Master's Thesis of Science in Biomodulation

Sulforaphene Attenuates Acne-related Responses by Inhibiting Bacterial Growth and Regulating *P. acnes*-induced Inflammation

설포라펜의 *Propionibacterium acnes*에 의해 유도되는 여드름 관련 반응에 대한 항균 및 항염증 효능

August, 2018

The Graduate School

Seoul National University

Biomodulation Major

Hwanju Hwang

ABSTRACT

Acne is one of the common skin diseases with inflammation caused by various pathogenic factors. There are currently several ways to treat acne, but these medications have a variety of side effects. Therefore, there is a need for studies on new components to treat acne with few side effects. Previous studies have shown that sulforaphene has multiple functions which are related to lipid metabolism, antibacterial, and anti-inflammation. However, there is no study of sulforaphene functionalities in acne disorders in which these phenomena work together. Therefore, in this study, I investigated the effects of sulforaphene on the growth of the bacteria and the production levels of inflammatory cytokines due to immune responses to acne bacteria. Sulforaphene inhibited the growth of *Propionibacterium acnes* (*P. acnes*). In addition, sulforaphene regulated the production of pro-inflammatory cytokines in *P. acnes*-treated HaCaT keratinocytes by inhibiting the activity of NF-κB-related pathway. Also, sulforaphene regulates the expression level of IL-1β, one

of the representative pro-inflammatory cytokines expressed in immune cells, induced by heat-killed *P. acnes* in co-cultured HaCaT keratinocytes and THP-1 monocytes. In conclusion, sulforaphene represents an antibacterial activity against *P. acnes* and regulates inflammatory reaction by controlling the inflammatory mechanism in keratinocytes and monocytes. These results support the availability of sulforaphene as a therapeutic compound to treat acne diseases.

Keywords:

Sulforaphene; Acne; *P. acnes*; Inflammation; HaCaT; THP-1;

Student ID: 2016-20486

CONTENTS

Ⅰ. INTRODUCTION

Acne vulgaris is a chronic skin disease that occurs in the hair follicles and sebaceous gland units. It is symptoms such as papules and nodules occur on the face or chest, in which there are a lot of sebaceous glands. As a result of acne, concave scars can appear [1]. There are about 650 million acne patients worldwide. Acne often has a negative psychological and social impact on patients' quality of life due to scarring and hyperpigmentation after inflammation [2]. Acne usually occurs in adolescents during puberty, when sebaceous glands are active. More than 85% of the world's teenagers experience acne. In recent years, acne also tends to occur a lot in adults with 20 to 30 years of age.

Acne has characteristics that are caused by a variety of causes. The pathogenesis of acne is very complex, but it is generally known to go through the multiple pathogenic processes of over-production of sebum,

hyperkeratinization, bacteria proliferation, and inflammation [3]. Because acne is caused by a combination of multiple factors, acne medication needs multi-functionality [4].

Propionibacterium acnes (*P. acnes*), a strain of bacterial flora in the normal skin causes acne inflammation when it overgrows [5]. *P. acnes* is a Gram-positive anaerobic bacteria and the pathogen-associated molecular pattern (PAMP) of *P. acnes* is recognized by toll-like receptor 2 (TLR2). This stimulates the production of pro-inflammatory cytokines such as interleukin (IL)-6, and IL-8 in keratinocytes, and tumor necrosis factor alpha (TNFα) and IL-1β in monocytic cells [6-10]. These inflammatory responses related to the activation of TLR2 are regulated by mitogen-activated protein kinase (MAPK) and the NF-κB transcription factor signaling pathways [11, 12].

Therapeutic agents such as antibiotics of erythromycin, clindamycin, tetracycline, benzoyl peroxide, azelaic acid, and retinoids are widely

used for inhibiting the growth of *P. acnes* and controlling the inflammatory response associated with the bacteria [13-15]. However, these agents have been known that there are various side effects such as skin irritation, antibiotic resistance, teratogenicity, and deterioration of liver function [16]. Therefore, it is necessary to develop new acne medicinal materials that can be safely applied to various causes of acne.

Radish seeds are multi-functional materials which can control lipogenesis, the growth of bacteria, and inflammation [17, 18]. Especially, sulforaphene, which is an active compound of radish seed exerts its functionalities related to lipid metabolism. Also, sulforaphene possesses antibacterial effects against various types of bacteria [19]. In addition, previous studies have shown that sulforaphene has anti-inflammatory and anti-cancer effects [20-22]. Because acne is caused by mechanisms related to these functions, sulforaphene may improve acne disorder.

In this research, I conducted the experiments to examine whether

sulforaphene inhibits the growth of *P. acnes* and inflammatory responses in HaCaT keratinocytes. In addition, I tested the hypothesis that sulforaphene inhibits the inflammatory response by modulating the NF-κB signaling pathway in HaCaT cells. Furthermore, I examined whether the sulforaphene regulation of HaCaT keratinocytes leads to inhibition of IL-1β production by THP-1 monocytes using a co-culture system.

Ⅱ. MATERIALS AND METHODS

1. Chemicals and reagents

Sulforaphene was purchased from LKT Laboratory (New York, USA). Benzoyl peroxide was purchased from Sigma Aldrich (MO, USA). Brain heart infusion agar and Brain heart infusion broth were purchased by BD Biosciences (CA, USA). Dulbecco's Modified Eagle Medium (DMEM) and RPMI-1640 were purchased from Welgene (Gyeongsan, South Korea). Fetal bovine serum (FBS) was purchased from VWR (PA, USA). Penicillin (10,000 IU)/streptomycin (10,000 μg/ml) solution was bought from Corning, Inc. (NY, USA). Antibodies against p-IKKα/β, p-IκBα, basal IκBα were purchased from Cell Signaling Biotechnology (MA, USA). The other antibodies against total IKKα/β and GAPDH were bought from Santa Cruz Biotechnology (CA, USA). Protein assay reagent kits were obtained from BioRad Laboratories (CA, USA).

2. Cell culture

HaCaT cells were maintained in DMEM, supplemented with 10% (v/v) FBS and 0.2% (v/v) penicillin/streptomycin at 37°C and in a 5% CO_2 atmosphere. Human THP-1 promonocytic cells were cultured in RPMI-1640 medium, supplemented with 10% (v/v) FBS, 1% (v/v) penicillin/streptomycin, and 0.05 mM mercaptoethanol at 37°C and in a 5% CO_2 atmosphere.

