Impact of Long-Wavelength UVA and Visible Light on Melanocompetent Skin

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The purpose of this study was to determine the effect of visible light on the immediate pigmentation and delayed tanning of melanocompetent skin; the results were compared with those induced by long-wavelength UVA (UVA1). Two electromagnetic radiation sources were used to irradiate the lower back of 20 volunteers with skin types IV–VI: UVA1 (340–400 nm) and visible light (400–700 nm). Pigmentation was assessed by visual examination, digital photography with a cross-polarized filter, and diffused reflectance spectroscopy at 7 time points over a 2-week period. Confocal microscopy and skin biopsies for histopathological examination using different stains were carried out. Irradiation was also carried out on skin type II. Results showed that although both UVA1 and visible light can induce pigmentation in skin types IV–VI, pigmentation induced by visible light was darker and more sustained. No pigmentation was observed in skin type II. The quality and quantity of pigment induced by visible light and UVA1 were different. These findings have potential implications on the management of photoaggravated pigmentary disorders, the proper use of sunscreens, and the treatment of depigmented lesions.

INTRODUCTION

Electromagnetic radiation exists as a spectrum. It is classified based on its wavelength into radio waves, microwaves, infrared (IR), visible light, UV, X-rays, and γ radiation. Studies on human photobiology have focused primarily on UV radiation, and more recently, on IR (Schieke *et al.*, 2003). The visible spectrum, used for general illumination, is defined as the portion of electromagnetic radiation visible to the human eye, which corresponds to wavelengths from 400 to 700 nm (Diffey and Kochevar, 2007). The absorption of visible light by chromophores in the skin is the principle for its use in laser therapy, intense pulse light therapy, and photodynamic therapy. However, the effect of visible light on pigmentary alterations has not been explored. This is especially relevant, as the visible spectrum comprises 38.9% of sunlight when it reaches the surface of the earth (Frederick *et al.*, 1989).

The limited information on visible light is partly due to the lack of readily available broad-spectrum light source that emits only in the visible spectrum without UV or IR components. In this study, we evaluated the effects of a light source that emits 98.3% visible light on cutaneous pigmentary alterations in individuals with Fitzpatrick skin types IV–VI, and compared these effects with those induced by UVA1 (340–400 nm). Furthermore, because it is known that responses to electromagnetic radiation in the UV range differ in individuals with different skin types (Jackson, 2003), the results obtained were compared with those in individuals with skin type II.

RESULTS

The spectral irradiance of the visible light source used in this study is shown in Figure 1; the dose range used is shown in Table 1. The UVA1 light source's spectral distribution was as follows: 99.7% of UVA1, 0.12% of UVA2, and 0.17% of visible light, and less than 0.00001% of UVB radiation. This made the effects of visible light, UVA2, and UVB radiation negligible because the highest UVA1 dose used in this study was 60 J cm^{-2}. The visible light source emitted 0.19% UVA1 (340–400 nm), 98.3% visible light (400–700 nm), and 1.5% IR (700–1800 nm) radiation. Considering that the highest dose for the visible light used in this study was 480 J cm^{-2}, there was less than 1 J cm^{-2} of UVA1 emitted.

Effects of long-wavelength UVA1 (340–400 nm) on skin types IV–VI

The lowest dose at which pigmentation developed for all patients was 5 J cm^{-2}; as the dose was increased, darker pigmentation was observed. Pigmentation was more intense in volunteers with darker skin, and was still evident after

