Ann Dermatol Vol. 33, No. 5, 2021
pISSN 1013-9087 · eISSN 2005-3894
https://doi.org/10.5021/ad.2021.33.5.409

ORIGINAL ARTICLE

Red Ginseng Acidic Polysaccharides Promote the Expression of Acne-Related Inflammatory Biomarkers in Lipopolysaccharide-Treated Sebocytes and Outer Root Sheath Cells and *Cutibacterium acnes*-Injected Mice

Hyun Ji Lee, Mi Hee Kwack[1], Weon Ju Lee

Departments of Dermatology and [1]Immunology, School of Medicine, Kyungpook National University, Daegu, Korea

Background: Although ginseng has beneficial effects largely related to their constituent ginsenosides, pharmacological effects of non-ginsenosides have been reported. Acidic polysaccharides of red ginseng (RGAP) are among the non-ginsenoside constituents that have characterized antioxidant properties. **Objective:** We investigated the impact of RGAP on sebocytes and outer root sheath (ORS) cells treated with lipopolysaccharide (LPS) and in mice with *Cutibacterium acnes* (*C. acnes*)-induced inflammatory nodules. **Methods:** Sebocytes and ORS cells were cultured and treated with either 0.1% dimethyl sulfoxide, 5 μg/ml LPS, 50 μg/ml RGAP or 5 μg/ml LPS+50 μg/ml RGAP for 6 and 24 hours. Real-time polymerase chain reaction, ELISA, Western blot analysis, and immunofluorescence staining were among the methods used to detect and quantify inflammatory cytokine production. Mice infected with *C. acnes* were treated with 2 weeks of RGAP provided in drinking water followed by immunohistochemical evaluation of inflammatory nodules. **Results:** Administration of RGAP to LPS-treated sebocytes and ORS cell cultures resulted in increased expression of inflammatory cytokines like interleukin (IL)-1 β, IL-6, IL-8, and tumor necrosis factor-α, toll-like receptor 2, p-c-jun, p-JNK and p-iKB ($p<0.05$). Administration of RGAP also resulted in increased expression of LL37 in LPS-treated sebocytes and ORS cells, and increased production of sebum in LPS-treated sebocytes ($p<0.05$). RGAP also promoted increased expression of inflammatory biomarkers in *C. acnes*-associated inflammatory nodules in mice ($p<0.05$). **Conclusion:** RGAP may exacerbate inflammatory pathology associated with acne vulgaris. Ginseng supplements may be contraindicated in patients diagnosed with inflammatory acne. **(Ann Dermatol 33(5) 409~418, 2021)**

-Keywords-
Acne, Outer root sheath cells, Red ginseng, Sebocytes

INTRODUCTION

Acne is an inflammatory follicular disease that frequently develops on the face and trunk during puberty. The pathogenesis of this disorder includes increased sebum production, folliculoinfundibular hyperkeratosis, and inflammation associated with proliferation of the gram-positive bacteria, *Cultibacterium ances* (*C. acnes*)[1]. Acne can be exacerbated by psychological stress, hormone levels, and poor nutrition. Adverse events associated with acne include atrophic or hypertrophic scarring, hyperpigmentation, and post-inflammatory erythema. Intensive treatment should be applied at the early stages when lesions first appear to prevent adverse events[2]. There are a variety of therapeutic options used to treat acne, including topical and oral medications as well as surgical devices. Additional therapeutic options are needed for safe and effective treatment of this

Received November 6, 2020, Revised January 6, 2021, Accepted for publication January 18, 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
ORCID: https://orcid.org/0000-0001-5708-1305

problem[3,4]. Toward this end, modalities introduced from complementary and alternative medicine have become important therapeutic options for acne patients.

Ginseng is among the most popular of the herbal medicines. Ginseng has been reported to improve general health, notably with respect to immunity and memory. Ginsenosides, which consists the main portion of ginseng, have been the target of a lot of studies showing potential health effects including immunomodulatory, anti-inflammatory, and antioxidant properties[5,6]. The unique pharmacological properties of non-ginsenoside constituents have also been reported. Among these, acidic polysaccharides of red ginseng (RGAP) which represent 4.5%～7.5% of the net weight of red ginseng, have profound actions in combating the negative sequelae of oxidative stress[7-10]. Acidic polysaccharides have an immunomodulating properties via their interactions with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein-1 (AP-1), c-Jun-N-terminal kinase (JNK), and toll-like receptor (TLR) 2[11]. RGAP induce splenocyte proliferation, limit the antibody-mediated generation of sheep red blood cell rosettes and stimulate nitric oxide (NO) production in murine peritoneal macrophages[12,13]. Nevertheless, they are not advantageous to certain conditions. Therefore, we investigated the impact of RGAP on primary cell cultures and *C. acnes*-injected mouse model.

MATERIALS AND METHODS

Sebocytes and outer root sheath cell culture

Sebaceous glands within occipital hairs were kept in Dulbecco's modified Eagle medium (Hyclone Laboratories, Logan, UT, USA) and EpiLife (Gibco, Grand Island, NY, USA) at 37°C under 5% CO_2 atmosphere. Outer root sheath (ORS) cells from hair shafts were kept in DMEM and EpiLife. Following the second passage, sebocytes and ORS cells were used in these experiments. Written informed consent form was obtained from patients, and this study was approved by Institutional Review Board of the Kyungpook National University Hospital (No. 2018-0155).

Acidic polysaccharides of red ginseng preparation

RGAP (64.3 mg/g ginseng) were prepared from College of Veterinary Medicine of Kyungpook National University.