To examine the effect of sulforaphene on HaCaT cells, cells were seeded in complete medium in 6-well plate at a density of 1.0×10^6 cells per well. Twenty-four hour later, the cells were changed to serum-free DMEM and incubated for 12 h. After serum-starvation, HaCaT cells were treated with serum-free medium containing sulforaphene, dissolved in DMSO, for 1 hour before the addition of heat-killed *P. acnes* (multiplicity of infection (MOI)=100).

In co-culture experiments, HaCaT cells were seeded at a density of

1.0×10^5 cells per wells in upper part of 12-well Transwell (Corning, NY, USA) and THP-1 cells were seeded at a density of 2.0×10^5 cells per wells in lower part of Transwell. Media in the upper wells were changed to serum-free media containing sulforaphene (5, 10, and 20 μM), and after 1 h, the cells were stimulated with the heat-killed *P. acnes* (MOI = 100)

3. Bacteria culture

Propionibacterium acnes ATCC 6919 were cultured on Brain Heart Infusion broth medium under anaerobic conditions at 37°C. GasPak (BD, CA, USA) was used to make an anaerobic environment. The OD value was measured at 600 nm and the colony-forming unit (CFU) of *P. acnes* was counted every 12 hours to obtain the growth curve. To obtain heat-killed *P. acnes*, the bacteria in the exponential phase of growth were centrifuged at 10,000 g at 4°C for 10 minutes. Pellets were washed three times with phosphate-buffered saline (PBS). The bacterial suspension

was heated at 65℃ for 30 min and was collected pellets by centrifugation at 10,000 g at 4°C for 10 minutes. The pellets were resuspended in serum-free DMEM and were stored at -80 °C until use.

4. Minimum inhibitory concentration (MIC) test

The MIC tests were conducted by Culture Collection of Antimicrobial Resistant Microbes (CCARM, Seoul, Korea). MIC was measured according to the guidelines of the Clinical Laboratory Standards Institute (CLSI). Sulforaphene were diluted to a concentration of 1.25 mM to 0.002 mM in the medium. MIC tests against *Escherichia coli* CCARM 0012, *Staphylococcus aureus* CCARM 3102 were performed by plate culture dilution using Muller Hinton I medium (BBL, Sparks, MD, USA). Norfloxacin was used as a control antibiotic. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as control strains. The MIC against *Propionibacterium acnes* CCARM 9008 was determined by microfluidic

dilution method with 5% lysed horse blood added to cation-adjusted Muller Hinton Ⅱ broth (BBL). Clindamycin was used as a control antibiotic for the quality control of microfluidic dilution method. *Streptococcus pneumoniae* CCARM 0031 was used as a control strain.

5. Cell viability assay

Cell viability of HaCaT cells was determined by MTT assay. HaCaT cells were cultured in the 96 well plates at 5.0×10^4 cells per wells and incubated in completed DMEM. Cells were starved in serum-free DMEM for 12 h. After serum-starvation, the cells were incubated with different concentrations of sulforaphene or heat-killed *P. acnes* for 22 h. After the addition of MTT solution (10% v/v) in serum-free DMEM, the cells were incubated for 2 h. The medium was removed and dimethyl sulfoxide (DMSO) was added to each well to dissolve formazan crystals. The absorbance at 570 nm was measured using a microplate reader.

6. Enzyme-linked immunosorbent assay (ELISA)

To identify IL-6, IL-8, and IL-1β contents in the conditioned media of HaCaT cells and THP-1 cells, human IL-6, human IL-8/CXCL8 and human IL-1β DuoSet ELISA (R&D system, MN, USA) were used according to the manufacturer's instructions.

7. Real-time quantitative PCR

HaCaT cells were treated with the indicated concentration of suforaphene and stimulated with the heat-killed *P. acnes*. RNA in HaCaT cells were harvested in RNAiso Plus (Takara Bio, Shiga, Japan). The concentration and purity of RNA samples were quantified using NanoDrop ND-2000 spectrophotometer.

After reverse transcription with oligo-dT primers using a PrimeScriptTM 1st strand cDNA Synthesis Kit (Takara Bio Inc.), real-time quantitative PCR was performed using IQ SYBR (Bio-Rad

Laboratories). 2 μl of cDNA was used in triplicate with GAPDH as an internal control. Before PCR amplification, the primers were denatured at 95 °C for 3 min. Amplification was made up of 44 cycles at 95 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds. PCR was performed by CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories). cDNA was probed by the following primer: IL-6 forward (5'- CAA TCT GGA TTC AAT GAG GAG AG -3'); IL-6 reverse (5'- CTC TGG CTT GTT CCT CAC TAC TC -3'); IL-8 forward (5'- TCT TGG CAG CCT TCC TGA TT -3'); IL-8 reverse (5'- TTT CGT GTT GGC GCA GTG T -3'); GAPDH forward (5'- TCC TCA CCC TGA AGT ACC CCA T –3'); GAPDH reverse (5'- AGC CAC ACG CAG CTC ATT GTA -3')

8. Luciferase reporter gene assay

The lentiviral expression vector, including pGF-NF-κB-mCMV-EF1-Puro were obtained from System Biosciences (CA, USA). Packaging vectors (pMD2.0G) and enveloping vector (psPAX) were purchased from Addgene (Cambridge, MA, USA). The vectors were transfected into HEK293T cells using jetPEI according to the manufacturer's instructions. The conditioned medium was collected and then infected into 50 % confluent HaCaT cells overnight. The cell culture medium was replaced with a fresh complete medium for 24 h before the infected cells were selected by using puromycin. The selected cells were used for the reporter gene assay.

The cell extracts were collected using a reporter lysis buffer (Promega, WI, USA) for luciferase assay. NF-κB transcription activity in HaCaT cells was determined by using a luciferase assay kit (Promega, WI, USA).

