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Original Article

Antioxidant cinnamaldehyde attenuates UVB-induced photoaging

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ABSTRACT

Background: Ultraviolet (UV) irradiation disrupts skin through several deleterious actions, such as induction of reactive oxygen species (ROS), DNA damage, and collagen degradation. Cinnamaldehyde (CIN) is a major constituent of the cinnamon and it possesses potent antioxidative activity; however, it is unclear whether CIN is capable of inhibiting the adverse effects of UVB.

Objective: To investigate protective effects of CIN against UVB-induced photodamage.

Methods: HaCaT keratinocytes were pretreated with CIN, irradiated with UVB, and assessed for the ROS production by flow cytometry and for the DNA damage by ELISA. As *in vivo* mouse model, Hos:HR-1 hairless mice were treated with ointments containing DMSO or CIN and irradiated multiple times with UVB. After 10 weeks of irradiation, wrinkle formation, epidermal thickness, infiltrating cell number, malondialdehyde amount, collagen amount, MAP kinase signaling, and related gene expressions (*Hmox1*, *Col1a1*, *Mmp1a*, and *Mmp13*) were analyzed.

Results: CIN significantly reduced the ROS production and accelerated the repair of DNA damage pyrimidine(6-4)pyrimidone photoproducts in UVB-irradiated human keratinocytes *in vitro*. In the mouse model, topical application of CIN significantly inhibited wrinkle formation, epidermal hyperplasia, and dermal inflammatory cell infiltration. The antioxidative process was significantly promoted in the CIN-applied site, as evidenced by upregulation of the antioxidative enzyme *Hmox1* as well as the reduced accumulation of malondialdehyde. In addition, topical application of CIN normalized the UVB-induced collagen/*Col1a1* downregulation and the UVB-induced *Mmp13* upregulation, implying the prevention of UVB-induced collagen degradation.

Conclusions: CIN and CIN-containing herbal agents may exert potent protective effects against UVB exposure on skin.

1. Introduction

Ultraviolet (UV) radiation impairs the outermost layer of the body, namely, the skin. Among UV rays, UVB is regarded as “the burning rays,” making up 4%–5% of UV light. It is 1000 times more potent at causing sunburn than UVA and is responsible for photo-induced skin damage [1,2]. UVB irradiation evokes complex and multifaceted signal networks that promote the intracellular formation of reactive oxygen species (ROS), which in turn damages lipids, proteins, and nucleic acids in keratinocytes [1–4]. In addition, UV exposure causes DNA damage by initiating the photoisomerization of two adjacent pyrimidine bases, such as pyrimidine(6-4)pyrimidone photoproducts (6-4PPs) [5,6]. The generated 6-4PPs are rapidly removed and repaired by DNA repair enzyme complexes, but this repair process is deterred by ROS [6]. ROS also induce lipid peroxidation as assessed by the accumulation of malondialdehyde (MDA) [7,8]. In addition, UV irradiation increases epidermal thickness, reduces collagen synthesis, and facilitates collagenolysis by upregulating the expression of matrix metalloproteases (MMPs) [9–16]. UVB-induced ROS activates mitogen-activated protein kinase (MAPK) signaling that further induces MMPs and eventually promotes collagen degradation [17,18]. These UVB-mediated biological responses are likely to contribute to the process of photocarcinogenesis and photoaging.

A number of plants are known to contain various constituents that exert beneficial effects on organisms, such as antioxidation, anti-inflammation, and anticarcinogenesis [19–22]. Among these plants, cinnamon has a long history of use as a traditional medicine for bronchitis, diarrhea, and dysmenorrhea [23,24].

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Cinnamaldehyde (3-phenyl-2-propenal; CIN) is the major constituent of the bark of *Cinnamomum cassia*, and is known to have various biological activities, including anti-inflammatory and antibacterial properties [23–25]. CIN also possesses potent antioxidative effects to inhibit ROS production in association with the upregulation of the antioxidative enzyme heme oxygenase 1 (HMOX1) [26]. However, it remains unknown whether CIN is capable of inhibiting the adverse effects of UVB.

In this study, we demonstrated that CIN 1) reduces UVB-induced ROS production in keratinocytes and 2) accelerates the clearance of the DNA damage 6-4PPs. Moreover, the topical application of CIN prevented UVB-induced photoaging *in vivo* by upregulating *Hmox1* expression and downregulating MDA accumulation. The topical application of CIN also restored the UVB-induced collagen/*Col1a1* downregulation and inhibited *Mmp13* upregulation.

2. Materials and methods (see also supplementary materials and methods)

2.1. Cell toxicity

HaCaT cells were seeded at density of 5000 cells/well of 96-well culture plates and incubated for 24 h at 37 °C. Then, the cells were treated with DMSO (0.1%, control), or chemicals (CIN, RES, or LIG; 6.25, 12.5, 25, 50, or 100 μM). At 24 h of treatment, cell toxicity was measured using Cell Counting Kit-8 (Dojindo Molecular Technologies Inc., Kumamoto, Japan) following the manufacturer's instructions. The absorbance of reaction products was measured with an iMark microplate reader (Bio-Rad Laboratories Inc., Hercules, CA) at 450 nm.

2.2. UVB irradiation experiments in vitro for RNA expression analysis

NHDFs were seeded at a density of 1.5×10^5/well of 12-well culture plates and incubated for 24 h. HaCaT keratinocytes were seeded at a density of 3×10^5/well of 6-well culture plates and incubated for 48 h. Cells were then treated with DMSO (0.1%, control) or CIN (25 or 50 μM) for 6 h, followed by irradiation with UVB at 300 J/m^2 (NHDFs) or 50 J/m^2 (HaCaT cells). At 24 h post-irradiation, cells were harvested and used for further analysis.

2.3. RNA extraction and quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

RNA extraction and qRT-PCR were performed following the methods described previously [27]. The sequences of the primers used are listed in Supplemental Table S1.

2.4. UVB irradiation experiments in vivo

Six-week-old female Hos:HR-1 hairless mice (Hoshino Laboratory Animals, Inc., Bando, Japan) were housed in a vivarium following the guidelines of the animal facility of Kyushu University. The mice were maintained under *ad libitum* feeding and handled in accordance with the Guidelines for the Care and Use of Laboratory Animals of Kyushu University. The mice were anaesthetized in 2%–3% sevoflurane (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) using a small-animal anesthetic machine (Muromachi Kikai Co., Ltd., Tokyo, Japan) and ointments containing DMSO (1%) or CIN (Low, 50 μM; and High, 500 μM) were applied to the dorsal skin 1 h prior to each UVB irradiation. Considering the difference between mono-layered keratinocytes and multiple-layered skin tissue, 10-times higher concentration of CIN than *in vitro* experiment was used as "High" and the effective dose *in vitro* was used as "Low". According to the published method inducing photoaging *in vivo* in hairless mice and with slight modification [28,29], the mice were irradiated with UVB three times a week at the following doses: 500 J/m^2 (minimal erythema dose: MED) for the first week, followed by increases of the dose by 500 J/m^2 per week up to the fourth week, after which the mice were exposed to 2000 J/m^2 (4 MED) for 6 weeks (total 10 weeks). Twenty-four hours after the final irradiation, the mice were sacrificed and the dorsal skin was collected for further analyses. Whole skin tissues (epidermis and dermis) were put in RNAlater for mRNA expression analysis or were frozen by liquid nitrogen and stored at −80 °C for the other analyses. This experiment was approved by the Animal Care and Use Committee, Kyushu University (Approval ID: A29-176-1).

