Journal of Dermatological Science 94 (2019) 220–228

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Original artcle

Effects of irradiance on UVA-induced skin aging

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ARTICLE INFO

Article history:
Received 7 March 2019
Received in revised form 22 March 2019
Accepted 24 March 2019

Keywords:
UVA
Irradiance
Photoaging

ABSTRACT

Background: Ultraviolet A (UVA) radiation is the most relevant component of solar radiation-induced skin aging. Sunscreens were used to minimize the harmful effects of UV radiation on our skin by reducing UV irradiance. We previously found that at equivalent fluence, UVB radiation at low irradiance (LI) has higher photocarcinogenic potential as compared to its high irradiance (HI) counterpart.
Objectives: To examine the effects of equivalent fluence of UVA radiation administered at different irradiance on photoaging.
Methods: Both the hairless mice (SKH-1) and human dermal fibroblasts were irradiated with high irradiance UVA (HIUVA) or low irradiance UVA (LIUVA; 50% irradiance of HIUVA) at equivalent fluence. Parameters related to skin photoaging were evaluated.
Results: For hairless mice receiving equivalent fluence of UVA radiation, LIUVA treated mice showed prominent skin aging as compared to its HIUVA treated counterpart. In addition, LIUVA radiation induced higher reactive oxygen species (ROS) production and c-Jun N-terminal kinases (JNK) phosphorylation as compared to their HIUVA treated counterparts. Pretreatment with N-acetylcysteine (NAC) abrogate the difference between HI and LIUVA radiation on fibroblasts in terms of intracellular ROS, JNK phosphorylation, MMP-1 expression and type I collagen expression.
Conclusion: UVA radiation administered at LI (a scenario similar to sunscreen use) led to more severe aging process as compared to its HI counterpart. Unexpected negative effect may be imposed on the skin if sunscreen use is accompanied by longer duration spent under the sun.

1. Introduction

Exposure of human skin to terrestrial solar radiation induces a variety of well-known acute and chronic responses. Among the different regions of solar radiation, ultraviolet (UV) radiation has been recognized as the major culprit for causing skin cancers and premature aging [1]. The major solar UV radiation reaching the earth consists of a combination of UVB (290–320 nm) and UVA (320–400 nm) wavelength. Due to the deeper penetration into the skin dermis, UVA radiation is regarded as the predominant factor leading to development of skin photoaging, characterized by formation of wrinkles, loss of skin tone and reduced elasticity [2,3]. Mechanistically, increased synthesis and expression of matrix metalloproteinase- 1 (MMP-1) by dermal fibroblasts after UV radiation due to increased ROS formation are believed to play a critical role in photoaging by enhancing degradation of type-1 and type-3 collagen [4,5]. Therefore, MMP-1 is thought to play a key role in photoaging of the human skin [6].

Sunburn represents an inflammatory erythema on the skin induced by UV radiation. Sunscreens were introduced to protect the skin from UV radiation-induced damages. However, sunscreen use has not been associated with reduced sunburn episodes. Association between sunscreen use and longer sun exposure provided a possible link between sunscreen use and more sunburns [7].

Previously, it has been shown that erythema response from the sun depend on fluence but not irradiance [8,9]. Therefore, development of sunburn indicates equivalent UV fluence has been received by the skin, with or without sunscreen use. As aforementioned, sunscreen use is not associated with reduction

Abbreviations: UV, ultraviolet; UVA, ultraviolet A; ROS, reactive oxygen species; MMP-1, matrix metalloproteinase- 1; HI, high irradiance; LI, low irradiance; HIUVA, high irradiance UVA; LIUVA, low irradiance UVA; HDFs, human dermal fibroblasts; DMEM, Dulbecco's minimal essential medium; NAC, N-acetylcysteine; FACS, fluorescence-activated cell sorting; MFI, mean fluorescence intensity; SA-β-gal, senescence-associated β-galactosidase; PCR, polymerase chain reaction; pJNK, phosphorylated-JNK.

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https://doi.org/10.1016/j.jdermsci.2019.03.005
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of sunburn episodes. Therefore, it is imperative to determine if sunburn episodes with sunscreen use produce similar damages to the skin as compared to sunburn episodes without sunscreen use. Sunscreens, either chemically or physically, reduce the UVA photons from penetrating the skin. This scenario is similar to reduced irradiance (photon density; mW/cm^2) from a UVA radiation source. Our previous study demonstrated that equivalent UVB fluence (mJ/cm^2) delivered at different irradiance has different impacts on UVB-induced photocarcinogenesis of the skin [10]. In this study, we use a neutral density physical filter to examine the effects of equivalent UVA radiation administered at different irradiance on the skin for clarifying how UVA-induced skin aging relates to sunscreen use when exposed equivalent fluence of UVA radiation.

2. Material and methods

2.1. Establishment of photoaging mouse model

6- to 8-week-old hairless SKH-1 mouse were purchased from Charles River Laboratories (Wilmington, MA). The treatments of hairless mice included: 1) sham radiation group (control group); 2) high irradiance (HI) UVA radiation group ($8 J/cm^2$, three times a week for 8 weeks); 3) low irradiance (LI) UVA radiation group ($8 J/cm^2$, three times a week for 8 weeks). The number of mouse was at least 3 for each experimental group. For UVA radiation, the CL-1000 L UVA light (UVP, Upland, USA; emission spectrum 320–400 nm with peak wavelength at 365 nm) was used. For LIUVA radiation, a physical filter that reduces 50% irradiance without altering its wavelength spectrum was used [10]. The wavelengths spectrum and the irradiance of the UVA irradiation with or without filter were shown in supplementary file (Fig S1). The animal studies were approved by the ethic committee of Kaohsiung Medical University.