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Abbreviations: DRS, diffuse reflectance spectroscopy; IPD, immediate pigment darkening; IR, infrared
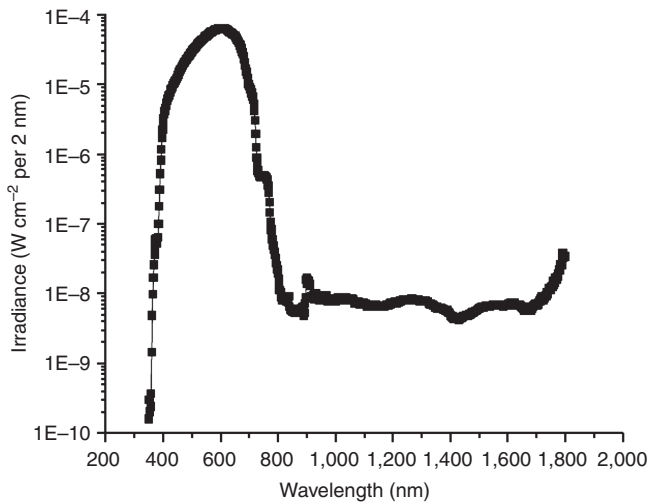
Figure 1. **Spectral irradiance of the visible light source.** Note that the vertical axis is in logarithmic scale.

Table 1. Irradiation doses and fluence rates

Visible light	UVA1
Fluence rate: 200 mW cm^{-2}	Fluence rate: 25 mW cm^{-2}
Dose (J cm^{-2})	Dose (J cm^{-2})
8	1
40	5
80	10
160	20
320	40
480	60

Abbreviation: UVA1, long-wavelength ultraviolet A.

diascopy, suggesting that the observed skin darkening was indeed due to pigmentary alteration rather than dilatation of cutaneous vasculature. The immediate pigmentation was characterized by being well defined and grayish in color (Figure 2a). With time, the skin color changed to brown and faded rapidly over the course of 2 weeks following irradiation. No erythema was observed at any time point following irradiation.

The diffuse reflectance spectroscopy (DRS) results for oxy-hemoglobin level and melanin content after irradiation with the UVA1 light source were analyzed and graphed. The assessment of oxy-hemoglobin levels is a reflection of erythema clinically, whereas the measurement of melanin content reflects cutaneous pigmentation. There was no dose–response or time-course relationship between UVA1 radiation and oxy-hemoglobin levels assessed using DRS; this finding correlated with our clinical observation of no erythema induced by UVA1 irradiation. In contrast, there was a dose–response correlation between the melanin content and the UVA1 dose delivered at all of the time points studied, corresponding to pigmentation observed clinically.

Confocal microscopy did not show any significant difference in melanocyte density or in the amount of melanin between irradiated and non-irradiated control sites.

Effects of visible light (400–700 nm) on skin types IV–VI
Immediately after visible light irradiation, there was an induction of immediate pigmentation; the lowest dose at which pigmentation developed was 40 J cm^{-2}. As the dose was increased, pigmentation became darker (Figure 2b). Similar to the results of UVA1, pigmentation was still evident after diascopy and more intense in skin type V volunteers. However, in contrast to the UVA1 effect, the immediate pigmentation was characterized by being dark brown from the start and surrounded by ill-defined erythema, which disappeared in less than 2 hours. Furthermore, pigmentation induced by visible light was sustained during the 2-week period of the study and did not fade away even at lower doses.

DRS results for oxy-hemoglobin and melanin after irradiation with the visible light source are illustrated in Figures 3 and 4, respectively. DRS findings showed a direct correlation between the concentration of oxy-hemoglobin and the visible light doses delivered, which correlates with the clinical finding that the higher the dose, the more intense the erythema (Figure 3). The threshold for a minimal perceptible erythema, based on clinical observation and correlation with DRS measurements, is approximately 0.45 in terms of relative difference in absorbance values (irradiated minus control sites). As shown in Figure 3, only the two highest doses, 320 and 480 J cm^{-2}, have values consistently above 0.45 for time points from immediately after exposure (0 hour) to 2 hours, which correlate with erythema observed clinically (Figure 2b). After 2 hours, even at 480 J cm^{-2}, oxy-hemoglobin levels dropped to less than 0.45, consistent with the clinical observation that the erythema was resolved beyond the 2-hour time point. DRS data show that the increase in melanin content was directly related to the dose delivered (Figure 4). For all the doses studied, the melanin contents at 1 day after irradiation were lower than those at the earlier time points; this difference was statistically significant for 160 and 320 J cm^{-2} ($P < 0.05$) doses. At 1 week and 2 weeks after irradiation, for all the doses studied, melanin contents increased above the levels observed at the 1-day time point. This DRS-measured decrease at 24 hours corresponds clinically to the transitional zone between persistent pigment darkening and delayed tanning due to new melanin formation.

