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Research Article

Maltol, a compound in Korean Red Ginseng, attenuates the *Staphylococcus aureus*–induced inflammasome activation in the skin

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ABSTRACT

Background: *Staphylococcus aureus* can cause local or systemic infections as an opportunistic pathogen and induce the activation of inflammasomes, leading to the secretion of interleukin (IL)-1β. Since *S. aureus* is part of the normal flora, it is essential to control it using safe, non-antibiotic substances like Korean Red Ginseng Extract (RGE). This study investigated the effects of maltol, a non-saponin compound found in RGE, on *S. aureus*-mediated inflammasome signaling.

Methods: Human keratinocytes (HaCaT) and macrophages were infected with *S. aureus* and treated with RGE and maltol. The secretion of IL-1β, an indicator of inflammasome activation, was analyzed. For the mechanistic studies, the HaCaT cells were infected with *S. aureus* in the presence of maltol or inflammasome inhibitors, and the generation of mitochondrial reactive oxygen species (mitROS) and IL-1β production were measured. The effect of maltol was also evaluated in *S. aureus*-injected mice.

Results: RGE and maltol inhibited *S. aureus*-mediated IL-1β secretion in HaCaT, but not in macrophages. In the mechanistic studies, maltol suppressed the production of mitROS and the priming step of inflammasome signaling resulting in attenuated *S. aureus*-mediated inflammasome activation in HaCaT. In mice, maltol inhibited the production of peritoneal IL-1β and IL-6 in response to the *S. aureus* injection.

Conclusion: Maltol selectively regulated skin inflammasome activation by inhibiting mitROS generation and the inflammasome priming step.

1. Introduction

Staphylococcus aureus, a Gram-positive bacterium, is found as a commensal in the skin, the gut mucosa, the upper respiratory tract, and the female lower reproductive tract [1]. Although *S. aureus* is a part of the normal flora, it can become an opportunistic pathogen, causing skin infections (e.g., pimples, boils, folliculitis, impetigo, and cellulitis), systemic diseases (e.g., pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis), and life-threatening antibiotic-resistant infections (i.e., methicillin-resistant *S. aureus* [MRSA]) [1]. In response to *S. aureus* infection, immune cells release pro-inflammatory cytokines. *S. aureus* exacerbates symptoms by triggering excessive production of these cytokines [2].

The inflammasome (e.g., nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing 3 [NLRP3]) is a multiprotein complex, that recognizes cytosolic danger signals and mediates the activation of caspase-1, that cleaves pro-forms of interleukin (IL)-1β (pro-IL-1β) into their active forms for release [3]. The virulence factors of *S. aureus* damage the cell membrane of macrophages, inducing potassium (K^+) efflux, thereby activating the NLRP3 inflammasome and promoting IL-1β secretion [4]. The NLRP3 inflammasome signaling involves a priming step, in which the transcription of *NLRP3* and *pro-IL-1β* is upregulated via toll-like receptor (TLR) signaling, preceding the activation step during which proteins assemble and activate caspase-1 [3]. While inflammasomes have been primarily studied in macrophages, keratinocytes (e.g., HaCaT) have also been found to express inflammasome components [3].

Korean Red Ginseng is a natural remedy with various therapeutic

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properties, including anti-inflammatory effects. The therapeutic roles of both, the saponin fractions (SF) represented by ginsenosides and the non-saponin fractions (NS) composed of polysaccharides and amino sugars of Korean Red Ginseng have been studied [5]. Maltol (3-hydroxy-2-methyl-4-pyrone) is the primary non-saponin component of Korean Red Ginseng extract (RGE), and it is produced through the Maillard reaction during the steaming process of Korean Red Ginseng [6,7]. Maltol is present in RGE at a concentration of 440 to 1160 μg/g [8,9]. Maltol demonstrates anti-inflammatory effects through its antioxidant activity, with documented therapeutic effects across various diseases [7,10].

Previous studies have investigated the differences in the inflammasome signaling between macrophages and keratinocytes in response to RGE [3]. In macrophages, the SF has been shown to inhibit the activation step of inflammasome signaling, while the NS has been shown to promote the priming step [3]. It has been demonstrated that maltol specifically inhibits reactive oxygen species (ROS) production and caspase-1 activity in macrophages, thereby disrupting NLRP3 and non-canonical inflammasomes [10]. Conversely, in HaCaT, SF does not affect the activation step induced by ultraviolet (UV) B radiation but inhibits the priming step induced by polyinosinic:polycytidylic acid (poly(I:C)), a TLR3 ligand [3]. However, studies have shown that maltol induces the priming step, leading to an increase in pro-IL-1β in macrophages [5,10].

