



Korean Red Ginseng attenuates ultraviolet-mediated inflammasome activation in keratinocytes



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ABSTRACT

Background: Keratinocytes form a physical barrier and act as an innate immune cell in skin. Keratinocytes secrete pro-inflammatory cytokines, such as interleukin (IL)-1 β , resulting from inflammasome activation when exposed to ultraviolet (UV) irradiation. Korean Red Ginseng extracts (RGE) have been well-studied as modulators of inflammasome activation in immune cells, such as macrophages. In the study, we elucidated the role of RGE on the UV-mediated inflammasome activation in keratinocytes compared with that in macrophages.

Methods: Human skin keratinocyte cells (HaCaT), human epidermal keratinocytes (HEK), human monocyte-like cells (THP-1), and mouse macrophages were treated with RGE or a saponin fraction (SF) or non-saponin fraction (NS) of RGE before and after UV irradiation. The secretion levels of IL-1 β , as an indicator of inflammasome activation, were analyzed.

Results: The treatment of RGE or SF in macrophages after UV irradiation inhibited IL-1 β secretion, but similar treatment in HaCaT cells did not. However, the treatment of RGE or SF in HaCaT cells in the presence of poly I:C, a toll-like receptor (TLR) 3 ligand, before UV exposure elicited the inhibition of the IL-1 β secretion. The inhibition was caused by the disruption by RGE or SF of the TLR mediating up-regulation of the *pro-IL-1 β* and *NLRP3* genes during the priming step.

Conclusion: RGE and its saponins inhibit IL-1 β secretion in response to UV exposure in both keratinocytes and macrophages. In particular, RGE treatment interrupted only the priming step in keratinocytes, although it did attenuate both the priming and activation steps in macrophages.

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1. Introduction

Keratinocytes, the major cell type of the epidermis, create a physical barrier that separates the internal and external environments. Additionally, keratinocytes are regarded as an innate immune cell active in skin immunity and inducing an inflammation response to mediate the adaptive immune response [1]. When inflammation occurs, blood monocytes are recruited into epidermis [2]. Generally, keratinocytes are resistant to ultraviolet (UV) radiation, one of the causative factors for skin inflammation [3], but

repeated UV exposure damages skin tissue [1]. UV-irradiated keratinocytes not only secrete pro-inflammatory cytokines, but also induce pyroptosis through inflammasome activation [1,4]. Inflammasomes, an intracellular multi-protein complex, are observed in immune cells, epithelial cells, and keratinocytes, and can activate inflammatory caspases, such as caspase-1 [5]. Inflammasomes are composed of caspase-1, an adaptor protein (apoptosis-associated speck-like protein containing a caspase recruitment domain [ASC]), and a sensor protein, such as nucleotide-binding oligomerization domain (NOD) or leucine rich repeat and pyrin domain containing 3 (NLRP3), which recognized cytosolic danger signals, such as the presence of reactive oxygen species (ROS). Human keratinocytes spontaneously express inflammasome components and secrete interleukin (IL)-1 β depending on inflammasome activation status [1]. In addition, keratinocytes induce gene expression of the

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inflammasome components through activation of toll-like receptors (TLRs) and via nuclear factor (NF)- κ B signaling [6]. UV exposure leads to an increase in cytosolic ROS and calcium ions, resulting in NLRP3 inflammasome activation [1].

Several studies have reported on the roles of Korean Red Ginseng extracts (RGEs) and the effects of RGE-derived molecules on skin healthiness; however, there have been no reports on the effect of RGE on inflammasome activation in keratinocytes. As well, the role of RGE on UV-mediated inflammasome activation has not been revealed. In this study, we investigated the efficacy of RGE on UV-mediated inflammasome activation in keratinocytes and compared the results with those from macrophages. Additionally, we elucidated how RGE regulates the gene expression of inflammasome components in skin cells.

2. Materials and methods

2.1. RGE and its sub-fractions

The RGE and two of its sub-fractions were provided by the Korea Ginseng Corporation (Daejeon, Republic of Korea). Briefly, the two RGE sub-fractions, a non-saponin fraction (NS) and a saponin fraction (SF), were processed as follows: 2 kg of RGE was subjected to sequential Diaion HP20 adsorption chromatography (Mitsubishi Chemical Co., Tokyo, Japan), and eluted by water, 20% ethyl alcohol (EtOH), and absolute EtOH [12,13]. The NS was obtained from the dried water and 20% EtOH eluents, while the SF was acquired from the evaporated matter from the absolute EtOH eluent [12,13]. Detailed information on RGE components, including the NS and the SF, is available in [Supplementary table 1](#) [12,13].