Confocal microscopy performed following the highest dose (480 J cm^{-2}) at 2 and 24 hours after irradiation showed redistribution of pigment, in the form of migration of melanin from basal cells to the upper epidermal cell layers, in the irradiated site compared with control.

Histopathological results
The histopathological examination carried out following exposure to the highest dose (480 J cm^{-2}) of visible light at 24 hours after irradiation showed no difference between irradiated and non-inrradiated control sites when stained with hematoxylin and eosin, Acid Orcein, or P53 stains. Specifically, no thermal or actinic damage was observed in the
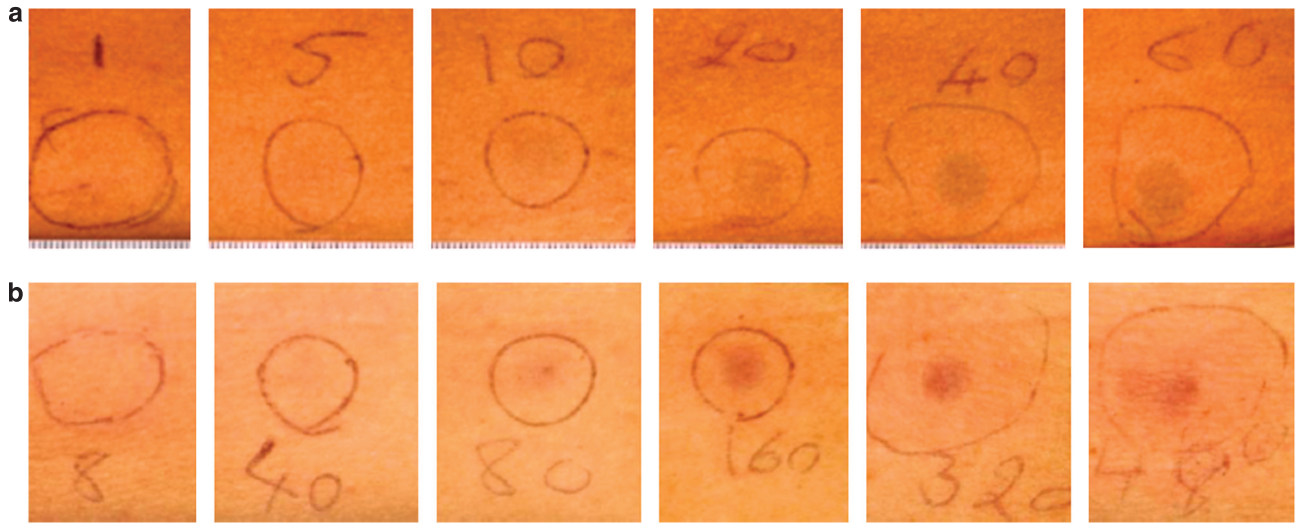
Figure 2. Clinical appearance of irradiated sites immediately after exposure. (**a**) Six photos showing sites irradiated with UVA1 at doses of 1, 5, 10, 15, 20, 40, and 60 J cm^{-2}, respectively, as indicated in the figure; (**b**) six photos showing sites irradiated with visible light at doses of 8, 40, 80, 160, 320, and 480 J cm^{-2}, respectively, as indicated in the figure.
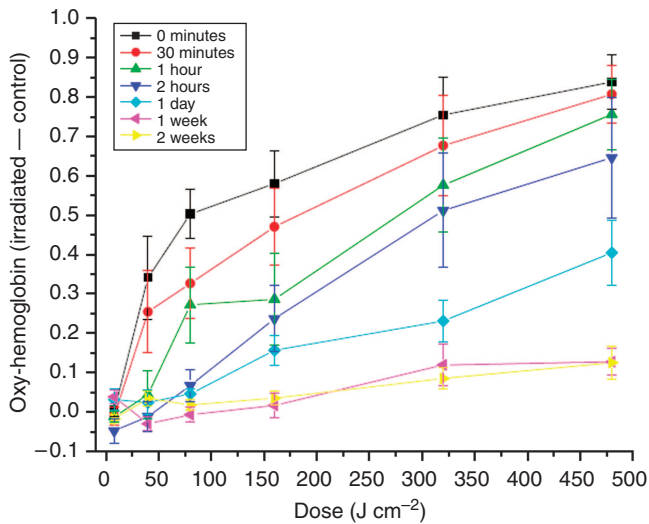

