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Original Article

α-Ionone protects against UVB-induced photoaging in epidermal keratinocytes

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ABSTRACT

Objective: To evaluate whether α-ionone, an aromatic compound mainly found in raspberries, carrots, roasted almonds, fruits, and herbs, inhibits UVB-mediated photoaging and barrier dysfunction in a human epidermal keratinocyte cell line (HaCaT cells).
Methods: The anti-photoaging effect of α-ionone was evaluated by detecting the expression of barrier-related genes and matrix metalloproteinases (MMPs) in HaCaT cells. The levels of reactive oxygen species, oxidation product, antioxidant enzyme, and inflammatory factors were further analysed to underline the protective effect of α-ionone on epidermal photoaging.
Results: It was found that α-ionone attenuated UVB-induced barrier dysfunction by reversing keratin 1 and filaggrin in HaCaT cells. α-Ionone also reduced the protein amount of MMP-1 and mRNA expression of MMP-1 and MMP-3 in UVB-irradiated HaCaT cells, implying protective effects on extracellular matrix. Furthermore, HaCaT cells exposed to α-ionone showed significant decreases in interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α as compared to UVB-irradiated HaCaT cells. α-Ionone treatment significantly inhibited the UVB-induced intracellular reactive oxygen species increase and malondialdehyde accumulation. Therefore, the beneficial effects of α-ionone on inhibiting MMPs secretion and barrier damage may be related to attenuated inflammation and oxidative stress.
Conclusion: Our results highlight the protective effects of α-ionone on epidermal photoaging and promote its clinic application as a potential natural anti-photodamage agent in future.

1. Introduction

The skin is the largest organ of the body, and skin aging is one of the main manifestations of body aging (Farage, Miller, Elsner, & Maibach, 2008). Skin aging includes natural aging, heat aging, and photoaging (Lee, Kang, & Chung, 2012). Among them, photoaging is the most crucial factor causing skin aging damage. Skin photoaging is caused by long-term exposure to ultraviolet (UV) (Debacq-Chainiaux, Leduc, Verbeke, & Toussaint, 2012), even leading to various benign or malignant tumors (Huang & Chien, 2020).

Skin consists of three parts: stratified epidermis, dermis, and subcutaneous tissue (Geng et al., 2022). The epidermis is mainly composed of keratinocytes (90%–95% of skin cells) (Stalder et al., 2014), which is the key to maintaining skin hydration and barrier function. Photoaging is mainly caused by UVA and UVB (Kang, Choi, & Park, 2019). Due to its short wavelength, UVB is mostly absorbed in the epidermis. Therefore, UVB are assumed to the main culprits behind photoaging of the epidermis (Kammeyer & Luiten, 2015). The macroscopic characteristics of skin photoaging include wrinkle formation, rough texture, pigmentation, and loss of skin elasticity (Molly & Elma, 2018). Epidermis thickening is typical during photoaging (Gilchrest, 2013). Besides, daily sunlight exposure damages the epidermal basement membrane and disrupts epidermal homeostasis (Iriyama et al., 2020). UV-irradiated keratinocytes frequently undergo apoptosis, followed by a wave of increased division by surrounding cells to replace lost cells and to increase epidermal thickness. Surviving mutated cells, including stem cells, may also begin to divide inappropriately, giving rise, for example, to actinic keratoses (Iriyama et al., 2020). Studies have

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shown that UV-induced mutations in keratinocytes and melanocytes ultimately promote development of skin malignancies (Huang & Chien, 2020).