2.2. Cell culturing

Unless otherwise indicated, all plastic wares were supplied by SPL Life Sciences (Gyeonggi-do, Republic of Korea), and the culturing materials were supplied from Capricorn Scientific GmbH (Ebsdorfergrund, Germany). The HaCaT cells (T0020001, Addexbio Technologies, San Diego, CA, USA) were cultured in DMEM, 10 % fetal bovine serum (FBS), and antibiotics (penicillin and streptomycin). Only HaCaT cells that underwent less than 10 passages after purchase were used in the current study. Human epidermal keratinocytes (HEK; MC1323, passage 2, Biosolution Co., Ltd., Seoul, Republic of Korea) were cultured in the supplied medium for 2 d after purchase before being used in the experiments. The THP-1 cells (40202, Korean Cell Line Bank, Seoul, Republic of Korea) were maintained in RPMI 1640 containing 10% FBS and antibiotics and were differentiated to macrophage-like cells in a medium containing phorbol 12-myristate 13-acetate (200 nM; PMA; Invivogen, San Diego, CA, USA) for 24 h. Mouse bone marrow-derived macrophages (BMDMs) were prepared from progenitor cells of mouse femurs (C57BL/6, Narabio Co., Seoul, Republic of Korea) using a 50% L929-conditioned medium containing a macrophage colony-stimulating factor for 7 d [14]. The mouse experiments related to BMDMs were conducted in accordance with laboratory animals' guidelines of the institutional animal care and use committee of Kangwon National University (approval no. KW-200210-2).

2.3. Inflammasome activation and treatment

Cells were seeded into 12-well culture plates 16 h or 24 h before use in the experiments. For the priming of inflammasome activation, cells were treated with poly I:C (10 μ g/mL, short synthetic analog of dsRNA; tlrl-picw, Invivogen), lipopolysaccharides (LPS, 10

ng/mL, L4130, Sigma-Aldrich Co., St. Louis, MO, USA), or flagellin (500 ng/mL, tlrl-stfla, Invivogen) for 16 h or 24 h as indicated in the figures. Before UV irradiation, media were changed to phosphate-buffered saline (PBS, 200 μ L per well), after which the cells were exposed to UVA (365 nm) or UVB (312 nm) using the BIO-LINK crosslinker (BLX, Vilber Lourmat, Collégien, France). For UV-mediated inflammasome activation, macrophages (THP-1 cells or BMDMs) were exposed to 0.05 J/cm² UVB, while keratinocytes (HaCaT or HEK cells) were irradiated by 0.4 J/cm² UVB or 20 J/cm² UVA [15]. The cells were treated with RGE (1 mg/mL), NS (1 mg/mL), SF (0.5 mg/mL), glyburide (150 μ M, Santa Cruz Biotechnology, Dallas, TX, USA), KCl (50 mM, Biosesang, Seoul, Republic of Korea), Z-VAD-FMK (10 μ g/mL, R&D Systems, Minneapolis, MN, USA), diphenyleiiodonium (DPI, 100 μ M, Tocris Bioscience, Bristol, UK), dimethyl sulfoxide (DMSO, 5%, Sigma-Aldrich Co.), BAPTA-AM (200 μ M, Abcam, Cambridge, UK), MCC950 (200 nM, Invivogen), TAK-242 (TAK, 5 μ M, Invivogen), or cycloheximide (50 μ g/mL, Cayman, Ann Arbor, MI, USA).

2.4. Cytokine assay

The IL-1 β secretion levels in cell culture media were measured by using a human or mouse IL-1 beta/IL-1F2 ELISA kit (DY201 or DY401, R&D Systems). The ELISA plates were quantified using a microplate reader (Synergy H1™ microplate reader, BioTek, Winooski, VT, USA).

2.5. RNA extraction and real-time PCR

The total RNA prepared by NucleoZOL (MN Co., Postfach, Düren, Germany) was reverse-transcribed into complementary DNA (cDNA) by using a cDNA synthesis kit (Enzynomics Co., Daejeon, Republic of Korea). Gene expressions were quantified using a qPCR pre-mixture kit (Enzynomics Co.) and a real-time PCR machine (Eco, Illumina, San Diego, CA, USA). The sequence of primers was informed in [Supplementary table 2](#).

2.6. Immunoblotting analysis

HaCaT cells were lysed with a lysis buffer containing a cocktail of proteinase inhibitors (Sigma-Aldrich Co.) [16,17]. The cellular lysates underwent electrophoresis (10% SDS-PAGE gel) and the products transferred to polyvinylidene fluoride membranes. The membranes were probed with anti-human IL-1 β antibody (R&D Systems) or anti-actin antibody (Santa Cruz Biotechnology) overnight at 4°C before being incubated with HRP conjugated 2nd antisera (Santa Cruz Biotechnology) for 3 h at room temperature. The immunoblotting bands were visualized using a chemiluminescence solution (ThermoFisher Scientific Korea, Seoul, Republic of Korea) and a chemiluminometer (EZ-Capture II, ATTO Technology, Tokyo, Japan).

2.7. Statistical analyses

Statistics analyses were executed by the software GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Comparisons of two or multiple groups were analyzed by using t-tests (Mann-Whitney test) or one-way ANOVA (Tukey's multiple comparisons test).