This study investigated the role of RGE and maltol in the *S. aureus*-mediated inflammasome activation in keratinocytes (HaCaT). RGE was evaluated for its impact on the *S. aureus*-mediated IL-1β secretion in HaCaT and this was compared with its effects on macrophages. Maltol, the NS compound of the RGE, was identified as a candidate compound. The mechanism of the *S. aureus*-mediated inflammasome activation and mitochondrial ROS (mitROS) production of maltol in HaCaT were further elucidated. Additionally, the inhibitory effect of maltol on inflammasome was validated *in vivo*.

2. Materials and methods

2.1. Cell culturing

Unless otherwise indicated, the cell culturing materials were purchased from Welgene (Gyeongsan-si, Gyeongsangbuk-do, Republic of Korea) and SPL Life Sciences (Pocheon-si, Gyeonggi-do, Republic of Korea). HaCaT was obtained from Addexbio Technologies (T0020001, San Diego, CA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing antibiotics and 10 % fetal bovine serum (Merck KGaA, Seoul, Republic of Korea). Bone marrow-derived macrophages (BMDM) were differentiated by culturing progenitor cells extracted from mouse femurs in 50 % L929-conditioned media for 7 d [11]. Cells were incubated at 37 °C with 5 % CO_2.

2.2. Inflammasome activation and treatment

HaCaT and BMDM were seeded in 12-well culture plates (1×10^6 cells/well) and primed for 6 h using poly(I:C) (20 μg/mL, short synthetic analog of dsRNA; tlrl-picw, Invivogen, San Diego, CA, USA) and for 3 h with lipopolysaccharides (LPS, 10 ng/mL, L4130, Sigma-Aldrich Co., St. Louis, MO, USA) [3]. The primed cells were then subjected to *S. aureus* (multiplicity of infection [MOI] 35, #40881, Korean Culture Center of Microorganisms, Seoul, Republic of Korea) inoculation or ultraviolet B (UVB, 312 nm, 0.1 J/cm^2, BIO-LINK crosslinker, BLX, Vilber Lourmat, Collégien, France) irradiation to induce inflammasome activation [3]. After 16 h, the cell culture supernatants were collected for analysis of interleukin (IL)-1β secretion as shown in Fig. 1A. During the inflammasome activation step, RGE (1 mg/mL), NS (1 mg/mL), synthetic maltol (Supplementary Fig. S1A, ESFOOD, #186785643, Gunpo-si,