Epidermal photoaging is a complex process. In recent years, the mechanisms underlying epidermal photoaging have been intensively studied. Multifaceted signaling pathways and molecules are found to play important regulatory roles in this process (Fitsiou, Pulido, Campisi, Alimirah, & Demaria, 2021). Matrix metalloproteinases (MMPs) are a group of endopeptidases that depend on metal ions such as Ca^{2+} or Zn^{2+}, and they are secreted by keratinocytes and dermal fibroblasts. Under various stimuli such as oxidative stress and UV radiation, the secretion of MMPs increases (Ham et al., 2014). Studies have shown that UV radiation activates MMPs and induces their expression in epidermal keratinocytes (Kwon, Alam, Park, Kim, & Lee, 2019; Zheng et al., 2021). MMPs reduce extracellular matrix (ECM), resulting in skin aging (Michalak, Pierzak, Krecisz, & Suliga, 2021). Among MMPs, MMP-1, MMP-3, and MMP-9 play major roles in epidermal photoaging (Lu et al., 2016). Furthermore, UV radiation induces the production of reactive oxygen species (ROS), which breaks the dynamic balance between oxidation and antioxidant systems in epidermis (Tobin, 2017), and promotes keratinocytes to release various cytokines that influence the immune balance in skin (Geng, Kang, Huang, & Tong, 2021). At the late stages of keratinocyte differentiation, the supra-basal keratins, such as keratin 1 and keratin 10, along with the soluble proteins, including filaggrin, form a scaffold on the cellular membrane. Thereafter, other barrier-related proteins are attached to this scaffold, completing the corneocyte backbone with lipids (Jensen & Proksch, 2009; Van Smeden, Janssens, Gooris, & Bouwstra, 2014). UV irradiation is the major cause for disruption of the epidermal barrier function, accompanied by decrease in keratin and filaggrin levels, even causing skin inflammation (Kang, Son, Park, Choi, & Park, 2021).

Increasing *in vivo* and *in vitro* evidence suggests that phytochemicals, such as carotenoid, allicin, and eugenol, can protect skin cells from photoaging damage (Geng et al., 2021; Kang et al., 2019; Kang, Choi, & Park, 2020), and are becoming attractive strategies to prevent photoaging damage. α-Ionone, an aroma compound with violet scent, is found in raspberries, carrots, roasted almonds, fruits, and herbs (Tong, Park, Moon, Kang, & Park, 2019). It is a ketone compound composed of 13 carbons with a monocyclic terpenoid backbone (Fig. 1). α-Ionone exhibits anti-inflammatory, antimicrobial, and anticancer effects (Aloum, Alefishat, Adem, & Petroianu, 2020). Our previous study showed that α-ionone has anti-photoaging effects in human dermal fibroblasts (Tong et al., 2019). Due to the key role of epidermal keratinocytes in photoaging process, we aimed to test whether α-ionone attenuates UVB-induced photodamage in human epidermal keratinocytes in this study and provide theoretical basis for the application of α-ionone in skin management.

2. Materials and methods

2.1. Reagents

α-Ionone (#W259403) and DMSO (cat. #D2650) were provided by Sigma-Aldrich (St. Louis, MO, USA). Modified eagle's medium (MEM, #C11095500BT), fetal bovine serum (#10270–106), penicillin–streptomycin solution (#15140–122), and 0.25% trypsin-ethylene diamine tetraacetic acid solution (#25200–056) were purchased from Gibco (Thermo Fisher Scientific, New York, USA). Cell counting kit (CCK)-8 (#C0037), ROS assay kit (#S0033S), malondialdehyde (MDA) assay kit (#S0131S), and superoxide dismutase (SOD) assay kit (#S0101S) were obtained from Beyotime Biotechnology Company (Shanghai, China). The human total MMP-1 ELISA (#DHYAL0) was provided by R&D systems (Minneapolis, MN, USA).

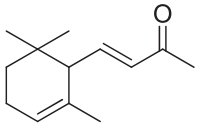

Fig. 1. Chemical structure of α-ionone.

2.2. Cell culture

HaCaT cells, a human epidermal keratinocyte cell line, were purchased from Cell Resource Center of Peking Union Medical College, China. Cells were grown in MEM supplemented with 10% FBS and 1% penicillin–streptomycin solution in incubator aerated with 5% CO_2 at 37 °C. Culture medium was changed twice a week.

2.3. UV irradiation

UVB was generated by using a UVB lamp (TL 20 W/12 RS SLV/25, Philips, China) at a distance of 30 cm, and its dose was accurately measured using a UVB irradiance meter (Beijing Normal University Optical Instrument Factory, Beijing, China). Detection using the UVB irradiance meter used an irradiation intensity of the UVB lamp of 0.126 mW/cm^2. According to the formula below:

$$\text{Irradiation dose } (\text{mJ/cm}^2) = \text{irradiation intensity } (\text{mW/cm}^2) \times \text{irradiation time } (\text{s}).$$

Using this formula, we calculated the corresponding irradiation time for the UVB irradiation dose ranging from 0 to 30 mJ/cm^2. During irradiation (ranging from 0 to 30 mJ/cm^2), cells were maintained in 50 μL phosphate buffer saline (PBS) instead of MEM.

