Effect of Intense Pulsed-Light Exposure on Lipid Peroxides and Thymine Dimers in Human Skin In Vivo

Olivier Sorg, PhD; Victor Janer, MD; Christophe Antille, MD; Pierre Carraux; Evelyne Leemans; Elizabeth Masgrau, MD; Jean-Hilaire Saurat, MD; Denis Salomon, MD

Background: Intense pulsed light (IPL) generates high-intensity short flashes of visible light and has been used for about 10 years to improve dermatological conditions such as telangiectasia, pigmented lesions, and skin aging. Although these systems deliver a moderate dose (10-30 J/cm²) of visible light, this dose is delivered during a short pulse (2-5 milliseconds), which implies a very high fluence rate (approximately 4000 W/cm²). For this reason, we speculated whether the Bunsen-Roscoe law of reciprocity could still be valid in these conditions.

Observations: Nine healthy volunteers were exposed to IPL or UV-A or simulated solar UV radiation, and then thymine dimer and lipid peroxide concentrations were determined in skin biopsy specimens of the exposed sites. Only exposure to solar UV radiation (7-J/cm² UV-A + 80-mJ/cm² UV-B) produced measurable amounts of thymine dimers in DNA from skin biopsy specimens, whereas UV-A radiation (40 J/cm²) and IPL (9 J/cm²) induced 3-fold and 6-fold increases of cutaneous lipid peroxides, respectively.

Conclusions: These preliminary results indicate that IPL, although filtered for wavelengths shorter than 500 nm, can generate oxidative stress, a typical hallmark of UV-A, but does not induce thymine dimers. This emphasizes the need for long-term studies involving IPL before using this technique in a recurrent manner.

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INTENSE PULSED LIGHT (IPL) GENERATES high-intensity short flashes of polychromatic light in the visible and near-infrared range (500-1200 nm), depending on the filters used. These systems are believed to act through the selective photothermolysis of targets such as melanin and hemoglobin, as described by Anderson and Parrish for the pulsed-dye laser. The therapeutic uses for IPL range from treating benign vascular lesions and pigmented lesions to hair removal and nonablative skin rejuvenation. The particularity of these systems is that a moderate dose (10-30 J/cm²) of polychromatic visible light (550-950 nm for most of the applications) is delivered in a very short time (2-5 milliseconds) using a very high fluence rate (approximately 2000-5000 W/cm²). For this reason, the Bunsen-Roscoe law of reciprocity, which states that the strength of a radiative stimulus only depends on the total energy dose irrespective of the administered regime, might no longer be valid. As a consequence, the flashes produced by these systems can induce biological effects, which cannot be observed when the same doses are delivered by common light sources that produce much lower fluence rates (0.1-50.0 mW/cm²).

In this study, we examined whether IPL could induce relevant biological markers of UV-induced tissue damage: we analyzed the concentrations of lipid peroxides and thymine dimers—markers of oxidative stress and DNA photodamage, respectively, in skin biopsy specimens of 9 healthy volunteers following a single exposure to IPL or UV-A or solar UV radiation.

METHODS

CHEMICALS AND IRRADIATION

Common chemicals were purchased from Sigma Chemical Co (St Louis, Mo). UV-A and solar UV radiation was delivered by a 16S solar simulator from Solar Light (Philadelphia, Pa), giving a 1-cm spot on the buttocks. The fluence rates were 90-mW/cm² UV-A + 15-µW/cm² for UV-A and 130-mW/cm² UV-A + 1.5-mW/cm² UV-B for solar UV radiation. Intense pulsed light was delivered by an Ellipse Flex flash lamp (Danish Dermatologic Development, Hoersholm, Denmark) with the photorejuvenation device (520-750 nm). The fluence of 9 J/cm² was given in a single 2.5-millisecond pulse, corresponding to a calculated fluence rate of 3600 W/cm².

Author Affiliations:
Dermatology Department,
University Hospital of Geneva,
Geneva, Switzerland.
HUMAN SUBJECTS AND STUDY PROTOCOL

Nine adult volunteers participated in the study; their age ranged from 17 to 60 years (mean±SD age, 38±5 years). All subjects had skin phototype II or III and were in good health with no evidence of acute, chronic illness, or cutaneous disease. Patients with a history of abnormal response to sunlight or taking medications with known photosensitizing properties were not included.

