltmann H, Neitzel H, Orfanos CE. Establishment and characterization of an immortalized human sebaceous gland cell line (SZ95). J Invest Dermatol 1999;113:1011–20. [PubMed: 10594745]

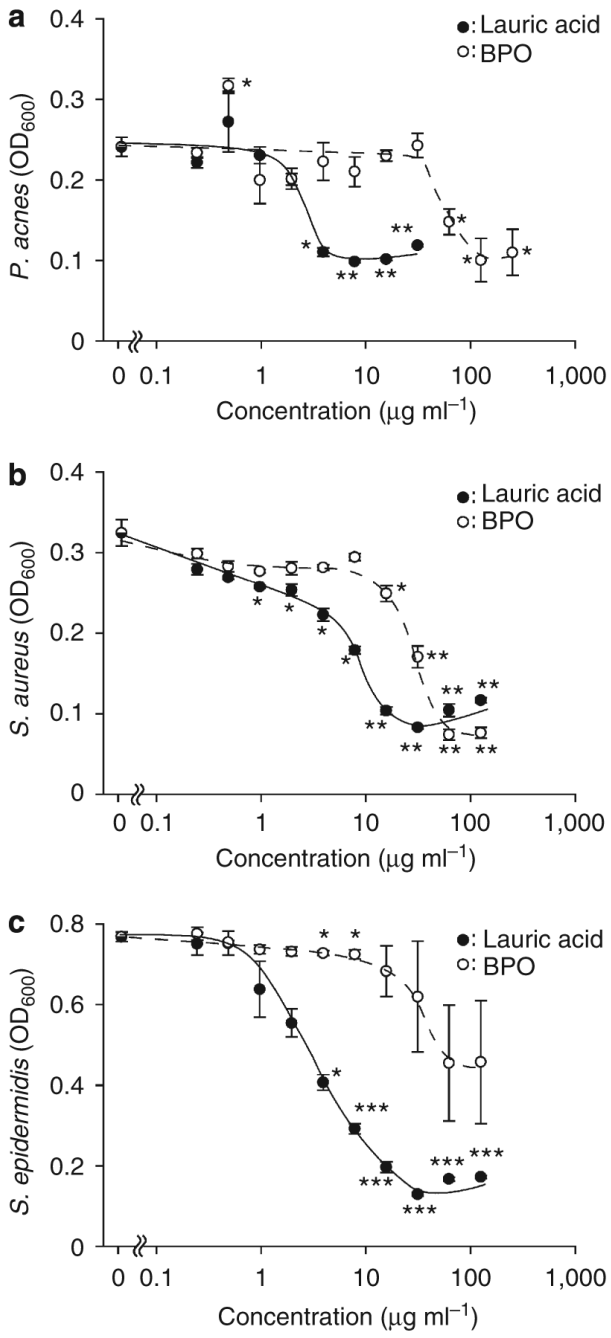

Figure 1. Inhibitory effects of lauric acid on bacterial growth

(**a**) *P. acnes* (1×10^6 CFU per ml), (**b**) *S. aureus*, ATCC 35556 (1×10^6 CFU per ml), and (**c**) *S. epidermidis*, ATCC 12228 (1×10^6 CFU per ml) were incubated with lauric acid (solid circles) and BPO (open circles), in 5% DMSO under anaerobic conditions at 37 °C for 72, 24, and 48 hours, respectively. After incubation, OD_{600} of each sample was measured by a microplate reader to determine bacterial growth. Data represent mean±SE of three individual experiments (**P*<0.05, ***P*<0.005, ****P*<0.0005 by Student's *t*-test).