2.5. Malondialdehyde (MDA) measurement

For the measurement of MDA, 10% w/v homogenate of skin tissues was prepared in cold assay buffer and cleared by centrifugation at 14,000 rpm for 10 min at 4 °C. Supernatants were then transferred to a new tube and used for the assay. MDA was measured by the thiobarbituric acid reactive substances (TBARS) assay using a malondialdehyde assay kit (Nikken Seil Co., Ltd., Tokyo, Japan), in accordance with the manufacturer's instructions.

2.6. Collagen quantification

The amount of total collagen in mouse skin was measured using Total Collagen Assay Kit (QuickZyme Biosciences, Leiden, Netherlands) following the manufacturer's instructions. Briefly, skin tissues were hydrolyzed with 6 M HCl for 20 h at 95 °C. The lysate were cleared by centrifugation at 12,000 rpm for 10 min and the supernatant was used for the assay. Absorbance of the reaction products at 570 nm was measured with an iMark microplate reader and the amount of collagen was calculated based on the standard curve provided in the kit.

2.7. Western blot

Mouse dorsal skin were frozen by liquid nitrogen, ground with pestle, lysed with lysis buffer (25 mM HEPES, 10 mM $Na_4P_2O_7 \cdot 10H_2O$, 100 mM NaF, 5 mM EDTA, 2 mM Na_3VO_4, 1% Triton X-100) at 4 °C for 2 h, and cleared by centrifugation at 14,000 rpm for 30 min. Then the supernatants were run by SDS-PAGE with Bolt 4–12% Bis-Tris Plus Gels (Thermo Fisher Scientific) and the proteins were transferred to PVDF membranes (Millipore, Burlington, MA). After blocking with 2% BSA, the membranes were probed with specific primary antibodies, and further treated with HRP-conjugated secondary antibodies. Proteins were then visualized with SuperSignal West Pico Chemiluminescence Substrate (Thermo Fisher Scientific), recorded by the ChemiDoc™ XRS Plus System (Bio-Rad Laboratories Inc.), and analyzed with Image Lab software (Bio-Rad Laboratories Inc.).

3. Results

3.1. Plant-derived chemicals reduced the UVB-induced ROS production in keratinocytes

We first examined whether CIN exerts antioxidative activity against UVB-induced ROS production in keratinocytes. Three antioxidants, NAC, RES, and LIG, served as functional controls [27,30–32]. Since CIN and LIG showed cell toxicity at 100 μM, we selected lower doses (25 and 50 μM) for the following analysis (Supplementary Fig. S1). All plant-derived chemicals (25 and 50 μM) could induce the expression of antioxidant enzyme *HMOX1*, and CIN exhibited the strongest induction compared to RES or LIG (Supplementary Fig. S2). HaCaT cells were then pre-

treated with these chemicals, irradiated with UVB, and assessed for ROS production by flow cytometry. At 12 h after UVB irradiation, UVB (200 J/m^2)-irradiation was able to induce ROS without causing entire cell death. The significant reduction of ROS production was observed in the cells pre-treated with CIN (25 and 50 μM, Fig. 1A), RES (25 μM, Fig. 1B), and LIG (25 and 50 μM, Fig. 1C). The levels of ROS reduction by CIN and LIG were comparable to that by NAC, while the capacity of RES to inhibit the UVB-induced ROS production was weaker than that of CIN or LIG.

3.2. Cinnamaldehyde accelerated the clearance of UVB-induced 6-4PPs

In addition to ROS, UVB exposure creates pyrimidine dimers such as 6-4PPs, a type of DNA damage. This damage prevents proper DNA replication and transcription, which eventually causes mutation, destabilization of chromosomes, and potentially cell death [33,34]. To assess the ability of CIN, RES, and LIG to prevent the formation or accelerate the clearance of DNA damage, the level of 6-4PPs in UVB-irradiated HaCaT cells pre-treated with CIN, RES, or LIG was measured. As shown in Fig. 2, UVB irradiation caused the formation of 6-4PPs in DMSO-treated controls, while pre-treatment with CIN (50 μM) significantly reduced the level of 6-4PPs compared with that of DMSO-treated control cells at 0 and 1 h post-irradiation. Moreover, at 3 h post-irradiation, the level of 6-4PPs almost recovered to the basal level (Fig. 2A). The level of 6-4PPs tended to be lower in the cells treated with RES (50 μM) or LIG (50 μM) than in the DMSO-treated control cells, although the differences were not statistically significant (Fig. 2B and C). These results imply that CIN, but not RES or LIG, significantly accelerates the clearance of UVB-induced DNA damage in keratinocytes.

3.3. Cinnamaldehyde reduced the UVB-induced photoaging in mice

As CIN downregulated the UVB-induced ROS generation and facilitated the repair of UVB-mediated DNA damage, we next examined its anti-photoaging effects *in vivo* using Hos: HR-1 hairless mice exposed to repetitive UVB radiation. Before each irradiation, CIN (Low, 50 μM; or High, 500 μM) or DMSO ointment was applied to the dorsal skin. At the 10th week of treatment, UVB exposure appeared to induce the formation of wrinkles on dorsal skin in the DMSO ointment-treated control group. However, the wrinkle formation was impaired in the CIN (High) ointment-treated group (Fig. 3A, left).

HE staining was performed to evaluate the epidermal thickness and number of infiltrating cells (Fig. 3A, right, B, and C). A significant increase of epidermal thickness was observed in the UVB-irradiated, DMSO-treated control group (140.9 ± 9.74 μm) compared with that in unirradiated normal skin (25.2 ± 1.14 μm) (Fig. 3B). Topical application of CIN (High) ointment significantly reduced the epidermal thickness (113.5 ± 11.5 μm) induced by UVB exposure compared with that of DMSO-treated controls (Fig. 3B). The inhibitory effect of topical CIN (Low) application was limited and did not reach statistical significance (Fig. 3B). The number of infiltrating cells in the dermis, including inflammatory cells, was also evaluated from the HE staining images. An increased number of infiltrating cells was observed in response to UVB irradiation (Fig. 3C). The UVB-irradiated groups treated with DMSO or CIN (Low) showed approximately the same numbers of infiltrating cells (3842 ± 462.5 and 3542 ± 606.7 cells/mm^2, respectively), which was slightly but significantly decreased in the CIN (High)-treated UVB-irradiated group (2963 ± 548.3 cells/mm^2) (Fig. 3C).

