Environmental Science & Technology

Article

pubs.acs.org/est

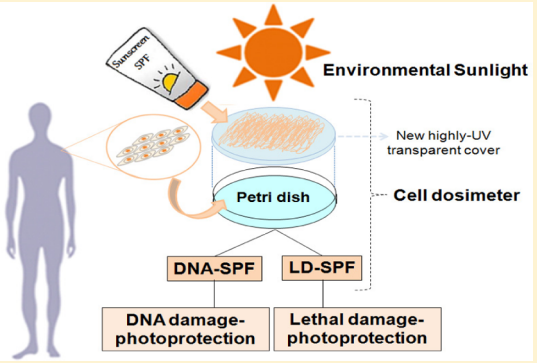
Highly Sensitive Biological Assay for Determining the Photoprotective Efficacy of Sunscreen

André P. Schuch, Maria Carolina S. Moraes, Teiti Yagura, and Carlos F. M. Menck*

Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 1374, São Paulo, SP 05508-000, Brazil

Supporting Information

ABSTRACT: The protective effect of sunscreens has been extensively evaluated *in vivo* as a measure of erythema induced in human skin and is expressed as Sun Protection Factor (SPF). *In vitro* alternatives that use human cells might overcome the limitations of testing on human beings. Here is proposed a broad and accurate *in vitro* approach for evaluating the efficacy of commercial sunscreens even under environmental conditions. This Cell dosimeter allowed the determination of Sun Protection Factor for DNA (DNA-SPF), using specific DNA repair enzymes and antibodies, and Sun Protection Factor for Lethal Damage (LD-SPF), by measuring cell viability and apoptosis induced after the irradiation of human cells. The use of xeroderma pigmentosum (XP) cells, which are deficient in DNA repair, rendered this assay more sensitive. The results revealed significant protection against the effects elicited by UVB radiation; however, there was no efficient protection from DNA lesions and cell death induced by UVA radiation or natural sunlight. This work demonstrates the environmental application of this biodosimeter for measuring UV-induced biological damage to human cells and supports the need for better evaluation of the UVA protection efficacy conferred by commercial sunscreens, in terms of induction of DNA lesions and cell death.



INTRODUCTION

The terrestrial ultraviolet (UV) component of sunlight, which corresponds to UVB (280−315 nm) and UVA (315−400 nm) wavelengths, is considered the predominant cause of several human dermal pathologies, ranging from sunburn to skin aging and the induction of skin tumors.[1] In fact, cell death and mutagenesis represent the most important cellular effects elicited by the UV irradiation of human skin.[2] Nonetheless, the chemical nature and the efficiency of DNA lesion formation significantly depends on the wavelength of incidental UV photons, which correlates directly with geographical location on the planet.[3] The direct excitation of DNA bases by solar UV radiation leads to well-known modifications that trigger dimerization reactions predominantly between adjacent pyrimidines, resulting in the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP).[4,5]

Conversely, sunlight can also indirectly damage DNA after the absorption of photons by other chromophores, generating reactive oxygen species that can oxidize nucleotide bases.[6−8] In contrast, recent studies have suggested that CPDs represent the most relevant UVA-induced type of DNA lesion observed in various biological models, including purified DNA samples,[5] cultured cells,[9] and whole-skin explants.[10] Recently, *in vivo* assays revealed that UVA1 (340−400 nm) can induce CPDs in the skin of healthy volunteers, indicating that UVA radiation might be more carcinogenic than previously assumed.[11] Moreover, as UVA penetrates more deeply, CPDs induced by UVA were found in internal human corneal tissues.[12] Therefore, in general, CPDs are considered the predominant type of DNA damage that is attributable for the deleterious effects elicited by sunlight on human cells.[13,14]

With regard to public healthcare, photoprotection has become a topic of increasing interest, and the use of sunscreen lotions is widely considered to be one of the main preventive measures against the harmful effects of UV radiation. The protective efficiency of sunscreens is predominantly evaluated *in vivo* by measuring the induction of erythema in human skin and is expressed as a Sun Protection Factor (SPF). In addition to SPF, other UVA protection parameters, such as *in vivo* Persistent Pigment Darkening (PPD) and *in vitro* UVA-PF, have recently been described.[15] An important issue concerning biological relevance is that these parameters do not reflect the deleterious effects of UV radiation, including immunosuppression, photoaging, and carcinogenesis.[1,16] Furthermore, it is evident that SPF labeling directly influences personal exposure time; people use sunscreens to intentionally prolong sun exposure.[17]

Received: May 20, 2014
Revised: September 4, 2014
Accepted: September 12, 2014
Published: September 12, 2014

Nonetheless, the severe depletion that the stratospheric ozone layer suffered over the last decades resulted in an increase of the incidence of UV radiation on the Earth's surface, which has been predicted to continue to increase throughout most of this century.[18,19] In addition, the synergistic interaction between ozone depletion and global warming will possibly enhance the incidence of skin cancer by about 20% in the UK alone, an increase estimated in 5,000 to 6,000 cases per year by 2050.[20] Furthermore, a similar but proportionally higher impact can be expected in tropical countries, such as Brazil, where increasing exposure to very high UV doses should deal a heavy blow in terms of public healthcare. Due to little access to pertinent information and the lack of necessary financial resources for adequate skin protection, people will be increasingly prone to develop skin tumors, thereby generating a greater demand for care as well as additional expenditure for appropriate treatment by public health systems.

