Melanin Photosensitization and the Effect of Visible Light on Epithelial Cells

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Abstract

Protecting human skin from sun exposure is a complex issue that involves unclear aspects of the interaction between light and tissue. A persistent misconception is that visible light is safe for the skin, although several lines of evidence suggest otherwise. Here, we show that visible light can damage melanocytes through melanin photosensitization and singlet oxygen (¹O₂) generation, thus decreasing cell viability, increasing membrane permeability, and causing both DNA photodamage and necro-apoptotic cell death. UVA (355 nm) and visible (532 nm) light photosensitize ¹O₂ with similar yields, and pheomelanin is more efficient than eumelanin at generating ¹O₂ and resisting photobleaching. Although melanin can protect against the cellular damage induced by UVB, exposure to visible light leads to pre-mutagenic DNA lesions (i.e., Fpg- and Endo III-sensitive modifications); these DNA lesions may be mutagenic and may cause photocaging, as well as other health problems, such as skin cancer.

Introduction

Humans and other animals produce melanin mainly for protection against exposure to ultraviolet B (UVB) radiation [1]. In contrast to UVB radiation, which is directly absorbed by DNA, UVA radiation acts essentially through photosensitization, which generates a triplet species, ¹O₂, and subsequently generates other radical species that can damage both DNA and other epithelial cell biomolecules [2–4]. UVA penetrates deeper in the dermis than does UVB, and it is the major UV source responsible for skin photoaging and the development of several types of skin cancer [5].

However, the visible portion of the spectra has garnered much less attention, despite several scientific reports that have described the effect of visible and IR irradiation on the skin [6,7]. Kielbassa and co-workers [8] and Kvam and Tyrrel [9] showed that irradiating Chinese hamster cells and dermal fibroblasts, respectively, with UVA and visible light induced oxidative damage in DNA. More recent studies showed that visible light disturbs the epidermal barrier, and this disturbance induces pigmentation and inflammatory responses [10,11]. However, a great deal of controversy remains concerning the effect of visible light on the skin, mostly because of the lack of a mechanism that explains the observed effects [12].

It has been shown that, in addition to UV [13], visible light also induces pigmentation in certain skin types. Mahmoud and co-workers [11] showed that visible light induces skin darkening in people with skin types IV and V but not in individuals with type II skin. The darkening induced by visible light depended on the pre-irradiation melanin content of the skin, suggesting that melanin may directly damage skin cells upon exposure to visible light.

The literature describes both protective and damaging roles for melanin [14,15]. Two independent studies using Xiphophorus, which is a fish that is highly susceptible to melanoma, showed that the action spectra for both melanoma induction [16] and the photo-induced generation of reactive species [17] extend to visible wavelengths and that the shapes of the action spectra correspond to the shape of the melanin absorption spectrum. Despite the lack of a mechanistic explanation for the observations, these articles highlight the importance of understanding the role of the excited species that are generated after melanin is excited by visible light. In an earlier publication, we showed that melanin can act as a photosensitizer, leading to ¹O₂ generation after excitation with visible light [18]. Singlet oxygen can react with proteins, nucleic acids and membranes [19] (Figure S1); consequently, melanin photosensitization is likely involved in the phototoxicity of visible light, which is the main hypothesis that we aim to demonstrate herein.
dosimeter (VLX-3W, France). The irradiances of the light source was measured at eight different areas in this irradiator using a and evaporation of the initial NaOH solid (1 g) with D2O (10 g).

Available. NaOD was prepared using three cycles of dissolution as received. All other materials were the best analytical grade and BSA from Sigma-Aldrich (either USA or Germany) were used.

purity value refers to the amount of DHPNO2 obtained relative to N, N-170 to 190 mmol/L with a purity of approximately 84%. This filtration. The solutions were obtained at concentrations between 170 to 190 mmol/L with a purity of approximately 84%. This purity value refers to the amount of DHPNO2 obtained relative to the total amount of (DHPNO2). Eumelanin and pheomelanin were synthesized as described by Haywood et al with modifications [21]. Eumelanin was prepared from L-tyrosine (2.5 mg/mL) in pH 7.4 phosphate buffer (50 mM) and mushroom tyrosinase (150 U/mL) in bovine serum albumin (BSA) solution (5 mg/mL). Pheomelanin was synthesized from L-dopa (0.5 mg/mL) and L-cysteine (1.5 mg/mL) in pH 7.4 phosphate buffer (50 mM) and mushroom tyrosinase (100 U/mL). The reactions were performed at room temperature with stirring for 24 h. We also used eumelanin and pheomelanin samples that were kindly provided by Dr. S. Ito [22]. The samples from our lab and those from Dr. Ito’s lab behaved identically with regard to 1O2 generation.