2.2. Wrinkle measurement

Dorsal skin wrinkling following the UVA exposure at week 6 and 8 was graded according to the method described by Bissett et al. [11] as follows: grade 0, no coarse wrinkles; grade 1, a few shallow coarse wrinkles; grade 2, some coarse wrinkles; grade 3, several deep coarse wrinkles.

2.3. Evaluation of skin elasticity

The skin elasticity was measured with Multi Skin Test Center® MC 900 (Courage + Khazaka electronic GmbH, Cologne, Germany) just before the animals were sacrificed, as previously described [12].

2.4. Immunofluorescence microscopy

The skin samples from treated mice were embedded in paraffin wax, and tissue sections of 4-μm thickness were prepared as described previously [10]. The skin sections were incubated with primary antibodies including type I collagen (1:1000 dilution; Invitrogen, CA, USA) and MMP-1 (1:1000 dilution; (Proteintech, Chicago, USA) over night at 4 °C, followed by 1 h-incubation at RT with a Alexa Fluor 488 goat anti-rabbit IgG secondary antibody (1:1000 dilution; Life Technologies, MD, USA). One hundred nanograms per milliliter of DAPI (Invitrogen) were then added and incubated for 10 min at room temperature for nuclear staining. Tissue sections were coverslipped with Vectashield mounting medium and then examined with an Olympus DP70 fluorescence microscope (Olympus Optical. Co., Ltd., Tokyo, Japan).

2.5. Cultured of dermal fibroblasts

Normal human dermal fibroblasts (HDFs) were obtained from healthy adult foreskin. Cell culture for dermal fibroblasts were performed as described elsewhere (see supplementary file) [13]. This study was approved by the Ethics Committee of Kaohsiung Medical University Hospital.

2.6. Treatment of fibroblasts

For UVA radiation, the CL-1000 L UVA light (UVP, Upland, USA) was used. The cultured cells were exposed to UVA radiation at fluences of 0, 1, 3, 5, and $10 J/cm^2$. Immediately after UVA radiation, PBS were removed and fresh media were added to the cells. In addition, a physical filter that reduce the irradiance of UVA radiation by 50% was used. N-acetylcysteine (NAC; 6 mM), a ROS scavenger involving in glutathione regeneration [14], was used to pretreat fibroblasts for 1 h before UVA radiation to testify the effects of ROS on skin aging.

2.7. Cell viability assay

The viability of cultured fibroblasts was determined using an MTS cell proliferation kit (CellTiter 961 AQueous One Solution Cell Proliferation Assay kit, Promega, Madison, WI) as described previously [15]. The detailed protocols were described in the supplementary file.

2.8. Determination of intracellular ROS by flow cytometry

Cultured fibroblast cells (4×10^5 cells) were seeded onto 6-well plate and pre-loaded with 10 mM of 2′,7′-dichlorodihydrofluorescein-diacetate (DCF-DA) solution (Sigma, St. Louis, MO) for 30 min. Immediately after indicated UVA treatments, 10,000 cells were sorted by fluorescence-activated cell sorting (FACS) (FACScan; Becton Dickinson, San Jose, CA, USA) and analyzed by CELLQuest Prosoftware (Becton Dickinson).

2.9. Senescence-associated β-galactosidase (SA-β-gal) staining

SA-β-gal activity was determined at 72 h after indicated UVA radiation by using a cellular senescence assay kit (Biovision, Milpitas, CA, USA) according to the manufacturer's instructions.

2.10. Real-time quantitative polymerase chain reaction (PCR)

Total RNA were extracted from treated fibroblasts using the Trizol method (Gibco) and processed as recommended by the manufacturer. Five microgram of RNA were reverse-transcribed to cDNA as the PCR template. The primers used for real-time PCR were the following: MMP-1, 5′-TGTGGCTCAGTTTGTCCTCACT-3′ (sense) and 5′-CAAATCTGGCGTG-TAATTTTCAAT-3′ (anti-sense); β-actin, 5′-AGTGTGACGTTGACATCCGT-3′ (sense) and 5′-GCAGCT-CAGTAACAGTCCGC-3′ (anti-sense). Amplification and detection were performed with an ABI Prism 7500 sequence detection system (Applied Biosystems, NJ, USA). The data were analyzed by use of the Delta-Delta CT method (Applied Biosystems).

2.11. Western blotting analysis

The total cellular proteins were extracted from indicated UVA radiation fibroblasts with lysis buffer (1.5% SDS, 0.0625 M Tris–HCl, and 1 mM Na3VO4, pH 6.8) containing protease inhibitor cocktail (Roche, Mannheim, Germany). For Western blot analysis, 50–70 μg of extracted proteins were subjected to 10% SDS-PAGE and electroblotted onto a piece of nitrocellulose membrane for 1 h

