Original Article
Annals of Dermatology 2022;34(2):95-104 • https://doi.org/10.5021/ad.2022.34.2.95

Effects of Black Ginseng Water Extract under the Inflammatory Conditions of Cultured Sebocytes and Outer Root Sheath Cells

Jun Young Kim[1], Mi Hee Kwack[2,3], Eun Hye Lee[1], Weon Ju Lee[1]

[1]Department of Dermatology, Kyungpook National University Hospital, School of Medicine, Kyungpook National University, [2]Department of Immunology, School of Medicine, Kyungpook National University, [3]BK21 FOUR KNU Convergence Educational Program of Biomedical Sciences for Creative Future Talents, School of Medicine, Kyungpook National University, Daegu, Korea

Received March 22, 2021
Revised September 14, 2021
Accepted September 30, 2021

Corresponding Author
Weon Ju Lee
Department of Dermatology, Kyungpook National University Hospital, 130 Dongdeok-ro, Jung-gu, Daegu 41944, Korea
Tel: +82-53-420-5838
Fax: +82-53-426-0770
E-mail: weonju@knu.ac.kr
https://orcid.org/0000-0001-5708-1305

Background: Ginseng has been used in Korea for a long time as a restorative herbal medicine. Black ginseng (BG) is made from red or white ginseng by multiple steamy and dry processes. Although BG has been reported to have anti-inflammatory potential, studies on its influence on inflammatory skin disorders are lacking.
Objective: To investigate the effects of BG under the inflammatory conditions of cultured sebocytes and outer root sheath (ORS) cells.
Methods: The cultured cells were treated with 0.1% dimethyl sulfoxide, 5 μg/ml lipopolysaccharide (LPS) or 5 μg/ml LPS+50 μg/ml BG for 6 hours and 24 hours. Reverse transcription-polymerase chain reaction (RT-PCR), real-time PCR, enzyme-linked immunosorbent assay, western blotting, immunofluorescence staining and Nile red staining were performed for analysis of inflammatory biomarkers and sebum-related biomarkers.
Results: BG brought out the increased gene and protein expression of inflammatory biomarkers such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α, in the LPS-treated sebocytes and ORS cells. In addition, BG induced increased expression of TLR4, p-c-jun, p-JNK and p-iκB in LPS-treated sebocytes and ORS cells. Furthermore, it significantly increased the expression of LL-37 and the production of sebum in LPS-treated sebocytes.
Conclusion: It may be possible for BG to increase the expression of inflammatory biomarkers in inflammatory skin disorders, such as acne.

Keywords: Black ginseng, Outer root sheath cells, Sebocytes

INTRODUCTION

Ginseng is an herbal medicine using plant roots of the genus *Panax*, which has bioactive metabolites[1]. Ginseng has been known in Korea for a long time as a restorative herbal medicine, health promoter and therapeutic agent for sexual dysfunction and infectious diseases. It is clinically proven to have various pharmacological effects on human health improvement. Immunological stimulation, induction of apoptosis and suppression of cell proliferation have been reported as effects of *Panax ginseng*[2]. A variety of laboratory and clinical studies have shown that ginseng can be a good therapeutic agent for metabolic diseases and degenerative disorders[3,4]. In addition, ginseng has been taken as dietary supplements in Korea[5].

Black ginseng is processed ginseng produced from white or red ginseng through repeated processes of steaming and drying[6-8]. This process leads to wide changes in the characteristics of secondary metabolites in ginseng. The main secondary bioactive metabolites of ginseng have been known to be ginsenosides, which is a kind of dammarane-type triterpene saponins. In black ginseng, they transform into less polar ginsenosides because of steaming. Additionally, significant changes occur

Copyright © The Korean Dermatological Association and The Korean Society for Investigative Dermatology

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

in other secondary metabolites. The amounts of phenolic compounds are increased. On the contrary, the contents of sugars and acidic polysaccharides are decreased. Additionally, the concentration of free amino acids and polysaccharides is decreased. In most comparative biological studies, these obvious chemical changes have been found to be associated noticeably more with black ginseng than with red or white ginseng. White ginseng is made by sunlight-drying after peeling of ginseng roots, and red ginseng is produced by steaming at around 100℃ before drying[9].

Despite numerous clinical studies, the effects of black ginseng in dermatologic disorders still remain unclear. Although it has been reported for black ginseng to have anti-inflammatory potential, studies on its influence on inflammatory skin disorders are lacking. Therefore, we conducted a study on the effects of black ginseng water extract (BG) under the inflammatory conditions of cultured sebocytes and outer root sheath (ORS) cells.

MATERIALS AND METHODS

Sebocyte and outer root sheath cell culture

The specimens of occipital scalp from patients undergoing hair transplantation surgery were obtained. Informed written consent was obtained. The Medical Ethical Committee of the Kyungpook National University Hospital approved this study (IRB Number KNU 2018-0155). The sebaceous glands were isolated using a binocular microscope and transferred to Biocoat collagen type I-coated tissue culture dishes (CORNING, Kennebunk, ME, USA) in Dulbecco's modified Eagle medium (DMEM; Hyclone Laboratories, Logan, UT, USA) at 37℃ in a humidified atmosphere of 5% CO_2. After 4 days, medium was changed to EpiLife (MEPI500CA; Gibco BRL, Grand Island, NY, USA). The medium was changed every 3 days. After cell outgrowth became sub-confluent, cells were harvested with 0.25% trypsin/10 mM EDTA in Hank's balanced salt solution (HBSS) and sub-cultured.

The hair shaft and hair bulb region of the hair follicles were cut and ORS were isolated. And then, hair shafts were immersed in DMEM supplemented with 20% fetal bovine serum. After culturing for three days, the medium was replaced with EpiLife. Cells from the second passage were used for the experiments in this study.

Black ginseng extracts

Ginseng roots were purchased from Daedong Korea Ginseng Corporation (Seoul, Korea), processed for black ginseng. The ginseng roots turned to black ginseng through boiling, cooling, filtering and condensing. After then, the black ginseng was lyophilized in a powdery form. Ginsenoside composition of black ginseng was investigated using high-performance liquid chromatography.