The study protocol was approved by the ethics committee of the Hôpitaux Universitaires de Genève, Genève, Switzerland. Written consent was obtained from all participants in the study. Each volunteer was exposed to a single dose of solar UV radiation corresponding to 80-mJ/cm² UV-B radiation (approximately 2 minimal erythema doses), a single dose of 40 J/cm² UV-A radiation, and a single dose of 9-J/cm² IPL; another area was left unexposed. For thymine dimers, 4-mm-diameter punch biopsy specimens were taken immediately after exposure. The skin was harvested and epidermis separated from the dermis by treatment with 2M sodium bromide for 20 minutes at 37°C. For lipid peroxides, 4-mm-diameter punch biopsy specimens were taken 15 minutes after exposure and frozen until assayed. Morphological examinations were performed on histological sections from 4-mm punch biopsy specimens taken 2 hours following irradiation.

PYRIMIDINE DIMERS AND LIPID PEROXIDATION

The spatial distribution of thymine dimers was visualized in histological sections using an antibody that binds to thymine dimers (mouse antithymine dimers clone KTM53; Kamiya, Seattle, Wash).

Figure 1. Thymine dimer formation in the skin. A, Immunohistochemical studies of thymine dimer sections of areas exposed to intense pulsed light (IPL) and solar UV radiation and nonirradiated areas. B, Immunoslot blot analysis of DNA from skin biopsy specimens of areas exposed to IPL and solar UV radiation and nonirradiated areas. C, Densitometric analysis of the immunoslot blots shown in panel B.
at a concentration of 1 µg/mL. For quantitation of thymine dimers, DNA was extracted from the epidermis using a Biorobot EZ1 (Qiagen, Basel, Switzerland) according to the manufacturer instructions, then thymine dimers were detected in 100 ng of DNA by immunoslot blots using the aforementioned antibody, as described by Smit et al.6 The quantitation of immunoslot blots was performed with a densitometer and the software ImageQuant from Molecular Dynamics (Redwood City, Calif).

Lipid peroxidation was assessed by determining the concentration of cutaneous lipid peroxides as previously described.7 Briefly, skin samples were minced with scissors and homogenized in ice-cold methanol containing 3mM butylated hydroxyltoluene, using a Polytron PT 3100 homogenizer (Polytron Inc, Norcross, Ga). Sample homogenates were then sonicated and centrifuged, and supernatant was harvested and separated into two 500-µL aliquots; one aliquot was incubated with 50 µL of 10mM triphenylphosphine in methanol (30 minutes at room temperature), while 50-µL methanol was added in the other aliquot. A 500-µL mixture containing 25mM sulfuric acid, 250µM ammonium ferrous sulfate (Mohr salt), 100µM xylene orange, and 4mM butylated hydroxyltoluene in 90% methanol was then added in both aliquots (room temperature), and optical density was read at 585 nm 1 hour later. The specific lipid peroxide signal was obtained by subtracting the nonspecific signal (triphenylphosphine aliquots) from the global signal. Lipid peroxide concentration was determined using cumene hydroperoxide as a standard (0.5-8.0 nmol).

MORPHOLOGICAL EXAMINATION AND STATISTICAL ANALYSIS

Specimens of full skin were fixed in 10% formalin solution and embedded in paraffin. Sections of 5-µm thickness were cut and stained with hematoxylin-eosin.

Proliferative epidermal cells were visualized in human histological sections using an anti-Ki67 antibody. The immunohistochemical staining was made using an Ultravision mouse tissue detection system (Labvision Corp, Fremont, Calif) in combination with a monoclonal anti-Ki67 at a concentration of 40 µg/mL (Dako, Glostrup, Denmark) according to the manufacturer instructions. Epidermal Ki67-positive cells were counted visually on sections (2 sections per patient and 4 consecutive fields per section).

Analysis of variance was performed to compare several series of data.

RESULTS

MORPHOLOGICAL EXAMINATION

The hematoxylin-eosin–stained histological sections from human skin did not show any morphological differences between the 4 conditions (unexposed, IPL, and UV-A and solar UV radiation). These sections did not show any alteration or modification of the tissue (not shown). Ki67-immunostained sections displayed similar numbers of positive cells for each of the 4 conditions, indicating that the various radiative treatments did not alter the proliferation of the exposed epidermis (not shown).

SOLAR UV-INDUCED THYMINE DIMERS IN HUMAN SKIN

The effect of the different radiative stimuli on the formation of thymine dimers on DNA was analyzed 15 minutes after irradiation. This period was long enough to ensure complete formation of thymine dimers but too short to initiate their repair,4 thus indicating the maximum levels of thymine dimers produced. As expected, DNA photoproducts were observed only in solar UV–irradiated areas (Figure 1).

