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Evaluation of Acute Reactions on Mouse Skin Irradiated with 222 and 235 nm UV-C

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ABSTRACT

Biological response and DNA damage following irradiation with shorter wavelengths in the UV-C range were evaluated to investigate the safety at three wavelengths because of the recent emergence of germicidal equipment emitting short-wavelength UV-C for various purposes, including medical uses. To estimate an acceptable safety dose for human skin in the UV-C range, especially short UV-C, we studied the biological effects of 207, 222 and 235 nm UV-C using albino hairless mice and evaluated the inflammatory reactions in the skin. To explore an appropriate indicator to evaluate the biological response, we employed determination of the minimal perceptible response dose (MPRD), by which any subtle cutaneous response; erythema, edema and scale could be observed by visual inspection. Erythema was rarely observed, but edema and scale formation were evident for short UV-C wavelengths. The MPRD at 207, 222 and 235 nm was determined to be > 15, 15 and 2.0 kJ m^{-2}, respectively. These values could be thresholds and indicators for possible safety assessments. Our data suggest that the current human exposure limits for short UV-C wavelengths below 254 nm are overly restrictive and should be reconsidered for future disinfection lamps with short UV-C wavelengths.

INTRODUCTION

Recently, as a result of the COVID-19 pandemic, the use of UV-C lamps for inactivation of microbes, including viruses, bacteria and spores, has drawn great attention. However, the application of conventional 254 nm UV-C germicidal lamps has been restricted for human safety reasons. Therefore, the acceptable dose of ultraviolet radiation (UV) on the human skin is based on the occupational and public health exposure limits (ELs). The most widely used ELs have been advised by the American Conference of Governmental Industrial Hygienists (ACGIH) as a threshold limit value (TLV) (1). In the range of UV-C wavelength (100–280 nm), the mercury 254 nm UV-C lamp has been most widely utilized for germicidal purposes, but its use is limited to locations outside of working spaces because of its irritative acute effects on the eyes (snow-blindness, painful photokeratitis), skin (redness of the skin, erythema), mutagenicity and potential carcinogenic consequences for the skin (2–6). Although evaluation of acute reactions to UV on skin using the erythema reaction (minimal erythema dose, MED) by ultraviolet radiation (UVR) has been commonly utilized, only a few studies of an erythema reaction at wavelengths below 254 nm have been reported. One of the reasons for this is the low level of interest, as the UV-C range is absorbed by the ozone layer and does not reach the surface of the earth. Therefore, *in vivo* studies of the effects of UV-C have received little attention without the application of UV-C lamps that expose the human body to such radiation (7). Conversely, various kinds of shorter wavelength UV-C have recently emerged for various purposes. Argon-fluoride 193 nm UV-C laser radiation has been proven to cause little DNA damage and is now utilized for human corneal ablation (8) and newly developed 222 nm UV-C lamps have been shown to have sterilizing efficacy similar to that of 254 nm UV-C lamps (9–11). We showed that 222 nm UV-C did not elicit erythema or photokeratitis in albino hairless mice (12) at the substantial levels we applied. Moreover, 222 nm UV-C exposure did not produce a single non-melanoma skin cancer with our fairly high-dose irradiation on even xeroderma pigmentosum complementation group A (*Xpa*)-knockout mice, an animal model that would have a more than 10 000-fold greater chance for developing non-melanoma skin cancer compared with the normal population (13). We considered this non-carcinogenic property to be largely attributed to the very short epidermal transmittance of 222 nm UV-C resulting from its short wavelength. This indicates that shorter wavelength UV-C lamps, including 222 nm UV-C, may be promising for application to the human body for sterilizing purposes (12). During the study, we noticed that erythema did not develop during single exposure of 10 kJ m^{-2} (1 J cm^{-2}) of 222 nm UV-C, whose indicated erythema-inducible dose was 40 times or more higher than what had been defined as TLV by ACGIH (0.23 kJ m^{-2}) (1). A previous study reported that intermittent irradiation of 4.5 kJ m^{-2} 222 nm UV-C did not cause erythema (14). In addition, we noticed scale formation (flaking) in the absence of erythema development after 222 nm UV-C irradiation. Because there is little information on dose limitations

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of UV-C for humans, our previous data suggest that more sensitive and appropriate evaluation than MED should be applied to estimate the threshold for UV-C around 222 nm. In the current study, we investigated inducible biological skin reactions in mice irradiated with several shorter UV-C wavelengths to estimate more reasonable dose limits for applying germicidal lamp irradiation of the human body. For this purpose, we used three wavelengths: 207 nm UV-C, the emission from which was provided with excimer lamps and has been reported to be effective for disinfection of bacteria and safety for human and mouse skin (15,16); 235 nm, as an example wavelength longer than 222 nm; and 222 nm. As a positive control for UV-induced inflammation, a 311 nm UV-B lamp was used.