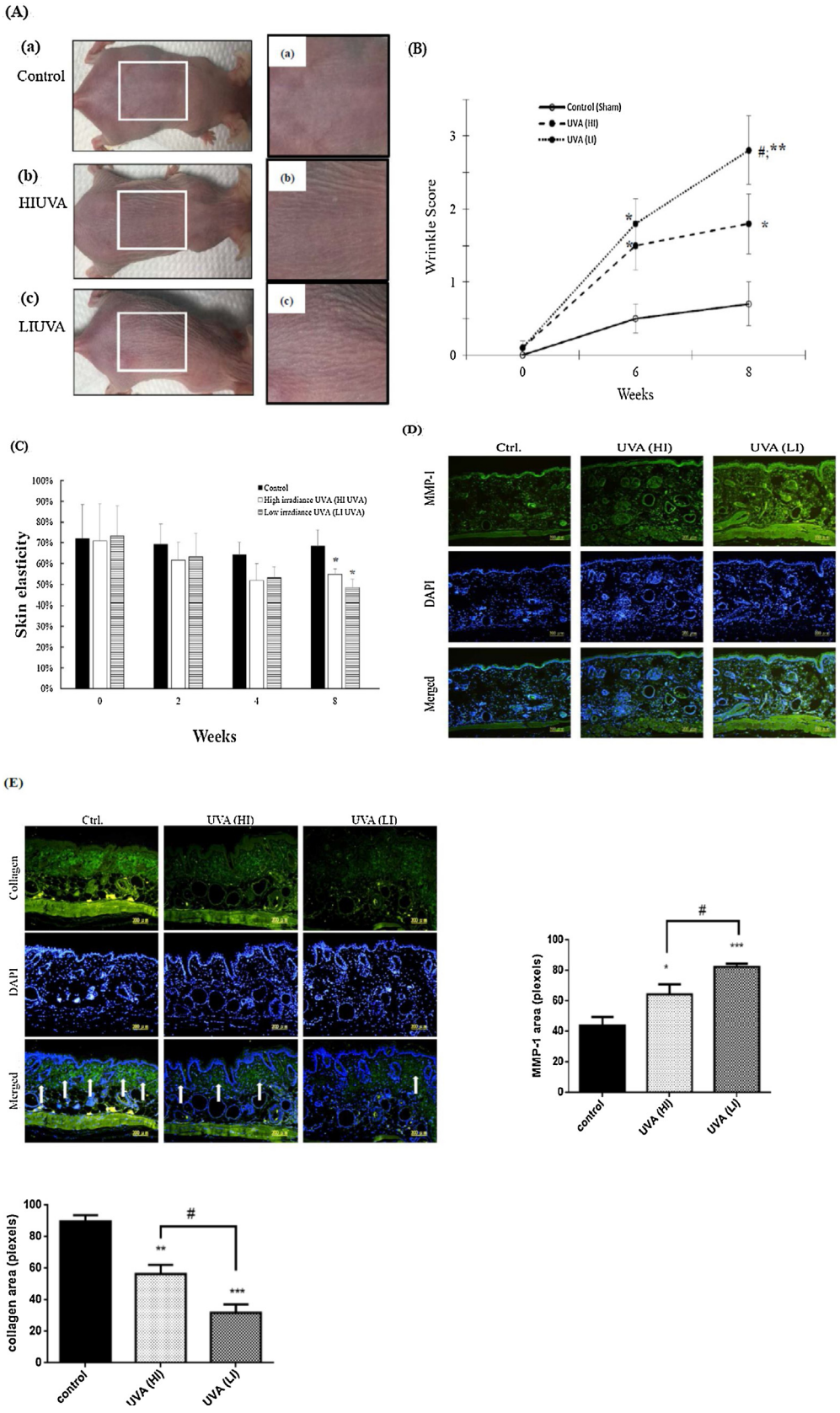

Fig. 1. Effects of irradiance on UVA-induced skin aging. The hairless mice were treated with high or low UVA irradiance at equivalent surface exposure (8 J/cm^2) three times per week for 8 weeks. (A) Photograph of wrinkle formation on the dorsal skin of hairless mice at week 8 after indicated UVA treatment. (B) Different irradiance with equivalent UVA fluence radiation on wrinkle formation was evaluated by wrinkle score. (C) The skin elasticity was measured by Multi Skin Test Center® MC 900 at week 0, 2, 4, and 8 after indicated UVA treatment. The expressions of MMP-1 (D) in mouse epidermis and dermis and type I collagen (E) in mouse dermis (as shown by white arrow) were evaluated by immunofluorescence staining after 8 weeks of UVA radiation. The intensity of fluorescence was measured by Image J software. The data showed one typical experiment from 5 animals. Scale bar =200 μm. *Indicates $P < 0.05$ as compared to control group; **Indicates $P < 0.01$ as compared to control group; ***Indicates $P < 0.001$ as compared to control group; #Indicates $P < 0.05$ as compared to HIUVA treated group.

using a Mini Trans-Blot Transfer membrane (Bio-Rad) at 90 V. After blocking and washing, the nitrocellulose membrane was incubated with anti-pro-COL1A1 (1:400 dilution; Santa Cruz Biotechnology, CA, USA), anti-MMP-1 (1:750 dilution; Millipore), anti-JNK (1:750 dilution; Cell Signaling), anti-p-JNK (1:750 dilution; BD Biosciences), and anti-GAPDH antibody (Sigma), followed by incubation with a 1:10,000 dilution of horseradish peroxidase-labeled secondary antibody (Jackson ImmunoReasearch Laboratories, PA, USA). The membranes were detected by ChemiDoc™ XRS (Bio-Rad Laboratories Inc., Hercules, CA).

2.12. Statistical analysis

All the experiments were repeated at least three independent experiments. The results were expressed as mean ± SD. The significance of the differences between control and experimental groups was evaluated using *Student's t-test*. For all the tests, a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Hairless mice exposing to LIUVA radiation are more susceptible to wrinkle formation as compared to their HIUVA treated counterpart when equivalent fluence was delivered

The results shown in Fig. 1B demonstrated that 6 weeks after both HIUVA and LIUVA radiation, visible signs of wrinkling on the dorsal skin of hairless mice were noticed as compared to the control group. At the end of 8 weeks, hairless mice receiving LIUVA demonstrated significantly more wrinkle formation when compared to their HIUVA counterpart (Fig. 1A and B). These results suggested that at equivalent fluence, LIUVA induced more prominent skin wrinkling when compared to its HIUVA counterpart.