9. Western blot assay

After removing cell culture media, cells were lysed with RIPA lysis buffer containing 10 mM Tris (pH 7.5), 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, 1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10% glycerol and a protease inhibitor cocktail tablet (Gendepot, TX, USA). The protein concentration of lysates was measured using a protein assay reagent kits as described by the manufacturer. 60 μg of protein lysates were separated by SDS-PAGE using a 10 % SDS-polyacrylamide gel and transferred onto Immobilon-P PVDF membrane (MERK Millipore). The membrane was blocked in 5 % fat-free skim milk for 1 h and then treated with the specific primary antibody at 4°C overnight. Protein bands were visualized using a chemiluminescence detection kit (GE Healthcare, London, UK) after hybridization with the HRP-conjugated secondary antibody (Life Technologies, Waltham, MA).

10. Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics ver. 23.0 (IBM, NY, USA) and *P* values of less than 0.05 were considered statistically different. Differences between controls and heat-killed *P. acnes*-treated groups were assessed with Student's *t*-test. The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD and expressed as a mean ± standard error of the mean (SEM).

Ⅲ. RESULTS

1. Sulforaphene inhibited the growth of *P. acnes*

To determine the exponential growth phase of *P. acnes*, the growth curve of the bacteria was drawn by measuring the absorbance at 600 nm of the cultured medium and colony-forming unit (CFU) of bacteria every 12 hours. According to the curve, *P. acnes* reached the exponential phase of growth after 96 h, so the 96 h-cultured bacteria were used for the experiments (Fig. 1A).

To identify the effects of sulforaphene on the growth of bacteria, Minimum inhibitory concentration tests were used against *E. coli* CCARM 3102, *P. acnes* CCARM 9008, or *S. aureus* CCARM 3102 (Table 1). The minimum inhibitory concentration of sulforaphene was 1.25 mM or less for all strains. Especially, the growth of *P. acnes* was inhibited at a lower concentration of sulforaphene than that for other bacteria.

Figure 1

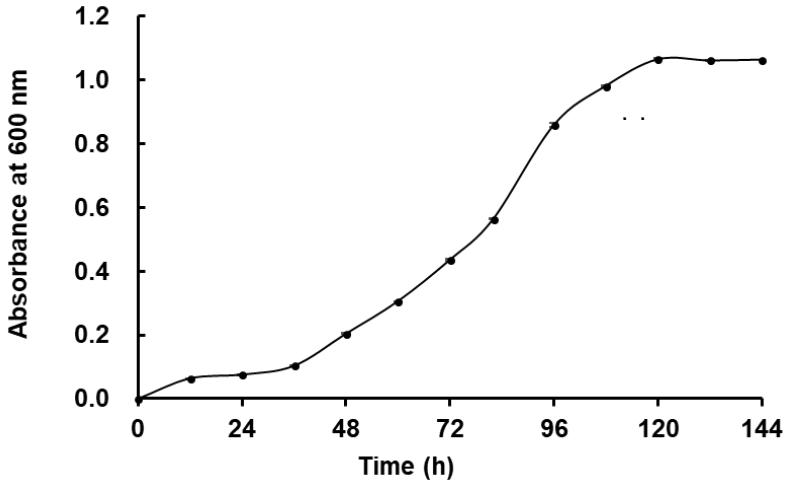


Figure 1. Growth curve of *P. acnes*

The absorbance of cultured media was measured at 600 nm, and the growth curve was obtained as described in MATERIALS AND METHODS.

Table 1

Table 1. Minimum inhibitory concentrations of sulforaphene

Microbial strains	Minimum inhibitory concentration (MIC)		
	Sulforaphene (mM)	Norfloxacin (㎍/ml)	Clindamycin (㎍/ml)
E. coli CCARM 0012	1.25	0.12	-
P. acnes CCARM 9008	0.31	-	≤0.06
S. aureus CCARM 3102	1.25	32	-
E. coli ATCC 25922	1.25	0.06	-
S. aureus ATCC 29213	1.25	1	-
S. pneumoniae CCARM 0031	1.25	-	0.03

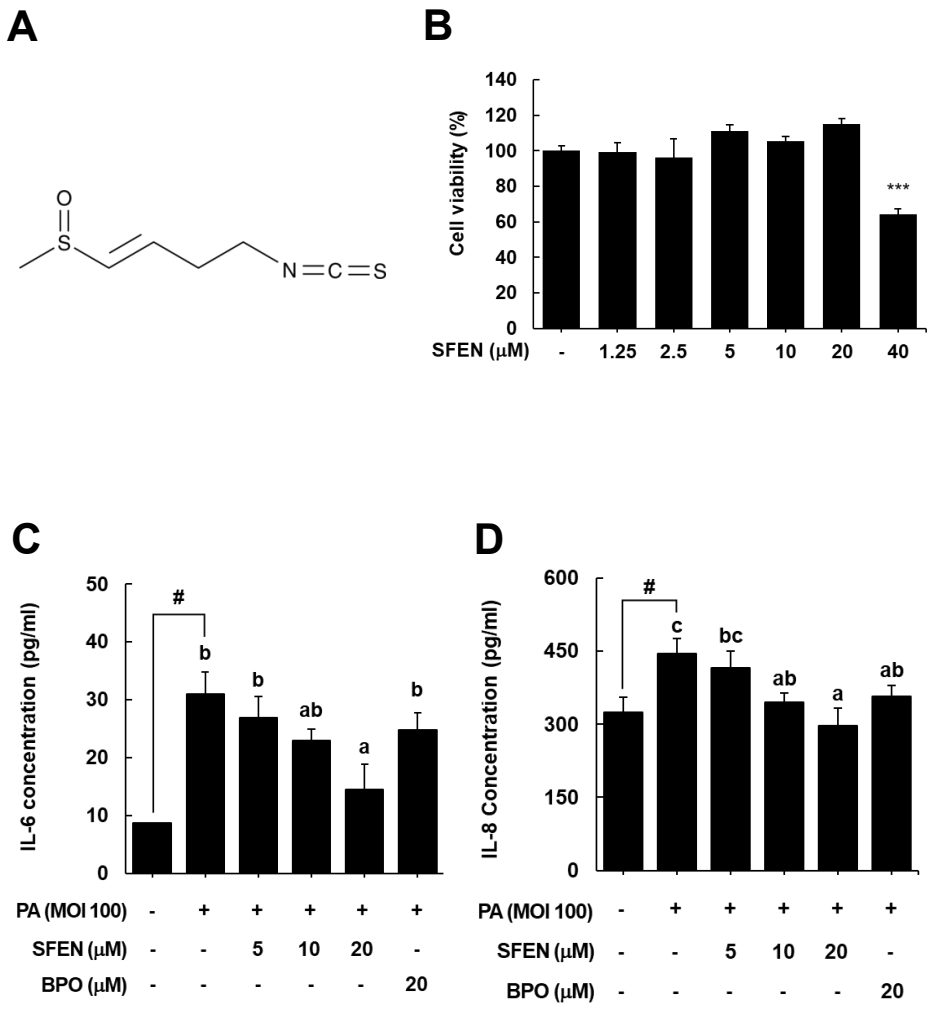
The MIC tests against various types of bacteria were conducted as described in MATERIALS AND METHODS.