MATERIALS AND METHODS

UV sources.

207-nm source. A krypton-bromine excimer lamp that emits primarily 207 nm radiation was used in this study (Fig. 1). The lamp unit is air-cooled and consists of a lamp, a custom bandpass filter that eliminates wavelengths over 230 nm and a mirror. The peak wavelength is 207 nm, and the full width at half maximum (FWHM) is 2 nm. The intensity was measured with a QEPro spectrometer (Ocean Insight. Rostock, Germany), at a location 1 cm below the exit window of 320 μW cm^{-2}.

222-nm source. A krypton-chloride excimer lamp with an optical filter that limits emission to 200–230 nm wavelength UV, with a maximum output wavelength of 222 nm and FWHM of 2 nm, was used in this study. The lamp unit consists of a lamp (SafeZone UVC®; Ushio Inc., Tokyo, Japan), an air-cooling fan, a mirror and a custom bandpass filter. The custom filter removes almost all UV wavelengths other than the main emission of 222 nm UV-C, as previously described. In this study, we utilized the irradiator named irradiator B in our previous study (12). The irradiance of 222 nm radiation was measured using an S-172/UIT250 cumulative UV meter (Ushio Inc. Tokyo, Japan) and was 3 mW cm^{-2} 10 cm from the emission window.

235-nm source. The irradiation system used in this study consists of a 300 W xenon lamp (Ushio Inc.), a cooling fan, a custom bandpass filter (225–245 nm reflection) and a monochromator (SPG-120U: Shimazu Corp. Kyoto, Japan) set at 235 nm. The central wavelength was 235 nm with a FWHM of 4 nm, and the light intensity was measured with a QEPro spectrometer (Ocean Insight). The irradiation intensity was 224 μW cm^{-2} at a position 5 cm from the exit of the monochromator.

311-nm source. Banks of 6 TL 20W/01RS fluorescent lamps (Philips, Eindhoven, Holland) were used in this study. The TL 20W/01RS lamps used in dermatology only emit light at a narrow peak around 311 nm. The irradiance at a distance 40 cm from the lamps was 1.2 mW cm^{-2}, as measured by a QEPro spectrometer (Ocean Insight.)

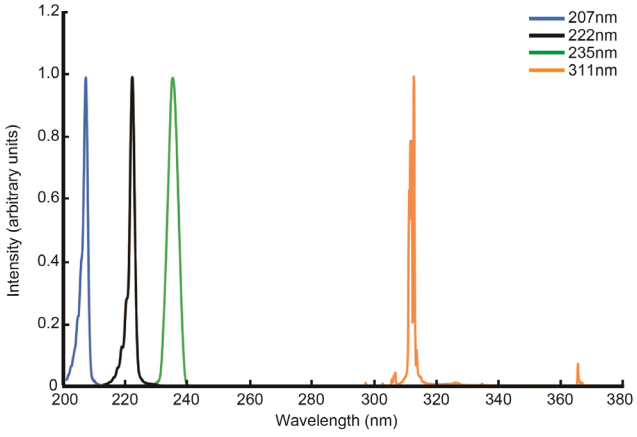

Figure 1. Measured spectra of irradiation at each wavelength. The vertical axis shows the normalized intensity. The peak wavelengths of each irradiator were 207, 222, 235 and 311 nm.

Mice. Albino hairless mice (Hos:HR-1) aged 12–18 weeks were used. The mice were housed under specific pathogen-free conditions, and all animal experiments were conducted according to the Guidelines for Animal Experimentation at Kobe University Graduate School of Medicine. All experimental protocols described in the materials and methods were approved by the Institutional Review Boards for Experimental Animals (Approval number: P180207-R2).

Evaluation of skin reaction after UV irradiation. Each radiation group consisted of five mice, and both sexes were used for each group (2–3 female and 2–3 male mice). The mice were sedated by isoflurane inhalation. Each mouse was placed below the UV lamps, followed by single exposure to UV light on the dorsal skin within a 1-cm square aperture mask. To minimize the variability in response to UV with respect to irradiation site, only the caudal area of the mouse was exposed. The distance between the UV source and the irradiated site was set at the same distance as the UV dose rate was measured for each UV source. The irradiation dose was designed to cover a broad enough range to provide both sub- and supra-threshold doses with each irradiation group, referring to the human exposure limit value defined by ACGIH. As for the evaluation methods, we refer to several previous reports; Cole *et al*. suggested that edema 24 h after UV irradiation is the most sensitive marker for the evaluation of monochromatic UV-C reaction in hairless mice (17), while Diffey *et al*. reported that minimal erythema similar to sunburn erythema could be developed by germicidal 254 nm UV-C at 0.1 kJ m^{-2} in humans (18). Therefore, we evaluated the skin reaction after irradiation with several UV-C wavelengths using two methods: visual inspection and measurement of ear swelling. Regarding visual inspection, all perceptible reactions (erythema, edema, scales and fissures) were evaluated before and 3, 24, 48 and 72 h after irradiation using the methods described by Cole *et al*. (17). Skin edema was evaluated as uplifted and observed from the side view. Edema was detected as the color of the skin increased in white tone by reducing the vascular permeability in the epidermis and dermis. The criteria for the scores by visual inspection were defined as modifications of criteria as follows: 0, absence of reaction; 0.5, very slight but discernible reaction (outline not clearly marked); and 1, easily perceptible, outline clear reaction (Figure S1). The score was determined by three independent dermatologists. Ear thickness was measured as previously described (19,20). The ear thickness of each mouse was measured on both sides of the ear with a digital micrometer (Mitutoyo Corp. Tokyo, Japan) before exposure and 24, 48 and 72 h after UV irradiation under mild sedation by a similar method. Ear swelling at a given time point was determined by the difference between the ear thickness after UV irradiation and that before irradiation.