MTT assay

Sebocytes and ORS cells were seeded in 96-well collagen-coated plates at a density of 5,000 cells per well (CORNING) for 24 hours. BG were added to the well plates of sebocytes and ORS cells for 3 days. MTT solution (3-[4,5]dimethylthiazol-2,5-diphenyltetrazolium bromide) was put at 70 μg/well for 3 hours. Optical density for formazan product was evaluated at 570 nm.

Reverse transcription polymerase chain reaction (RT-PCR) analysis and real-time PCR

Cells were treated with 0.1% dimethyl sulfoxide (DMSO), 5 μg/ml lipopolysaccharide (LPS; Sigma-Aldrich, St. Louis, MO, USA), or 5 μg/ml LPS+50 μg/ml BG for 6 hours and 24 hours. The dose of BG was determined by MTT assay. Total RNA was gained with an RNeasy Mini Kit (Qiagen, Hilden, Germany), and cDNA was synthesised from 3 μg of total RNA with a cDNA synthesis kit containing ImProm-II™ reverse transcriptase and oligo-dT primers based on protocol suggested by manufacturer (Promega, Madison, WI, USA). One microliter of cDNA was amplified using forward and reverse primers. The primers used in the study are shown in Supplementary Table 1. Electrophoresis with PCR products were done on a 1% agarose gel and visualized with ultraviolet light.

The real-time PCR was conducted with Step one Plus real-time PCR Assay (Applied Biosystems, Waltham, MA, USA). All reactions were conducted with Power SYBR Green premix (Applied Biosystems) using 50 ng cDNA and 10 pM of specific oligonucleotide primers. The primers used in the study are shown in Supplementary Table 1. Cycling conditions for amplification were as follows: 95℃ for 10 minutes and 40 cycles at 95℃ for 15 seconds and 60℃ for 60 seconds. The products of PCR were evaluated with the Step one Plus real-time PCR analysis software (Applied Biosystems).

Enzyme-linked immunosorbent assay (ELISA)

Expression of interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α (R&D Systems Inc., Minneapolis, MN, USA) was performed with ELISA, following recommendation

of the manufacturer. For measurement of protein levels in the conditioned media of sebocytes and ORS cells, they were seeded overnight at a density of 30,000 cells/24-well culture dish and were washed three times with phosphate-buffered saline (PBS). To examine protein induction in cells in response to the BG, the cells were treated with BG in serum-free media for 6 hours or 24 hours, and protein concentrations in the conditioned medium were measured.

Western blot analysis

Cells were treated with 0.1% DMSO, 5 μg/ml LPS or 5 μg/ml LPS and 50 μg/ml BG for 6 hours. Ten μg of protein per lane were gained with 10% SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) and transferred to nitrocellulose membranes. The membrane was incubated with a blocking buffer (5% skim milk in PBS) for 1 hour. They were detected with rabbit polyclonal antibody dilutions of p-c-jun (1:1,000; Cell Signaling, Danvers, MA, USA), p-JNK (1:1,000; Cell Signaling) or p-iκB (1:1,000; Cell Signaling). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit immunoglobulin G (Jackson ImmunoResearch, West Grove, PA, USA) was bound as the secondary antibody at a 1:7,000 dilution. The bands were visualised with SuperSignal West Femto (Thermo Scientific, Waltham, MA, USA). The nitrocellulose membranes were also probed with mouse monoclonal antibody dilution of actin (1:5,000; Chemicon, Temecula, CA, USA).

Quantification of lipid production

The supernatant of BG-treated sebocytes for 24 hours was transferred to a clean 1.5 ml e-tube and stored at –20℃. Neutral lipids were measured using the TG-S reaction kit (Asan Pharm. Co., Seoul, Korea) based on protocol suggested by manufacturer.

AdipoRed assay reagent (Lonza, Walkerville, MD, USA) was used for Nile red. Slides were incubated in Adipo red solution (1:100 dilution) for 10 minutes at dark. The slides were washed with water and mounted.