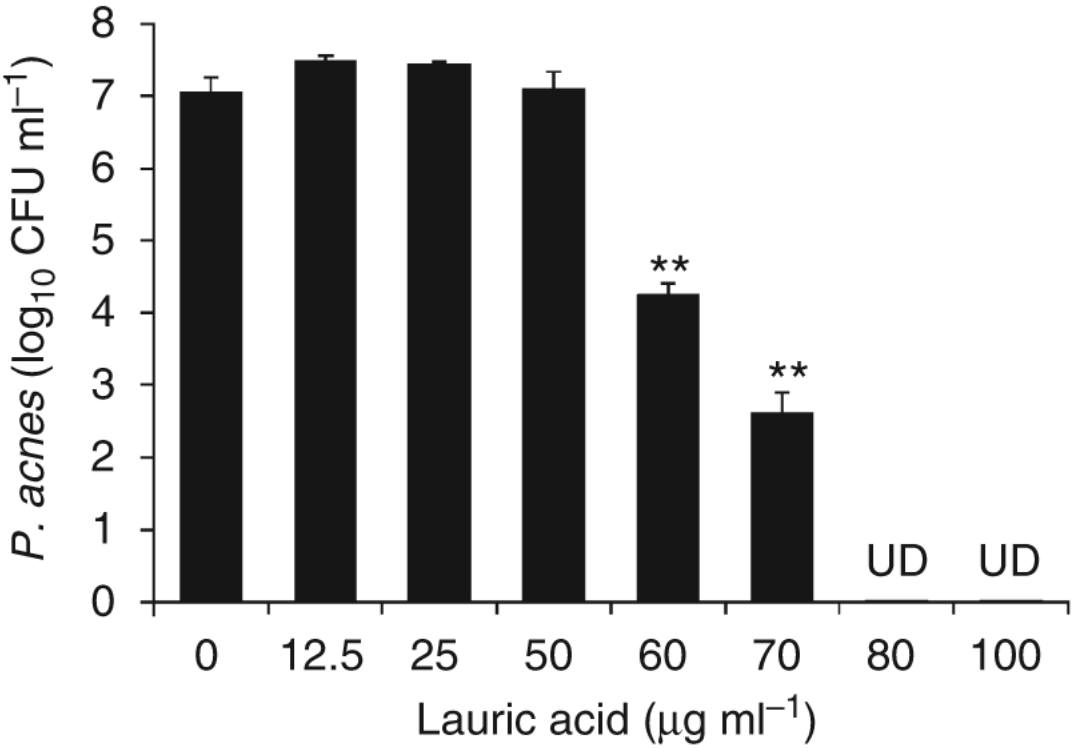

Figure 2. Bactericidal effects of lauric acid on *P. acnes*

P. acnes (1×10^7 CFU per ml) was incubated with 0-100 μg ml^{-1} of lauric acid in 5% DMSO in PBS for 5 hours under anaerobic conditions. After incubation, *P. acnes* suspension was diluted 1:10-1:10^6 with PBS, and 5 μl of the dilutions was spotted on a Brucella broth agar plate supplemented with 5% defibrinated sheep blood and hemin and vitamin K. After liquid in the *P. acnes* suspension was absorbed into the agar, the plate was incubated under anaerobic conditions to quantify CFU of *P. acnes*. Data represent mean±SE of three individual experiments (**$P<0.005$ by Student's *t*-test). UD: undetectable.