Figure 3. Oxy-hemoglobin concentration. Oxy-hemoglobin concentration, as measured using DRS, at sites irradiated with visible light radiation (8–480 J cm^{-2}) and with measurements taken immediately after irradiation (0 minutes) to 2 weeks later. Oxy-hemoglobin values higher than 0.45 correspond to visually perceptible erythema. Values represent the average difference between the irradiated and control site values of 20 healthy volunteers having skin types IV–VI. Error bars denote standard error.

dermis of the irradiated skin. On the other hand, using the Fontana–Mason stain, there was a redistribution of melanin pigment. This means that in the non-irradiated control site, the pigment was seen just in the basal keratinocytes; whereas in the irradiated site the pigment was redistributed into the keratinocytes in the upper spinous cell layers (Figure 5).

Effects of UVA1 and visible light on skin type II

In contrast to pigmentation induced in individuals with skin types IV–VI, no pigmentation was induced in subjects with skin type II using the same light sources of UVA1 and visible light as well as the same doses that were used for volunteers with skin types IV–VI (Figure 6). DRS results also confirmed our clinical observation, for there was no significant difference between oxy-hemoglobin and melanin concentration measured at the irradiated site compared with the control site in subjects with skin type II.

DISCUSSION

Studies on cutaneous pigmentary changes have focused primarily on UV radiation, especially UVA. Whereas visible light has been used extensively in laser therapy, intense pulse light therapy, and photodynamic therapy, very little is known about its effect on the time course and quality of pigmentation, and its effect on different skin types. Furthermore, the majority of studies done on the effect of UV have focused on Caucasian population. Population statistics in the United States show significantly changing demographics in the past decade. According to the 2000 census, 29% of the United States population, representing approximately 85 million people, are individuals of color (Taylor and Cook-Bolden, 2002).

In 1983, Kollias and Baqer (1984) conducted an *in vivo* study on the changes in pigmentation induced by visible and near-IR light using a polychromatic light source of 390–1,700 nm. They observed that pigmentation could occur without significant UV component. Porges *et al.* (1988) exposed 20 healthy individuals with skin types II, III, and IV to a visible light source of a compact 150-W xenon-arc solar simulator, with a spectral distribution between 385 and 690 nm. Both IPD and immediate erythema faded over a 24-hour period. The residual tanning response remained unchanged for the remaining 10-day observation period. The threshold dose for IPD with visible light was between 40 and 80 J cm^{-2}, whereas the threshold dose for delayed tanning was closer to 80–120 J cm^{-2}. Owing to the lack of

