Differences in visible light-induced pigmentation according to wavelengths: a clinical and histological study in comparison with UVB exposure

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Summary
The visible light spectrum is wide, and it can be hypothesized that all the wavelengths between 400–700 nm do not induce the same photobiological effects on pigmentation. We assessed the potential pro-pigmenting effects of two single wavelengths located at both extremities of the visible spectrum: the blue/violet line ($\lambda = 415$ nm) and the red line ($\lambda = 630$ nm). We made colorimetric, clinical, and histological assessments with increasing doses of those lights on healthy volunteers. Then, we compared these irradiations to non-exposed and UVB-exposed skin. Colorimetric and clinical assessments showed a clear dose effect with the 415-nm irradiation, in both skin type III and IV subjects, whereas the 630 nm did not induce hyperpigmentation. When compared to UVB irradiation, the blue–violet light induced a significantly more pronounced hyperpigmentation that lasted up to 3 months. Histological examination showed a significant increase of keratinocyte necrosis and p53 with UVB, as compared to 415- and 630-nm exposures.

Until recent years, photodermatology studies were mainly focused on the ultraviolet (UV) domain of the electromagnetic spectrum which is known to have sufficient energy to produce biological effects in the skin (Svobodova et al., 2006). Because the damage induced by short UVA (320–340 nm) is similar to those of the UVB, the UVA domain was divided into UVA1 (340–400 nm) and UVA2 (320–340 nm) (Suh et al., 2007). Visible light (400–700 nm) was considered, until recently, as having no significant cutaneous photobiological effect other than a thermal effect. Since the last two decades, the developments in the field of photodynamic therapy and various dermatological treatments using lasers and light-emitting diodes in the visible gave rise to numerous studies and to reconsider the cutaneous effects of visible light. In a recent study, the quality of the pigmentation observed after irradiation with UVA1 was compared with irradiation with visible light on skin type IV–VI subjects (Mahmoud et al., 2010). It was noted that the visible light induced pigmentation that lasted for the 2-week study

Significance
This study demonstrates that various wavelengths of the visible part of solar spectrum have different effects on skin pigmentation. Although inducing a potent and long-lasting hyperpigmentation, the 415 nm light does not lead to a significant increase of p53, suggesting that other pathways are involved in this induced melanogenesis. The doses of blue-violet light that stimulate the pigmentation are achieved in less than 2 h of sun exposure, suggesting that the shorter wavelengths of visible light could play a key role in the worsening of some pigmentary disorders after sun exposure, despite the use of sunscreens with UVB and UVA protection.
period, whereas the pigmentation produced by UVA1 quickly faded during the study period. Neither pigmentation nor erythema was observed on one subgroup of skin type II subjects using the same experimental conditions. However, the visible light spectrum is wide, and it can be hypothesized that all the wavelengths between 400–700 nm do not induce the same photobiological effects on pigmentation. The in vitro data obtained, for example, on fibroblasts, clearly show that various wavelengths of the visible or the infrared can have opposite biological effects (McDaniel et al., 2010). Therefore, it is crucial to evaluate the photobiological effect of different wavelengths of the visible spectrum on pigmentation. We assessed in healthy subjects the potential pro-pigmenting effects of two single ‘pure’ wavelengths located at both extremities of the visible domain: the blue/violet line ($\lambda = 415$ nm) that is potentially pro-pigmenting and the red line ($\lambda = 630$ nm) that was shown active on proliferation and differentiation of melanocytes in vitro (Lan et al., 2006). To have the UV-induced pigmentation as reference, a UVB-exposed zone was also included in the study design.

In a first step, we determined the minimal pigmentary dose (MPD) for 415- and 630-nm irradiations. The colorimetry ITA angle that is inversely correlated with pigmentation (Del Bino and Bernerd, 2013) displayed a clear dose effect with the 415-nm irradiation (Figure 1A). When angle ITA is displayed for skin types separately, both sets of curves are similar but shifted by 20 ITA units with lower values for skin type IV and higher for skin type III (Figure S1A, B). The pronounced decrease of ITA angle observed one hour after exposure is probably due to a mix between immediate pigmentation and erythema (which darkens the skin color). No effect on the ITA angle was observed with the 630-nm irradiation (Figure 1B and Figure S1C, D). A substantial IPD (immediate pigment darkening) was observed at 1 h after exposure to blue light, followed by a brown color hyperpigmentation that was maintained till the end of the study (Day 22) (Figure 1C). The pigmentation level was higher on skin type IV subjects compared with skin type III ones (Figure 1D–K). One hour after exposure, the pigmentation was accompanied by weak-to-moderate erythema. This erythema disappeared after 24 or 48 h, depending on the subjects. On the 630-nm exposed zones, no pigmentation and no erythema were observed whatever the time point is. Therefore, the MPD could not be determined on the 630-nm exposed zones, and the dose planned for the step 2 was fixed to 150 J/cm$^2$. The MPD and MED values are summarized in Table S1 for both skin types.

