



Dieckol Inhibits the Effects of Particulate Matter 10 on Sebocytes, Outer Root Sheath Cells, and *Cutibacterium Acnes*–Pretreated Mice

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Background: Particulate matter (PM) is an air pollutant that can impair the human skin. Antioxidants have been tested to improve PM-induced skin inflammation.

Objective: In this study, we investigated the effects of dieckol on PM-induced inflammation on cultured human sebocytes, outer root sheath (ORS) cells, and mice pretreated with *Cutibacterium acnes*.

Methods: We cultured and treated the sebocytes and ORS cells with 5 μ M of dieckol and 100 μ g/ml of PM10 for 24 h. The *C. acnes*–pretreated mice received 5 μ M of dieckol and 100 μ g/ml of PM10. We measured cell viability using MTT assay. Real-time PCR and measurement of reactive oxygen species (ROS) and sebum production analyzed the effects.

Results: Dieckol inhibited the upregulation of the gene expression of the inflammatory cytokines, matrix metalloproteinase (MMP), aryl hydrocarbon receptor, and nuclear factor kappa-light-chain-enhancer of activated B cells by PM10 in the cultured sebocytes and ORS cells and inhibited an increase in ROS production by PM10 in the cultured sebocytes. In addition, dieckol decreased the inflammatory cytokines, MMP, and sebum production in *C. acnes*–pretreated mice.

Conclusion: Dieckol effectively reduced the expression of inflammatory biomarkers and the production of sebum in cultured sebocytes, ORS cells, and *C. acnes*–pretreated mice.

Keywords: Antioxidant, Dieckol, Outer root sheath cells, Particulate matter, Sebocytes

INTRODUCTION

Human skin is exposed to environmental stressors such as particulate matter (PM), particularly in urban areas. PM is a complex mixture originating from factories, power plants, automobiles, construction activities, and naturally windblown dust^{1,2}. PM of <10 μ m in size (PM10) is a harmful air pollutant, carrying toxic compounds such as transitional metals, endotoxins, organic chemicals, and ultrafine components.

PM10 damages the skin through the synthesis of reactive oxygen species (ROS). The generation of oxidative stress can cause extrinsic skin aging and inflammatory skin disorders³⁻⁶.

Antioxidants have been used to inhibit oxidative stress and improve inflammatory skin conditions. Several polyphenolic compounds have antioxidative and anti-inflammatory effects⁷⁻⁹. Marine algae are rich in biological polyphenolic compounds such as eckol, 6,6'-bieckol, dieckol, phlorofuroeckol, and triphlorethol-A. In particular, *Ecklonia cava* dieckol exhibits strong antioxidant activity¹⁰⁻¹².

In this study, we investigated the effects of dieckol on the PM10-induced inflammatory biomarkers of cultured human sebocytes and outer root sheath (ORS) cells. In addition, we examined the effects of dieckol on PM10-induced inflammatory biomarkers in *Cutibacterium acnes*–pretreated mice. The



C. acnes-pretreated mouse is an animal model with acneiform inflammation.

MATERIALS AND METHODS

Sebocytes and ORS cell cultures

We transferred the sebaceous glands of the occipital hairs to a tissue culture dish and cultured these in a human sebocyte basal medium (Cell Application, San Diego, CA, USA) containing sebocyte growth supplement in a 5% CO₂ incubator at 37°C. After 2 weeks of isolation, we harvested these cells with 0.25% trypsin/10 mM ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA) in Hank's balanced salt solution and sub-cultured the cells at EpiLife (Gibco BRL, Rockville, MD, USA) with supplements.

For the ORS cells' culture, we cut off the hair shaft and bulb regions of the follicle to prevent contamination with the other cells. We immersed the trimmed hair follicles in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum. On day 3 of the culture, we changed the medium to EpiLife (Gibco BRL) keratinocyte growth medium containing supplements. After the second passage, we used these sebocytes and ORS cells in the study.

We obtained informed written consent from patients. The Medical Ethical Committee of the Kyungpook National University approved this study (IRB no. KNUH 2021-03-006).

Animal study

Six-week-old female HR-1 mice were purchased from SLC Inc. (Hamamatsu, Japan) and acclimatized for 1 week. We obtained and isolated *C. acnes* from the pustules of the Korean patients with acne. We injected *C. acnes* as 10⁹ colony-forming units/20 µl at four sites on the backs of the mice using a 30-gauge needle. One week after injection, we applied the back skin of the mice with 100 µl of 100-µg/ml PM10 (PM10-like European reference material ERM-CZ120; Sigma, Burlington, MA, USA) or 5 µM of dieckol for 2 weeks. In addition, mice were also applied separately with 100 µl of 100-µg/ml PM10 in the morning and with 5 µM of dieckol in the afternoon to observe changes in the PM-induced gene expression by Dieckol. We measured the size of the *C. acnes* suspension-induced inflammatory nodules at weeks 1 and 2 after treatment.

