A Chemiluminescence Study of UVA-Induced Oxidative Stress in Human Skin *In Vivo*

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Oxidative stress is defined as an imbalance between pro-oxidants and antioxidants in favor of pro-oxidants. Photon emission (also called chemiluminescence) has been widely used to study oxidative stress in biological systems *in vitro*. *In vivo* chemiluminescence has been proposed as a non-invasive method to assess oxidative stress in the skin. UVA (320–400 nm part of the ultraviolet radiation) exposure is generally accepted as a source of oxidative stress in the skin. In this study, UVA-induced oxidative stress was studied by using an *in vivo* chemiluminescence detection method. First, the dose response and the fluence rate response of the UVA-induced oxidative stress in human skin were investigated by examining the decay kinetics of the chemiluminescence signal following UVA exposure. A kinetic model was proposed to help differentiate these two responses. We found that the initial burst of the chemiluminescence signal depended on the UVA fluence rate, whereas the decay of the signal following exposure can be related to the UVA dose involved. Second, a significant reduction of UVA-induced chemiluminescence signal was observed after tape-stripping, indicating that stratum corneum is a major source of UVA-induced oxidative stress in the skin. Furthermore, the oxygen dependence of UVA-induced chemiluminescence signal was also confirmed by application of a pressure cuff, implying that some of the oxidative stress occurs in the deeper layers of the skin. Finally, topical application of vitamin C before exposure significantly reduced the UVA-induced chemiluminescence signal. We thus conclude that chemiluminescence is an effective method to assess the oxidative stress induced by UVA in human skin *in vivo.*

Key words: antioxidant/oxidant/oxygen supply/tape-stripping/UVA
J Invest Dermatol 122:1020–1029, 2004

Ultraviolet radiation is an external stressor to the skin and can induce a number of reactions. UVB radiation is believed to interact directly with molecular species in the epidermis and dermis to produce alterations in biological structures and processes. UVA (320–400 nm part of the ultraviolet radiation), on the other hand, is believed to act through the generation of reactive oxygen/nitrogen species (free radicals), which then interact with various molecular species such as nucleic acids, proteins, and lipids (Krutmann, 2000). Previous studies have shown that depletion of either skin surface oxygen supply or blood oxygen supply to the skin may reduce the UVA-induced pigmentation (Gange, 1988), indicating the importance of oxygen in UVA-induced skin reactions.

Oxidative stress is a phenomenon closely associated with life. Even under normal circumstances, most biological processes include the generation of free radicals with simultaneous photon emission. The number of emitted photons, however, is insufficient to be detected with current instrumentation. Pro-oxidants continuously produced in the skin under normal conditions can be easily accommodated by the endogenous antioxidant systems. Noxious agents, such as UVA, peroxides, and hypochlorite, can generate oxidative stress at a rate exceeding the rate of constitutive antioxidant regeneration, thereby leading to an elevated level of reactive species in the skin. The reaction between this excess and skin constituents results in the emission of photons with low quantum efficiency but detectable with current methods (Evelson *et al*, 1997; Sauermann *et al*, 1999; Stab *et al*, 2000; Yasui and Sakurai, 2000). Photon emission in the visible wavelength range has been associated with reactive oxygen species (Boveris *et al*, 1980, 1981; Evelson *et al*, 1997).

In this study, we have applied chemiluminescence to investigate UVA-induced oxidative stress in human skin *in vivo* by asking: (a) How does the skin recover from the UVA-induced oxidative stress? Chemiluminescence signals undergo a fast decay immediately following UVA exposure. A detailed study of the decay kinetics may provide an insight into the interaction between UVA photons and the skin and how the skin antioxidant system responds to UVA. (b) As UVA penetrates well into the dermis, where do the oxidative reactions occur in the skin during and following UVA exposure? It has been suspected that the stratum corneum as well as the viable epidermis and dermis may contribute to the detected photons (Sauermann *et al*, 1999; Tian *et al*, 2001). (c) If the *in vivo* chemiluminescence is directly related to oxidative stress in the skin, how does the change in the oxygen supply to the skin affect the signal?

Abbreviation: ESR, electron spin resonance; UVA, 320–400 nm part of the ultraviolet radiation

More importantly, does topical application of antioxidants reduce the UVA-induced skin chemiluminescence?

Results

Chemiluminescence could be used to evaluate oxidative stress in human skin *in vivo* and UVA-induced skin chemiluminescence signal decays exponentially with time The spontaneous photon emission from normal skin is very weak (Fig 1*a–c*). The chemiluminescence signal is only 20–40 counts per second higher than the instrument background level (Fig 1*a*). Both UVA exposure and topical application of oxidizing chemical agents induce oxidative stress in the skin. As shown in Fig 1*a–c*, the i*n vivo* chemiluminescence signals were significantly elevated when the skin is exposed to UVA or low concentrations of hydrogen peroxide (Fig 1*b*) and sodium hypochlorite solutions (Fig 1*c*). For UVA, the signal starts from its maximum value immediately following the exposure (2 J per cm^2 delivered at 20 mW per cm^2) and then undergoes a rapid decay to reach its original level in a few minutes. The data can be fitted to a two-parameter exponential decay function with $R^2 > 0.95$.

Rate of chemiluminescence decay decreases with increasing UVA doses UVA dose responses on the chemiluminescence signal were studied to better under-