This situation is further complicated for people suffering from diseases that are associated with extreme sensitivity to sunlight, such as the genetic syndrome called xeroderma pigmentosum (XP). This disease is caused by mutations in any of the XP genes (*XPA* to *XPG* and the variant *XPV*), the products of which play multiple roles in the Nucleotide Excision Repair (NER) and Translesion Synthesis (TLS) pathways.[21−23] In general, these individuals exhibit an increased frequency of cancers in areas of skin that are exposed to sunlight, and their cells unveil an elevated mutation rate when irradiated with UV light, which is directly associated with tumor formation.[23,24] In addition, the fact that NER-deficient cells are extremely sensitive to UV light is a clear indication that unrepaired UV-induced DNA lesions constitute the main cell death triggering signal after UV irradiation.[25−27] Therefore, these features make this type of cell very attractive for development of *in vitro* alternative approaches to evaluate sunscreen photoprotection, overcoming the limitations of current methodologies.

Extensive evidence of DNA protection conferred by sunscreen exists and has been demonstrated using various biological models, including the skin of human volunteers,[28] mice,[29] human skin explants,[1] a reconstructed human skin model,[30] and *in vitro* cultured human cells.[31,32] Recently, our group introduced the use of DNA dosimetry in the assessment of genotoxic damage for environmental sunlight of different locations (latitudes) in South America[3] and protection conferred by sunscreen.[33] In this work a relatively simple and economically feasible *in vitro* method was designed to quickly and accurately measure the protective properties of sunscreens directly in human cells, quantifying different types of DNA damage and cell death by artificial UV lamps or natural sunlight. This dosimeter provides the quantification of the Sun Protection Factor for Lethal Damage (LD-SPF), measuring cell viability after UV treatment, and the determination of the Sun Protection Factor for genomic DNA (genomic DNA-SPF), using a CPD-specific antibody. These analyses were based on the use of the human skin fibroblast cell line XP4PA (mutated at the *XPC* gene), deficient in the global genome repair of NER (GGR-NER). The results were compared with a DNA dosimeter, which determines the Sun Protection Factor for DNA (DNA-SPF) using purified plasmid DNA samples to measure the induction of both CPDs and oxidized DNA bases.

In this work, we demonstrate, through the use of these biosensors, that it is possible to both qualitative and quantitatively assess the protective properties of commercial sunscreens against the genotoxic impact induced by artificial UV-light and also environmental solar radiation. It is reasonable to assume that this technology can be broadly used to evaluate the efficacy of commercial sunscreens for the general human population.

EXPERIMENTAL SECTION

Cell Dosimeter and Quantification of UV-Induced Lethal Damage. The Cell dosimeter was constructed out of the same material as the DNA dosimeter. This made it possible to create a new highly UV-transparent cover for Petri dishes (60 mm in diameter) that promotes an airtight seal on the dish for cell culture and allows the exposure of human cultured cells directly to sunlight in a hermetically sealed, sterile, and highly transparent environment for UVB/UVA wavelengths.

To calculate the Biologically Effective Dose (BED) induced by irradiation, cellular viability was measured with the colorimetric assay XTT Cell Proliferation Kit II (Roche, CH) (please see details in the Supporting Information, page S5). BED values provide the average level of lethal damage induced per cell after UV treatment, as obtained from the following equation

$$\mathrm{BED} = -\ln(n_{\mathrm{exposed}}/n_{\mathrm{control}})$$

where n_{exposed} represents the absorbance signal of the cell samples irradiated with UV light (490 nm−750 nm), and n_{control} represents the absorbance signal of the nonirradiated control samples (490 nm−750 nm).

UV-Irradiation of Biodosimeters in the Presence or Absence of Sunscreens. Before each irradiation, sunscreens were applied to the surface of each biosensor using a fine brush, at a density of 2 mg/cm^2, in accordance with the recommendations of the COLIPA/CTFA-SA/JCIA/CTFA International Sun Protection Factor (SPF) Test Method Guideline (see Supporting Information, Figure S1A-B for a representative example of the use of Cell and DNA dosimeters as well as their transmittance spectra).

Due to the large variety of brands, formulations, prices, and SPFs available in the market, four sunscreens of the same brand (SPFs ranged from 8, 15, 35, and 60) and a moisturizing lotion without sunscreen in the formulation (vehicle) were tested. The chosen brand represents a widely available popular commercial product that is available in supermarkets, pharmacies, and department stores. The composition of these products is described in the Supporting Information, pages S6−S8.

The treatment with artificial UVA and UVB radiation sources was made under controlled conditions inside the laboratory, as described in the Supporting Information, pages S2−S3.

The Sun Protection Factor for Lethal Damage (LD-SPF) and Its Respective Percentage of Protection against the Induction of Lethal Damage. The calculation of LD-SPF was adapted from the COLIPA/CTFA-SA/JCIA/CTFA International Sun Protection Factor (SPF) Test Method Guideline.[34] This was determined using the arithmetic averages of individual LD-SPF values obtained after UV irradiation treatments (n), as described by the following equation

$$\text{LD-SPF} = (\Sigma\text{LD-SPFi})/n$$

where LD-SPFi was calculated by the ratio between the BED induced by UV light in the exposed cells without protection

(vehicle) and the BED verified in the irradiated cells that were protected with sunscreen.

It was also of interest to evaluate this new protective factor to clearly demonstrate the biological protective efficacy of a specific LD-SPF provided by a sunscreen. Therefore, for each LD-SPFi obtained, the corresponding percentage of photoprotection against the induction of lethal damage (% Lethal damage photoprotection) was calculated. The total % Lethal damage photoprotection was determined by the arithmetic average of the individual values for the % Lethal damage photoprotection.

Determination of Genomic DNA-SPF by Immunoblot Assays. The measurement of UV-induced CPDs was performed using a anti-CPD antibody, as described in the Supporting Information, pages S5−S6.