3.4. Cinnamaldehyde upregulated antioxidative Hmox1 expression and downregulated MDA accumulation

As CIN is known to upregulate the expression of the antioxidant enzyme *HMOX1* [26], we next measured *Hmox1* expression in the skin tissue of four groups of mice (Fig. 4A). *Hmox1* expression in the skin of the DMSO-treated UVB-irradiated group was comparable to that of the unirradiated control group. However, the topical application of CIN (High), but not CIN (Low), significantly enhanced the *Hmox1* expression, even under the UVB-treated conditions (Fig. 4A).

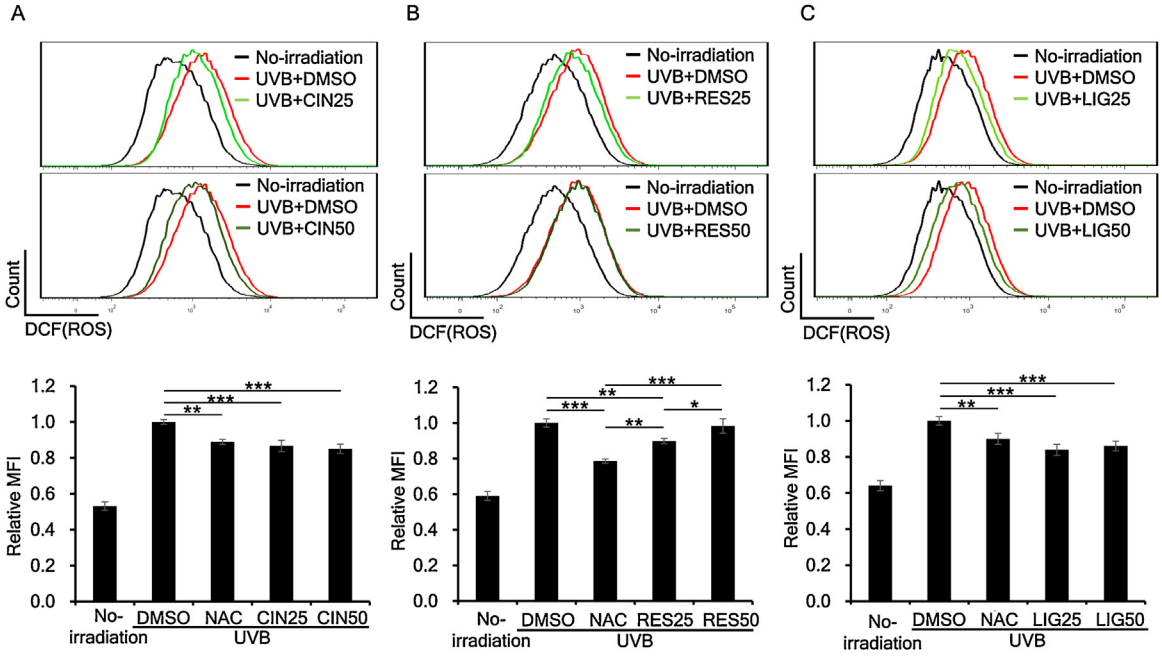

Fig. 1. CIN, RES, and LIG reduce the UVB-induced ROS production in keratinocytes. HaCaT cells were treated with DMSO (0.1%, control), N-acetyl-L-cysteine (NAC, 5 mM), (A) cinnamaldehyde (CIN, 25 or 50 μM), (B) resveratrol (RES, 25 or 50 μM), or (C) z-ligustilide (LIG, 25 or 50 μM), irradiated with UVB (200 J/m^2), and assessed for ROS production by flow cytometry at 12 h post-irradiation. Representative histograms of DCF fluorescence (upper panels) are shown, along with the mean fluorescence intensity (MFI) of DCF (lower panels). Data are presented as mean ± standard deviation (n = 3 per group). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

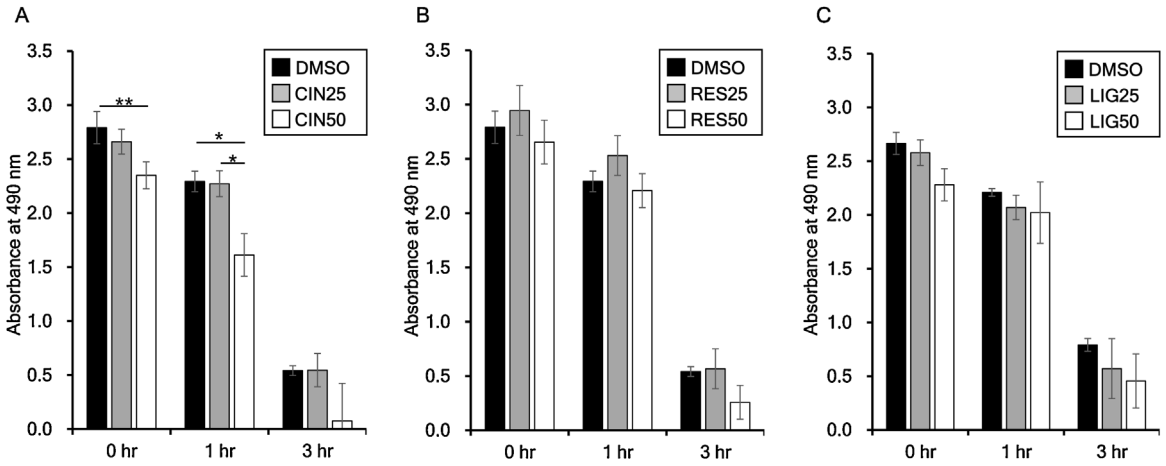

Fig. 2. CIN, but not RES and LIG, accelerates the clearance of 6-4PPs in keratinocytes. HaCaT cells were treated with DMSO (0.1%, control), (A) cinnamaldehyde (CIN, 25 or 50 μM), (B) resveratrol (RES, 25 or 50 μM), or (C) z-ligustilide (LIG, 25 or 50 μM), irradiated with UVB (300 J/m^2), and assessed for the level of 6-4PPs at 0, 1, and 3 h post-irradiation using ELISA. Data are presented as mean ± standard deviation (n = 3 per group). * $P < 0.05$ and ** $P < 0.01$.

