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DNA damage profiles induced by sunlight at different latitudes

Research Article

DNA Damage Profiles Induced by Sunlight at Different Latitudes

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Despite growing knowledge on the biological effects of ultraviolet (UV) radiation on human health and ecosystems, it is still difficult to predict the negative impacts of the increasing incidence of solar UV radiation in a scenario of global warming and climate changes. Hence, the development and application of DNA-based biological sensors to monitor the solar UV radiation under different environmental conditions is of increasing importance. With a mind to rendering a molecular view-point of the genotoxic impact of sunlight, field experiments were undertaken with a DNA-dosimeter system in parallel with physical photometry of solar UVB/UVA radiation, at various latitudes in South America. On applying biochemical and immunological approaches based on specific DNA-repair enzymes and antibodies, for evaluating sunlight-induced DNA damage profiles, it became clear that the genotoxic potential of sunlight does indeed vary according to latitude. Notwithstanding, while induction of oxidized DNA bases is directly dependent on an increase in latitude, the generation of 6-4PPs is inversely so, whereby the latter can be regarded as a biomolecular marker of UVB incidence. This molecular DNA lesion-pattern largely reflects the relative incidence of UVA and UVB energy at any specific latitude. Hereby is demonstrated the applicability of this DNA-based biosensor for additional, continuous field experiments, as a means of registering variations in the genotoxic impact of solar UV radiation. Environ. Mol. Mutagen. 53:198–206, 2012. © 2012 Wiley Periodicals, Inc.

Key words: solar ultraviolet radiation; DNA photoproducts; biological dosimetry; DNA-dosimeter

INTRODUCTION

Human industrial activities, as well as the release of destructive gases, have been deranging atmospheric balance over the last centuries. In a scenario of stratospheric ozone depletion, compounds, such as chlorofluorocarbons (CFCs), have been playing a leading role. The discovery of the ozone ''hole'' in the 1980's has led to grave concern worldwide, since intensified UVB radiation (280–315 nm), through inducing greater damage to a wide range of organic molecules, including DNA, generally enhances the harm to several biological and physical processes [Takahashi and Ohnishi, 2004; Andrady et al., 2007; Caldwell et al., 2007; McKenzie et al., 2007; Zepp et al., 2007].

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The most important cellular effects arising from UV radiation, namely, cell-death and mutagenesis, are directly related to a chain of events that mainly involve the induction of DNA lesions. Nonetheless, the chemical nature, as well as the efficiency in the formation of DNA lesions, greatly depends on the wavelength of incidental UV photons. Hence, different wavelengths of UV light induce different types of DNA damage. For example, through direct excitation by sunlight UV components, mainly UVB wavelengths, the DNA molecule undergoes well-known modifications that trigger dimerization reactions between adjacent pyrimidines. The main products resulting from these photochemical reactions are cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) [Schuch et al., 2009]. In addition, on further irradiation with UVA wavelengths (315–400 nm) at around 320 nm, normal isomers of 6-4PPs can be converted into their respective Dewar valence isomers (DewarPPs) [Perdiz et al., 2000]. On the other hand, UV radiation can also indirectly damage DNA after the absorption of its photons by other chromophores, thereby generating reactive oxygen species. Oxidatively generated DNA damage, such as 7,8-dihydro-8-oxoguanine (8oxodG), which is more effectively induced with UVA than UVB [Schuch and Menck, 2010], has often been considered as a premutagenic lesion in UVA mutagenesis [Piette et al., 1986; Agar et al., 2004; Kozmin et al., 2005; Dahle et al., 2008], whereas single strand breaks (SSBs) are probably not involved [Dunn et al., 2006; Schuch et al., 2009]. Therefore, several alternative biological consequences can arise from an altered increase in incident UV irradiance.

Fortunately, with global efforts addressed to diminishing ozone-depleting substances, recent measures have revealed a constant increase in ozone levels worldwide. Nevertheless, even though atmospheric ozone levels are undergoing restoration, it remains unclear whether climate change will delay or accelerate ozone recovery [McKenzie et al., 2007]. Hence, directed studies are essential to guiding health-care decisions, as well as future policy programs related to variations in UV radiation caused by climate change and ozone depletion [UNEP, 2010].