MTT assay

We seeded sebocytes and ORS cells in 96-well plates (5,000 cells in each well; Corning, Inc., New York, NY, USA). After 24 hours, sebocytes and ORS cells were transferred to supplement-free medium and were added RGAP or not for 3 days. We kept MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution at a dose of 70 μg in each well for 3 hours. Optical density for formazan product was evaluated at 570 nm.

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

We treated cells in triplicate with 0.1% DMSO, 5 μg/ml LPS (Sigma-Aldrich, St. Louis, MO, USA) or 50 μg/ml RGAP for 6 and 24 hours. A RNeasy Mini Kit (Qiagen, Hilden, Germany) was used for total RNA and a cDNA synthesis kit (Promega, Madison, WI, USA) for cDNA. We amplified One microliter of cDNA using forward and reverse primers of TLR2, TLR4 and LL37. Electrophoresis was done on a 1% agarose gel.

Real-time PCR was conducted in triplicate with Power SYBR Green premix (Applied Biosystems, Waltham, MA, USA) using 50 ng cDNA and 10 pM interleukin (IL)-1β (Qiagen; Hs-IL1B-SG), IL-6 (Qiagen; Hs-IL6-SG), IL-8 (Qiagen; Hs-IL8-SG), and tumor necrosis factor (TNF)-α specific oligonucleotide primers (Qiagen; Hs-TNF-SG). Cycling conditions for amplification were as follows: 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 60 seconds. The products of PCR were evaluated with a real-time PCR analysis software, the Step one Plus (Applied Biosystems).

Enzyme-linked immunosorbent assay (ELISA)

We conducted ELISA in triplicate for levels of IL-1β, IL-6, IL-8, and TNF-α (R&D Systems, Minneapolis, MN, USA), following recommendation of the manufacturer. For measurement of protein levels in the conditioned media of sebocytes and ORS cells, we seeded them overnight as 30,000 cells/24-well culture dish. We treated cells with various concentrations of RGAP in serum-free medium for 6 or 24 hours to examine induction by RGAP in cells, and specific protein concentrations were measured.

Cell death ELISA

This analysis was conducted in triplicate with a cell death ELISA kit (Roche, Mannheim, Germany), based on the recommendation by the manufacturer. Briefly, after treatment with 5 μg/ml LPS or 50 μg/ml RGAP for 24 hours, cytoplasmic extracts were prepared from 20,000 passage-2 sebocytes or ORS cells. The assay detects DNA-histone complexes resulting from apoptotic nucleosomes measured by reading at optical density at 405 nm using a microplate reader.

Western blot analysis

We treated cells in triplicate with 0.1% DMSO, 5 μg/ml LPS (Sigma-Aldrich) or 50 μg/ml RGAP for 6 hours. After

electrophoresis of extracted proteins (10 μg/lane), they were transferred to nitrocellulose membranes, which were blocked by 1 hour preparation with 5% skim milk. Rabbit polyclonal antibodies against p-c-jun (1:1,000 dilution; Cell Signaling, Beverly, MA, USA), p-JNK (1:1,000 dilution; Cell Signaling) or p-iKB (1:1,000 dilution; Cell Signaling) were used. The secondary antibody was Horseradish peroxidase-conjugated donkey anti-rabbit Ig (1:7,000; Jackson ImmunoResearch, West Grove, PA, USA). Super signal West Femto (Thermo Scientific, Rockford, IL, USA) presented the bands. Mouse monoclonal antibody of β-actin (1:5,000; Chemicon, Temecula, CA, USA) was used.

Quantification of lipid production

The supernatants of RGAP-treated sebocytes for 24 hours were transferred to a clean 1.5 ml e-tubes and kept at −20°C. Neutral lipids were measured in triplicate using the TG-S reaction kit (Asan Pharm. Co., Seoul, Korea) by the manufacturer protocol.

Immunofluorescence staining

For immunofluorescence staining of p-c-jun, we cultured cells in the presence of 5 μg/ml LPS (Sigma-Aldrich) or RGAP for 6 hours, fixed, blocked as above, and maintained at 4°C overnight with rabbit polyclonal p-c-jun antibody (1:100; Cell Signaling) and incubated using a secondary antibody (Molecular Probes, Eugene, OR, USA). Lastly, cells were counterstained with DAPI for 10 minutes. The images were taken by fluorescence microscopy (Olympus BX51; Olympus, Tokyo, Japan).

Animal study

C. acnes was prepared from the pustules of Korean moderate acne patients. *C. acnes* suspensions were made as 10^9 colony forming units /20 μl and intradermally injected in 20-μl aliquots at 4 points on the back of 6-week-old female HR-1 mice (SLC Inc., Hamamatsu, Japan). Two mice were treated with RGAP in water for 2 weeks starting on day 1 after the *C. acnes* injection. The other 2 mice were not treated with RGAP in water as a control group. The sizes of inflammatory nodules were monitored and measured. Specific mice within this cohort were euthanized after 2, 4, and 8 weeks and the injected back skin were stained with H&E. This study was approved by Institutional Animal Care and Use Committee of Kyungpook National University (No. 2018-0167).