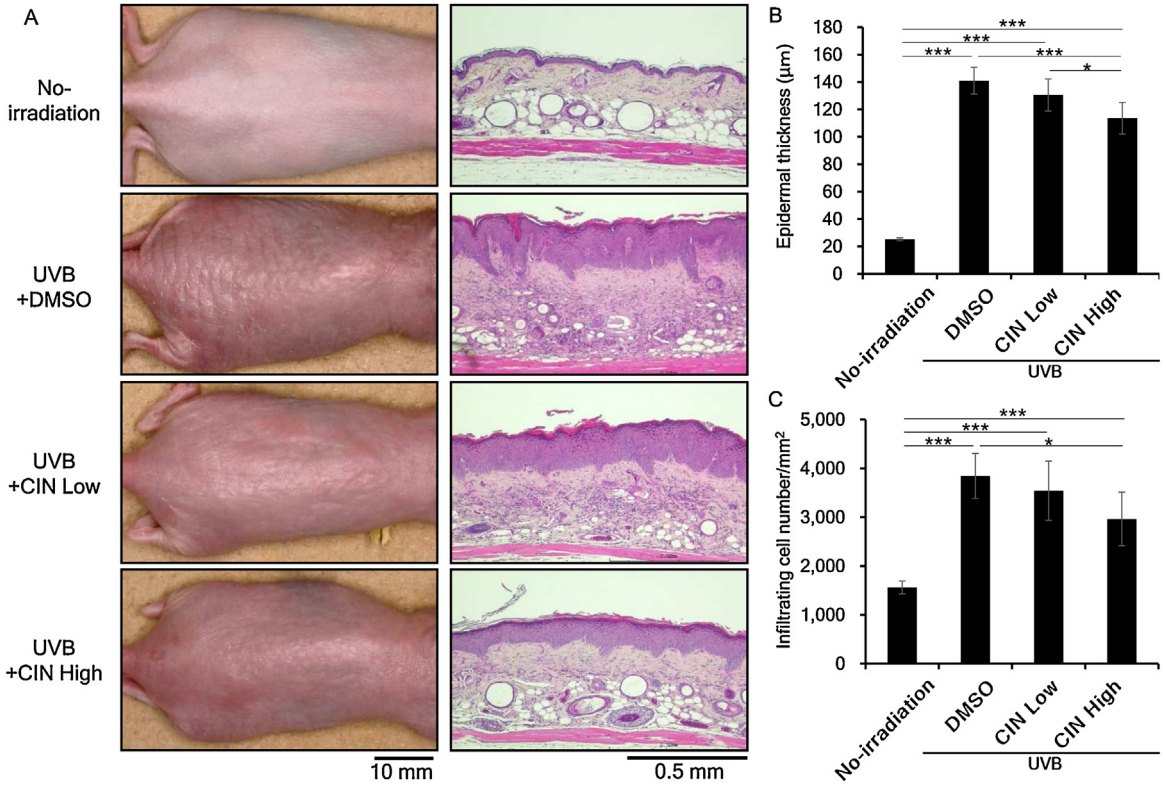

Fig. 3. UVB-induced wrinkle formation, epidermal thickness and inflammatory cell infiltration in dermis are attenuated by CIN *in vivo*. Hos:HR-1 hairless mice were treated with ointments containing DMSO (1%) or cinnamaldehyde: CIN (Low, 50 μM; or High, 500 μM), and further exposed to UVB three times a week for 10 weeks. Tissues were obtained from the dorsal skin and used for further analysis. (A) Representative images of mouse dorsal skin (left, scale bar =10 mm) and representative HE staining images of mouse dorsal skin tissue (right, scale bar =0.5 mm) at 10th week are shown. (B) Epidermal thickness of skin tissue was measured from HE staining images. (C) The number of infiltrating cells in dermis was counted and is presented as cell number per mm^2. Data are presented as mean ± standard deviation (n = 6 per group). * $P < 0.05$ and ** $P < 0.01$.

The amount of MDA, a lipid peroxidation marker, was also measured. As shown in Fig. 4B, significantly more MDA was produced in the DMSO-treated UVB-irradiated group (45.60 ± 10.05 nmol/100 mg skin tissue) than in the unirradiated group (27.68 ± 6.80 nmol/100 mg skin tissue). The UVB-mediated MDA production was inhibited in the CIN (High)-treated group (30.68 ± 4.80 nmol/100 mg skin tissue), but not in the CIN (Low)-treated group (43.38 ± 16.76 nmol/100 mg skin tissue). These results suggested that the topical application of CIN may exert antioxidative effects by upregulating *Hmox1* expression and inhibiting lipid peroxidation *in vivo*.

3.5. Cinnamaldehyde restored the UVB-mediated collagen downregulation and Mmp13 upregulation

UVB reduces collagen production and accelerates collagenolysis by induction of MMPs, especially *MMP13* (a homolog of mouse *Mmp13*) and *MMP1* (a homolog of mouse *Mmp1a* [35,36]) in

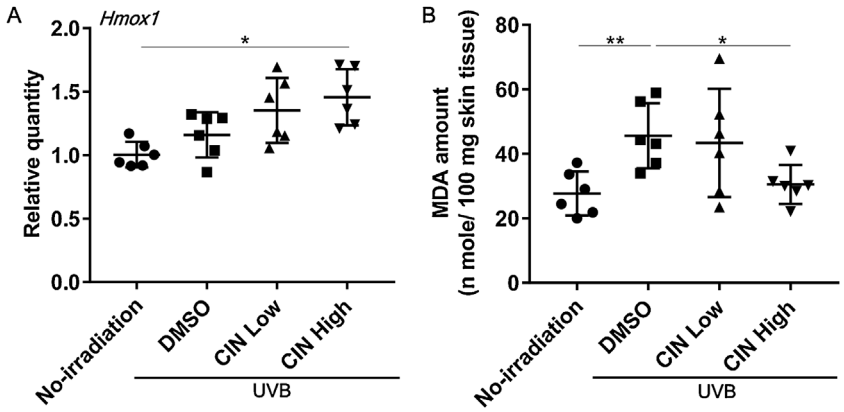

Fig. 4. CIN exerts antioxidative effects *in vivo*. (A) The mRNA expression of *Hmox1* in the dorsal skin of mice was measured by qRT-PCR. (B) The amount of MDA in 100 mg (wet weight) of dorsal skin of mice was quantified by TBARS assay. Data are presented as mean ± standard deviation (n = 6 per group). * $P < 0.05$ and ** $P < 0.01$.

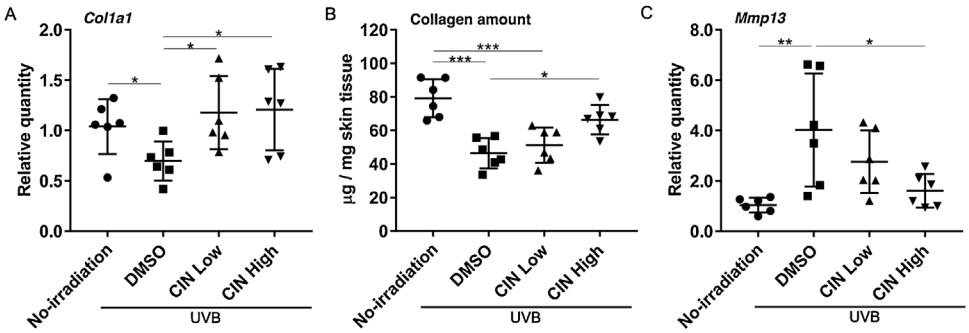

Fig. 5. UVB-induced collagenolysis is prevented by CIN. (A) *Col1a1* mRNA expression and (B) total collagen amount (μg per mg skin tissue) in the dorsal skin of mice were evaluated. (C) *Mmp13* mRNA expression in the dorsal skin of mice was measured by qRT-PCR. Data are presented as mean ± standard deviation (n = 6 per group). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