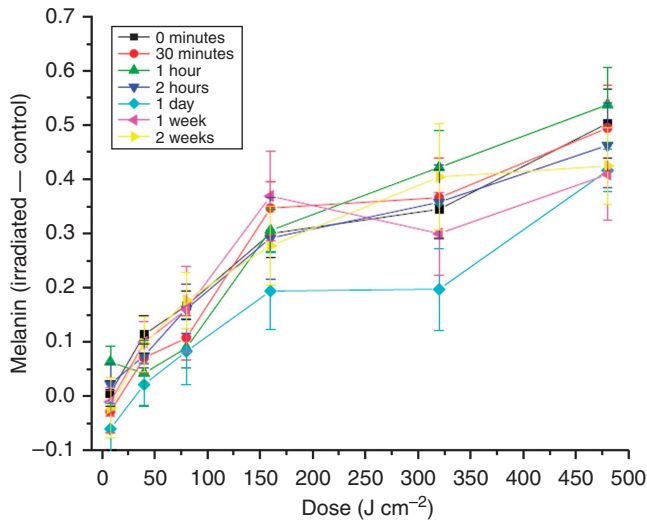**Figure 4. Melanin concentration.** Melanin concentration as measured using DRS at sites irradiated with visible light radiation (8–480 J cm^{-2}) and with measurements taken immediately after irradiation (0 minutes) to 2 weeks later. Values represent the average difference between the irradiated and control site values of 20 healthy volunteers having skin types IV–VI. Error bars denote standard error.

Figure 5. Histological changes at 24 hours after irradiation with 480 J cm^{-2} of visible light. (**a**) Unirradiated control site, pigment was seen just in the basal keratinocytes; (**b**) irradiated site, the circled area highlights the redistribution of melanin pigment into the keratinocytes in the upper spinous cell layers. Fontana-Mason stain; bar = 0.33 mm.

standardization pertaining to the spectrum of visible light producing sources in the aforementioned two studies, it is difficult to compare the results. The filter used in the latter study was a 3-mm Schott GG385 (Schott Optical Company, Duryea, PA), which should have removed most of the short-wavelength UV radiation; however, a part of the long UVA spectrum, together with visible light, was still present in this filtered light source.

In our study, a light source emitting 98.3% visible light was used to evaluate the effect of visible light on skin. In addition, melanocompetent volunteers with skin types IV–VI were used, which are different from the samples evaluated in the aforementioned studies. The results obtained for skin types IV–VI were then compared with those obtained for skin type II. Although there was evident pigmentation following UVA1 and visible light irradiation with threshold doses of 5 and 40 J cm^{-2}, respectively, no pigmentation was induced on skin type II for all doses used and at all the time points studied (Figure 6). These results were also confirmed by DRS, which objectively assess the degree of pigmentation in skin.

When comparing the quality of pigmentation observed following UVA1 and visible light irradiation in skin types IV–VI, it was noted that pigmentation induced by UVA1 was initially gray in color and then turned brown after 24 hours, whereas pigmentation induced by visible light was dark brown from the start (Figure 6). Also, UVA1-induced pigmentation was well defined and not surrounded by erythema at any point of time. This was confirmed by DRS, which showed no increase in the levels of oxy-hemoglobin at any point of time following irradiation. On the other hand, following exposure to visible light, erythema appeared immediately after irradiation surrounding the pigmentation. It started to fade after half an hour and completely disappeared 2 hours after irradiation (Figure 6).
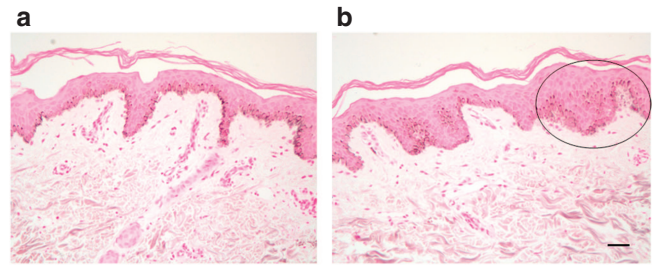
The light source used in this study had three KG5/3 mm filters to block IR radiation, which generates heat. Therefore, only a minimal IR component (1.5%) was detected (Figure 1). It is possible that the erythema following visible light irradiation in skin types IV–VI is due to the fact that heat is produced within melanocompetent skin from the absorption of visible light by melanin pigment. Heat in turn can lead to dilatation of deep dermal vessels. In this scenario, the heat generated would be independent of the heat in the light source; however, it would depend on the concentration of the melanin chromophore and the amount of visible light delivered to the skin. This could be the reason why no skin response was seen in subjects with skin type II.

