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Cryptomeria japonica Essential Oil Inhibits the Growth of Drug-Resistant Skin Pathogens and LPS-Induced Nitric Oxide and Pro-Inflammatory Cytokine Production

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Abstract

In this study, the chemical composition of *Cryptomeria japonica* essential oil (CJE) was analyzed and its biological activities were tested. CJE was obtained by steam distillation from leaves collected from Jeju Island and analyzed by gas chromatography (GC)-flame ionization detection (FID) and GC-MS. Kaurene (17.20 %), elemol (10.88 %), γ-eudesmol (9.41 %), and sabinene (8.86 %) were the major components in CJE. The antibacterial and anti-inflammatory activities of CJE against drug-susceptible and -resistant skin pathogens have been not reported previously. Thus, we determined the anti-bacterial activities of CJE using the disk diffusion method and minimum inhibitory concentration (MIC) values. CJE showed excellent antibacterial activities against *Propionibacterium acnes* and *Staphylococcus epidermidis*, which are acne-causing bacteria. The MIC of CJE against drug-susceptible and -resistant *P. acens* and *S. epidermidis* ranged from 0.16 to 10.0 μl/ml. In addition, the effects of CJE on nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages were also examined. Pro-inflammatory cytokine and mediator tests indicated that CJE has excellent dose-dependent inhibitory activities. Therefore, based on these results, we pro pose that CJE is an attractive acne-mitigating candidate for skin health.

Key word: *Propionibacterium acnes*, *Staphylococcus epidermidis*, acne, essential oil, skin pathogen,

Introduction

Acne is a significant clinical problem with severe social, psychological, and emotional implications. The disease affects up to 80% of teenagers and frequently continues into adulthood. Clinically, acne is characterized by distended sebaceous follicles seen as open or closed comedones and inflamed lesions presenting as papules, pustules, and cysts (Bek-Thomsen *et al.*, 2008). The pathogenesis of acne appears to be multifactorial yet is only partly understood. A mainly genetically determined host response pattern combined with bacterial "triggering" is generally accepted as being important for the apparently unbalanced inflammatory activity in acne (Bataille *et al.*, 2002; Bek-Thomsen *et al.*, 2008). Normal skin commensals, including *Propionibacterium acnes* and *Staphylococcus epidermidis*, proliferate rapidly during puberty and are often involved in the development of acne. *P. acnes* has been described as an obligate anaerobic organism. These organisms produce neutrophil chemotactic factors, which attract neutrophils to release inflammatory mediators such as reactive oxygen species (ROS) and lysosomal enzymes that disrupt the integrity of the follicular epithelium (Webster and Leyden, 1980). ROS are considered one of the most potent stimuli for inflammation. They also stimulate monocyte/macrophages to increase production of pro-inflammatory cytokines such as TNF-α, IL-8 and IL-1β, which induce many of the hallmark mediators of inflammatory response. In contrast, *S. epidermidis*, an aerobic organism, is usually involved in superficial infections within the sebaceous unit (Burkhart *et al.*, 1999; Chomnawang *et al.*, 2005). Therefore, *P. acnes* is considered to play an important role in acne development by secreting inflammation-inducing factors.

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Therapeutic agents for acne are usually employed to inhibit inflammation or kill the bacteria. However, these remedies have been known to induce side effects. Benzoyl peroxide and retinoid cause xerosis cutis and skin irritation if they are used excessively (Zesch, 1988), and several reports also suggest that prolonged use of tetracycline, erythromycin, macrolide and clindamycin can lead to the appearance of resistant bacteria, organ damage, and immunohypersensitivity (Eady, 1998; Wawruch *et al.*, 2002; Park *et al.*, 2004). In addition, triclosan is converted into an environmental hormone when exposed to light, inducing severe environmental pollution (Park *et al*., 2004). Therefore, many researchers have tried to develop therapeutic agents for acne that have high antibacterial and anti-inflammatory activities with no side effects (Jain and Basal, 2003; Lim *et al.*, 2007; Joo *et al*., 2008; Kim *et al.*, 2008; Leyden, 2008; Marcinkiewicz *et al.*, 2008).