In a second part of the study, new test zones were used for light combination evaluation (Figure S2) and were assessed at 24 h and 2 weeks after exposure. The evolution of ITA angle for combined skin type and for the different types of exposure is illustrated in Figure 2A. Statistical analyses allowed establishing the following ranking of exposure types on the induced pigmentation:

![Figure 1](image-url)

**Figure 1.** (A) Evolution of the ITA angle as a function of the 415-nm doses (n = 12); (B) and 630-nm doses (n = 12); (C) evolution of the clinical pigmentation score as a function of the 415-nm doses (for combined skin types, n = 12). (D) Clinical example of pigmentation induced with increased doses of 415 nm light in skin type III subject 24 h after irradiation; (E) 7 days after irradiation; (F) 14 days after irradiation; (G) 21 days after irradiation; (H) in skin type IV subject 24 h after irradiation; (I) 7 days after irradiation; (J) 14 days after irradiation; (K) 21 days after irradiation.
A group containing the control zone, whose pigmentation is significantly lower than all others (except after week 1 where it is not different from R100%).

The group R100% and R70/B30%.

The group R50/B50%, the UVB zone, and R30/B70%.

The group containing the zone B100% that is not always different from the previous group (in particular from the zone R30/B70%).

The evolution of clinical score of pigmentation as a function of types of exposure and skin types is illustrated in Figure 2B. The pigmentation score was somewhat higher for skin type IV compared with skin type III whatever the type of exposure is. On the other hand, as expected, the UVB-induced pigmentation was weak at 24 h after exposure, whereas all other light combinations induced higher levels of pigmentation. Overall, the higher levels of pigmentation score were produced by the B100% and the R30/B70%. Statistical comparisons, using combined or separated skin types, indicate that pigmentation score observed on the non-exposed control zone was always significantly lower than that measured on all exposed zones including the R100% one. Statistically, one and 2 weeks after exposure, the UVB-induced pigmentation was not different from that of the B100%, the R30/B70% and R50/B50% scores. Erythema was observed 24 h after exposure with the highest scores for the UVB-exposed zone (mean ± SD = 1.4 ± 0.7), followed by the B100% (0.5 ± 0.5), the B70/R30% (0.1 ± 0.2) and the B50/R50% (0.04 ± 0.1). No erythema was observed on other test zones at 24 h and on all test zones at one week and two weeks after exposure. Histology results indicated that cellular damages were mainly produced by UVB exposure (higher keratinocyte necrosis, higher number of melanophages and higher p53 positivity in keratinocytes) and were significantly more important than those induced by B100% and other types of exposure (Figure 3 and Figure S4). No significant changes in melanin content can be found using Fontana–Masson staining. Using MITF staining, we did not observe significant difference in melanocyte number between control and UVB, blue or red irradiations at 24 h and 7 days, respectively (Figure S5). There was no difference either with the Ki67 labeling. The oxidative stress was assessed by the 8-oxoguanine labeling (Figure S3). It showed a mild-to-moderate positive mitochondrial staining concerning the basal keratinocytes and perivascular fibroblasts, at 24 h post-irradiation, and only for the UVB and the 630-nm

Figure 2. (A) Evolution of ITA angle as a function of exposure types. If bars are associated with different letters, that means they are significantly different (P < 0.05). For example, 24 h after exposure, control zone (a) is different from 100% R (b) and 70% R30% B (b) which are not different. (B) Evolution of the pigmentation score as a function of exposure types and skin types. Clinical example of pigmentation after exposure (upper left, 70% blue and 30% red; upper right, non-exposed; lower left, UVB; lower right, 100% blue); (C) 24 h after irradiation; (D) after 1 week; (E) after 2 weeks. CTL, non-exposed zone; R, red light (630 nm); B, blue–violet light (415 nm).


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![Figure 3. Histological analysis with hematoxylin and eosin staining and p53 staining at 24 h for control, 415-nm, 630-nm, and UVB irradiation.](image)

conditions. Synthesis of histopathology and immunohistochemistry results is presented in Table S2.