Equipment
The visible and UV light irradiator (Novatec, Brazil) included temperature and humidity sensors. Both variables were maintained during the experiments. The irradiation (mW/cm²) was measured at eight different areas in this irradiator using a dosimeter (VLX-3W, France). The irradiances of the light source were 3.0 mW/cm² and 3.3 mW/cm² in the UVB and visible regions, respectively. For UVB, 25 min of irradiation provided 4.5 J/cm². For the visible region, 30 min of irradiation provided 6.7 J/cm². 30 min of irradiation provided 36 J/cm², and 300 min of irradiation provided 72 J/cm².

Cell absorption/emission was quantified using a plate reader (Tecan Infinite 200M USA). The 1O2 measurements were performed in a specially designed instrument [18,19,23] consisting of a Surelite III laser (355 nm and 392 nm, 5-ns pulses, 10 pulses/ s, 1 nJ/pulse; Continuum Lasers), cuvette holder, silicon filter, monochromator, liquid-nitrogen-cooled near infrared photomultiplier tube (NIR-PMT R5509) from Hamamatsu (Hamamatsu Co., Bridgewater, NJ, USA) and a fast multiscaler analyzer card with a resolution of 5 ns/channel (MSA-300; Becker & Hickl, Berlin, Germany). The signal was acquired either from a cell cuvette or directly under a fluorescence microscope (Nikon Eclipse Ti, USA). Fluorescence/transmission microscopy images were acquired from an Axiovert 200 microscope or an LSM 510 laser confocal microscope (Zeiss, Germany). The comet assay images were obtained using fluorescence microscopy (Olympus BH-2, USA). ImageJ Launcher was used for the confocal image analyses (National Institutes of Health, Bethesda).

Methods
Reagents
All solvents were spectroscopic grade. Water was distilled from an all-glass apparatus and further purified via a Millipore Milli-Q system. D2O (99%), tyrosine (Ty), ammonium chloride (NH4Cl), the enzymes Fpg and Endo III, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), KCl, Na2EDTA, HEPES and BSA from Sigma-Aldrich (either USA or Germany) were used as received. All other materials were the best analytical grade available. NaOD was prepared using three cycles of dissolution and evaporation of the initial NaOH solid (1 g) with D2O (10 g).

Melanogenesis, irradiation and viability
The cells were plated (2×10^4 cells/mL) and, after 24 h, were treated with 0.5 mM Tyr (Sigma-Aldrich, Germany) and 10 mM NH4Cl (Labsynth, Brazil) for 48 h. This protocol increases the melanin production of melanocytes, and the resulting melanocytes are referred to as M-+++. The cells were irradiated in PBS (8 g/L NaCl, 0.20 g/L KCl, 1.15 g/L Na2HPO4, and 0.2 g/L KH2PO4). We applied 4.5 J/cm² of UVB irradiation and 36 J/cm² of visible irradiation. The cell density was evaluated using acridine orange fluorescence (excitation 488 nm, emission 513 nm). The cell viability was evaluated using MTT colorimetric and crystal violet assays [29]. Damage to the cytoplasmic membrane was quantified using propidium iodide incorporation. Apoptotic cell death was characterized using caspase-3 activation (Cell Signaling Technology, USA).

B16-F10 cell viability after 24 h of a DHPNO2 treatment
The B16-F10 cells (CT and M-+++) were treated with a solution containing RPMI and 10 mM DHPNO2 in the absence of serum for 2 h. In control assays, the cells were treated with a solution containing RPMI and 10 mM DHPNO2 (decomposition product of DHPNO2) in the absence of serum for the same time period. After this period, the cell medium was changed to a normal culture medium (RPMI 1640 (Cultilab) supplemented with fetal cell serum (7.5%) from Gibco and with sodium bicarbonate (0.8 mM), HEPES (20 mM), and gentamicin (50 mg/mL) from Sigma-Aldrich. Cell viability was determined using an MTT assay 24 h after the DHPNO2 treatment.