Measurement of transmittance of the skin with UV irradiation. For measurement of the transmittance of skin tissue, the 15-μm-thick human primary stratum corneum (KAC Co. Ltd., Kyoto, Japan) was measured with respect to linear and diffuse transmittance with a 5-mm-diameter circle aperture using a V-7200 double monochromatic spectrophotometer, measurement wavelength: 200–400 nm (JASCO, Tokyo, Japan). Diffuse transmittance and reflectance were measured by equipped with an integrating sphere VISN-7090 (measurement wavelength: 235–400 nm) to the spectrophotometer. The diffuse transmittance was outside the measurement range for wavelengths shorter than 235 nm, so it was estimated by multiplying the measured linear transmittance by the ratio of diffuse transmittance/linear transmittance at 235 nm or greater. The transmittance of the mouse stratum corneum was determined after reflectance was set to 10%, which is measurement value, with the abovementioned stratum corneum transmittance, and the mouse stratum corneum was simulated as a 10-μm stratum corneum (21).

Immunohistochemistry. For detection of cyclobutane pyrimidine dimers (CPDs) in mouse skin, specimens were collected following UV irradiation. Skin specimens were fixed in 10% neutralized formalin and embedded in paraffin. Immunohistochemical staining for CPD detection was performed using a monoclonal antibody against CPD (TDM-2 1:5,000 dilution) as described previously (22). Specimens were observed using a Biozero BZ-X710 microscope (Keyence, Osaka, Japan).

Statistics. Differences between groups were assessed for significance using the Mann–Whitney test for the grading scale of erythema, edema and scale, and Student's *t*-test for ear swelling. *P* values less than 0.05 were considered statistically significant.

RESULTS

Biological reactions other than erythema were observed with UV-C irradiation

According to previous reports, several investigations have been conducted to evaluate the threshold for several UV-C wavelengths, leading to estimation of the minimal perceptible response dose (MPRD) (17), that is the minimal dose causing any perceptible reaction (erythema, edema, scales and fissure). We also attempted to evaluate the MPRD by visual inspection together with measurement of the ear thickness after UV irradiation. Figure 2a represents the evaluated score for visual inspection for each radiation wavelength. Because no skin change was observed initially, the score was 0 before UV irradiation for all the mice, and only scores after UV irradiation were plotted. Each dot represents the average of three dermatologists' evaluations, in which the sum of scores of five mice at each time point was taken. As described below, and as is self-explanatory, each skin reaction was correlated, and the purpose of this study was to determine the lowest dose that caused any subtle but definite biological response. We tentatively set the MPRD to be the dose with which the total score of five mice was 2, namely if at least four 0.5-scored mice out of five mice were recorded at any time point and with any reaction.

After irradiation with 222 nm UV-C, erythema was difficult to recognize at any time point, even after irradiation with the highest dose of 30 kJ m^{-2}, which confirmed our previous findings that 10 kJ m^{-2} of 222 nm UV-C did not induce erythema at all (12). Although edematous changes were detectable immediately and 24 h after irradiation with all doses, they soon diminished, with a wide variance in evaluation scores among investigators; a dose of 30 kJ m^{-2} led to a total score > 2. Conversely, scale formation appeared 48 h after irradiation with all doses, and a dose of 15 kJ m^{-2} led to a total score > 2 (Fig. 2a and b). Taken together, edema at 24 h and scale formation at 48 h were considered more reliable indicators to determine the MPRD than erythema, and scale formation appeared to be more sensitive and had a smaller variance among the investigators. Thus, the MPRD for 222 nm UV-C was considered to be 15 kJ m^{-2}. Similarly, the reaction propensity of 235 nm UV-C was similar, showing that edema developed immediately after UV irradiation, followed by scaly changes 48 h after irradiation, whereas erythema was very faint with even the presumably highest dose for all time points after irradiation, so the MPRD was considered to be 2.0 kJ m^{-2}. Regarding the 207 nm UV-C exposure, because the irradiance was too weak to be assessed with an even high doses, only the same dose as the MPRD of 222 nm UV-C, that is 15 kJ m^{-2}, was applied. No erythema, edema or scale formation was observed after 207 nm UV-C irradiation.