Pigmentation induced by visible light was darker and more sustained than the pigmentation induced by UVA1 (Figure 6). It should be noted that the UVA1 and visible light doses used in this study are those easily obtained from daily sun exposure. The fluence rate of the solar spectrum of visible light, during clear sky conditions at sea level, is about 15 times higher than that of UVA. Therefore, a dose of 20 J cm^{-2} of UVA would correspond to about 300 J cm^{-2} of visible light for an exposure time of about 1 hour. At 20 J cm^{-2}, pigmentation induced by UVA1 was faint and faded rapidly over the course of 2 weeks; on the other hand, at 320 J cm^{-2} of visible light pigmentation was much darker and remained unchanged until the end of the study period, which was 2 weeks (Figure 6). Thus, our results strongly suggest that visible light could potentially have a significant role in producing darker and longer-lasting pigmentation in populations with skin types IV–VI than UV1 radiation.

When specifically looking for thermal and actinic DNA damage after a single irradiation, histopathological examination of irradiated sites compared with non-irradiated control sites showed no difference using hematoxylin and eosin, Acid Orcein, and P53 stains. A noticeable change observed in our histopathological examination was the redistribution of melanin pigments, in the form of migration of melanin from basal cells to the upper layers of the epidermis, in comparison with the non-irradiated control sites. This redistribution corresponds clinically to the persistent pigment darkening seen in our volunteers 24 hours following irradiation, which was the time when biopsies were taken.
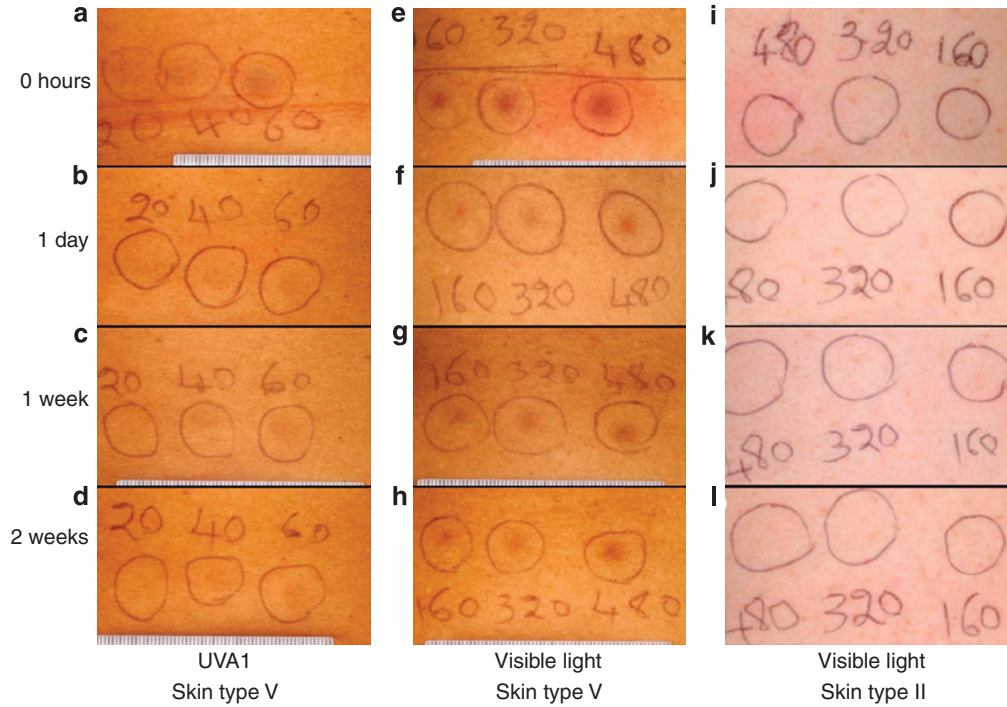
Figure 6. **Clinical photos of skin type V irradiated with UVA1 and visible light and skin type II irradiated with visible light at different time points.** Cross-polarized images of sites irradiated with UVA1 (**a–d**) and visible light (**e–l**) at different times on both type V (**e–h**) and type II (**i–l**) skin. (**a, e**, and **i**) Immediately after irradiation, (**b, f**, and **j**) 1 day after irradiation, (**c, g**, and **k**) 1 week after irradiation, (**d, h**, and **l**) 2 weeks after irradiation.