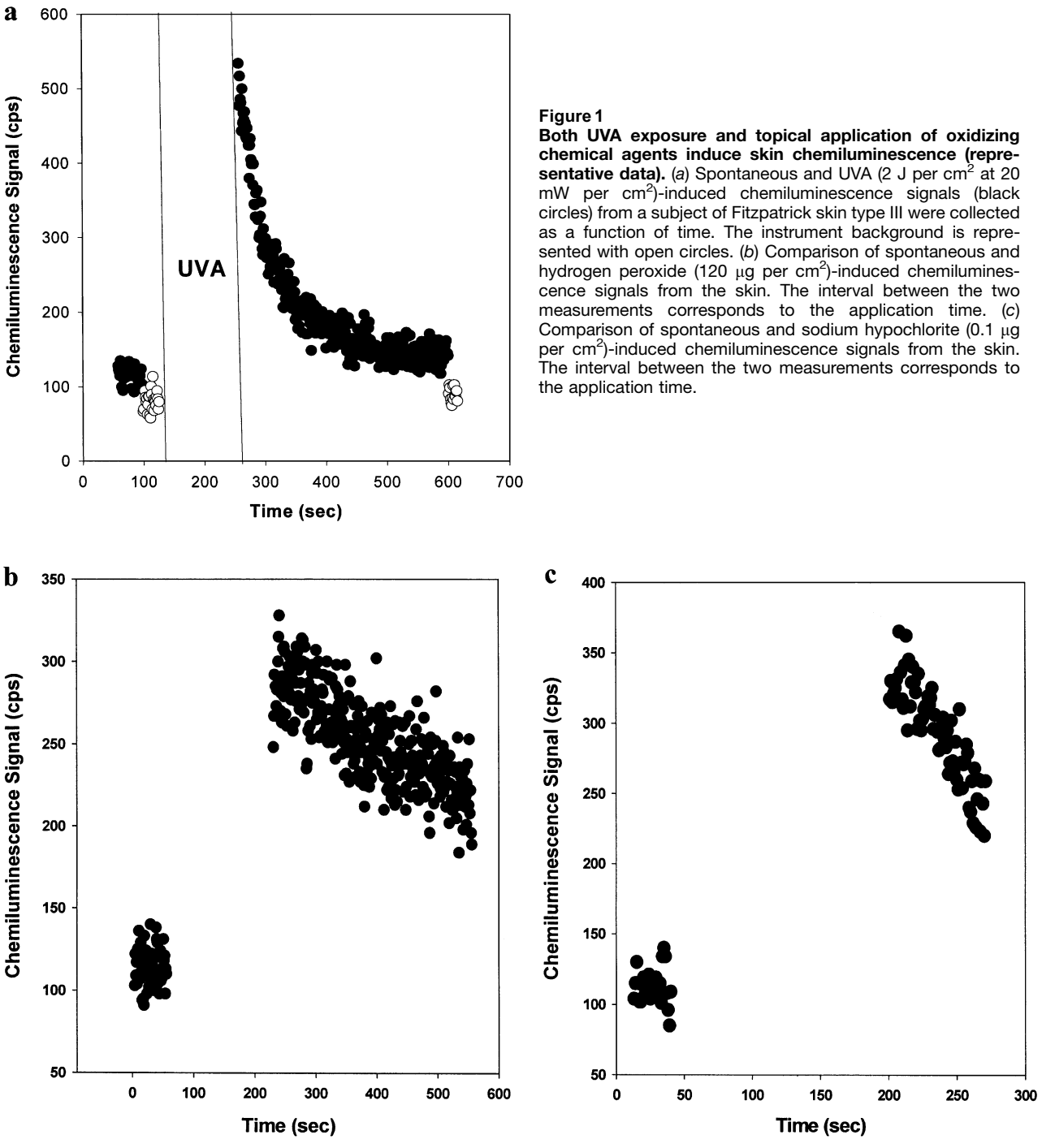

Figure 1
Both UVA exposure and topical application of oxidizing chemical agents induce skin chemiluminescence (representative data). (*a*) Spontaneous and UVA (2 J per cm^2 at 20 mW per cm^2)-induced chemiluminescence signals (black circles) from a subject of Fitzpatrick skin type III were collected as a function of time. The instrument background is represented with open circles. (*b*) Comparison of spontaneous and hydrogen peroxide (120 μg per cm^2)-induced chemiluminescence signals from the skin. The interval between the two measurements corresponds to the application time. (*c*) Comparison of spontaneous and sodium hypochlorite (0.1 μg per cm^2)-induced chemiluminescence signals from the skin. The interval between the two measurements corresponds to the application time.

stand the decay kinetics. The UVA doses ranging from 0.2 J per cm^2 to 10 J per cm^2 were all delivered to the skin with a fluence rate of 20 mW per cm^2. It is obvious that the skin recovers differently with different doses (Fig 2*a*). The total counts of the first 300 sec of chemiluminescence signal showed a monotonic increase with the dose (Fig 2*b*). The dose response tends to reach a plateau between 2 and 5 J per cm^2. All the curves were fitted to an exponential decay function and their decay constants (parameter "B") were calculated and averaged for all the nine subjects. As a general trend, chemiluminescence signals decayed more slowly with higher UVA doses (Fig 2*c*). The decay rate tends to drop very fast from 0.5 to 2 J per cm^2, and this change of decay rate slows down after 2 J per cm^2. The data in Fig 2*c* can be fitted to an equation $y = 0.82 + 1.64 \times e^{-0.79x}$ with $R^2 = 0.96$. We also found that the initial burst of the decay (parameter "A") does not correlate significantly with the dose and varies from person to person.

Initial chemiluminescence signal from the skin increases with UVA fluence rate Chemiluminescence decay curves with different UVA fluence rates were measured

