Journal of Dermatological Science 93 (2019) 116–122

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Original Article

The impact of irradiance on UVB-induced cutaneous immunosuppression: Implications on administering most efficient phototherapy

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ARTICLE INFO

Article history:
Received 18 September 2018
Received in revised form 26 December 2018
Accepted 8 January 2019

Keywords:
Phototherapy
Irradiance
Immune suppression
aryl hydrocarbon receptor

ABSTRACT

Background: Ultraviolet B (UVB) is commonly used for treating dermatologic conditions. Recently, high irradiance UVB (HIUVB) has been suggested to be more effective for treating skin conditions as compared to its low irradiance (LI) counterpart. The biological impact of UVB radiation emitted at different irradiance on cutaneous immunity remains obscure.
Objective: This study aimed to explore the impacts of UVB radiation administered at equivalent fluence (mJ/cm^2) but different irradiance (mW/cm^2) on cutaneous immune response.
Methods: Cultured bone marrow derived dendritic cell (BMDC) were treated with equivalent fluence of UVB radiation with HIUVB or LIUVB. The phenotypic and functional alterations of BMDCs were documented. Animal models were used to validate the *in vitro* results *in vivo* and explore the mechanisms involved.
Results: After equivalent fluence of UVB radiation, the HIUVB treated BMDC showed significantly lower MHCII and CD86 expressions, reduced capacity to stimulate T cell proliferation, and enhanced activation of aryl hydrocarbon receptor (AhR)-activated genes as compared to control while their LIUVB treated counterpart showed no significant change. Using animal model, the HIUVB induced significantly higher immune suppressive effect in mice as compared to their LIUVB counterpart after equivalent fluence of UVB treatment. The superior immune suppressive effect of HIUVB over LIUVB radiation was not observed when similar experiments were performed using AhR-deficient mice.
Conclusion: We propose irradiance played an important role modulating UVB-induced cutaneous immune suppression. Future works on UVB phototherapy, both clinical and research, should incorporate this important parameter into consideration.

1. Introduction

Phototherapy is commonly used for treating various skin disorders, and currently, UVB light sources, including narrow band UVB (NBUVB) and excimer light, are most frequently used. Although both emitting UVB radiation with wavelengths at close proximity (308 nm for excimer and 311 nm for NBUVB), excimer light sources emit radiation at much higher irradiance (W/cm^2) as compared to NBUVB. In other words, to deliver equivalent fluence (J/cm^2) of UVB radiation, the excimer light needs shorter exposure duration as compared to NBUVB. Many clinical reports have documented the therapeutic differences between excimer and NBUVB for treating different skin conditions including vitiligo (a depigmenting condition involving skin and occasionally the hair) and psoriasis (an

Abbreviations: AD, atopic dermatitis; AhR, aryl hydrocarbon receptor; BMDC, bone marrow derived dendritic cell; C, challenge; CD40, cluster of differentiation 40; CD86, cluster of differentiation 86; CHS, contact hypersensitivity; cyp1a1, cytochrome P450, family 1, subfamily A, member 1; cyp1b1, cytochrome P450, family 1, subfamily B, member 1; DC, dendritic cell; DNFB, 2,4-dinitrofluorobenzene; FCS, fetal calf serum; gapdh, glyceraldehyde-3-phosphate dehydrogenase; HIUVB, high irradiance UVB; LIUVB, low irradiance UVB; LPS, lipopolysaccharide; MHC II, major histocompatibility complex class II; NBUVB, narrow band UVB; PCR, polymerase chain reaction; S+C, sensitize +challenge; Treg, regulatory T cells.

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https://doi.org/10.1016/j.jdermsci.2019.01.003
0923-1811/

inflammatory condition of skin with systemic involvement) [1,2]. For treating stable vitiligo, activation and functional development of melanocyte stem cells are required, while for clearing psoriasis, depletion of skin-infiltrating immune cells is needed. Therefore, biostimulation and immune suppression are important biological events that account for the therapeutic effects of UVB treatment. In the context of biostimulation, using primitive pigment cell development as a model for investigation, we had shown that after equivalent fluence, high irradiance UVB (HIUVB) emitted from excimer light device contributes to more effective vitiligo repigmentation clinically by inducing more efficient pigment cell differentiation via aryl hydrocarbon receptor (AhR)-related pathway as compared to its low irradiance UVB (LIUVB) counterpart [3]. In terms of cutaneous immune suppression, it has been demonstrated that HIUVB suppresses cutaneous immunity more effectively as compare to its LIUVB counterpart after equal fluence exposure using contact hypersensitivity (CHS) animal models, although the mechanism involved remains unexplored [4,5].