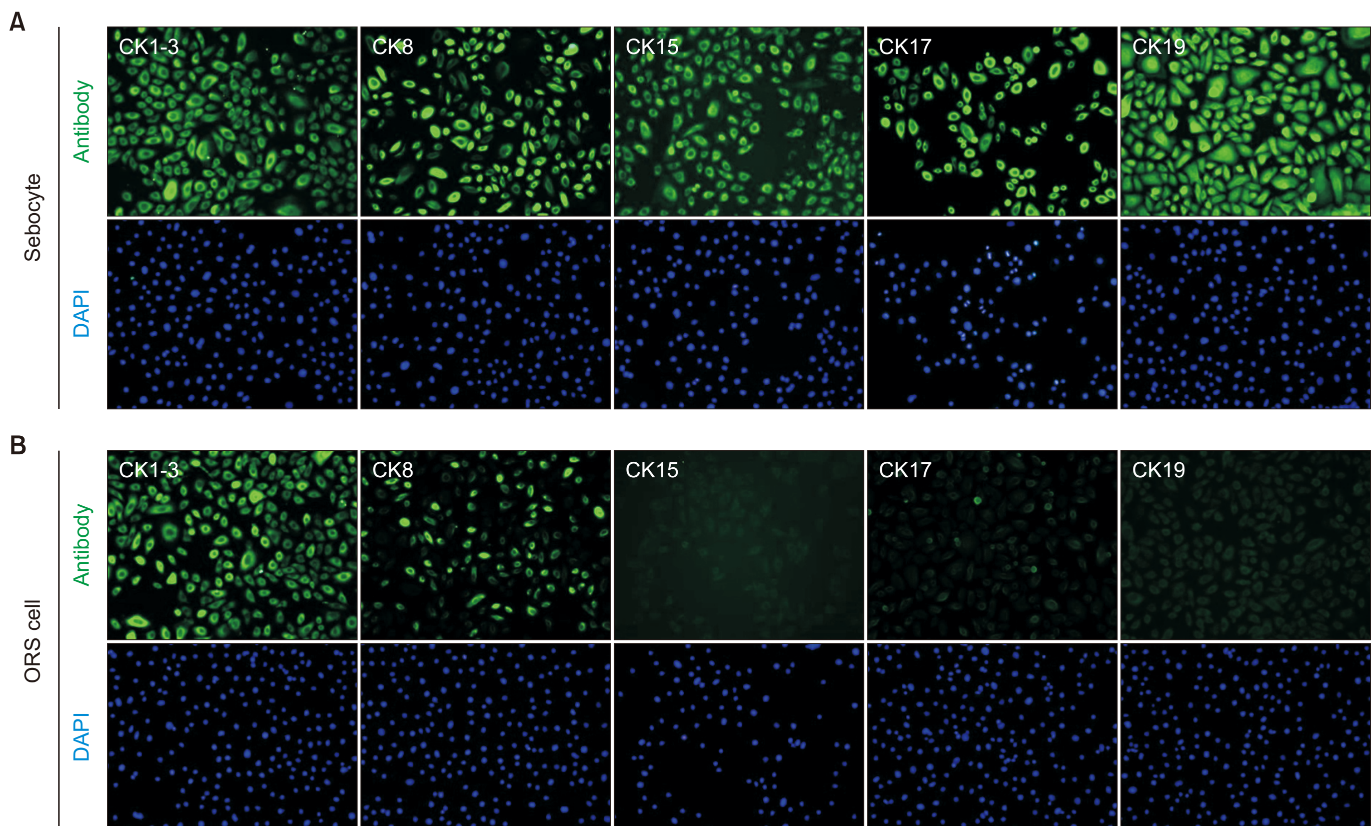

Fig. 1. Immunofluorescence staining was performed in cultured sebocytes and ORS cells. (A) Sebocytes were positive for CK1-3, CK8, CK15, CK17, and CK19. (B) ORS cells were positive only for CK1-3 and CK8. ORS: outer root sheath.

Immunofluorescence staining for cells

Sebocytes and ORS cells in an 8-well chamber slide (Nunc Lab-Tek, Roskilde, Denmark) were fixed in 4% paraformaldehyde for 10 minutes, blocked with 5% normal donkey serum for 1 hour, and then incubated with antibody dilutions of CK1-3 (1:100; Chemicon), CK8 (1:100; Chemicon), CK15 (1:100; Chemicon), CK17 (1:100; Abcam, Cambridge, UK), and CK19 (1:100; Chemicon) at 4℃ overnight. After washing with PBS, the cells were incubated using Alexa Fluor 488-labeled donkey anti-rabbit or mouse secondary antibody (Molecular Probes, Eugene, OR, USA) for 1 hour. Finally, the cells were counterstained using 4,6-diamidino-2-phenylindole (DAPI) for 10 minutes.

For immunofluorescence staining of p-c-jun, cells treated with 5 μg/ml LPS or 5 μg/ml LPS and 50 μg/ml BG for 6 hours were fixed and blocked as above, maintained with rabbit polyclonal p-c-jun antibody dilution (1:100; Cell Signaling) at 4℃ overnight, and incubated using Alexa Fluor 488-labeled donkey anti-rabbit secondary antibody (Molecular Probes). Finally, the cells were counterstained using DAPI for 10 minutes.

Statistical analysis

Data are expressed as follows: means±standard deviation. Statistical analysis for data was done with analysis of variance (ver. 18.0; IBM Corp., Armonk, NY, USA). *p*-value less than 0.05 was considered statistically significant.

RESULTS

Sebocytes were different from outer root sheath cells in the expression of cytokeratin

Immunofluorescence was performed in cultured sebocytes and ORS cells using antibodies against CK1-3, CK8, CK15, CK17, and CK19. Sebocytes were positive for CK1-3, CK8, CK15, CK17, and CK19 (Fig. 1A), but ORS cells were positive only for CK1-3 and CK8 (Fig. 1B). Sebocytes were different from ORS cells in the expression of cytokeratin in immunofluorescence.

BG increased the expression of inflammatory cytokines in lipopolysaccharide-treated sebocytes and outer root sheath cells

To determine the cytotoxic effects of BG, sebocytes and ORS cells were treated with various concentrations of BG. No cytotoxicity with BG was showed up to concentrations 50 μg/ml comparing with control (Fig. 2A). Furthermore, the effects of LPS and BG on cell viability in sebocytes and ORS cells were observed. It showed that cell viability did not change when 5 μg/ml LPS and 50 μg/ml BG were simultaneously treated in sebocytes and ORS cells (Fig. 2B).

The gene expression of IL-1β and IL-8 was significantly increased after treatment of sebocytes and ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 6 hours rather than treatment of