2. Sulforaphene regulated the production of pro-inflammatory cytokines in *P. acnes*-treated HaCaT keratinocytes

To determine the cytotoxicity of sulforaphene, MTT assay was performed at different concentrations. The viability of HaCaT cells was reduced at a concentration of 40 μM sulforaphene (Fig. 2B). Therefore, the maximum concentration of sulforaphene used in this research was set to 20 μM.

IL-6 and IL-8 cytokines were produced by HaCaT cells as a result of immune responses to *P. acnes*. I investigated the amount of production of cytokines using ELISA. Sulforaphene significantly reduced *P. acnes*-induced secretion of IL-6 (Fig. 2C) and IL-8 (Fig. 2D) at the concentration of 20 μM. Also, the ability of sulforaphene to inhibit the secretion of IL-6 was better than that of benzoyl peroxide. When the mRNA expression levels of these cytokines were assayed by real-time qPCR, the expression induced by *P. acnes* was also reduced in a dose-dependent manner by sulforaphene with a similar tendency to protein secretion (Fig. 2E, 2F)

Figure 2


A
O
S
N=C=S
B
Cell viability (%)
140
120
100
80
60
40
20
0
***
SFEN (μM) - 1.25 2.5 5 10 20 40
C
IL-6 concentration (pg/ml)
50
40
30
20
10
0
#
b
b
ab
a
b
PA (MOI 100) - + + + + +
SFEN (μM) - - 5 10 20 -
BPO (μM) - - - - - 20
D
IL-8 Concentration (pg/ml)
600
450
300
150
0
#
c
bc
ab
a
ab
PA (MOI 100) - + + + + +
SFEN (μM) - - 5 10 20 -
BPO (μM) - - - - - 20

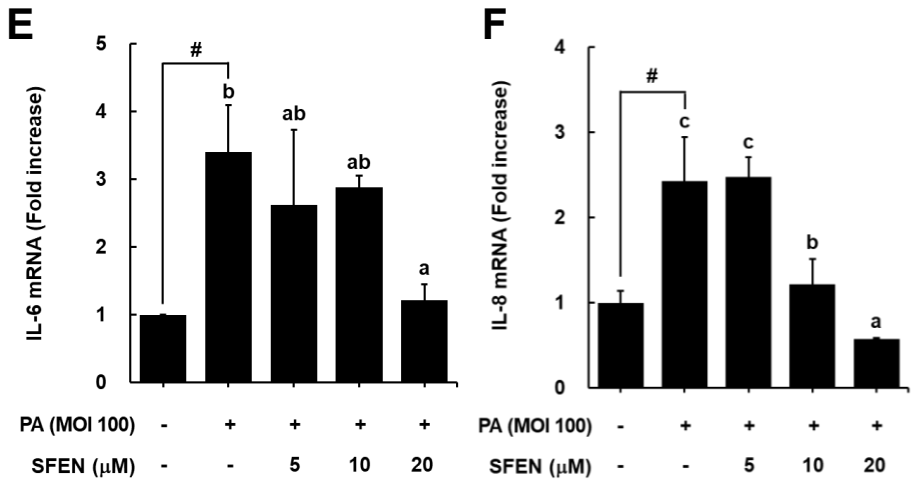


Figure 2. Effects of Sulforaphene on the production of pro-inflammatory cytokines in HaCaT cells

(A) Chemical structure of sulforaphene. (B) Cell viability was evaluated using MTT assay. The secretion levels of (C) IL-6, and (D) IL-8 were detected by ELISA. The expression levels of (E) IL-6 mRNA, and (F) IL-8 mRNA were detected using real-time quantitative PCR described in MATERIALS AND METHODS. All graphs represent the means ± SEM (n=3). Mean values without the same letters (a-c) are significantly different among the different concentration of sulforaphene (p<0.05).

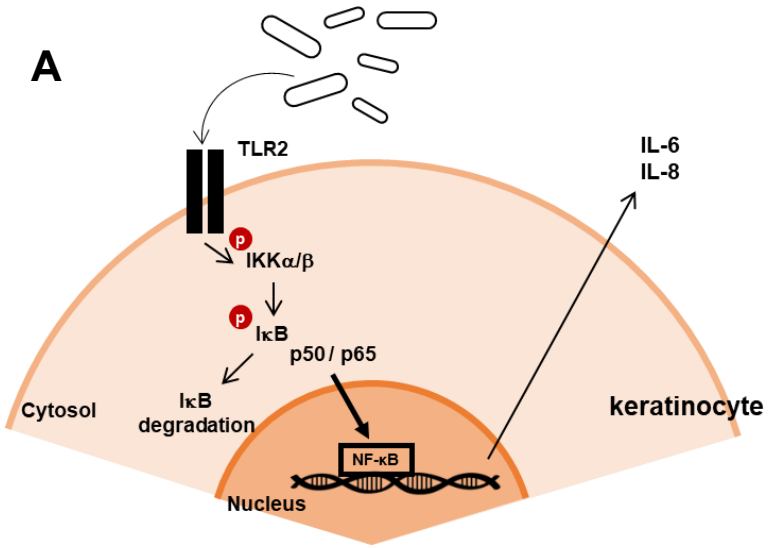
$^{***}p<0.001$ between the non-treated group and sulforaphene–treated groups, $^{\#}p<0.05$ between the non-treated group and heat-killed *P. acnes*–treated group without sulforaphene.