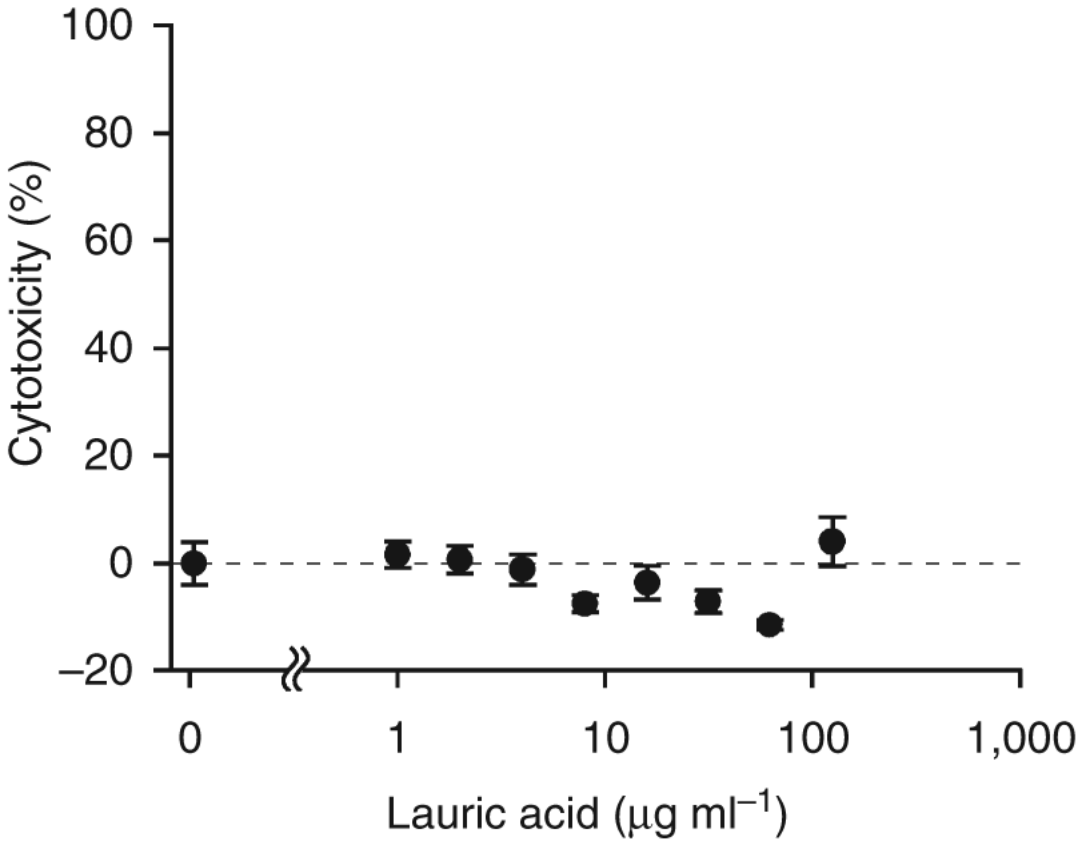

Figure 3. Cytotoxicity of lauric acid on human sebocytes

The immortalized human SZ95 sebocytes (1×10^5 cells) were incubated with the indicated concentrations of lauric acid in Sebmed supplemented with 1% fetal bovine serum, 5 ng ml^{-1} EGF at 37 °C for 18 hours. As a background, Triton X-100 [0.1% (v v^{-1})] was added to achieve 100% of cell cytotoxicity. After incubation, cell viability of sebocytes was determined with p-nitrophenyl phosphate, and the cytotoxicity of a neutralizing mixture was calculated as described in Materials and Methods. Data represent mean±SE of five individual experiments.