Cryptomeria japonica is one of the most commercially important conifers in Asia. It has been reported that various parts of *C. japonica* have various activities including anti-inflammatory, and hepatoprotective and antifeeding activities (Shyur *et al*., 2008; Wu *et al.*, 2008). The essential oil of *C. japonica* (CJE) possesses several medicinal functions including anti-termitic, anti-ulcer, antifungal, insecticidal and antimicrobial activities against several microorganisms (Matsunaga *et al.*, 2000; Cha *et al*., 2007; Cheng *et al*., 2005; 2009). However, the anti-inflammatory effects and antibacterial activities of CJE against drug-resistant skin pathogens have not yet been described. Therefore, based on the previously known chemical composition and biological activities of CJE, its effectiveness as a possible acne treatment was investigated.

Experimental

Material and Methods

Extraction, isolation and identification of the oil components. The fresh leaves of *C. japonica* were subjected to hydrodistillation, producing a yield of 0.6% (v/w). The oil was analyzed by GC-FID and GC/MS. The GC apparatus was the Agilent Technology (HP) 5890 system with a capillary DB-HT column (30 m×0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 40°C, held for 5 min, then ramped up to 250°C at a rate of 10°C/min and held for 28 min. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The relative percentages of the oil constituents were expressed as percentages following peak area normalization. Components of the essential oil were identified by comparing their retention indices to a series of *n*-alkane indices on the DB-1HT capillary column and GC-MS spectra from the Wiley 6.0 MS data and literature data.

Microbial strains. Gram-positive bacterial species, *S. epidermidis* SK4, *S. epidermidis* SK 9, *S. epidermidis* SK19, *P. acne* ATCC 3314, *P. acne* SKA 4, and *P. acne* SKA 7, which are involved in acne, were selected as test microorganisms according to their pathological capacities. The microorganisms used in this study were obtained from Culture Collection of Antibiotics Resistant Microbes (CCRM). *Propionibacterium* sp. was cultured at 37°C for 24 h in GAM broth (Nissui Pharmaceutical Co. Tokyo, Japan) under anaerobic conditions before the assay. *S. epidermidis* sp. KCTC3958 was cultured at 37°C for 24 h in *Corynebacterium* media before the assay.

Antibacterial activities against drug-resistant skin pathogens. The microbial growth inhibitory potential of the extracts was determined using the agar disk diffusion method. Culture suspensions densities were adjusted to 4 McFarland. The wells (Ø 7.0 mm) were prepared, and the essential oil was diluted in ethanol to the test concentration and added to the wells (20 μl); an equal volume (20 μl) of ethanol was used as a control. Although the results of the disk diffusion assay cannot always be compared to MIC data, CJE showed positive antimicrobial activity against most of the microorganisms tested in the disk diffusion bioassay and was further tested to determine the MIC. A stock solution of essential oil was prepared in 10% dimethylsulfoxide (DMSO) and then serially diluted to concentrations ranging from 0.078 to 20 μl/ml. The 96-well plates were prepared by dispensing 95 μl of culture broth, 100 μl of essential oil and 5 μl of the inoculants into each well. The inoculums of microorganisms were prepared from 24-h cultures, and suspensions were adjusted to 4 McFarland standard turbidity. The final volume in each well was 200 μl. A positive control (containing inoculum but no essential oil) and a negative control (containing essential oil but no inoculum) were included on each microplate. The contents of the wells were mixed and the microplates were incubated at 30°C for 24 h. The MIC was defined as the lowest concentration of CJE to inhibit the growth of microorganisms.

Cell culture. The murine macrophage cell line RAW 264.7 was purchased from the Korean Cell Line Bank (KCLB; Seoul, KOREA). RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Inc., NY) supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin and 10% fetal bovine serum (FBS; GIBCO, Inc., NY). The cells were incubated in an atmosphere of 5% CO_2 at 37°C and were subcultured every 3 days.