Contact hypersensitivity model is frequently used to evaluate UV-induced immune suppression. More specifically, animals showed reduced reaction (swelling) to challenge of the ear if UV radiation was administered prior to sensitization [6]. This immune suppression induced by UV radiation involved generation of regulatory T cells (Tregs). It has been shown that UV-induced DNA damage plays an important role for induction of Tregs, as upon reduction of DNA damage, generation of Tregs is also reduced [7]. More recently, AhR-related signaling has also been demonstrated to play an essential role in UV-induced immunosuppression [8]. The AhR is a ubiquitously expressed ligand-triggered transcription factor mostly responsible for the detoxification of aromatic hydrocarbons [9]. Additionally, AhR signaling participates in a variety of cellular processes including cell growth, differentiation, and inflammation. The AhR has been identified as a molecular target of UVB radiation [10] and modulation of dendritic cells (DC) via AhR cascade was shown to play a pivotal role responsible for Treg-associated immune suppression [11].

Dendritic cells are the main antigen presenting cells that act as sentinels to activate immune response in the skin. Immature DCs are specialized in capturing and processing antigens while mature DCs are critical for the initiation of proper immunity [12]. Previous studies showed that lipopolysaccharide (LPS)-treated DC undergoes maturation, migrates to the draining lymph nodes, and activates T cells. Additionally, DC also modulates the induction Treg cells after UVB radiation [11].

Recently, we reported that UVB radiation at equivalent fluence but emitted through different irradiance has different photocarcinogenic potential on the skin [5]. In that study, we also showed that UVB irradiance has significant impact on UVB-induced immunosuppression. The current study was launched to explore the impacts of UVB irradiance on immune suppression of the skin after same fluence was administered. We hypothesized that at equivalent fluence, HIUVB will activate the AhR cascade in DC more effectively as compared to its LIUVB counterpart, and the enhanced AhR signaling will result in more efficient immunosuppression of the skin.

2. Materials & methods

2.1. Culture of bone-marrow derived dendritic cell (BMDC)

Six- to eight-weeks-old pathogen-free C3H/HeN mice were obtained from the BioLASCO Tawian, Co., Ltd. and maintained at the Animal Center of Kaohsiung Medical University. All animal studies were approved by the Animal Care and Use Committee of the Kaohsiung Medical University. BMDCs were obtained by culturing of bone marrows cells from tibial and femoral bones of C3H/HeN mice. Briefly, erythrocytes were lyzed with ACK buffer and the remaining cells were passed through a mesh to remove small pieces of debris. Cells in suspension were washed with HBSS and cultured in 10% decomplemented fetal calf serum (FCS) RPMI 1640 medium supplemented with recombinant mouse GM-CSF (20 ng/ml) in six-well plates (5×10^5 cells per well) for 5 days. Subsequently, the cultured cells were stimulated with lipopolysaccharide (LPS) (1ug/ml; Sigma-Aldrich) for another 24 h.

2.2. UVB treatment

UVB lamp (MEL@308 nm DEKA, Firenze, Italy) with peak wavelength at 308 nm was used and the irradiance was measured by UVB meter (National Biological Corporation, Twinsburg, OH). During UVB irradiation, the lids of culture dishes or plates were removed and the culture medium was replaced with PBS to avoid the formation of medium-derived toxic photoproducts induced by UV exposure [13]. In addition, neutral density filter that reduces 50% UVB irradiance without altering its wavelength spectrum was used [5]. The wavelengths spectrum and the irradiance of the UVB irradiation with or without filter were shown in supplementary file (Fig S1).

2.3. Cell viability

A commercially available kit (CellTiter 96 aqueous proliferation assay kit, Promega, Madison, WI) was used to determine cell viability according to the manufacturer's instructions.

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

Total RNA was extracted from BMDCs using the Trizol method (Gibco BRL, Gaithersburg, MD, U.S.A.) and processed as recommended by the manufacturer. Five μg of RNA was reverse-transcribed to cDNA as the PCR template. The gene expression level of *cyp1a1*, *cyp1b1* and internal control *gapdh* were determined using Tagman probes by real-time PCR. Amplification and detection were performed with an ABI Prism step-one plus sequence detection system (Applied Biosystems, New Jersey, USA). Results were analyzed using a comparative critical threshold (Ct) method where the amount of target gene was normalized to the amount of endogenous control.

2.5. Phenotypic change of DC: surface markers MHCII, CD 86, CD 40

To validate the phenotypic change of BMDC after LPS stimulation, the markers of BMDCs were determined by the respective fluorochrome-conjugated mAb labels (MHC II-FITC, CD86-PE, CD40-PE, all from BD Pharmingen, San Diego, CA), and the cells were sorted by fluorescence-activated cell sorting (FACS) (FACScan; Becton Dickinson, San Jose,CA, USA) that analyzed 10,000 events by CELLQuest Prosoftware (Becton Dickinson).