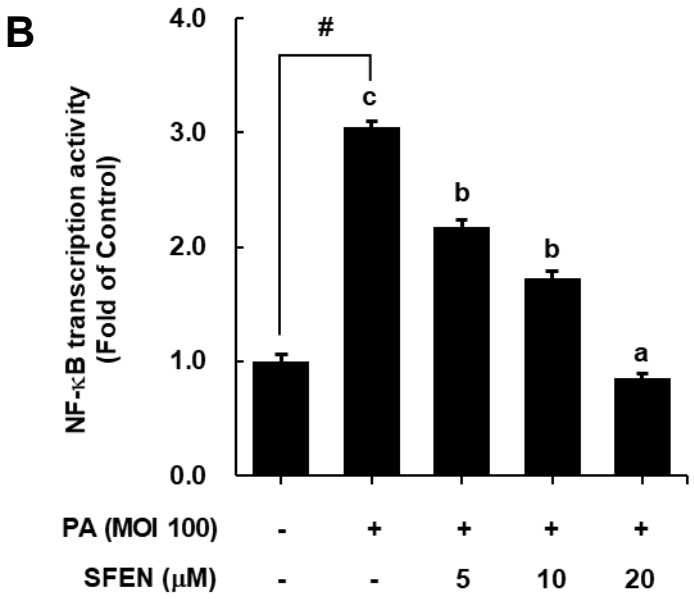
3. Sulforaphene inhibited the inflammatory responses by regulating the NF-κB signaling pathway in HaCaT keratinocytes

The cytokines are regulated by the NF-κB transcription factor in HaCaT keratinocytes (Fig. 3A). To investigate the activity of NF-κB transcription factor, I performed luciferase reporter gene assay. When HaCaT were treated with *P. acnes*, the transcription activity of NF-κB was increased compared to the control. With sulforaphene, the *P. acnes*-induced activity was significantly reduced in a dose-dependent manner (Fig. 3B).

Also, sulforaphene inhibits phosphorylation of IκBα and IKKα/β (Fig. 3C), which are upstream regulators of NF-κB (Fig. 3A).

Figure 3


A
TLR2
IL-6
IL-8
IKKα/β
IκB
p50 / p65
IκB
degradation
Cytosol
keratinocyte
NF-κB
Nucleus


B
NF-κB transcription activity
(Fold of Control)
4.0
3.0
2.0
1.0
0.0
#
c
b
b
a
PA (MOI 100) - + + + +
SFEN (μM) - - 5 10 20

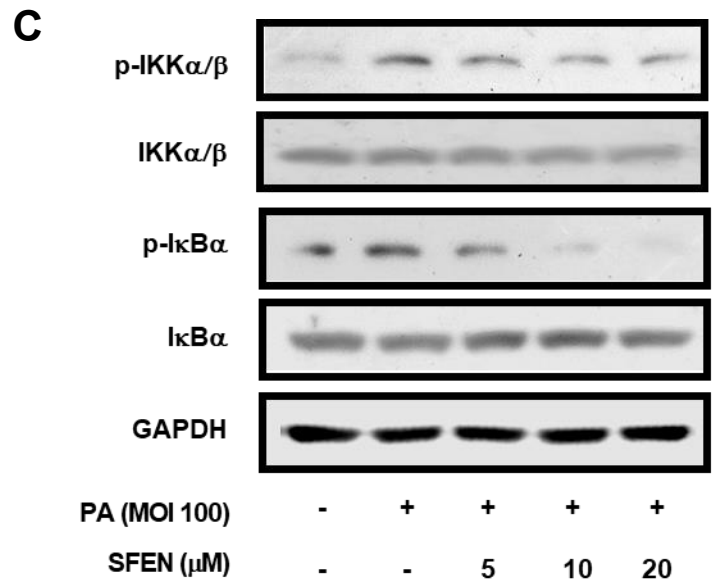


Figure 3. Inhibitory effects of sulforaphene on the transcription activity of NF-κB and the upstream regulator proteins of NF-κB

(A) NF-κB signaling pathway was described. (B) Transcription activity of NF-κB was analyzed by luciferase reporter gene assay described in the MATERIALS AND METHODS. Data (n=6) represented the mean values ± SEM. $^{\#}p<0.05$ between the non-treated group and heat-killed *P. acnes*–treated group without treatment of sulforaphene. Means without the same letters (a-c) are significantly different among the

different concentration of sulforaphene (p<0.05). (C) Phosphorylated and total forms of IKKα/β and IκBα proteins were determined by western blot assay described in MATERIALS AND METHODS.

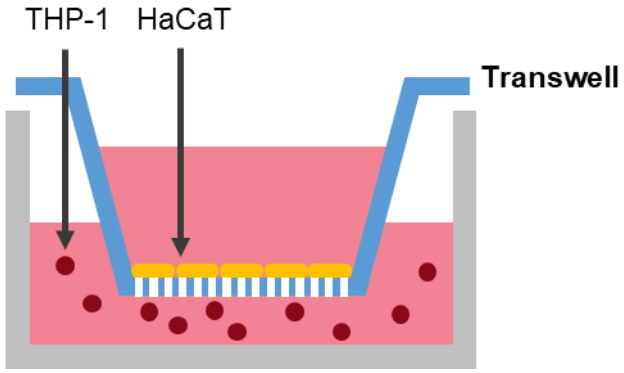
4. *P. acnes*-induced the secretion of IL-1β in a co-culture system of keratinocytes and monocytic cells

The cytokines expressed in keratinocytes act on immune cells and promote the expression of another proinflammatory cytokine such as IL-1β [23]. The interaction between these cells has been reported in previous studies [24]. Therefore, I used a co-culture experimental model of HaCaT keratinocytes and THP-1 monocytes to identify changes in the secretion of IL-1β.

To examine the secretion level of IL-1β in the co-culture model, I divided into three conditioned groups; HaCaT only, THP-1 only, and HaCaT and THP-1 were seeded. Then, each group is induced by P. acnes. The secretion of IL-1β was significantly increased when HaCaT and THP-1 were co-cultured, which was further induced by the *P. acnes*.