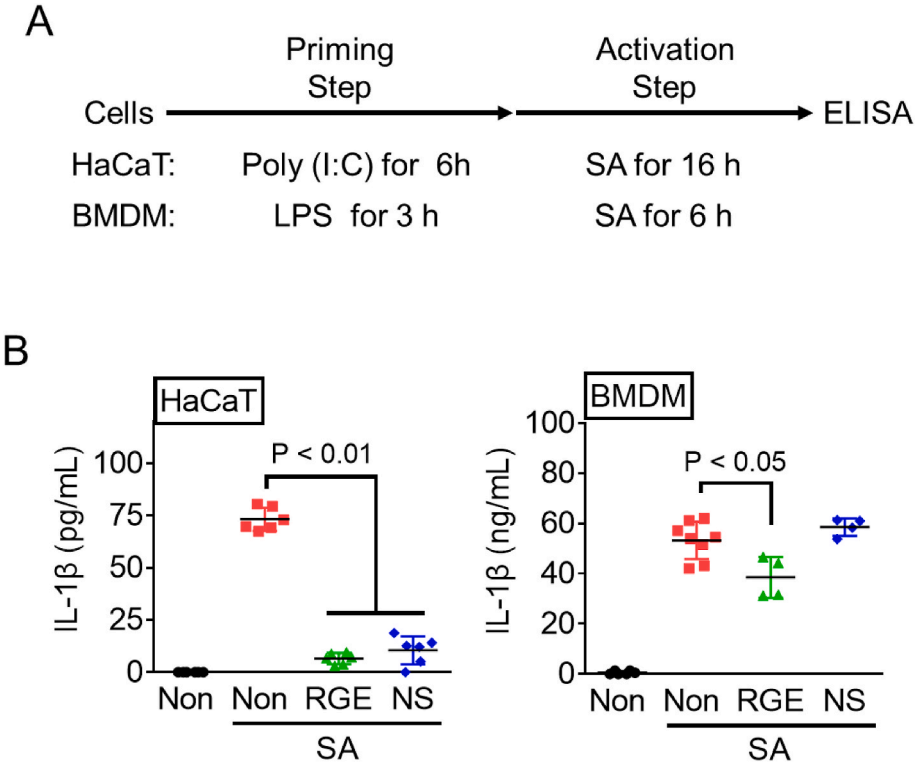

Fig. 1. Effects of RGE and NS on *S. aureus*-mediated inflammasome activation in HaCaT and BMDM.
A, Schematic diagram depicts the priming and activation steps of inflammasome signaling. **B**, HaCaT and BMDM were primed with 20 μg/mL of poly(I:C) and 10 ng/mL of LPS. *S. aureus* (SA, MOI 35) was then administered to activate the inflammasome. During the activation step, the cells were treated with 1 mg/mL of RGE and NS, and the IL-1β secretion was measured by ELISA to assess the activation of inflammasomes. Bar graphs indicate mean ± standard deviation (SD) values with at least three independent experiments.

Gyeonggi-do, Republic of Korea, or W265608, Sigma-Aldrich Co.), diphenyleneiodonium (DPI, 100 μM, Tocris Bioscience, Bristol, UK), MCC950 (200 nM, Invivogen), and extracellular high potassium solution (KCl, 50 mM; Biosesang, Seoul, Republic of Korea) were administered in the presence of *S. aureus*, grown in Luria-Bertani broth (KisanBio, Seoul, Republic of Korea) at 37 °C with shaking. The preparation and components of RGE and NS are detailed in an earlier study [3]. For the priming studies, HaCaT was treated with poly(I:C) or *S. aureus* in combination with maltol. The cellular lysates were then collected and used for the immunoblotting assay [5].

2.3. Animal study

Mice (female, C57BL/6, 7-week-old, Narabio Co., Seoul, Republic of Korea) were acclimated for one week at 18–24 °C with a 12 h light/dark cycle. The mice were provided with a normal chow diet and tap water *ad libitum*. As shown in Fig. 4A, maltol (100 mg/kg) was administered orally and intraperitoneally [10]. Additionally, *S. aureus* (5×10^8 colony-forming unit [CFU]) was injected intraperitoneally, and the mice were sacrificed 3 h later [12]. The peritoneal lavage was collected by injecting 5 mL of saline into the peritoneal cavity and shaking gently. The lavage (4 mL) was then collected to measure cytokine secretion. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (the Guide), and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The experimental protocol for the animal study was approved by the Institutional Animal Care and Use Committee of Kangwon National University (IACUC; approval no. KW-230328-1 and KW-220401-4).

2.4. Assay to assess cytokine secretion and mitochondrial ROS generation

Cytokines (i.e., IL-1β and IL-6) in cell culture supernatants or peritoneal lavages were measured using ELISA kits (DY201, DY401, DY406, R&D Systems, Minneapolis, MN, USA). HaCaT was seeded in a 96-well plate (1.25×10^5 cells/well) and primed with poly(I:C) for 3 h, followed by staining with MitoSOX™ (M36008, ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Subsequently, the cells were treated with rotenone (160 μM, Santa Cruz Biotechnology, Dallas, TX, USA), *S. aureus* (MOI 35), and maltol. Six hours later, mitochondrial ROS production was measured at 510/580 nm (Ex/Em). The plates were analyzed using a microplate reader (Synergy H1 microplate reader, BioTek, Winooski, VT, USA).

2.5. Immunoblotting analysis

The cellular lysate of HaCaT was separated using electrophoresis (10 % sodium dodecyl-sulfate polyacrylamide [SDS-PAGE] gel), and the proteins of the gel were transferred to a polyvinylidene fluoride (PVDF) membrane [3,5,10]. The membrane was then probed with a primary antibody (anti-human IL-1β antibody [R&D Systems] or anti-actin antibody [Santa Cruz Biotechnology]) followed by a secondary antibody (Santa Cruz Biotechnology). The antibody-bound membrane was visualized using a chemiluminometer (EZ-Capture II, ATTO Technology, Tokyo, Japan) and a chemiluminescence solution (Thermo Fisher Scientific).

2.6. Statistical analyses

Statistical analysis was conducted using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). The T-test (Mann-Whitney test) was used for comparing two groups, and one-way ANOVA (Kruskal-Wallis test) was employed for comparing multiple groups.