3.2. LIUVA treated mice skin showed a higher decrease in skin elasticity as compared to their HIUVA treated counterpart

In addition to wrinkle formation, loss of skin elasticity is another important clinical character in photoaging. In our study, the measurements of the skin elasticity were made using a Cutometer® after 2, 4, and 8 weeks of UVA radiation. The results shown in Fig. 1C demonstrated that there was no significant difference in skin elasticity between control and experimental groups at 2 and 4 weeks after treatment. However, both HIUVA and LIUVA radiation significantly decreased skin elasticity when compared to control group at 8 weeks after UVA radiation. Furthermore, it was noticed that LIUVA radiation induced higher reduction in skin elasticity at 8 weeks after UVA radiation as compared to their HIUVA counterpart (although not reaching statistical significance).

3.3. After exposure to equivalent fluence of UVA radiation, the dermis of the LIUVA treated mice skin demonstrated significantly higher expression of MMP-1 and lower expression of type I collagen as compared to their HIUVA treated counterpart

The results shown in Fig. 1D and E indicated that an increased in MMP-1 and a decreased in type I collagen expressions were noticed in the dermis, respectively, in both HIUVA and LIUVA treated mice when compared to control mice. Additionally, it was interesting to find that the dermis of mice skin receiving LIUVA

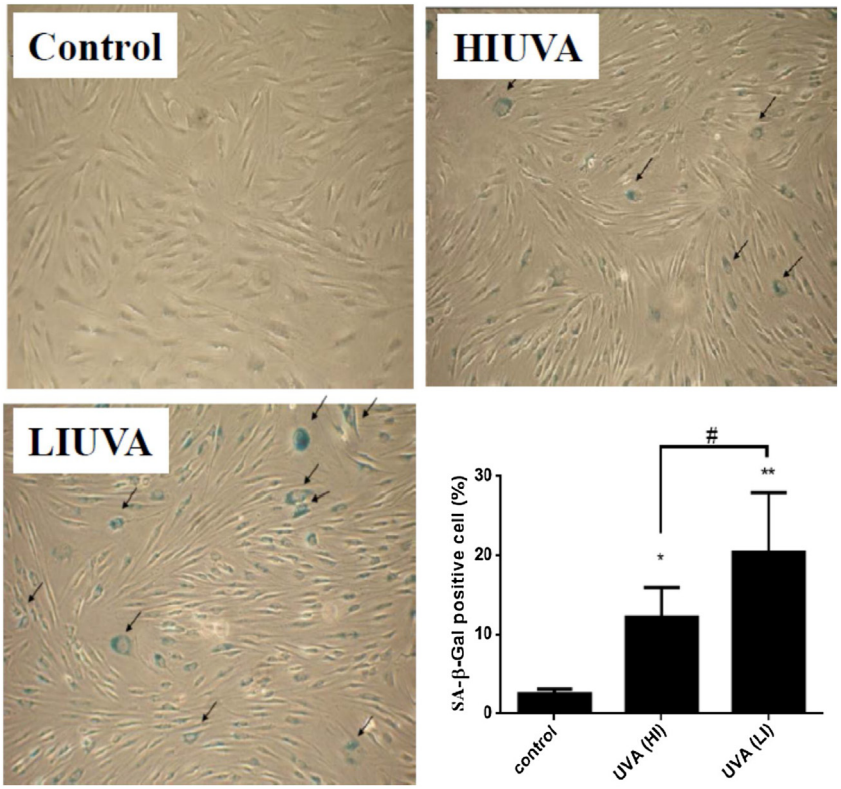

Fig. 2. Appearance of senescence-associated β-galactosidase after indicated UVA radiation in cultured fibroblasts. Cells in culture were observed under phase-contrast microscopy. The blue color staining cells indicated by black arrows represented the positive cells. Six randomly selected views were counted and the results were expressed as the percentage of senescence-associated β-galactosidase-positive cells. The quantitative analyses of results was shown in the lower-right figure. *Indicates $P < 0.05$ as compared to control group; **Indicates $P < 0.01$ as compared to control group; #Indicates $P < 0.05$ as compared to HIUVA treated group.