Because evaluation methods for shorter wavelength UV-C exposure have not yet been established, we also evaluated the reaction using methods other than visual inspection, that is, measurement of ear swelling after UV exposure. We observed a similar tendency to that from visual inspection, although ear swelling appeared to be less sensitive than visual inspection. Negligible ear swelling was observed after 207 and 222 nm irradiation, and very slight swelling was observed after irradiation with the currently determined MPRD of 235 nm UV-C for all time points, while the ears exposed to 311 nm irradiation were significantly swollen 24 h after irradiation (Fig. 2c). Considering all the data from the visual inspection and measurement of ear swelling, we determined the MPRD using the evaluation assessed by visual inspection.

Histopathological findings

Hematoxylin and eosin staining of skin specimens showed pronounced hyperkeratosis 48 h after irradiation with 222 and 235 nm UV-C at the MPRD, which was consistent with the results of gross observations, that is macroscopically detected scale formation (Fig. 3). In contrast, scaly changes started to appear 24 h after 311 nm irradiation at 3.7 kJ m^{-2}, that is the MED (23). Furthermore, epidermal hyperplasia was pronounced 24 h after 311 nm with the MED and was also noticed 48 h and 72 h after 235 nm irradiation with the MPRD, whereas epidermal hyperplasia was not observed with either 207 or 222 nm at any time point (Fig. 3).

Detection of cyclobutene pyrimidine dimers

We investigated how deep CPDs, DNA lesions caused by UV, were formed in the epidermis after UV-C irradiation. Only the uppermost layer of epidermis was positive for CPDs with the MPRD for 207 nm or 222 nm, whereas some weakly stained CPD-positive cells were present down to the basal layer after 235 nm UV-C irradiation with the MPRD, although the large majority of strongly positive cells were found in the upper layers of the epidermis. The number of CPD-positive cells and the intensity of staining signals in the epidermis irradiated by 235 nm UV-C at the MPRD were obviously smaller than those produced at 311 nm (Fig. 3). Strong CPD-positive cells were observed in the entire epidermis and hair follicle epithelium after 311 nm irradiation (Fig. 3).

Minimal perceptible response dose in shorter wavelengths in the UV-C

Finally, in addition to direct evaluation of mouse skin reactions at short UV-C wavelengths, the UV spectral transmittance of the stratum corneum was measured to estimate an alternative to the MED using the primary human stratum corneum. The diffuse transmittance (actual total transmittance) of the human stratum corneum (15 μm) was 44.3% at 254 nm and 0.027% at 222 nm, respectively, which is consistent with a previous report (24). The diffuse reflectance human stratum corneum (15 μm) of 250–300 nm was measured to be 10%, as previously described in the Materials and Methods section (25). Then, the absorbance (absorption; ABS) was calculated with -Log (transmittance/(100-10)) and found to be 0.31 at 254 nm and 3.52 at 222 nm, respectively. In the same way, when the mouse stratum corneum is estimated to be 10 μm thick (21), the ABS of mouse stratum corneum was 0.21 at 254 nm and 2.35 at 222 nm, respectively. As a result, the estimated transmittances of mouse stratum corneum were obtained as 10^((Log(44.3/90))*10/15)*90, namely 56.0%, at 254 nm and as 10^((Log(0.027/90))*10/15)*90, namely 0.40%, at 222 nm.

The transmittance difference of the measured 15 μm human stratum corneum was significant below 240 nm and increased as the wavelength became shorter. Similar curves were obtained for