2.6. BrdU cell proliferation assay

The T cell proliferation was assessed by 5-bromo-2′-deoxyuridine (BrdU) incorporation assay using a commercially available BrdU-enzyme-linked immunosorbent assay (ELISA) cell proliferation assay kit (Roche, Basel, Switzerland) according to manufacturer's instructions [14].

2.7. Contact hypersensitivity (CHS) model

The shaved back of the mice was irradiated UVB 200 mJ/cm^2 for 4 consecutive days. Twenty-four hours after the last UVB irradiation, the mice were sensitized by painting 50 ul 2,4-

dinitrofluorobenzene (DNFB; 0.5% in acetone/olive oil, 4/1) on their back. Five days later, ear challenge was performed by applying 20 μl of 0.3% DNFB to the right ear and an acetone/ olive oil vehicle was applied to the left ear. Ear swelling was measured with a spring-loaded micrometer (Mitsutoyo, Kawasaki, Kanagawa, Japan) at 24 h after the challenge. CHS was defined as the amount of swelling of the hapten-challenged ear compared with the thickness of the vehicle-treated ear in sensitized animals and expressed as millimeters $\times 10^{-3}$ (mean ± SD). The ear-swelling response was measured in a blinded manner. Mice that received ear challenge without prior sensitization served as negative controls [8,15].

2.8. Transgenic AhR mice experiments

Mice were obtained from Jackson laboratory (AhR-deficient; Bar Harbor, ME) and maintained at the Animal Center of Kaohsiung Medical University. At 3–4 weeks of age, the mice received genotyping by PCR for identification genotype. The CHS model was employed to evaluate UVB-induced cutaneous immunosuppression as aforementioned. The AhR transgenic mice were separated into four treatment groups: 1). Challenge (C) only, 2). sensitize + challenge (S + C), 3). HIUVB followed by S + C, and 4). LIUVB followed by S + C.

2.9. Statistical analyses

For each study, at least three independent experiments were performed. The results were expressed as mean ± SEM. Student *t*-test was used for statistical evaluation between control and experimental groups, and $P < 0.05$ was considered statistically significant.

3. Results

To determine if UVB radiation administered at same fluence but different irradiance imparts significant impact on BMDC, we first determined the viability of BMDC after indicated treatment. At UVB exposure up to 20 mJ/cm^2, the viability of BMDCs was not significantly reduced as compared to the control 48 h after the treatment regardless of the irradiance (Fig. 1). On the other hand, the viability of BMDCs was significantly reduced as compared to the control 24 h after 40 mJ/cm^2 UVB treatment (data not shown). Therefore, 48 h after 20 mJ/cm^2 UVB irradiation was used as the time point to study the functional alterations of BMDC induced by equivalent fluence of UVB radiation emitted at different irradiance.

3.1. After UVB exposure at equivalent fluence, HIUVB treated BMDCs showed significant reduction in their surface co-stimulatory molecule expressions as compared to control

The surface co-stimulatory molecules on DC are known to interact and activate T cells. Previous studies demonstrated that surface co-stimulatory molecules on DC are modulated by UV radiation [16]. Therefore, we first examined the impacts of UVB radiation administered at same fluence but different irradiance on expressions of co-stimulatory molecules on BMDCs. The major histocompatibility complex (MHC) class II, CD86 and CD40 on the DC surface proteins are essential for priming T cell proliferation [12,17]. Twenty-four hours after equivalent 20 mJ/cm^2 UVB exposure, the expression of CD40 was significantly reduced on the surface of BMDCs ($p < 0.05$), regardless of the irradiance. However, it is noted that the MHCII and CD86 expressions were also significantly reduced on the HIUVB treated BMDCs as compared to control ($p < 0.05$; Fig. 2). On the contrary, no significant difference was found between the LIUVB treated BMDC as compared to the control in terms of MHCII and CD86 expressions. These result suggested that at equivalent fluence, HIUVB may suppress DC-mediated immune modulation more effectively as compared to its LIUVB counterpart.

3.2. HIUVB exposed BMDC showed reduced capacity to stimulate T cell proliferation as compared to control

Since DCs are capable of stimulating T cell proliferation [12] and equivalent fluence (20 mJ/cm^2) of UVB exposure induced different phenotypic changes on DC when administered at different irradiance, we next examined if UVB radiation at equivalent fluence but different irradiance induces modifications on BMDCs that alter their capacity to stimulate T cell proliferation. As demonstrated in Fig. 3, lipopolyscharride (LPS) -treated BMDCs significantly upregulated T cell proliferation as compared to BMDC without LPS stimulation. This is expected as matured DCs are more capable of immune stimulatory effect. After equivalent UVB fluence irradiation, the HIUVB treated BMDCs showed significant reduction in their capacity to stimulate T cells ($p < 0.05$) while no significant alteration was found for their LIUVB treated counterpart as compared to control (Fig. 2). These results corroborated with previous finding regarding the impact of HIUVB on surface co-