Figure 4

A


THP-1
HaCaT
Transwell

B

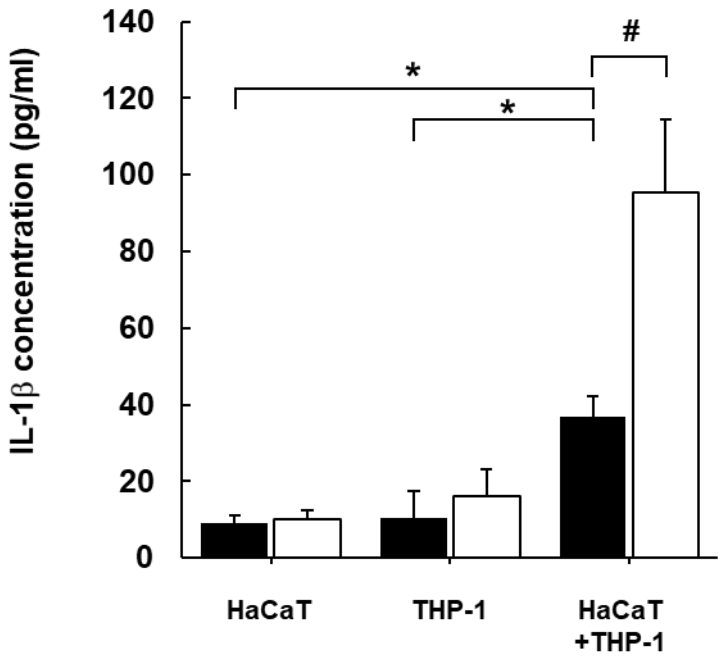

IL-1β concentration (pg/ml)
140
120
100
80
60
40
20
0
*
*
#
HaCaT
THP-1
HaCaT
+THP-1

Figure 4. Effects of *P. acnes* on the production of IL-1β cytokines by the interaction between HaCaT cells and THP-1

(A) The co-culture method of HaCaT cells and THP-1 cells using transwells as described in MATERIALS AND METHODS. (B) The concentration of IL-1β in conditioned media was detected by ELISA. $^{*}P<0.05$ between non-treated HaCaT only or THP-1 only groups and non-treated HaCaT and THP-1-seeded group. $^{\#}P<0.05$ between non-treated cells and heat-killed *P. acnes*–treated cells of the co-cultured group.

5. Sulforaphene reduced *P. acnes*-induced secretion of IL-1β in a co-culture of HaCaT keratinocytes and THP-1 monocytes

I identified that the production level of IL-1β was increased by co-culturing both two cells and treating with *P. acnes*. Therefore, using this experimental model, I examined the changes in the amount of IL-1β secreted during sulforaphene treatment. The amount of IL-1β increased by heat-killed *P. acnes* was decreased by sulforaphene in a dose-dependent manner.

Figure 5

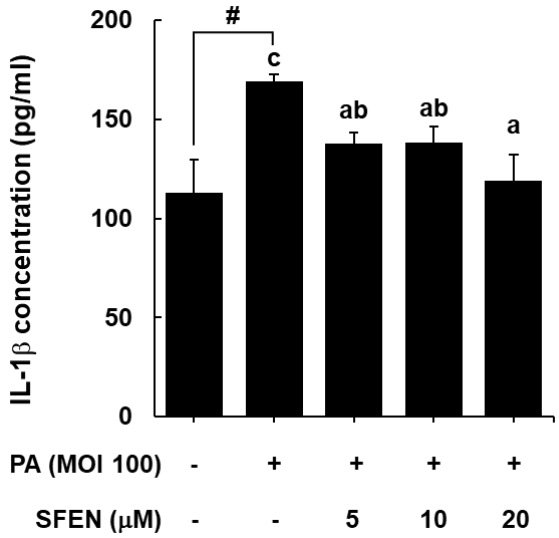


Figure 5. Effects of sulforaphene on the production of IL-1β cytokine in a co-culture model of HaCaT cells and THP-1 cells

The protein level of IL-1β cytokine was determined by ELISA as described in the Materials and Methods. Data (n=3) represented the mean values ± SEM. Means without the same letters (a-c) are significantly different among the different concentration of sulforaphene (p<0.05). $^{\#}p$<0.05 between the non-treated group and heat-killed *P. acnes*–treated group without sulforaphene.