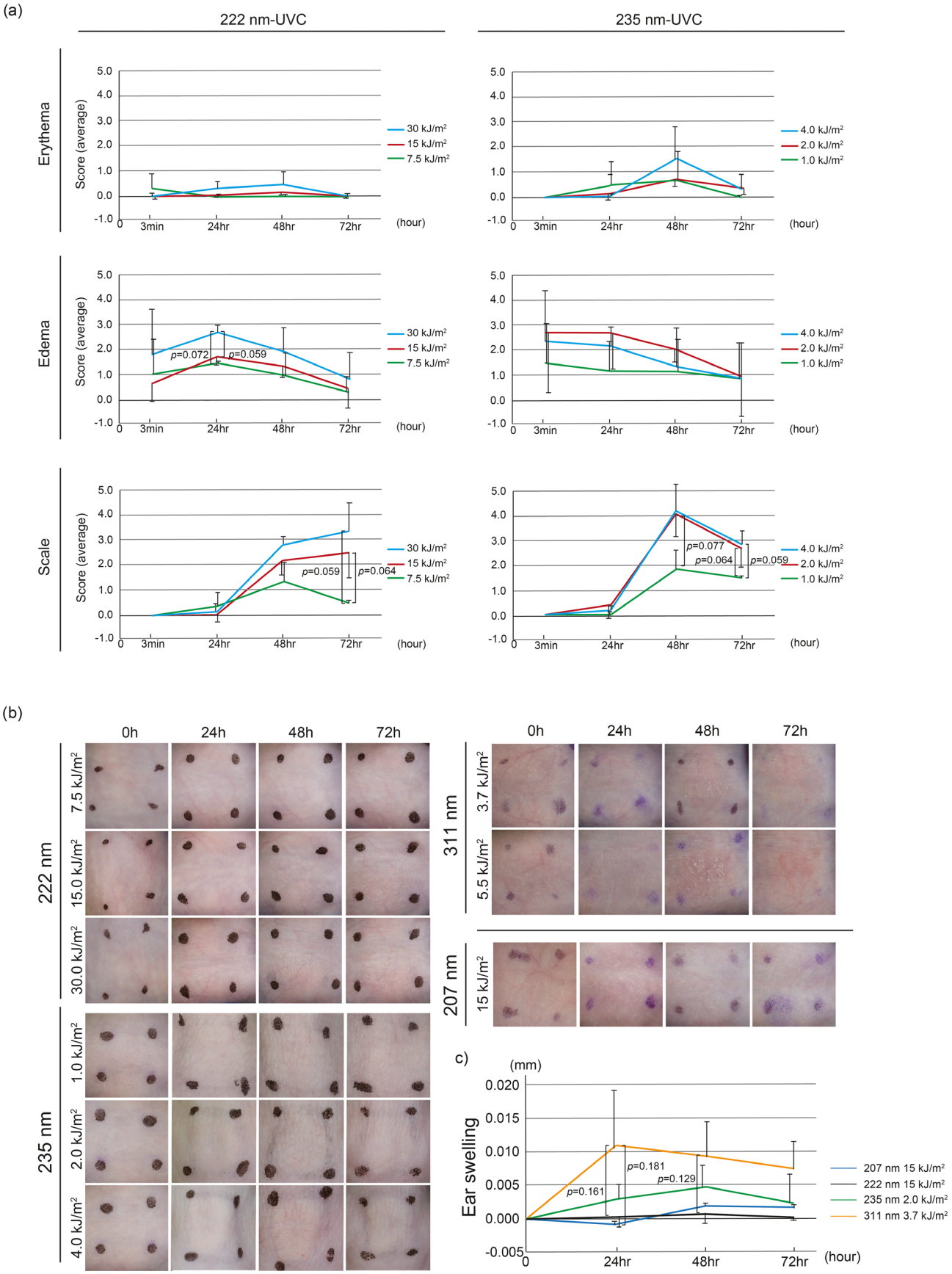
(a)
222 nm-UVC
235 nm-UVC
Erythema
Edema
Scale
Score (average)
(hour)
3min
24hr
48hr
72hr
30 kJ/m²
15 kJ/m²
7.5 kJ/m²
4.0 kJ/m²
2.0 kJ/m²
1.0 kJ/m²
p=0.072
p=0.059
p=0.064
p=0.077
(b)
0h
24h
48h
72h
222 nm
235 nm
311 nm
207 nm
7.5 kJ/m²
15.0 kJ/m²
30.0 kJ/m²
1.0 kJ/m²
2.0 kJ/m²
4.0 kJ/m²
3.7 kJ/m²
5.5 kJ/m²
15 kJ/m²
c)
(mm)
Ear swelling
p=0.181
p=0.129
p=0.161
207 nm 15 kJ/m²
222 nm 15 kJ/m²
235 nm 2.0 kJ/m²
311 nm 3.7 kJ/m²

Figure 2. Mice skin responses following UV-C irradiation. (a) Each graph shows responses at 3 min and 24, 48 and 72 h after several doses of 222 nm and 235 nm UV-C irradiation. Each score was determined by three independent dermatologists according to the protocol described in the Materials and Methods section. Representative features of erythema, edema and scale are shown in Figure S1. Error bars represent standard error. (b) Each panel shows a 1 × 1 cm sample of hairless mice back skin with determination of any visual reaction (erythema, scales, edema and fissures). The threshold of 311 nm was determined by the minimal erythema dose, as previously reported (23). The dose of 207 nm irradiation follows the dose of 222 nm because of its poor output. (c) Ear swelling following irradiation at each wavelength. Error bars represent standard error.

humans and mice between 240 and 340 nm, when the mouse stratum corneum was estimated to be 10 μm thick (Fig. 4a). Based on the fact that the action spectra of erythema induction are well correlated with thymidine dimer formation in the upper epidermis between 280 and 340 nm (26), we overlaid our curve of MPRD data over an estimated MED spectrum multiplied by two factors, that is, the reciprocal of the action spectrum of the induction of thymine dimers (CPDs) (27) to the reciprocal of the transmittance value of both mouse and human through the stratum corneum (Fig. 4b) and compared it with previously reported mouse, rabbit and human spectra of MED (7,18,26,28–31). The MPRDs in this study are consistent with the estimated MED spectra and have higher thresholds than the spectra from previous animal experiments and TVLs for acute reactions by UV exposure (Fig. 4b) (7,32), suggesting that the evaluation of MPRDs, especially short UV-C wavelengths, would be justified as an alternative evaluation of the MED and a proposal that TLVs is considered to be higher than previously assessed (1,32).