3. Results

3.1. RGE and SF inhibit IL-1 β secretion in response to UV irradiation in macrophages

Although activation of inflammasomes is achieved in two steps, the activation step, in which cytokines are matured without protein synthesis, is the target for the development of an inflammasome regulator [18,19]. Previously, RGE and ginsenoside have been shown to attenuate the activation step [20]. In this study, macrophages were adopted to study the effect of RGE and its sub-fractions on UV-mediated inflammasome activation. PMA-treated THP-1 cells, macrophage-like cells, were irradiated by UVB to activate inflammasomes before RGE, NS, or SF treatment (Fig. 1A). The RGE and SF treatments significantly reduced the level of IL-1 β secretion in THP-1 cells, while the NS treatment did not (Fig. 1B). In addition, murine macrophages (BMDMs) were primed with LPS and then treated with RGE, NS, or SF after UVB irradiation (Fig. 1C). As seen in Fig. 1D, BMDMs treated with RGE and SF had attenuated IL-1 β secretion levels in response to UVB. Taken together, treatment with RGE or SF during the activation step blocked UV-mediated IL-1 β maturation in macrophages.

3.2. RGE and SF inhibit UV-mediated IL-1 β secretion in keratinocytes

Next, the effect of RGE on inflammasome activation in HaCaT cells, a human skin keratinocyte cell line, was examined. As shown in Fig. 2A, HaCaT cells were treated with poly I:C during the priming step, exposed to UV, and finally treated with RGE, NS, or SF. The results show that the UVB-mediated IL-1 β secretion level was not altered by the RGE, NS, or SF treatments (Fig. 2B). In addition, RGE and its sub-fractions did not change the level of IL-1 β release from UVA exposed HaCaT cells (Fig. 2C). Next, we were curious about whether NLRP3 inflammasomes were elicited by IL-1 β secretion in response to UV exposure in keratinocytes [1]. Poly I:C-primed HaCaT cells were exposed to UVB and then treated with several

inhibitors of NLRP3 inflammasomes, which had been previously established as inhibitors in macrophages [2,21,22]. As a result (Fig. 2D), UVB-mediated IL-1 β secretion was significantly attenuated by the caspase inhibitor (Z-VAD-FMK) and several NLRP3 inhibitors (glyburide, DPI, BAPTA-AM, and DMSO). As well, two additional NLRP3 inhibitors (KCl and MCC950) also inhibited IL-1 β secretion. Although NLRP3 inflammasome activation in HaCaT cells induced the secretion of IL-1 β in response to UV irradiation, RGE treatment after UV exposure did not affect the level of IL-1 β secretion.

The maturation of IL-1 β resulting from inflammasome activation is preceded by the priming step [5,22]. In a previous study using macrophages [12], SF treatment inhibited IL-1 β secretion through disruption of the priming step; in contrast, NS treatment induced inflammasome activation by stimulating the priming step. To elucidate the effect of RGE on the priming step in HaCaT cells, the cells were treated with poly I:C in the presence of RGE, NS, or SF before UV irradiation (Fig. 2E). The results showed that treatments by RGE and its two sub-fractions significantly reduced the UVB- and UVA-mediated IL-1 β secretion levels (Fig. 2F and G). The inhibition by NS of IL-1 β release, as shown in Fig. 2F and G, might have been affected by contamination of NS by ginsenosides (Supplementary table 1) [12]. To elucidate the role of protein synthesis during the priming step of IL-1 β secretion, HaCaT cells were treated with cycloheximide, which blocks protein synthesis, during the poly I:C priming step (Fig. 2H). As expected, cycloheximide interrupted the UV-mediated IL-1 β secretion. Thus, the saponin components of RGE might block inflammasome activation by inhibiting the priming step.

3.3. Conversely, RGE and SF treatment without poly I:C stimulates IL-1 β secretion

In the previous study [12], NS induced the priming step in macrophages through the activation of TLR4-NF- κ B signaling. However, SF treatment of macrophages inhibited the expression of