LDH cytotoxicity assay. RAW 264.7 cells (1.5×10^5 cells/ml) plated in 96-well plates were pre-incubated for 18 h and then treated with LPS (1 μg/ml)

plus aliquots of CJE at 37°C for 24 h. The release of lactate dehydrogenase (LDH) from RAW 264.7 cells was measured to detect cytotoxicity at the end of each proliferation experiment. LDH leakage is a means of measuring membrane integrity as a function of the amount of cytoplasmic LDH released from the cytosol into the medium. LDH activity was determined from the production of NADH during the conversion of lactate to pyruvate and was determined using an LDH cytotoxicity detection kit (Promega, Madison, WI, USA). Briefly, culture medium was centrifuged at 12 000 rpm for 3 min at room temperature to ensure accumulation of cells. The cell-free culture medium (50 µl) was collected and then incubated with 50 µl of the reaction mixture from the cytotoxicity detection kit for 30 min at room temperature in the dark. 1N HCl (50 µl) was added to each well to stop the enzymatic reaction. The optical density of the solution was then measured using an ELISA plate reader at a wavelength of 490 nm. Percent cytotoxicity was determined relative to the control group. All experiments were performed in triplicate.

Determination of nitric oxide (NO) production. After pre-incubation of RAW 264.7 cells (1.5×10^5 cells/ml) with LPS (1 µg/ml) for 24 hours, the quantity of nitrite accumulated in the culture medium was measured as an indicator of NO production. Briefly, 100 µl of cell culture medium was mixed with 100 µl of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid), the mixture was incubated at room temperature for 10 min, and the absorbance at 540 nm was measured in a microplate reader. Fresh culture medium was used as a blank in every experiment. The quantity of nitrite was determined from a sodium nitrite standard curve. All experiments were performed in triplicate.

Measurement of pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) production. The inhibitory effect of CJE on pro-inflammatory cytokine (IL-1β, IL-6, and TNF-α) production in LPS-treated RAW 264.7 cells was determined by ELISA as described in the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). All experiments were performed in triplicate.

Determination of PGE_2 production. CJE was diluted with DMEM before treatment. Cells were treated with LPS (1 µg/ml) for 24 h to allow cytokine production. The PGE_2 concentration in the culture medium was quantified using a competitive enzyme immunoassay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. PGE_2 production was measured relative to that following control treatment. All experiments were performed in triplicate.

RNA isolation and RT-PCR analysis. Total RNA from LPS-treated RAW 264.7 cells was prepared with Tri-Reagent (MRC, Cincinnati, OH, USA), according to the manufacturer's protocol. RNA was stored at –70°C until use. One µg RNA was reverse transcribed with M-MuLV reverse transcriptase (Promega, Madison, WI, USA), oligo dT-18 primer, dNTP (0.5 µM) and 1 U RNase inhibitor. After this reaction cocktail was incubated at 70°C for 5 min, 25°C for 5 min, and 37°C for 60 min in series, M-MuLV reverse transcriptase was inactivated by heating at 70°C for 10 min. Polymerase chain reaction (PCR) was performed in reaction buffer [cDNA, 1.25 U Taq DNA polymerase (Promega, Madison, WI, USA), 3'- and 5'-primer (50 µM each) and 200 mM dNTP in 200 mM Tris-HCl buffer (pH 8.4) containing 500 mM KCl and 1–4 mM $MgCl_2$]. The PCR was performed in a DNA gene cycler (BIO-RAD, Hercules, CA, USA) with amplification by 30 cycles of 94°C for 45 s (denaturation), 60–65°C for 45 s (annealing) and 72°C for 1 min (primer extension).