3. Results

3.1. Non-saponin compounds of RGE inhibit the activation of skin inflammasomes induced by S. aureus

To determine the effect of *S. aureus* on skin inflammasomes and the regulation of inflammasome activation by compounds derived from RGE, HaCaT and BMDM, representing keratinocytes and macrophages, respectively, were utilized. HaCaT and BMDM were primed with TLR ligands, such as poly(I:C) and LPS, and then inoculated with *S. aureus* to activate inflammasomes (Fig. 1A). Both the cells secreted IL-1β, a readout of inflammasome activation, in response to the *S. aureus* inoculation (Fig. 1B). The IL-1β secretion induced in both the cells was attenuated during the activation step when RGE was co-administered with *S. aureus*. However, the inhibitory effect of RGE on HaCaT was much more potent than that on BMDM. We further investigated whether the inhibitory effect persisted when the cells were co-administered NS fractions of RGE and *S. aureus* during the activation step. It was revealed that NS inhibited *S. aureus*-mediated IL-1β secretion in HaCaT, while NS had no effect on IL-1β secretion in BMDM (Fig. 1B). Overall, this suggests that NS compounds derived from RGE can selectively inhibit the activation of skin inflammasomes in response to *S. aureus*.

3.2. Maltol, a non-saponin compound of RGE, inhibits the activation of skin inflammasomes

We selected maltol as a candidate compound based on published literature [5,10], and investigated its role in the activation of skin inflammasomes. In HaCaT, maltol inhibited IL-1β secretion induced by *S. aureus* inoculation in a concentration-dependent manner. However, it did not affect IL-1β secretion in BMDM (Fig. 2A). Additionally, primed HaCaT and BMDM were irradiated with UVB, a representative trigger for activating skin inflammasomes [3], and the inhibitory effect of maltol was investigated. The results (Supplementary Fig. S1B) showed that UVB irradiation increased IL-1β secretion as expected, and maltol treatment significantly prevented the IL-1β increase in both cells. We also investigated inflammasome activation in another constituent cell type of the skin, the skin fibroblasts (CCD-986sk). However, we did not observe IL-1β secretion due to inflammasome activation (Supplementary Fig. S1C). The maltol concentration used in this study was not toxic to skin cells (Supplementary Figs. S1D and S1E). Taken together, maltol was identified as a candidate compound for selectively inhibiting skin inflammasome activation in response to *S. aureus* infection.

3.3. Maltol attenuates IL-1β secretion by inhibiting the generation of mitochondrial ROS in S. aureus-mediated inflammasome signaling

We investigated the mechanism by which *S. aureus* activates the skin inflammasome and maltol inhibits *S. aureus*-mediated IL-1β secretion in HaCaT. In general, the generation of mitochondrial ROS (mitROS) and the increase in K^+ efflux are well-defined mechanisms of NLRP3 inflammasome activation in macrophages [13]. As expected, HaCaT treated with rotenone resulted in an increase in mitROS generation, and maltol prevented the increase in mitROS production in a dose-dependent manner (Fig. 3A). Additionally, HaCaT inoculated with *S. aureus* showed an increase in mitROS generation, and maltol inhibited mitROS production (Fig. 3B). This implies that *S. aureus* activates the skin inflammasome through the generation of mitROS, and maltol inhibits NLRP3 inflammasome activation by attenuating mitROS production. This mechanism was further elucidated with inhibitors of ROS production (i.e., DPI), NLRP3 assembly (i.e., MCC950), and K^+ efflux (high KCl solution) in the primed HaCaT [14]. As shown in Fig. 3C, DPI and MCC950 significantly blocked IL-1β secretion in response to *S. aureus* infection. However, these effects were not observed with KCl. Overall, maltol inhibited mitROS production, resulting in the inhibition of NLRP3 inflammasome activation in HaCaT infected with *S. aureus*.