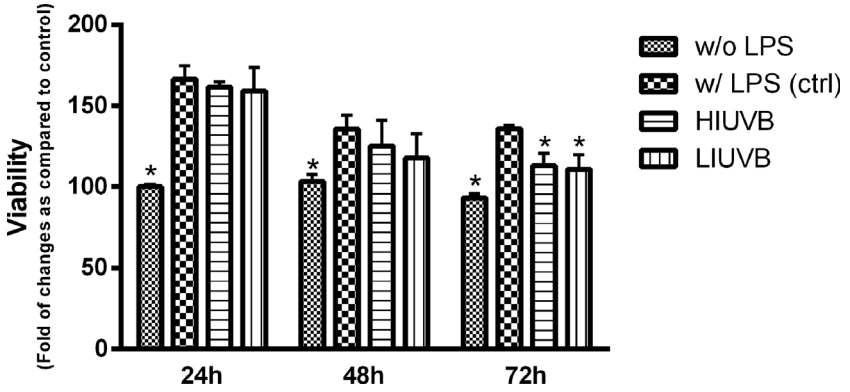

Fig. 1. Cultured bone marrow derived dendritic cells (BMDC) were treated with indicated lipopolysaccharide (LPS; 1ug/ml) and UVB radiation (20 mJ/cm^2). The details of treatment were described in the method section. Briefly, BMDCs were cultured with or without LPS for 24 h and exposed to indicated UVB irradiation. All the UVB treated groups were stimulated with LPS prior to UVB irradiation. At 24 h, 48 h, and 72 h after UVB exposure, the viability of cells was determined. The results were obtained from 8 independent experiments and presented as mean + SEM.
w/LPS: control; * indicates $P <$ 0.05 as compared to control.