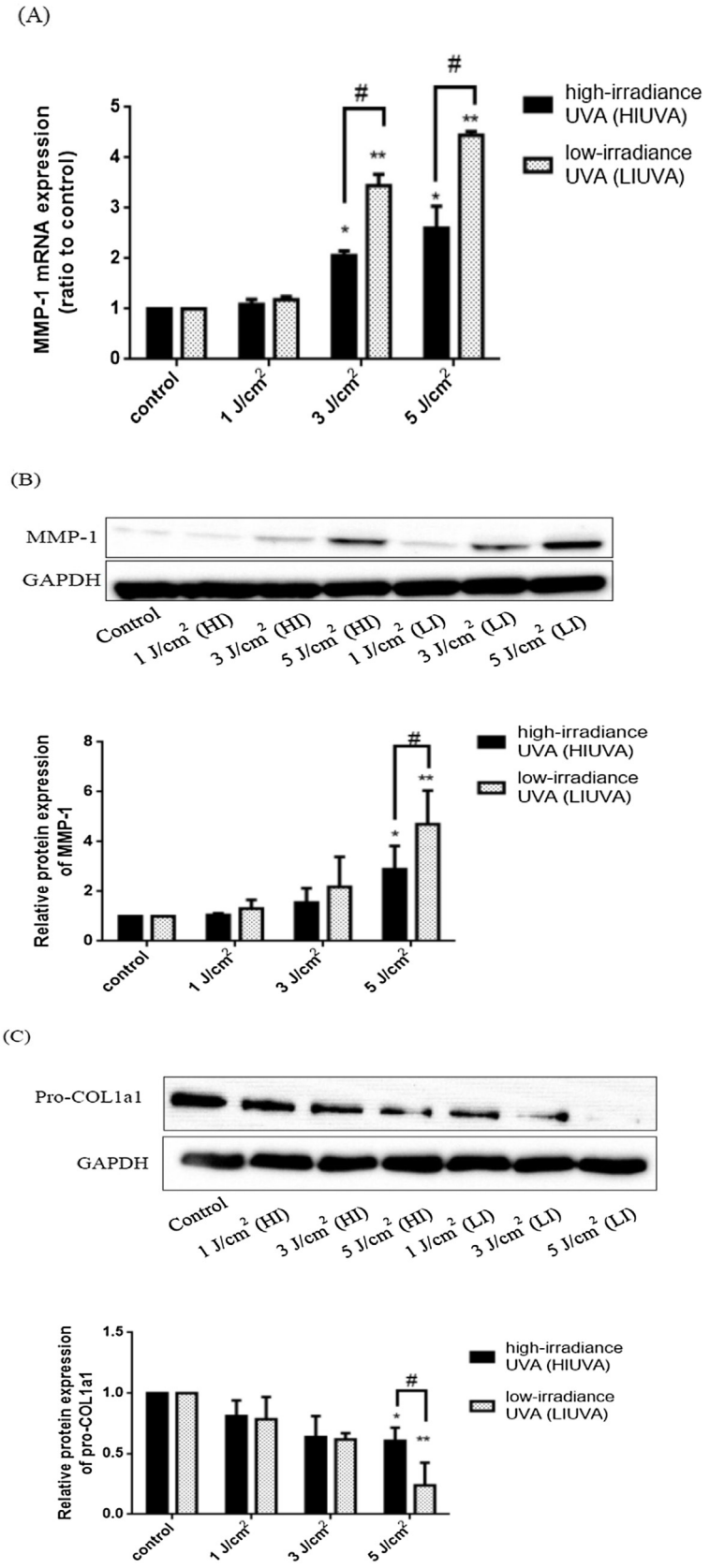

Fig. 3. The expressions of MMP-1 and type I collagen after indicated UVA treatment in cultured fibroblasts. (A) The MMP-1 mRNA expression in cultured fibroblasts 18 h after indicated UVA radiation. (B) The MMP-1 expression in cultured fibroblasts 24 h after indicated UVA radiation. (C) The expression of type I collagen in cultured fibroblasts 48 h after indicated UVA radiation. *Indicates $P < 0.05$ as compared to control group; **Indicates $P < 0.01$ as compared to control group; #Indicates $P < 0.05$ as compared to HIUVA treated group.

radiation demonstrated significantly higher MMP-1 and a lower type I collagen expressions as compared to their HIUVA treated counterparts. These results suggested that at equivalent fluence, LIUVA treatment is more likely to facilitate photoaging process.

3.4. UVA radiation higher than 10 J/cm^2 significantly decreased the viability of cultured dermal fibroblasts

In our experimental conditions, both HIUVA radiation and LIUVA radiation reduced fibroblast viability at all given fluences in a dose-dependent manner. More specifically, the viability of 1, 3, 5, 10 J/cm^2 HIUVA-irradiated fibroblasts after 24 h were 100.8 ± 1.3%, 90.3 ± 6.3%, 71.7 ± 1.7%, and 14.3 ± 3.2%, respectively, as compared to sham irradiated group. On the other hand, the viability of 1, 3, 5, 10 J/cm^2 LIUVA-irradiated fibroblasts after 24 h were 94.9 ± 5.4%, 93.3 ± 5.9%, 70.7 ± 3.7%, and 3.7 ± 1.3%, respectively, as compared to sham irradiated group. Since the viability of the cultured fibroblasts was reduced more than 50% after UVA 10 J/cm^2 radiation, UVA radiation fluence higher than 5 J/cm^2 was not selected for the subsequent experiments. In addition, it should be noted that after equivalent fluence exposure, no significant difference in cell viability was found between the HIUVA and LIUVA treated groups up to 5 J/cm^2.

3.5. LIUVA radiation induced higher SA-β-gal staining in cultured fibroblasts as compared to their HIUVA treated counterpart

SA-β-gal staining was performed to determine SA-β-gal activity, a recognized biomarker of senescence. Our study revealed prominent perinuclear SA-β-gal staining in both HI and LIUVA-treated fibroblasts when compared to control group (Fig. 2). It was observed that more cells were positively stained in the LIUVA treated group as compared to its HIUVA treated counterpart. As shown in the lower right corner of Fig. 2, the proportion of positive cells treated with LIUVA was markedly higher than their HIUVA counterpart after exposed to equivalent fluence.