The primers used in this study were:

β-actin (forward primer 5'-GTGGGCCGCCCTAGGCACCAG-3' and reverse primer 5'-GGAGGAAGAGGATGCGGCAGT-3'),

iNOS (forward primer 5'-CCCTTCCGAAGTTTCTGGCAGCAGC-3' and reverse primer 5'-GGCTGTCAGAGCCTCGTGGCTTTGG-3'),

COX-2 (forward primer 5'-CACTACATCCTGACCCACTT-3' and reverse primer 5'-ATGCTCCTGCTTGAGTATGT-3').

The PCR products were electrophoresed in 1.5% agarose gels and stained with ethidium bromide. The β-actin, iNOS and COX-2 primers produced the expected amplified products of 603, 496 and 696 bp, respectively.

Immunoblotting. RAW 264.7 cells were pre-incubated for 18 h and then stimulated with LPS (1 µg/ml) in the presence of test materials for 24 h. After incubation, the cells were collected and washed twice with cold PBS (Phosphate-Buffered Saline). Cells were lysed in lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, 1 mM EGTA, 1 mM $NaVO_3$, 10 mM NaF, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 25 µg/ml aprotinin, 25 µg/ml leupeptin] and kept on ice for 30 min. The cell lysates were centrifuged at 12,000×g at 4°C for 15 min and the supernatants were stored at –70°C before use. Protein concentration was measured by the Bradford method. Aliquots of the lysates (30~50 µg of protein) were separated on 8~12% SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride (PVDF) membranes (BIO-RAD, HC) with glycine transfer buffer [192 mM glycine, 25 mM Tris-HCl (pH 8.8), 20% MeOH (v/v)]. After blocking nonspecific sites with 5% nonfat dried milk, the membrane was incubated with specific primary mouse monoclonal anti-iNOS Ab (1:1000, Calbiochem,

La Jolla, CA) or rabbit polyclonal anti-COX-2 Ab (1:1000, BD Biosciences Pharmingen, San Jose, CA) at 4°C overnight. Each membrane was further incubated for 30 min with secondary peroxidase-conjugated goat anti-mouse or -rabbit IgG (1:5000, Amersham Pharmacia Biotech, Little Chalfont, UK), respectively. The immunoactive proteins were detected using an enhanced chemiluminescence (ECL) Western blotting detection kit (Amersham Biosciences, Piscataway, NJ, USA).

Statistical analysis. The Student's t-test and one-way ANOVA were used to determine the statistical significance of differences between the values for the various experimental and control groups. Data are expressed as means ± standard errors (S.E.M.) and the results are taken from at least three independent experiments performed in triplicate. *p*-values of 0.05 or less were considered statistically significant.

Results

Chemical composition of essential oil. GC-MS analyses of the oil led to the identification of 24 different components, representing 89.41% of the total oil. The identified compounds are listed in Table I according to their elution order on a ZB-1 capillary column. The oil was a complex mixture consisting of olefinic hydrocarbons and mono- and sesquiterpene hydrocarbons along with some other essential phytochemicals. The major components detected in the oil were kaurene (17.20%), elemol (10.88%), γ-eudesmol (9.41%), sabinene (8.86%), α-eudesmol (5.26%), β-eudesmol (5.13%), terpene-4-ol (4.10%), lendene (3.80%), α-pinene (3.48%), δ-cadinene (3.31%), widdrene (2.56%), α-cadinol (2.20%), γ-terpinene (1.92%), δ-3-carene (1.73%), Germacrene D (1.48%) limonene (1.22%), β-myrcene (1.17%), and α-terpinolene (1.13%). Terpenoid hydrocarbons were the characteristic constituents of the CJE. α-Cedrol (0.99%), α-terpinene (0.97%), 10-epi-γ-eudesmol (0.93%), β-himachalene (0.66%), α-muurolene (0.52%), and caphene (0.50%) were the trace or minor components of CJE.