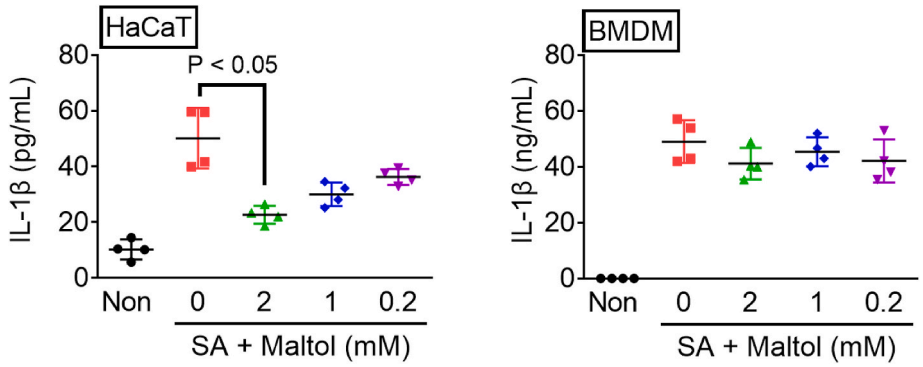

Fig. 2. Effects of maltol on *S. aureus*- and UVB-mediated inflammasome activation in HaCaT and BMDM.
Poly(I:C)-primed HaCaT and LPS-primed BMDM were infected with *S. aureus* (SA, MOI 35) in the presence of maltol. The secretion of IL-1β was measured by ELISA. Bar graphs indicate mean ± standard deviation (SD) values with at least three independent experiments.

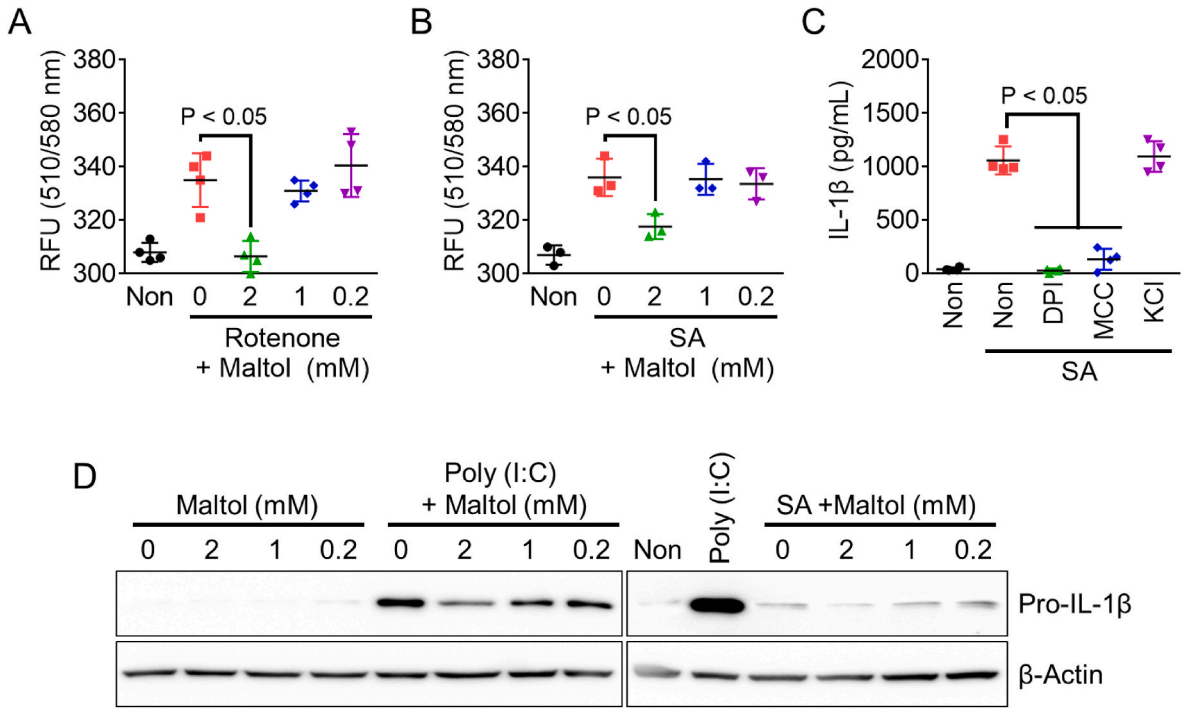

Fig. 3. Effects of maltol on mitochondrial ROS generation and the priming step in *S. aureus*-mediated inflammasome signaling.
A, Poly(I:C)-primed HaCaT was treated with rotenone in the presence of maltol. **B**, The primed HaCaT was infected with *S. aureus* (SA, MOI 35) in the presence of maltol. The mitochondrial ROS production was measured using MitoSOX™. **C**, HaCaT was primed with 20 μg/mL of poly (I:C) and then inoculated with *S. aureus* in the presence of inflammasome inhibitors (i.e., DPI [100 μM], MCC950 [MCC, 200 nM], and KCl [50 mM]). IL-1β secretion was measured by ELISA. **D**, HaCaT was treated with maltol, 20 μg/mL of poly (I:C), and *S. aureus* (SA) as indicated. The pro-IL-1β expression was measured by immunoblotting. Bar graphs indicate mean ± standard deviation (SD) values with at least three independent experiments. RFU, relative fluorescence units.