3.6. LIUVA induced significantly higher MMP-1 and lower collagen expressions in cultured fibroblasts as compared to their HIUVA treated counterpart

Our present results shown in Fig. 3A demonstrated that regardless of irradiance, UVA radiation at 3 J/cm^2 and 5 J/cm^2 significantly enhanced MMP-1 mRNA expressions as compared to control group. Furthermore, the expressions of MMP-1 mRNA in fibroblasts exposed to LIUVA 3 J/cm^2 and 5 J/cm^2 were higher than their HIUVA counterparts by approximately 1.67-fold and 1.73-fold, respectively (Fig. 3A). The findings from Western blot analysis revealed that LIUVA radiation at 5 J/cm^2 markedly increased MMP-1 protein expression by approximately 1.67-fold when compared to their HIUVA counterpart (Fig. 3B). Since MMP-1 upregulation was associated with degradation of collagen, we further examined the protein expression of collagen after UVA radiation. The result shown in Fig. 3C revealed that LIUVA radiation at 5 J/cm^2 significantly decreased type I collagen expression by approximately 2.55-fold when compared to its HIUVA counterpart.

3.7. LIUVA induced significantly higher JNK phosphorylation in cultured fibroblasts as compared to its HIUVA Counterparts

The activation of the MAPK pathway is important for the production of MMPs in UV-mediated skin aging [16]. More specifically, it has been shown that UV induced MMP-1 upregulation in human dermal fibroblasts involves JNK-related pathway [17]. Therefore, we were interested in investigating the expression of JNK signaling in our experimental conditions. As shown in Fig. 4A and B, both HIUVA and LIUVA radiation significantly increased phosphorylated-JNK (pJNK) expression, peaking at 15 min after radiation. Moreover, at equivalent fluence, the increase in pJNK expression was significantly higher in LIUVA treated dermal fibroblasts as compared to their HIUVA treated counterpart while no significant difference was found between the total JNK expression.

3.8. LIUVA radiation induced significantly higher intracellular ROS production in cultured fibroblasts as compared to their HIUVA treated counterpart

UVA-induced production of ROS is considered to be the principle cause of photoaging [18]. To address whether equivalent fluence of UVA radiation delivered at different irradiance has significant impact on this important factor, we measured the intracellular ROS levels after HIUVA or LIUVA treatments. As presented in Fig. 5A, both HIUVA and LIUVA radiation at 3 J/cm^2 and 5 J/cm^2 stimulated intracellular ROS production as compared to the control group. Additionally, it is noted that LIUVA radiation at 3 J/cm^2 and 5 J/cm^2 increased the intracellular ROS production by approximately 1.28-fold and 1.74-fold, respectively, compared with their HIUVA treated counterparts (Fig. 5B).

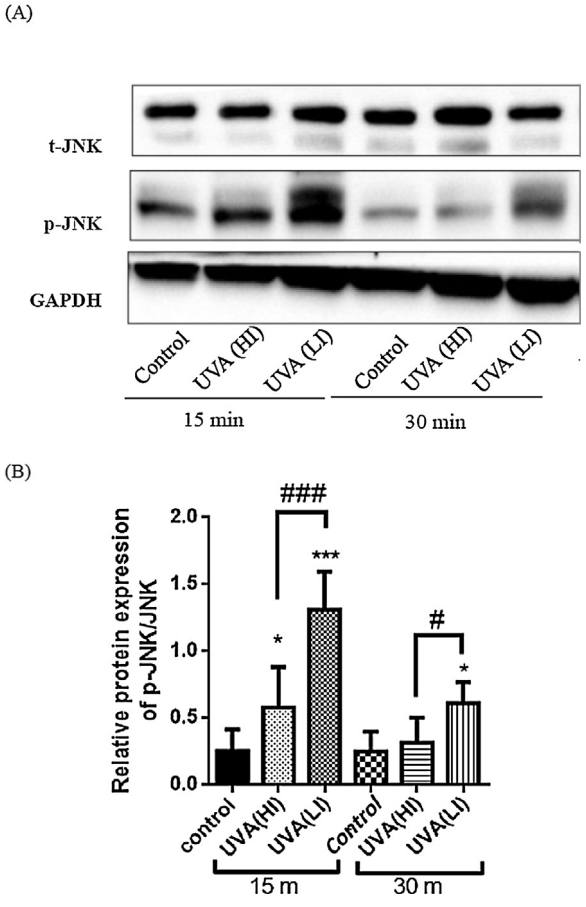

Fig. 4. The expression of JNK pathway after indicated UVA treatment. (A) Cultured fibroblasts were treated with high irradiance (HI) and low irradiance (LI) UVA (5 J/cm^2) radiation. The protein extracts were collected at 15 and 30 min after indicated treatment and analyzed with western blotting using antibodies against phosphorylated JNK (p-JNK), total ERK (t-JNK), and GAPDH. (B) Densitometric analyses of (A). *Indicates $P < 0.05$ as compared to control group; ***Indicates $P < 0.001$ as compared to control group; #Indicates $P < 0.05$, ### Indicates $P < 0.001$ as compared to HIUVA treated group.