We followed the experiment guidelines for the use of labo-

ratory animals. The study was permitted by the Institutional Animal Care and Use Committee of Kyungpook National University (IACUC no. KNU 2021-0068).

MTT assay

We used the MTT assay to check cell viability. We seeded the sebocytes and ORS cells in 96-well collagen-coated plates at a density of 5,000 cells/well (Becton Dickinson, Franklin, NJ, USA) for 24 h. We added various concentrations of dieckol (Sigma) to the well plates of the sebocytes and ORS cells for 3 days and the MTT solution (3-[4,5]dimethylthiazol-2,5-diphenyltetrazolium bromide) at 70 µg/well for 3 h. We solubilized the formazan produced with dimethyl sulfoxide (DMSO) and measured the optical density at 570 nm.

Real-time PCR

We treated the cells with 5 µM of dieckol or 100 µg/ml of PM10 for 24 h. We also treated the cells with 5 µM of dieckol and 100 µg/ml of PM10 simultaneously for 24 h. Subsequently, we obtained the total RNA using an RNeasy Mini Kit (Qiagen, Hilden, Germany) and synthesized the cDNA from 3 µg of the total RNA with a cDNA synthesis kit containing ImProm-IITM reverse transcriptase and oligo-dT primers based on the manufacturer's suggested protocol (Promega, Madison, WI, USA).

We conducted the real-time PCR with the StepOnePlusTM Real-Time PCR Assay (Applied Biosystems, Foster City, CA, USA) and all reactions with the Power SYBR Green premix (Applied Biosystems) using 50 ng of the cDNA and 10 pM of the primers (The PCR primer sequences are summarized in Supplementary Table 1).

The cycling conditions for amplification were as follows: 95°C for 10 min and 40 cycles at 95°C for 15 s and at 60°C for 60 s. We evaluated the PCR products using the StepOnePlus (Applied Biosystems).

ROS measurement

We assessed the ROS cellular production by measuring 2',7'-dichlorodihydrofluorescein diacetate. We plated the cells onto 6-well collagen-coated plates (Becton Dickinson) at 5×10⁵ cells/well for 24 h. We pre-labeled the cells with 10 µM of DCF-DA (Invitrogen, Carlsbad, CA, USA) for 30 min. We washed the plates with phosphate-buffered saline (PBS) and then treated them with 5 µM of dieckol and/or 100 µg/ml of

PM10, incubating them at 37°C for 2 h, protecting them from light. We extracted the cells with 20 mM of Tris-Cl buffer containing 1% sodium dodecyl sulfate and 2.5 mM of EDTA. We centrifuged the extracts at 13,000 rpm for 15 min, detecting the supernatants using a fluorescence microplate reader (Molecular Devices, Sunnyvale, CA, USA) excitation at 485 nm and emission at 538 nm.

H&E staining

We obtained the tissue samples from the mice's inflammatory nodules, placing these into cryomolds with an embedding medium (optimal cutting temperature compound) at -80°C. We sliced the samples (7- μ m slices) with a cryostat (Leica CM3050 S; Leica Biosystems, Nussloch, Germany). For the H&E staining, we fixed the sections of the skin from each group with methanol and stained hematoxylin (DAKO Inc., Santa Clara, CA, USA) for 1 min and 1% eosin (Muto, Tokyo, Japan) for 30 s.

Inflammatory nodules' measurement

We measured the inflammatory nodules' diameters from four sites on the back using a Mitutoyo micrometer at weeks 1 and 2 after the PM10 and/or dieckol treatment. We assessed the tissue thickness in slides with the H&E staining, measuring the thicknesses as the distance from the bottom of the skin to the epidermis at sections of 200 \times magnification.

Lipid production quantification

We used the Oil Red O Stain Kit (Abcam, Cambridge, UK) following the manufacturer's instructions. We incubated the slides in propylene glycol for 2 min and in the solution for 30 min. We immersed the slides in 85% propylene glycol for 1 min and washed them twice in water and stained hematoxylin for 2 min. Then, we washed the slides with water and mounted them.

We used the AdipoRed assay reagent (Lonza, Walkersville, MD, USA) for Nile red. We incubated the slides in this solution (1:100 dilution) for 10 min in the dark, washed with them with water, and mounted them.

Statistical analysis

We expressed the data as means \pm standard deviations. We performed the experiment three times independently. We used analysis of variance (SPSS Statistics ver. 18.0; SPSS Inc., Chicago, IL, USA) for the statistical analysis, considering $p < 0.05$

to be statistically significant.