2.4. Cell viability assay

The activity of cells was measured via a CCK8 assay. HaCaT cells (8×10^3 cells/well) were transferred into 96-well plates and cultured for 24 h. Then each well was treated with either the vehicle (DMSO) or 12.5–50 μmol/L α-ionone and incubated for 24 h. In addition, the blank group was set to medium only and no cells were seeded. Subsequently, plates were incubated with a CCK8 solution for 3 h. Finally, the OD values of each well in the 96-well plate were measured using a microplate reader at 450 nm. Cell viability was calculated using the following formula: Cell viability (%) = $[OD450_{(UVB)} - OD450_{(blank)}]/[OD450_{(control)} - OD450_{(blank)}] \times 100\%$.

2.5. Determination of ROS

HaCaT cells were seeded in 96-well plates. After 24 h of incubation (50%–60% confluence), the cells were exposed to UVB at a dose of 5 mJ/cm^2 in 50 μL PBS and treated with 50 μmol/L α-ionone for 24 h in complete medium. The generation of ROS was examined by the fluorescent staining with 2′-7′-dichlorofluorescin diacetate (DCFH-DA). After cell uptake, DCFH-DA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into 2′-7′-dichlorofluorescein. The level of intracellular ROS was measured by fluorescence microplate reader (Thermo VARIOSKAN FLASH) at the excitation and emission wavelengths of 480 and 530 nm, respectively.

2.6. Determination of MDA and SOD and MMP-1 quantification

HaCaT (3×10^5) cells were seeded in 6-well plates for 24 h. After 24 h of incubation (50%–60% confluence), the cells were exposed to UVB at a dose of 5 mJ/cm^2 in 0.5 mL PBS and treated with 50 μmol/L α-ionone for 24 h in complete medium. The lipid peroxidation assay is based on the reaction of MDA with thiobarbituric acid, forming a MDA-thiobarbituric acid adduct that absorbs strongly at 532 nm. Based on WST-8 method, SOD assay was then performed using an SOD assay kit and measured the absorbance at 450 nm. Total MMP-1 contents in the supernatants were then estimated using the human total MMP-1 enzyme-linked immunosorbent assay kit, following the manufacturers' protocols. Absorbance was measured at 450 nm using a microplate reader. The final MDA levels, SOD levels, and total MMP-1 contents were normalized to the total cellular protein content.

2.7. RNA extraction and PCR

HaCaT cells (3×10^5) were seeded in 6-well plates for 24 h. After 24 h of incubation (50%–60% confluence), the cells were exposed to UVB at a dose of 5 mJ/cm^2 in PBS and treated with 50 μmol/L α-ionone for 24 h in complete medium. The cell RNA was extracted by Trizol reagent (Thermo fisher scientific, New York, USA). The RNA concentration was measured with a Nano 300 spectrophotometer (Allsheng Co., ltd., Hangzhou, China). The cDNA was synthesized by TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). Quantitative real-time PCR (qPCR) was performed with SuperReal PreMix Plus (SYBR Green) (TransGen Biotech, Beijing, China). Primer sequences for qPCR are displayed in Table 1. Expression of β-actin was used to normalize the mRNA expression.

2.8. Statistical analysis

Data is presented as mean ± standard error of mean (SEM). For statistical analyses, significance of the differences between two groups was determined using Student's *t*-test. GraphPad Prism 8 software (GraphPad, San Diego, CA, USA) was used to perform statistical analyses. In all statistical tests, significance was set at $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant ($P > 0.05$).