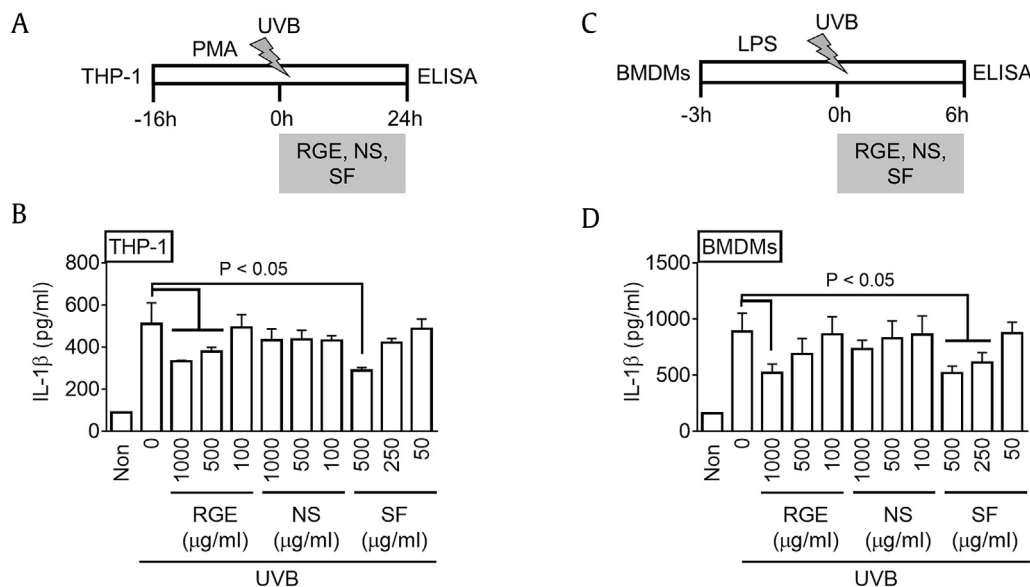


Fig. 1. Effects of RGE, NS, and SF on UV-mediated IL-1 β secretion in macrophages. A, Schematic diagram indicating the experimental process used to obtain the results in panel B. B, PMA-treated THP-1 cells were irradiated with UVB, and treated with RGE, NS, or SF during the activation step. The human IL-1 β secretion level was measured by ELISA. C, A schematic diagram shows the experimental process used to obtain the results in panel D. D, LPS-primed BMDMs were exposed to UVB followed by the treatment of RGE, NS, or SF. The mouse IL-1 β releases were determined by ELISA. Bar graphs indicate mean \pm standard deviation (SD) values. All data shown are representative of at least three independent experiments.