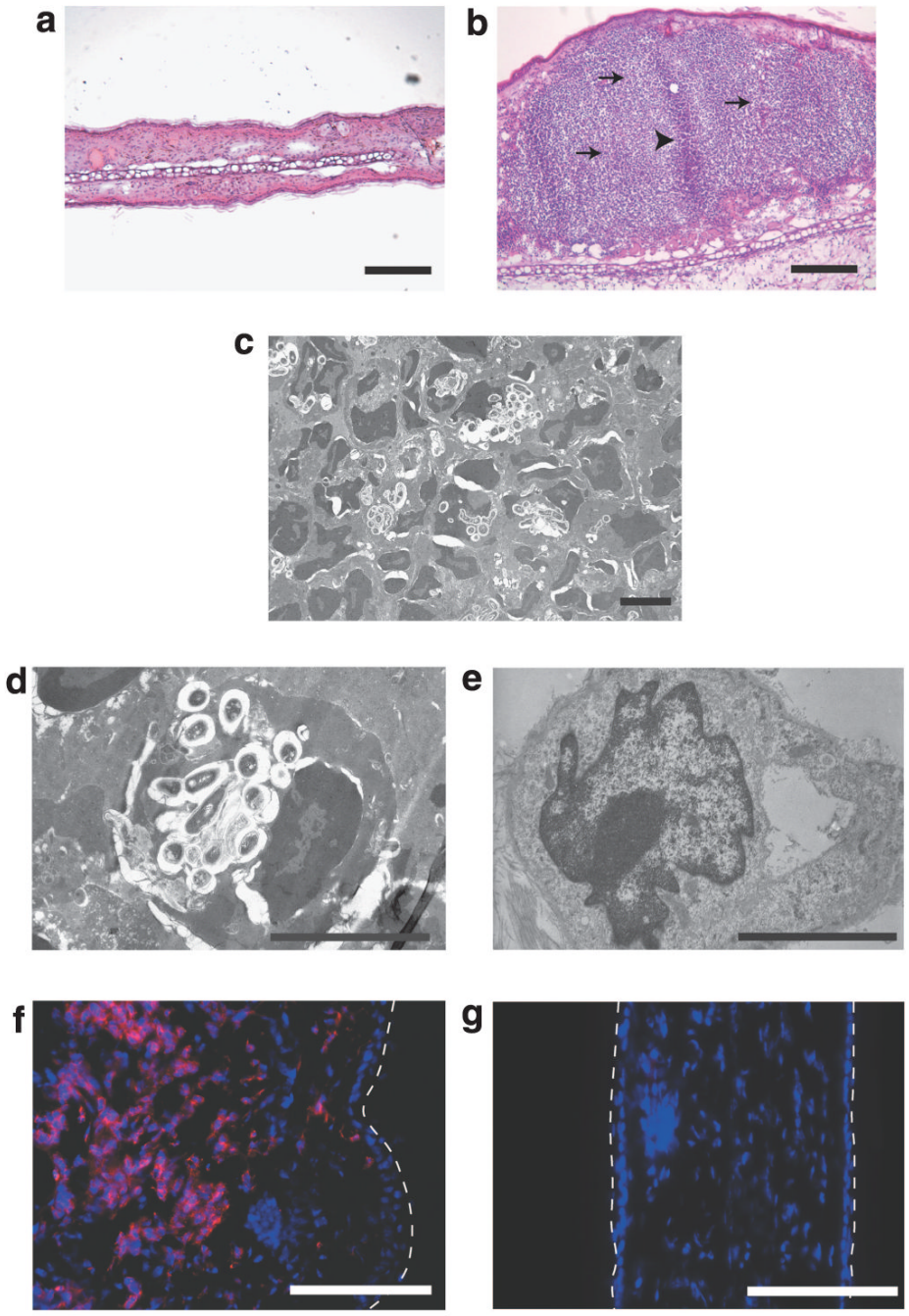

Figure 4. Inflammatory acne model using mouse ears

Ears of ICR mice were injected intradermally with 1×10^7 CFU per 20 μl of *P. acnes* (left ear), or 20 μl of PBS (right ear) and observed by hematoxylin and eosin (H&E) staining (**a, b**), transmission electron microscopy, (**c-e**), and fluorescence immunohistochemistry (**f, g**) 24 hours after *P. acnes* injection. (**a, b**) Increase in ear thickness and infiltrated inflammatory cells (arrows) surrounding the injection site of *P. acnes* (arrowhead) were observed at an H&E-stained frozen section of the *P. acnes*-injected ear (**b**), but not the PBS-injected ear (**a**). Scale bar = 200 μm. (**c-e**) Colonized and/or phagocytized *P. acnes* were observed in macrophage-like cells (**c** and **d**; ×8,000 and ×24,000 magnifications, respectively), but not observed in PBS-injected control ear (**e**; ×24,000 magnification) (**e**). Scale bar = 4 μm. (**f, g**) The sections were

stained with antimouse CD11b IgG, a conventional macrophage marker, and TRITC-streptavidin conjugate (red), followed by 4′-6-Diamidino-2-phenylindole (blue). Infiltration of numerous CD11b-positive macrophages was observed in the *P. acnes*-injected ear (**f**), but not in PBS-injected ear (**g**). Broken lines indicate the outlines of ear sections. Data are representative of four separate experiments with similar results. Scale bar = 200 μm.