3. Results

3.1. UVB irradiation reduces proliferation of HaCaT cells dose-dependently

To select the proper dose of UVB irradiation, we exposed HaCaT cells to UVB (0, 3, 5, 7, 10, 20, and 30 mJ/cm^2) and evaluated the resulting cell viability using a commercially available CCK8 kit. The corresponding irradiation method was calculated using the formula described in materials and method. As shown in Fig. 2, when compared to HaCaT cells without irradiation, UVB irradiation ranging from 3 to 30 mJ/cm^2 reduced cell proliferation dose-dependently. We used 5 mJ/cm^2 for UVB dose in all following experiments.

Table 1
Primer sequences used for qPCR.

Target genes	Forward primers (5′–3′)	Reverse primers (5′–3′)
IL-1β	AAACAGATGAAGTGCTCCT TCCAGG	TGGAGAACACCACTTGTT GCTCCA
IL-6	GCCTTCGGTCCAGTTGGCTT	GCAGAATGAGATGAGTTGTC
IL-8	ACTGTGTGTAAACATGACTTCC	CACTGGCATCTTCACTGATTCT
TNF-α	CTTGTTCCTCAGCCTCTTC	GCTGGTTATCTCTCAGCTC
MMP-1	CACTGGCATCTTCACTGATTCT	ATGTCCTTGGGGTATCCGTGTAG
MMP-3	TGAGGACACCAGCATGAACC	ACTTCGGATGCCCAGGAAAG
MMP-9	TTGACAGCGACAAGAAGTGG	GCCATTCACGTCGTCCTTAT
Keratin 1	CCTTCTTCAGCCCCTCAATGTG	AGCAGCTCCCATTTTGTTTGC
Keratin 10	CAACTCACATCAGGGGGAGC	CAGCTCATCCAGCACCCTAC
Filaggrin	AGGCTCCTTCAGGCTACATTC	CAGGAGAGTAGACATCTTTT GGCA
β-actin	AGCGAGCATCCCCCAAAGTT	GGGCACGAAGGCTCATCATT

3.2. α-Ionone shows no toxicity to HaCaT cells

To investigate the effect of α-ionone on cell viability, the CCK8 assay was performed. We found that up to 50 μmol/L α-ionone treatment showed no significant cytotoxicity to HaCaT cells (Fig. 3). Therefore, the concentration of α-ionone used in the following experiments was 50 μmol/L.

3.3. α-Ionone attenuates UVB-induced barrier dysfunction in HaCaT cells

As UVB could damage the barrier function (Kang et al., 2021), we detected the mRNA expression variation of barrier-related proteins in UVB-irradiated and non-UVB-irradiated HaCaT cells. Our results showed that UVB irradiation significantly decreases barrier-related genes, containg *keratin 1*, *filaggrin*, and *keratin 10* (Fig. 4). Nevertheless, α-ionone significantly reversed *keratin 1* (Fig. 4A) and *filaggrin* (Fig. 4B) in UVB-exposed HaCaT cells. The

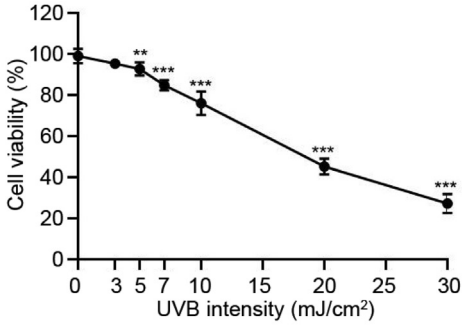

Fig. 2. Cell viability of HaCaT cells irradiated with different UVB doses. OD values of each well in 96-well plate were measured using a microplate reader at 450 nm. Values are presented as means ± SEM ($n = 8$). Significant differences between groups are indicated as: $^{**}P < 0.01$; $^{***}P < 0.001$.

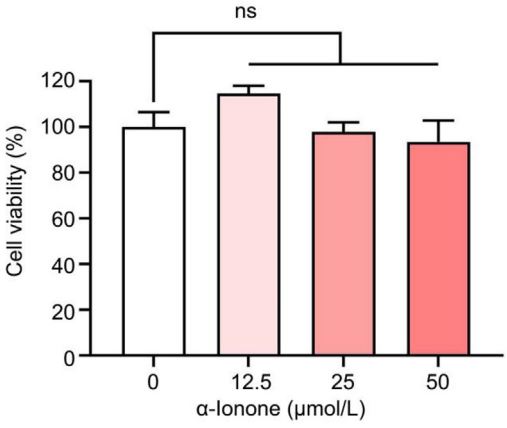

Fig. 3. Effect of α-ionone on cell viability in HaCaT cells. The cell viability of non-UVB-exposed HaCaT cells was determined after incubation with 12.5–50 μmol/L α-ionone for 24 h. Values are presented as means ± SEM ($n = 3$). Significant differences between groups are indicated as: ns, not significant ($P > 0.05$).