To activate NLRP3 inflammasomes, a priming step is required, during which the expression of pro-IL-1β is increased [14]. To assess the effect of maltol on the priming step, HaCaT was treated with maltol and poly(I:C), and the pro-IL-1β expression was measured. The results (Fig. 3D) showed that maltol dose-dependently reduced poly(I:C)-mediated pro-IL-1β expression. Additionally, maltol also inhibited the pro-IL-1β expression induced by *S. aureus* inoculation. In contrast to the current data in HaCaT, maltol was observed to promote the priming step in macrophages in an earlier study [5]. Therefore, it may be concluded that maltol selectively inhibits the priming step of inflammasome signaling in keratinocytes.

3.4. Maltol reduces cytokine secretion in *S. aureus*-mediated peritonitis in mice

S. aureus is a part of the skin microbiota, and as an opportunistic pathogen, induces abscesses [1]. Mice were injected with *S. aureus* in the dorsal skin to induce an abscess model (Supplemental Fig. S2A). Subsequently, maltol was topically applied to the abscess region to observe scar formation as part of the healing process. The injection of *S. aureus* successfully induced abscess formation, but no significant changes in scar formation were observed with the application of maltol (Supplemental Figs. S2B and S2C). Additionally, *S. aureus* was injected into the mouse skin, and maltol was administered either intraperitoneally (i.p.) or orally (p.o.) as shown in Supplemental Fig. S3A. When IL-1β levels were measured in the abscess tissues, no significant difference in

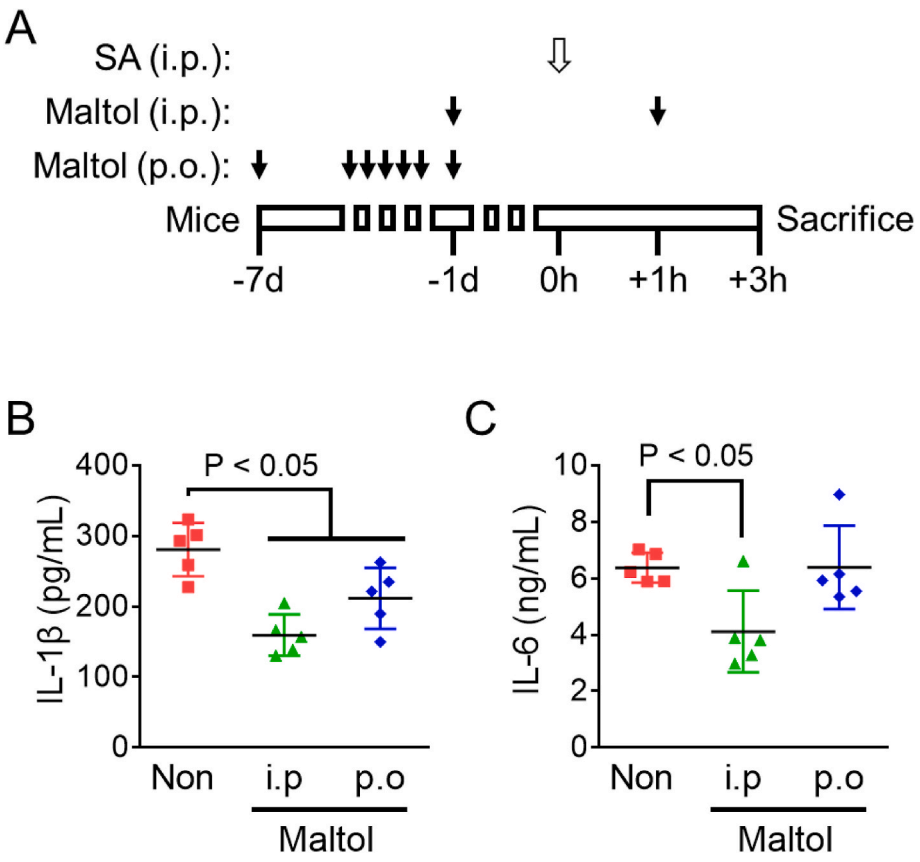

Fig. 4. Effects of maltol on the secretion of peritoneal IL-1β and IL-6 in *S. aureus*-injected mice.
A, The schematic diagram depicts the timelines for the initiation of infection with *S. aureus* (SA, 5×10^8 CFU) and administration of maltol (100 mg/kg) to the mice (n = 5 per group, total n = 15). The secretion of peritoneal IL-1β (**B**) and IL-6 (**C**) was measured by ELISA. Bar graphs represent the mean, and each dot indicates the value for each mouse.