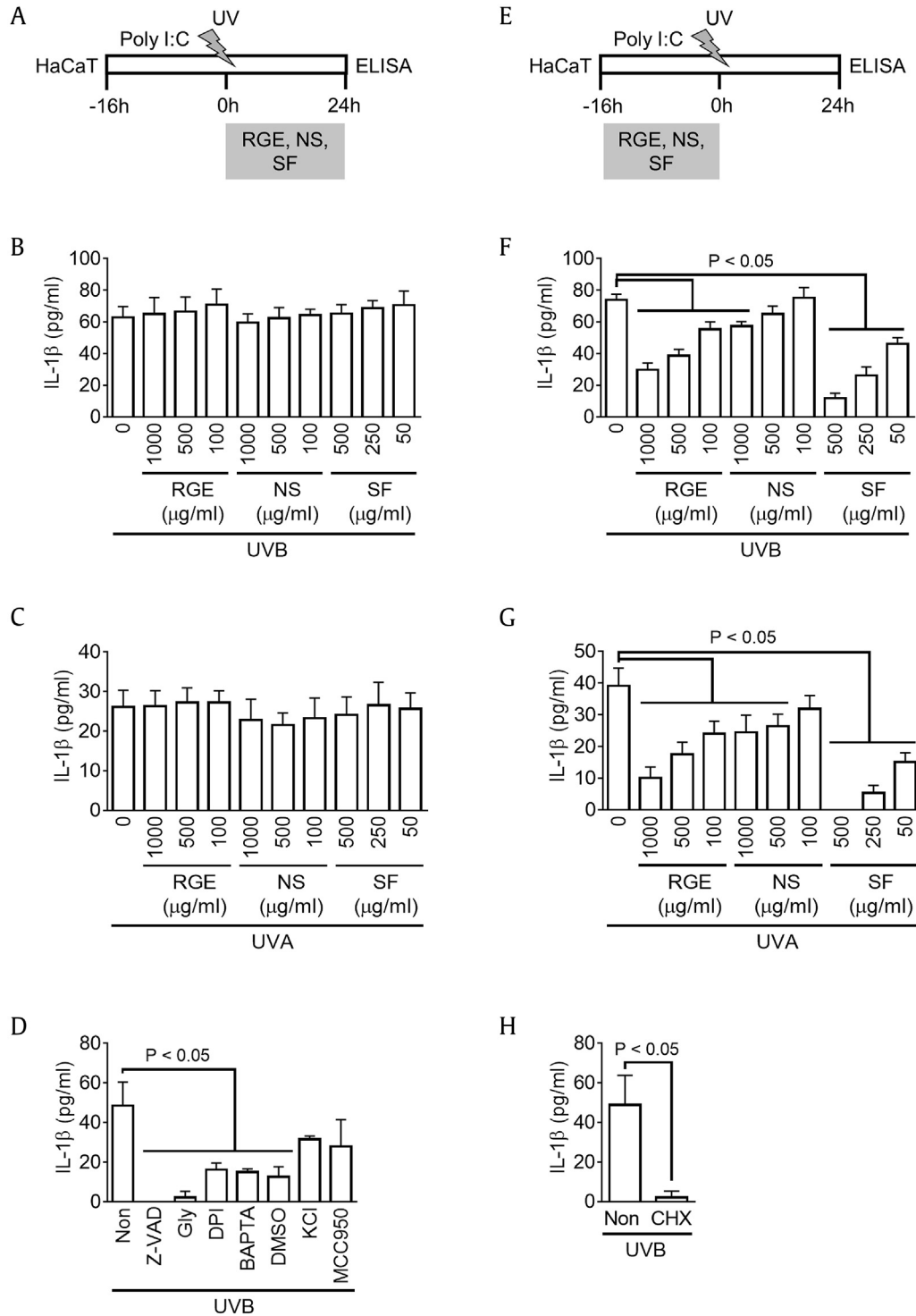


Fig. 2. Effect of RGE, NS, and SF on UV-mediated IL-1 β secretion in HaCaT cells. A, Schematic diagram indicating the experimental model used to obtain the results in panels B and C. Poly I:C-primed HaCaT cells were exposed to UVB (B) or UVA (C) and then treated with RGE, NS, or SF during the activation step. D, Poly I:C-primed HaCaT cells were irradiated with UVB followed by treatment of Z-VAD-FMK (Z-VAD), glyburide (Gly), diphenyleneiodonium (DPI), BAPTA-AM (BAPTA), dimethyl sulfoxide (DMSO), KCl, or MCC950. E, Diagram showing the treatment sequence used to obtain the results in panels F and G. HaCaT cells primed with poly I:C in the presence of RGE, NS, or SF during the priming step and then exposed to UVB (F) or UVA (G). H, HaCaT cells were treated with poly I:C and/or cycloheximide (CHX) followed by UVB exposure. The IL-1 β secretion level was determined by ELISA. Bar graphs indicate mean \pm SD values. All data shown are representative of at least three independent experiments.

pro-IL-1 β and the activation of inflammasomes [12,20]. Thus, RGE showed two contrasting effects on the priming step.

To elucidate the role of RGE on the priming step, HaCaT cells were treated with RGE, NS, or SF followed by UV exposure (Fig. 3A).

HaCaT cells barely secreted IL-1 β in the absence of UV irradiation with or without RGE treatment (Fig. 3B). As shown in Fig. 3C, RGE or SF treatment during the priming step significantly elicited UVB-mediated IL-1 β secretion. Similar results were observed in the