To estimate the amount of CPDs induced per kbp of genomic DNA in the XP4PA cells, these experiments were normalized using purified plasmid DNA samples that were irradiated in a DNA dosimeter under the same experimental conditions. This was performed by determining the ratio between the number of T4-endo V sensitive sites (T4-endo V-SS = CPDs) and the intensity of the bands detected by the antibody. Based on this information, it was possible to estimate number of CPDs per kbp of genomic DNA and to determine the genomic DNA-SPF for CPD induction provided by these products.

Induction of Apoptosis by Flow Cytometry. In parallel to determining the BED and Cell-SPF, the induction of apoptosis at 48 h after UV treatment was also quantified by flow cytometry, identifying sub-G1 cells, as described in the Supporting Information, page S6.

RESULTS AND DISCUSSION

The Use of a Cell Dosimeter in Environmental Exposures to Sunlight. Prior to the determination of photoprotection properties of commercial sunscreens, it was of interest to demonstrate the environmental relevance for the use Cell dosimeter by performing irradiations directly to the natural sunlight in the city of São Paulo, Brazil (23°3′S; 46°4′W). The purpose of this experiment was to quantify the genotoxic and cytotoxic impact of solar UV radiation in DNA repair proficient and deficient human cell lines. These environmental exposures were performed in parallel to physical radiometers that monitor solar UVB/UVA radiation incidence. The MRC5 (proficient in DNA repair) and XP4PA (mutated at the *XPC* gene, deficient in NER) cell lines were irradiated for three different treatment periods under the midday sun on a clear sky day (May/2011, fall in the Southern Hemisphere). The measurements of solar UVB/UVA radiation doses as well as the induction of lethal damage by sunlight in both cell lines are shown in Figure 1.

Relatively high UVB and UVA doses reach the ground at the latitude of São Paulo. Additionally, these high UV doses are directly correlated with an increase in the induction of lethal damage in both the MRC5 and XP4PA cell lines. As expected, the XP4PA cell line, which is deficient in DNA repair, exhibited increased sensitivity to solar radiation compared to the DNA repair-proficient MRC5 cell line.

In order to investigate the protective efficiency of commercially available sunscreens for this genetic background that leads to hypersensitivity to solar UV radiation, further experiments were performed through the use of UVB and UVA lamps inside laboratory, with doses similar to 1 h exposure at

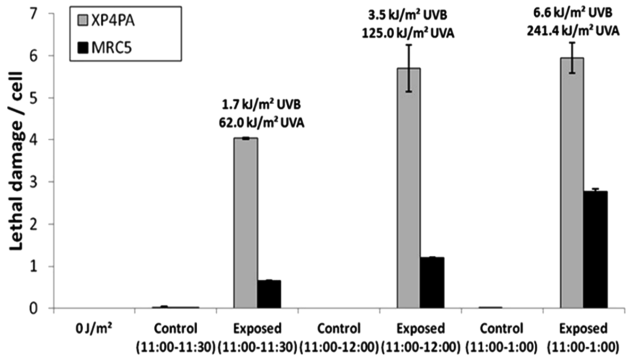


Figure 1. Quantification of lethal damage induced by sunlight in the MRC5 (black) and XP4PA (gray) cell lines. ″0 J/m^{2}″ indicates the nonirradiated control that remained inside the laboratory (37 °C) during the experiment. "Control" indicates the Cell dosimeters that were exposed to ambient temperatures during the same period of irradiation but that were covered with aluminum foil (temperature control). The average and standard deviation of experiment performed in triplicate.

midday in Sao Paulo city. It is important to emphasize that the choice of a single UVB/UVA dose higher than the median lethal dose (LD50%) was made as this leads to less than 1% viability (the sunlight exposure, from 11:00 to 12:00 a.m., resulted in 0.4% of viable cells), thus allowing quantification of almost 100% of photoprotection against lethal damage. The same reasoning is used for the calculation of DNA damage, since the applied UVB/UVA doses reach the peak of formation of DNA damage in DNA dosimeter (e.g., > 1.0 CPDs/kbp), and the amount of plasmid DNA molecules without CPDs is almost null. Additionally, this purified DNA sample irradiated with DNA dosimeter is used for the calibration of the assay, which determines the amount of CPDs/kbp induced in the genomic DNA.

Genotoxic and Cytotoxic Photoprotection for UVB and UVA Radiation. The current SPF method is a ratio calculated from the energy required to induce a minimum erythemal response with and without sunscreen applied to the skin of human volunteers, using UV radiation from an artificial source. However, the UV-induced erythema in unprotected human skin is mainly generated by wavelengths between 295 and 320 nm, with a maximum effectiveness around 308 nm.[34] Therefore, this methodology lacks a fundamental environmental underpinning, since these UV wavelengths correspond to less than 5% of total solar UV radiation that human beings are naturally exposed outdoor. Consequently, new alternative methods are required to evaluate the sunscreen protective effectiveness for all UV wavelengths of natural sunlight.

The use of highly sensitive DNA repair deficient cells from XP patients might be very beneficial for these approaches since it can reduce the limits of detection of DNA damage and cell death resulting from sunlight exposure, making the quantification of genotoxicity and cytotoxicity precise parameters to reflect the decrease of UV dose provided by the sunscreen. However, one limitation of our alternative *in vitro* assay is the relative poor connection with carcinogenesis, and improvements would be important to achieve this critical point. Notwithstanding, the actual SPF methods have a much lower connection with carcinogenesis because they are only based on the evaluation of erythema induction or skin pigmentation.

