Imaging of ultraweak photon emission for evaluating the oxidative stress of human skin

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\textbf{A B S T R A C T}

Ultraweak photon emission (UPE) is generally observed in living organisms and often designated as biophoton emission. UPE is detectable from human skin, and its intensity increases by external stress such as ultraviolet (UV) irradiation. Presently, UPE measurement is used to evaluate oxidation status. The fact that the electronically excited species responsible for UPE are formed by reactive oxygen species (ROS)-induced lipid peroxidation and protein and nucleic acid oxidation is well known. The human skin undergoes oxidative stress by UV irradiation, resulting in various skin complications; therefore, it is essential to know the oxidation status of the skin. In this study, we assessed the characteristics of UV-induced UPE in the skin by the imaging and spectroscopy systems. Two-dimensional images obtained by a highly sensitive imaging system using a cooled charge-coupled device (CCD) camera revealed that UPE intensity increases with the amount of UV and is suppressed by antioxidants. Additionally, it is indicated that UPE is generated not only from the epidermis but also from the dermis. The spectra of UPE induced by UVA or UVB showed similar peaks in the visible light region. Furthermore, we confirmed the efficiency of sunscreen by the imaging technique. UPE measurement is a useful method to evaluate UV-induced oxidation in the human skin, and UPE imaging is an effective method to visually evaluate oxidative stress in the human skin.

\section*{1. Introduction}

Ultraweak photon emission (UPE) produces a very weak luminescence and is emitted from living organisms \cite{1,2}, including microorganisms, plants, and humans \cite{3,4}. It is often designated as biophoton emission. Presently, UPE measurement is used to evaluate oxidation status and can potentially be used in an optical biopsy to detect tumor viability \cite{5}. UPE is detectable from the human skin, and its intensity increases by external stress such as ultraviolet (UV) irradiation \cite{6–8}. The emitted photons are primarily in the visible light range of wavelength, and it was proposed that the electronically excited species responsible for UPE are formed by reactive oxygen species (ROS)-induced lipid peroxidation and protein and nucleic acid oxidation \cite{2}. The oxidation of these biomolecules leads to the formation of high-energy intermediates \cite{9}. The decomposition of these intermediates generates electronically excited species that undergo electronic transition. UPE is accompanied by electronic transition from the singlet or triplet excited state to the ground state. UPE intensity is extremely weak at approximately $10^{-16}$ W/cm$^2$ ($10^3$ photon/s/cm$^2$) or less, which is weaker by 1/1000 times the sensitivity of the human eye. UPE can be distinguished from black-body radiation because the intensity black-body radiation is weaker by 1/1000 times the UPE intensity \cite{10}.

UPE from human skin can be visualized using highly sensitive cooled charge-coupled device (CCD) cameras \cite{11}, offering a label-free and non-invasive method for detecting oxidation.

In daily life, UV irradiation causes various skin complications. UV irradiation leads to increased ROS production \cite{12,13}, which alters gene and protein functions \cite{14}. This results in dysregulation of intracellular and extracellular homeostasis causing impaired skin function. ROS can interact with intracellular and extracellular components, thus causing oxidative reactions. It is well known that UV irradiation induces skin oxidation, as indicated by the squalene oxidation \cite{15}, protein carbonylation in the stratum corneum \cite{16}, and protein oxidation \cite{17}. UV irradiation has also been reported to induce UPE from the skin; however, this has not been studied in detail. If better understood, evaluations of UV-induced UPE from the skin may help in replacing the present methods used for evaluating UV-induced oxidation, which require labeling.

In this study, we have reported the characterization of UV-induced UPE in the human skin by the imaging and spectroscopy systems.

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Additionally, we have confirmed the UV-protective efficacy of sunscreen using imaging technique.

2. Material and Methods

2.1. UV Irradiation

UV irradiation was generated using a Dermaray 200 (Canon Medical Supply, Tokyo, Japan) system with a UVA source (TOREX FL20SBL/DMR, 300–430 nm, peak 352 nm; Toshiba Medical Supply, Tokyo, Japan) and a UVB source (TL20W/12 RS, 280–380 nm, peak 311–313 nm; Philips, Eindhoven, The Netherlands). UVA and UVB intensities were measured by DMR-UV-ABBNB-1 (Gigahertz-Optik GmbH, Puchheim, Germany).