Thus, the increment of research projects with interdisciplinary approaches, and focused on the evaluation of biological effects induced by natural sunlight, is becoming an urgent necessity, in an attempt to avoid serious consequences for coming decades. In this sense, various simple test systems have already been developed for use as biological dosimeters of UV components in sunlight. Most largely reflect UV sensitivity of the main target of UV radiation in living organisms, by measuring, directly or indirectly, the DNA damaging capacity of solar UV radiation, as the initiating event in various harmful effects to human health and life in general [Yagura et al., 2011].

Hereby, the environmental application of a highly UV-transparent DNA-based biosensor [Schuch et al., 2009] is described after exposure of sunlight at different latitudes in Brazil and Chile, parallel to the use of physical UVB/UVA radiometers. The DNA damage profiles induced by sunlight at each location were determined by DNA repair enzymes [*E. coli* formamidopyrimidine-DNA glycosylase (Fpg) recognizes mainly oxidized purine bases; the T4 bacteriophage endonuclease V (T4-endo V) recognizes mainly CPD; and the yeast ultraviolet damage endonuclease (UVDE) recognizes large distortions in DNA double helix, such as, CPDs, 6-4PPs, and DewarPPs]. Specific antibodies were also used in immunoblot assaying to confirm the induction of CPDs and 6-4PPs after exposure to sunlight. The use of this DNA-dosimeter to measure the daily genotoxic impact of sunlight revealed the variations in DNA damage profiles related to solar UVB/UVA incidence, observed at several latitudes.

MATERIAL AND METHODS

Plasmid

For plasmid DNA purification the *E. coli* strain *DH10b* (F⁻, mcrA (mrr-hsdRMS-mcrBC), 80lac-ZM15, lacX74, deoR, recA1, endA1, ara139, galU, galK, rpsL, nupG, tonA, STM^R) was made electrocompetent [Datsenko and Wanner, 2000] and transformed with *pCMUT* vector (1,762 bp; *C*, chloramphenicol resistance and *MUT*, supF mutation target gene) [Schuch et al., 2009], which is the target DNA molecule for solar UV radiation. Purification of DNA samples was prepared by using Qiagen Plasmid Maxi Kit (Valencia, CA), and stored in TE buffer [10 mM Tris-HCl (pH = 8.0), 1 mM EDTA] at −20°C, until the beginning of the experiments.

Exposures of DNA Samples to Sunlight and Measurements of Solar UV Doses

With the exception of São Paulo, Brazil, where experiments were carried out throughout the year (2008), daily environmental exposures of plasmid DNA samples to sunlight were carried out in triplicate in other towns throughout South America, during the southern hemisphere summer, to so evaluate the genotoxic impact of sunlight under the maximum incidence of UV radiation. Hence, sunlight exposures in Punta Arenas, Chile, were anticipated to the southern hemisphere spring, due to the occurrence of the ozone hole phenomenon at this latitude. Briefly, the studied locations were Punta Arenas, extreme south Chile (53°1′S; 70°9′O), where three exposures (October 6, 8, 9, 2008) were undertaken; São Martinho da Serra, south Brazil (29°4′S; 53°8′O), with three exposures (December 27 and 29, 2006, and January 4, 2007); São Paulo, southeast Brazil (23°3′S; 46°4′O), with the largest number of exposures (April 25, June 3, July 1, July 21, August 8, December 5, and December 8, all 2008, and July 8, 2010); and Natal, northeast Brazil (5°5′S; 35°1′O), with two exposures (February 27 and 28, 2009). All the exposures, continuous from 10:00 A.M. to 2:00 P.M., took place only on days with a clear sky. Parallel to DNA biosensor exposure, solar UVB and UVA doses were obtained from the integration of irradiance values measured by continuous UVB/UVA radiometers installed at each location (UVB and UVA Radiometers, EKO Instruments Trading, Tokyo, Tokyo-to, Japan).