On the other hand, it is well-known that the UV-induced DNA damage is the basis of skin carcinogenesis, and XP

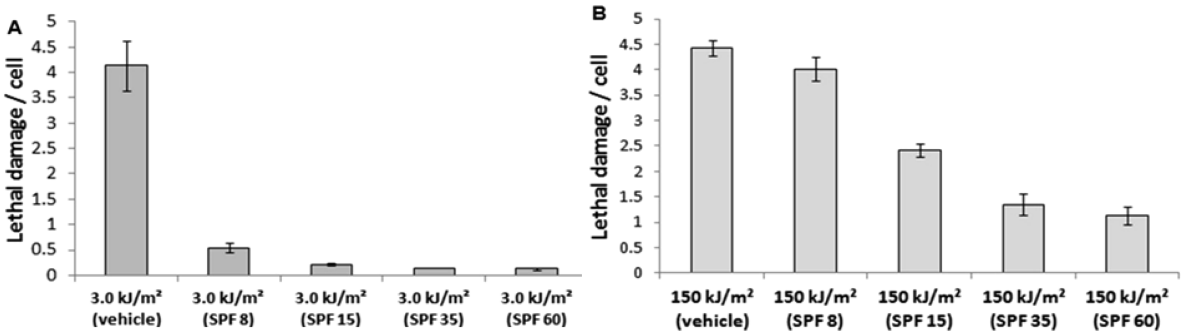


Figure 2. Quantification of lethal damage induced by UVB (A) and UVA (B) radiation in the absence (vehicle) or presence of 4 different sunscreens. The average and standard deviation of three independent experiments performed in triplicate.

patients develop tumors with high frequency due to their deficiency on DNA damage processing. Therefore, the evaluation of sunscreen photoprotection efficacy against the induction of DNA lesions in this UV-hypersensitive cellular model provides indirect indication of protection against carcinogenesis (at least against mutagenesis) that would be much more informative than the protection against erythema or skin pigmentation, especially for those consumers presenting high sensitive to sunlight.

The XP4PA cells were exposed to UVB and UVA lamps in the presence or absence of sunscreens. Figure 2 shows the protective efficacy of these sunscreens against the induction of lethal damage by both UVB and UVA exposures. The LD-SPF and the percentages of photoprotection against lethal damage are presented in Table 1.

Table 1. Lethal Damage Photoprotection Properties Provided by Sunscreens for UVB and UVA Radiation[a]

sunscreens	LD-SPF (95% CI)	% lethal damage photoprotection (95% CI)
SPF 8 (UVB)	7.7 (3.4 to 12.1)	86.6 (78.3 to 94.8)
SPF 15 (UVB)	19.4 (18.6 to 20.2)	94.8[b] (94.6 to 95.7)
SPF 35 (UVB)	27.9 (17.2 to 38.5)	96.4[b] (95.1 to 97.7)
SPF 60 (UVB)	32.4 (26.2 to 38.5)	96.9[b] (96.3 to 97.5)
SPF 8 (UVA)	1.1 (0.9 to 1.3)	12.7 (8.6 to 16.8)
SPF 15 (UVA)	1.8 (1.5 to 2.1)	45.3[b] (36.5 to 54.0)
SPF 35 (UVA)	3.3 (1.9 to 4.7)	69.5[b,c] (56.1 to 82.8)
SPF 60 (UVA)	4.0 (2.3 to 5.6)	74.3[b,c] (63.3 to 85.3)

[a]LD-SPF - Sun Protection Factor for Lethal Damage; % Lethal damage photoprotection—percentage of protection against lethal damage; and 95% CI − 95% confidence interval of three independent experiments performed in triplicate. [b]Statistically different from SPF 8. [c]Statistically different from SPF 15 ($p < 0.05$).

Taken together, these data show that the majority of these products were effective in protecting the XP-C cells against lethal damage induced by UVB but not by UVA light (Figure 2). Although sunscreens of SPF 15, 35, and 60 were significantly more efficient than the SPF 8 sunscreen, it was not possible to statistically distinguish which was the most effective in protecting against UVB-induced cell death, even though LD-SPF increased according to the labeled SPF of the product. Surprisingly, none of the tested sunscreen exhibited photoprotection, as expected from the SPF label, against the lethal damage induced by UVA light in the XP-C cells, presenting LD-SPF values much lower than one-third of the SPF label. Despite the low LD-SPF values and lethal damage photoprotection observed for UVA, the SPF 15 was significantly more efficient than the SPF 8 sunscreen, and the SPF 35 and 60 were more efficient than the SPF 8 and 15 sunscreens. There were no statistically significant differences observed between SPF 35 and 60 sunscreens.

These viability results were consistent with the observations of UV-induced apoptosis. The quantification of sub-G1 populations after UVB and UVA light treatment is presented in Figure 3. With the exception of SPF 8, sunscreens with SPFs greater than or equal to 15 elicited satisfactory protection against UVB-induced apoptosis. However, these results confirm that, in fact, none of these products efficiently protected against UVA-induced apoptosis.

To confirm that the cellular photoprotection of the XPC cells observed was directly attributed to the prevention of formation of genomic DNA lesions by the application of sunscreen on the surface of Cell dosimeter, immunoblot analysis was performed using a specific antibody anti-CPD immediately after the irradiation to avoid DNA repair (time 0 h) (see Supporting Information, Figure S2). The number of CPDs per kbp of genomic DNA induced by either UVB or UVA radiation as well as the values of genomic DNA-SPF together with the percentage of CPD-photoprotection are presented in Table 2. These experiments were normalized using the purified plasmid DNA irradiated in the DNA dosimeter, as described in the Experimental Section (see Supporting Information, Table S1 and Figure S3).