Immunohistochemistry

Tissue samples were obtained from the inflammatory nodules, and put in cryomolds with embedding medium, frozen at −80°C. Samples were sliced (7 μm thick), fixed with 4% paraformaldehyde and 0.1% Triton X-100 for 10 minutes. After 1 hour preparation with 5% normal donkey serum (Jackson ImmunoResearch), they were incubated at 4°C overnight with antibodies (Abcam, Cambridge, UK) for integrin α6 (1:200), CD4 (1:300), CD8 (1:100), neu-

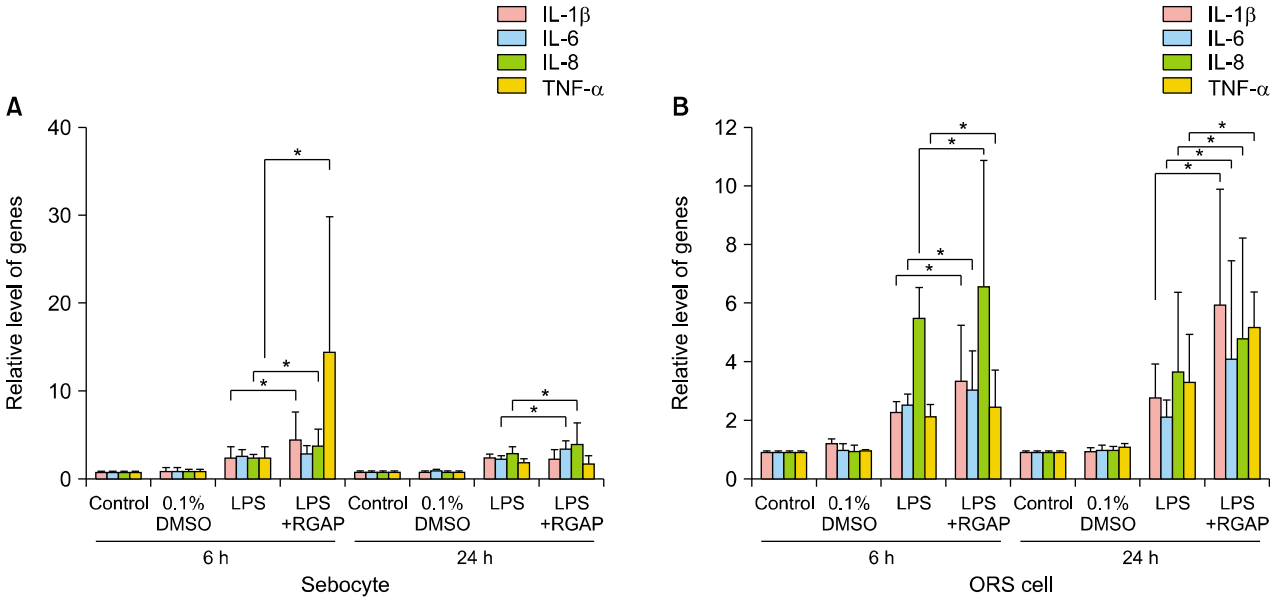

Fig. 1. RGAP promoted expression of transcripts encoding proinflammatory cytokines in the LPS-treated sebocytes (A) and ORS cell cultures (B). RGAP promoted synthesis of proinflammatory cytokines in the LPS-treated sebocytes and ORS cell cultures. Values are presented as mean±standard deviation. RGAP: acidic polysaccharides of red ginseng, LPS: lipopolysaccharide, ORS: outer root sheath. *Statistically significance ($p<0.05$).

trophil (1:80), myeloperoxidase (MPO) (1:200), IL-1β (1:150), matrix metalloproteinase (MMP)-2 (1:300), MMP-3 (1:100), MMP-9 (1:250), TLR2 (1:500), and LL37 (1:300) and with secondary antibody (1:100; Molecular Probes) for 1 hour. The slides counterstained with DAPI for 10 minutes. Histological evaluation was done in triplicate for the degree of inflammation. The images were taken by fluorescence microscopy.

Statistical analysis

Our data were shown as mean±standard deviation (SD). ANOVA was done and *p*-value less than 0.05 was regarded to have statistical significance.