Table I
Chemical composition of the essential oils from *C. japonica*

RT	RI	Components	Peak areas
5.321	918.7	α-pinene	3.48
5.662	926.9	caphene	0.50
6.792	954.3	sabinene	8.86
7.805	978.8	β-myrcene	1.17
8.422	993.8	δ-3-carene	1.73
8.703	1000.4	α-terpinene	0.97
9.315	1011.1	limonene	1.22
10.907	1039.0	γ-terpinene	1.92
12.504	1067.0	α-terpinolene	1.13
17.412	1147.6	terpene-4-ol	4.10
32.754	1392.4	widdrene	2.56
35.767	1437.5	Germacrene D	1.48
36.770	1452.3	β-himachalene	0.66
37.508	1463.2	α-muurolene	0.52
39.237	1488.7	δ-cadinene	3.31
40.978	1518.3	elemol	10.88
42.713	1550.9	α-cedrol	0.99
43.925	1573.7	10-epi-γ-eudesmol	0.93
44.900	1592.0	γ-eudesmol	9.41
45.319	1599.9	β-eudesmol	5.13
45.589	1607.8	α-eudesmol	5.26
45.754	1612.8	α-cadinol	2.20
45.941	1618.3	lendene	3.80
55.047	1978.3	kaurene	17.20

Antibacterial activity of CJE against skin pathogens. To assess the antibacterial activity of CJE against drug-susceptible and -resistant *P. acens* and *S. epidermidis*, we used the disk diffusion method (Table II). Erythromycin was used as a positive control. CJE exhibited significant susceptibility, with >10 mm inhibition diameter towards drug-susceptible and -resistant *P. acens* ATCC3314, *P. acens* SKA4 and *P. acens* SKA7. The effect on drug-susceptible and – resistant *S. epidermidis* was weak (<10 mm inhibition diameter). The antibacterial activities of CJE were further evalu-

Table II
Antimicrobial activity of the essential oils from *C. japonica*

Strains	Drug resistance patterns of skin pathogens (MIC; μg/ml)	Inhibition zones (mm)		MIC values (μl/ml)
		3.3 μl / disk	6.6 μl /disk	
S. epidermidis SK4	Susceptible	8	9	0.156
S. epidermidis SK 9	Erythromycin (>32), Clindamycin (>16), Chloramphenicol (64)	8	9	0.156
S. epidermidis SK19	Tetracycline (>32)	8	9	10.00
P. acne ATCC 3314	Susceptible	12	13	0.156
P. acne SKA 4	Clindamycin (64)	12	12	0.625
P. acne SKA 7	Clindamycin (64)	11	13	0.312