Figure 6

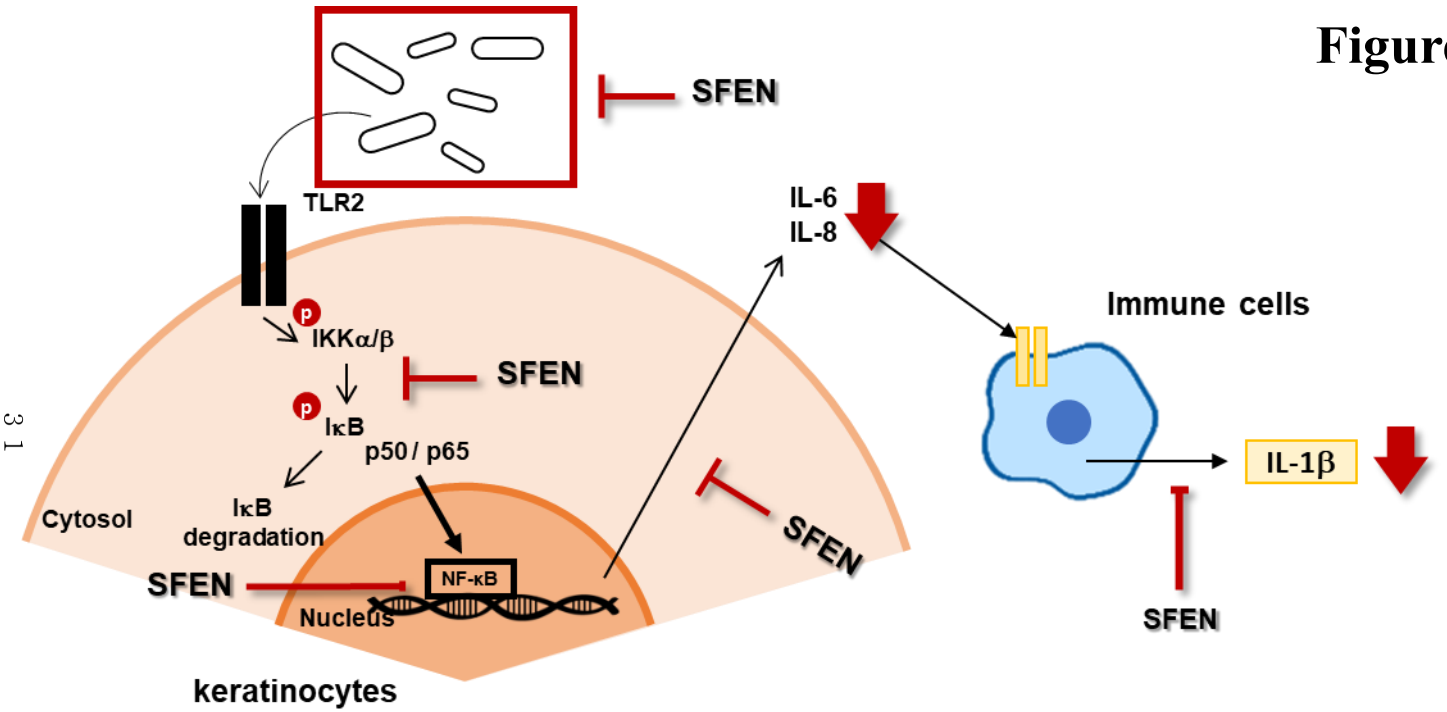


Figure 6. Summary

Ⅳ. DISCUSSION

Acne is a disease caused by various causes. Thus, to treat acne effectively, it is necessary to control a variety of factors. In particular, acne inflammation is associated with increased bacterial growth and immune response by bacteria. Therefore, it is important to control these factors to treat acne after inflammation has occurred.

In previous studies about sulforaphene, it shows antibacterial effects against various types of bacteria. Also, in this experiment, sulforaphene exhibited antibacterial activity against various bacterial strains such as *E. coli*, *S. aureus*, and *P. acnes*. Especially, *P. acnes* is more sensitive to sulforaphene than other bacteria. Therefore, it can be expected that sulforaphene specifically suppresses the growth of *P. acnes*.

P. acnes is one of the critical factors that cause acne-related inflammatory reactions. In previous studies, it is known that acne

inflammation is caused by immune responses between TLR2 and *P. acnes*. Activation of TLR2 induces the production and release of proinflammatory cytokines or chemokines such as IL-6 and IL-8 in keratinocytes [25]. These cytokines have a role to regulate inflammatory responses in keratinocytes or monocytes [26, 27].

Activation of TLR2 induces NF-κB and MAPK signaling pathways. Activated NF-κB transcription factor promotes the expression level of the gene of proinflammatory cytokines, production and secretion of inflammatory cytokines [28, 29]. In this study, sulforaphene inhibited the transcription activity of NF-κB by suppressing phosphorylation of IκBα and IKKα/β, which are upstream regulators of NF-κB in HaCaT cells.

The cytokines and chemokines produced by *P. acnes*-induced keratinocyte act on the immune cells in the skin and cause secretion of other pro-inflammatory cytokines such as IL-1β [23]. In particular, the IL-1β cytokine is known to be produced and secreted by NLRP3

inflammasome activation, a pattern recognition receptor (PRR) in immune cells[30]. Thus, in this study, I conducted a co-culture experiment with three conditioned groups to determine whether IL-1β production is induced by interactions between immune cells and keratinocytes; 1) only HaCaT cells, 2) only THP-1 cells, and 3) both HaCaT and THP-1 cells. My results clearly showed that the amount of IL-1β secretion increased when co-cultured, compared to when two cell lines were cultured alone. In addition, when the *P. acnes* was added to the co-culture well, the amount of IL-1β was further increased. Moreover, when the co-culture cells were treated with sulforaphene, the secretion level of IL-1β was decreased. Taken together, these results confirmed that the interaction of keratinocytes and monocytes in the *P. acnes* infection site plays an important role in post-infection inflammatory response, which can be prevented by sulforaphene

However, in this research, the mechanism which sulforaphene

regulates the secretion of IL-1β was not identified. Also, it is not known which mechanism influences cytokine secretion in the co-culture model. Therefore, it is necessary to study the mechanism of IL-1β secretion through further experiments.

In order to effectively control acne, multi-functionality such as inhibition of hyperkeratosis and sebum production are required in addition to the antimicrobial and anti-inflammatory effects observed in this experiment. Therefore, additional experiments related to hyperkeratosis and sebum production should be conducted. In addition, the molecular mechanism that plays a major role in the pathogenesis of acne has not yet been clarified, and the precise molecular target of sulforaphene for acne inflammation is not clear. Therefore, the molecular mechanism of sulforaphene should be identified.

In conclusion, I identified the protective and therapeutic effects of sulforaphene on the growth of *P. acnes* and the *P. acnes*-induced

inflammatory responses. These results show that sulforaphene has a potential to apply sulforaphene for the prevention or treatment of acne diseases induced by *P. acnes*.

Ⅴ. REFERENCES

1. Williams, H.C., R.P. Dellavalle, and S. Garner, *Acne vulgaris.* The Lancet, 2012. **379**(9813): 361-372.
2. Hay, R.J., et al., *The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions.* J Invest Dermatol, 2014. **134**(6): 1527-1534.
3. Zouboulis, C.C., et al., *What is the pathogenesis of acne?* Experimental Dermatology, 2005. **14**(2): 143-143.
4. Kurokawa, I., et al., *New developments in our understanding of acne pathogenesis and treatment.* Exp Dermatol, 2009. **18**(10): 821-32.
5. Tanghetti, E.A., *The Role of Inflammation in the Pathology of Acne.* The Journal of Clinical and Aesthetic Dermatology, 2013. **6**(9): 27-35.
6. Dessinioti, C. and A.D. Katsambas, *The role of Propionibacterium acnes in acne pathogenesis: facts and controversies.* Clin Dermatol, 2010. **28**(1): 2-7.
7. Lheure, C., et al., *TLR-2 Recognizes Propionibacterium acnes CAMP Factor 1 from Highly Inflammatory Strains.* PLoS One, 2016. **11**(11): e0167237.
8. Vowels, B.R., S. Yang, and J.J. Leyden, *Induction of proinflammatory cytokines by a soluble factor of Propionibacterium acnes: implications for chronic inflammatory acne.* Infection and Immunity, 1995. **63**(8): 3158-3165.
9. Beylot, C., et al., *Propionibacterium acnes: an update on its role in the pathogenesis of acne.* J Eur Acad Dermatol Venereol, 2014. **28**(3): 271-8.
10. Shibata, M., et al., *Glucocorticoids enhance Toll-like receptor 2 expression in human keratinocytes stimulated with Propionibacterium acnes or proinflammatory cytokines.* J Invest Dermatol, 2009. **129**(2): 375-82.
11. Grange, P.A., et al., *Nicotinamide inhibits Propionibacterium acnes-induced IL-8 production in keratinocytes through the NF-kappaB and MAPK*

pathways. J Dermatol Sci, 2009. **56**(2): 106-12.