2.2. Human Skin Tissue

Human (Caucasian) skin samples were purchased from Biopredic International (Rennes, France) and Analytical Biological Services (Wilmington, DE) via KAC (Kyoto, Japan). Subcutaneous tissues were removed prior to the experiments. The epidermis and dermis layers were obtained from the skin tissue by heating at 60 °C. Human stratum corneum (Caucasian) sheets were purchased from Biopredic International (Rennes, France) via KAC (Kyoto, Japan).

2.3. UPE Measuring Systems

2.3.1. Imaging System

UPE images were captured using a CCD camera system \[11,18\] in a dark chamber (Fig. 1). The skin tissues were placed on the stage, and the human hands were inserted through an input port in vivo. UPE images were continuously taken with 5-min exposures using the CCD camera (SI 850 s; Spectral Instruments Inc., USA, equipped with e2v CCD42-40). A specially designed high-throughput lens system, bearing 0.5 numerical aperture on the CCD side with 1/3 magnification, was used. Imaging was performed on a surface area of 75 × 75 mm. In this experiment, the CCD was operated in the 8 × 8 binning mode, and the actual pixel number was 256 × 256. The resulting images were edited using ImageJ (NIH) and UPE intensity was calculated.

2.3.2. Spectroscopy System

UPE spectra of excised human skin were taken using a polychromatic spectrum analysis system, which consists of a transmission-type diffraction grating, a lens system, and a CCD camera (SI 600 s; Spectral Instruments Inc., USA) [19]. The exposure time of the CCD camera for spectral measurement of UV-induced UPE was set at 20 min. The exposure time for spontaneous UPE was set at 30 min and its photon intensity was multiplied by 2/3 to compare with the intensity of UV-induced UPE. UPE spectra were measured by blocking the skin tissue using a cooled charge-coupled device (CCD) camera.

2.4. UPE Imaging of Human Skin

To evaluate the UV dose dependence of UPE, human skin was irradiated with various doses of UVA or UVB. To confirm the origin of UPE, human skin was separated into epidermis and dermis layers. The separated layers of human skin and human stratum corneum were irradiated with UVA (1100 mJ/cm², 2.3 mW/cm²) or UVB (234 mJ/cm², 0.65 mW/cm²). UPE images were immediately taken.

Once human skin was separated into the epidermis and dermis layers, the epidermis was placed on the dermis. After UVA (1100 mJ/cm², 2.3 mW/cm²) or UVB (234 mJ/cm², 0.65 mW/cm²) irradiation to this reconstructed skin, human skin was separated into epidermis and dermis layers again, and UPE images of each layer were immediately taken.

To evaluate the effects of antioxidants against UV-induced UPE, 5% antioxidant solutions of sodium L-ascorbate (Kanto Chemical), 1-glutathione (Sigma-Aldrich) and d-δ-tocopherol (Tama Biochemical) were prepared. Sodium L-ascorbate and 1-glutathione solutions were prepared with PBS (−), whereas d-δ-tocopherol solution was prepared with 50% EtOH. After UVA (1100 mJ/cm², 2.3 mW/cm²) or UVB (234 mJ/cm², 0.65 mW/cm²) irradiation, 5% antioxidant solutions were applied to the skin surfaces, and UPE images were immediately taken.

2.5. UPE from Human Skin In Vivo

After recruitment, human subjects (Asian, 7 healthy males in their 20s) provided written informed consent to participate in the study. Before measurement, subjects were asked to wash their hands. Subsequently, they were asked to wait for 10 min in a dark room to allow decrease in the effect by external light. Spontaneous UPE images of the skin of the back of fingers were taken for 5 min. The backs of the fingers were exposed to UVA at 800 mJ/cm² (1.33 mW/cm²) via a SiO₂ plate with or without a sunscreen (SPF 50+, PA ++++) coating (2 mg/cm²). Irradiated skin areas measured 1 × 1 cm². Immediately after UVA irradiation, UVA-induced UPE images of the skin at the back of fingers were taken for 5 min.