Melanin quantification
The melanin content was quantified as previously described [30]. The B16-F10 cells were seeded in 96-well plates (1×10^4 cells/mL), and after 24 h, they were treated with 0.5 mM Tyr (Sigma-Aldrich Germany) and 10 mM NH4Cl (Labsynth Brazil) for 48 h. After incubation, a portion of the cells was centrifuged and suspended in 1 M NaOH (Labsynth Brazil). The other portion was maintained in PBS (8 g/L NaCl, 0.20 g/L KCl, 1.13 g/L Na2HPO4, and 0.2 g/L KH2PO4) for protein quantification. Both aliquots were lysed in a Branson Sonifier 450 (USA)

Cell culture
Several cell lines, which are available commercially, were received as gift: B16-F10 [24]; HaCaT [25]; J774 [26] and SK-mel 28 [27]. B16-F10, HaCaT and Hela cells were cultivated in Dulbecco’s Eagle (DMEM) culture medium (Sigma-Aldrich). J774 and SK-mel cells were cultivated in RPMI 1640 culture medium (Sigma-Aldrich). Both media were supplemented with 10% FSB (Gibco/BRL, Life Technologies), 4 mM L-glutamine (Sigma USA), 100 U/mL penicillin (Sigma USA) and 100 mg/mL of streptomycin (Nissui Setyaku) and inoculated at 5% CO2 and 37°C. Primary skin cell cultures (melanocytes) were obtained from the foreskins of University Hospital (Hospital Universitario – HU-USP) patients [28]. The project was reviewed and approved by the Research Ethics Committee of the University Hospital (Av. Prof. Lineu Prestes, 2, 2563-Cidade Universitária-CEP 05508-000; + (55)1/30919200, São Paulo, Brazil) protocol# 943/09. The experiments were performed with each subject’s understanding and written consent, and the study methodologies conformed to the standards set by the Declaration of Helsinki. The melanocytes were maintained in 254 CF medium (SKU# M-500-254CF; Cascade Biologies, USA) with human melanocyte growth supplement (HMGS – SKU# S-002-5; Cascade Biologies, USA).
Melanin was quantified by measuring the absorption at 470 nm in a Tecan Infinite 200M plate reader using a standard curve for commercial melanin (Sigma Aldrich Germany) [30]. The total protein content was determined using the Bradford method [31]. Melanin is expressed as mg of melanin/mg of protein.

Comet assay

Comet assays were performed according to optimized protocols [32,33]. B16-F10 cells were plated at 1x10^5 cells/mL in culture medium. Twenty-four hours after seeding, the cells were treated with 0.5 mM Tyr (Sigma-Aldrich, Germany) and 10 mM NH4Cl (Labsynth, Brazil) for 48 h. After incubation, the cells were irradiated in PBS (8.20 g/L NaCl, 1.15 g/L Na2HPO4, and 0.2 g/L KH2PO4) using 36 J.cm^-2 or 6 J.cm^-2 of visible light. We mixed 30 μL of a 1x10^6 cell suspension with 100 μL of agarose (Sigma USA) (0.5% concentration in PBS) and distributed the mixture on slides that were pre-coated with agarose (Sigma USA) (1.5% concentration in PBS) and incubated on ice.

After solidifying, the cells were lysed in the dark using a high-salt alkaline buffer (0.5 M NaCl, 0.1 M EDTA, 0.01 M Tris, and 1% Triton X-100, pH 10). For samples irradiated with 36 J.cm^-2 or 6 J.cm^-2, the slides were placed in electrophoresis buffer (0.3 M NaOH and 1 mM EDTA, pH 13, cooled in a refrigerator) in the dark for 30 min. Electrophoresis was performed in a cold-storage room, in the dark, using a power supply (ESP 301; GE USA) with the same buffer for 30 min at 25 V. After electrophoresis, the slides were neutralized using 0.4 M Tris at pH 7.5 and fixed in ethanol. In the protocol used to evaluate the direct DNA damage from melanin photosensitization, the cells were treated with 6 J.cm^-2 of visible light, and before electrophoresis, the slides were treated with 0.2 U of Fpg or Endo III enzymes (Sigma-Aldrich, USA) in buffer (0.1 M KCl, 0.5 mM Na2EDTA, 40 mM HEPES and 0.2 mg/mL BSA at pH 8.0) for 30 minutes at 37°C. Subsequently, the DNA was stained with ethidium bromide (10 μg.mL^-1), excited at 515 nm and observed using a fluorescence microscope (Olympus BH-2, USA) [34].
1O2 generation

The B16-F10 cells were seeded in six-well plates (2×10^5 cells/mL), and after 24 h, they were treated with 0.5 mM Tyr (Sigma-Aldrich, Germany) and 10 mM NH4Cl (Labsynth, Brazil) for 48 h. The cells were washed in PBS (8 g/L NaCl, 0.20 g/L KCl, 1.15 g/L Na2HPO4, and 0.2 g/L KH2PO4), removed from the plates using a cell scraper and resuspended in D2O saline solution. We obtained 1O2 emission spectra by measuring the emission intensities from 1180 to 1360 nm with 1- to 5-nm steps using the equipment described above.