keratinocytes and fibroblasts *in vitro* [10,37], and *Mmp13 in vivo* in mice [10]. As shown in Fig. 5A, UVB exposure reduced the *Col1a1* expression in the skin compared with that in the unirradiated control group. Topical application of both CIN (Low) and CIN (High) restored the UVB-induced *Col1a1* downregulation (Fig. 5A). We also quantified the amount of collagen protein in the skin of our experimental mice. In accordance with gene expression, total collagen amount was reduced in DMSO-treated UVB-irradiated group, which was significantly restored in CIN (High)-treated UVB-irradiated group (Fig. 5B). In contrast to the expression pattern of collagen, UVB treatment significantly upregulated the expression of *Mmp13* compared with that in the untreated control group. The UVB-induced *Mmp13* upregulation was significantly inhibited by the topical application of CIN (High) (Fig. 5C). Although MMP1 is known as a responsible enzyme for collagen degradation in human, it is reportedly not responding to UVB irradiation in rodents and we did not observe the upregulation of *Mmp1a* by UVB irradiation in our *in vivo* model (Supplementary Fig. S3).

Since *Mmp1a* was not responding to UVB exposure in mice, we further examined whether CIN inhibits the UVB-induced *MMPs* in human fibroblasts and keratinocytes *in vitro*. CIN did not decreased the baseline *MMP1* and tended to inhibit the baseline *MMP13* expression in NHDFs, but the differences were not statistically significant (Supplementary Fig. S4A and S4B). UVB irradiation significantly augmented the *MMP1* and *MMP13* expression, which was susceptible to inhibition by CIN in NHDFs (Supplementary Fig. S4C and S4D). The expressions of *MMPs* were also assessed in HaCaT keratinocytes. CIN did not change baseline *MMP1* but significantly decreased baseline MMP13 in HaCaT cells (Supplementary Fig. S5A and S5B). Furthermore, UVB-induced *MMP1* and *MMP13* expression was significantly inhibited by CIN (Supplementary Fig. S5C and S5D). These results show that CIN is able to exert protective effect against UVB exposure and photoaging both *in vitro* and *in vivo*. It is also indicated that UVB-induced *MMP13* expression might be used as a marker for investigating UVB-mediated photodamage and that CIN inhibits UVB-induced *MMP13* expression in both mouse and human.

3.6. Effects of cinnamaldehyde on MAPK pathway in the mouse skin

UV-induced oxidative stress is known to activate the MAPK signaling pathway, which further regulates the expression of MMPs [17,18]. To investigate the effect of CIN on UVB-induced MAPK pathway, phosphorylation status of 3 kinds of signaling molecules, i. e. p38 MAPK, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) were assessed. As shown in Fig. 6, the phosphorylation of p38 MAPK was significantly upregulated in the skin of DMSO-treated UVB-irradiated mouse, which was significantly decreased in CIN (Low, High)-treated UVB-irradiated mice (Fig. 6B). The phosphorylation of ERK and JNK were varied among samples and we did not observe significant induction in UVB-irradiated DMSO-treated mice, whereas it was noted that CIN (Low, High)-treatment also decreased the phosphorylation of ERK and JNK. Taken together, it seems that CIN inhibits MAPK signaling and it contributes to prevent the adverse effects caused by UVB-irradiation.

4. Discussion

Various salubrious phytochemicals possess antioxidative activity through the induction of antioxidative enzymes including HMOX1, the expression of which is upregulated by CIN, a potent antioxidative phytochemical [22,26]. It is also known that CIN and

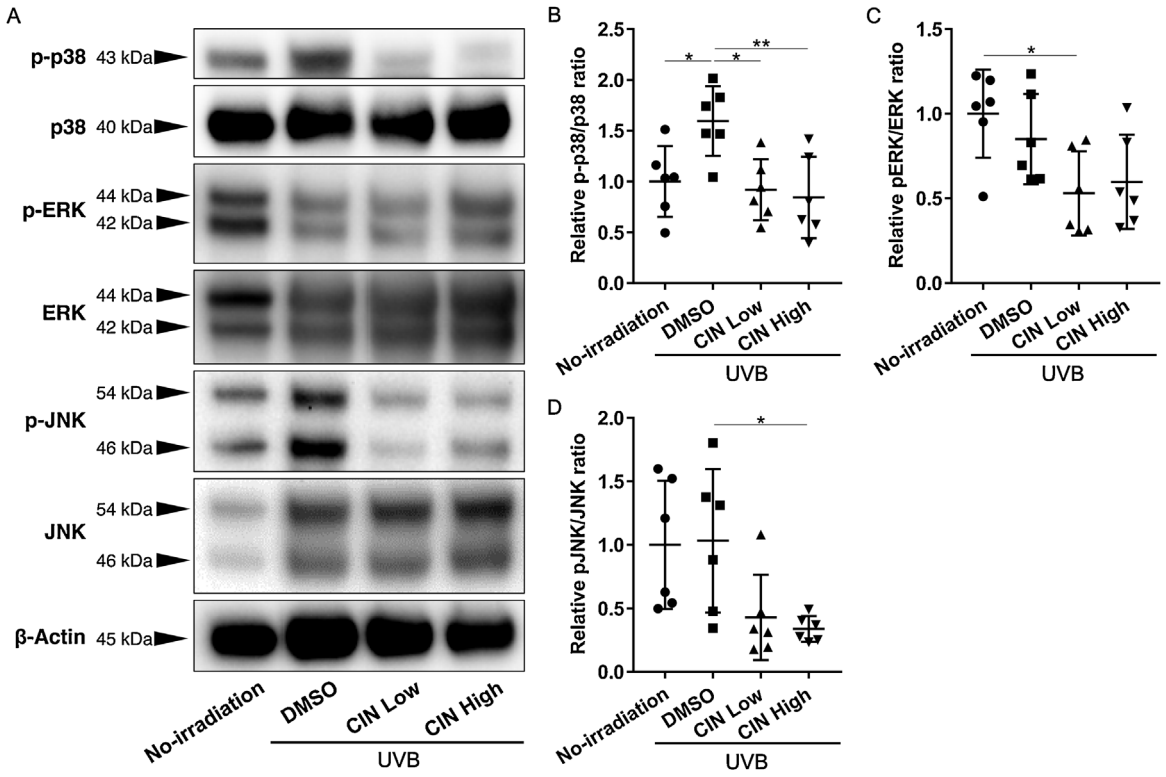

Fig. 6. CIN inhibits the UVB-induced MAPK p38 activation *in vivo*. The phosphorylation status of MAPK signaling molecules; p38, ERK, and JNK in the dorsal skin of experimental mice was assessed by western blot. (A) The representative blots, the value of (B) p-p38/p38 ratio, (C) pERK/ERK ratio, and (D) pJNK/JNK ratio are shown. Data are presented as mean ± standard deviation (n = 6 per group). * $P < 0.05$ and ** $P < 0.01$.