DISCUSSION

Because lamps emitting UV-C wavelengths have been generally considered to be harmful for intentional human exposure, it is necessary to take protective measures and stay away from UV irradiation when sterilizing with 254 nm UV-C. However, the recent usage of short UV-C wavelengths has drawn greater attention for the application of UV inactivation of several types of viruses and bacteria. Conversely, the biological basis for TLVs at short UV-C wavelengths is limited compared with the biological data for UV-A and UV-B lamps, which provide a number of benefits for various skin diseases and provide supporting data for defining other TLVs.

First, we attempted to evaluate the reaction by scoring with visual inspection, using the method to determine the MED after UV-B exposure. However, this was rather difficult because we could not mark scores higher than 2, namely all the reactions were 0, 0.5 or 1 at all serially increasing doses. Erythema was especially difficult to observe, except for 311 nm irradiation. Without a change in color without palpation, it was difficult to differentiate the clear outlines of edema. Therefore, we determined the MPRD that rendered at least four out of five mice to develop any of the skin changes (erythema, edema, scale or fissure), even when the reaction was subtle, that is a score of 0.5.

We referred to the method applied by Cole *et al.*, in which minimal mouse edema effective action spectrum (MEE) or MPRD for an acute action spectrum and TLVs were determined by the absence of any reaction, that is erythema, edema, scale or fissuring, in hairless mice (17). Although we scarcely observed any erythema, we could detect slight edema both by palpation and visual inspection and measurement of ear thickness, and scale formation was much more sensitively visualized than edema at the analyzed short UV-C wavelengths (Fig. 2b and c); thus, we preferred the terms of MPRD rather than MEE. Histological findings were also consistent with the macroscopic