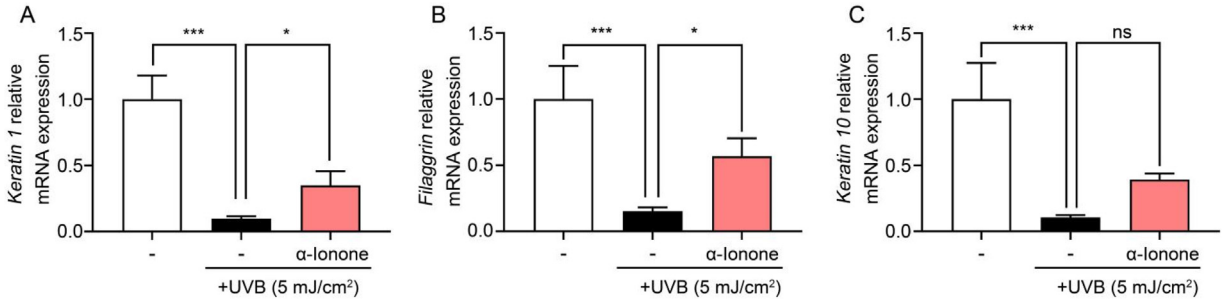

Fig. 4. α-Ionone attenuates UVB-induced skin barrier dysfunction in HaCaT cells. (A) mRNA expression of *keratin 1*. (B) mRNA expression of *filaggrin*. (C) mRNA expression of *keratin 10*. Values are presented as means ± SEM (n = 3). Significant differences between groups are indicated as: $^{*}P < 0.05$; $^{***}P < 0.001$; ns, not significant ($P > 0.05$).

level of *keratin 10* tended to be restored after α-ionone treatment, although there was no statistical difference (Fig. 4C). Overall, α-ionone could attenuate barrier damage after UVB irradiation in HaCaT cells.

3.4. α-Ionone inhibits UVB-induced MMP increase in HaCaT cells

MMPs, the key enzymes in the photoaging process, could degrade almost all ECMs (Quan et al., 2009). Similar to previous reports, we also observed that UVB irradiation increases MMP-1 protein secretion. The expression of MMP-1 protein detected by ELISA was significantly restored after α-ionone treatment in UVB-induced HaCaT cells (Fig. 5A). Besides, we examined the mRNA expression of MMP-1, MMP-3, and MMP-9 based on the ELISA results in HaCaT cells. Irradiation of HaCaT cells with UVB significantly upregulated *MMP-1*, *MMP-3*, and *MMP-9* (Fig. 5B). In contrast, treatment of HaCaT cells with α-ionone resulted in decrease of *MMP-1* and *MMP-3*, and the expression of *MMP-9* tended to reduce although there was no statistical difference. α-Ionone treatment could inhibit UVB-induced MMP increase in HaCaT cells.

3.5. α-Ionone inhibits UVB-induced inflammation in HaCaT cells

UVB-induced pro-inflammatory factors have been demonstrated to induce MMP secretion (Ranzato et al., 2017) and barrier damage (Jia & Zeng, 2020) in epidermal keratinocytes. In the present study, we observed that UVB irradiation significantly increased the mRNA expression of *interleukin* (*IL*)-*1β*, *IL-6*, *IL-8*, and *tumor necrosis factor* (*TNF*)-*α* in HaCaT cells (Fig. 6). While, α-ionone treatment significantly decreased the expression of these genes as compared to UVB irradiation, showing the anti-inflammatory effects on HaCaT cells.