Cinnamomum cassia extract inhibit dioxin-mediated ROS generation [26], and Kampo herbal medicine containing *Cinnamomum cassia* extract was reported to improve dioxin-related symptoms in patients exposed to high concentrations of dioxins [38,39]. In this study, we investigated whether the antioxidative function of CIN can be used to inhibit UVB-mediated damage of the skin.

We first demonstrated that CIN inhibits UVB-induced ROS production, and also accelerates the repair of UVB-induced DNA damage, in the form of 6-4PPs. Other antioxidative phytochemicals, RES and LIG did inhibit the UVB-induced ROS production, however, they were insufficient to accelerate the repair of 6-4PPs. To our knowledge, the DNA photorepair capacity found for CIN has not been documented for other antioxidative phytochemicals. However, in a recent publication by An et al., DNA photorepair capacity was reported in psychrophilic microalga, *Chlamydomonas* sp. ICE-L, isolated from floating ice in the Antarctic [40]. Considering the DNA photorepair capacity, CIN may exert a greater protective function against UVB-induced damage than RES and LIG. Furthermore, UV radiation induces transformation of *trans*-RES into *cis*-isomeric form which has decreased biological activity [41]. LIG is also known as an unstable compound and is degraded under the sunlight radiation through oxidation, hydrolysis, and isomerization [42]. Therefore, it is possible that the antioxidative effects of RES and LIG were attenuated by UV radiation in the condition of current study.

The antioxidative activity of CIN was operative *in vivo*. Topically applied CIN significantly augmented the expression of the antioxidative enzyme *Hmox1 in vivo*. In parallel with the *Hmox1* upregulation, the UVB-induced oxidative stress was significantly inhibited by CIN, as assessed by MDA accumulation. The accumulation of MDA is a useful marker to evaluate tissue oxidation [7,8]. Macroscopically, irradiation with UVB multiple times induced the appreciable formation of wrinkles on the skin of hairless mice and the wrinkle formation was inhibited by the topical application of CIN. Microscopic observation confirmed a significant reduction of UVB-induced epidermal hyperplasia and dermal inflammatory cell infiltration by CIN. These microscopic alterations by UVB exposure are associated with UVB-mediated oxidative stress since they are known to be aggravated in mice deficient in NF-E2-related factor 2 (NRF2), which is a master transcription factor of antioxidative enzymes [19,43,44].

UVB irradiation reduces collagen production and accelerates the degradation of collagen through the induction of MMPs, which eventually causes the destruction of tissue structure and promotes wrinkle formation. The deleterious effects of UVB on collagen metabolism are also the major cause of photoaging [10–12,15,16]. In the present mouse model, we found that UVB irradiation reduced collagen amount/*Col1a1* expression and induced *Mmp13* expression, and topically applied CIN again significantly restored the UVB-induced collagen metabolism. In human skin, MMP1 is upregulated by UVB exposure and is known as a responsible enzyme for collagen degradation. In accordance with this, *MMP1* was significantly upregulated by UVB exposure in human cells (*i.e.* NHDFs and HaCaT cells) *in vitro*, and CIN treatment significantly suppressed the UVB-induced *MMP1* upregulation (Supplementary Fig. S4 and S5). On the other hand, the expression of *Mmp1a* was not elevated by UVB exposure in mouse skin *in vivo* (Supplementary Fig. S3). This inconsistency of MMP1 expression in human and in rodents had been recognized by previous reports and it is suggested that MMP1 is not responsible for UVB-induced collagenolysis in rodents and it may be functionally substituted by other enzymes with collagenolytic activity, such as MMP13 [15,45]. In concurrence with this, our results showed that *Mmp13* was upregulated in response to UVB exposure in mouse skin. Thus, we assume that MMP13, but not MMP1 serves as a suitable marker for investigating UVB-mediated photodamage *in vivo*.

Oxidative stress is related to the *MMP* upregulation by UVB since it is downregulated by the antioxidative chemical NAC [46,47]. UV-induced oxidative stress activates the MAPK signaling pathway, which further regulates the expression of MMPs [17,18,48]. When we assessed the effect of CIN on MAPK signaling in mouse skin, phosphorylation of ERK and JNK were suppressed by CIN and the UVB-induced p38 MAPK phosphorylation was significantly inhibited, suggesting that CIN downregulates MMP expression through the suppression of MAPK pathway, especially p38 MAPK. Previous studies indicated that transforming growth factor β (TGFβ) and its receptor play crucial roles in *COL1A1* regulation [12,49,50]. How ROS directly contribute to *COL1A1* downregulation remains unclear, however, UVB-induced premature senescence might be involved in the *COL1A1* regulation [50]. Our previous study demonstrated that CIN is a potent activator of the NRF2 signal pathway [26]. The CIN-NRF2-antisenescence pathway might thus be an interesting topic for future research in relation to *COL1A1* regulation. In conclusion, this study shows that CIN is a salubrious phytochemical that inhibits UVB-induced ROS production and accelerates the repair of DNA damage 6-4PPs. When applied topically, CIN restores UVB-mediated macroscopic, microscopic, and biological photodamage.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Appendix A. Supplementary data