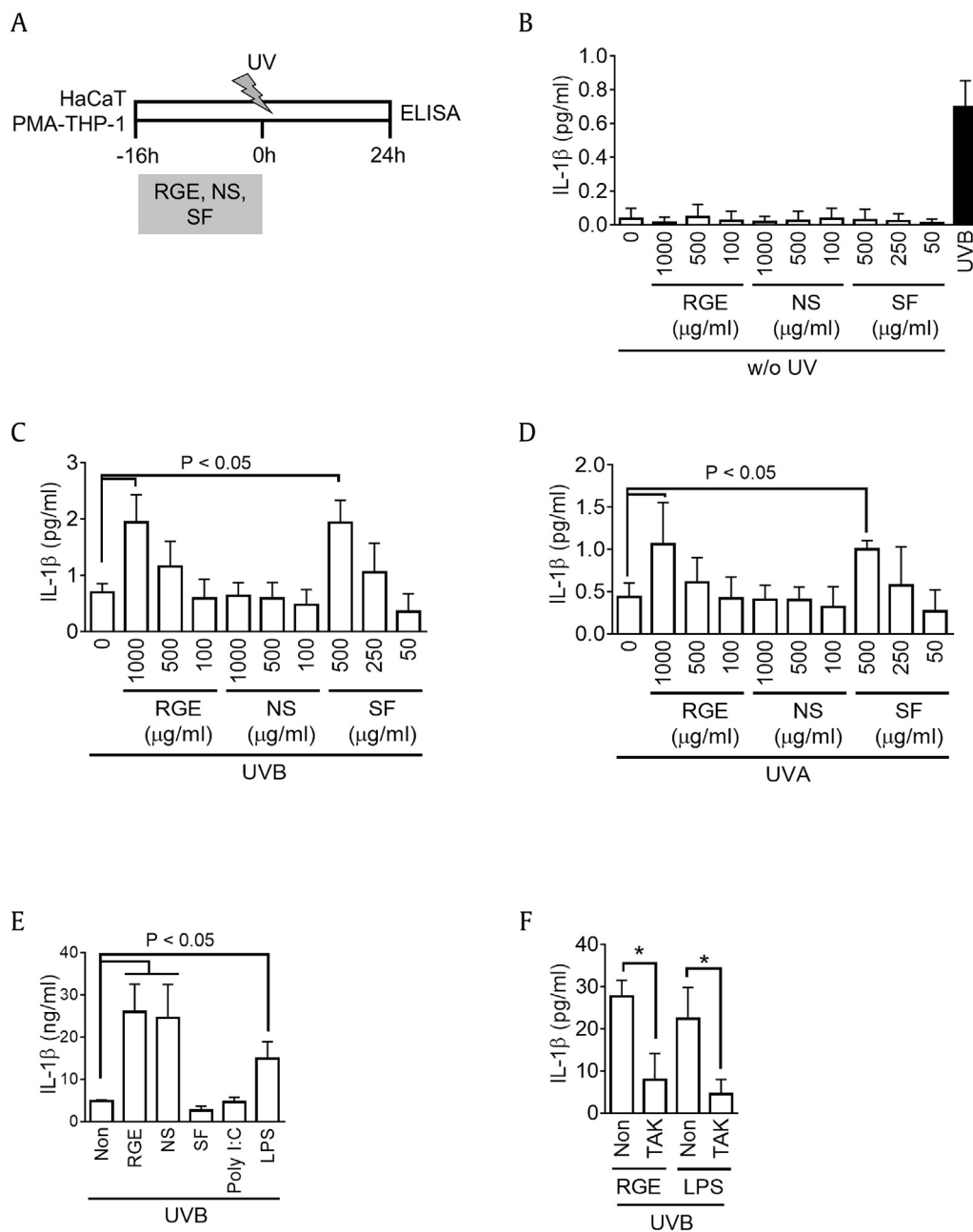


Fig. 3. Effects of RGE and its sub-fractions on the priming step. **A**, Schematic diagram showing the experimental model used to obtain the results in panels **B**, **C**, **D**, **E**, and **F**. **B**, HaCaT cells were treated with RGE, NS, or SF, and the secretion of IL-1 β was observed without UV exposure. HaCaT cells were treated with RGE, NS, or SF during the priming step and then exposed to UVB (**C**) or UVA (**D**) irradiation. **E**, PMA-treated THP-1 cells were primed with RGE, NS, SF, poly I:C, or LPS and then exposed to UVB. **F**, PMA-treated THP-1 cells were treated with RGE or LPS with or without TAK-242 (TAK) during the priming step and then irradiated with UVB. The release of IL-1 β was measured by ELISA. Bar graphs indicate mean \pm SD values. *, $P < 0.05$. All data shown are representative of at least three independent experiments.

UVA exposure experiment (Fig. 3D). Thus, priming with RGE or SF increased IL-1 β secretion. In addition, THP-1 cells were treated with RGE, NS, SF, poly I:C, or LPS followed by UVB irradiation, and as shown in Fig. 3E, significantly enhanced IL-1 β secretion levels were measured in the RGE-, NS-, or LPS-primed THP-1 cells. As expected [12], the priming property of RGE was blocked by the TLR4 inhibitor TAK-242 (Fig. 3F). Taken together, the results indicate that RGE treatment could be replaced by a TLR ligand in both HaCaT and THP-1 cells during the priming step. However, HaCaT cells were primed by SF, not by NS, implying a difference in TLR signaling between cell types (Supplementary Fig. 1A).

3.4. RGE attenuates UV-mediated IL-1 β secretion in HEK cells

Human epidermal keratinocytes (HEK) were used to confirm the effect of RGE on UV-mediated inflammasome activation. Initially, HEK cells were irradiated by UVB and then treated with RGE, NS, or SF (Fig. 4A). UVB exposure induced IL-1 β secretion that was subsequently inhibited by RGE, NS, or SF treatment (Fig. 4B). However, the level of inhibition in the HEK cells was not more potent than that in macrophages. Next, RGE or its sub-fractions were treated to HEK cells before UVB exposure (Fig. 4C). As a result (Fig. 4D), IL-1 β secretions in response to UVB were significantly attenuated by the RGE, NS, or SF treatments. Based on a previous study [4], the

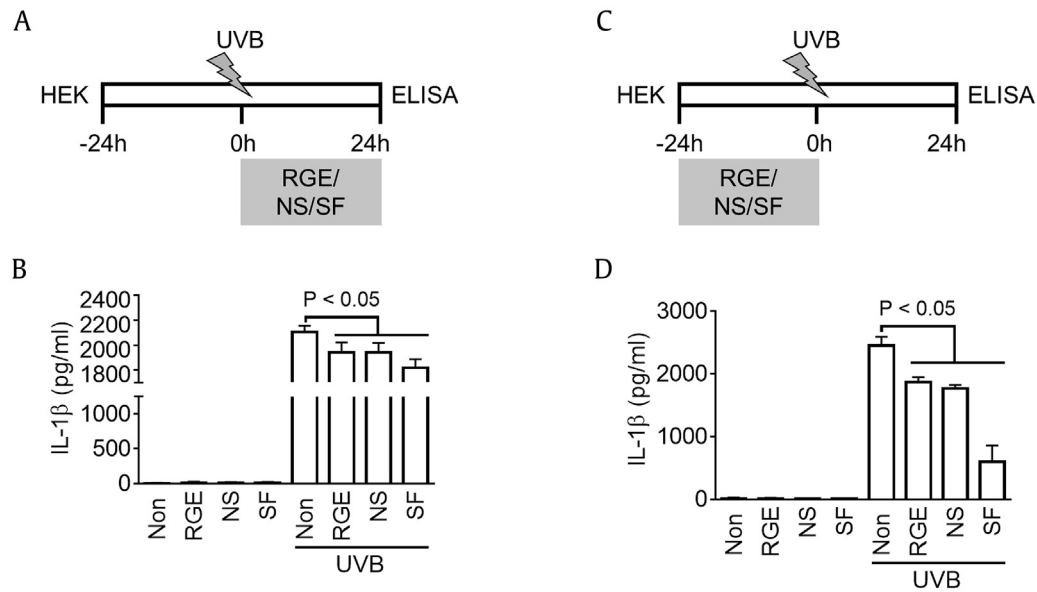


Fig. 4. Effects of RGE and its sub-fractions on UV-mediated inflammasome activation in HEK cells. A. Schematic diagram indicating the experimental process used to obtain the results in panel B. B. HEK cells were treated with RGE, NS, or SF during the activation step. C. Schematic diagram indicating the experimental process used to obtain the results in panel D. D. HEK cells were treated with RGE, NS, or SF before UVB exposure. Human IL-1 β secretion level was determined by ELISA. Bar graphs indicate mean \pm SD values. All data shown are representative of at least two independent experiments.

priming and activation steps in HEK cells simultaneously occur following UV exposure. Taken together, the results indicate that RGE treatment in HEK cells might attenuate UV-mediated IL-1 β maturation by inhibiting the priming step.