3.6. α-Ionone protects against UVB-induced oxidative stress in HaCaT cells

Studies have shown that intracellular oxidative stress induced by UV irradiation promotes MMP secretion (Oh et al., 2017) and barrier dysfunction (Marunaka, Kobayashi, Shu, Matsunaga, & Ikari, 2019) in HaCaT cells. We therefore evaluated whether α-ionone can inhibit UVB-induced oxidative stress in HaCaT cells by measuring ROS, MDA, and SOD levels. In HaCaT cells exposed to UVB, the contents of ROS and lipid oxidation product MDA were significantly increased (Fig. 7A and B), and the level of intracellular antioxidant enzyme SOD was significantly decreased (Fig. 7C), indicating that UVB irradiation induced oxidative damage in HaCaT cells. α-Ionone treatment significantly inhibited the UVB-induced intracellular ROS increase and MDA accumulation. Intracellular SOD levels showed a trend of recovery after α-ionone treatment, although there was no statistical difference. Taken together, these results indicate that α-ionone treatment is capable of alleviating UVB-induced oxidative stress in HaCaT cells.

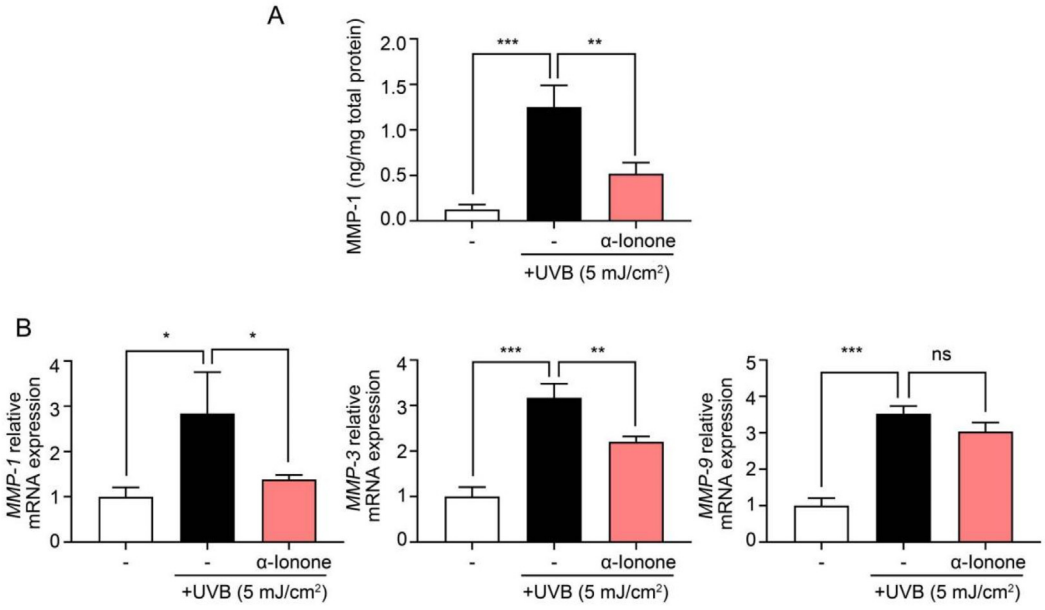

Fig. 5. α-Ionone decreased the protein amount and mRNA expression of MMP in HaCaT cells. (A) Protein amount of MMP-1. (B) mRNA expression of *MMP-1*, *MMP-3*, and *MMP-9*. Values are presented as means ± SEM (n = 3). Significant differences between groups are indicated as: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant ($P > 0.05$).

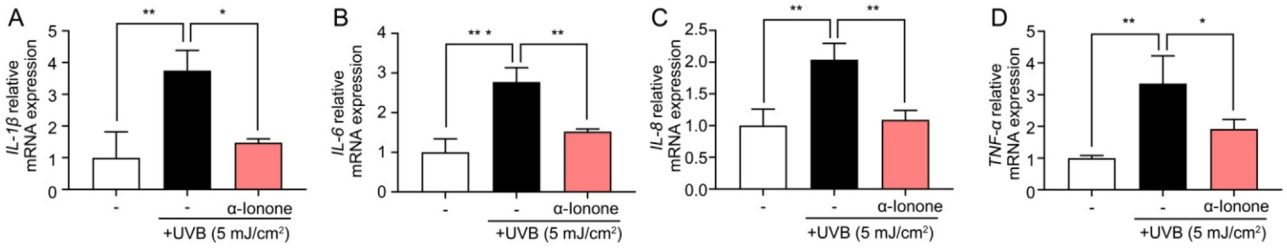

Fig. 6. α-Ionone protects against UVB-induced inflammation in HaCaT cells. mRNA expression of *IL-1β* (A), *IL-6* (B), *IL-8*(C) and *TNF-α*(D). Values are presented as means ± SEM (n = 3). Significant differences between groups are indicated as: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