This work has implications on the use of sunscreens. UV filters are divided into organic (also known as chemical) and inorganic (also known as physical) filters. There is no effective organic filter for visible light. As only optically opaque filters are able to absorb visible light, only optically opaque inorganic filters can protect against visible light. The two generally available inorganic sunscreen agents are zinc oxide and titanium dioxide (Lim and Honigsmann, 2007). Considering that the results of this study showed that visible light can produce sustained dark pigmentation in individuals with skin types VI–VI, there may be a need for the development of filters that protect against visible light. Such filters could be useful for the management of patients with photoaggrevated dermatoses, such as melasma.

In conclusion, this study described a method to assess pigmentation induced by pure UVA1 and visible light, and then applied this method to both type II and IV–VI skin. We have also developed a visible light source that can produce these wavelengths with minimal UV and IR contamination. A difference exists between the quality, time course, and duration of pigmentation produced by UVA1 and pigmentation produced by visible light. Furthermore, the response to UVA1 and visible light irradiation depends on skin type, as no pigmentation was induced on skin type II. Although currently no standardized visible light source is used in all studies, it would be ideal if such a light source could be agreed upon and used in future studies. The fact that visible light can induce dark and relatively sustained pigmentation has a clinical implication on the treatment of photoderma-toses. In addition, it shows the need for sunscreens with better coverage in the visible light range.

MATERIALS AND METHODS

This study was reviewed and approved by the Institutional Review Board, Henry Ford Hospital. Study procedures were followed in accordance with the ethical standards of the Institutional Review Board and the principles of the Helsinki Declaration of 1975. Informed consent was obtained from all participants before the initiation of the study.

Volunteers

To be included in the study, volunteers had to be at least 18 years old, and not taking any photosensitizing drugs. Women who were lactating, pregnant, or planning to become pregnant, and patients with serious systemic disease, immunosuppression, skin cancers, with recent history of vitiligo, melasma, other disorders of pigmentation, and photosensitivity were excluded. The study was conducted throughout the calendar year.

In all, 22 normal healthy volunteers were recruited, 20 of them had Fitzpatrick skin types IV–VI (4 type IV, 12 type V, and 4 type VI) and 2 volunteers had skin type II. Regarding gender, there were 4 males and 18 females, with a mean age of 36 years (20–60 years). We chose the lower back as a non-sun-exposed area to conduct our study, and a non-irradiated nearby skin site served as a control. Volunteers were instructed to avoid sun exposure or tanning beds to the irradiated as well as control areas during the period of the study.

Materials

Light sources and irradiation steps. We used two targeted light sources, a UVA1 light source and a visible light source. The UVA1 light source was a Hamamatsu LightingCure UV Spot Light 200, 240–400 nm, 200 W (Hamamatsu photonics K.K., Shimokanzo, Toyooka-village, Iwata-gun, Shizuoka-ken, Japan). For irradiation

purposes a 9-mm Hamamatsu liquid light guide was used. The UVA1 light source was filtered using one UV Hot Mirror/3-mm (Newport Thin Film Laboratories, Chino, CA), UG11/1-mm, and WG 345/3-mm filter (Schott Optical Company). The light source irradiance spectrum was measured using a calibrated Optronics OL754 spectroradiometer (Optronics, Orlando, FL).