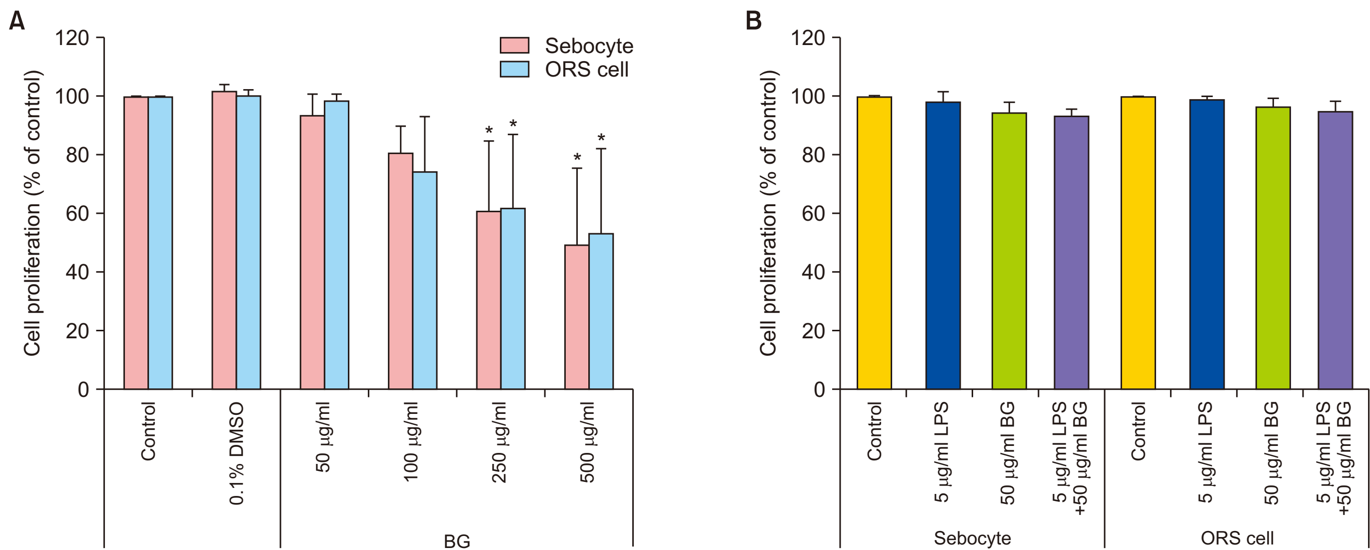

Fig. 2. MTT assay was performed in cultured sebocytes and ORS cells. (A) Cells were treated with BG of various concentrations for 3 days. The cell viability decreased after treatment with BG over 100 μg/ml comparing with control. (B) A combination of 5 μg/ml LPS and 50 μg/ml BG induced no change in cell viability for 3 days. The data in the bar graphs represent the mean±standard deviation from three independent experiments (*$p<0.05$). ORS: outer root sheath, LPS: lipopolysaccharide, BG: black ginseng, DMSO: dimethyl sulfoxide.

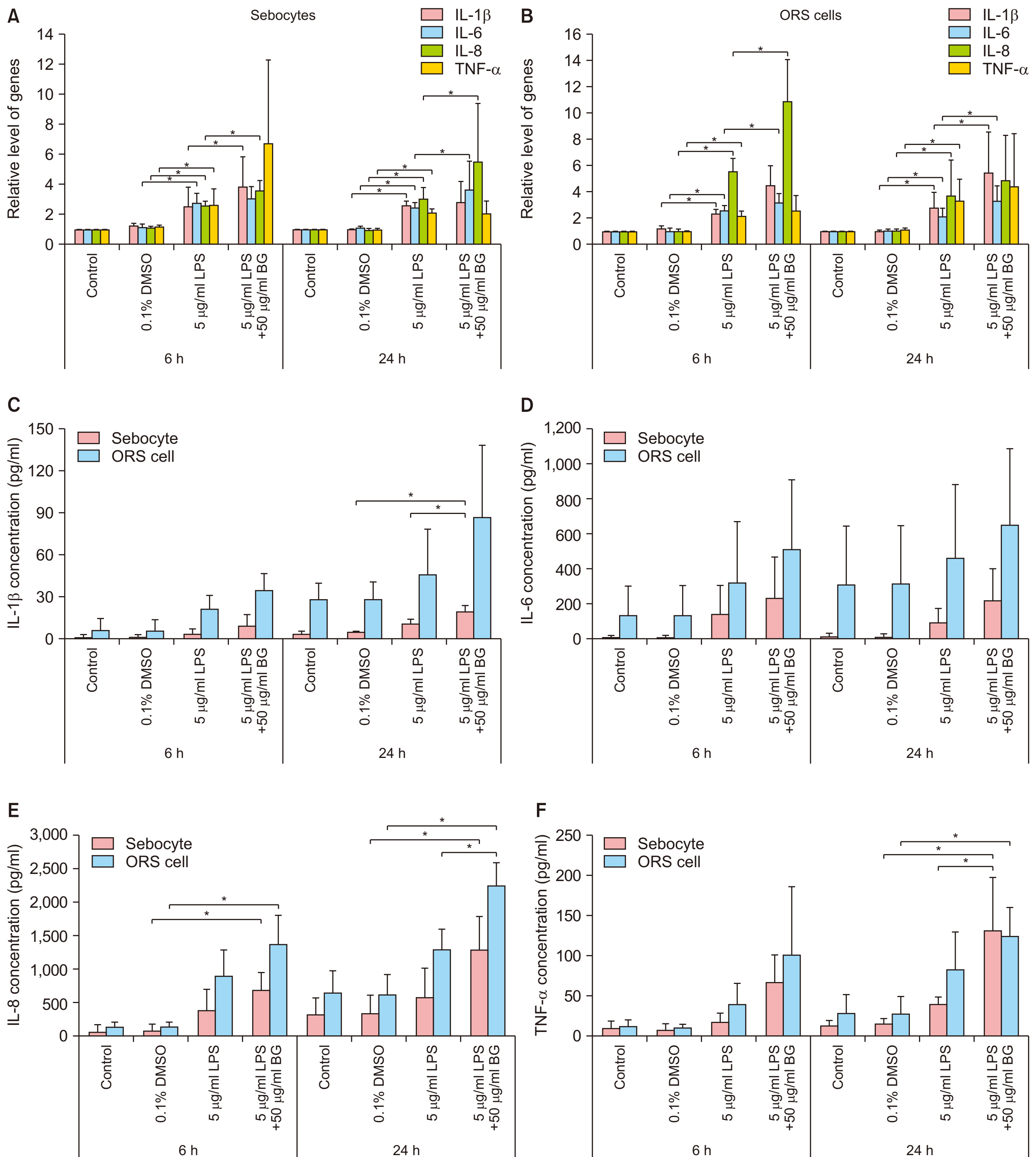

Fig. 3. A combination of BG and LPS showed increased gene expression of inflammatory cytokines in (A) sebocytes and (B) ORS cells. A combination of BG and LPS induced increased protein expression of inflammatory cytokines, including (C) IL-1β, (D) IL-6, (E) IL-8, and (F) TNF-α in sebocytes and ORS cells. The data in the bar graphs represent the mean±standard deviation from three independent experiments (*$p<0.05$). BG: black ginseng, LPS: lipopolysaccharide, ORS: outer root sheath, DMSO: dimethyl sulfoxide, IL: interleukin, TNF: tumor necrosis factor.

sebocytes and ORS cells with 5 μg/ml LPS ($p<0.05$) (Fig. 3A, B). In addition, the gene expression of IL-6 was significantly increased after treatment of sebocytes and ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 24 hours rather than treatment of sebocytes and ORS cells with 5 μg/ml LPS ($p<0.05$) (Fig. 3A, B). The gene expression of the others was insignificantly increased after treatment of sebocytes and ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 6 hours and 24 hours rather than treatment of sebocytes and ORS cells with 5 μg/ml LPS (Fig. 3A, B).