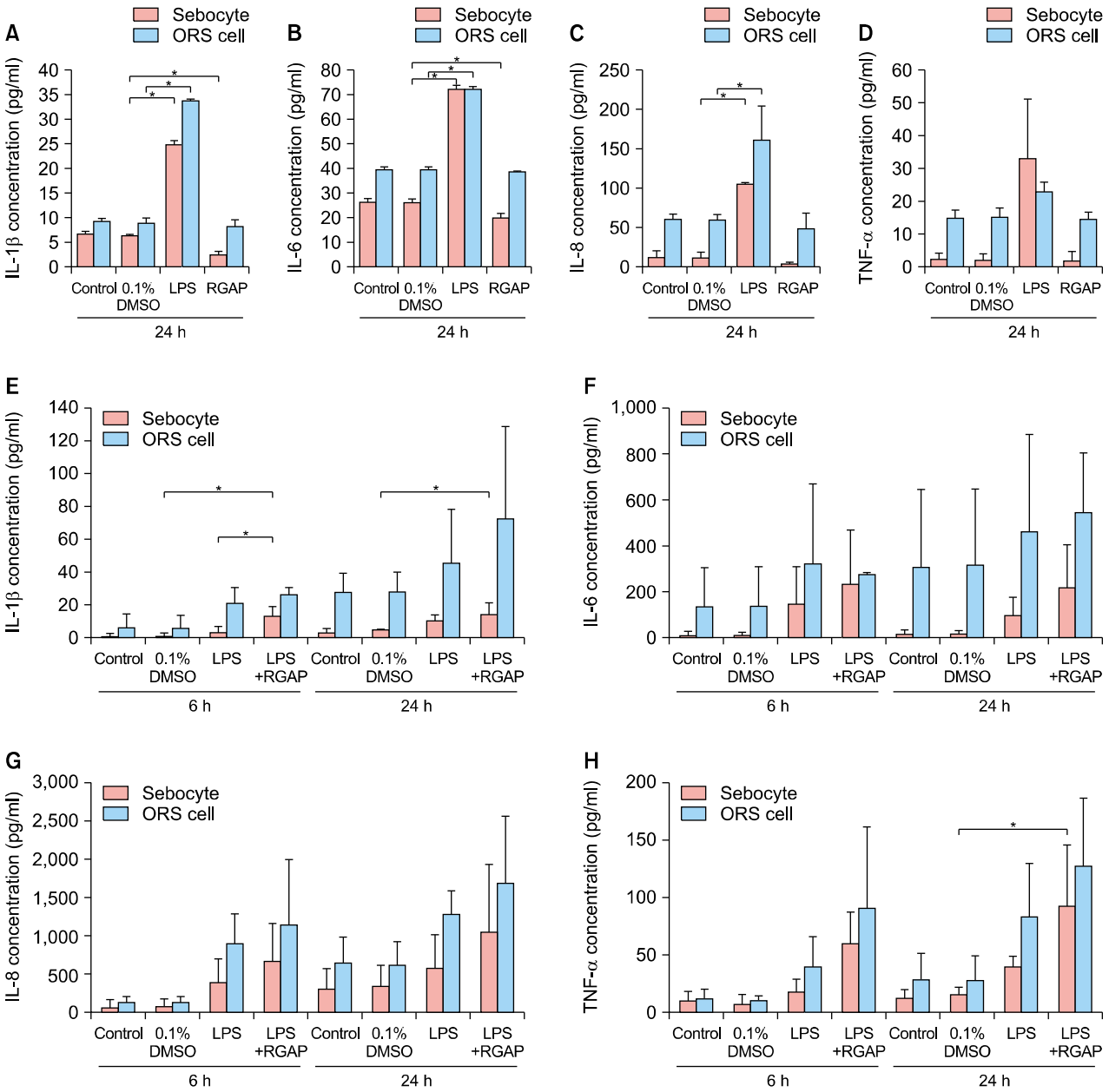

Fig. 2. (A~D) RGAP alone decreased synthesis of IL-1β, IL-6, IL-8, and TNF-α in sebocytes and ORS cell cultures. (E~H) RGAP promoted the synthesis of proinflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α in the LPS-treated sebocytes and ORS cell cultures. Values are presented as mean±standard deviation. RGAP: acidic polysaccharides of red ginseng, IL: interleukin, TNF: tumor necrosis factor, LPS: lipopolysaccharide, ORS: outer root sheath. *Statistically significance ($p<0.05$).

RESULTS

Acidic polysaccharides of red ginseng increased inflammatory cytokines expression in the lipopolysaccharide-treated sebocytes and outer root sheath cell cultures

Expression of transcripts encoding IL-1β, IL-6, IL-8, and TNF-α was elevated in sebocyte cultures after 6 and 24 hours of treatment with LPS and RGAP compared to results from sebocyte cultures treated with LPS alone (Fig. 1A). In addition, expression of transcripts encoding IL-1β, IL-6, IL-8, and TNF-α was increased in ORS cell cultures after 6 and 24 hours of treatment with LPS and RGAP compared to results from ORS cell cultures treated with LPS alone (Fig. 1B).

Immunoreactive IL-1β ($p < 0.05$), IL-6 ($p<0.05$), IL-8, and TNF-α levels in sebocytes and ORS cell cultures after 24 hours of treatment with RGAP were lower than those detected in response to the diluent control (Fig. 2A~D). However, immunoreactive IL-1β ($p<0.05$), IL-6 ($p<0.05$), IL-8 ($p<0.05$), and TNF-α levels in sebocytes and ORS cell cultures were elevated after 24 hours of treatment with LPS (Fig. 2A~D). In addition, immunoreactive IL-1β ($p<0.05$), IL-8, and TNF-α levels were elevated in both sebocytes and ORS cell cultures after 6 or 24 hours of treatment with both LPS and RGAP; elevated levels of immunoreactive IL-6 were detected after 24 hours but not after 6 hours of treatment with LPS and RGAP (Fig. 2E~H).

Acidic polysaccharides of red ginseng induced apoptosis of lipopolysaccharide-treated sebocytes and outer root sheath cells

The fraction of apoptotic cells in sebocytes and ORS cell cultures after treatment with RGAP alone was similar to that detected in cultures treated with the diluent control. Elevated levels of apoptosis were detected in LPS-treated sebocytes and ORS cell cultures ($p<0.05$) (Fig. 3A). Moreover, apoptosis was elevated in LPS and RGAP-treated sebocytes and ORS cell cultures compared to those with LPS alone ($p<0.05$) (Fig. 3B).

Acidic polysaccharides of red ginseng promoted expression of TLR2, p-c-jun, p-JNK and p-iKB in lipopolysaccharide-treated sebocytes and outer root sheath cell cultures

Expression of p-c-jun in the LPS and RGAP-treated sebocytes and ORS cell cultures was elevated compared to that detected in response to LPS alone in Western blot analysis ($p<0.05$; Fig. 4A, B). Expression of p-JNK was also elevated in LPS and RGAP-treated sebocyte cultures ($p<0.05$) and in ORS cells than those with LPS alone (Fig. 4A, B). A small increase in p-iKB was detected in the LPS and RGAP-treated sebocyte cultures compared to those treated with LPS alone, although it was not statistically significant (Fig. 4A, B). By immunofluorescence staining, expression of p-c-jun was elevated in the LPS and RGAP-treated sebocytes and ORS cell cultures than those with LPS alone (Fig. 4C). Via RT-PCR, we found that expression of transcripts encoding TLR2 ($p<0.05$) and TLR4 was elevated in LPS and RGAP-treated sebocyte cultures than those with LPS alone (Fig. 4D, E).