RESULTS

MTT assay to determine dieckol concentration

To investigate dieckol's effects on the cell viability of the human sebocytes and ORS cells, we performed an MTT assay with varying concentrations for 3 days (Fig. 1) using 5 μ M of dieckol.

Dieckol prevented the upregulation of the inflammatory biomarkers in the cultured sebocytes and ORS cells by PM10

In real-time PCR, the expressions of interleukin (IL)-1 α , IL-1 β , IL-6, IL-8, and tumor necrosis factor (TNF)- α (Fig. 2A); matrix metalloproteinase (MMP)1, MMP3, and MMP12 (Fig. 2B); and aryl hydrocarbon receptor (AhR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (Fig. 2C) all significantly increased in the 100 μ g/ml PM10-treated sebocytes and ORS cells for 24 h ($p < 0.05$). The 5 μ M of Dieckol inhibited the upregulation of these expressions ($p < 0.05$). The 100 μ g/ml of PM10 also increased ROS production in the sebocytes and ORS cells. However, dieckol inhibited the upregulation of the ROS expression ($p < 0.05$) (Fig. 2D).

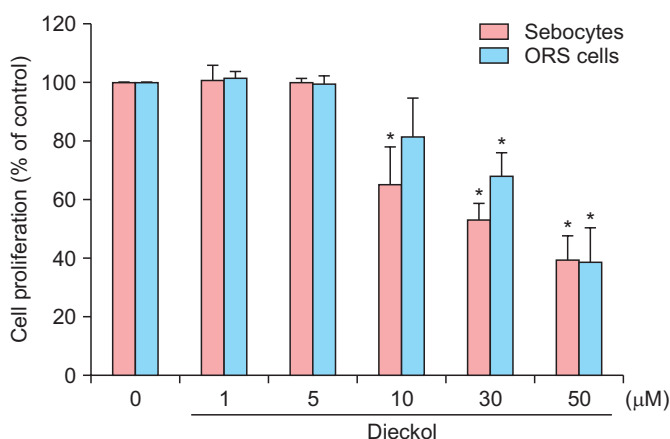


Fig. 1. Effects of dieckol in human-cultured sebocytes and outer root sheath (ORS) cells. We treated the cells with dieckol for 3 days, using the 3-[4,5]dimethylthiazol-2,5-diphenyltetrazolium bromide assay to measure cell viability. Data in the bar graphs represent the means \pm standard deviations from the three independent experiments (* $p < 0.05$).