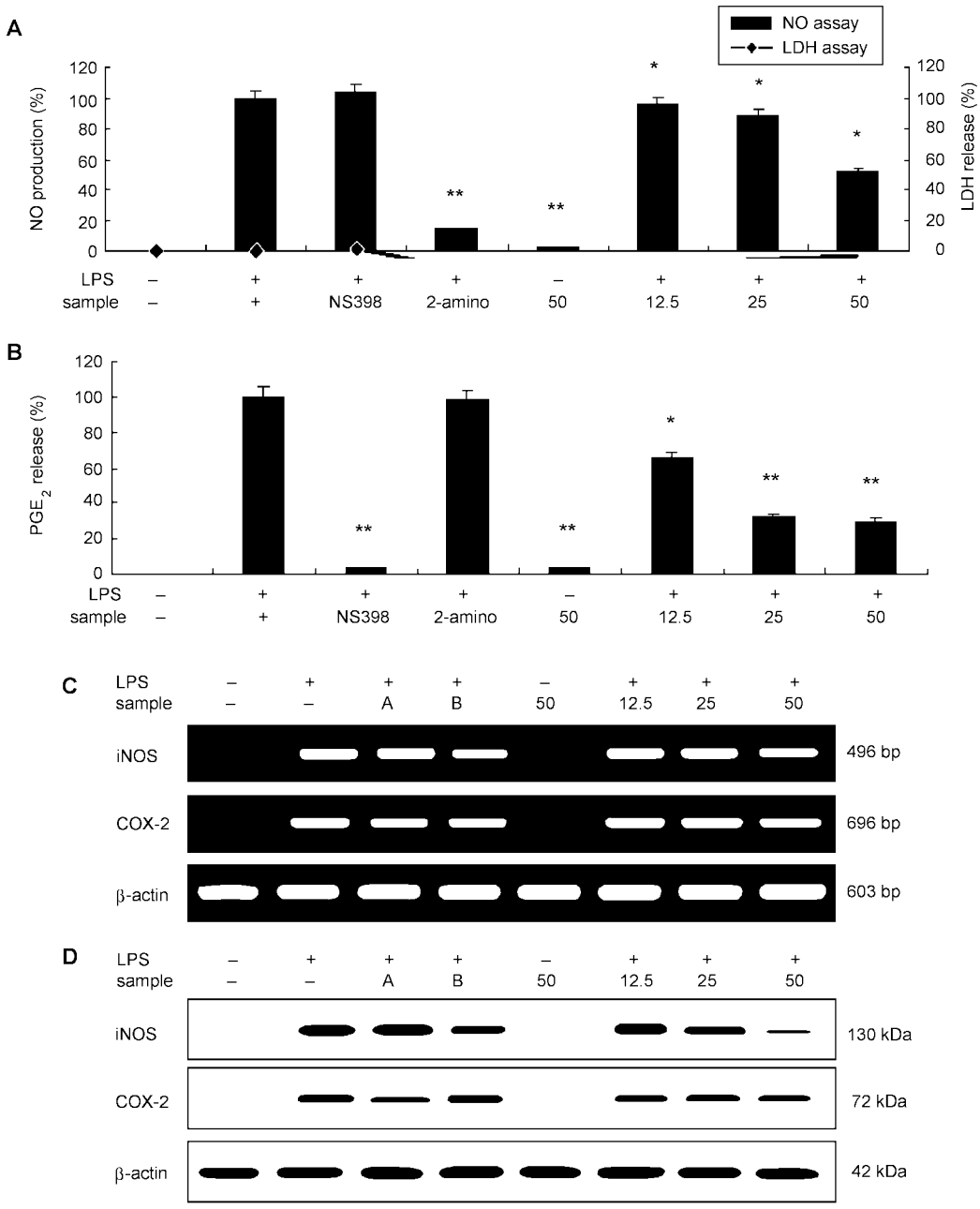

Fig. 1. Inhibitory effect of CJE on iNOS and COX-2 expression in macrophage RAW cells.

Nitric oxide production was assayed in the culture medium of cells stimulated with LPS (1 μg/ml) for 24 h in the presence of CJE. Cytotoxicity was determined using the LDH method (A). Supernatants were collected and PGE_2 concentration in the supernatants was determined by ELISA (B). iNOS and COX-2 mRNA expression (C) and protein levels (D) were determined by RT-PCR and immunoblotting, respectively. Values are the mean ± SEM of triplicate experiments. *, $P<0.05$; **, $P<0.01$

ated by determining the MIC, which is the lowest concentration yielding no growth. The MIC of CJE was determined using a two-fold serial dilution method. As seen in Table II, CJE exhibited inhibitory effects towards all of the test organisms. The MICs for CJE ranged from 0.156 μl/ml to 0.625 μl/ml for all test microorganisms except tetracycline-resistant *S. epidermidis* SK19 (MIC, 10 μl/ml).