Studies on light-induced skin pigmentation have focused mainly on the UV part of the solar spectrum. Up to now, very few studies have been carried out to study visible light effects on skin pigmentation (Kollias and Baqer, 1984; Mahmoud et al., 2010; Porges et al., 1988; Ramasubramaniam et al., 2011). In the previous studies, the spectral bands were fixed using optical filters. This process allowed eliminating a great proportion of UV light from the visible exposures, but due to the transmittance profile of the filters, traces of UV light, in particular UVA, could have been still present. Moreover, while it is now clearly demonstrated and accepted that UV A1, A2, B, and C induce significant different biological effects on the skin, most studies on visible light have used the entire visible part of the solar spectrum (Kollias and Baqer, 1984; Mahmoud et al., 2010; Porges et al., 1988; Ramasubramaniam et al., 2011). We show here for the first time that the blue/violet irradiation induces a dose-correlated hyperpigmentation, while the red line induces no or very slight modification of the pigmentation of the skin in vivo. Although red light was reported to enhance the melanocyte proliferation in vitro (Lan et al., 2006), our results show that it does not have significant effect on pigmentation in vivo, at least with physiological doses, and the combination of red and blue light did not lead to a synergistic effect on pigmentation. These results support the fact that the wavelengths of the visible part of the solar spectrum do not have similar biological properties in the skin, especially on pigmentation. As the hyperpigmentation was still highly pronounced 2 weeks after the 415-nm irradiation, we asked the subjects to come back after 3 months. Eleven subjects accepted to return. All of them still presented a marked hyperpigmentation on the area exposed to the blue–violet light (Figure S6). This blue light-induced pigmentation remained more pronounced than the UVB-induced hyperpigmentation (Figure S7). Interestingly, while 1.5 MED of UVB induces a significant necrosis of keratinocytes, and a strong increase in p53 expression, those effects were found significantly less pronounced with the blue–violet irradiation. When those histological markers were compared at doses inducing a comparative pigmentation (1.5 DEM for UVB and 50% dose of the 415 nm light), keratinocyte necrosis and p53 expression were almost absent and significantly lower as compared to those induced by UVB. Quite surprisingly, we did not observe any increase in 8-oxoguanine expression after one 415-nm irradiation at the doses used in this study. Although the evaluation of the oxidative stress is less accurate in tissue as compared to cultured cells, the 8-oxoguanine expression has been reported to be a good marker of this oxidative stress in the skin (Kunisada et al., 2005). It has been demonstrated that mitochondrial DNA (mtDNA) is a critical cellular target for ROS. Thus, ROS exposure leads to increased mitochondrial-generated ROS (Yakes and Van Houten, 1997), and mtDNA damage is a powerful marker of photoinduced skin aging (Birch-Machin et al., 2013; Tulah and Birch-Machin, 2013). Our results suggest that the oxidative stress should not play a significant role in the blue–violet light-induced hyperpigmentation. The p53 protein is known to play a key role in UVB-induced hyperpigmentation (Cui et al., 2007). As p53 appears to be significantly less induced after blue–violet irradiation, the hyperpigmentation observed should rely on other mechanisms. The biological pathway involved in this blue–violet light-induced hyperpigmentation warrants to be further studied as it could provide new therapeutic targets to prevent this visible light-induced hyperpigmentation. The mean solar intensity received at ground level is about 1000 W/m² with a fraction of 44% of visible light (i.e., 440 W/m²) [ASTM Standard, 2008]. If we consider the visible spectrum from 400 to 700 nm, the blue–violet part represents about one-fifth of the visible energy and thus 8.8 mW/cm². Importantly, the 100% dose of blue–violet light used in this study was 87.5 J/cm², which corresponds to about 2 h and 45 min of sun exposure during summer. The 50% dose that induced similar pigmentation as 1.5 MED of UVB was 43.8 J/cm², which corresponds to 83 min of sun exposure. As a comparison, the 1.5 MED of UVB corresponded to 25 and 45 min of sun exposure during the peak of UVB emission for skin type III and skin type IV, respectively. Although the length

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of sun exposure is higher to reach these doses for blue–violet light as compared to UVB, it remains clinically relevant. Many pigmented disorders, and in first places melasma and post-inflammatory hyperpigmentation, worsen after sun exposure and especially in the summer period despite the use of sunscreens with a broad and efficient UVB and UVA protection. It might be hypothesized that the blue–violet part of the visible light could play a key role in this phenomenon. Using sunscreens effective against the entire visible light spectrum is almost impossible to use in daily practice. By showing that different wavelengths of the visible part of the solar spectrum have different effects on the skin pigmentation, our results suggest that it is probably not necessary to get a protection against the entire part of the visible light and that a tinted sunscreen providing a protection in the shorter wavelengths of the visible light could be helpful in those pigmented disorders. These data open and foster new research avenue in the mechanisms involved in melanogenesis and in the prevention of pigmented disorders.

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Conflict of interest

This study was conducted with the technical and financial support of Deleo luminotherapy, St Raphael, France.

References


Supporting information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Evolution of the ITA angle as a function of the 415 nm doses [(A) for skin type III, n = 6; (B) for skin type IV, n = 6] and 630 nm doses (C) for skin type III, n = 6; (D) for skin type IV, n = 6.

**Figure S2.** Test zones locations.

**Figure S3.** Immunohistochemical analysis for Oxo-8.

**Figure S4.** Immunohistochemical analysis for p53.

**Figure S5.** Immunohistochemical analysis for MITF.

**Figure S6.** Clinical example of pigmentation induced with increased doses of 415 nm light (10, 30, 60, 90, 110 and 150 J/cm2) in skin type IV subject, 3 months after one single irradiation.

**Figure S7.** Clinical example of pigmentation 3 months after exposure in skin type IV (upper left, 70% blue and 30% red; upper right, non-exposed; lower left, UVB; lower right, 100% blue).

**Data S1.** Methods.

**Table S1.** MED and MPD values as a function of skin types (mean ± SD).

**Table S2.** Synthesis of histology results.