Treatment of sebocytes with 5 μg/ml LPS and 50 μg/ml BG for 24 hours showed a significant increase in protein expression levels of IL-1β and TNF-α rather than treatment of sebocytes with LPS ($p<0.05$) (Fig. 3C~F). In addition, treatment of ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 24 hours showed a significant increase in protein expression levels of IL-8 rather than treatment of ORS cells with LPS ($p<0.05$) (Fig. 3C~F). Treatment of sebocytes and ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 6 hours and 24 hours showed an insignificant increase in protein expression levels of the others rather than treatment of sebocytes and ORS cells with LPS (Fig. 3C~F). However, it was observed that there was no change of the expression of inflammatory cytokines by 50 μg/ml BG in LPS untreated cells (Fig. 4).

Treatment of lipopolysaccharide-treated sebocytes and outer root sheath cells with BG increased protein expression of p-c-jun, p-JNK, p-iκB, and TLR4

In western blot analysis, treatment of sebocytes and ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 6 hours increased the protein expression of p-c-jun ($p<0.05$) and p–JNK ($p<0.05$) rather than treatment of sebocytes and ORS cells with 5 μg/ml LPS (Fig. 5A, B).

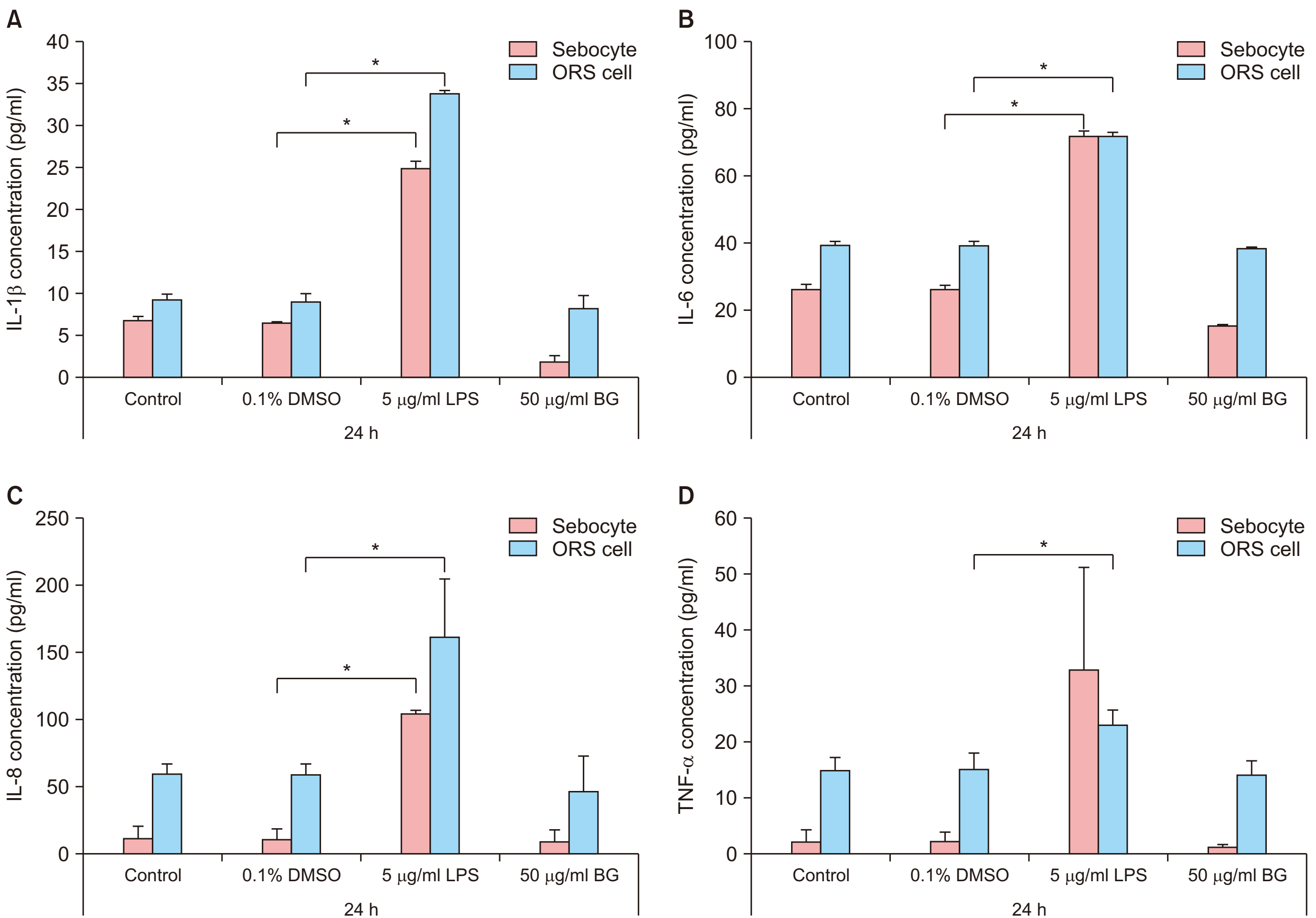

Fig. 4. BG weakly reduced the expression of inflammatory cytokines in LPS-untreated sebocytes and ORS cells. The protein expression of inflammatory cytokines, including (A) IL-1β, (B) IL-6, (C) IL-8, and (D) TNF-α decreased after BG for 24 hours. The data in the bar graphs represent the mean±standard deviation from three independent experiments (*$p<0.05$). BG: black ginseng, LPS: lipopolysaccharide, ORS: outer root sheath, DMSO: dimethyl sulfoxide, IL: interleukin, TNF: tumor necrosis factor.