3.5. RGE inhibits gene expression of *pro-IL-1 β* and *NLRP3*

In macrophages, the treatment of RGE alone induced the expression of *pro-IL-1 β* and *NLRP3*, but co-treatment of RGE and LPS blocked both the mRNA and protein expressions of those genes [12]. In the current study, UV-mediated IL-1 β secretion was enhanced when HaCaT cells were primed by RGE or SF; however that secretion was blocked when HaCaT cells were primed by poly I:C in the presence of RGE or SF. To elucidate whether RGE or SF disrupt gene expression during the priming step, HaCaT cells were treated with RGE or its sub-fractions with or without poly I:C. As the results show, RGE or SF alone treatment in HaCaT cells induced the transcription of the *pro-IL-1 β* (Fig. 5A) and *NLRP3* (Fig. 5B) genes. However, co-treatment of poly I:C with RGE or SF attenuated the *pro-IL-1 β* (Fig. 5A) and *NLRP3* (Fig. 5B) gene expression levels. Similarly, the treatment of RGE or SF alone increased the protein levels of *pro-IL-1 β* , but that treatment decreased the translation of *pro-IL-1 β* in response to poly I:C (Fig. 5C). These results coincide with those for IL-1 β secretion (Fig. 2F and G). In addition, RGE alone treatment in THP-1 cells up-regulated *pro-IL-1 β* gene expression, but RGE down-regulated the LPS-mediated *pro-IL-1 β* transcription level (Fig. 5D). Taken together, the results show that RGE and SF regulate the IL-1 β secretion resulting from inflammasome activation through disruption of the priming step.

4. Discussion

In this study, we examined the role of RGE on UV-mediated inflammasome activation in skin-origin cells and compared the results with those for macrophages. Inflammasome activation consists of a priming step, when the genes related to inflammasomes are up-regulated, and an activation step when cytokines mature. For assessment of the priming step, HaCaT cells were

treated with poly I:C, while macrophages were treated with LPS. UV irradiation acted as the trigger to activate inflammasomes and result in IL-1 β maturation in both skin cells and macrophages. In HaCaT cells, the results of our inhibitor experiments showed that the target inflammasome of UV exposure was NLRP3. Treatment with RGE during the activation step did not change the UVA- or UVB-mediated IL-1 β secretion levels in HaCaT cells. By contrast, RGE treatment during the priming step reduced the UV-induced IL-1 β release in poly I:C-primed HaCaT cells. However, RGE or SF treatment during the priming step without poly I:C led to the secretion of IL-1 β . In HEK cells, RGE treatment decreased the secretion of IL-1 β in response to UVB irradiation. The effect of RGE on the IL-1 β secretion was the result of RGE regulation of the priming step. Both RGE and SF treatments induced *pro-IL-1 β* and *NLRP3* mRNA expression in non-primed HaCaT cells, but these gene expressions were blocked in poly I:C-treated HaCaT cells. Taken together, the results indicate that RGE and its sub-fractions interrupt the priming step during inflammasome activation.

NLRP3 inflammasome activation needs two steps [5]. The first step is the priming step, which is induced by the signaling pathways of TLRs, NOD-like receptors (NLRs), and cytokine receptors; subsequently, the expressions of NLRP3 and *pro-IL-1 β* are stimulated via NF- κ B signaling [23]. The second step is the activation step, which is triggered by cytosolic danger signals. The activation step elicits assembly of inflammasomes, maturation of IL-1 β and IL-18 by caspase-1, and pyroptosis [23]. However, sometimes only the priming signal may sufficient to activate caspase-1 and mature IL-1 β in the absence of inflammasome triggers [23]. In such instances, NF- κ B-mediation of the up-regulation of NLRP3 and *pro-IL-1 β* should not be interrupted [23]. In macrophages, an RGE alone treatment increases the expressions of *pro-IL-1 β* , TNF α , and IL-1 α , whereas RGE treatment attenuates the LPS-mediated cytokine expression levels [12]. When macrophages were treated with NS, the expression of *pro-IL-1 β* and *NLRP3* genes and their protein levels increased, while SF treatment inhibited their LPS-induced transcription and translation [12]. The up-regulation of *pro-IL-1 β* and *NLRP3* genes in response to NS treatment can be blocked by TLR4 and NF- κ B inhibitors [12,20]. As well, macrophages have been