DNA Photoproduct Quantification

On defining the average number of DNA photoproducts generated by sunlight at each latitude, the relative amounts of supercoiled and circular

plasmid DNA forms were measured through densitometry analysis, following separation by 0.8% agarose gel electrophoresis (ImageQuant 300, GE Healthcare, Little Chalfont, Buckinghamshire, UK). Samples with 200 ng of DNA were first preincubated with either 0.8 U of Fpg protein (New England Biolabs, Ipswich, MA), 70 ng of T4-endo V (produced in this laboratory), or 250 ng of UVDE (Trevigen, Gaithersburg, MD), so as to discriminate the different types of DNA lesions. They were then incubated for 60 min at 37°C (Fpg and T4-endo V) or 30°C (UVDE). The enzymes, previously tested up to saturation, were used in amounts where no nonspecific cleavage was observed. SSBs induction was also quantified through the same densitometry analysis, although without pretreatment with DNA repair enzymes. The number of enzyme-sensitive sites and SSBs per kbp of plasmid DNA was calculated, assuming Poisson distribution adapted to this technique, as previously described [Schuch et al., 2009].

Identification of CPDs and 6-4PPs by Immunoblot Assays

The formation of CPDs and 6-4PPs was also measured immunologically, as follows. A total of 200 ng of pCMUT vector previously exposed to sunlight was mixed with 800 ng of salmon sperm DNA (Sigma-Aldrich, St. Louis, MO), boiled for 10 min at 100°C, immediately transferred to ice, and then spotted onto nitrocellulose membranes (BIO-RAD, Hercules, CA) by using a slot-blot apparatus (Omniphor, San Jose, CA). The membranes were subsequently incubated with 5X SSC (750 mM NaCl; 75 mM sodium citrate) for 15 min at room temperature, dried, also at room temperature, and baked for 2 hr at 80°C. Blocking was carried out in 5% milk diluted in a PBS buffer (137 mM NaCl; 2.7 mM KCl; 8 mM Na_2HPO_4; 1.5 mM KH_2PO_4; pH = 7.6) for 18 hr at 4°C, whereupon the membranes were incubated with anti-CPD and anti-6-4PP primary antibodies (Cosmo Bio, LTDA, Tokyo, Tokyo-to, Japan; diluted 1:2,000 in 5% milk diluted in a PBS buffer) under constant shaking for 3 hr at room temperature. All primary antibodies were removed, and the membranes washed six times (5 min each) with PBST (0.1% of Tween 20 in PBS). The secondary antibody, Antimouse IgG HRP conjugate (R&D Systems, Minneapolis, MN), was diluted 1:2,000 in 5% milk-PBS, and the membranes incubated under constant shaking for 2 hr at room temperature. The secondary antibody was removed by six washes with PBST. Detection of DNA lesions was by adding a chemiluminescence reagent (Amersham ECL Western blotting detection reagents and analysis system, GE Healthcare, Little Chalfont, Buckinghamshire, UK), followed by chemiluminescence detection (ImageQuant 300, GE Healthcare, Little Chalfont, Buckinghamshire, UK).

RESULTS

Applicability of DNA-Dosimeter for Daily-UV Measurements

The main reason for using this biosensor was to determine and quantify the amount of DNA damage induced by sunlight at a specific place during a predetermined exposure period. The DNA damage profile was further defined as the percentage of certain types of induced-DNA lesions. Thus, environmental exposure using the DNA-dosimeter was carried out on a platform situated at the University of São Paulo, São Paulo, Brazil, on July 8, 2010 (winter in the southern Hemisphere) from 7:00 A.M. to 5:00 P.M. Specific UVB/UVA radiometers were also installed in the same locale. Quantification of DNA lesions is shown in Figure 1.
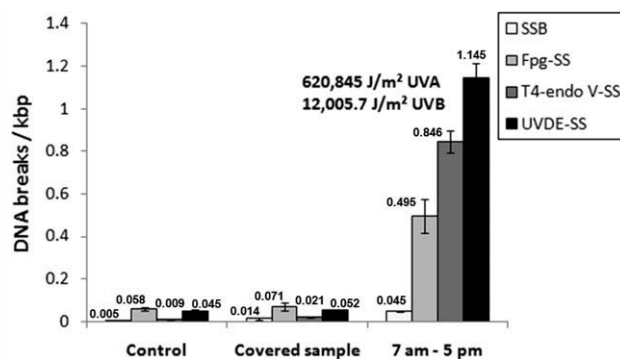