Acidic polysaccharides of red ginseng promoted the expression of LL37 in the lipopolysaccharide-treated sebocytes and outer root sheath cell cultures and the production of sebum in lipopolysaccharide-treated sebocytes

As shown in our RT-PCR experiments, expression of transcript encoding LL37 was elevated in LPS and RGAP-treated sebocytes and ORS cell cultures compared to those

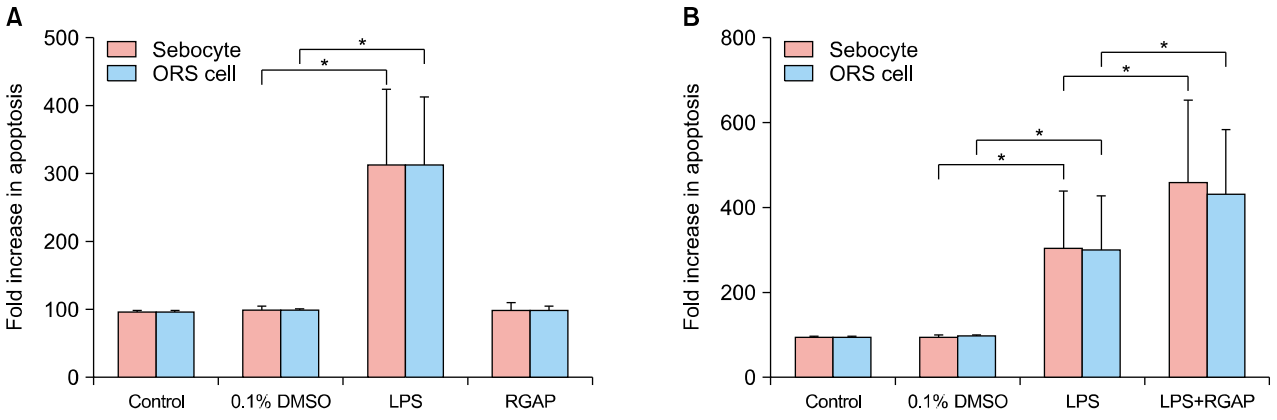

Fig. 3. (A, B) LPS promoted apoptosis in sebocytes and ORS cell cultures. RGAP alone did not promote apoptosis, but RGAP promoted apoptosis after 24 hours in LPS-treated sebocytes and ORS cell cultures Values are presented as mean±standard deviation. RGAP: acidic polysaccharides of red ginseng, LPS: lipopolysaccharide, ORS: outer root sheath. *Statistically significance ($p<0.05$).

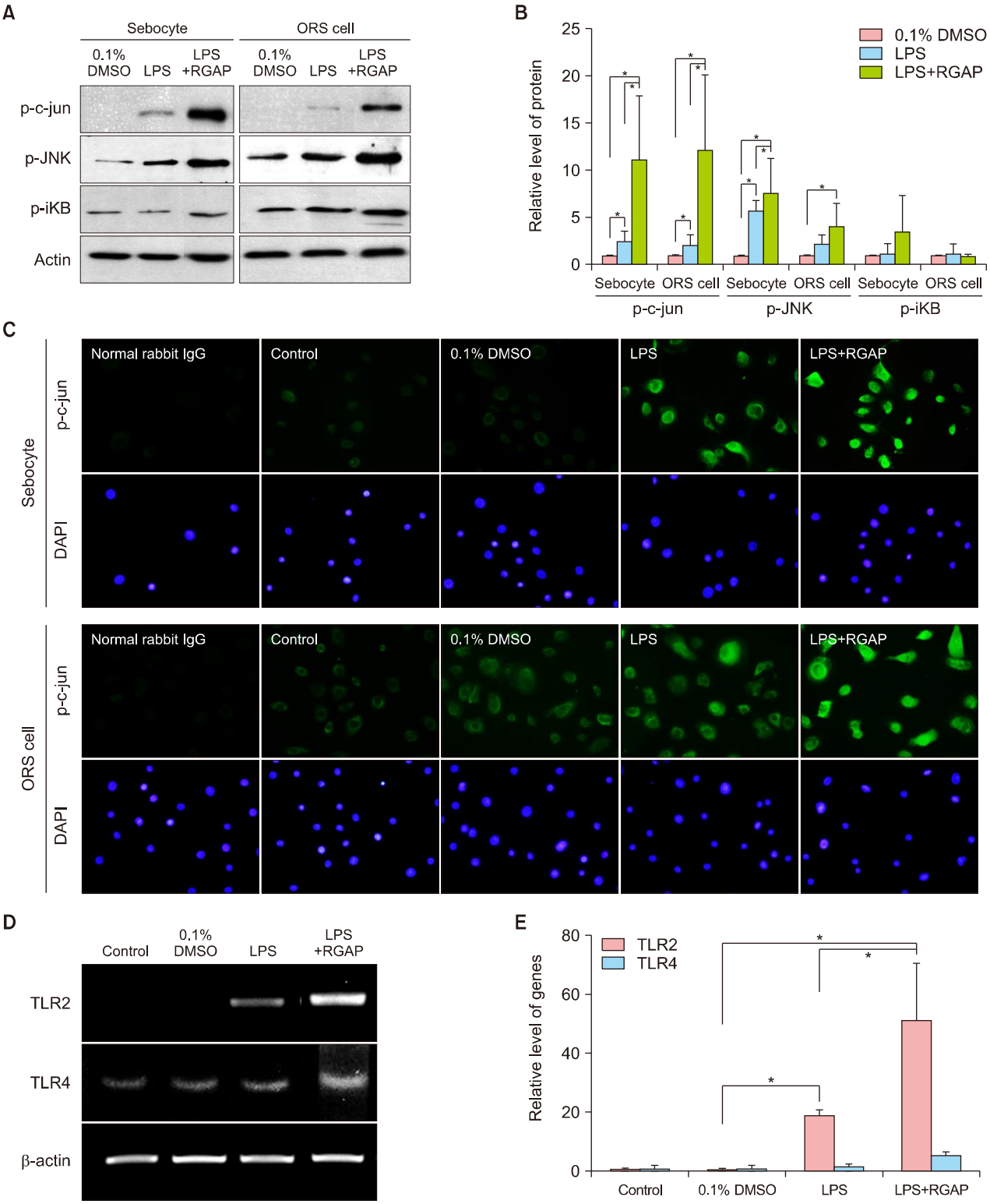

Fig. 4. (A~C) RGAP promoted synthesis of p-c-jun, p-JNK, and p-iKB in the LPS-treated sebocytes and ORS cell cultures. (D, E) RGAP promoted expression of transcripts encoding TLR2 and TLR4 in LPS-treated sebocytes culture. Values are presented as mean±standard deviation. RGAP: acidic polysaccharides of red ginseng, LPS: lipopolysaccharide, ORS: outer root sheath, TLR: toll-like receptor. *Statistically significance ($p<0.05$).