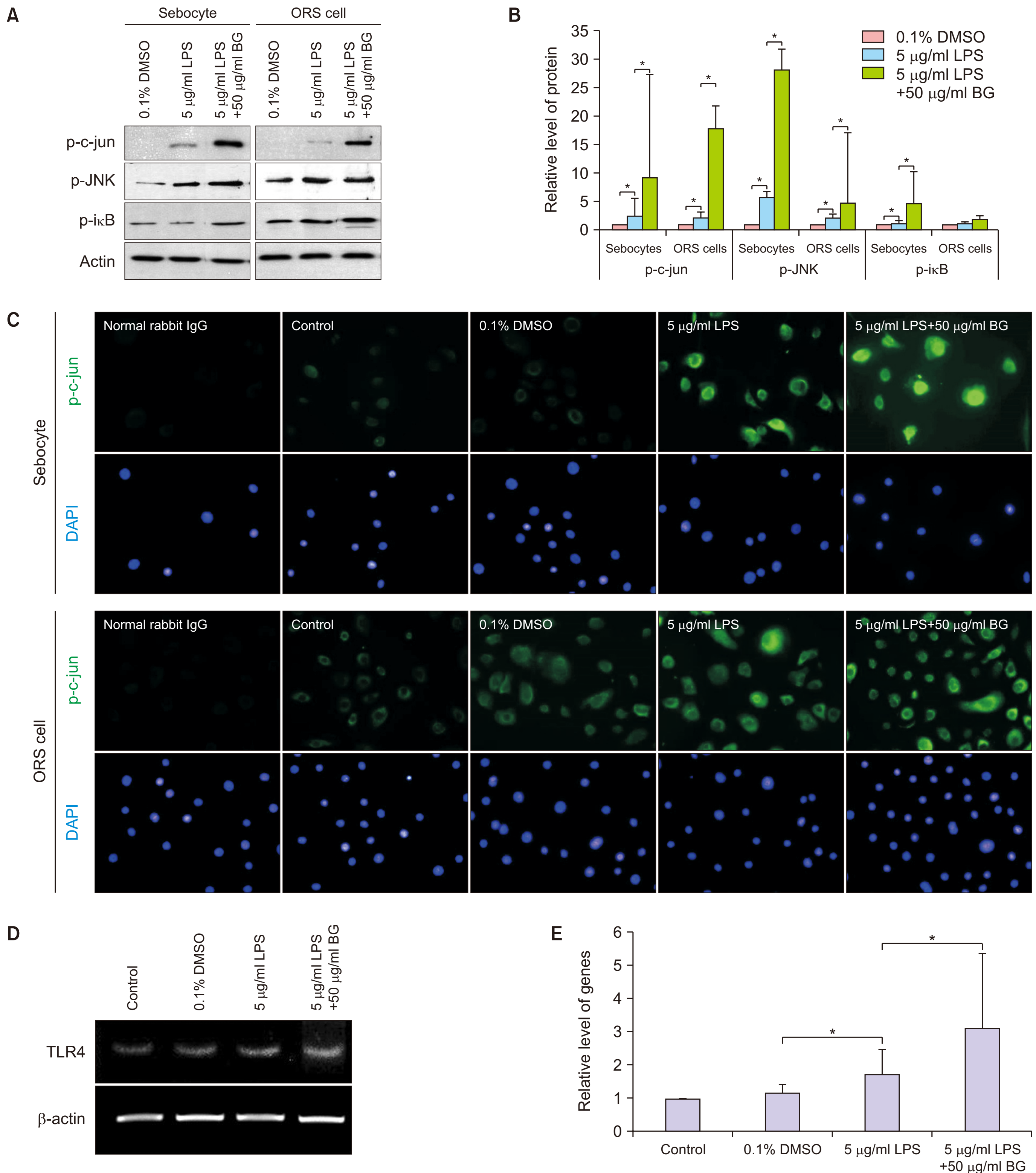

Fig. 5. (A, B) A combination of BG and LPS induced increased protein expression of p-c-jun, p-JNK, and p-ικB in sebocytes and ORS cells in western blot analysis. (C) A combination of BG and LPS induced increased protein expression of p-c-jun in sebocytes and ORS cells in immunofluorescence staining. (D, E) A combination of BG and LPS induced increased gene expression of TLR4 in sebocytes. The data in the bar graphs represent the mean±standard deviation from three independent experimcents (*$p<0.05$). BG: black ginseng, LPS: lipopolysaccharide, ORS: outer root sheath, DMSO: dimethyl sulfoxide, Ig: immunoglobulin.