Fig. 1. Quantification of DNA lesions induced by sunlight in São Paulo (23°3'S; 46°4'O – Brazil) during a winter day (July 8, 2010). 'Control' means DNA samples kept at −20°C inside the laboratory. 'Covered samples' means DNA samples exposed to sunlight, but covered with aluminum foil. SSB – single strand breaks; Fpg-SS – Fpg sensitive sites (oxidized DNA bases); T4-endo V-SS – T4-endo V sensitive sites (CPD); UVDE-SS – UVDE sensitive sites (CPD + 6-4PP). The raw numbers are indicated above each type of DNA lesion.

It was clearly shown that temperature (22°C) exerts no influence on DNA lesion induction, since the amount of all types of damage quantified in the covered control (unexposed samples) was very similar to that of DNA lesions observed in the unexposed control kept at −20°C in the laboratory. The daily DNA damage profile observed after exposure was characterized by 2.7% of SSBs, 29.4% of oxidized DNA bases (Fpg-SS), 50.2% of CPDs (T4-endo V-SS), and 17.7% of 6-4PPs (UVDE-SS - T4-endo V-SS). Evaluation of any variation in solar-UV genotoxic impact was through shorter exposures (only 2 hr), on the same day. Data on directly induced DNA photoproducts, as well as solar UVB/UVA doses measured by UV-radiometers, are shown in Figure 2.

Although there was a clear variation in DNA damage induction, according to the time of day of DNA exposure, this biological response was entirely in accordance with the physical data provided by the radiometers, that is, the highest UV doses induced the highest amount of DNA lesions. A summary of DNA damage profile and percentage of UVB/UVA incidence is presented in Table I.

As expected, although very low early in the morning and late in the afternoon, the incidence of UVB light reached a maximum at midday. Accordingly, at the peak there was a decrease in the percentage of single-strand breaks and oxidized DNA bases, and a clear increase in 6-4PPs. Notwithstanding, although the absolute amount of CPDs increased during midday exposure, the relative frequency of this DNA damage remained invariable throughout the day. Thus, the relative frequency of 6-4PPs in the DNA damage profile is concomitant with increased UVB-light incidence. These results indicate the DNA-dosimeter as being a suitable system for measuring variations in the genotoxic impact of sunlight in a specific location.
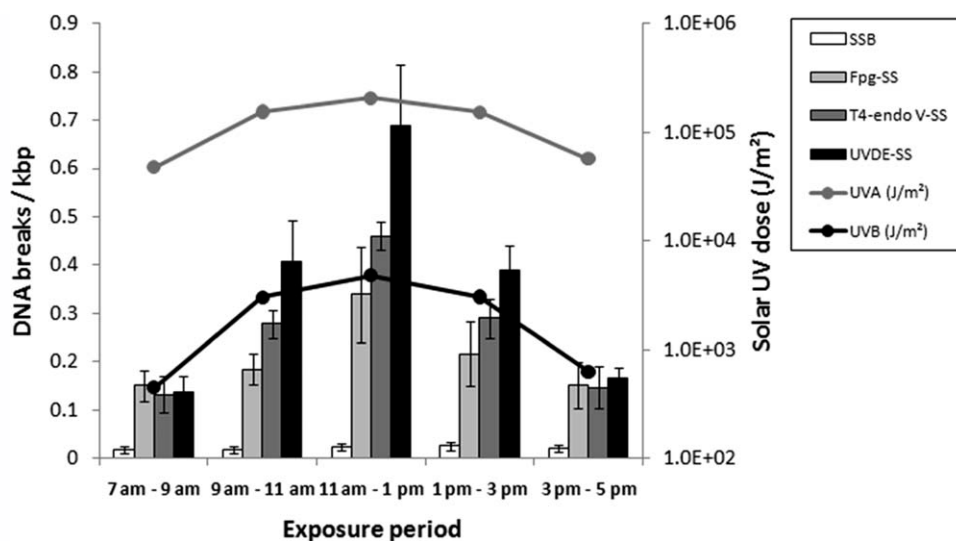
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Fig. 2. Variation in DNA damage during a winter day (July 8, 2010) in São Paulo (23°3′S; 46°4′O – Brazil). Comparison of quantified DNA damage with measurements of solar UVB (black line) and UVA (grey line) doses, following 2-hr sunlight-exposure (23°3′S; 46°4′O – Brazil).