These results clearly demonstrate that the tested products are generally effective in protecting against UVB-induced DNA damage in purified plasmid DNA as well as in genomic DNA, although the same protective effect was not observed after UVA exposure (Figures S2 and S3). As a consequence, both high and low values of DNA-SPF and percentages of DNA photoprotection were observed for UVB and UVA radiation, respectively (Table S1). A similar trend was observed for the induction of CPDs in the cellular genome (Table 2). Notably, the cells examined were unable to remove significant amounts of DNA damage, and the DNA lesions detected were not masked by DNA repair, as it would be expected in the case of MRC5 cells. Therefore, these results confirm that both the high and the low protective efficacies observed against the cytotoxic effects induced by the UVB and UVA doses, respectively, are directly associated with the capacity of the sunscreens to prevent genomic CPD formation in the XP4PA cells.

Genotoxic and Cytotoxic Photoprotection for Natural Sunlight. The main innovative contribution as well as the environmental appeal of this work is to propose an alternative methodology that makes it possible to investigate the sunscreen photoprotective efficiency against the genotoxic and cytotoxic

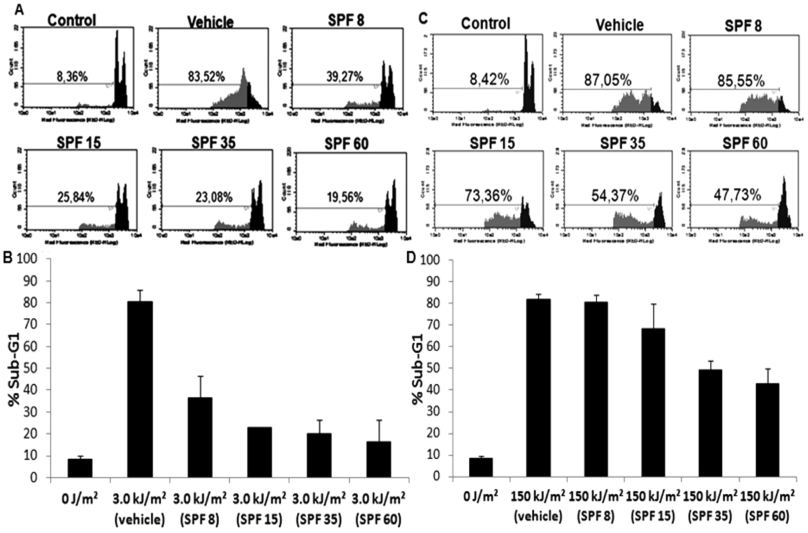


Figure 3. Photoprotection provided by 4 different sunscreens against UVB- or UVA-induced apoptosis. A representative example of the histograms of sub-G1 population following UVB irradiation (A). The percentage of sub-G1 population of nonirradiated (0 J/m^2) and UVB-irradiated cells in the absence (vehicle) or presence of 4 different sunscreens (B). A representative example of the histograms of sub-G1 population following UVA irradiation (C). The percentage of sub-G1 population of nonirradiated (0 J/m^2) and UVA-irradiated cells in the absence (vehicle) or presence of 4 different sunscreens (D). The average and standard deviation of three independent experiments performed in triplicate.

Table 2. Genomic DNA Photoprotection Provided by Sunscreens against CPD Induced by UVB and UVA Radiation[a]

sunscreens	CPD/kbp-genomic DNA	genomic DNA-SPF	% CPD photoprotection
vehicle (UVB)	1.52	1	0
SPF 8 (UVB)	0.13	12.1	91.6
SPF 15 (UVB)	0.07	20.7	95.1[b]
SPF 35 (UVB)	0.04	35.3	97.1[b,c]
SPF 60 (UVB)	0.03	48.8	97.9[b,c]
vehicle (UVA)	0.91	1	0
SPF 8 (UVA)	0.51	1.8	43.5
SPF 15 (UVA)	0.33	2.7	63.5[b]
SPF 35 (UVA)	0.24	3.7	72.9[b,c]
SPF 60 (UVA)	0.15	6.2	83.7[b,c,d]

[a]CPD/kbp-genomic DNA – CPD induced per kbp of genomic DNA; Genomic DNA-SPF – Sun Protection Factor for Genomic DNA; % CPD photoprotection–percentage of CPD photoprotection. [b]Statistically different from SPF 8. [c]Statistically different from SPF 15. [d]Statistically different from SPF 35 ($p < 0.05$).

effects elicited directly by environmental sunlight. This opens new perspectives for the determination of *in loco* environmental photoprotection patterns of commercial sunscreen, which could improve the benefits of its use (in terms of sun protection) for population living in a specific latitude on the planet.[16] This concern is very important because the incidence of UVB doses inversely increases dramatically with the decrease in latitude, while this is not true for UVA doses, where it increases only slight. Thus, given the totally different incidence of UV light in each region, sunlight exerts a variable genotoxic pressure, inducing different patterns of DNA damage according to geographical location.[3]

The solar UVB and UVA doses measured during this treatment were 1.3 and 57.3 kJ/m^2, respectively (corresponding to 30 min of exposure from 11:30 to 12:00 a.m., Sao Paulo city, May/2011, fall in the Southern Hemisphere). At 48 h after the environmental exposures, the induction of lethal damage was evaluated. This result is presented in Figure 4, and the percentages of photoprotection against lethal damage are listed