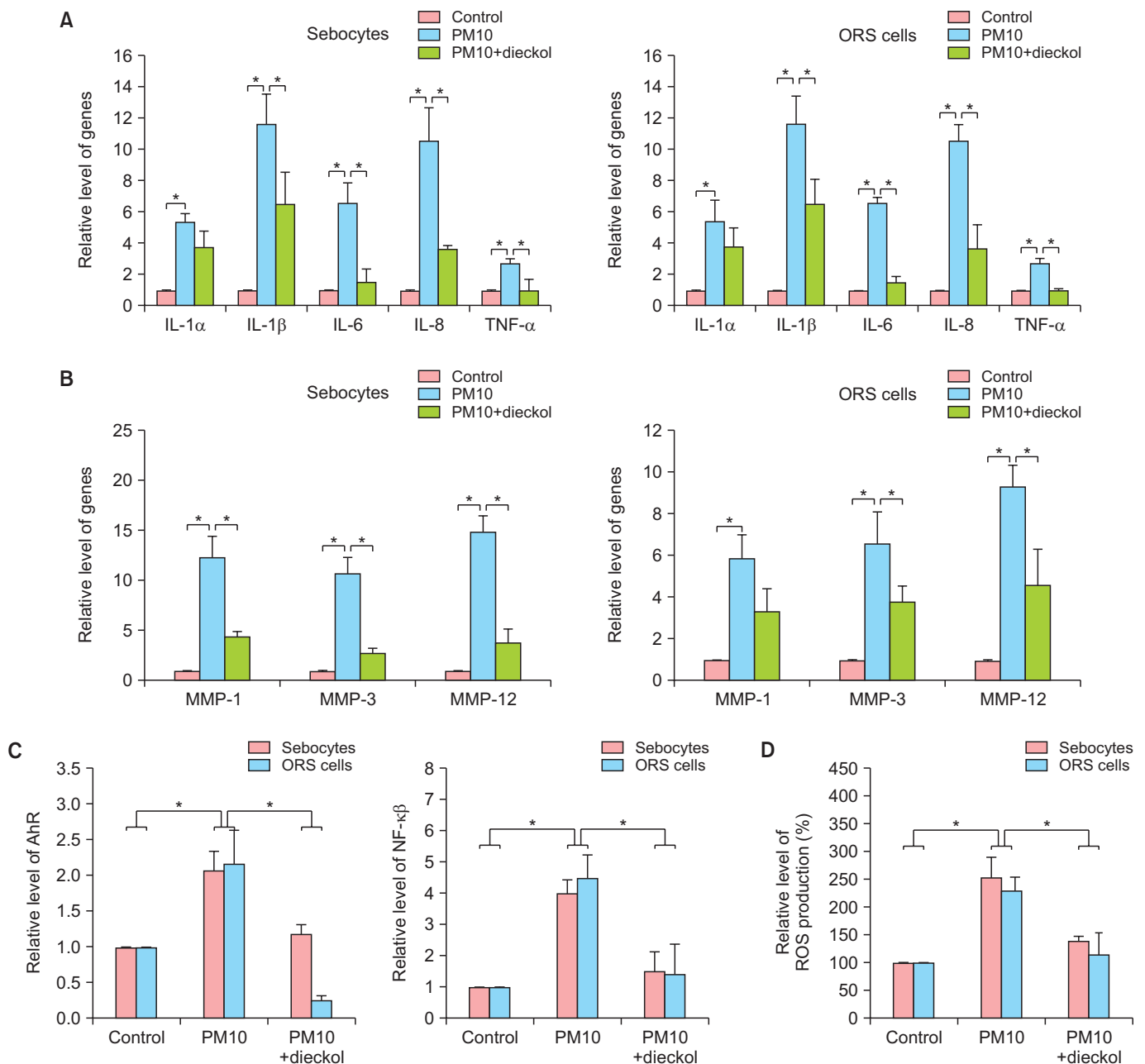


Fig. 2. Dieckol's preventive role against the upregulation of inflammatory biomarkers in the cultured sebocytes and outer root sheath (ORS) cells by particulate matter (PM) 10. (A) Dieckol 5 μ M inhibited the upregulation of the expression of the inflammatory genes interleukin (IL)-1 α , IL-1 β , IL-6, IL-8, and tumor necrosis factor (TNF)- α by 100 μ g/ml of PM10 in the cultured sebocytes and ORS cells. (B) Dieckol 5 μ M inhibited the upregulation of the gene expressions of matrix metalloproteinase (MMP)1, MMP3, and MMP12 by 100 μ g/ml of PM10 in the cultured sebocytes and ORS cells. (C) Dieckol 5 μ M inhibited the upregulation of the gene expressions aryl hydrocarbon receptor (AhR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) by 100 μ g/ml of PM10 in the cultured sebocytes and ORS cells. (D) Dieckol 5 μ M inhibited the increase in reactive oxygen species production by 100 μ g/ml of PM10 in the cultured sebocytes and ORS cells. Data in the bar graphs represent the means \pm standard deviations from the three independent experiments (* p <0.05).

Dieckol prevented an increase in the size and thickness of the *C. acnes*-induced inflammatory nodules by PM10

PM10 100 μ g/ml increased the diameter of the *C. acnes*-induced inflammatory nodules in the mice, and dieckol 5

μ M inhibited the aggravation of these nodules at weeks 1 and 2 (Fig. 3A). PM10 100 μ g/ml increased the thickness of these nodules in the mice, and dieckol 5 μ M inhibited the aggravation of these nodules at week 2 (p <0.05) (Fig. 3B). It