2.6. Statistical Analysis

Tukey–Kramer test was used for UPE images of each skin layer directly irradiated with UV and paired t-test was used for other experiments. The level of significance was defined as *P < .05, **P < .01, and ***P < .001.

3. Results

3.1. Imaging of UV-Induced UPE in Human Skin Tissue

Intensities of UV-induced UPE were increased in a dose-dependent manner (Fig. 2), clearly representing a reaction of skin to UV. The data in Fig. 3 show that UV-induced UPE was detectable from the epidermis and dermis, and intensity from the dermis was higher than that from the epidermis. UVA induced higher UPE in the dermis than UVB. We also confirmed UV-induced UPE from the stratum corneum. After UV irradiation to the reconstructed skin with the epidermis placed on the dermis, UV-induced UPE was detected from the dermis but the intensities were lower than intensities that were UV irradiated to dermis directly (Fig. 4). The lower intensities indicated that the epidermis partially absorbed UV radiation.

Fig. 5 shows the effects of antioxidants against UV-induced UPE. UV-induced UPE was suppressed by antioxidant treatments confirming that UV-induced UPE are related to the oxidative stress of skin. The present antioxidants did not emit UPE and did not absorb UV-induced UPE (data not shown).
3.2. Spectral Patterns of UV-Induced UPE

Polychromatic spectrum analyses revealed that UV irradiation induces increase in UPE within the visible spectrum (Fig. 6). Irradiation with UVA or UVB led to a similar peak UPE at approximately 550 nm. Spectral pattern of spontaneous UPE peaked at 600–650 nm. These experiments were performed in the visible range and UPE in the infrared range was not considered.

Fig. 2. Relationship between UV dose and UPE intensity. Human skin was irradiated with several doses of UVA or UVB. The graph on the right side of the figure shows intensity of UV-induced UPE determined from imaging data on the left side of the figure. The intensity of spontaneous UPE was subtracted. Images were captured using a cooled CCD camera with 5-min exposures; scale bar, 1 cm. The color scale indicates signal intensity from 0 (blue) to 100 (white). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. UV-induced UPE from separated skin layers. Separated skin layers were irradiated with UVA (1100 mJ/cm²) or UVB (234 mJ/cm²). UPE images were captured using a cooled CCD camera with 5-min exposures; scale bar, 0.5 cm. Data of epidermis and dermis are presented as means ± SD, (UVA) N = 5, (UVB) N = 4; **P < .01. The color scale indicates signal intensity from 0 (blue) to 280 (white). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Fig. 4. UPE from dermis after UV irradiation via epidermis. Dermis samples with or without overlying epidermis were irradiated with UVA (1100 mJ/cm²) or UVB (234 mJ/cm²). The upper portions of the images show dermis after UV irradiation via epidermis [Epidermis (+)]. The lower portions of the images show dermis that was directly irradiated by UV [Epidermis (−)]. Imaging data were taken using a cooled CCD camera with 5-min exposures; scale bar, 0.5 cm. Data are presented as means ± SD, N = 4; **⁎⁎⁎ P < .001. The color scale indicates signal intensity from 0 (blue) to 280 (white). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. The effect of antioxidants against UV-induced UPE. Human skin was irradiated with UVA (1100 mJ/cm²) or UVB (234 mJ/cm²). After UV irradiation, antioxidants were applied to the right sides of skin samples and the respective solvents were applied to the left sides of skin samples. Imaging data were taken using a cooled CCD camera with 5-min exposures; scale bar, 1 cm. Data are presented as means ± SD, N = 5; * P < .05, ** P < .01. The color scale indicates signal intensity from 0 (blue) to 100 (white). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
3.3. Suppression of Oxidative Stress by Sunscreen In Vivo

UVA-irradiated areas of finger skin showed UV-induced UPE, whereas UVA irradiation through sunscreen failed to increase the intensity of UPE (Fig. 7). Because the sunscreen was indirectly applied, these data indicate actual UV protection by the sunscreen. Accordingly, UV-induced UPE significantly differed ($P < .01$) between parts with and without sunscreen.