12. Wang, Y.Y., et al., *Chlorin e6-Mediated Photodynamic Therapy Suppresses P. acnes-Induced Inflammatory Response via NFkappaB and MAPKs Signaling Pathway.* PLoS One, 2017. **12**(1): e0170599.
13. Zaenglein, A.L., et al., *Guidelines of care for the management of acne vulgaris.* J Am Acad Dermatol, 2016. **74**(5): 945-73 e33.
14. Sato, T., et al., *Adapalene suppresses sebum accumulation via the inhibition of triacylglycerol biosynthesis and perilipin expression in differentiated hamster sebocytes in vitro.* J Dermatol Sci, 2013. **70**(3): 204-10.
15. Hun Suh, D., *Pharmacologic Treatment of Acne*. Vol. 53. 2010.
16. Walsh, T.R., J. Efthimiou, and B. Dréno, *Systematic review of antibiotic resistance in acne: an increasing topical and oral threat.* The Lancet Infectious Diseases, 2016. **16**(3): e23-e33.
17. Jadoun, J., et al., *Identification of a New Antibacterial Sulfur Compound from Raphanus sativus Seeds.* Evidence-based Complementary and Alternative Medicine : eCAM, 2016. **2016**: 9271285.
18. Sham, T.T., et al., *A review of the phytochemistry and pharmacological activities of raphani semen.* Evid Based Complement Alternat Med, 2013. **2013**: 636194.
19. Lim, S., S.-W. Han, and J. Kim, *Sulforaphene identified from radish (Raphanus sativus L.) seeds possesses antimicrobial properties against multidrug-resistant bacteria and methicillin-resistant Staphylococcus aureus.* Journal of Functional Foods, 2016. **24**: 131-141.
20. Bao, C., et al., *Sulforaphene Interferes with Human Breast Cancer Cell Migration and Invasion through Inhibition of Hedgehog Signaling.* J Agric Food Chem, 2016. **64**(27): 5515-24.
21. Byun, S., et al., *Sulforaphene suppresses growth of colon cancer-derived tumors via induction of glutathione depletion and microtubule depolymerization.* Mol Nutr Food Res, 2016. **60**(5): 1068-78.
22. Yang, M., et al., *The natural compound sulforaphene, as a novel anticancer*

reagent, targeting PI3K-AKT signaling pathway in lung cancer. Oncotarget, 2016. **7**(47): 76656-76666.

23. Kistowska, M., et al., *IL-1beta drives inflammatory responses to propionibacterium acnes in vitro and in vivo.* J Invest Dermatol, 2014. **134**(3): 677-85.
24. Hennen, J., et al., *Cross talk between keratinocytes and dendritic cells: impact on the prediction of sensitization.* Toxicol Sci, 2011. **123**(2): 501-10.
25. Selway, J.L., et al., *Toll-like receptor 2 activation and comedogenesis: implications for the pathogenesis of acne.* BMC Dermatology, 2013. **13**: 10-10.
26. Hernandez-Quintero, M., et al., *Interleukin-6 promotes human epidermal keratinocyte proliferation and keratin cytoskeleton reorganization in culture.* Cell Tissue Res, 2006. **325**(1): 77-90.
27. Dixit, N. and S.I. Simon, *Chemokines, selectins and intracellular calcium flux: temporal and spatial cues for leukocyte arrest.* Front Immunol, 2012. **3**: 188.
28. Kim, J., et al., *Activation of Toll-Like Receptor 2 in Acne Triggers Inflammatory Cytokine Responses.* The Journal of Immunology, 2002. **169**(3): 1535-1541.
29. Chen, Q., et al., *Propionibacterium acnes-induced IL-8 production may be mediated by NF-κB activation in human monocytes.* Journal of Dermatological Science, 2002. **29**(2): 97-103.
30. Qin, M., et al., *Propionibacterium acnes Induces IL-1beta secretion via the NLRP3 inflammasome in human monocytes.* J Invest Dermatol, 2014. **134**(2): 381-8.

국문 초록

여드름은 다양한 병인에 의해 발생하는 흔한 염증성 피부 질환 중 하나이다. 현재 여드름을 치료할 수 있는 여러가지 방법들이 존재하지만, 대부분의 여드름 치료법은 다양한 부작용을 보인다. 따라서 부작용이 적게 여드름을 치료할 수 있는 신물질에 대한 연구의 필요성이 늘어나고 있다.

한편, 설포라핀(sulforaphene)은 여러 선행연구를 통해서 지질 대사, 항균, 항염증과 관련된 효능이 알려져 있다. 하지만, 앞에서 언급되었던 기작들이 서로 연계되어 발생하는 여드름 질환에서의 기능성에 대한 연구는 진행되지 않았다. 따라서, 본 연구에서는 여드름 유발 균주인 프로피오니박테리움 아크네스(*P. acnes*) 균의 성장 및 균에 의해 발생하는 염증반응에서의 설포라핀의 항균 및 항염증 효능 평가를 실시하여, 설포라핀의 여드름 완화 효능을 *in vitro* 실험을 통해 평가하였다.

설포라핀은 여드름을 유발하는 프로피오니박테리움 아크네스 균의 성장을 억제한다. 그리고 설포라핀은 NF-κB와 관련된 기작을 조절하여, *P. acnes*를 처리한 HaCaT 각질화세포에서의 염증성 사이토카인의 분비를 억제한다. 또한, 설포라핀이 면역세포에서 생성되는 IL-1β의 생성을 억제함을 HaCaT 세포와 THP-1 단핵구세포의 공동배양 모델을 통해 밝혀내었다. 결론적으로, 설포라핀은 *P. acnes* 균에 대한 항균효능을 보이고, 각질세포와 단핵구세포에서의 염증 기작을 조절하여 염증반응을 억제한다. 이 결과를 통해 설포라핀은 여드름 치료 소재로서의 개발 가치가 있을 것으로 사료된다.