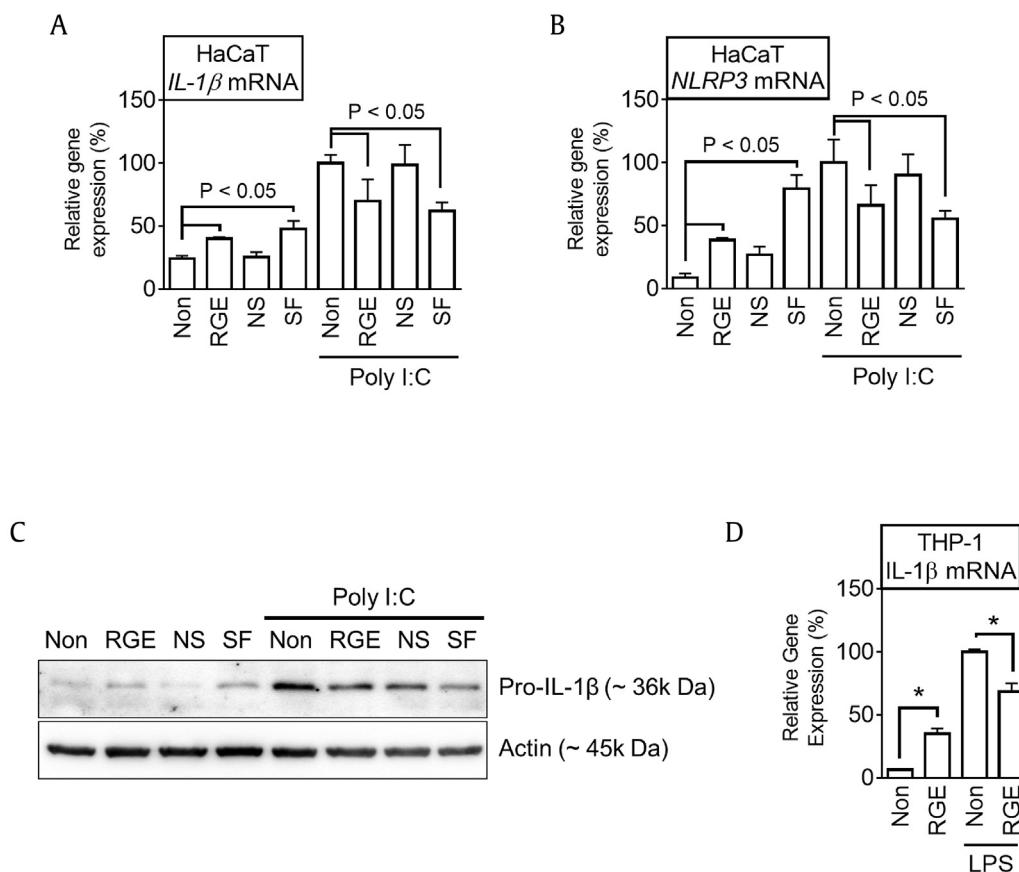


Fig. 5. Effect of RGE and its sub-fractions on the gene expressions of inflammasome components. HaCaT cells were treated with RGE, NS, or SF with or without poly I:C for 16 h. The gene expression of *pro-IL-1β* (A) and *NLRP3* (B) were quantified by real-time PCR. C, The protein levels of pro-IL-1β in treated HaCaT cells were assessed by immunoblotting. D, PMA-treated THP-1 cells were treated with RGE and/or LPS for 3 h, and the expression level of *pro-IL-1β* mRNA was measured by real-time PCR. Bar graphs indicate mean \pm SD values. *, $P < 0.05$. All data shown are representative of at least three independent experiments.

primed with NS instead of LPS, triggering the assembly of inflammasomes and presenting successful IL-1β secretion [12]. Ginsenosides, major components of SF, attenuate cytokine expressions by selectively inhibiting NF-κB signaling [20]. In the current study, RGE and SF treatment reduced the effect of poly I:C during the priming step, resulting in a decrease in the release of IL-1β in response to UV irradiation. However, the inhibitory property of RGE and SF on the activation step was not observed in HaCaT cells. In HEK cells, the RGE treatment after UV exposure moderately attenuated IL-1β secretion, but treatment of RGE before UV irradiation strongly inhibited cytokine maturation. Overall, the results indicate that IL-1β secretion in skin cells may be inhibited by SF, which blocks NF-κB signaling during the priming step, as was observed in macrophages.

In the present study, a major difference in inflammasome activation between HaCaT and THP-1 cells was related to the effects of TLR signaling during the priming step. THP-1 cells were induced during the priming step by LPS, not by poly I:C (Supplementary Fig. 1B and 1C). In contrast, HaCaT cells were primed by poly I:C, not by LPS (Supplementary Fig. 1D). This difference might be related to the expression pattern of TLRs. As shown in Supplementary Fig. 1A, THP-1 cells expressed most TLRs (exception TLR3), while HaCaT cells were observed to express only TLR3. These observations are consistent with those in a previous study [24] that reported on the expression of TLRs in several cell lines originating from keratinocytes. Most keratinocytes have been shown to express TLR1–TLR6 and TLR9, but the expression patterns differed by

cell line [12]. Based on those results, it may be that most keratinocytes cannot be primed by poly I:C. In addition, IL-1β secretion resulting from UV-mediated inflammasome activation was observed in THP-1 cells primed with NS, acting as a TLR4 ligand, but such secretion was not observed in NS-primed HaCaT cells. Indeed, HaCaT cells were shown to be relatively sensitive to a TLR3 ligand and insensitive to a TLR4 signal, while THP-1 cells did respond to a TLR4 activator but not to a TLR3 ligand (Supplementary Fig. 1C and 1D). Although we did not reveal the mechanism involved, SF-primed HaCaT cells were able to secrete IL-1β in response to UV irradiation; presumably because SF, like poly I:C, might activate TLR3 signaling.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2021.02.002>.

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