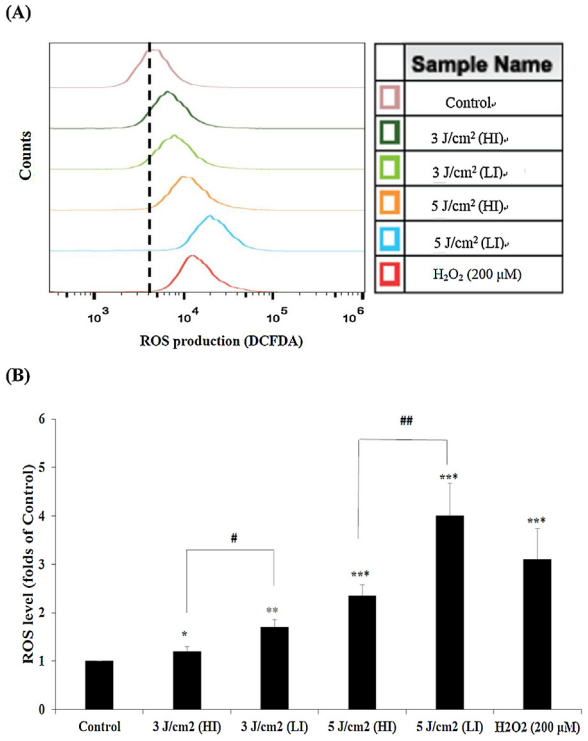

Fig. 5. The levels of intracellular reactive oxygen species (ROS) after indicated UVA radiation. (A) The 2′,7′-dichlorodihydrofluorescein-diacetate fluorescence intensities measured by flow cytometry were demonstrated immediately after indicated treatment conditions. (B) Statistical analysis of the intracellular ROS expression in indicated UVA treated conditions. The cultured fibroblasts treated with H_2O_2 200 μM was used as a positive control. *Indicates $P < 0.05$, **Indicates $P < 0.01$, ***Indicates $P < 0.001$ as compared to control group; #Indicates $P < 0.05$, ##Indicates $P < 0.01$ as compared to HIUVA treated group.

3.9. Antioxidant pretreatment abrogated the difference in MMP-1 and collagen protein expressions induced by UVA exposure at equivalent fluence but different irradiance

Previous studies have demonstrated that upregulation of MMP-1 expression by UVA is dependent on ROS formation [19,20]. Since our present results indicated that after equivalent fluence exposure, LIUVA stimulated higher ROS formation in cultured fibroblasts as compared to their HIUVA counterpart, we next investigated the effects of antioxidant pretreatment on MMP-1 protein expression after UVA radiation at equivalent fluence but different irradiance. The results shown in Fig. 6A and B demonstrated that antioxidant N-acetylcysteine (NAC) pretreatment reduced the LIUVA-induced and HIUVA-induced MMP-1 protein expressions by approximately 2.00-fold and 1.30-fold, respectively. In addition, NAC pretreatment upregulated the reduced type I collagen protein expression after LIUVA and HIUVA exposure by approximately 1.68-fold and 1.27-fold, respectively (Fig. 6A and C). No significant difference was found between LIUVA and HIUVA treated groups after pretreatment with NAC on MMP-1 and type I collagen protein expressions in cultured fibroblasts.

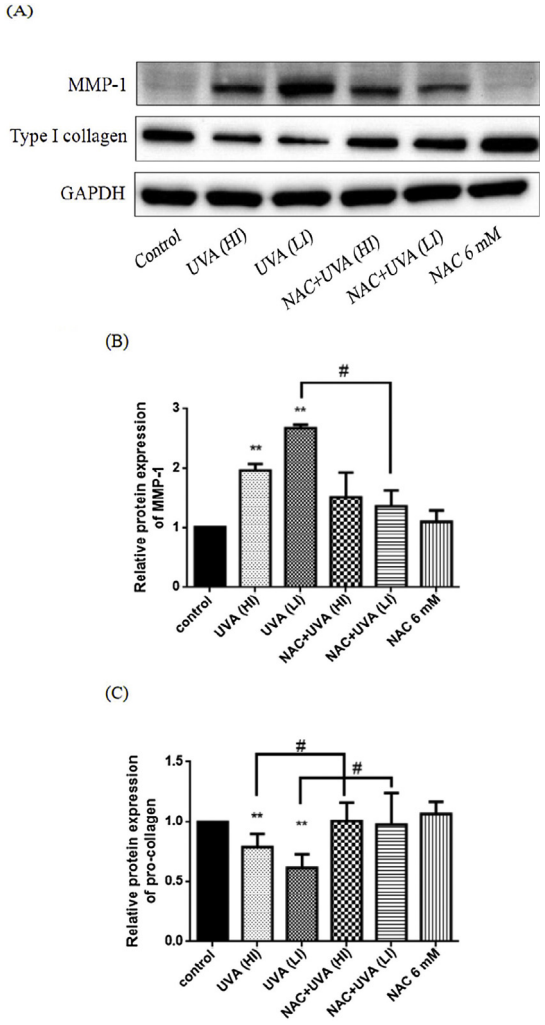

Fig. 6. The expressions of MMP-1 and type I collagen in N-acetylcysteine (NAC)-pretreated cultured fibroblasts after indicated UVA radiation. (A) The cultured fibroblasts were pretreated with NAC for 1 h followed by indicated UVA treatment. The protein extracts were collected at 24 h (for MMP-1) and 48 h (for type I collagen) after treatments and analyzed with western blotting using antibodies against MMP-1, type I collagen, and GAPDH. (B) Densitometric analyses of MMP-1 in.(A). (C) Densitometric analyses of type I collagen in (A). **Indicates $P < 0.01$ as compared to control group; #Indicates $P < 0.05$ as compared to HIUVA or LIUVA treated groups.

4. Discussion

Our previous study demonstrated that equivalent UVB fluence delivered at HI showed a superior efficacy on inducing primitive melanoblast differentiation [21] as compared to its LI counterpart and therefore, provided an explanation for more favorable results in treating vitiligo using a HIUVB emitting device. Recently, we showed that equivalent UVB fluence delivered at HI demonstrated a lower photocarcinogenic potential [10] on skin as compared to their LI counterpart. These findings elicited our interests to examine the effects of UVA radiation delivered at equivalent fluence but different irradiance on skin aging since this scenario mimics the situation when sunscreen was used. Results from our *in vivo* animal study demonstrated that at equivalent fluence, a prominent skin wrinkle formation was noticed after UVA radiation administered at LI as compared to its HI counterpart. This result showed a conflicting concept to the conventional reciprocity law in which one assumes the effect of radiation will be proportional to the total dose delivered ((Dose (mJ/cm^2) = Irradiance (mW/cm^2) × exposure time (seconds)). Indeed, a "reciprocity failure" on UVB-induced skin cancer has been reported by Kelfkens et al. [22] and our study [10].