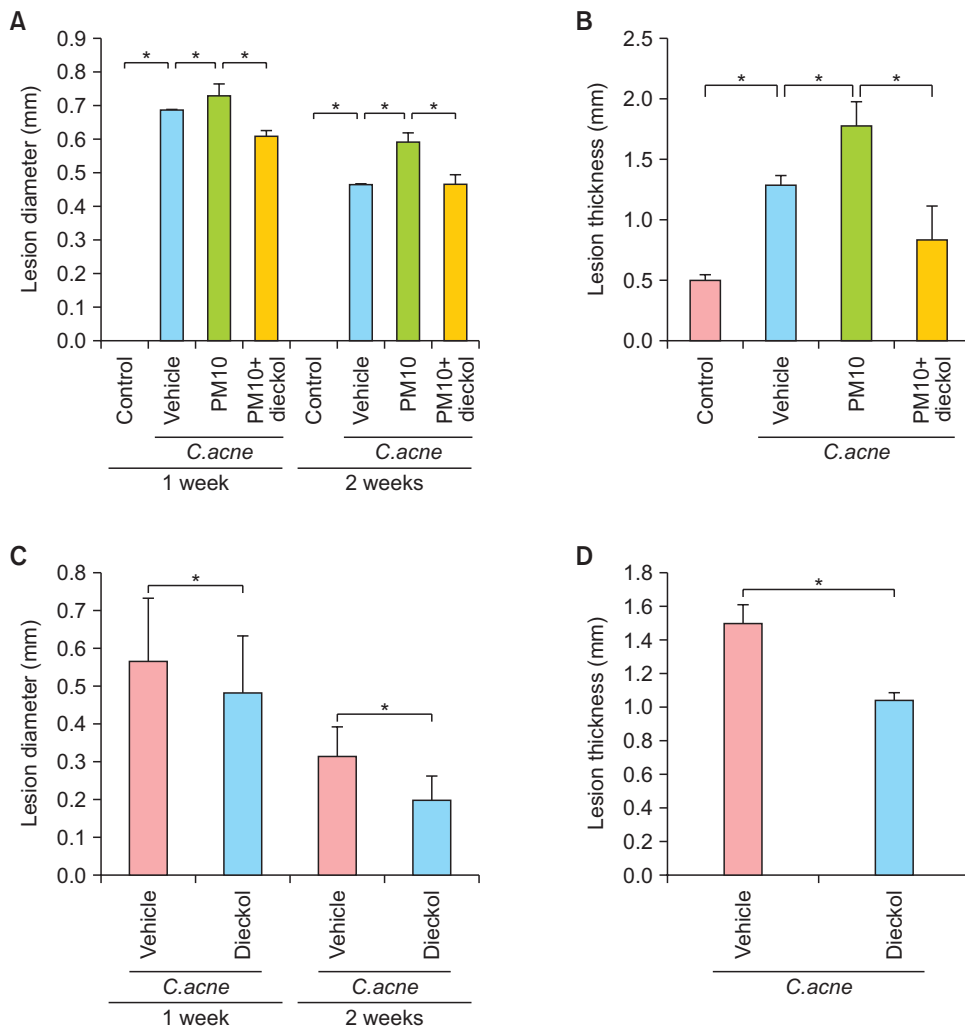


Fig. 3. Dieckol's preventive role against an increase in the size and thickness of *Cutibacterium acnes*-induced inflammatory nodules by particulate matter (PM) 10. (A) PM10 100 $\mu\text{g/ml}$ increased the diameter of the *C. acnes*-induced inflammatory nodules in mice, and dieckol 5 μM inhibited the aggravation of these nodules at weeks 1 and 2. (B) PM10 100 $\mu\text{g/ml}$ increased the thickness of the *C. acnes*-induced inflammatory nodules in mice, and dieckol 5 μM inhibited the aggravation of these nodules at week 2. (C) Dieckol 5 μM inhibited aggravation of the *C. acnes*-induced inflammatory nodules at week 1 and week 2. (D) Dieckol 5 μM also decreased thickness of the *C. acnes*-induced inflammatory nodules in mice at week 2. Data in the bar graphs represent the means \pm standard deviations from the three independent experiments (* $p < 0.05$).

was found that dieckol 5 μM inhibited aggravation of the *C. acnes*-induced inflammatory nodules at week 1 and week 2 ($p < 0.05$) (Fig. 3C). Dieckol 5 μM also decreased thickness of the *C. acnes*-induced inflammatory nodules in mice at week 2 ($p < 0.05$) (Fig. 3D).

Dieckol prevented the upregulation of the inflammatory biomarkers in the *C. acnes*-induced inflammatory nodules by PM10

PM10 100 $\mu\text{g/ml}$ upregulated the expression of toll-like receptor (TLR) 2 in the *C. acnes*-induced inflammatory nodules in the mice, and dieckol 5 μM downregulated this expression in these nodules at week 2 ($p < 0.05$) (Fig. 4A). PM10 100 $\mu\text{g/ml}$ upregulated the expressions of IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α in the *C. acnes*-induced inflammatory nodules in the mice, and dieckol 5 μM downregulated these expressions in

these nodules at week 2 ($p < 0.05$) (Fig. 4B). PM10 100 $\mu\text{g/ml}$ upregulated the expressions of MMP1, MMP3, and MMP12 in the *C. acnes*-induced inflammatory nodules in the mice, and dieckol 5 μM downregulated these expressions in these nodules at week 2 ($p < 0.05$) (Fig. 4C). Dieckol 5 μM downregulated the expression of TLR2 in the *C. acnes*-induced inflammatory nodules at week 2 ($p < 0.05$) (Fig. 4D). Dieckol 5 μM downregulated the expression of IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α in the *C. acnes*-induced inflammatory nodules at week 2 ($p < 0.05$) (Fig. 4E). Dieckol 5 μM downregulated the expression of MMP1, MMP3, and MMP12 in the *C. acnes*-induced inflammatory nodules at week 2 ($p < 0.05$) (Fig. 4F).