Anti-inflammatory effects of CJE in murine macrophage RAW 264.7 cells. To investigate the effect of CJE on NO production, we used the Griess assay to measure the accumulation of nitrite, a stable oxidized product of NO, in culture media. As shown in Fig. 1A, CJE markedly inhibited LPS-induced NO production by RAW 264.7 cells in a dose-dependent manner. The numbers of viable activated macrophages were not altered by CJE as determined by LDH assays, indicating that the inhibition of NO synthesis by CJE was not due simply to cytotoxic effects. As a positive control, we used 2-amino-4-methyl pyridine, which inhibits NO production. We next examined the effects of CJE on PGE_2 production. As shown

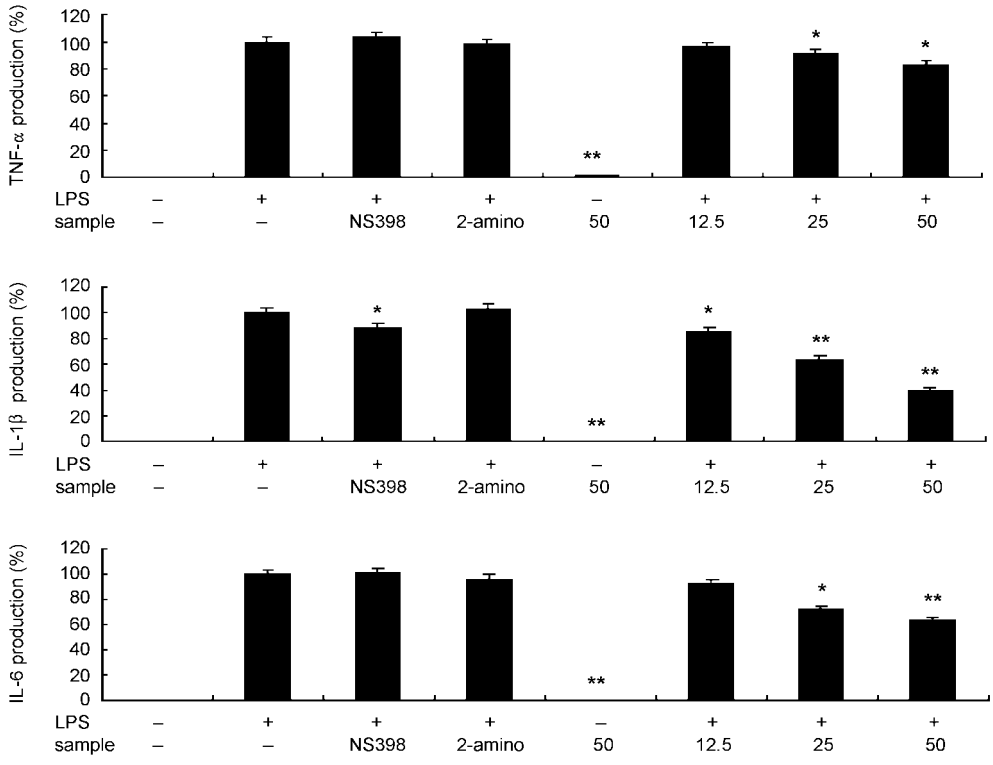

Fig. 2. Inhibitory effect of CJE on TNF-α, IL-1β and IL-6 production in RAW 264.7 cells.
Cells (1.5×10^5 cells/ml) were stimulated with LPS (1 μg/ml) for 24 h in the presence of CJE (12.5, 25, and 50 μg/ml). Supernatants were collected, and the pro-inflammatory cytokine concentrations in the supernatants were determined by ELISA. Values are the mean ± SEM of triplicate experiments. *, *P<0.05*; **, *P<0.01*

in Fig. 1B, CJE and NS398 (positive control) suppressed LPS-induced PGE_2 production in a dose-dependent manner. Western blot and RT-PCR analyses were also performed to determine whether the inhibitory effects of CJE on NO and PGE_2 were related to the modulation of iNOS and COX-2 expression. As shown in Fig. 1C, CJE reduced the expression of iNOS protein. Since the amount of iNOS protein correlated with NO accumulation, these results suggest that CJE inhibited NO production by reducing iNOS protein expression. We also found that the amount of the 72-kDa COX-2 protein was increased by LPS, and this increase was antagonized by CJE. To further determine whether the inhibition of LPS-stimulated NO and PGE_2 production by CJE was mediated by the regulation of iNOS and COX-2 expression, RT-PCR analyses were performed. As shown in Fig. 1D, CJE reduced iNOS and COX-2 mRNA without affecting the mRNA of β-actin, a housekeeping protein. Therefore, an inhibitory effect on iNOS and COX-2 gene expression appears to be one of the mechanisms responsible for the anti-inflammatory action of CJE. Since CJE potently inhibited the pro-inflammatory mediators, we further investigated its effects on LPS-induced IL-1β, IL-6 and TNF-α release by enzyme immunoassay. After 24-hour incubation with both LPS (1 μg/mL) and CJE, there was remarkable inhibition of TNF-α, IL-1β and IL-6 production (Fig. 2).