treated with LPS alone (Fig. 5A). Production of sebum was also elevated in LPS and RGAP-treated sebocyte cultures compared to those treated with LPS alone ($p<0.05$; Fig. 5B).

Acidic polysaccharides of red ginseng aggravated inflammatory nodules in mice

Inflammatory nodules developed on the back of Hos:HR-1 mice inoculated intradermally with *C. acnes*. The inflammatory nodules were larger in the RGAP-treated mice at 1 to 8 weeks compared to controls (Fig. 6A, B). H&E staining revealed more prominent and severe inflammation at the nodules of RGAP-treated mice compared to controls at weeks 2, 4, and 8 (Fig. 6C). Inflammatory nodules included both neutrophils and T cells. In addition, immunohistochemistry revealed elevated expression of inflammatory biomarkers, including integrin α6, neutrophil ($p<0.05$), MPO ($p<0.05$), MMP-2, MMP-3 ($p<0.05$), MMP-9 ($p<0.05$), IL-1β ($p<0.05$), TLR2 ($p<0.05$), and LL37 ($p<0.05$), in RGAP-treated mice compared to controls at week 2 (Fig. 6D, E).

DISCUSSION

Acidic polysaccharides derived from red ginseng (RGAP) promote a variety of effects due to their innate immunomodulatory and anti-oxidative activities[14,15]. The immunomodulatory impact of RGAP is achieved via their interactions with the NF-κB and AP-1 pathways and also with JNK and TLR2[11]. RGAP are recognized by TLR2, expressed on monocytes, macrophages, neutrophils, B and T cells. As such, RGAP may be capable of stimulating a wide variety of host immune cells via interactions with the TLR2-mediated signaling pathway, transcription factors including NF-κB and AP-1, and upstream signaling enzymes, including extracellular signal-regulated kinase and JNK[16]. Red ginseng has 3 times higher content of acidic polysaccharides than white ginseng; this may result from differential activation of metabolic processing pathways. Therefore, red ginseng is considered to be a more effective in modulating host immune responses than is white ginseng.

In earlier studies, ginseng S-IIA was found to induce IL-8 production from the human monocytic THP-1 cell line; this was by increased expression of transcript encoding IL-8[16]. Likewise, RGAP treatment resulted in increased biosynthesis of IL-1, IL-6, and NO by macrophages, whereas levels of TNF-α and reactive oxygen species (ROS) remained unchanged. But the treatment of macrophages combined with RGAP and recombinant interferon-γ resulted in activated NF-κB signaling pathway and marked upregulation of IL-1, IL-6, TNF-α, and NO production[17].

Our study showed RGAP increased proinflammatory cytokines like IL-1β, IL-6, IL-8, and TNF-α, in both sebocytes and ORS cell cultures after treatment with LPS. In the skin, TLRs can be found on keratinocytes and sebaceous glands. The combination of RGAP and LPS might stimulate sebocytes and ORS cells via a TLR-mediated pathway and ultimately activate nuclear transcription factors. Expression of transcripts encoding TLR2 and TLR4 was increased in sebocyte cultures in response to LPS and elevated levels were detected in response to the combination of RGAP and LPS. Furthermore, the combination of RGAP and LPS activated the NF-κB/AP-1 pathway in sebocyte and ORS cell cultures; expression of p-c-jun, p-iKB, and p-JNK was increased by RGAP treatment of the LPS-treated sebocyte and ORS cell cultures. Interestingly, there was no response (i.e., no proinflammatory cytokine production) to RGAP alone in sebocytes or ORS cell cultures.

LPS enhances apoptosis of target cells. In this study, LPS alone resulted in an elevated level of apoptosis in sebocytes and ORS cell cultures. RGAP alone did not promote apoptosis, but addition of RGAP to these cell cultures treated with LPS resulted in a substantial increase in apop-

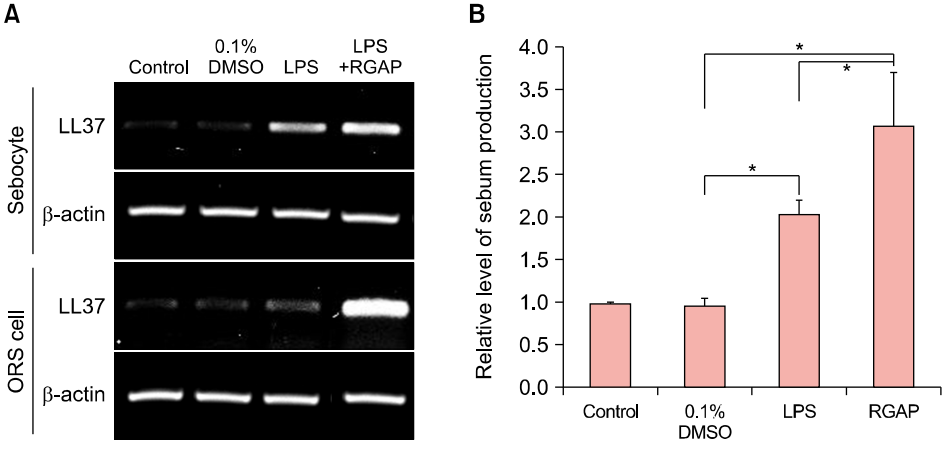

Fig. 5. (A) RGAP promoted expression of LL37 in the LPS-treated sebocytes and ORS cell cultures. (B) RGAP promoted the production of sebum in LPS-treated sebocyte cultures. Values are presented as mean±standard deviation. RGAP: acidic polysaccharides of red ginseng, LPS: lipopolysaccharide, ORS, outer root sheath. *Statistically significance ($p<0.05$).