Accumulating evidences have confirmed that increased expression of MMP-1 and reduced expression of type I collagen by dermal fibroblasts are prominent features of skin photoaging characterized by formation of wrinkles and reduction of skin elasticity [17,23]. In the

present study, we demonstrated that after equivalent exposure fluence, LIUVA radiation induced a significantly higher increase in MMP-1 and a more reduction in type I collagen expressions in mice skin dermis as compared to their HIUVA treated counterpart. Furthermore, we demonstrated that at 8 weeks after UVA radiation, LIUVA treatment showed a trend of higher reduced skin elasticity in mice as compared to their HIUVA treated counterpart. These *in vivo* animal findings suggested that LIUVA radiation is more likely to induce more severe photoaging as compared to its HIUVA counterpart when equivalent fluence is delivered. Based on the scientific evidence *in vivo*, an *in vitro* cultured dermal fibroblasts model was used to clarify the mechanisms involved between different biological effects of HIUVA and LIUVA radiation at equivalent fluence in the context of photoaging.

UVA radiation is known to induce fibroblast senescence [24]. The increase in SA-β-gal activity levels within senescent cells is an indicator of cellular senescence and aging [25]. Our result demonstrated that regardless of irradiance, UVA radiation significantly increased SA-β-gal-positive fibroblasts as compared to control cells. Additionally, we found that after equivalent fluence, LIUVA radiation induced more SA-β-gal-positive fibroblasts as compared to its HIUVA counterpart. This result suggested that when compared to HIUVA, LIUVA radiation may have a higher potential to induce dermal fibroblast into senescent status, further contribute to UVA-induced skin photoaging.

More than induction of cellular senescence, UVA radiation also resulted in reduction of collagen in fibroblasts. This reduction could result from enhanced protein degradation by MMPs and/or from reduced collagen synthesis [26]. In this study, we demonstrated that at equivalent fluence, LIUVA radiation significantly increased MMP-1 and decreased type I collagen expressions in cultured fibroblasts as compared to their HIUVA treated counterpart. These results strongly suggest that LIUVA radiation is more likely to induce photoaging as compared to its HI counterpart when equivalent fluence is delivered. UVA radiation activates several signaling pathways and transcription factors [27–29]. Recently, we and other study have demonstrated that UVA induced MMP-1 upregulation in human dermal fibroblasts involved JNK-related pathway [19,30]. In our present study, it was noticed that LIUVA radiation significantly induced higher pJNK expression, the upstream regulator of MMP-1, as compared to its HIUVA treated counterpart in dermal fibroblasts. Since our previous study demonstrated that pretreating fibroblasts with JNK inhibitor SP600125 significantly reduced the UVA-induced MMP-1 expression [19], it was reasonable to suggest that the increase in pJNK expression in cultured fibroblasts after LIUVA radiation as compared to their HIUVA treated counterpart results in higher MMP-1 expression and subsequently enhanced collagen degradation. It was reported that UVA radiation may act through a mechanism that involves the absorption of photons by endogenous photosensitizers, which subsequently cause photo-oxidation reactions to produce ROS [30–32]. It is recognized that the generation of ROS after UVA radiation is a key step that increase MMP-1 production and subsequently leads to increased collagen breakdown, and eventually results in premature skin aging [4,33]. In this study, we demonstrated that at equivalent fluence, LIUVA radiation induced higher ROS production in cultured fibroblasts as compared to its HIUVA treated counterpart. Furthermore, pretreatment with antioxidant NAC abrogated the differences in MMP-1 and type I collagen expressions induced by equivalent fluence of LIUVA and HIUVA radiation. Taken together, these results showed that at equivalent fluence, LIUVA radiation induce more MMP-1 and less type I collagen expression as compared to its HIUVA counterpart in cultured fibroblasts through a ROS-initiated and JNK-mediated cascade.

It is noted that the mechanisms for inducing higher ROS level after LIUVA radiation as compared to its HIUVA treated counterpart when equivalent fluence was delivered in cultured fibroblasts remain unclear. One possible explanation is that when crossing the threshold for ROS generation, the LIUVA radiation may have resulted in more ROS production in cultured fibroblasts by allowing a longer duration of exposure for the reaction to take place as compared to its HIUVA counterpart. This hypothesis was supported by the results from Iida et al. [9] demonstrating that at equivalent fluence, single LIUVB treatment induced more oxidative stress in the epidermis of hairless mice as compared to their HIUVB treated counterpart. Further experiments are warranted to clarify this issue.

Our findings demonstrated for the first time that if same fluence of UVA (similar to sunburn reaction with development of erythema) is administered, LI will result in higher ROS formation (as in scenario where sunscreen is used), and more severe aging process will result. Therefore, we emphasize the notion that by more frequent reapplying of sunscreen or by addition of antioxidant to the sun-protection strategy will definitely serve to provide protection against UVA-induced photoaging. This information should be made aware to the general public who may have a sense of “false security” when sunscreen is used.

Funding source

None.

Conflict of interest disclosures

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.03.005.

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