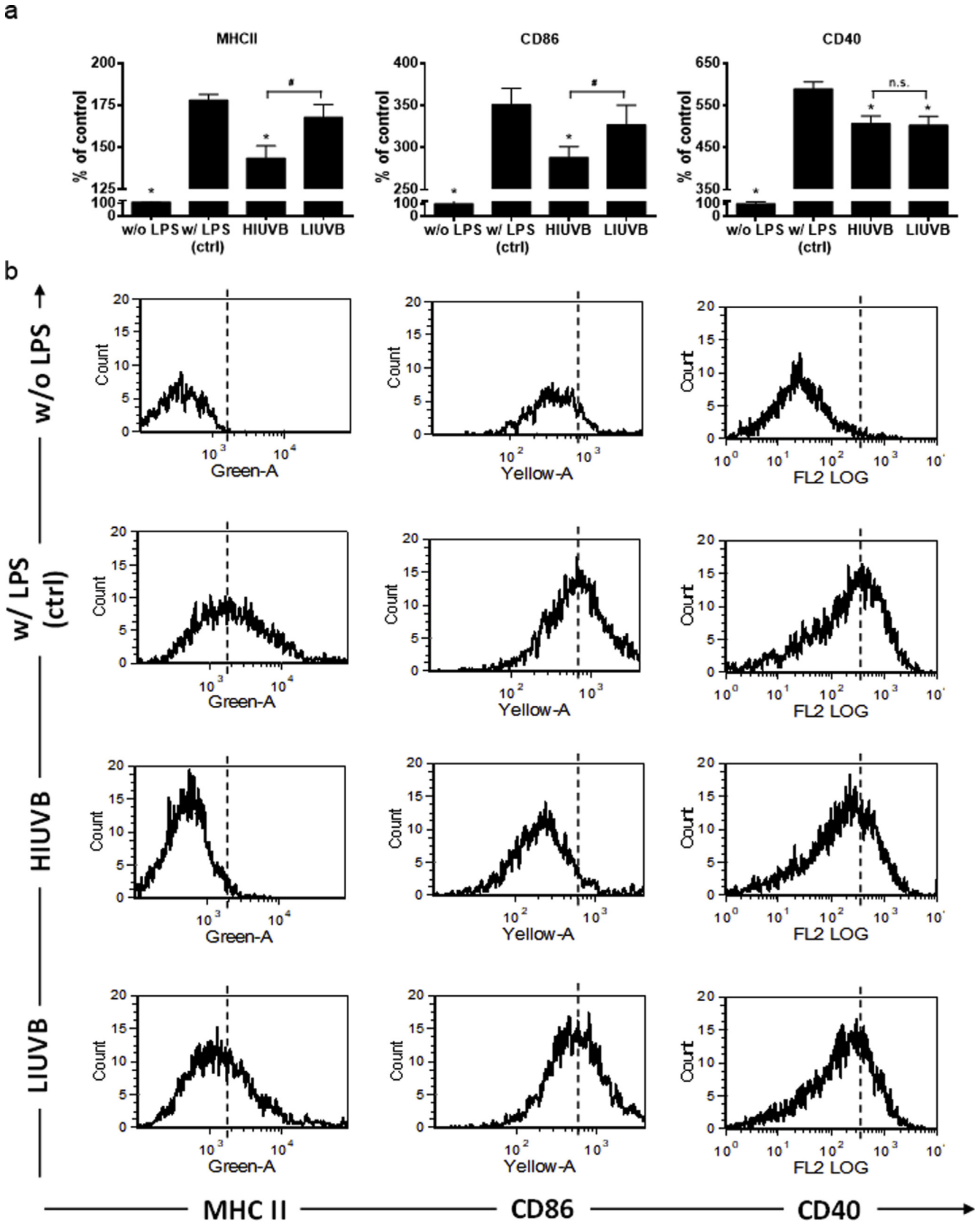

Fig. 2. Bone marrow-derived dendritic cells (BMDC) were exposed to high irradiance UVB (HIUVB) or low irradiance UVB (LIUVB) at equivalent fluence (20 mJ/cm^2). Briefly, BMDCs were cultured with or without lipopolysaccharide (LPS; 1ug/ml) for 24 h and exposed to indicated UVB irradiation. All the UVB treated groups were stimulated with LPS prior to UVB irradiation. Twenty-four hours after irradiation, BMDCs were harvested and stained with antibodies against MHCII, CD86 or CD40, and measured by FACS analysis. A). Mean fluorescence intensity analyses of the surface marker expressions on BMDCs after indicated experimental condition. The results were obtained from 8 independent experiments and presented as mean + SEM. B). Representative histograms of BMDCs after different experimental conditions.
w/LPS: control; * indicates $P < 0.05$ as compared to control; # for $P < 0.05$.

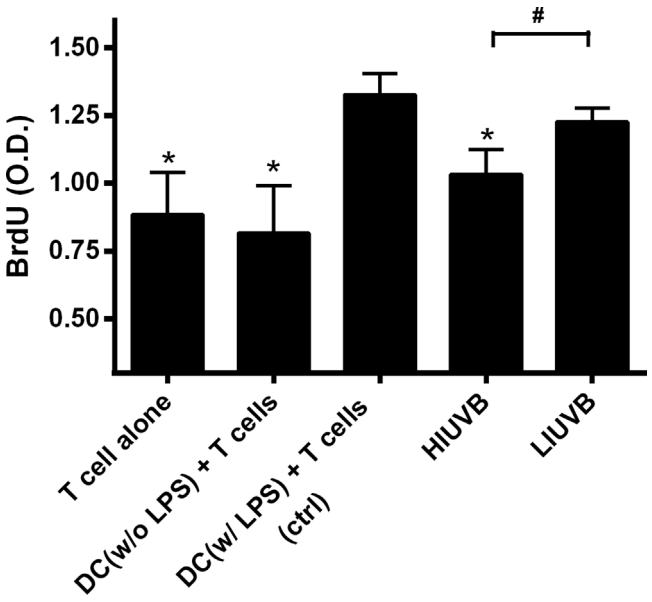

Fig. 3. The cultured bone marrow-derived dendritic cells (BMDC) were treated with indicated lipopolysaccharide (LPS; 1ug/ml) and UVB (20 mJ/cm^2) radiation. Subsequently, BMDCs were co-cultured with T cells. The details of treatment were described in the method section. All the UVB treated groups were stimulated with LPS prior to UVB irradiation. Proliferative response was determined by Brdu assay. The results were obtained from 8 independent experiments and presented as mean + SEM.
w/LPS: control; * indicates $P < 0.05$ as compared to control; # for $P < 0.05$.

stimulatory molecules of DC and supported the notion that at equivalent fluence, the HIUVB has significantly higher impact on modulating BMDCs as compare to its LIUVB counterpart.

3.3. *HIUVB induced* cyp1a1 *and* cyp1b1 *mRNA expression in BMDCs*

Previous studies demonstrated that modulation of AhR signaling in DC played an important role in UVB-induced immunosuppression [8,18]. Therefore, we next examined the expression of AhR signature genes *cyp1a1* and *cyp1b1* after equivalent fluence of UVB (20 mJ/cm^2) exposure delivered at different irradiance. As demonstrated in Fig. 4, HIUVB treated BMDCs showed significant increase in *cyp1a1* and *cyp1b1* transcription levels in BMDC ($p < 0.05$) while their LIUVB treated counterpart showed no significant change as compared to control. These results indicated that differential activation of AhR cascade may contribute to the difference in UVB-induced immunosuppression induced by UVB radiation administered at equivalent fluence but different irradiance.