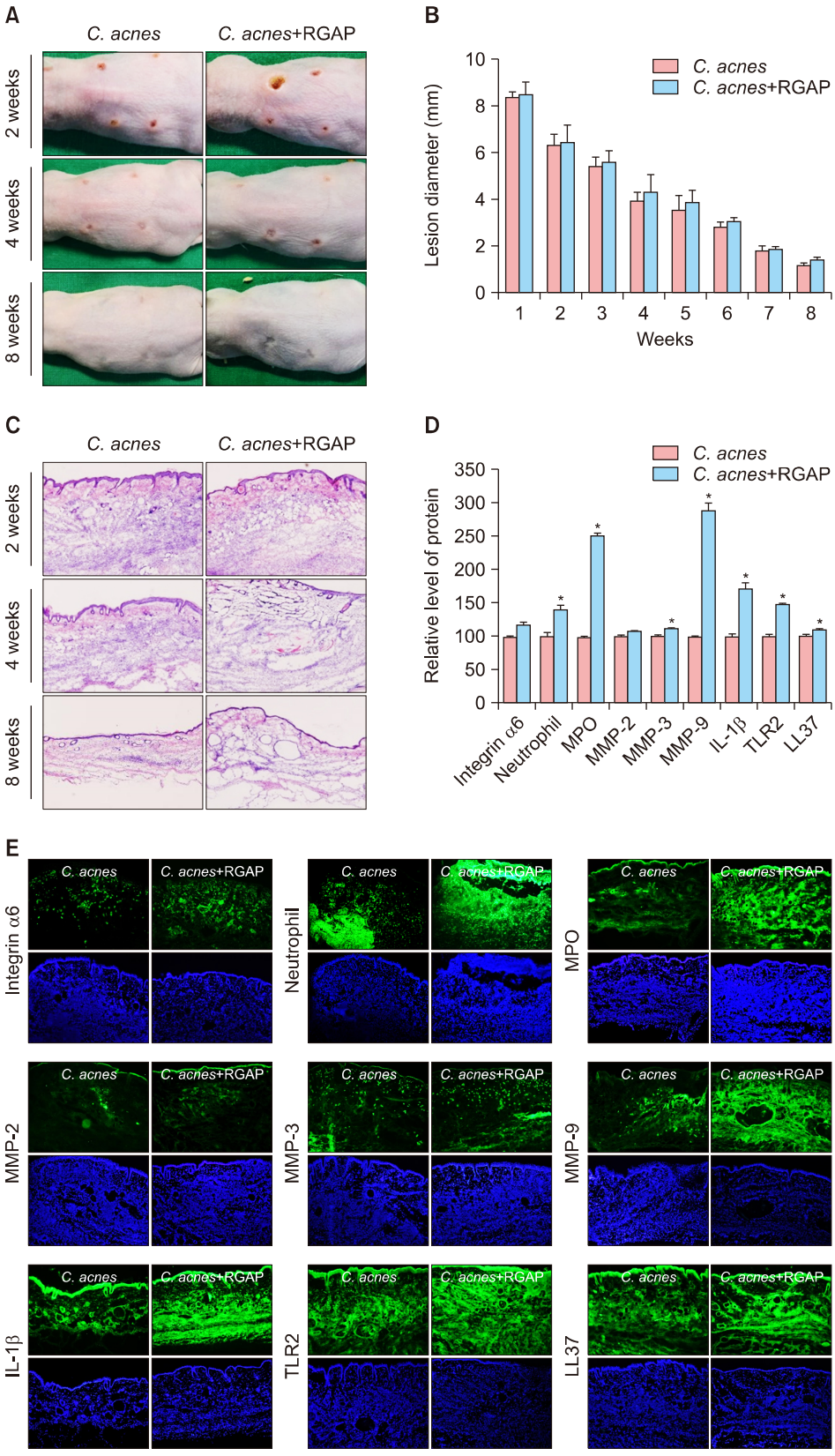

Fig. 6. (A, B) Inflammatory nodules generated by injection of *Cutibacterium acnes* were larger in RGAP-treated mice than in the controls. (C) H&E staining revealed more severe inflammatory responses at these nodules in RGAP-treated mice than in control (×40). (D, E) Inflammatory biomarkers are present in elevated levels in RGAP-treated *C. acnes*-inoculated mice compared with control. Values are presented as mean±standard deviation. RGAP: acidic polysaccharides of red ginseng, MMP: matrix metalloproteinase, MPO: myeloperoxidase, IL: interleukin, TLR: toll-like receptor.

tosis of sebocytes and ORS cells over that observed in response to LPS alone. Apoptosis is critical in normal growth, aging, and maintaining homeostasis and is also a defense mechanism for eliminating cells damaged by disease or noxious agents[18]. Although numerous stimuli and conditions are capable of triggering apoptosis, not all cells respond to the same stimulus in the same fashion. Interestingly, RGAP also enhanced the expression of LL37 in the LPS-treated sebocytes and ORS cell cultures. LL37 (cathelicidin) is important in development of acne vulgaris and especially of unique types of acne[19]. RGAP also promoted the production of sebum from LPS-pretreated sebocytes. This is an interesting finding given that the hydrophobic fraction in a red ginseng ethanol extract was reported to reduce oxidized sebum contents and eliminate erythema[20]. The relationship between RGAP and production of sebum should be investigated further.