IPL-INDUCED LIPID PEROXIDES

The levels of lipid peroxides—a marker of oxidative stress—were similar in nonirradiated skin and skin exposed to solar UV radiation, whereas they were 3 times higher after exposure to UV-A radiation and 6 times higher after exposure to IPL (Figure 2). This confirms the ability of UV-A irradiation to promote an oxidative stress and shows a new biological action of IPL in the skin. Values for irradiated skin were significantly (P<.05) different from those for nonirradiated skin.

Although IPL technology has been used for more than 10 years by many physicians for dermatological and cosmetic purposes,2,9 very few studies were aimed at documenting its adverse events. The most common reported adverse reactions were transient erythema, occasionally accompanied by edema, light to mild transient pain, and superficial burning,2,10 but the action of pure IPL on the skin has, to our knowledge, not been addressed at the molecular level. This might be because this technology uses only visible and near infrared light, which causes much less concern than UV radiation, and moderate doses (approximately 10-40 J/cm²) are delivered. Because it has been often stated, although without any scientific evidence, that the biological effect of a radiative stimulus only depends on the dose, according to the Bunsen-
Roscoe law, most IPL users were not concerned by the exposure to a moderate dose of visible light. However, if the Bunsen-Roscoe law can be verified on certain applications of visible light, in particular on the physical responses of physicochemical systems (eg, photography), living systems are much more intricate and can be more sensitive to the fluence rates of the radiative stimuli. The highest fluence rate in the visible range we can receive at sea level at noon in tropical regions is approximately 50 mW/cm², whereas common IPL lamps deliver fluence rates of 2000 to 5000 W/cm² (ie, approximately 100,000 times higher). In these conditions, we should not be surprised to observe biological events after IPL treatments that can never be observed after exposures of similar doses delivered by natural fluence rates.

In the present study, which aimed at opening a new trend of research on the biological consequences of IPL treatments, we analyzed 2 known markers of tissue photodamage, DNA thymine dimers and lipid peroxidation, hallmarks of UV-B[12,13] and UV-A radiation, respectively, on human skin in vivo. If the absence of thymine dimer formation by IPL is comforting, which is not surprising because the action spectrum for pyrimidine dimer formation is centered on the absorption spectrum of pyrimidine bases (260 nm), the ability of IPL to induce a significant oxidative stress in the skin should induce IPL users to be careful when using IPL treatment in a recurrent manner. Oxidative stress is a well-documented consequence of UV-A irradiation, although the primary chromophores have not yet been identified. It is unlikely that the lipid peroxidation observed in this study was due to UV-A radiation because the IPL system was well filtered below 550 nm. On the other hand, Applegate and colleagues showed that infrared light (700-4000 nm) was not capable of inducing frank damage to DNA or oxidative stress proteins in fibroblasts in vitro, indicating that the small infrared component of our IPL system (800-950 nm) was probably not responsible for the lipid peroxidation shown in skin biopsy specimens. This oxidative stress was thus probably due to a moderate dose of visible light delivered through a very high fluence rate. This is a new observation, to our knowledge, and, as for UV-A, the primary chromophores remain to be identified. The histological examination of the biopsy specimens does not show any morphological alteration of both the dermis and the epidermis; moreover, no modulation of the proliferative state was observed, as shown by Ki67 staining. Although the biopsy specimens were taken only 2 hours after irradiation, which precludes the detection of delayed biological events such as apoptosis, epidermal hyperplasia, or leukocyte infiltration, this period would have been long enough to show acute reactions such as burning, swelling, or acute epidermal necrosis.

In conclusion, our data stress the need for studies to analyze the biological consequences of IPL treatments and a careful follow-up of patients treated in a recurrent manner by IPL to guarantee the best level of safety for this equipment with constantly expanding uses.

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Correspondence: Denis Salomon, MD, Clinique de Dermatologie, Hôpital Cantonal Universitaire, 24 Rue Michel-du-cres, 1211 Genève 14, Switzerland (Denis.Salomon@hcuge.ch).

Author Contributions: Dr Salomon had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Sorg, Antille, Saurat, and Salomon. Acquisition of data: Sorg, Janer, Carraux, Leemans, Masgra, and Salomon. Analysis and interpretation of data: Sorg. Drafting of the manuscript: Sorg, Janer, Leemans, and Salomon. Critical revision of the manuscript for important intellectual content: Sorg, Antille, and Carraux. Statistical analysis: Sorg. Obtained funding: Sorg and Saurat. Administrative, technical, and material support: Sorg, Janer, Carraux, and Leemans. Study supervision: Sorg.

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