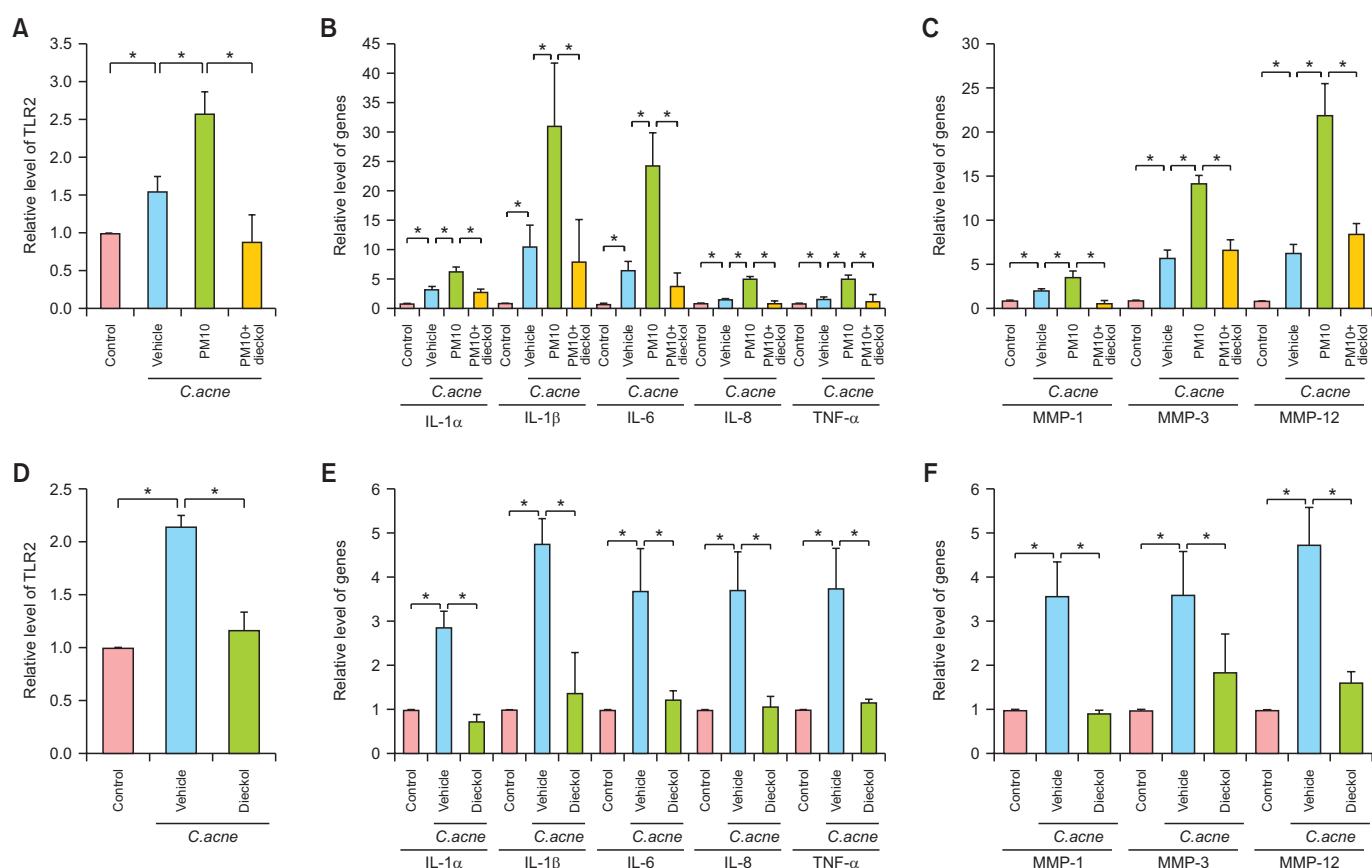


Fig. 4. Dieckol's preventive effects against the upregulation of the inflammatory biomarkers in the *Cutibacterium acnes*-induced inflammatory nodules by particulate matter (PM) 10. (A) PM10 100 $\mu\text{g/ml}$ upregulated the expression of toll-like receptor (TLR) 2 in the *C. acnes*-induced inflammatory nodules in mice, and dieckol 5 μM downregulated this expression in these nodules at week 2. (B) PM10 100 $\mu\text{g/ml}$ upregulated the expressions of interleukin (IL)-1 α , IL-1 β , IL-6, IL-8, and tumor necrosis factor (TNF)- α in the *C. acnes*-induced inflammatory nodules in the mice, and dieckol 5 μM downregulated these expressions in these nodules at week 2. (C) PM10 100 $\mu\text{g/ml}$ upregulated the expressions of matrix metalloproteinase (MMP)1, MMP3, and MMP12 in the *C. acnes*-induced inflammatory nodules in mice, and dieckol 5 μM downregulated these expressions in these nodules at week 2. (D) Dieckol 5 μM downregulated the expression of TLR2 in the *C. acnes*-induced inflammatory nodules at week 2. (E) Dieckol 5 μM downregulated the expression of IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α in the *C. acnes*-induced inflammatory nodules at week 2. (F) Dieckol 5 μM downregulated the expression of MMP1, MMP3, and MMP12 in the *C. acnes*-induced inflammatory nodules at week 2. Data in the bar graphs represent the means \pm standard deviations from the three independent experiments (* $p < 0.05$).