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References

[1] G.J. Fisher, S. Kang, J. Varani, Z. Bata-Csorgo, Y. Wan, S. Datta, J.J. Voorhees, Mechanisms of photoaging and chronological skin aging, Arch. Dermatol. 138 (11) (2002) 1462–1470.
[2] M. Perluigi, F. Di Domenico, C. Blarzino, C. Foppoli, C. Cini, C. Grillo, F. De Marco, D.A. Butterfield, M.E. Schinina, R. Coccia, Effects of UVB-induced oxidative stress on protein expression and specific protein oxidation in normal human epithelial keratinocytes: a proteomic approach, Proteome Sci. 8 (2010) 13.
[3] G.M. Halliday, Inflammation, gene mutation and photoimmunosuppression in response to UVR-induced oxidative damage contributes to photocarcinogenesis, Mutat. Res. 571 (1-2) (2005) 107–120.
[4] K. Takei, A. Hashimoto-Hachiya, M. Takahara, G. Tsuji, T. Nakahara, M. Furue, Cynaropicrin attenuates UVB-induced oxidative stress via the AhR-Nrf2-Nqo1 pathway, Toxicol. Lett. 234 (2) (2015) 74–80.
[5] D. Yamada, H.M. Dokainish, T. Iwata, J. Yamamoto, T. Ishikawa, T. Todo, S. Iwai, E.D. Getzoff, A. Kitao, H. Kandori, Functional conversion of CPD and (6-4) photolyases by mutation, Biochemistry. 55 (30) (2016) 4173–4183.
[6] G. Yang, Y. Ibuki, α,β-Unsaturated aldehyde-induced delays in nucleotide excision repair and the contribution of reactive oxygen species, Chem. Res. Toxicol. 31 (2) (2018) 145–155.
[7] B.Y. Chen, D.P. Lin, C.Y. Wu, M.C. Teng, C.Y. Sun, Y.T. Tsai, K.C. Su, S.R. Wang, H.H. Chang, Dietary zerumbone prevents mouse cornea from UVB-induced photokeratitis through inhibition of NF-κB, iNOS, and TNF-α expression and reduction of MDA accumulation, Mol. Vis. 17 (2011) 854–863.
[8] A. Gęgotek, P. Rybałtowska-Kawałko, E. Skrzydlewska, Rutin as a mediator of lipid metabolism and cellular signaling pathways interactions in fibroblasts altered by UVA and UVB radiation, Oxid. Med. Cell. Longev. 2017 (2017)4721352.
[9] A.R. Im, Y.M. Kim, Y.W. Chin, S. Chae, Protective effects of compounds from Garcinia mangostana L. (mangosteen) against UVB damage in HaCaT cells and hairless mice, Int. J. Mol. Med. 40 (6) (2017) 1941–1949.
[10] J.K. Lee, S.H. Ko, S.K. Ye, M.H. Chung, 8-Oxo-2′-deoxyguanosine ameliorates UVB-induced skin damage in hairless mice by scavenging reactive oxygen species and inhibiting MMP expression, J. Dermatol. Sci. 70 (1) (2013) 49–57.
[11] M. Murai, K. Yamamura, A. Hashimoto-Hachiya, G. Tsuji, M. Furue, C. Mitoma, Tryptophan photo-product FICZ upregulates AHR/MEK/ERK-mediated MMP1 expression: implications in anti-fibrotic phototherapy, J. Dermatol. Sci. 91 (1) (2018) 97–103.
[12] M. Murai, G. Tsuji, A. Hashimoto-Hachiya, Y. Kawakami, M. Furue, C. Mitoma, An endogenous tryptophan photo-product, FICZ, is potentially involved in photo-aging by reducing TGF-β-regulated collagen homeostasis, J. Dermatol. Sci. 89 (1) (2018) 19–26.
[13] T. Quan, T. He, S. Kang, J.J. Voorhees, G.J. Fisher, Solar ultraviolet irradiation reduces collagen in photoaged human skin by blocking transforming growth factor-beta type II receptor/Smad signaling, Am. J. Pathol. 165 (3) (2004) 741–751.
[14] Z. Ujfaludi, A. Tuzesi, H. Majoros, B. Rothler, T. Pankotai, M. Boros, Coordinated activation of a cluster of MMP genes in response to UVB radiation, Sci. Rep. 8 (1) (2018) 2660.
[15] H.S. Cho, M.H. Lee, J.W. Lee, K.O. No, S.K. Park, H.S. Lee, S. Kang, W.G. Cho, H.J. Park, K.W. Oh, J.T. Hong, Anti-wrinkling effects of the mixture of vitamin C, vitamin E, pycnogenol and evening primrose oil, and molecular mechanisms on hairless mouse skin caused by chronic ultraviolet B irradiation, Photodermatol. Photoimmunol. Photomed. 23 (5) (2007) 155–162.
[16] Y. Cheong, C. Kim, M.B. Kim, J.K. Hwang, The anti-photoaging and moisturizing effects of Bouea macrophylla extract in UVB-irradiated hairless mice, Food Sci. Biotechnol. 27 (1) (2018) 147–157.
[17] S.H. Xuan, Y.M. Park, J.H. Ha, Y.J. Jeong, S.N. Park, The effect of dehydroglyasperin C on UVB-mediated MMPs expression in human HaCaT cells, Pharmacol. Rep. 69 (2017) 1224–1231.
[18] K.R. Kwon, M.B. Alam, J.H. Park, T.H. Kim, S.H. Lee, Attenuation of UVB-induced photo-aging by polyphenolic-rich Spatholobus Suberectus stem extract via modulation of MAPK/AP-1/MMPs signaling in human keratinocytes, Nutrients 11 (2019) 1341.
[19] L. Carrera-Quintanar, R.I. López Roa, S. Quintero-Fabián, M.A. Sánchez-Sánchez, B. Vizmanos, D. Ortuño-Sahagún, Phytochemicals that influence gut microbiota as prophylactics and for the treatment of obesity and inflammatory diseases, Mediators Inflamm. 2018 (2018)9734845.
[20] S. Chikara, L.D. Nagaprashantha, J. Singhal, D. Horne, S. Awasthi, S.S. Singhal, Oxidative stress and dietary phytochemicals: role in cancer chemoprevention and treatment, Cancer Lett. 413 (2018) 122–134.
[21] M. Furue, H. Uchi, C. Mitoma, A. Hashimoto-Hachiya, T. Chiba, T. Ito, T. Nakahara, G. Tsuji, Antioxidants for healthy skin: the emerging role of aryl hydrocarbon receptors and nuclear factor-erythroid 2-related factor-2, Nutrients 9 (3) (2017) pii: E223.
[22] M. Furue, A. Hashimoto-Hachiya, G. Tsuji, Antioxidative Phytochemicals accelerate epidermal terminal differentiation via the AHR-OVOL1 pathway: implications for atopic dermatitis, Acta Derm. Venereol. 98 (10) (2018) 918–923.
[23] J.S. Muhammad, S.F. Zaidi, S. Shaharyar, A. Refaat, K. usmanghani, I. Saiki, T. Sugiyama, Anti-inflammatory effect of cinnamaldehyde in Helicobacter pylori induced gastric inflammation, Biol. Pharm. Bull. 38 (1) (2015) 109–115.
[24] J. Zhao, X. Zhang, L. Dong, Y. Wen, X. Zheng, C. Zhang, R. Chen, Y. Zhang, Y. Li, T. He, X. Zhu, L. Li, Cinnamaldehyde inhibits inflammation and brain damage in a mouse model of permanent cerebral ischaemia, Br. J. Pharmacol. 172 (20) (2015) 5009–5023.
[25] G. Singh, S. Maurya, M.P. DeLampasona, C.A. Catalan, A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins, and their constituents, Food Chem. Toxicol. 45 (9) (2007) 1650–1661.
[26] H. Uchi, M. Yasumatsu, S. Morino-Koga, C. Mitoma, M. Furue, Inhibition of aryl hydrocarbon receptor signaling and induction of NRF2-mediated antioxidant activity by cinnamaldehyde in human keratinocytes, J. Dermatol. Sci. 85 (1) (2017) 36–43.
[27] Y. Tanaka, H. Uchi, A. Hashimoto-Hachiya, M. Furue, Tryptophan photoproduct FICZ upregulates IL1A, IL1B, and IL6 expression via oxidative stress in keratinocytes, Oxid. Med. Cell. Longev. 2018 (2018)9298052.
[28] B. Bai, Y. Liu, Y. You, Y. Li, L. Ma, Intrapertineally administered biliverdin protects against UVB-induced skin photo-damage in hairless mice, J. Photochem. Photobiol. 144 (2015) 35–41.
[29] T.R. Kwon, C.T. Oh, E.J. Choi, S.R. Kim, Y.J. Jang, E.J. Ko, K.H. Yoo, B.J. Kim, Conditioned medium from human bone marrow-derived mesenchymal stem cells protects skin moisturization and effacement of wrinkles in UVB-irradiated SKH-1 hairless mice, Photodermatol. Photoimmunol. Photomed. 32 (3) (2016) 120–128.
[30] L. Subedi, T.H. Lee, H.M. Wahedi, S.H. Baek, S.Y. Kim, Resveratrol-enriched rice attenuates UVB-ROS-induced skin aging via downregulation of inflammatory cascades, Oxid. Med. Cell. Longev. 2018 (2018)6052623.
[31] G. Tsuji, M. Takahara, H. Uchi, T. Matsuda, T. Chiba, S. Takeuchi, F. Yasukawa, Y. Moroi, M. Furue, Identification of ketoconazole as an AhR-Nrf2 activator in