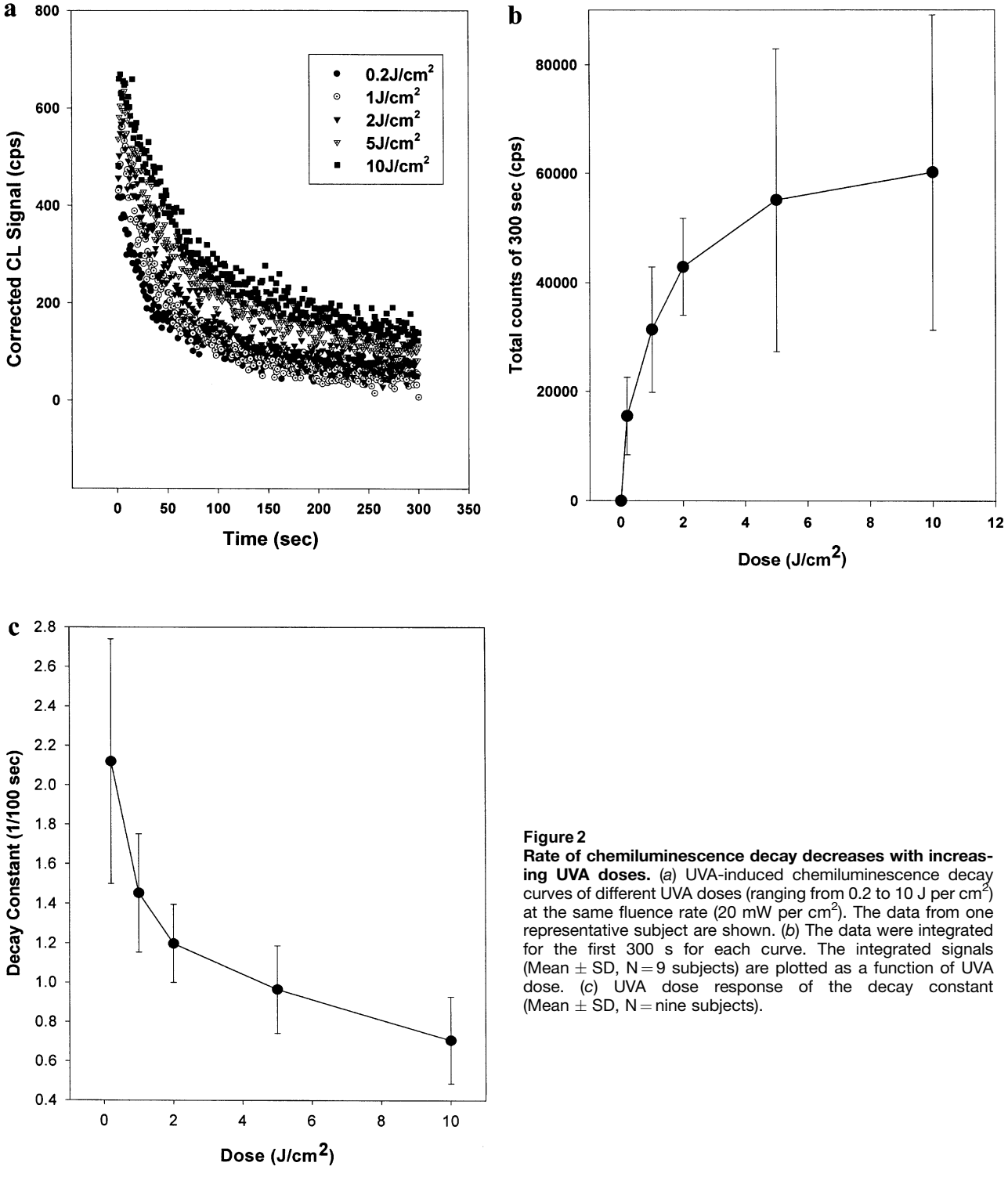

Figure 2
Rate of chemiluminescence decay decreases with increasing UVA doses. (*a*) UVA-induced chemiluminescence decay curves of different UVA doses (ranging from 0.2 to 10 J per cm^2) at the same fluence rate (20 mW per cm^2). The data from one representative subject are shown. (*b*) The data were integrated for the first 300 s for each curve. The integrated signals (Mean ± SD, N = 9 subjects) are plotted as a function of UVA dose. (*c*) UVA dose response of the decay constant (Mean ± SD, N = nine subjects).

(Fig 3*a*). The UVA dose used in this set of experiments was fixed at 2 J per cm^2, with fluence rates ranging from 2 to 20 mW per cm^2. The total counts of the first 300 s of the chemiluminescence signal as a function of the fluence rate also exhibit a monotonic increase (Fig 3*b*). The initial burst of the decay (parameter "A") is dependent on the fluence rate (Fig 3*c*). A quadratic relation ($y = 15.8 + 34.9x - 0.8x^2$ with $R^2 = 0.98$) can be found between the fluence rate and the initial burst of the chemiluminescence signals. The decay constant (parameter "B") for the same UVA dose (2 J per cm^2) is about 0.01 per s and is independent of the fluence rate.

UVA-induced skin chemiluminescence originates in part in the stratum corneum Tape-stripping is a widely used method to remove the superficial layers of the stratum corneum. To determine how much skin was removed, we applied confocal microscopy to monitor the thickness of the stratum corneum before and after 10 tape-strips. The result showed a change of 3.5 ± 1.7 μm, indicating that only part

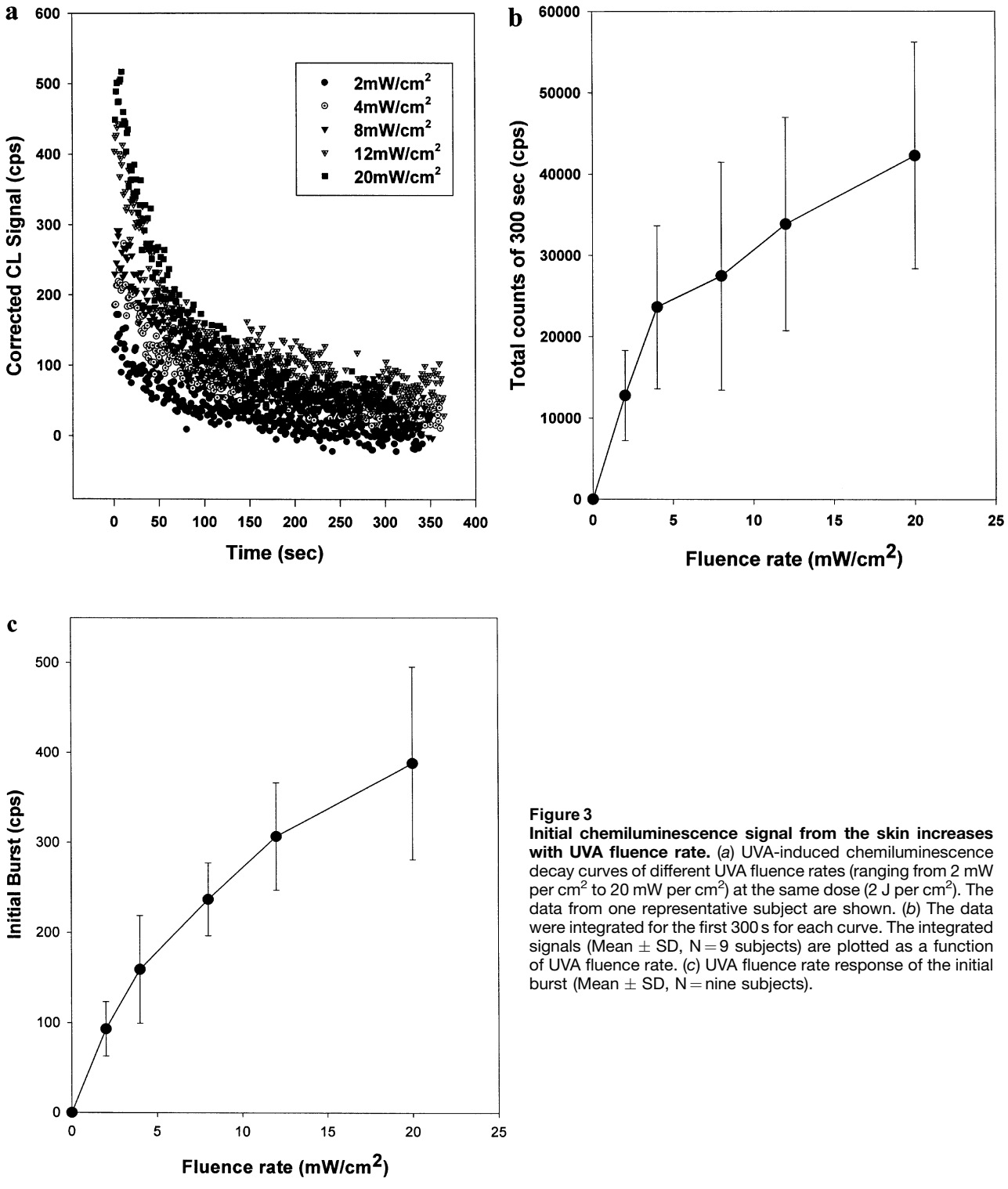

Figure 3
Initial chemiluminescence signal from the skin increases with UVA fluence rate. (*a*) UVA-induced chemiluminescence decay curves of different UVA fluence rates (ranging from 2 mW per cm^2 to 20 mW per cm^2) at the same dose (2 J per cm^2). The data from one representative subject are shown. (*b*) The data were integrated for the first 300 s for each curve. The integrated signals (Mean ± SD, N = 9 subjects) are plotted as a function of UVA fluence rate. (*c*) UVA fluence rate response of the initial burst (Mean ± SD, N = nine subjects).

of the stratum corneum had been removed. Increasing the number of tape-strips may remove more layers, but it may also induce an inflammatory response (erythema). Indeed, in this study, 10 tape-strips did not generate any visible or measurable skin inflammatory response. Comparison of the initial burst of the decay curves for the same skin site before and after 10 tape-strips (Fig 4*a* and *b*) reveals that about 30% of the chemiluminescence signal came from the outermost layers of the stratum corneum.