IL-1β secretion was observed with both routes of maltol administration. Furthermore, histological examination did not reveal any differences in inflammatory cell infiltration or other changes in the abscess with maltol treatment (Supplemental Fig. S4C). In conclusion, maltol did not demonstrate any efficacy in the *S. aureus*-mediated cutaneous abscess model.

Next, we investigated the effects of maltol in a peritonitis model injected with *S. aureus*. Mice were subjected to intraperitoneal (i.p.) or oral (p.o.) administration of maltol before and/or after being infected intraperitoneally with *S. aureus* as shown in Fig. 4A. Subsequently, IL-1β secretion was measured in the peritoneal cavity. The increased production of peritoneal IL-1β levels induced by *S. aureus* was significantly reduced by maltol administration (Fig. 4B). Furthermore, the secretion of IL-6, which regulated by IL-1β [15], was also significantly reduced in the group that received the intraperitoneal administration of maltol (Fig. 4C). Overall, maltol significantly reduced the secretion of IL-1β and IL-6 in the *S. aureus*-peritonitis mice model.

4. Discussion

Maltol is widely utilized not only in the food industry but also in the cosmetics sector due to its flavor-enhancing properties. Additionally, maltol has gained attention for its anti-inflammatory effects mediated through antioxidative properties [10]. Maltol also exerts the anti-inflammatory effects by regulating various inflammatory signaling pathways (e.g., nuclear factor erythroid 2–related factor 2 [Nrf2], nuclear factor kappa B [NF-κB], phosphatidylinositol 3-kinase [PI3K]/-protein kinase B [Akt], and mammalian target of rapamycin [mTOR]) leading to the inhibition of pro-inflammatory cytokine expression (e.g., IL-1β, IL-6, and TNF-α) [16,17]. In recent studies, maltol derivatives isolated from RGE demonstrated anti-inflammatory properties by inhibiting the cytokine expression in macrophages and keratinocytes [9]. Furthermore, maltol targets the inflammasome, which is responsible for the maturation and secretion of IL-1β. The effects of maltol on various inflammasomes (NLRP3, NLR Family CARD Domain Containing 4 [NLRC4], Absent in Melanoma 2 [AIM2], and non-canonical inflammasomes) in macrophages have been well-studied [10]. Notably, maltol selectively inhibited NLRP3 and non-canonical inflammasomes by impairing ROS generation [10]. Additionally, maltol inhibited the activity of recombinant caspase-1 [10,18]. In the current study, maltol selectively inhibited *S. aureus*-mediated skin inflammasomes in HaCaT cells by suppressing mitochondrial ROS generation. Maltol also exhibited cell- or tissue-specific regulation of the priming step in inflammasome activation. In macrophages, maltol has been shown to promote the priming step, thereby increasing the expression of NLRP3 and pro-IL-1β [10]. Conversely, in the nucleus pulposus cells of the spine, maltol reduced the expression of NLRP3 and pro-IL-1β [18]. In the current study, maltol inhibits the priming step in keratinocytes, unlike in macrophages, implying that maltol selectively regulates inflammasome signaling depending on the cell type and the inflammasome triggers.

The effect of ginseng on *S. aureus* infection has been studied earlier. RGE treatment inhibits the intracellular invasion of bovine mammary epithelial cells by *S. aureus*, the pathogen causing mastitis in dairy cows [19]. In an earlier study, black ginseng inhibited the growth of *S. aureus* and the secretion of virulence factors [20]. Additionally, substances derived from ginseng were found to inhibit *S. aureus* growth [21]. In the current study, the effect of maltol on *S. aureus* growth was analyzed. However, maltol was found to have no impact on *S. aureus* growth (Supplementary Fig. S1F). In an earlier study ginsan, an acidic polysaccharide of RGE, reduced the expression of pro-inflammatory

cytokines and TLR signaling genes induced by *S. aureus* infection in mice, thereby decreasing the sepsis-induced death caused by *S. aureus* [22]. Similarly, in this study, maltol reduced the secretion of IL-1β and IL-6 induced by the intraperitoneal administration of *S. aureus*. It implies that maltol in RGE might control local and systemic *S. aureus* infections. Based on Fig. 4, the intake of RGE also appears to inhibit inflammasome signaling induced by *S. aureus* infection. As expected, oral administration may result in relatively lower systemic concentrations compared to intraperitoneal administration, as it undergoes absorption and metabolism in the digestive system. The oral administration of maltol showed a lower inhibitory effect on IL-1β secretion compared to intraperitoneal administration. Therefore, it is assumed that the impact on IL-6 secretion, which is regulated by IL-1β [15], is also reduced.