cultured human keratinocytes: the basis of its anti-inflammatory effect, J. Invest. Dermatol. 132 (1) (2012) 59–68.
[32] Z. Wu, H. Uchi, S. Morino-Koga, W. Shi, M. Furue, Z-ligustilide ameliorated ultraviolet B-induced oxidative stress and inflammatory cytokine production in human keratinocytes through upregulation of Nrf2/HO-1 and suppression of NF-κB pathway, Exp. Dermatol. 24 (9) (2015) 703–708.
[33] H.L. Lo, S. Nakajima, L. Ma, B. Walter, A. Yasui, D.W. Ethell, L.B. Owen, Differential biologic effects of CPD and 6-4PP UV-induced DNA damage on the induction of apoptosis and cell-cycle arrest, BMC Cancer 5 (2005) 135.
[34] J.D. Mallet, M.M. Dorr, M.C. Drigeard Desgarnier, N. Bastien, S.P. Gendron, P.J. Rochette, Faster DNA repair of ultraviolet-induced cyclobutane pyrimidine dimers and lower sensitivity to apoptosis in human corneal epithelial cells than in epidermal keratinocytes, PLoS One 11 (9) (2016)e0162212.
[35] M. Balbín, A. Fueyo, V. Knäuper, J.M. López, J. Alvarez, L.M. Sánchez, V. Quesada, J. Bordallo, G. Murphy, C. López-Otín, Identification and enzymatic characterization of two diverging murine counterparts of human interstitial collagenase (MMP-1) expressed at sites of embryo implantation, J. Biol. Chem. 276 (2001) 10253–10262.
[36] C.J. Foley, A. Kuliopulos, Mouse matrix metalloprotease-1a (Mmp1a) gives new insight into MMP function, J. Cell. Physiol. 229 (12) (2014) 1875–1880.
[37] J.E. Oh, M.S. Kim, W.K. Jeon, Y.K. Seo, B.C. Kim, J.H. Hahn, C.S. Park, A nuclear factor kappa B-derived inhibitor tripeptide inhibits UVB-induced photoaging process, J. Dermatol. Sci. 76 (3) (2014) 196–205.
[38] C. Mitoma, H. Uchi, K. Tsukimori, T. Todaka, J. Kajiwara, T. Shimose, M. Akahane, T. Imamura, M. Furue, Current state of yusho and prospects for therapeutic strategies, Environ. Sci. Pollut. Res. Int. 25 (17) (2018) 16472–16480.
[39] M. Furue, Y. Fuyuno, C. Mitoma, H. Uchi, G. Tsuji, Therapeutic agents with AHR inhibiting and NRF2 activating activity for managing chloracne, Antioxidants Basel (Basel) 7 (7) (2018) pii:E90.
[40] M. An, Z. Zheng, C. Qu, X. Wang, H. Chen, C. Shi, J. Miao, The first (6-4) photolyase with DNA damage repair activity from the Antarctic microalga Chlamydomonas sp, ICE-L, Mutat. Res. 809 (2018) 13–19.
[41] C.B. Detoni, G.D. Souto, A.L. da Silva, A.R. Pohlmann, S.S. Guterres, Photostability and skin penetration of different E-resveratrol-loaded supramolecular structures, Photochem. Photobiol. 88 (4) (2012) 913–921.
[42] A.H. Zuo, M.C. Cheng, R.J. Zhuo, L. Wang, H.B. Xiao, Structure elucidation of degradation products of z-ligustilide by UPLC-QTOF-MS and NMR spectroscopy, Acta Pharm. Sin. 48 (6) (2013) 911–916.
[43] A. Hirota, Y. Kawachi, M. Yamamoto, T. Koga, K. Hamada, F. Otsuka, Acceleration of UVB-induced photoageing in nrf2 gene-deficient mice, Exp. Dermatol. 20 (8) (2011) 664–668.
[44] C.L. Saw, M.T. Huang, Y. Liu, T.O. Khor, A.H. Conney, A.N. Kong, Impact of Nrf2 on UVB-induced skin inflammation/photoprotection and photoprotective effect of sulforaphane, Mol. Carcinog. 50 (6) (2011) 479–486.
[45] M. Balbin, A. Fueyo, V. Knäuper, J.M. Lopez, J. Alvarez, L.M. Sanchez, V. Quesada, J. Bordallo, G. Murphy, C. Lopez-Otin, Identification and enzymatic characterization of two diverging murine counterparts of human interstitial collagenase (MMP-1) expressed at sites of embryo implantation, J. Biol. Chem. 276 (13) (2001) 10253–10262.
[46] L. Lei, Q. Zeng, J. Lu, S. Ding, F. Xia, J. Kang, L. Tan, L. Gao, L. Kang, K. Cao, J. Zhou, R. Xiao, J. Chen, J. Huang, MALAT1 participates in ultraviolet B-induced photoaging via regulation of the ERK/MAPK signaling pathway, Mol. Med. Rep. 15 (6) (2017) 3977–3982.
[47] I. Santhakumaran, S.K. Shanuja, R. Narayanaswamy, A. Gnanamani, Asperyellone prevents HDF cells from UVB irradiation damages: an elaborated study, J. Cell. Biochem. (2018) Epub ahead of print.
[48] H.S. Han, J.S. Shin, D.B. Myung, H.S. Ahn, S.H. Lee, H.J. Kim, K.T. Lee, *Hydrangea serrata* (thumb.) ser. Extract attenuate UVB-induced photoaging through MAPK/AP-1 inactivation in human skin fibroblasts and hairless mice, Nutrients 11 (2019) 533.
[49] C.L. Chen, S.F. Liou, S.J. Chen, M.F. Shih, Protective effects of Chlorella-derived peptide on UVB-induced production of MMP-1 and degradation of procollagen genes in human skin fibroblasts, Regul. Toxicol. Pharmacol. 60 (1) (2011) 112–119.
[50] F. Debacq-Chainiaux, C. Borlon, T. Pascal, V. Royer, F. Eliaers, N. Ninane, G. Carrard, B. Friguet, F. de Longueville, S. Boffe, J. Remacle, O. Toussaint, Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway, J. Cell. Sci. 118 (Pt 4) (2005) 743–758.