UVA-induced skin chemiluminescence is oxygen dependent Oxygen in skin tissue can be supplied via diffusion from the surface of the skin or via the blood circulation (Stucker *et al*, 2002). Applying a pressure cuff on the upper arm can regulate the oxygen supply to the tested skin sites. Previous studies have shown that blood oxygen supply plays an important role in UVA-induced erythema and pigmentation (Gange, 1988). Here, we found that increasing the occlusion pressure (with a pressure cuff) results in a decrease in the chemiluminescence signal (up to 40% for 60 mmHg) following UVA exposure (Fig 5*a*). The portion of the chemiluminescence signal affected by the blood oxygen supply is probably from the deeper layers of the skin, since outermost layers of the skin are still supplied with atmospheric oxygen during the UVA exposure. Furthermore, in a separate experiment, tape-stripping was combined with the application of a pressure cuff. This combination resulted in a greater reduction of the UVA-induced chemiluminescence signal than either tape-stripping or pressure cuff alone (Fig 5*b*). Together, these results confirm that UVA induces an oxidative stress in both the stratum corneum and viable skin layers.

Topical application of vitamin C can reduce the UVA-induced skin chemiluminescence To further determine the direct relationship between oxidative stress in skin and chemiluminescence measurements, vitamin C, a known antioxidant was applied on the skin 4 hrs prior to UVA exposure. Vitamin C does not absorb in the UVA wavelength range (data not shown) and consequently has no UVA-filtering effect. The significant reduction in the chemiluminescence signal by vitamin C (Fig 6) confirms the occurrence of reactive oxygen intermediates in the skin induced by UVA exposure.

Discussion

It has been established that UVA exposure can induce reactive intermediate species, which can further react with nucleic acids, lipids, and proteins in the skin (Krutman, 2000). Electron spin resonance (ESR) studies have shown that lipid alkyl and alkoxyl radicals can be generated in skin biopsies following UVA exposure (Jurkiewicz and Buettner, 1996; Herrling *et al*, 2002). From a different aspect, Packer and coworkers have shown that the cutaneous antioxidant defense system can be impaired by UVA irradiation (Fuchs *et al*, 1989; Thiele *et al*, 1999). These studies strongly support the argument that UVA induces oxidative stress in human skin (Klein-Szanto and Slaga, 1982; Panasenko, 1997; Thiele *et al*, 1999).

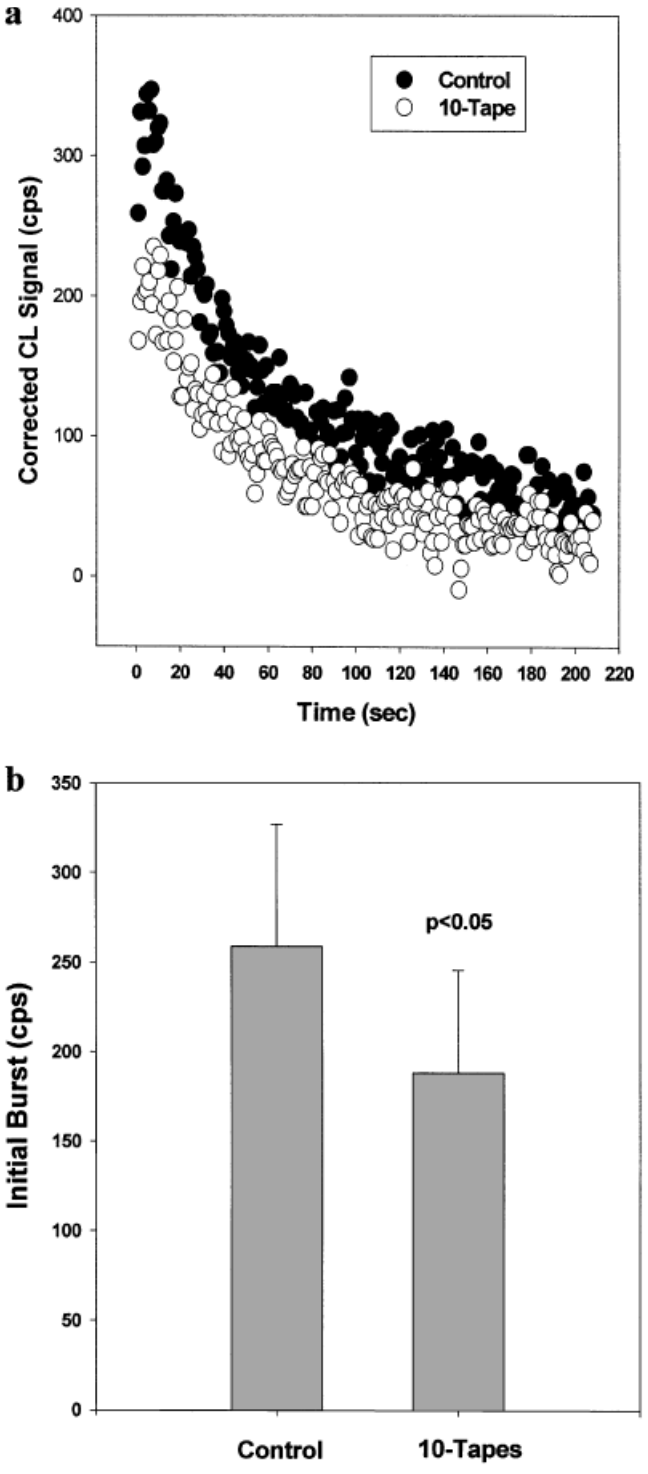

Figure 4
UVA-induced skin chemiluminescence originates in part in the stratum corneum. (*a*) UVA-induced chemiluminescence decay curves before and after 10 tape-strips (UVA dose = 2 J per cm^2 and fluence rate = 15 mW per cm^2). Representative data from one subject are shown. (*b*) Initial burst of chemiluminescence (Mean ± SD, N = 5 subjects) for the skin site before and after 10 tape-strips. p value was calculated from paired *t* test.