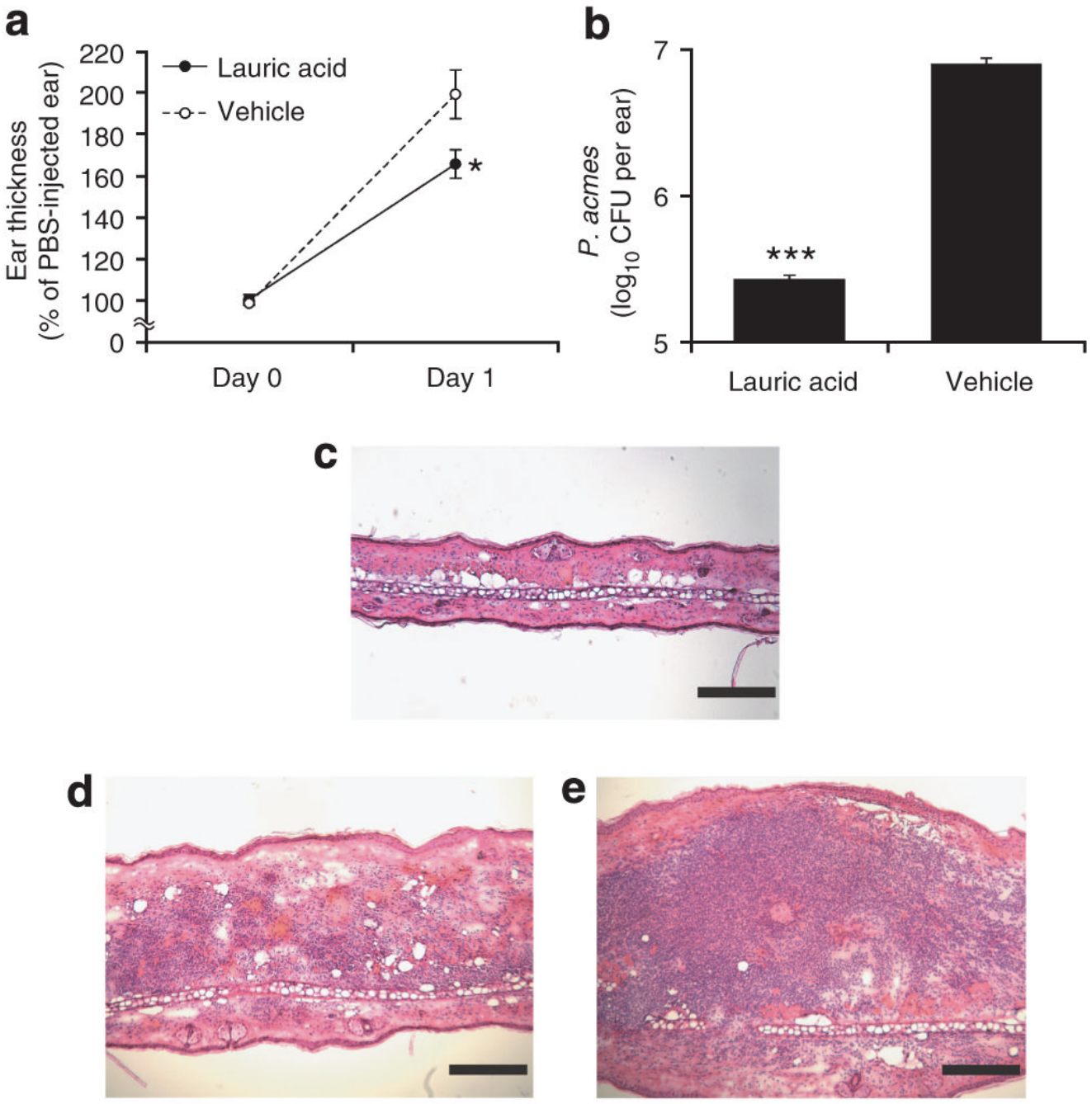

Figure 5. Effects of intradermal injection of lauric acid on *P. acnes* growth *in vivo* and *P. acnes*-induced inflammation

Left ears of ICR mice were intradermally injected with *P. acnes* (1×10^7 CFU per 20 μl in PBS). Right ears of the same mice were injected with 20 μl of PBS. Subsequently, the *P. acnes*- and PBS-injected sites were intradermally injected with lauric acid (2 μg per 20 μl in 5% DMSO in PBS). As a control, an equal volume (20 μl) of 5% DMSO in PBS was injected into both ears. (**a**) The increase in ear thickness was measured using a micro caliper before and 24 hours after the bacterial injection. (**b**) The *P. acnes*-injected ear was punched with an 8 mm biopsy punch 24 hours after *P. acnes* injection and homogenized in 200 μl of sterile PBS with a tissue grinder. CFUs of *P. acnes* were enumerated by plating serial dilutions of the

homogenate on an agar plate. Data represent mean±SE of four individual experiments (**P*<0.05, ****P*<0.0005 by Student's *t*-test). (**c-d**) Ear injected with lauric acid only (**c**), ear injected with both *P. acnes* and lauric acid (**d**), and ear injected with both *P. acnes* and vehicle (5% DMSO in PBS) (**e**) were cross-sectioned, stained with H&E. Increase in ear thickness and infiltrated inflammatory cells (arrows) surrounding the injected site of *P. acnes* (arrowhead) were observed in an H&E-stained frozen section of *P. acnes* injected ear (**e**), and were decreased in the presence of lauric acid (**d**). Data are representative of four separate experiments with similar results. Scale bar = 200 μm.