In the skin abscess experiments (Supplementary Figs. S2 and S3), the topical application of maltol and its oral and intraperitoneal administration showed no effect. When *S. aureus* naturally causes abscesses in the skin, the amount of *S. aureus* is likely to be relatively low, and the process of abscess formation may progress more slowly than in the abscess model. For this reason, it was suspected that the amount of *S. aureus* injected during the creation of the abscess model was excessively high for maltol to demonstrate its inhibitory effects.

S. aureus activates the NLRP3 inflammasome through various virulence factors, such as pore-forming toxins (PFTs) and phenol-soluble modulins (PSMs) [4]. PFTs damage the plasma membrane, leading to K^+ efflux, and they cause the destabilization of phagolysosome during *S. aureus* phagocytosis [4,23]. Also, PFTs activate cathepsin B and induce caspase-1-independent cell death, thereby activating the NLRP3 inflammasome [24]. In addition, PSMs promote the secretion of IL-1β through a caspase-1-independent mechanism [4]. Bacterial RNA, lipoproteins, and peptidoglycans of *S. aureus* are also known to be involved in inflammasome activation [4]. *S. aureus* induced the priming step of inflammasome signaling through lipoteichoic acid, a major constituent of the cell wall, which acts as a TLR2 ligand, promoting the transcription of pro-IL-1β [4]. *Nlrp3* knockout macrophages impaired IL-1β secretion in response to *S. aureus* [25]. Mechanistically, *S. aureus* damages the plasma membrane of the host cell by secreting virulence factors and induces K^+ efflux through the pores or damaged membrane leading to the activation of the NLRP3 inflammasome [4,23]. Also, extracellular high potassium treatment blocks NLRP3 inflammasome activation in macrophages [23]. However, in this study a high KCl solution did not inhibit *S. aureus*-mediated IL-1β secretion in HaCaT as shown in Fig. 3C. This could be attributed to the tissue-specific pathogenesis of *S. aureus* [4]. This study revealed that *S. aureus*-mediated inflammasome activation in keratinocytes is governed by mitROS generation, which is targeted by RGE and maltol. Although not revealed in this study, maltol may regulate the early inflammatory response through pathways other than mitROS-related pathways, such as other cell signaling pathways (e.g., Nrf2, NF-κB, PI3K/Akt, and mTOR).

5. Conclusion

In this study, we examined the selective inhibitory efficacy of RGE and its non-saponin fraction (NS) on skin inflammasomes activated by the opportunistic pathogen, *S. aureus*. *S. aureus* is known to activate NLRP3 inflammasomes in innate immune cells, including macrophages. It was revealed that RGE and NS can selectively regulate *S. aureus*-mediated inflammasome activation in keratinocytes. This study began by observing the contrasting responses of RGE and NS to *S. aureus*-mediated inflammasome activation in keratinocytes (HaCaT) and macrophages (BMDM). RGE, NS, and maltol inhibited *S. aureus*-mediated IL-1β secretion in HaCaT. However, the inhibition was weak or absent in BMDM. On the other hand, UVB induced IL-1β secretion in both HaCaT and BMDM, and maltol suppressed the elevated IL-1β secretion in both cells. Mechanistic studies have shown that *S. aureus* promotes mitROS production in HaCaT and maltol inhibits mitROS production, thereby inhibiting the activation of skin inflammasomes. Additionally, a pharmacological approach demonstrated that maltol inhibits NLRP3 inflammasome activation by suppressing *S. aureus*-mediated ROS generation in HaCaT. Although maltol is known to promote the priming step of NLRP3 inflammasome activation in macrophages, it inhibited the priming step in keratinocytes. Based on these findings, it can be concluded that maltol selectively inhibits skin inflammasome activation. Finally, the inhibitory effect of maltol on *S. aureus*-mediated inflammasome activation was validated using animal experiments. Maltol did not show significant efficacy in the skin abscess model mice induced by *S. aureus*. However, in the systemic infection model induced by the intraperitoneal administration of *S. aureus*, maltol effectively suppressed IL-1β and IL-6 production. Taken together, this study revealed that RGE and NS are effective modulators of *S. aureus*-mediated skin inflammasome activation. Maltol, a key component of RGE, was identified as the active compound and was shown to inhibit skin inflammasome activation by suppressing mitROS generation and the priming step.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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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.2024.09.008.

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