Chemiluminescence is a widely accepted method to study oxidative reactions in cells and in whole organs (Boveris *et al*, 1980, 1981). Using a similar instrument, we have investigated the oxidative stress in human skin *in vivo*. We have focused on the measurement of UVA-induced

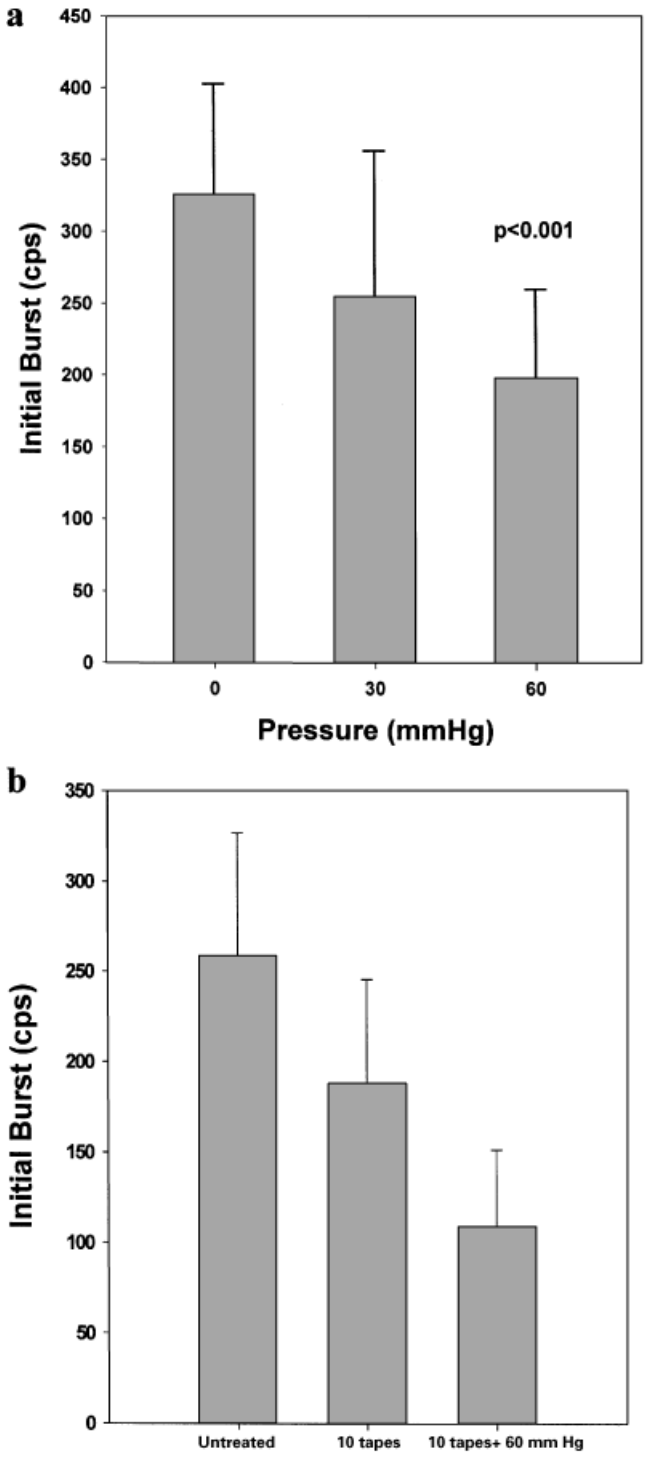

Figure 5
(*a*) UVA-induced skin chemiluminescence is oxygen dependent. The initial burst of UVA-induced chemiluminescence (Mean ± SD, N = 5 subjects) was measured in the absence or presence of a pressure cuff (set at 30 or 60 mmHg-pressure with UVA dose = 2 J per cm^2 and fluence rate = 15 mW per cm^2). p value was calculated from paired *t* test. (*b*) UVA-induced oxidative stress occurs in both the stratum corneum and the viable skin layers. Tape-stripping was combined with the application of a pressure cuff to further reduce the UVA-induced skin chemiluminescence signals. The initial burst of UVA-induced skin chemiluminescence (mean ± SD, N = 5 subjects) was compared for the various conditions: untreated control, tape-stripped ten times, tape-stripped ten times plus the application of a pressure cuff (60 mmHg) during the UVA exposure.

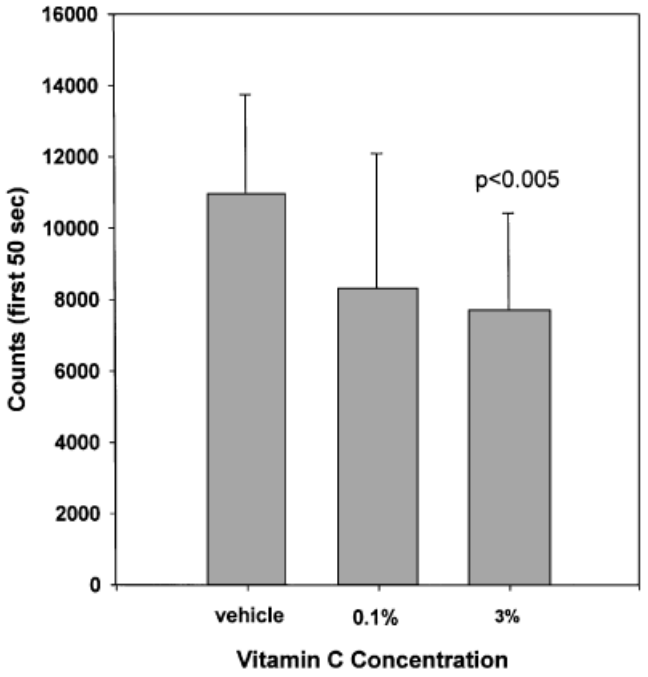

Figure 6
Topical application of vitamin C can reduce the UVA-induced skin chemiluminescence. The total counts of the first 50 s of UVA (dose = 2 J per cm^2 and fluence rate = 15 mW per cm^2)-induced chemiluminescence signals were averaged (mean ± SD, N = 7 subjects) and compared for skin sites treated with vehicle, 0.1% of vitamin C, and 3% of vitamin C. p value was calculated from paired *t* test.

oxidative stress in this study, but the same method can also be used to monitor the oxidative stress induced by other oxidizing agents. In this study, we have shown that the emission of chemiluminescence increases with the application of known oxidative agents (peroxides and chlorine) in Fig 1*b* and *c*. Therefore, the observed difference between spontaneous chemiluminescence and UVA-induced chemiluminescence (Fig 1*a*) may be related directly to the skin oxidative stress.

A major advantage of the *in vivo* chemiluminescence measurement method is that it is non-invasive. ESR that has the potential of identifying the reactive intermediates in the skin *in vivo* (He *et al*, 2002). *In vivo* measurement of reactive intermediates in the skin by ESR, however, requires topical application of spin-traps. Tape-stripping and subsequent chemical analysis of the skin tissues on the tape have also been used to monitor the oxidative stress in the stratum corneum (Thiele *et al*, 1998). In contrast, *in vivo* skin chemiluminescence method can be used to assess the oxidative stress in both the stratum corneum and the viable epidermis and dermis (Figs 4 and 5).