SSB – single strand breaks; Fpg-SS – Fpg sensitive sites (oxidized DNA bases); T4-endo V-SS – T4-endo V sensitive sites (CPD); UVDE-SS – UVDE sensitive sites (CPD + 6-4PP).

TABLE I. DNA Damage and UV Incidence Profiles After Exposure to Sunlight During a Winter Day (July 8, 2010) in São Paulo (23°3′S; 46°4′O—Brazil)

Environmental exposures 07/08/2010	UVB (%)	UVA (%)	SSB (%)	Oxidized DNA bases (%)	CPD (%)	6-4PP (%)
7:00 A.M. to 9:00 A.M.	0.9	99.1	5.7	49.3	43.3	1.7
9:00 A.M. to 11:00 A.M.	1.9	98.1	3.0	30.2	45.6	21.2
11:00 A.M. to 1:00 P.M.	2.3	97.7	2.3	32.2	43.7	21.8
1:00 P.M. to 3:00 P.M.	2.0	98.0	4.0	34.2	46.0	15.8
3:00 P.M. to 5:00 P.M.	1.0	99.0	6.2	44.9	43.5	5.4
Whole day—7:00 A.M. to 5:00 P.M.	1.9	98.1	2.7	29.4	50.2	17.7

UVB/UVA incidence profile was defined as the percentage of both UVB and UVA doses during exposure. Single strand breaks – SSB; oxidized DNA bases – Fpg-SS (Fpg sensitive sites); CPD – T4-endo V-SS (T4-endo V sensitive sites); 6-4PP – UVDE-SS (UVDE sensitive sites) - T4-endo V-SS.

## Variations in Solar UVB/UVA Radiation Incidence at Different Latitudes

In-the-field DNA-dosimeter exposures also took place on sunny days at other locations in the southern Hemisphere, namely, Punta Arenas, in the extreme south of Chile (53°1′S; 70°9′O), São Martinho da Serra, south Brazil (29°4′S; 53°8′O), and Natal, northeast Brazil (5°5′S; 35°1′O) and very close to the Equator, all in combination with measurements of solar UVB and UVA radiation doses by continuous UVB/UVA radiometers. Data on solar UVB/UVA doses are presented in Table II, together with the percentages of UVB/UVA incidence, and the maximum temperatures registered during exposure periods, as well as stratospheric ozone concentration data obtained from the Total Ozone Mapping Spectrometer available at NASA's website (http://jwocky.gsfc.nasa.gov/teacher/ozone_overhead_v8.html) [NASA, 2010], for each day of field experiments.