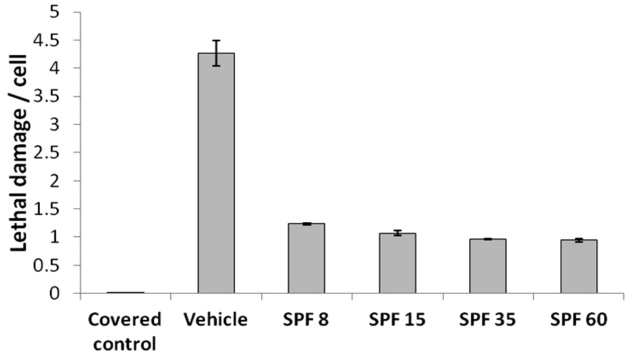


Figure 4. Quantification of lethal damage induced by sunlight in the absence (vehicle) or presence of 4 different sunscreens. The average and standard deviation of experiments performed in triplicate.

in Table 3. To complement these data, the induction of apoptosis by natural sunlight and the photoprotection provided by the sunscreens are presented in Figure 5.

To more accurately detect sunlight-induced DNA lesions, the sun exposure period was extended to 2 h, followed by the

Table 3. Lethal Damage Photoprotection Properties Provided by Sunscreens for Natural Sunlight[a]

sunscreens	LD-SPF (95% CI)	% lethal damage photoprotection (95% CI)
SPF 8	3.5 (3.2 to 3.7)	71.1 (68.7 to 73.5)
SPF 15	4.0 (3.9 to 4.1)	74.9[b] (74.5 to 75.4)
SPF 35	4.4 (4.1 to 4.8)	77.3[b] (75.5 to 79.1)
SPF 60	4.5 (4.0 to 5.0)	77.9[b,c](75.5 to 80.3)

[a]LD-SPF - Sun Protection Factor for Lethal Damage; % Lethal damage photoprotection–percentage of protection against lethal damage; and 95% CI – 95% confidence interval of three independent experiments performed in triplicate. [b]Statistically different from SPF 8. [c]Statistically different from SPF 15 ($p < 0.05$).

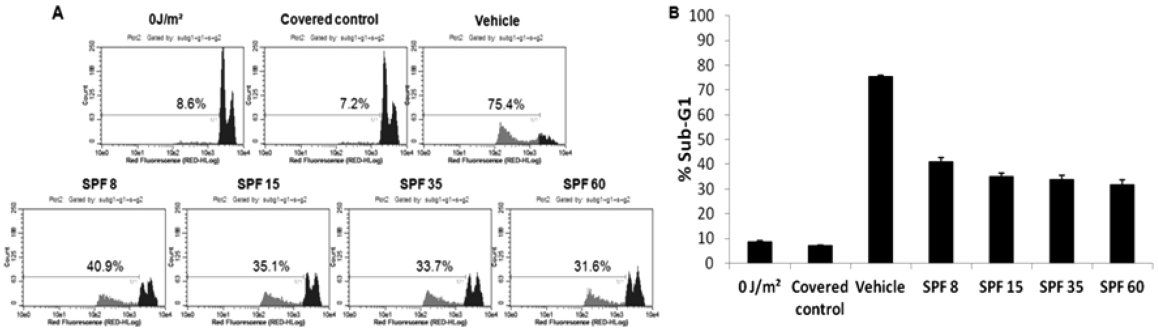


Figure 5. Photoprotection provided by 4 different sunscreens against the sunlight-induced apoptosis. A representative example of the histograms of sub-G1 population after sunlight exposure (A). The percentage of sub-G1 population of nonirradiated (0 J/m^2 - kept at 37 °C indoor), temperature control (covered control—exposed, but covered with aluminum foil), and irradiated cells in the absence (vehicle) or presence of 4 different sunscreens (B). The average and standard deviation of independent experiments performed in triplicate.

determination of the DNA-SPF with the DNA dosimeter (see Supporting Information, Table S2 and Figure S4) because the induction of DNA lesions in the plasmid DNA after shorter sun exposure was not sufficient for reproductive CPD detection (data not shown). Thus, both DNA and Cell dosimeters were exposed to sunlight from 11:00 a.m. to 1:00 p.m., and the solar doses of UVB and UVA measured were 6.6 and 241.3 kJ/m^2, respectively. The results of the protective efficacy of different sunscreens against the induction of CPDs by sunlight are shown in Table 4 (see also Supporting Information, Figure S5).

Table 4. Genomic DNA Photoprotection Provided by Sunscreens against CPD Induced by Natural Sunlight[a]

sunscreens	CPD/kbp-genomic DNA	genomic DNA-SPF	% CPD photoprotection
vehicle	0.59	1	0
SPF 8	0.08	1.5	35.2
SPF 15	0.05	1.8	44.5[b]
SPF 35	0.03	3.0	66.7[b,c]
SPF 60	0.02	4.3	76.9[b,c,d]

[a]CPD/kbp-genomic DNA − CPD induced per kbp of genomic DNA; Genomic DNA-SPF − Sun Protection Factor for Genomic DNA; % CPD photoprotection−percentage of CPD photoprotection. [b]Statistically different from SPF 8. [c]Statistically different from SPF 15. [d]Statistically different from SPF 35 ($p < 0.05$).

Surprisingly, the solar UVB and UVA measurements in São Paulo indicated that the overall incidence of UV radiation corresponded to 2.2 and 97.8% of UVB and UVA light, respectively. Therefore, most likely due to the vast majority of UVA photon incidence during the environmental experiments, the commercial sunscreens exhibited similar levels of protection against the induction of DNA damage (Table 4 and also Table S2, Figures S4 and S5) to those observed after exposures to UVA lamp inside the laboratory (Table 2 and also Table S1, Figures S2 and S3). In addition, the levels of photoprotection against lethal damage (Figure 4 and Table 3) and apoptosis (Figure 5) remained insufficient to ensure safe sun exposure for skin. For most of the sunscreens, the LD-SPF and genomic DNA-SPF for sunlight photoprotection were lower than one-third of the product SPF. The efficacies of sunscreens of SPF 15, 35, and 60 were significantly higher than that of the SPF 8 sunscreen; however, only SPF 60 sunscreen was more effective than SPF 15 sunscreen.