The decay of chemiluminescence emission measured on human skin immediately following UVA exposure has been previously shown (Sauermann *et al*, 1999; Rougier and Richard, 2002). A straightforward method to correlate the level of oxidative stress in the skin and the chemiluminescence signal is to integrate the area under the decay curve (Figs 4*b* and 5*b*). Another approach is to consider the chemiluminescence signal at the same time point for different conditions (Evelson *et al*, 1997). These methods, however, do not consider the characteristics of the decay

kinetics (i.e., initial burst and decay constant). A model has been introduced in this study to facilitate our understanding of the UVA-induced skin chemiluminescence decay. The experiment includes two distinct steps:

$$\text{Exposure}: \quad \mathbf{R} \leftrightarrow \mathbf{R}\bullet$$
$$\text{Decay}: \quad \mathbf{R}\bullet \rightarrow \mathbf{R} \quad (\mathbf{k}_1),$$

where k_1 represents the rate constant for the decay. If we assume a steady state during the exposure, i.e., the chemiluminescence emission reaches equilibrium quickly at the onset of the exposure and remains constant during the exposure, the kinetic of the chemiluminescence decay can be expressed as

$$[R\bullet] = [R\bullet]_0 e^{-k_1 t}, \qquad (1)$$

where $[R\bullet]_0$ corresponds to the initial burst (parameter "A") of the decay and is influenced only by the exposure condition (i.e., fluence rate) and the recovery capacity of the skin during the exposure. The rate constant k_1 corresponds to the decay constant (parameter "B") and has been related to UVA dose. Compared with other methods, this model can be used to differentiate the dose response and the fluence rate response of the chemiluminescence decay (Figs 2 and 3) and provides an insight into how the skin antioxidant system may respond to the UVA-induced oxidative stress.

The proposed model takes into consideration both the exposure period and the decay period, although no chemiluminescence measurements could be made during the exposure period (the UVA source would saturate the detector). For one given UVA dose, varying the fluence rate means varying the exposure time. A question then arises as to how do the exposure conditions (fluence rate and exposure time) affect the total amount of skin chemiluminescence induced by UVA exposure. In this study, the initial burst of the decay was found to be related to the fluence rate; however, the exposure time is also important for estimating skin chemiluminescence during the exposure if a steady state is assumed (Fig 7). The total amount of light emission from the skin induced by UVA should be the sum of the chemiluminescence during the exposure and the decay periods. It corresponds to the integrated area under the curve in Fig 7 and can be expressed as

$$\begin{aligned}\text{total chemiluminescence} = {} & (\text{initial burst} \\ & \times \text{exposure time}) \\ & + \text{decay integration} \qquad (2)\end{aligned}$$

The dose and fluence rate response curves of the total UVA-induced skin chemiluminescence are presented in Figs 8*a* and *b* using Equation (2). Interestingly, the total UVA-induced chemiluminescence depends strongly on the dose, but is practically independent of the fluence rate. Thus we can reduce the analysis to a single parameter model by considering both the exposure step and the decay step.

Although it has been proposed that singlet oxygen and excited carbonyls may be involved in the process of photon emission (Boveris *et al*, 1980, 1981; Barnard *et al*, 1993), the exact origin of the emitted photons still remains unknown. In fact, information about the spectral distribution of the *in vivo* skin chemiluminescence could be very useful in the characterization of reactive intermediates; however, the

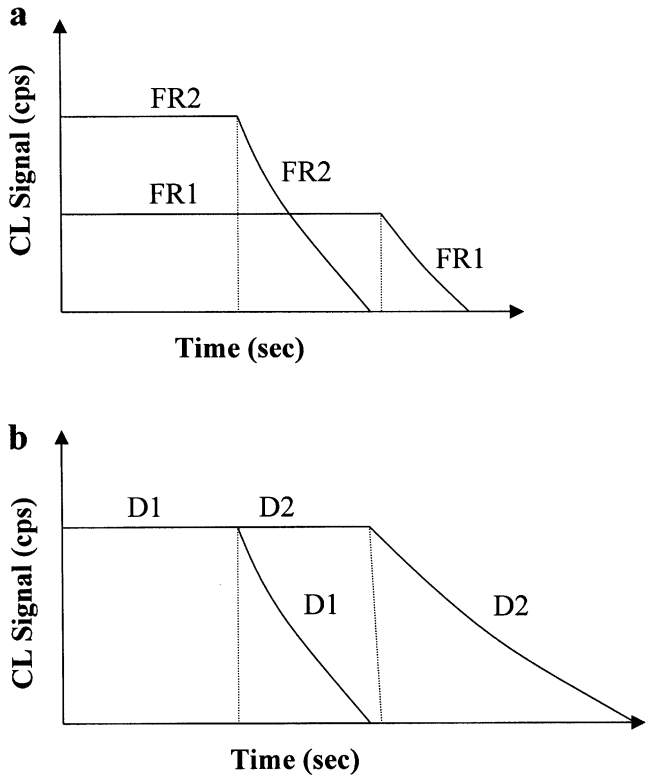

Figure 7
Proposed model for UVA-induced chemiluminescence of skin. The total UVA-induced skin chemiluminescence (including both the decay period and the exposure period) can be characterized by the integrated area under the chemiluminescence curve. (*a*) FR1 and FR2 represent two experiments with the same UVA dose, but different fluence rates (FR1 < FR2). The dotted line separates the exposure period from the decay period. (*b*) D1 and D2 represent two experiments with the same fluence rate but different UV doses (D1 < D2). The dotted line separates the exposure period from the decay period.

weak photon emission from the skin limits the use of filters or other light-discriminating devices (Evelson *et al*, 1997). An alternative approach to characterize the source of UVA-induced skin chemiluminescence is to use antioxidants to quench the signal. An earlier report on UVA-induced *in vivo* chemiluminescence in mouse skin has shown that topical application of α-tocopherol (10% wt/vol in acetone) or β-carotene (1 mM in acetone) can block the signal (Evelson *et al*, 1997). In this study, topical application of vitamin C (3% wt/vol in a skin-compatible vehicle) to human skin significantly reduced the UVA-induced chemiluminescence. Unlike α-tocopherol or β-carotene, vitamin C does not absorb UVA and its quenching activity arises primarily due to its capacity to scavenge the reactive species in the skin. This reduction of the UVA-induced chemiluminescence emission by an antioxidant further demonstrates that the UVA-induced chemiluminescence emission is related to reactive species.