Except for Punta Arenas, where exposures were during the Antarctic spring, due to the concurrent decrease in the stratospheric ozone layer, no appreciable differences as regards ozone concentration were registered at the other study locations on the stipulated days. Even though ozone depletion in Punta Arenas was the highest on October 6, the radiometers failed to register a clear local increase in incident UVB radiation in comparison to the other 2 days of exposure. Notwithstanding, there was a slight increase in UVA. On the other hand, it is absolutely clear that the increase in UVB doses is directly dependent on the decrease in latitude (Table II). To better illustrate this, ratios between the averages of UVB and UVA doses measured during DNA-dosimeter exposures for each location were calculated, with Punta Arenas as reference location for comparison (Fig. 3). As expected, the incidence of UVB radiation increased dramatically according to the decrease in latitude. UVB doses reached 1.9-, 5.3-, and 12.1-fold higher in São Martinho da Serra, São Paulo and Natal, respectively, than in the reference location. Interestingly, this appreciable increase was not observed for UVA doses, that is, only 1.3- and 2.3-fold higher in São

TABLE II. Measurements of Solar UVB and UVA Doses, Percentage of UVB/UVA Incidence, Stratospheric Ozone Concentration, and Maximum Temperature During Exposure of DNA-Dosimeter to Sunlight at Different Latitudes

Environmental exposures	10:00 A.M. to 2:00 P.M. UVB (kJ/m^2) 280–315 nm	10:00 A.M. to 2:00 P.M. UVA (kJ/m^2) 315–400 nm	(%) UVB/UVA incidence	Stratospheric ozone concentration (DU)	Maximum temperature (°C)
10/06/2008 (53°1′S)	2.9	401	0.7/99.3	222	10
10/08/2008 (53°1′S)	2.7	362	0.7/99.3	246	12
10/09/2008 (53°1′S)	2.3	357	0.6/99.4	303	11
12/27/2006 (29°4′S)	5.2	191	2.6/97.4	268	23
12/29/2006 (29°4′S)	4.8	177	2.6/97.4	267	26
01/04/2007 (29°4′S)	2.3	81	2.7/97.3	246	24
04/25/2008 (23°3′S)	14.6	518	2.7/97.3	246	27
06/03/2008 (23°3′S)	10.2	397	2.5/97.5	247	17
07/01/2008 (23°3′S)	8.0	341	2.3/97.7	255	18
07/21/2008 (23°3′S)	9.3	357	2.5/97.5	243	20
08/18/2008 (23°3′S)	11.8	443	2.6/97.4	255	25
12/05/2008 (23°3′S)	23.7	780	3.0/97.0	259	29
12/08/2008 (23°3′S)	19.4	625	3.0/97.0	276	27
02/27/2009 (5°5′S)	32.5	867	3.7/96.3	253	39
02/28/2009 (5°5′S)	31.5	819	3.7/96.3	256	36
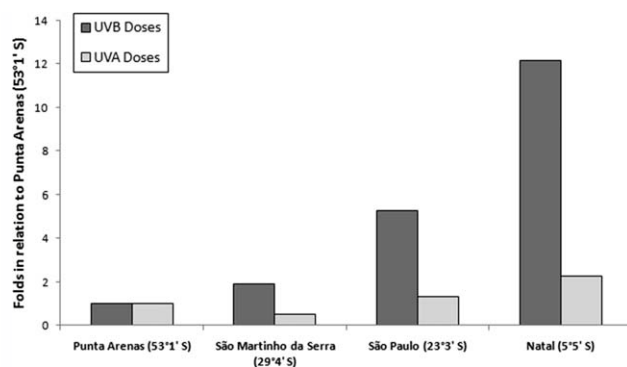
Fig. 3. Ratio between averages of UVB and UVA doses at each latitude, considering averages of UVB and UVA doses observed at Punta Arenas (53°1′S), as a pattern for comparison. The data represent the average of all measurements taken in each location.

Paulo and Natal, respectively, compared to Punta Arenas. Curiously, UVA doses observed in São Martinho da Serra were lower than those measured in Punta Arenas. There is no reasonable explanation for this observed effect, although there could have been a calibration problem in the UVA radiometer. Nonetheless, and worthy of note: operation of the UVB and UVA radiometers installed in each site is checked twice a year (February and August).