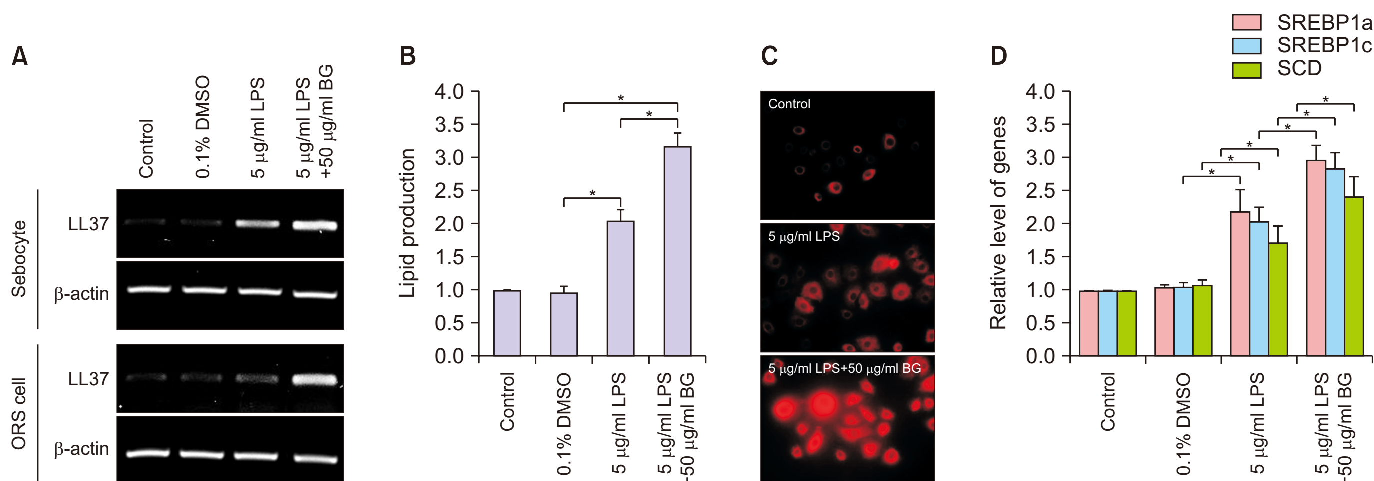

Fig. 6. (A) A combination of BG and LPS increased the gene expression of LL37 in sebocytes and ORS cells. (B, C) A combination of BG and LPS increased the production of sebum in sebocytes. (D) A combination of BG and LPS upregulates the expression of sebum production-related genes in cultured sebocytes. The data in the bar graphs represent the mean±standard deviation from three independent experiments (*$p<0.05$). ORS: outer root sheath, LPS: lipopolysaccharide, BG: black ginseng, DMSO: dimethyl sulfoxide.

In addition, the protein expression of p-iκB ($p<0.05$) was increased in treatment of sebocytes with 5 μg/ml LPS and 50 μg/ml BG for 6 hours more than in treatment of sebocytes with 5 μg/ml LPS (Fig. 5A, B). In immunofluorescence, the expression of p-c-jun was increased in treatment of sebocytes and ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 6 hours more than in treatment of sebocytes and ORS cells with 5 μg/ml LPS (Fig. 5C). In RT-PCR, the gene expression of TLR4 was increased in treatment of sebocytes with 5 μg/ml LPS and 50 μg/ml BG for 24 hours more than in treatment of sebocytes with 5 μg/ml LPS (Fig. 5D, E).

Treatment of sebocytes and ORS cells with LPS and BG increased the expression of LL37 and lipid production

In RT-PCR, the gene expression of LL37 was increased in treatment of sebocytes and ORS cells with 5 μg/ml LPS and 50 μg/ml BG for 24 hours more than in treatment of sebocytes and ORS cells with 5 μg/ml LPS (Fig. 6A). In addition, lipid production and sebum production-related genes, such as SREBP1a, SREBP1c and SCD, were increased in treatment of sebocytes with 5 μg/ml LPS and 50 μg/ml BG for 24 hours more than in treatment of sebocytes with 5 μg/ml LPS ($p<0.05$; Fig. 6B~D).

DISCUSSION

Pharmacological effects of black ginseng on malignancy, liver function, metabolic diseases, oxidative stress, and neurovascular and immune system have been studying with various molecular and biochemical methods[7,10-14]. It was shown that black ginseng has intense anti-inflammatory effects[14]. In addition, it has known that black ginseng can suppress the release of pro-inflammatory biomarkers, including IL-1β, IL-6, and TNF-α[13,15]. However, BG increased the gene and protein expression of inflammatory cytokines in LPS-treated sebocytes and ORS cells in our study.

Acne is an inflammatory skin disorder with the increased expression of inflammatory cytokines. Acne is primarily developed by colonization of *Cutibacterium acnes*[16]. The major cell wall components of *C. acnes*, such as lipoteichoic acid and peptidoglycan, can release inflammatory mediators through activation of TLR2[17]. In addition lipoteichoic acid can trigger TLR4 activation. Both TLR2 and TLR4 are active in sebaceous glands and play a central role in the occurrence of acne[18]. LPS, a component of gram-negative bacteria, also plays an important role in chronic inflammatory reactions through TLR2- and TLR4-dependent signalling pathways, although TLR2 meditates inflammatory signalling to component of gram-positive bacteria and TLR4 is required for cellular signalling induced by gram-negative bacteria[19,20]. The activation of TLR2 and TLR4 results in signalling cascades that produce inflammatory cytokines via NF-κB[19]. In the other way, the activation of TLR2 and TLR4 can meditate inflammatory responses through JNK and c-jun signalling pathway[21-24].

Although the mechanisms of the regulation of LL37 expression remain poorly understood, TLRs are thought to be involved in the regulation of LL37 expression[25]. In our study,

a combination of BG and LPS enhanced the expression of TLR4, p-JNK, p-c-jun, p-iκB, and LL37 in sebocytes and ORS cells. Nagy et al.[26] reported that C. acnes and LPS induced the expression antimicrobial peptides in human sebocytes, showing their role in acne pathogenesis. Lee et al.[27] also proved that *C. acnes* stimulated the mRNA expression of LL37 in keratinocytes. However, it remains to be determined what the increased gene expression of LL37 means.

Iinuma et al.[28] reported the intradermal administration of *Escherichia coli*-derived LPS to hamster auricle skin did not influence sebaceous morphology or sebum accumulation in sebaceous glands. In addition, BG did not affect the production of sebum in our study. However, sebum production was upregulated in sebocytes after treatment with LPS or a combination of BG and LPS in our study. Increased sebum production in the sebocytes was caused by upregulation of sebum production-related genes, including SREBP1a, SREBP1c, and SCD.

In conclusion, BG induces an increase in the expression of inflammatory biomarkers in sebocytes and ORS cells after treatment with LPS. This suggests that black ginseng be an aggravating factor of inflammatory skin disorders, such as acne, and black ginseng should be avoided in patients with inflammatory acne.

ACKNOWLEDGMENT

Authors would like to thank Prof. M.H. Rhee for providing black ginseng.

SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-34-095-s001.pdf.

FUNDING SOURCE

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health and Welfare, Republic of Korea (grant number: HP20C0004).