Discussion

The innate immune system plays an essential role in host defense against infections by microorganisms. Immune responses known as pathogen associated molecular patterns are recognized and regulated by cellular receptors, including TLRs (Medzhitov and Janeway, 1997). Pathogen-associated molecular patterns are antigenic and able to activate monocytes/macrophages to secrete various inflammatory cytokines including TNF, IL-1 and IL-6 (Takeda *et al.*, 2003; Chao *et al*., 2008). While mediation of inflammation against pathogen infection by these inflammatory cytokines could be beneficial to the host, over-expression of these cytokines may cause inflammatory diseases, including inflammatory acne vulgaris. Acne inflammation has been associated with several factors, including increased production of sebum, abnormal cornification of the pilosebaceous duct, and proliferation of commensal bacteria, especially *P. acnes* (Choi *et al*., 2008). *P. acnes* is a Gram-positive, non-sporeforming, pleomorphic, and anaerobic bacterium that is ubiquitous in nature and commonly found as a normal flora in the human gut, and this strain has been recognized as a bacterium that causes acne and skin inflammation. Other Gram-positive cocci such as *S. epidermidis* are also known to be the predominant bacteria that cause infectious skin

diseases such as furuncle and pyoderma (Leyden and Kligman, 1976). Clindamycin and erythromycin are topical antibiotics that are commonly used to treat acne (Webster and Graber, 2008). However, one of the major problems in the use of topical antibiotics is the development of resistance.

Since ancient times, herbs and plant species have been added to different types of foods to improve their flavor and organoleptic properties. Currently, much research is being performed on antimicrobial and anti-inflammatory compounds from plant extracts and essential oils to identify novel lead structures with significant biological activities. Among these various natural compounds, essential oils from aromatic plants are receiving special attention. Due to the increasing resistance to antibiotics of many bacteria, these essential oils are of new interest as antiseptics and antimicrobial agents in dermatology (Ben *et al*., 2007).

As previously mentioned, we identified the antibacterial effect of CJE against acne-inducing drug-susceptible and -resistant skin pathogens. The effects of CJE on NO, PGE_2, TNF-α, IL-1β, and IL-6 production in LPS-activated RAW 264.7 macrophages were also examined. Pro-inflammatory cytokine and mediator tests indicated that CJE has excellent inhibitory activities. Based on these results, we conclude that CJE may be introduced as a possible therapeutic agent for acne. However, although the antimicrobial and anti-inflammatory effects of CJE against acne-inducing bacteria were identified, their mechanism of action was not determined. Especially, their possible inhibitory mechanisms against pro-inflammatory cytokines remain to be evaluated in further studies. Nuclear factor-κB (NF-κB) has been reported to be involved in maximal transcription of many cytokines, including TNF-α, IL-1, IL-6, and IL-8, which are thought to be important in the generation of acute inflammatory responses. Therefore, CJE may inhibit NF-κB activation induced by LPS.

In conclusion, acne vulgaris is the result of the combined action of *P. acnes* infection and the inflammatory response to that infection. CJE presents a potentially interesting treatment because our results show that it inhibits *P. acnes* growth and others have shown that it has anti-inflammatory properties. Because of its ability to inhibit *P. acnes*, its anti-inflammatory properties and its ready availability, CJE may benefit those suffering from *P. acnes*-associated skin disease.

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