Variations in the DNA Damage Profiles at Different Latitudes

Following environmental exposure at each location, DNA samples were treated with the DNA repair enzymes Fpg, T4-endo V, and UVDE, in order to quantify the amount of specific DNA lesions induced by natural sunlight. Sample analysis through electrophoretic migration in agarose gels was to discriminate supercoiled DNA (form I) from either open-circular relaxed DNA present-

ing breaks or nicks caused by enzymatic cleavage (form II), or direct SSBs that had been discriminated without pretreatment with enzymes. The illustration in Figure 4 refers to experiments for defining DNA photoproducts resulting from exposures in Punta Arenas (53°1′S), São Paulo (23°3′S), and Natal (5°5′S), since data on exposures carried out in São Martinho da Serra (29°4′S) had already been published [Schuch et al., 2009].

The presence of DNA form II only in samples exposed to sunlight was a clear indication of the noninvolvement of environmental temperature in DNA lesion induction. In order to qualify and better evaluate the genotoxic impact of sunlight at these different latitudes, the averages of DNA damage profiles obtained from overall local exposure were calculated (Fig. 5).

As presented in Figure 5, any increase in latitude is accompanied by a corresponding increase in the induction of both oxidized DNA bases and SSBs, followed by a decrease in the formation of 6-4PPs. On the other hand, a decrease in latitude favors the formation of pyrimidine dimers in relation to oxidized DNA bases. Worthy of note, although the absolute amount of CPD varied among locales, their induction profiles were similar in the various locations, thereby indicating the facile generation of this type of DNA damage by sunlight at any latitude.

Furthermore, even though exposures in São Paulo took place in different seasons throughout the year of 2008, there was little change in DNA damage profiles observed at this latitude (Fig. 5), although the absolute amount of DNA lesions (breaks/kbp) can vary among exposures undertaken at different dates, with higher induction in late spring, when compared with fall and winter (Supporting Information, Fig. 1S). Interestingly, this correlates well with the variation of UVA and UVB incidence in São Paulo (Table II). Unfortunately, the high frequency of rainfall during the summer in São Paulo, made it impossi-
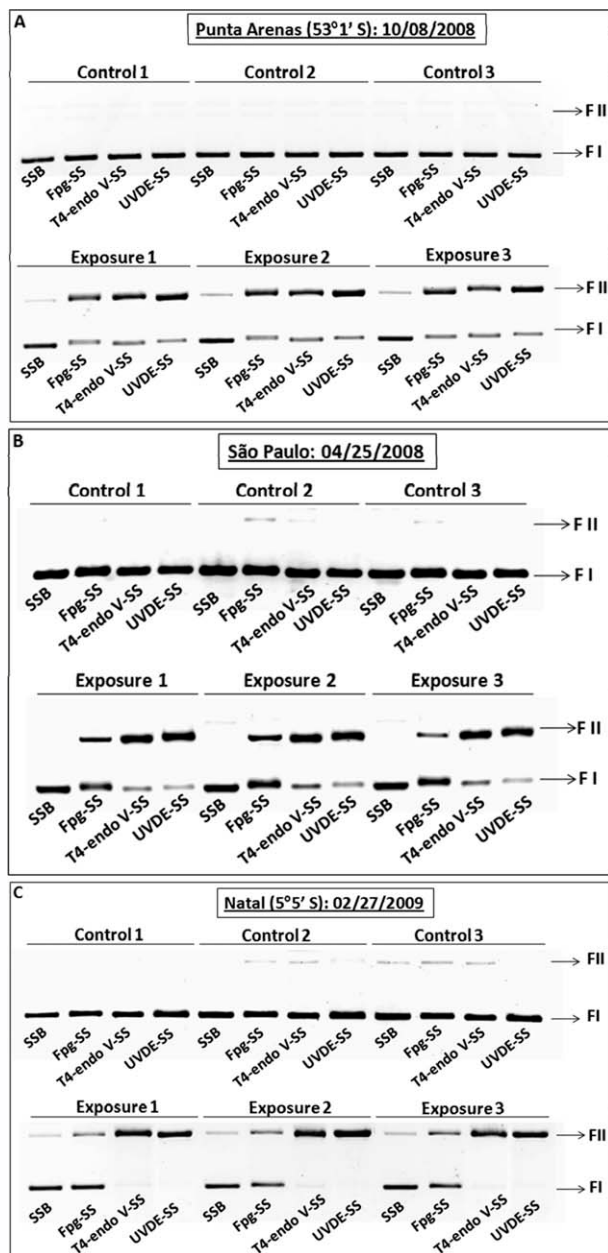
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Fig. 4. Representative example of experiments for defining DNA lesions after environmental exposures of the DNA-dosimeter in the cities of Punta Arenas (53°1'S; 70°9'O – Chile), São Paulo (23°3'S; 46°4'O – Brazil), and Natal (5°5'S; 35°1'O – Brazil).