Statistical analysis

The experiments were performed with at least as three independent repetitions. The statistical analyses were assessed using Student’s t test and Microcal Origin software (version 7.0); P<0.05 was considered statistically significant.

Results

The excitation of melanin with either UVA (355 nm) or visible (532 nm) light generated the characteristic 1O2 NIR emission spectra with a maximum centered at 1270 nm, which is a fingerprint of the O2 (a’18A→O2(X3^2Σg+)) transition (Fig. 1). Sodium azide suppression confirmed this assignment (Fig. 1B, insert) [18,19]. The emission intensity was stronger after melanin was excited by UVA than after excitation by visible light, but this difference is due to the greater melanin absorption at 355 compared with that at 532 nm; thus, the 1O2 generation efficiency does not vary depending on source of irradiation (UVA or visible).

Furthermore, the 1O2 emission from pheomelanin was 30% more intense than that from eumelanin (Fig. 1B), and visible light irradiation substantially decreased the absorption (Fig. 1C) and the 1O2 generation by eumelanin (Fig. 1B); in contrast, the decrease (~7%) in 1O2 generation by pheomelanin was substantially smaller (Fig. 1B). The chemical reaction underlying eumelanin photobleaching is the addition of 1O2 to the double bond at the C3 of the indole group with the consequent hydroperoxide formation [18]. This type of photoproduction was not detected from the pheomelanin photolysis.

To understand the potential effects of melanin photosensitization on epithelial cells, we compared the UVB and visible light photosensitivity using cell lines that express different amounts of melanin (Fig. 2). The irradiation doses were selected to mimic the exposure of an individual to ~10 minutes of a sunny day in Brazil. Notably, the cells expressing more melanin had a higher survival rate after they were challenged with UVB, which is consistent with previous results [30]. However, the darker cells suffered from high phototoxicity from visible irradiation (Fig. 2), providing strong evidence that the phototoxicity from visible irradiation is related to the amount of melanin.

We then tested the visible light toxicity in two melanocompetent cell lines (B16-F10 and human Caucasian melanocytes) under two different melanin production regimens (Fig. 3; i.e., basal level or control (CT) and induced melanogenesis (M+++)) [30]. Melanocytes in a culture clearly differ from skin melanocytes; thus, this system is a well-known, good experimental model for testing the cellular response to environmental challenges [35].

UVB exposure reduced the viability by 40% in B16-F10 CT (left), while pigmented cells (M+++; right) showed only a ~9% reduction (Fig. 3). Therefore, the higher melanin content protected the pigmented cells from UVB damage, which is consistent with previous data from the literature [30,36,37]. The effect of visible light was opposite to the effect observed for UVB irradiation. Furthermore, upon irradiation with visible light (36 J·cm⁻²), the control cells for both the Caucasian melanocytes (H36) and the B16-F10 (M36) cells (left side, Fig. 3B) only showed a ~5% decrease in cell viability. At 72 J·cm⁻², the viability decrease was also small (i.e., 15%; M72, left side, Fig. 3B). However, when the cells were pigmented and treated with 36 or 72 J·cm⁻² of visible light, both cell lines exhibited substantial decreases in viability (50% for H36, 25% for M36 and 40% for M72), which clearly demonstrates that the presence of melanin increases visible light phototoxicity. Moreover, the comet assay showed that the level of DNA fragmentation was higher in the pigmented cells than in the CT cells (Fig. 3B-images), which is consistent with increased visible light phototoxicity for the increased level of intracellular melanin. The mechanism of cell death was mainly necro-apoptosis; structural levels of propidium iodide were incorporated (cytoplasmic membrane damage, Fig. 3C), and caspase 3 was activated (Fig. 3D).