4. Discussion

UV causes skin disorders via oxidative stress. To counter this, various antioxidants and sunscreens have been developed. For development of effective antioxidants and sunscreens, better methods of evaluating oxidative stress are required. In this study, we focused on UPE and characterized UV-induced UPE in human skin by conducting UPE imaging and spectral analysis. Although previous studies reported UPE induced by either UVA or UVB, we evaluated and compared UPE induced by both UVA and UVB.

In UPE imaging, acute changes in UPE intensity were observed following UV irradiation, and these were dependent on the UV dose. Photon counts following UVA irradiation showed dose-dependent increases [7]. In Fig. 3, we present data showing that each skin layer includes components that emit UV-induced photons. Moreover, our findings showed that UVA induced higher UPE in the dermis than UVB. We propose that UVA can oxidize the dermis more than UVB under sunlight because the UVA/UVB intensity ratio of sunlight is higher than that of our experimental condition [20]. Furthermore, it was indicated that UV-induced UPE from human skin includes UPE from not only the epidermis but also the dermis layer (Fig. 4) and that epidermis attenuates the UV intensity. Considering transmittance of UV to skin [21,22], the contributions of dermis against total induced UPE is approximately 60%–80% for UVA and 20%–40% for UVB. The contribution of the epidermis against total induced UPE is approximately 20%–40% for UVA and 50%–70% for UVB. Differing contributions of UVA and UVB are reasonable because the transmittance of UVA to the dermis is higher than that of UVB.

Several studies reported that antioxidants suppress UPE photon counts [8,23–25]. UPE imaging in our study also showed that three different antioxidants (sodium L-ascorbate, L-glutathione, and d-δ-tocopherol) suppress UV-induced UPE. It supports the view that UPE is associated with oxidative stress. Because the hydroxyl radical and superoxide anion radical induce more UPE [26], it is speculated that these antioxidants suppress UPE by radical-scavenging. Because imaging was immediately performed after applying the antioxidant solutions, it was suggested that antioxidant effects were mainly observed in the epidermis.

We showed spectral pattern of spontaneous and UVA-induced UPE from human skin as previously reported [27]. The spectral pattern of UVA-induced UPE peaked at approximately 550 nm and UVB-induced UPE showed a similar peak with UVA-induced UPE. Several researchers have reported the origin of UPE from human skin. The origin of UV-induced UPE was previously shown to comprise spectra from $3(R = O)^*$ in the near UVA, blue–green regions (350–550 nm), and singlet and triplet excited pigments in the green–red region (550–750 nm) [2]. Additionally, it is assumed that the human skin includes the components as the origin of UV-induced UPE. Mei’s group proposed the contribution of excited amino acids (predominantly tryptophan) by
excited carbonyl \([R = O]\) and singlet oxygen \([O_2] \) via type I or type II reactions, which emit photons in the visible wavelength region. Also, the components such as collagen and melanin [30,31], which can be excited, are proposed as a source of UV-induced UPE in the visible wavelength region. For instance, it is suggested that alteration of the cross-links of collagen by UV irradiation [32] induces ROS via photoreinization reaction and that the pigments such as melanin [19] may cause photon emission (550–750 nm) through energy transfer from the triplet excited carbonyl.

Finally, the in vivo test shows that sunscreen is protective against UV. Previously, protection of skin from UVB by sunscreen was determined by measuring the photon count [33]; however, the antioxidant effect of sunscreen can be visually evaluated using imaging systems. These data led us to conclude that UPE imaging is a useful method for evaluating the oxidative stress of human skin and anti-oxidant effects. Imaging with the CCD camera was less sensitive than photon counting using PMT but it provides the advantage of visual evaluation of the oxidation status of the whole skin.

It is advantageous to non-invasively evaluate the oxidation status of microorganisms, plants, and humans. Also, studies involving evaluation of the oxidation status are important in the field of dermatological science because oxidative damage by UV to the skin is medically and cosmetically relevant. In particular, the imaging method may become a useful tool for evaluating the oxidation status of the skin. In this study, we focused on acute oxidation, but we believe that chronic oxidation can also be adequately evaluated. Although it is important to suppress oxidation of the skin, we believe that observation of the oxidation status is important as well.

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**Declaration of Competing Interest**

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**References**