A better understanding of the effects of UVA radiation on different skin layers may help to design better defense schemes. This study demonstrated that part of the UVA-induced chemiluminescence comes from the stratum corneum, thereby confirming that the outermost layer of the skin is an important UVA target and thus a source of oxidative stress. Keratin cross-links, which are present in corneocytes, may be the primary absorbers of UVA in the

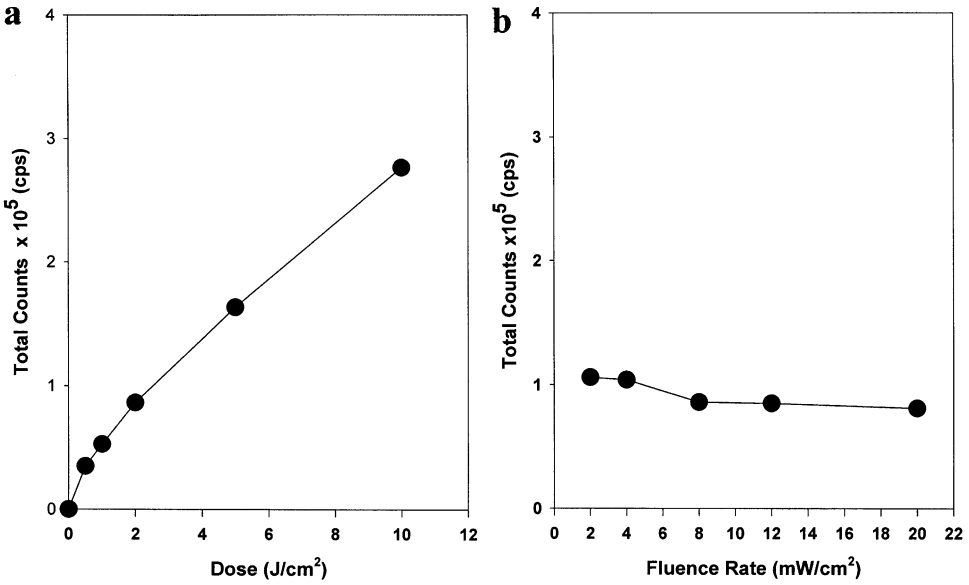

Figure 8
Integrating the chemiluminescence signal over the times during and following UVA exposure results in a single parameter model (i.e., removes the fluence rate dependency). (*a*) UVA dose response (fluence rate = 20 mW per cm^2) of estimated total chemiluminescence signals. The dose response curve presents a stronger linear correlation compared with Fig 2*b* (*b*) UVA fluence rate response (dose = 2 J per cm^2) of estimated total chemiluminescence signals. The fluence rate response curve is practically independent of the fluence rate.

stratum corneum. Oxidative stress in the stratum corneum could be a mechanism for the UVA-induced changes of stratum corneum barrier function described by McAuliffe and Blank (1991).

UVA-induced oxidative stress involves more than the superficial layer of the skin. The correlation between chemiluminescence signals and blood oxygen supply reveals that some of the signals may originate from the viable layers of the skin. Oxygen can be supplied to the skin tissue either by the atmosphere or by the blood vessels. A 30%–40% reduction of the initial burst was observed when a pressure cuff on the upper arm was inflated to a pressure of 60 mmHg, strongly suggesting that at least this portion of the chemiluminescence signal came from the vascularized dermis and the viable epidermis. This is possible because chemiluminescence emission is believed to occur at wavelengths longer than the hemoglobin absorption bands at 540–590 nm; therefore the attenuation of chemiluminescence by blood vessels is not significant. Interestingly, the combination of tape-stripping and application of a pressure cuff further reduced the chemiluminescence signal (Fig 5*b*), indicating that the origins of the chemiluminescence that can be modulated by tape-stripping and by application of a pressure cuff are additive and therefore to a great degree independent. In fact, UVA photons penetrate into the dermis and induce structural changes in collagen. *In vivo* fluorescence spectroscopy has revealed that the intensity of the intrinsic collagen cross-link fluorescence decreases following UVA exposure (Kollias *et al*, 1998; Tian *et al*, 2001) at doses similar to the ones used in this study, suggesting that photochemical reactions may occur readily in the dermis. Dermal collagen cross-links are possible targets for UVA photons and their break-down may contribute to the overall chemiluminescence signal.

In this study, we used chemiluminescence emission to assess the UVA-induced oxidative stress in human skin *in vivo*. We established the dependence of chemiluminescence signal on the UVA dose and fluence rate. The model proposed, considering the chemiluminescence emission during both the exposure and the decay times, allows the estimation of the total UVA-induced skin chemiluminescence emission. We demonstrated that the oxidative interaction between UVA photons and the skin is partially oxygen-dependent and may be reduced by topical application of antioxidants. This interaction occurs both in the stratum corneum and the deeper layers. Moreover, this study highlights the advantages of chemiluminescence as a non-invasive method to study *in vivo* cutaneous oxidative stress in human skin. The presented method can be easily adapted to measure the protective properties of antioxidants against photo- and chemical-induced oxidative stress in human skin.

Materials and Methods

Chemicals Hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl) and L-ascorbic acid (vitamin C) were purchased from Sigma Chemicals (St. Louis, Missouri). H_2O_2 and NaOCl were diluted in water/propylene glycol (70:30, vol/vol). Ascorbic acid was dissolved in water/ethanol/propylene glycol (50:35:15, vol/vol).

Instrumentation

UVA light source The UVA light source used throughout this study was a Hamamatsu LightingCure 200 UV Spot light source (Hamamatsu, Bridgewater, New Jersey) with a 150 W Xenon lamp and a UV dichroic reflector fixed behind the lamp to direct and filter the light (filter range from 300 to 450 nm with maximum at 365 nm). The light source was further filtered with Schott UG11 (1 mm) and WG335 (3 mm) glass filters (Schott, Elmsford, New York). The output was delivered to a beam conditioner (Hamamatsu E5147–06) by a quartz fiber bundle (Hamamatsu A2873, 10 mm in diameter). Condenser lenses were used to provide large areas of uniform irradiation (up to 100 × 100 mm). The spot size of UVA illumination on the skin was adjusted to be 2.5 cm in diameter so

that it matched the size of the photomultiplier import port. The spectral distribution of this source (Fig 9) was characterized with a spectroradiometer (Optronic Laboratories, Orlando, Florida, model 752). The spectral output of the source was in the UVA range with maximum output in 340–380 nm and less than 0.5% of the maximum irradiance at wavelengths below 320 nm. The output of this light source was routinely monitored with a thermopile (Oriel, Stratford, Connecticut, Model 71140), which was calibrated by Eppley Laboratory (New Port, Rhode Island).

Chemiluminescence setup (Fig 10) Photons emitted from the skin were measured by a red-sensitive, tri-alkali photomultiplier (PMT) with a 2.5 cm diameter photocathode surface (Electron Tubes, Rockaway, New Jersey, Model 9828SA). The photomultiplier was in a Peltier-cooled housing unit (Electron Tubes, Model CDM30) and was routinely cooled to $-18^{\circ}C$–$20^{\circ}C$ to reduce detector noise. The housing unit can be held with one hand as a probe and placed on the skin area to be measured. The surface of the photocathode was only protected by a manual shutter (Products for Research, Inc. Danvers, Massachusetts, Model PR318). The shutter is opened for measurements and closed for instrument background.