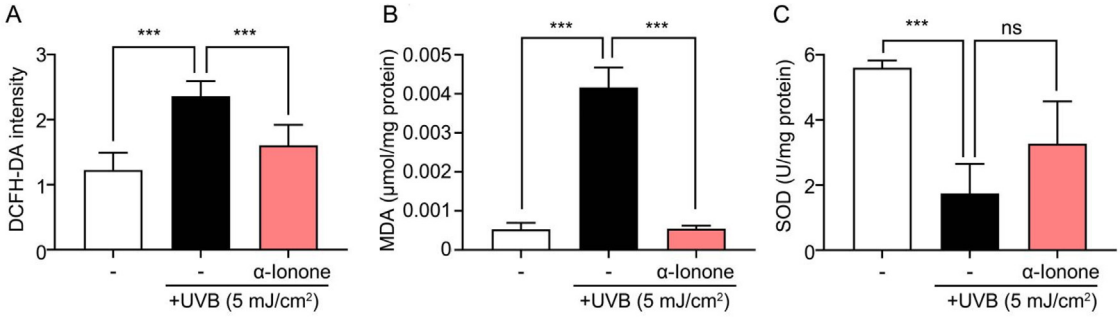

Fig. 7. α-Ionone inhibits UVB-induced oxidative stress in HaCaT cells. (A) ROS level. (B) MDA level. (C) SOD level. Values are presented as means ± SEM (n = 3). Significant differences among groups are indicated as: $^{***}P < 0.001$; ns, not significant ($P > 0.05$).

4. Discussion

In this study, we chose UVB as the light source to induce photoaging in HaCaT cells. Our results suggested that UVB irradiation causes dose-dependent decrease in cell viability of epidermal keratinocytes. Exposure to instant high-dose or chronic low-dose UV radiation is harmful to body health and is associated with the clinical hallmarks of skin aging (Debacq-Chainiaux et al., 2012; Young, 2006). The impact of UVA can reach the deep layer of the dermis due to its greater penetration ability, but its irradiation energy is much lower than UVB (Krutmann, Schalka, Watson, Wei, & Morita, 2021). Compared with UVA, UVB is the major factor in epidermal photoaging due to its shorter wavelength. Besides, UVB could damage intracellular DNA and proteins directly (Geng et al., 2021). In addition, similar to previous studies (Cui et al., 2021), there is also a dose-dependent relationship between cell viability and UVB irradiation dose. However, HaCaT cells irradiated by the same UVB dose showed different cell viability in different studies. In this study, the cell viability was −30% after 30 mJ/cm^2 dose of UVB irradiation. While Cui et al. showed that the survival rate of HaCaT cells was −50% after 30 mJ/cm^2 dose of UVB irradiation (Cui et al., 2021), which might be related to the type of UVB lamp and the volume of buffer during irradiation. In the present study, up to 50 μmol/L α-ionone treatment showed no significant cytotoxicity to HaCaT cells and our previous study demonstrated that α-ionone, up to a concentration of 100 μmol/L, has no toxicity toward human Hs68 dermal fibroblasts (Tong et al., 2019). Overall, α-ionone shows high safety in both epidermal and dermal cells.