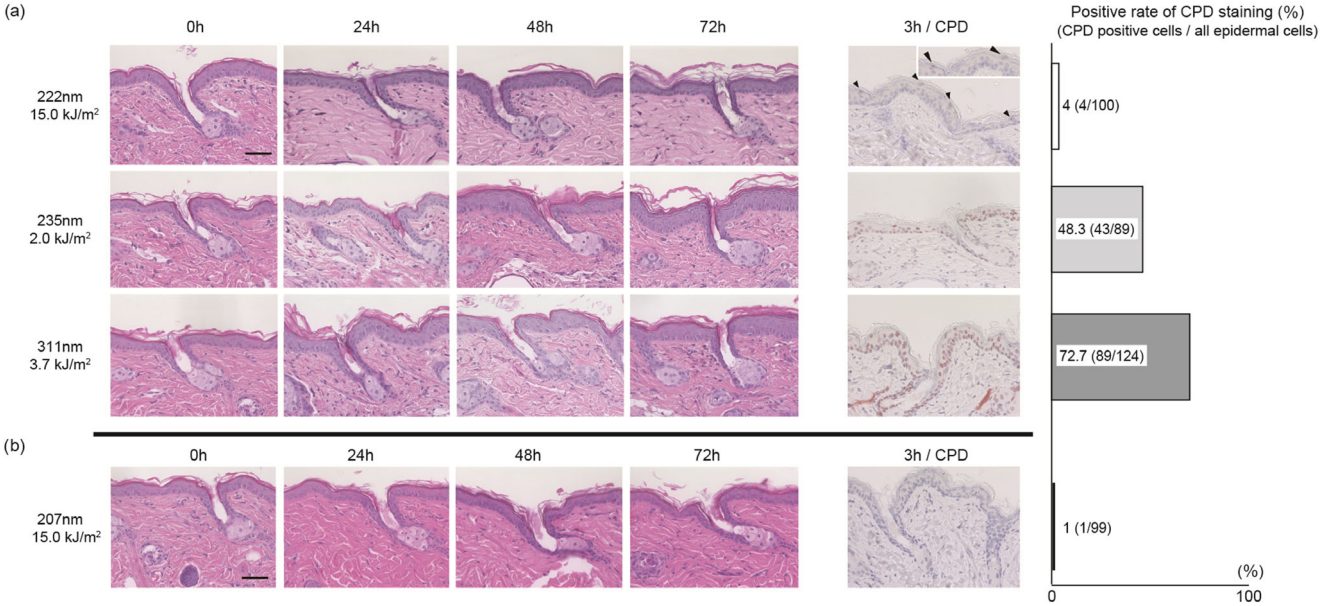

Figure 3. Histological evaluations of effects on mice skin after UV irradiation. Left panels: Histological features (hematoxylin and eosin) following exposure on hairless mice skin for each wavelength with threshold limits. The dose of 207 nm irradiation follows the dose of 222 nm because of its poor output. Right panels: Immunohistochemical detection of CPDs in hairless mice irradiated with each lamp 3 h after exposure. Arrowheads indicate CPD-positive cells. Scale bar: 50 μm. CPD stained cells in the epidermis were counted, and the rate of positive cells in all epidermal cells can be visualized in the right graphs.