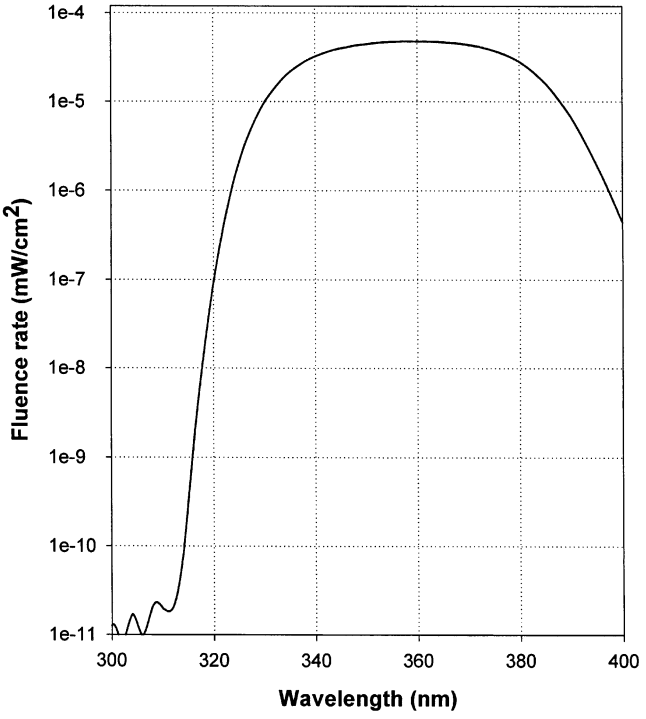

Figure 9
UVA light source. The spectral distribution of the light source used in this study.

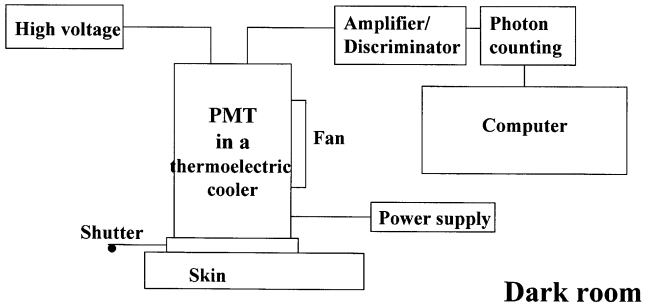

Figure 10
Chemiluminescence measurement setup. PMT refers to the photomultiplier.

The distance between the skin and the photocathode surface was about 1.5 cm. The PMT was supplied with a potential of -1.15 kV of high voltage (Brandenburg, Brierley Hill, UK, Model 477) with a variation less than ± 2 V. After going through an amplifier and discriminator (Electronic Tubes, Model AD6), the output signals from the PMT were measured in a photon-counting module (Electronic Tubes, Model CT1) and recorded by a computer. All measurements were conducted in a positively dark room, otherwise the background would overwhelm the signal. Volar forearms were used for all the *in vivo* measurements.

Measurements

General procedure An independent review board approved the study. Nine human volunteers of Fitzpatrick skin type II-IV participated in the study and they gave written consent prior to the experiments. Baseline chemiluminescence measurements were acquired from a skin site on the volar forearm before the site was exposed to UVA irradiation. Immediately following UVA, the same skin site was presented to the detector to collect the UVA-induced chemiluminescence. Data were collected over 5 min following each UVA exposure to monitor the skin recovery process. In the experiments involving H_2O_2 and NaOCl, the solutions were applied on a skin area of 2.5 cm in diameter (to match the detector size) using a pipette. Typically, the final concentrations of 12–120 μg per cm^2 of H_2O_2 and 0.01–0.1 μg per cm^2 of NaOCl solutions were applied topically on the skin. The subsequent measurements were conducted within 3–5 min following the application of H_2O_2 and NaOCl. In the vitamin C experiments, 20 μL of the vehicle, 0.1%, and 3% vitamin C (wt/vol) solutions were applied on three different but adjacent skin sites (2.5 cm in diameter) of each subject. These sites were exposed to UVA 4 h later and the chemiluminescence measurements were subsequently taken.

Dose response A series of UVA doses (ranging from 0.2 to 10 J per cm^2) were delivered to the volar forearm of each subject with a constant fluence rate of 20 mW per cm^2. No erythema was detected for any subject either immediately or 24 h after the exposure. For some people, immediate pigment darkening was observed at doses greater than 2 J per cm^2 but faded away in a few minutes.

Fluence rate response A series of exposures of the same UVA dose (2 J per cm^2) and different fluence rates (ranging from 2 to 20 mW per cm^2) were applied on the volar forearm of each subject, and chemiluminescence measurements were conducted before and immediately after UVA exposure.

Tape-stripping Adhesive D-squame tapes (2.5 cm in diameter, Cuderm Corporation, Dallas, Texas) were applied on the skin of five subjects and gently removed after one minute of application. The procedure was repeated ten times for each subject. The tape-stripped site was subsequently exposed to UVA (dose $=2$ J per cm^2 and fluence rate $=15$ mW per cm^2), and chemiluminescence was recorded for the same skin site. The thickness of the stratum corneum was measured before and after 10 tapes by an *in vivo* confocal microscope (Lucid, Henrietta, New York, Vivascope 1000). The detailed operating procedure of confocal microscope has been described elsewhere (Rajadhyaksha *et al*, 1999).

Oxygen dependency Oxygen supply to the skin can be modulated by arterial occlusion and this can be accomplished by applying a subdiastolic pressure. A pressure cuff was applied on the upper arms of each of the five subjects during UVA exposure (dose $=2$ J per cm^2 and fluence rate $=15$ mW per cm^2). For each subject, three different pressures settings (0, 30, and 60 mmHg) were used to regulate the blood oxygen supply to the skin on the lower forearm (measurement site). In each experiment, the pressure was kept in place 5 min before and during the exposure. The pressure was released immediately after the exposure to eliminate any possible attenuation of the signals from concentration variations of

deoxy-hemoglobin in skin tissues. The chemiluminescence measurement started after the release of the pressure and continued for 5 min.

In another set of experiments, a skin site on the volar forearm of each of the five subjects was tape-stripped ten times before a pressure cuff was applied. Then the same procedure was followed to examine the combination effect of tape-stripping and applied pressure.

Data analysis The chemiluminescence signals collected following UVA exposure were first corrected by the baseline spontaneous chemiluminescence signal and then integrated over the decay time. The UVA-induced chemiluminescence decay curves were also fitted to a two-parameter exponential function $y = A \times e^{-Bt}$ after the baseline correction, where "t" represents the time immediately following the UVA exposure. Parameter "B" represents the decay constant of the curve and parameter "A" represents the initial burst of the signal. The data were expressed as mean $\pm$ SD for five to nine subjects. Paired *t* tests were conducted to compare the mean values of different data sets.

DOI: 10.1111/j.0022-202X.2004.22405.x

Manuscript received April 28, 2003; revised November 4, 2003; accepted for publication December 11, 2003

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