To correlate the visible light phototoxicity with the melanin and 1O2 contents, we quantified the amount of 1O2 generated in cells by measuring the near-infrared emission spectrum (1270 nm) after excitation with visible light (532 nm) [18] (Fig. 4A). The control cells only exhibited background signals (Fig. 4A, insert). However, the pigmented B16-F10 cells (M+++; Fig. 4A) showed the characteristic 1O2 spectra with a maximum intensity at approximately 1270 nm (the darker line in the Fig. 4A insert). Thus, the higher intracellular melanin content in the pigmented cells corresponded to more 1O2 generation and higher phototoxicity in response to visible irradiation compared with those for the control cells. To establish a definitive relationship between the 1O2 level and the cell toxicity, we also generated this species in the intracellular environment using a clean 1O2 source (i.e., thermal decomposition of DHPN[O]), as shown in Fig. 4B. The cells treated with DHPN[O] exhibited a substantial decrease in viability (~50%) compared with the viability of both types of control (cells without treatment and cells challenged with DHPN), which demonstrates that 1O2 plays a role in the reduced cell viability (Fig. 4B). Furthermore, the generation of 1O2 slightly reduced the viability of CT cells (49.7%) compared with that of M+++ cells (53.3%). This difference was not statistically significant, possibly because melanin can suppress 1O2. As reported earlier, melanin can exert both types of effects, but in the presence of sufficient visible light illumination, melanin will stimulate the generation of 1O2 [14,18,38].

The excitation of melanin using visible light generates 1O2 and, consequently, the triplet species derived from melanin. Therefore, cellular damage can occur by both a type I mechanism (direct reaction between the triplet photosensitizer and biological targets, typically through an electron transfer reaction) and a type II mechanism (energy transfer reaction between the triplet photosensitizer and oxygen-forming 1O2) [19]. Depending on the severity of the damage, cell death will be the main outcome from visible light exposure. Another potential outcome, which is potentially more dangerous, is the generation of oxidative DNA products, which could lead to mutagenic compound accumulation, genomic instability and cancer [39].

To demonstrate the direct damage to nuclear DNA by the melanin photosensitization that occurs in response to visible light irradiation, we performed a comet assay under low-dose conditions (i.e., a light dose that does not measurably decrease the cell viability for both CT and M+++ cells (6 J·cm⁻²)). After irradiation, the cells were treated with endonuclease enzymes (Fpg and Endo III) that recognize specific types of oxidative damage in DNA (Fig. 5). Fpg recognizes 8-oxoguanine, 8-hydroxyguanine and formamidopyrimidine, and Endo III recognizes strand breaks, abasic sites and additional oxidative pyrimidine modifications [40]. For a control, the comet assay was repeated in the absence of endonucleases. As expected, under this mild condition, both the CT and pigmented cells in the
Figure 2. The effects of UVB and visible light on cell viability. The cell viability (MTT) of HaCaT, SK-mel and B16-F10 cells maintained in the dark (control) or irradiated with UVB (4.5 J.cm\(^{-2}\)) or visible (36 J.cm\(^{-2}\)) light. The color of the bars indicates the (qualitative) levels of melanin naturally produced by each cell line. Other cell lines, which are not melanocOMPETent (Hela, J774), were also tested and behaved similarly to the HaCaT cells (i.e., exhibited no phototoxicity from visible light at this dose (36 J.cm\(^{-2}\))).

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Figure 3. The effects of UVB and visible light in B16-F10 and human melanocyte cells. Left side: (no extra pigmentation); right side: cells subjected to the pigmentation protocol (M++++). (A) Viable cells (%) determined using the acridine orange fluorescence of B16-F10 cells that were maintained in the dark or with 4.5 J.cm\(^{-2}\) of UVB irradiation. The images are confocal optical microscopy images of the B16-F10 cells, CT (left) and M++++ (right). (B) The viable cells (%) were determined using the MTT colorimetric assay in the dark or with 36 J.cm\(^{-2}\) or 72 J.cm\(^{-2}\) of visible irradiation; murine B16-F10 cells are marked as either M36 or M72 depending on the light dose received. The human melanocytes (H36) only received a light dose of 36 J.cm\(^{-2}\). The images on the sides show comet assays performed using the CT and M++++ B16-F10 cells, 180 min after irradiation with a light dose of 36 J.cm\(^{-2}\). (C) Propidium iodide incorporation in B16-F10 CT and M++++ cells in the dark and after exposure to 72 J.cm\(^{-2}\) of visible light. The images on the sides show typical images used for quantifying the PI incorporation. (D) Caspase 3 activation in B16-F10 CT and M++++ cells in the dark and after exposure to 72 J.cm\(^{-2}\) of visible light. The images on the side are typical for quantifying caspase 3 activation. (*) p<0.001.