DATA SHARING STATEMENT

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

ORCID

Jun Young Kim, https://orcid.org/0000-0002-2999-1018
Mi Hee Kwack, https://orcid.org/0000-0002-7381-6532
Eun Hye Lee, https://orcid.org/0000-0002-4886-5439
Weon Ju Lee, https://orcid.org/0000-0001-5708-1305

REFERENCES

1. Yun TK. Brief introduction of Panax ginseng C.A. Meyer. J Korean Med Sci 2001;16(Suppl):S3-S5.
2. Shin HR, Kim JY, Yun TK, Morgan G, Vainio H. The cancer-preventive potential of Panax ginseng: a review of human and experimental evidence. Cancer Causes Control 2000;11:565-576.
3. Lee CH, Kim JH. A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. J Ginseng Res 2014;38:161-166.
4. Kim HJ, Kim P, Shin CY. A comprehensive review of the therapeutic and pharmacological effects of ginseng and ginsenosides in central nervous system. J Ginseng Res 2013;37:8-29.
5. Chung HS, Lee YC, Rhee YK, Lee SY. Consumer acceptance of ginseng food products. J Food Sci 2011;76:S516-S522.
6. Metwaly AM, Lianlian Z, Luqi H, Deqiang D. Black ginseng and its saponins: preparation, phytochemistry and pharmacological effects. Molecules 2019;24:1856.
7. Jin Y, Kim YJ, Jeon JN, Wang C, Min JW, Noh HY, et al. Effect of white, red and black ginseng on physicochemical properties and ginsenosides. Plant Foods Hum Nutr 2015;70:141-145.
8. Nam KY, Lee NR, Moon BD, Song GY, Shin HS, Choi JE. Changes of ginsenosides and color from black ginsengs prepared by steaming-drying cycles. Korean J Med Crop Sci 2012;20:27-35.
9. Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, et al. Characterization of Korean Red Ginseng (Panax ginseng Meyer): history, preparation method, and chemical composition. J Ginseng Res 2015;39:384-391.
10. Chen G, Li H, Gao Y, Zhang L, Zhao Y. Flavored black ginseng exhibited antitumor activity via improving immune function and inducing apoptosis. Food Funct 2017;8:1880-1889.
11. Seo YS, Shon MY, Kong R, Kang OH, Zhou T, Kim DY, et al. Black ginseng extract exerts anti-hyperglycemic effect via modulation of glucose metabolism in liver and muscle. J Ethnopharmacol 2016;190:231-240.
12. Kim JH, Pan JH, Cho HT, Kim YJ. Black ginseng extract counteracts streptozotocin-induced diabetes in mice. PLoS One

2016;11:e0146843.

13. Han MS, Han IH, Lee DH, An JM, Kim SN, Shin MS, et al. Beneficial effects of fermented black ginseng and its ginsenoside 20(S)-Rg3 against cisplatin-induced nephrotoxicity in LLC-PK1 cells. J Ginseng Res 2016;40:135-140.
14. Lee YY, Saba E, Irfan M, Kim M, Chan JY, Jeon BS, et al. The anti-inflammatory and anti-nociceptive effects of Korean black ginseng. Phytomedicine 2019;54:169-181.
15. Kim AJ, Kang SJ, Lee KH, Lee MS, Ha SD, Cha YS, et al. The chemopreventive potential and anti-inflammatory activities of Korean black ginseng in colon26-M3.1 carcinoma cells and macrophages. J Korean Soc Appl Biol Chem 2010;53:101-105.
16. 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;169:1535-1541.
17. Seo HS, Michalek SM, Nahm MH. Lipoteichoic acid is important in innate immune responses to gram-positive bacteria. Infect Immun 2008;76:206-213.
18. Törőcsik D, Kovács D, Póliska S, Szentkereszty-Kovács Z, Lovászi M, Hegyi K, et al. Genome wide analysis of TLR1/2- and TLR4-activated SZ95 sebocytes reveals a complex immune-competence and identifies serum amyloid A as a marker for activated sebaceous glands. PLoS One 2018;13:e0198323.
19. Chavarría-Velázquez CO, Torres-Martínez AC, Montaño LF, Rendón-Huerta EP. TLR2 activation induced by H. pylori LPS promotes the differential expression of claudin-4, -6, -7 and -9 via either STAT3 and ERK1/2 in AGS cells. Immunobiology 2018;223:38-48.
20. Suvanprakorn P, Tongyen T, Prakhongcheep O, Laoratthaphong P, Chanvorachote P. Establishment of an anti-acne vulgaris evaluation method based on TLR2 and TLR4-mediated Interleukin-8 Production. In Vivo 2019;33:1929-1934.
21. Lee HS, Hua HS, Wang CH, Yu MC, Chen BC, Lin CH. *Mycobacterium tuberculosis* induces connective tissue growth factor expression through the TLR2-JNK-AP-1 pathway in human lung fibroblasts. FASEB J 2019;33:12554-12564.
22. Adhikary G, Sun Y, Pearlman E. C-Jun NH2 terminal kinase (JNK) is an essential mediator of Toll-like receptor 2-induced corneal inflammation. J Leukoc Biol 2008;83:991-997.
23. Luo W, Wang CY, Jin L. Baicalin downregulates Porphyromonas gingivalis lipopolysaccharide-upregulated IL-6 and IL-8 expression in human oral keratinocytes by negative regulation of TLR signaling. PLoS One 2012;7:e51008.
24. Kim SB, Kang OH, Joung DK, Mun SH, Seo YS, Cha MR, et al. Anti-inflammatory effects of tectroside on UVB-induced HaCaT cells. Int J Mol Med 2013;31:1471-1476.
25. Kim BJ, Rho YK, Lee HI, Jeong MS, Li K, Seo SJ, et al. The effect of calcipotriol on the expression of human beta defensin-2 and LL-37 in cultured human keratinocytes. Clin Dev Immunol 2009;2009:645898.
26. 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;8:2195-2205.
27. Lee SE, Kim JM, Jeong SK, Jeon JE, Yoon HJ, Jeong MK, et al. Protease-activated receptor-2 mediates the expression of inflammatory cytokines, antimicrobial peptides, and matrix metalloproteinases in keratinocytes in response to Propionibacterium acnes. Arch Dermatol Res 2010;302:745-756.
28. Iinuma K, Sato T, Akimoto N, Kurihara H, Ito A. Induction of inflammatory reactions by lipopolysaccharide in hamster sebaceous glands and pilosebaceous units in vivo and in vitro. Exp Dermatol 2010;19:1107-1109.