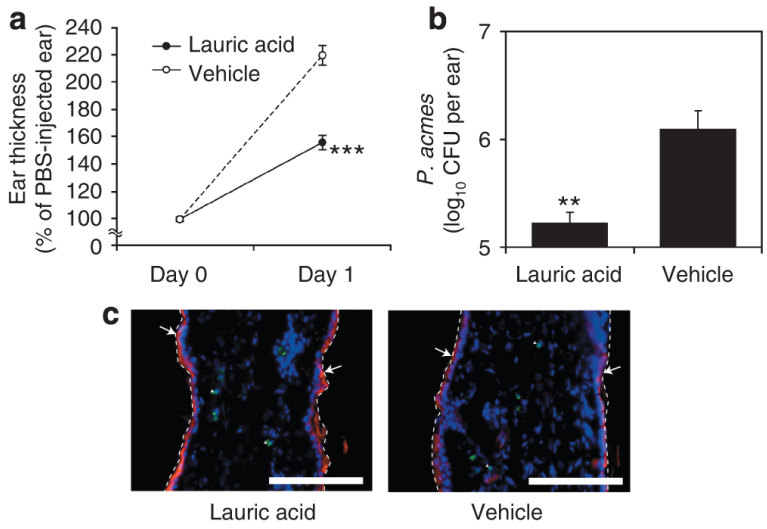

Figure 6. Effects of epicutaneous application of lauric acid on *P. acnes* growth *in vivo* and *P. acnes*-induced inflammation

Left ears of ICR mice were intradermally injected with 1×10^7 CFU per 20 μl of *P. acnes*. Right ears received an equal amount of PBS serving as a control. Lauric acid (150 μg in 5% acetone mixed with 15 mg of Vaseline) and 5% acetone mixed with 15 mg of Vaseline (vehicle) were epicutaneously applied on the left and right ears, respectively. (**a**) The increase in ear thickness was measured using a micro caliper before and 24 hours after the bacterial injection. The increase in ear thickness of *P. acnes*-injected ear was normalized to a PBS-injected control. (**b**) Ears with *P. acnes* injection were punched with an 8 mm biopsy punch 24 hours after bacterial injection and homogenized in 200 μl of sterile PBS. CFUs were enumerated by plating serial dilutions of the homogenate on an agar plate. The data represent mean±SE of six individual experiments (***P*<0.005, ****P*<0.0005 by Student's *t*-test). (**c**) To examine *in vivo* cytotoxic effect of epicutaneous application of lauric acid on the keratinocytes, ear sections were detected by TUNEL assays and stained with rabbit anti-K10 (a differentiated keratinocyte marker) IgG, followed by goat anti-rabbit IgG-TRITC conjugate (red). Nuclei were counterstained with 4′-6-Diamidino-2-phenylindole (blue). No apoptotic differentiated keratinocytes (arrows) were detected on lauric acid-treated skins. Few apoptotic cells (light blue arrowheads) occurred naturally in dermis were detected in both vehicle- and lauric acid-treated skins. Broken lines indicate the outline of the surface of epidermis. Data are representative of six separate experiments with similar results. Scale bar = 200 μm.

Table 1

MICs and EC_{50}s of lauric acid and BPO on *P. acnes, S. aureus, and S. epidermidis*

Bacteria	MIC (μgml^{-1})[1]			EC_{50} (μgml^{-1})[1]		
	P. acnes	*S. aureus*	*S. epidermidis*	*P. acnes*	*S. aureus*	*S. epidermidis*
Lauric acid	3.9	0.97	3.9	2	6	4
BPO	62.5	15.6	>100	60	30	ND

ND, Not determined.

[1] MIC and EC_{50} were determined from dose-dependent curves of Figure 1.