Notably, the cell death observed during these environmental exposures to natural sunlight are exclusively generated by the action of the incidental radiation because the covered controls (temperature control) exhibited levels of lethal damage and percentages of sub-G1 population that were similar to those observed in nonirradiated cells (0 J/m^2), which were maintained inside the laboratory at 37 °C (Figures 1, 4, and 5).

Taken together, these data also indicate that the UVA- and sunlight-induced DNA damage and cell death require further attention for the general population, as actual sunscreen protection provided by consumer products might be lower than commercially indicated.

Implications. The current *in vivo* methodologies for the determination of SPF values provide relevant public information on the photoprotection of UV light induced skin erythema and persistent pigmentation darkening in the skin of human beings.[15] These end points are related to inflammatory and possibly other cells' responses to light induced damage, but they may be not enough for the identification of deleterious long-term effects of sunlight exposure, such as skin carcinogenesis and aging. Moreover, these methodologies are limited, not providing good inference for the photoprotection of sunscreens for UVA or, more importantly, natural light. The Cell dosimeter proposed here offers the possibility to test DNA damage and cell death as potential measurements to identify new end points induced by sun exposure. By applying this biosensor it was possible to demonstrate that the effectiveness of SPF of commercially available sunscreens remains to be improved, particularly in terms of protection against the induction of deleterious effects in human cells by the action natural sunlight.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details about plasmid, cell lines and culture conditions, exposures of biodosimeters to artificial UVB and UVA sources and to environmental sunlight, DNA dosimeter and the quantification of DNA photoproducts, determination of cell viability, detection of CPD by immunoblot assay, induction of apoptosis by flow cytometry, sunscreen compositions, and statistical analysis as well as additional data (figures and tables). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 55.11.3091-7499. Fax: 55.11.3091-7354. E-mail: cfmmenck@usp.br.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by FAPESP (São Paulo, Brazil), CAPES and CNPq (Brasília, Brazil).

■ REFERENCES

(1) Mouret, S.; Bogdanowicz, P.; Haure, M. J.; Castex-Rizzi, N.; Cadet, J.; Favier, A.; Douki, T. Assessment of the photoprotection properties of sunscreens by chromatographic measurement of DNA damage in skin explants. *Photochem. Photobiol.* **2011**, *87* (1), 109−116.

(2) Yagura, T.; Makita, K.; Yamamoto, H.; Menck, C. F.; Schuch, A. P. Biological sensors for solar ultraviolet radiation. *Sensors (Basel)* **2011**, *11* (4), 4277−4294.

(3) Schuch, A. P.; Yagura, T.; Makita, K.; Yamamoto, H.; Schuch, N. J.; Agnez-Lima, L. F.; Macmahon, R. M.; Menck, C. F. DNA damage profiles induced by sunlight at different latitudes. *Environ. Mol. Mutagen* **2012**, *53*, 198−206.

(4) Runger, T. M. How different wavelengths of the ultraviolet spectrum contribute to skin carcinogenesis: the role of cellular damage responses. *J. Invest. Dermatol.* **2007**, *127* (9), 2103−2105.

(5) Schuch, A. P.; da Silva Galhardo, R.; de Lima-Bessa, K. M.; Schuch, N. J.; Menck, C. F. Development of a DNA-dosimeter system for monitoring the effects of solar-ultraviolet radiation. *Photochem. Photobiol. Sci.* **2009**, *8* (1), 111−120.

(6) Agar, N. S.; Halliday, G. M.; Barnetson, R. S.; Ananthaswamy, H. N.; Wheeler, M.; Jones, A. M. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (14), 4954−4959.

(7) Kozmin, S.; Slezak, G.; Reynaud-Angelin, A.; Elie, C.; de Rycke, Y.; Boiteux, S.; Sage, E. UVA radiation is highly mutagenic in cells that are unable to repair 7,8-dihydro-8-oxoguanine in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (38), 13538−13543.

(8) Schuch, A. P.; Menck, C. F. The genotoxic effects of DNA lesions induced by artificial UV-radiation and sunlight. *J. Photochem. Photobiol., B* **2010**, *99* (3), 111−116.

(9) Douki, T.; Reynaud-Angelin, A.; Cadet, J.; Sage, E. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. *Biochemistry* **2003**, *42* (30), 9221−9226.

(10) Mouret, S.; Baudouin, C.; Charveron, M.; Favier, A.; Cadet, J.; Douki, T. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (37), 13765−13770.

(11) Tewari, A.; Sarkany, R. P.; Young, A. R. UVA1 induces cyclobutane pyrimidine dimers but not 6−4 photoproducts in human skin in vivo. *J. Invest. Dermatol.* **2012**, *132* (2), 394−400.

(12) Mallet, J. D.; Rochette, P. J. Wavelength-dependent ultraviolet induction of cyclobutane pyrimidine dimers in the human cornea. *Photochem. Photobiol. Sci.* **2013**, *12* (8), 1310−1318.

(13) Besaratinia, A.; Yoon, J. I.; Schroeder, C.; Bradforth, S. E.; Cockburn, M.; Pfeifer, G. P. Wavelength dependence of ultraviolet radiation-induced DNA damage as determined by laser irradiation suggests that cyclobutane pyrimidine dimers are the principal DNA lesions produced by terrestrial sunlight. *FASEB J.* **2011**, *25* (9), 3079−3091.