3.4. *At equivalent fluence, HIUVB radiation induced higher immunosuppression in mice as compared to its LIUVB counterpart*

Using cell model, we demonstrated that at equivalent fluence, HIUVB significantly lowered MHCII and CD86 expressions on BMDC and reduced their capacity to stimulate T cell proliferation as compared to control while the LIUVB treated BMDC showed no significant alteration. We next validated these *in vitro* findings using animal models. Contact hypersensitivity model is frequently used for evaluating UVB-induced immunosuppression of the skin. Mice were exposed to equivalent fluence (200 mJ/cm^2) of HIUVB or LIUVB for 4 days prior to sensitization, and CHS was performed. Our results showed that after equivalent fluence of UVB exposure, the HIUVB treated mice showed reduced ear thickness increase after challenging with sensitizer, while the LIUVB treated mice showed no significant change as measured by micrometer (Fig. 5A and B).

3.5. *Knockdown of AhR signaling in mice abrogated the effects of irradiance on UVB-induced immunosuppression*

Since our cell study suggested that AhR signaling contributed to different levels of immune modulation imparted by equivalent fluence of UVB radiation delivered at different irradiance, we next aimed to confirm if AhR signaling contributes to different levels of immunosuppression found between HIUVB and LIUVB treated mice after irradiation at equivalent fluence using AhR transgenic mice. As demonstrated in Fig. 6, after exposure to UVB radiation at equivalent fluence followed by CHS assay, the AhR-sufficient mice ($AhR^{+/-}$) showed significantly lower ear thickness increase after HIUVB radiation as compared positive control group while no significant change was found between the LIUVB radiation treated group and the positive control group. For the AhR-deficient mice ($AhR^{-/-}$), opposite result was found. While HIUVB treated mice showed no significant difference in ear thickness increase with the positive control group, the LIUVB treated mice demonstrated significantly lower ear thickness increase as compared to the positive control group.

4. Discussion

In clinical settings, UVB phototherapy is frequently used to treat inflammatory skin disorders. Previously, wavelength and fluence

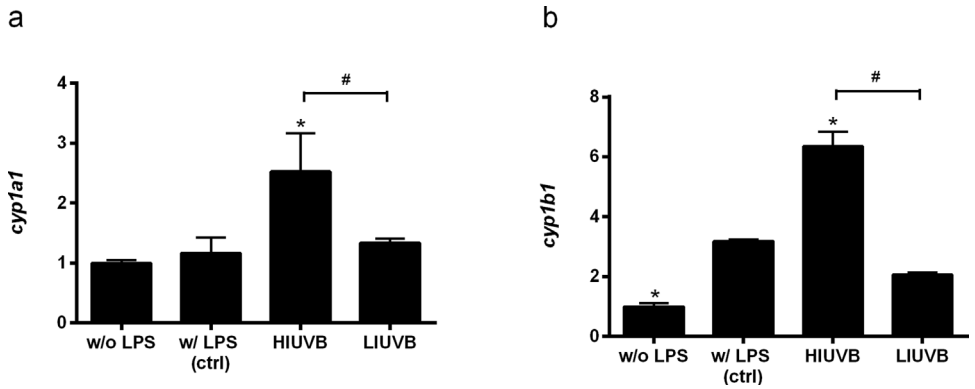

Fig. 4. Cultured BMDCs were treated with equivalent fluence (20 mJ/cm^2) of high irradiance UVB (HIUVB) or low irradiance UVB (LIUVB). All the UVB treated groups were stimulated with lipopolysaccharide (LPS; 1ug/ml) prior to UVB irradiation. The *cyp1a1* and *cyp1b1* mRNA expression were evaluated by RT-QPCR. The results were obtained from 8 independent experiments and presented as mean + SEM.
w/LPS: control; * indicates $P < 0.05$ as compared to control; # for $P < 0.05$.