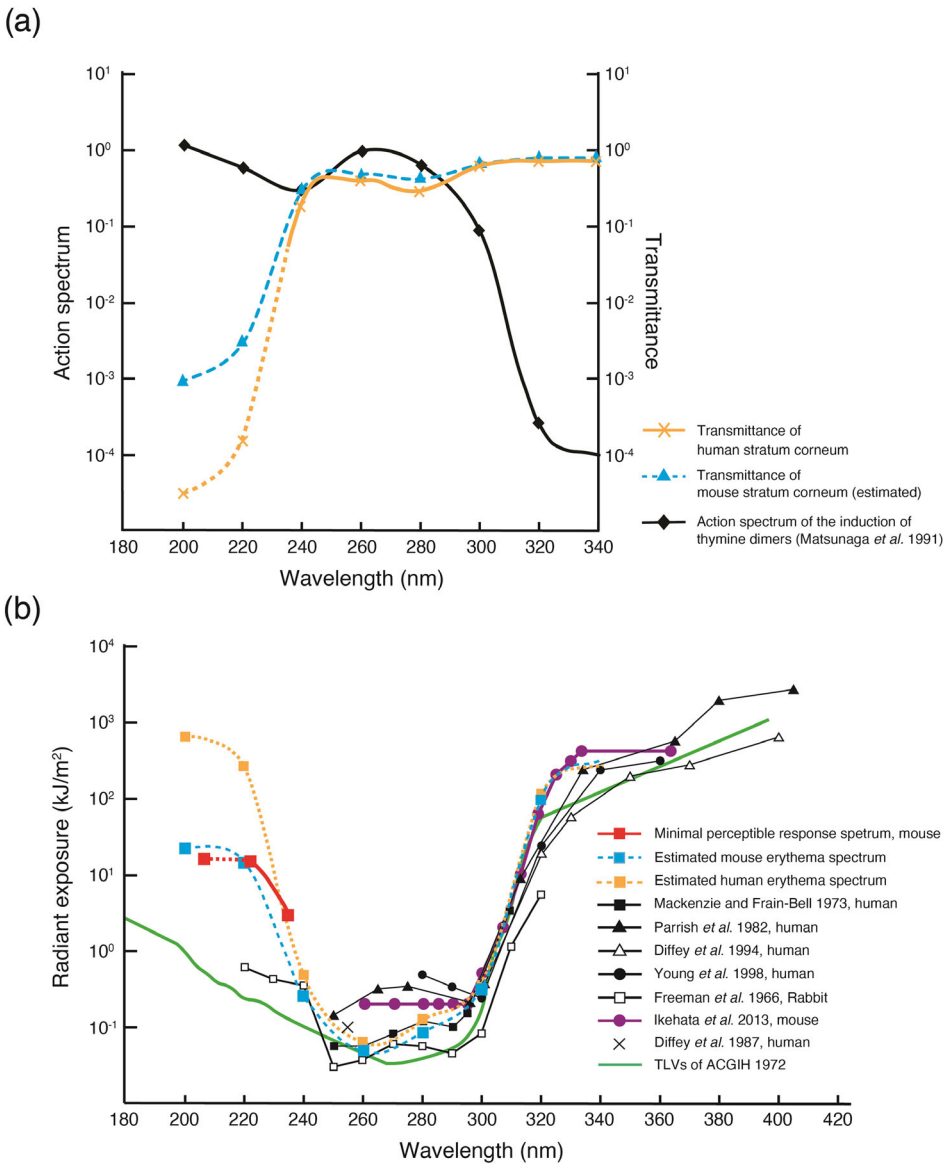

Figure 4. Ultraviolet radiation threshold limit and transmittance of stratum corneum around 222 nm wavelength in the skin. (a) The action spectrum of the induction of thymine dimers (CPDs) (27) in naked DNA and transmittance curve of presumable mouse stratum corneum (10 μm) and human (15 μm) obtained using the primary human stratum corneum transmission with various UV wavelength sources. The dotted yellow line of action spectrum shorter than 235 nm is distinguished from the solid yellow line because its diffuse transmittance was estimated using the ratio of diffuse transmittance/linear transmittance at 235 nm or greater. (b) The estimation of the MED spectrum was obtained (mouse; blue line and human; dotted orange line, respectively) by multiplying the reciprocal of the action spectrum of the induction of thymine dimers (CPDs) (Fig. 4a; black line) to the reciprocal of the transmittance value of mouse (Fig. 4a; blue line) and human stratum corneum (Fig. 4a; dotted orange line), respectively. The minimal perceptible response spectrum is delineated with the red line, except for the dotted red line between 207 and 222 nm, which is an estimated line. Previously reported action spectra in mouse (purple line) (31), human and rabbit are shown (black line) (7,18,26,28–30). The threshold limit value (TLV; ACGIH) is shown with green line (32).

observation of scale formation; slight hyperkeratosis could be observed 48 and 72 h after irradiation with 222 nm UV-C, while prominent hyperkeratosis and epidermal hyperplasia were observed 48 and 72 h after 235 nm and 311 nm irradiation. Scaly changes in the epidermis could be formed by failure of normal keratinization and/or an increase in epidermal turnover, which could substitute for the barely visible erythema induced by an equal dose at 222 nm.

The action spectrum of thymine dimer formation in the upper level of the epidermis is correlated with the inducible action of erythema (26). Therefore, we evaluated CPD formation using shorter UV-C wavelengths in the upper epidermal cells. Previous reports showed that irradiation (4.5 kJ m^{-2}) repeated for eight times and one shot of 1.6 kJ m^{-2} of 222 nm UV-C did not produce CPDs in the epidermis (33,14). Our previous and current studies presented compatible results with only small amounts of CPD-positive cells in the uppermost layer of epidermis with 5.0 and 15.0 kJ m^{-2} of 222 nm UV-C, respectively (12). In contrast, CPD formation in the upper epidermis along with a very faint erythematous reaction with 2.0 kJ m^{-2} of 235 nm UV-C was

observed (Fig. 2a and b). These results are consistent with the relationship between the action spectrum of CPD formation in the upper epidermis and that of the subsequent erythema reaction. The formation of CPDs in the basal layer of the epidermis, in turn, is an indication of skin tumorigenesis. The risk of nonmelanoma skin cancer (NMSC) owing to skin tumor production has been obtained based on the results of studies that induced skin tumors in mice (standard for skin cancer Utrecht-Philadelphia in hairless mice: SCUPm) and has been applied to possible prediction in humans (SCUPh; in white Caucasians) considering the thicker epidermis (5,34,35), and Commission International de l'Eclairage established the prediction curve of NMSC using SCUPh (36). In the previous and current studies with 222 nm UV-C, CPDs did not form in the basal layer (12,14). The filter installed in the lamps used in these studies could remove all but the dominant 222 nm emission wavelength. In contrast, skin erythema and CPD formation in basal cells with a smaller dose (less than 1.0 kJ m^{-2}) was observed in human skin (37). The lamps in this study, however, have a specificity showing emission peaks at 234 and 257 nm without a filter system. Another simulation study using Monte Carlo radiation transfer codes and a 5-layer skin model indicated that the 222 nm wavelength does not result in CPDs formation in the basal layer of the epidermis (24). Taken together, these results suggest that 222 nm UV-C solely does not cause DNA damage in the basal layer of the epidermis but does with UV-C ranging between 230 and 280 nm.

In summary, for future clinical applications of short UV-C wavelength lamps on humans, including 193 and 222 nm, we evaluated the acute biological reaction by irradiation with these wavelengths. Our data suggest that the MPRD could be a reliable alternative to the MED, and the threshold limit value is considered to be higher than previously assessed. More precise mechanisms and the biological properties of human skin at short UV-C wavelengths should be investigated.

CONFLICT OF INTERESTS

We have read the journal's policy and the authors of this manuscript. A.NS., H.O. and T.I., have the following competing interests: A.NS., H.O. and T.I. are provided support in the form of salaries from Ushio Inc., Tokyo, Japan. We declare no other employments, consultancies and patents of this funder, Ushio Inc.. No further intellectual property relating to this paper will be developed. This does not alter our adherence to *Photochemical and Photobiology* policies on sharing data and materials.

AUTHOR CONTRIBUTIONS

M.K, T.I. and C.N. involved in conceptualization, investigation and writing–review and editing. N.Y. and A.NS. involved in data curation. M.K., H.O., T.I. and C.N. involved in formal analysis and methodology. T.I. involved in funding acquisition. M.K., A.NS., H.O., T.I. and C.N. involved in resources. A.NS., H.O. and T.I. involved in software. T.I. and C.N. involved in supervision. N.Y. involved in writing–original draft.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its Supporting Information file).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. Evaluation of the minimal perceptible response following UV-C irradiation. Representative features of erythema, edema, and scale are shown corresponding to each score in albino hairless mice.

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