Dieckol prevented sebum production in the cultured sebocytes and *C. acnes*-induced inflammatory nodules by PM10

PM10 100 $\mu\text{g/ml}$ upregulated the expressions of peroxisome proliferator-activated receptor (PPAR)- γ , stearoyl-CoA desaturase, sterol regulatory element-binding protein 1a (SREBP1a), and SREBP1c in the cultured sebocytes, and dieckol 5 μM downregulated these expressions in these sebocytes ($p < 0.05$) (Fig. 5A). PM10 100 $\mu\text{g/ml}$ upregulated the sebum production in the *C. acnes*-induced inflammatory nodules in the mice, and dieckol 5 μM downregulated sebum production in these nodules at week 2 (Fig. 5B). Dieckol 5 μM downregulated the

production of sebum in the *C. acnes*-induced inflammatory nodules at week 2 (Fig. 5C).

DISCUSSION

Acne is a common inflammatory skin disease involving the elevated expression of proinflammatory cytokines. Environmental factors, including PM10, can aggravate this disease. In addition, PM10 activates AhR, leading to the release of increased amounts of proinflammatory cytokines through the creation of ROS¹³. Oxidative stress induces complex biological processes that can lead to the activation of transcription fac-

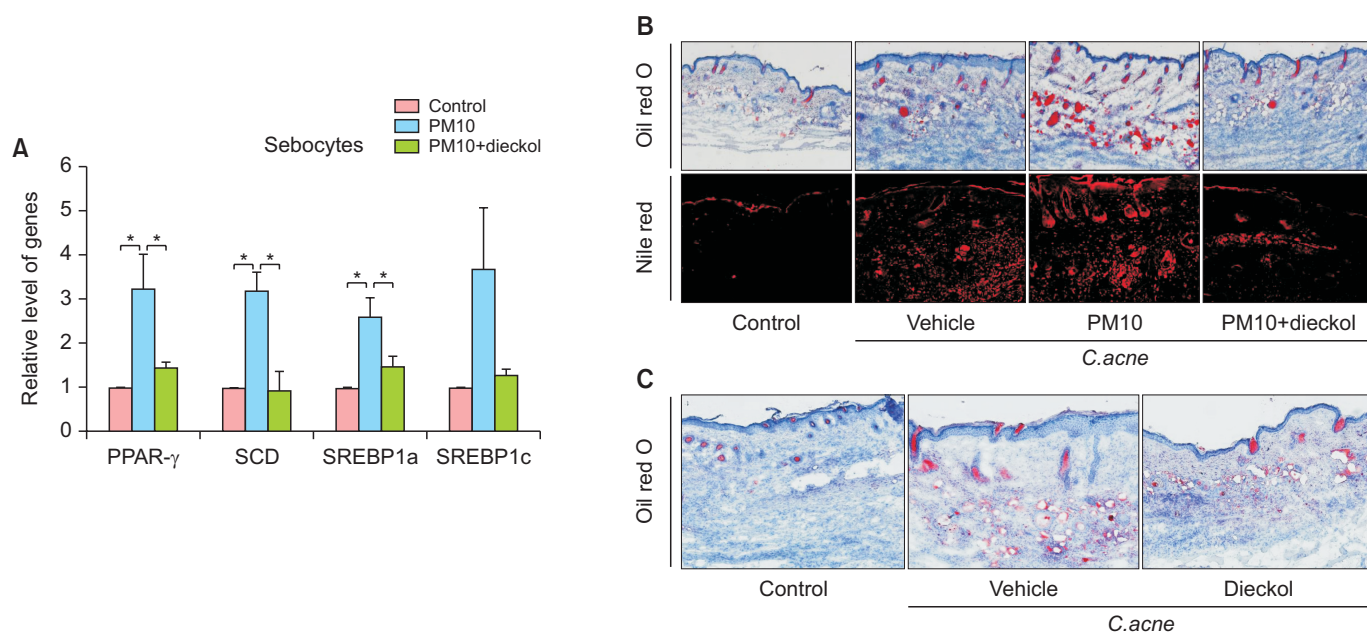


Fig. 5. Dieckol's preventive effects against sebum production in the cultured sebocytes and the *Cutibacterium acnes*-induced inflammatory nodules by particulate matter (PM) 10. (A) PM10 100 $\mu\text{g}/\text{ml}$ upregulated the expressions of peroxisome proliferator-activated receptor (PPAR)- γ , stearoyl-CoA desaturase (SCD), sterol regulatory element-binding protein 1a (SREBP1a), and SREBP1c in the cultured sebocytes, and dieckol 5 μM downregulated these expressions in the cultured sebocytes. Data in the bar graphs represent the means \pm standard deviations from the three independent experiments ($*p < 0.05$). (B) PM10 100 $\mu\text{g}/\text{ml}$ upregulated the production of sebum in the *C. acnes*-induced inflammatory nodules in the mice, and dieckol 5 μM downregulated this production in these nodules at week 2 ($\times 20$). (C) Dieckol 5 μM downregulated the production of sebum in the *C. acnes*-induced inflammatory nodules at week 2 ($\times 20$).

tors such as activator protein 1 (AP-1) and NF- κB ¹⁴.

Dieckol has various biological activities, including anti-inflammatory, antioxidative, and antiviral. Kwak et al.¹⁵ has shown the effects of dieckol on the expression of proinflammatory cytokines in the lipopolysaccharide-stimulated RAW 264.7 macrophages. Dieckol inhibited the inflammatory mediators, such as nitric oxide, TNF- α , and IL-6, in these macrophages, as well as the mitogen-activated protein kinases (MAPKs) and NF- κB . Both MAPKs and NF- κB are important transcription factors involved in controlling the expression of proinflammatory mediators.

Wang et al.¹⁶ has reported that dieckol can suppress airborne PM-induced skin aging by inhibiting the expressions of proinflammatory cytokines and MMP through regulating NF- κB , AP-1, and MAPKs signaling pathways in the human dermal fibroblast cells and *in vivo* in zebrafish. Dieckol and eckol attenuated the expression of inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and IL-8 in the human epidermal keratinocytes stimulated with PM10¹⁷.