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presence and absence of visible light did not show direct strand breaks, as indicated by the comet assay (data not shown, except for the M++++ cells with visible light irradiation, Fig. 5D). The first three controls (i.e., CT in the presence and absence of light and M++++ in the absence of light) also showed no DNA fragmentation after treatment with Fpg and Endo III (Fig. 5A–C). As mentioned above, the M++++ cells treated with light in the absence of these enzymes did not show DNA fragmentation (Fig. 5D). However, when Fpg or Endo III were applied to M++++ cells, which were irradiated with 6 J.cm$^{-2}$ of visible light, the comet assay showed a considerable increase in the number of strand breaks (Fig. 5 graph and images E–F), which were absent in the controls (Fig. 5A–D). The presence of strand breaks demonstrated that melanin photosensitization by visible irradiation induces direct oxidative damage to nuclear DNA. The ratio of Fpg- to Endo III-sensitive modifications indicate that oxidative damage in DNA is most likely due to both type II and type I mechanisms [32,33] (Fig. 5, scheme).

Discussion

Although melanin is biosynthesized to protect against UVB, it will damage skin cells in the presence of UVA and visible light. At sufficiently high light doses, melanin causes extensive necro-apoptotic cell death. The lower light doses were able to mimic the potential chronic consequences of visible light exposure. Melanin induces the formation of pre-mutagenic DNA lesions (Fpg- and Endo III-sensitive modifications) (Fig. 4, scheme). We also showed that pheomelanin is a more efficient photosensitizer than eumelanin because it generates more $^{1}$O$_2$ and better withstands photobleaching (i.e., it continuously generates $^{1}$O$_2$ for longer periods than does eumelanin).

Therefore, in the presence of melanin, the effects of visible light irradiation do not differ from those of UVA irradiation; consequently, it should be considered with care, and further investigations must be performed to evaluate whether it may be a class I carcinogen [41]. These data indicate a causal relationship between visible light irradiation and the development of genome instability in melanocompetent cells and, consequently, the development of melanoma [17,18,36] and the higher skin cancer prevalence in individuals with red hair [15,37]. Other authors have also concluded that visible light causes effects similar to those of UVA, such as inflammation and ROS production [6,10,17].

The current beliefs regarding the protection of skin against photoinduced damage are similar to those from 30 years ago but with a shift in the problematic wavelength region. In the early 1980s, photobiologists knew that UVA induced cellular responses [2,3]. However, people were convinced sunbathing with UVB-only protection was safe. The consequences of this strategy are felt today: the resulting deeper skin tumors have a higher prevalence of DNA mutations than those induced by UVA exposure [5,42]. Clearly, visible light affects skin health, but people are encouraged to stay under the sun if they use sufficient amounts of “good sunscreen” (i.e., sunscreens that provide effective protection against UVA and UVB). This recommendation is clearly a mistake because it ignores the effects of visible light, which penetrates more deeply into skin than does UVB and UVA [43], and because it disregards the effects of other wavelength regions, such as the infrared region [44].

The toxicity of visible light raises concerns about other situations in addition to the direct sun photosensitivity to the skin, e.g., clinical protocols that use visible light, such as blue light therapy, in jaundiced babies [45]; indoor tanning [46]; the
exposure of eyes to the toxicity of high levels of visible environmental light [47].
In the presence of melanin, visible light generates singlet oxygen and causes direct DNA damage. We hope this information will guide health professionals and the general population to safer interactions with the sun and, specifically, with visible light. We also hope that this information encourages companies to develop new sunscreen products that also provide protection against visible radiation.

Supporting Information

Figure S1 Scheme of the melanin photosensitization mechanisms that generate \( ^{1}\text{O}_2 \). This \( ^{1}\text{O}_2 \) can react with the following to form several products: lipids mainly through an ene reaction that forms hydroperoxide, nucleic acids via a guanine residue to form 8-oxo-guanine, and amino acids (the scheme shows the amino acids that are most reactive with \( ^{1}\text{O}_2 \)). The right side of the scheme shows the thermal decomposition of DHPNO, which is also used to generate \( ^{1}\text{O}_2 \) in the intracellular environment.

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Author Contributions

Conceived and designed the experiments: MSB. Performed the experiments: OCN EA FFF. Analyzed the data: PDM MGM GRM SSME. Contributed reagents/materials/analysis tools: MHM GRM MSB. Contributed to the writing of the manuscript: MSB OCN. Read and provided suggestions and corrections: ASF WKM CP DS FFF SSME GRM EA PDM MHM.
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