Finally, RGAP increased the severity of *C. acnes*–induced inflammatory nodules in Hos:HR-1 mice and immunohistochemical analysis revealed an increasingly expressed inflammatory biomarkers in RGAP-treated mice compared with control. These findings are consistent with the results obtained in sebocytes and ORS cell cultures.

In conclusion, RGAP promotes expression of inflammatory biomarkers in sebocytes and ORS cell cultures after challenge with LPS and inflammatory nodules generated in response to *C. acnes* were more severe in mice treated with RGAP. Therefore, we conclude that RGAP may exacerbate inflammatory acne. As such, patients with active acne or history of acne should be advised to avoid intake of and therapies that include red ginseng.

ACKNOWLEDGMENT

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

CONFLICTS OF INTEREST

The authors have nothing to disclose.

FUNDING SOURCE

None.

DATA SHARING STATEMENT

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

ORCID

Hyun Ji Lee, https://orcid.org/0000-0002-4222-1835
Mi Hee Kwack, https://orcid.org/0000-0002-7381-6532
Weon Ju Lee, https://orcid.org/0000-0001-5708-1305

REFERENCES

1. Hazarika N. Acne vulgaris: new evidence in pathogenesis and future modalities of treatment. J Dermatolog Treat 2021; 32:277-285.
2. Katsambas A, Papakonstantinou A. Acne: systemic treatment. Clin Dermatol 2004;22:412-418.
3. Zouboulis CC, Bettoli V. Management of severe acne. Br J Dermatol 2015;172 Suppl 1:27-36.
4. Bhat YJ, Latief I, Hassan I. Update on etiopathogenesis and treatment of Acne. Indian J Dermatol Venereol Leprol 2017; 83:298-306.
5. Christensen LP. Ginsenosides chemistry, biosynthesis, analysis, and potential health effects. Adv Food Nutr Res 2009;55: 1-99.
6. Yu T, Yang Y, Kwak YS, Song GG, Kim MY, Rhee MH, et al. Ginsenoside Rc from Panax ginseng exerts anti-inflammatory activity by targeting TANK-binding kinase 1/interferon regulatory factor-3 and p38/ATF-2. J Ginseng Res 2017;41: 127-133.
7. Ansari NA, Moinuddin, Alam K, Ali A. Preferential recognition of Amadori-rich lysine residues by serum antibodies in diabetes mellitus: role of protein glycation in the disease process. Hum Immunol 2009;70:417-424.
8. Arif B, Ashraf JM, Moinuddin, Ahmad J, Arif Z, Alam K. Structural and immunological characterization of Amadori-rich human serum albumin: role in diabetes mellitus. Arch Biochem Biophys 2012;522:17-25.
9. Ha KS, Jo SH, Kang BH, Apostolidis E, Lee MS, Jang HD, et al. In vitro and in vivo antihyperglycemic effect of 2 amadori rearrangement compounds, arginyl-fructose and arginyl-fructosyl-glucose. J Food Sci 2011;76:H188-H193.
10. 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.
11. Byeon SE, Lee J, Kim JH, Yang WS, Kwak YS, Kim SY, et al. Molecular mechanism of macrophage activation by red ginseng acidic polysaccharide from Korean red ginseng. Mediators Inflamm 2012;2012:732860.
12. Park KM, Kim YS, Jeong TC, Joe CO, Shin HJ, Lee YH, et al. Nitric oxide is involved in the immunomodulating activities of acidic polysaccharide from Panax ginseng. Planta Med 2001;67:122-126.
13. Shin HJ, Kim YS, Kwak YS, Song YB, Kim YS, Park JD. Enhancement of antitumor effects of paclitaxel (taxol) in combination with red ginseng acidic polysaccharide (RGAP). Planta Med 2004;70:1033-1038.
14. Shin JY, Song JY, Yun YS, Yang HO, Rhee DK, Pyo S. Immunostimulating effects of acidic polysaccharides extract

of Panax ginseng on macrophage function. Immunopharmacol Immunotoxicol 2002;24:469-482.
15. Yang X, Wang R, Zhang S, Zhu W, Tang J, Liu J, et al. Polysaccharides from Panax japonicus C.A. Meyer and their antioxidant activities. Carbohydr Polym 2014;101:386-391.
16. Sonoda Y, Kasahara T, Mukaida N, Shimizu N, Tomoda M, Takeda T. Stimulation of interleukin-8 production by acidic polysaccharides from the root of Panax ginseng. Immunopharmacology 1998;38:287-294.
17. Choi HS, Kim KH, Sohn E, Park JD, Kim BO, Moon EY, et al. Red ginseng acidic polysaccharide (RGAP) in combination with IFN-gamma results in enhanced macrophage function through activation of the NF-kappaB pathway. Biosci Biotechnol Biochem 2008;72:1817-1825.
18. Norbury CJ, Hickson ID. Cellular responses to DNA damage. Annu Rev Pharmacol Toxicol 2001;41:367-401.
19. Ozlu E, Karadag AS, Ozkanli S, Oguztuzun S, Kilic M, Zemheri E, et al. Comparison of TLR-2, TLR-4, and antimicrobial peptide levels in different lesions of acne vulgaris. Cutan Ocul Toxicol 2016;35:300-309.
20. Hou JH, Shin H, Jang KH, Park CK, Koo B, Shin H, et al. Anti-acne properties of hydrophobic fraction of red ginseng (Panax ginseng C.A. Meyer) and its active components. Phytother Res 2019;33:584-590.