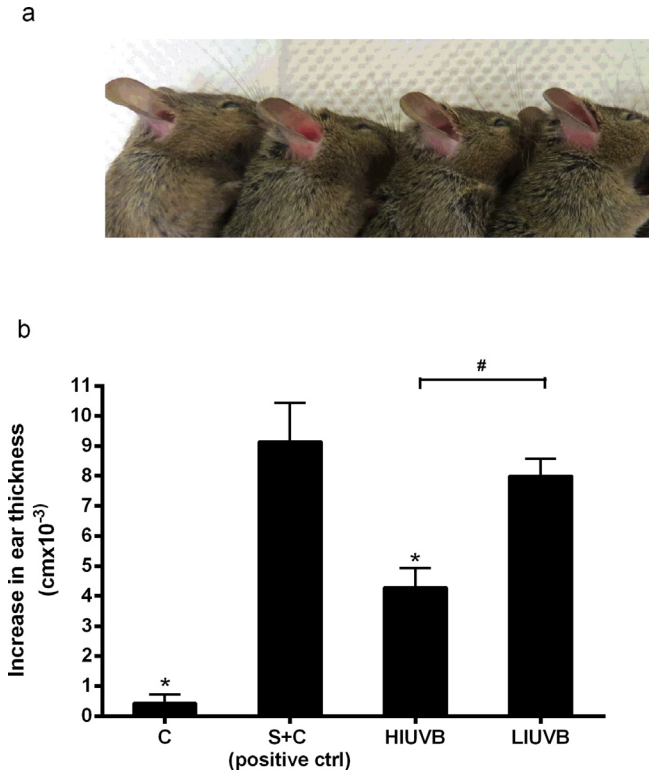

Fig. 5. The level of immunosuppression as evaluated by contact hypersensitivity (CHS) model after equivalent fluence of UVB administered at different irradiance. C3H/HeN mice were subjected equivalent fluence (200 mJ/cm^2) of high irradiance UVB (HIUVB) or low irradiance UVB (LIUVB) and ear thickness after challenge was determined as described in the method section. Briefly, the shaved back of the mice were irradiated with indicated UVB for 4 consecutive days. Twenty-four hours after the last irradiation, the mice were sensitized with DNFB on the shaved back. Five days later, ear challenge was performed and ear swelling was measured 24 h later. Each experimental group contained 7 mice, and the results were presented as presented as mean + SEM. (A) Representative mouse ear skin after treatment. Left to right: control (C) with no prior sensitization; Positive control (S + C) with prior sensitization; HIUVB treated mouse before sensitization; LIUVB treated mouse before sensitization. (B) The quantitative analyses of ear thickness after different treatment condition were measured by spring-loaded micrometer.
*indicates $p < 0.05$; ** indicates $p < 0.01$; # for $P < 0.05$

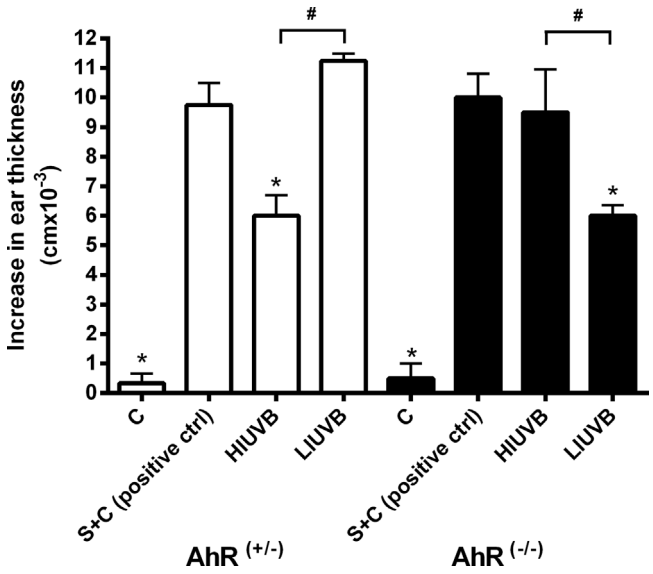

Fig. 6. Functional roles of arylhydrocarbon receptor (AhR) signaling in UVB-induced immunosuppression delivered at different irradiance using transgenic animal model. AhR-sufficient (AhR $^{+/-}$) and -deficient (AhR $^{-/-}$) mice were exposed to indicated UVB irradiation as described in the method section. The mice were subjected equivalent fluence (200 mJ/cm^2) of high irradiance UVB (HIUVB) or low irradiance UVB (LIUVB) and ear thickness after challenge was determined as described in the method section. Briefly, the shaved back of the mice was irradiated with UVB for 4 consecutive days. Twenty-four hours after the last irradiation, the mice were sensitized with DNFB on the shaved back. Five days later, ear challenge was performed, and ear swelling was measured 24 h later. For AhR (+/-) studies, each experimental group contained 4 mice except control (C) group that contained 3 mice. For AhR (-/-) studies, the experimental group contained at least 4 mice (the UVB treated groups each contained 6 mice) except control (C) group that contained 2 mice. The results were presented as presented as mean + SEM. (C): control with no prior sensitization; (S + C): Positive control with prior sensitization.
* indicates $p < 0.05$ as compared to S + C (positive control) group.
# indicates $P < 0.05$

delivered were the two important parameters considered when UVB phototherapy is administered. However, with the recent introduction of excimer light devices, it has become clear that irradiance of UVB radiation is another important factor to consider when UVB phototherapy is contemplated.