The visible light source used was the Fiber-Lite Model 170-D (Dolan-Jenner Industries, Boxborough, MA) with a 150 W quartz-halogen lamp; a straight 8-mm Dolan-Jenner glass optical fiber was used for irradiation. In all three KG5/3-mm Schott glass filters and one GG400/3-mm (Schott Optical Company) were used to filter IR and UV radiation from the light source, respectively. Spectral irradiance of the visible light source was measured using a calibrated Optronics OL750 spectroradiometer (Figure 1).

The fluence rate for both light sources was adjusted to 25 and 200 mW cm^{-2} for UVA1 and visible light, respectively, using a calibrated Oriel Thermopile Model 71767 (Oriel, Stamford, CT).

An acrylic holder, to which the optical fibers and the liquid light guide were attached, was specifically designed for the study. Next, the optical fiber holder was fixed on the lower backs of volunteers during the period of delivery of the required doses.

The delivered ranges for the irradiation doses are shown in Table 1. Sites were irradiated with visible light ranging from 8 J cm^{-2} to 480 J cm^{-2} and UVA1 from 1 J cm^{-2} to 60 J cm^{-2}.

Clinical and instrumental assessment

Clinical and instrumental assessment for immediate pigment darkening, persistent pigment darkening, erythema, and edema was done at seven time points: immediately after irradiation, and then at 30 minutes, 1, 2 hours, 1 day, 1 week, and 2 weeks following irradiation.

For each time point, cross-polarized digital photography was used to document the exposed sites. The advantage of a cross-polarized filter is that it prevents the glare coming from the skin surface and thus leads to a better visualization of sub-surface chromophores. Erythema and pigmentation were also assessed by measuring the levels of oxy-hemoglobin and melanin, respectively, using DRS. Biopsies were carried out at sites exposed to the highest visible light dose used at 24 hours; biopsies from an unexposed surrounding site served as control specimens.

Diffuse reflectance spectroscopy. DRS is an analytical tool used to investigate the optical properties of an absorbed molecule, and to measure the scattering and absorption properties of the skin in which a beam of light penetrates. The DRS instrument consisted of a quartz halogen light source (Ocean Optics, Boca Raton, FL), a bifurcated fiber bundle (Multimode Fiber Optics, East Hanover, NJ), an S2000 spectrometer (Ocean Optics), and a laptop computer. Before data acquisition, the spectrophotometer was calibrated using pure white tile and a dark background for compensation. Apparent concentrations of hemoglobin and melanin were calculated from the diffuse reflectance spectra as described by Kollias *et al.* (1992). Measurements were taken from the irradiated site and the adjacent unirradiated control site. In order to calculate the changes in the chromophore concentrations, the value for the control site was subtracted from that for the irradiated site. Therefore, if there is no

detectable change in the irradiated site, the value would be zero. Based on clinical correlation, the threshold for minimal perceptible erythema that correlated with DRS measurements was 0.45 in terms of relative difference in absorbance values (irradiated minus control sites).

Confocal microscopy. Pigmentation induced by the highest dose of UVA1 and visible light as well as the control site was then assessed using a hand-held confocal microscope (VivaScope 3000, Lucid, Rochester, NY) designed specifically for clinical imaging of the skin, at 2 and 24 hours after the highest dose of visible light irradiation.

Statistical analysis

DRS measurements were taken in triplicate with the 2.5-mm-diameter fiber on the irradiated as well as control skin sites. The results were presented as means and standard deviation; standard least square statistical model analysis was carried out using JMP 7 (SAS Institute, Cary, NC).

Histopathology and immunohistochemistry

Histological changes induced by the highest dose of visible light were assessed by histopathological examination 24 hours after irradiation, for the irradiated site and for the non-irradiated area (control) using hematoxylin and eosin, Fontana-Masson, AcidOrcein, and P53 stains.

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