(14) Sproul, C. D.; Mitchell, D. L.; Rao, S.; Ibrahim, J. G.; Kaufmann, W. K.; Cordeiro-Stone, M. Cyclobutane pyrimidine dimer density as a predictive biomarker of the biological effects of ultraviolet radiation in normal human fibroblast. *Photochem. Photobiol.* **2013**, DOI: doi: 10.1111/php.12194.

(15) Moyal, D.; Alard, V.; Bertin, C.; Boyer, F.; Brown, M. W.; Kolbe, L.; Matts, P.; Pissavini, M. The revised COLIPA in vitro UVA method. *Int. J. Cosmet. Sci.* **2013**, *35* (1), 35−40.

(16) Schuch, A. P.; Garcia, C. C.; Makita, K.; Menck, C. F. DNA damage as a biological sensor for environmental sunlight. *Photochem. Photobiol. Sci.* **2013**, *12* (8), 1259−1272.

(17) Young, A. R.; Boles, J.; Herzog, B.; Osterwalder, U.; Baschong, W. A sunscreen's labeled sun protection factor may overestimate protection at temperate latitudes: a human in vivo study. *J. Invest. Dermatol.* **2010**, *130* (10), 2457−2462.

(18) Lubin, D.; Jensen, E. H. Effects of clouds and stratospheric ozone depletion on ultraviolet radiation trends. *Nature* **1995**, *377*, 710−713.

(19) Tabazadeh, A.; Santee, M. L.; Danilin, M. Y.; Pumphrey, H. C.; Newman, P. A.; Hamill, P. J.; Mergenthaler, J. L. Quantifying denitrification and its effect on ozone recovery. *Science* **2000**, *288* (5470), 1407−1411.

(20) Diffey, B. Climate change, ozone depletion and the impact on ultraviolet exposure of human skin. *Phys. Med. Biol.* **2004**, *49* (1), R1−11.

(21) Costa, R. M.; Chigancas, V.; Galhardo Rda, S.; Carvalho, H.; Menck, C. F. The eukaryotic nucleotide excision repair pathway. *Biochimie* **2003**, *85* (11), 1083−1099.

(22) Lindahl, T.; Wood, R. D. Quality control by DNA repair. *Science* **1999**, *286* (5446), 1897−1905.

(23) Menck, C. F. M.; Munford, V. DNA repair diseases: What do they tell us about cancer and aging? *Genet. Mol. Biol.* **2014**, *37* (1), 220−233.

(24) de Laat, W. L.; Jaspers, N. G.; Hoeijmakers, J. H. Molecular mechanism of nucleotide excision repair. *Genes Dev.* **1999**, *13* (7), 768−785.

(25) Lehmann, A. R. DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. *Biochimie* **2003**, *85* (11), 1101−1111.

(26) Dunkern, T. R.; Fritz, G.; Kaina, B. Ultraviolet light-induced DNA damage triggers apoptosis in nucleotide excision repair-deficient cells via Bcl-2 decline and caspase-3/-8 activation. *Oncogene* **2001**, *20* (42), 6026−6038.

(27) Batista, L. F.; Kaina, B.; Meneghini, R.; Menck, C. F. How DNA lesions are turned into powerful killing structures: insights from UV-induced apoptosis. *Mutat. Res.* **2009**, *681* (2−3), 197−208.

(28) Liardet, S.; Scaletta, C.; Panizzon, R.; Hohlfeld, P.; Laurent-Applegate, L. Protection against pyrimidine dimers, p53, and 8-hydroxy-2′-deoxyguanosine expression in ultraviolet-irradiated human skin by sunscreens: difference between UVB + UVA and UVB alone sunscreens. *J. Invest. Dermatol.* **2001**, *117* (6), 1437−1441.

(29) Horiki, S.; Miyauchi-Hashimoto, H.; Tanaka, K.; Nikaido, O.; Horio, T. Protective effects of sunscreening agents on photocarcinogenesis, photoaging, and DNA damage in XPA gene knockout mice. *Arch. Dermatol. Res.* **2000**, *292* (10), 511−518.

(30) Bernerd, F.; Vioux, C.; Lejeune, F.; Asselineau, D. The sun protection factor (SPF) inadequately defines broad spectrum photoprotection: demonstration using skin reconstructed in vitro exposed to UVA, UVBor UV-solar simulated radiation. *Eur. J. Dermatol.* **2003**, *13* (3), 242−249.

(31) Duale, N.; Olsen, A. K.; Christensen, T.; Butt, S. T.; Brunborg, G. Octyl methoxycinnamate modulates gene expression and prevents cyclobutane pyrimidine dimer formation but not oxidative DNA damage in UV-exposed human cell lines. *Toxicol. Sci.* **2010**, *114* (2), 272−284.

(32) Gulston, M.; Knowland, J. Illumination of human keratinocytes in the presence of the sunscreen ingredient Padimate-O and through an SPF-15 sunscreen reduces direct photodamage to DNA but increases strand breaks. *Mutat. Res.* **1999**, *444* (1), 49−60.

(33) Schuch, A. P.; Lago, J. C.; Yagura, T.; Menck, C. F. DNA dosimetry assessment for sunscreen genotoxic photoprotection. *PLoS One* **2012**, *7* (6), e40344.

(34) COLIPA; CTFA-SA; CTFA; JCIA *International Sun Protection Factor (SPF) Test Method*; In COLIPA; CTFA-SA; CTFA; JCIA; Eds.; Brussels, 2006; p 46.