Few studies have examined the impact of irradiance on UVB-induced immunosuppression. Previous studies have suggested that different irradiances of UV treatment may be associated with different levels immune suppression [4,5,19,20]. These previous studies suggested that at equivalent fluence, the impact of UVB-induced immunosuppression may be significantly more effective when delivered via HIUVB as compared to its LIUVB counterpart. However, previous studies were mostly descriptive and the mechanism involved remains unexplored. In this study, we used BMDCs and CHS animal model to demonstrate that at equivalent fluence, HIUVB has significantly higher suppressive effect on cutaneous immunity as compared to its LIUVB counterpart. In our *in vitro* studies, we demonstrated that at equivalent fluence, HIUVB treatment significantly reduced MHCII and CD86 expressions on BMDCs as compared to its LIUVB counterpart (Fig. 3). Similar observation was reported by Denfeld et al [16] in which UVB radiation downregulated the co-stimulatory molecules of murine Langerhans cells. It is noted that at equivalent UVB fluenece, while HIUVB radiation reduced surface markers (MHC II and CD86) of BMDCs, its LIUVB counterpart did not significantly alter the expression of these co-stimulatory molecules on BMDCs. These results suggest that the irradiance of UVB radiation has a significant impact on the biological effects of exposed cells in the context of immune regulation. This notion was confirmed and validated when after equivalent UVB exposure, the HIUVB treated BMDCs showed significantly lower capacity to stimulate T-cell proliferation as compared to control while their LIUVB treated counterpart showed no significant modification.

Aryl hydrocarbon receptor pathway has been demonstrated to be an important immune regulator after UVB radiation [8]. It has been demonstrated that AhR signaling is involved in UVB-induced immunosuppression via modulation of DCs [11]. Previously, we had shown that at equivalent fluence, the HIUVB radiation is more efficient in inducing primitive cell differentiation as compared to its LIUVB counterpart through more efficient activation of AhR cascade [3]. These results suggest that AhR pathway may be involved in the mechanisms that contribute to different levels of immune modulation imparted by UVB radiation administered at equivalent fluence but different irradiance. Therefore, we next determined the effect of UVB irradiance on activation of AhR signature genes including *cyp1a1* and *cyp1b1*. As demonstrated in our results, at equivalent fluence, the HIUVB radiation induced significantly higher AhR pathway activation in BMDC as compared control while its LIUVB counterpart demonstrated no significant impact. Taken together, these results suggested that at equivalent fluence, HIUVB may provide more effective immunosuppression as compared to its LIUVB counterpart through AhR related pathway. In our previous work [3], we had shown that at equivalent fluence, radiations emitted from exicmer light (which has much higher

irradiance than NBUVB) are more readily absorbed by tryptophan as compared to NBUVB. This finding may provide a possible rationale explaining why HIUVB, but not LIUVB, activates AhR signaling in this study since after absorbing UVB photon, tryptophan turns into a ligand for AhR complex and subsequently activates AhR cascade. To validate this hypothesis, CHS animal model were employed. Using transgenic animal model, HIUVB-induced immunosuppression was abrogated in the AhR deficient mice. However, at equivalent fluence, the LIUVB treated AhR-deficient mice showed significant reduction in ear thickness increase as compared to the positive control group. Different pathways have been shown to be involved in UVB-induced immunosuppression including direct DNA damages and formation of reactive oxygen species [21]. Previously, we and Iida et al demonstrated that at equivalent fluence, the LIUVB induces more DNA damages and higher reactive oxygen species levels as compared to its HIUVB counterpart, respectively [4,5]. Therefore, it is likely that after damping the role of AhR cascade, other pathways, including DNA damage and oxidative stress, involved in the UVB-induced responses may contribute more significantly to immune modulation. Further studies are warranted to confirm and validate this hypothesis. Several limitations of this study should be mentioned. The immunosuppressive effects of HIUVB on LPS-treated BMDCs were post-activation effect (i.e., LPS treatment for 24 h first, then UVB treatment, then analyzed after 24 h). However, in CHS model, the back skins of mice were pre-exposed to UVB for 4 days before CHS model was started (preventive model). Therefore, the designs of *in vitro* experiments and *in vivo* studies contained certain intrinsic differences. Additionally, it is noted that regarding the results of CHS study using transgenic mice, there is no direct evidence indicating whether the changes in ear thickness in AhR-deficient mice are direct consequence resulting from phenotypic and functional alterations of DCs, in which AhR are not expressed. Further studies addressing these limitations are warranted in the near future.

In summary, this study demonstrated that irradiance of UVB radiation contributes significantly to the UVB-induced immunosuppression. This is an academically important and clinically relevant discovery as current practice in phototherapy presumes that equivalent fluence of UVB delivered will produce equivalent clinical response, and therefore, decay of UVB irradiance is usually compensated by increasing duration of exposure to achieve same fluence in clinical practice. Since this study demonstrated that irradiance of UVB radiation has significant impact on its immunosuppressive properties, the current concept regarding efficient UVB phototherapy should be revisited in the near future.

Founding source

This study was supported by research grants from Ministry of Science and Technology, Taiwan (MOST 105-2628-B-037-008-MY2).

Conflict of interest disclosures

None.

Acknowledgments

This study was supported by research grants from Ministry of Science and Technology, Taiwan (MOST 105-2628-B-037-008-MY2) and Ministry of Health and Welfare (MOHW106-TDU-B-212-113006; MOHW107-TDU-B-212-123006))

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.2019.01.003.

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