In addition, *E. cava* extract and dieckol can attenuate cellular lipid peroxidation in keratinocytes exposed to PM10¹⁷.

Ha et al.¹⁸ has revealed that the marine alga *E. cava* extract and dieckol attenuated prostaglandin E2 production in the HaCaT keratinocytes exposed to airborne PM. Lee et al.¹⁹ has reported that dieckol's protective effects isolated *E. cava* against high-glucose-induced oxidative stress in human umbilical vein endothelial cells.

Taken together, dieckol has an anti-inflammatory effect through various mechanisms. Dieckol can decrease NF- κB translocation by an increase in heme oxygenase-1 (HO-1)/nuclear factor erythroid 2-related factor 2 (Nrf-2) signaling²⁰. In addition, dieckol can inhibit phosphorylation of MAPKs which are strong regulators of inflammation²¹. It is revealed that dieckol has antioxidant potency through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and intracellular ROS scavenging²². In addition, dieckol shows high antioxidant activity through the induction of enzymes including HO-1, NAD(P)H:quinine oxidoreductase 1 (NQO1), and glutathione S-transferases (GST) by upregulation of Nrf2-antioxidant response element (ARE) signaling pathway²³.

Other antioxidants, such as punicalagin and epigallocatechin gallate (EGCG), also can attenuate the inflammatory

responses of the human epidermal keratinocytes exposed to PM10. EGCG is more potent than is punicalagin at the same concentration in suppressing PM10's proinflammatory effects, while punicalagin exhibits a wider therapeutic window²⁴. In addition, punicalagin and EGCG are antioxidants that effectively scavenge ROS²⁵. MMPs in the skin degrade macromolecules in the extracellular matrix, including Type I collagen²⁶⁻²⁸. MMP1, secreted by dermal fibroblasts and epidermal keratinocytes, degrades collagen in the skin^{29,30}. PM10-induced MMP1 expression effectively is suppressed by punicalagin and EGCG²⁴. Resveratrol serves as an AhR antagonist and can reverse the induction of CYP1B1 and c-Jun but is ineffective in inhibiting IL-6 and IL-8 secretion³¹.

In our study, dieckol inhibited ROS production in the cultured sebocytes and ORS cells by PM10, as well as reduced the expression of AhR and NF- κ B. In addition, dieckol down-regulated the expression of proinflammatory mediators such as IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α and of MMPs in the cultured sebocytes and ORS cells by PM10. Furthermore, dieckol reduced the expression of the sebum production-related biomarkers in the cultured sebocytes. Dieckol inhibited the expression of the proinflammatory mediators and MMPs in the mice with the *C. acnes*-induced inflammatory nodules by PM10 and reduced sebum production.

In conclusion, PM10 may be an aggravating factor for acne. Dieckol can be an effective anti-inflammatory and antioxidative material for improving this skin disease.

SUPPLEMENTARY MATERIALS

Supplementary data can be found via <http://anndermatol.org/src/sm/ad-34-182-s001.pdf>.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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