MMPs are critical in skin photoaging and can degrade almost all ECMs. Twenty-eight members of the human MMP family have been isolated and identified (Quan et al., 2009). MMP-1 can degrade type I and type III collagen, almost destroying collagen completely; MMP-3 extensively cuts type IV collagen, proteoglycans, and fibronectin; MMP-9 further degrades the collagen fragments produced by MMP-1 (Quan et al., 2009). Our previous study showed that α-ionone significantly reduces UVB-induced elevation of MMP-1, MMP-3, and MMP-9 gene expression in human dermal fibroblasts (Tong et al., 2019). While, in the present study, α-ionone showed no significant effect on the UVB-induced increase of *MMP-9* expression in keratinocytes. α-Ionone exhibited different effects on the same subtype of MMPs in different skin cells, which might be due to different irradiation doses and cell sensitivities to UV. Similarly, treatment with the same concentration of syringaresinol exhibited different suppression rates of MMP-1 secretion in human epidermal keratinocytes and dermal fibroblasts (Oh, Joo, Karadeniz, Ko, & Kong, 2020).

Various stimuli such as UV irradiation can trigger the secretion of pro-inflammatory cytokines (Jandova et al., 2021). UVB induces the expression of the nuclear factor-κB (NF-κB), which upregulates several kinds of pro-inflammatory cytokines in epidermal keratinocytes, including IL-1α, IL-1β, IL-6, IL-8, and TNF-α (Chen et al., 2019). These pro-inflammatory factors may lead to the degradation of ECM and result in skin photoaging by promoting the expression of MMP members (Pillai, Oresajo, & Hayward, 2005). For example, it was reported that IL-1α upregulates MMP-9 expression through NF-κB pathway in HaCaT cells (Ranzato et al., 2017). In the present study, α-ionone treatment inhibits the mRNA expression of *IL-1β*, *IL-6*, *IL-8*, and *TNF-α* in UVB-irradiated HaCaT cells. Besides, pro-inflammatory factors could also induce barrier damage in epidermal keratinocytes. IL-13 was reported to down-regulate the epidermal barrier related proteins in HaCaT cells, including filaggrin, loricrin, and keratin (Jia & Zeng, 2020). Notably, α-ionone significantly upregulated *keratin 1* and *filaggrin* in UVB-exposed HaCaT cells. Thus, as a natural anti-inflammatory substance, the beneficial effects of α-ionone on inhibiting MMPs secretion and barrier damage may be related to reducing the expression of pro-inflammatory factors.

Cellular redox balance is maintained by a series of enzyme antioxidants. Both *in vitro* and *in vivo* research have shown that UVB irradiation disrupts the redox balance and generates ROS markedly in HaCaT cells, resulting in MDA accumulation and the

decreased SOD activity in the cell (Cui et al., 2021; Im et al., 2019). ROS are important signaling molecules during photoaging (Pourang et al., 2022). Under UV irradiation, ROS accumulate in the body, causing the abnormal activation of signaling pathways such as NF-κB, affecting mitochondrial membrane potential, and inducing mitochondrial DNA damage and cell apoptosis (Gu, Han, Jiang, & Zhang, 2020). Furthermore, ROS can also activate the mitogen-activated protein kinase/activating protein-1 pathway and stimulate the expression of MMPs, promoting the degradation of collagen and resulting in skin photoaging (Park, 2013). In the present study, α-ionone was capable of scavenging oxidation products such as MDA, leading to the relief of oxidative stress in UVB-damaged HaCaT cells. Moreover, intracellular oxidative stress induced by UV promotes MMP secretion (Oh et al., 2017) and barrier dysfunction (Marunaka et al., 2019). Thus, decreased MMP levels and barrier function recovery after α-ionone treatment might be attributed to improved oxidative stress in UVB-exposed HaCaT cells.

5. Conclusion

In summary, we demonstrated that α-ionone could attenuate photoaging in HaCaT cells, as evidenced by barrier function recovery and MMPs decrease. Furthermore, the anti-photoaging effects of α-ionone may be related to the inhibition of inflammatory reaction and oxidative damage caused by UVB irradiation. Our results revealed the potential of α-ionone for the prevention and treatment of UVB-induced cellular damage in human keratinocytes, highlighting the usefulness of α-ionone for the protection of skin epidermis. Further studies are needed to determine the efficacy of α-ionone *in vivo* for developing α-ionone as an anti-skin-photoaging agent in the future.

Authors' contributions

R.G. and T.T. wrote the manuscript; R.G., S.-G.K., K.H. and T.T. reviewed the manuscript; all authors approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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