Fig. 5. Determination of DNA damage profiles after exposure of sunlight at different latitudes. These profiles were defined as the average and standard deviation in percentage of each type of sunlight-induced DNA lesion at each studied location. SSB – single strand breaks; oxidized DNA bases – Fpg-SS (Fpg sensitive sites); CPD – T4-endo V-SS (T4-endo V sensitive sites); 6-4PP – UVDE-SS - T4-endo V-SS (UVDE sensitive sites). The data represent the average of all measurements taken in each location.

for these DNA lesions. Figures 6 and 7 present the results of CPDs and 6-4PPs detection, respectively, for the exposures performed in Punta Arenas (53°1'S), São Paulo (23°3'S), and Natal (5°5'S). Data on exposures in São Martinho da Serra (29°4'S) had already been published [Schuch et al., 2009].

These results basically confirm the induction of both CPDs and 6-4PPs by sunlight at the latitudes investigated. Surprisingly, 6-4PPs induction proved to be much higher at the lowest latitude (5°5' S), when compared to the others. Notwithstanding, an analogous large difference was not observed for CPDs induction, notably less heterogeneous among the various locations. Hence, besides added support to the idea that sunlight genotoxicity changes according to geographic location, there is every indication that 6-4PPs are apparently a form of biomolecular marker of the amount of UVB radiation that reaches the ground at a specific place.

DISCUSSION

In general, a biosensor integrates incident UV wavelengths of sunlight, thereby weighting them according to their biological effectiveness. Thus, considering that one of the most important criteria for biosensor validity is the relevance of pertinent photobiological/photochemical effects, DNA-based biological dosimeters have a genuine biological appeal [Berces et al., 1999]. In this sense, our group developed an environmentally suitable DNA-dosimeter system, based on the exposure of a plasmid DNA solution to artificial UV lamps and sunlight [Schuch et al., 2009; Schuch and Menck, 2010]. Currently, the application of this system has focused on evaluating the genotoxic effects of solar UVB and UVA radiation directly within

ble to carry out continuous experiments (10:00 A.M. to 2:00 P.M.) during this season and at this location, due to the cloudy conditions at this time of day.

Confirming the Induction of CPDs and 6-4PPs by Immunoblot Assays

Confirmatory experiments for detecting CPDs and 6-4PPs induction, using the DNA repair enzymes T4-endo V and UVDE, were carried out with specific antibodies
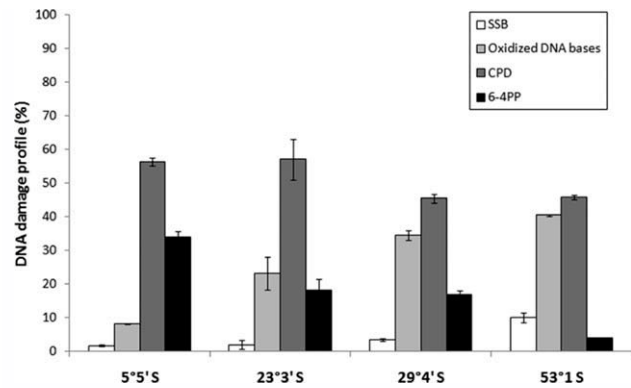
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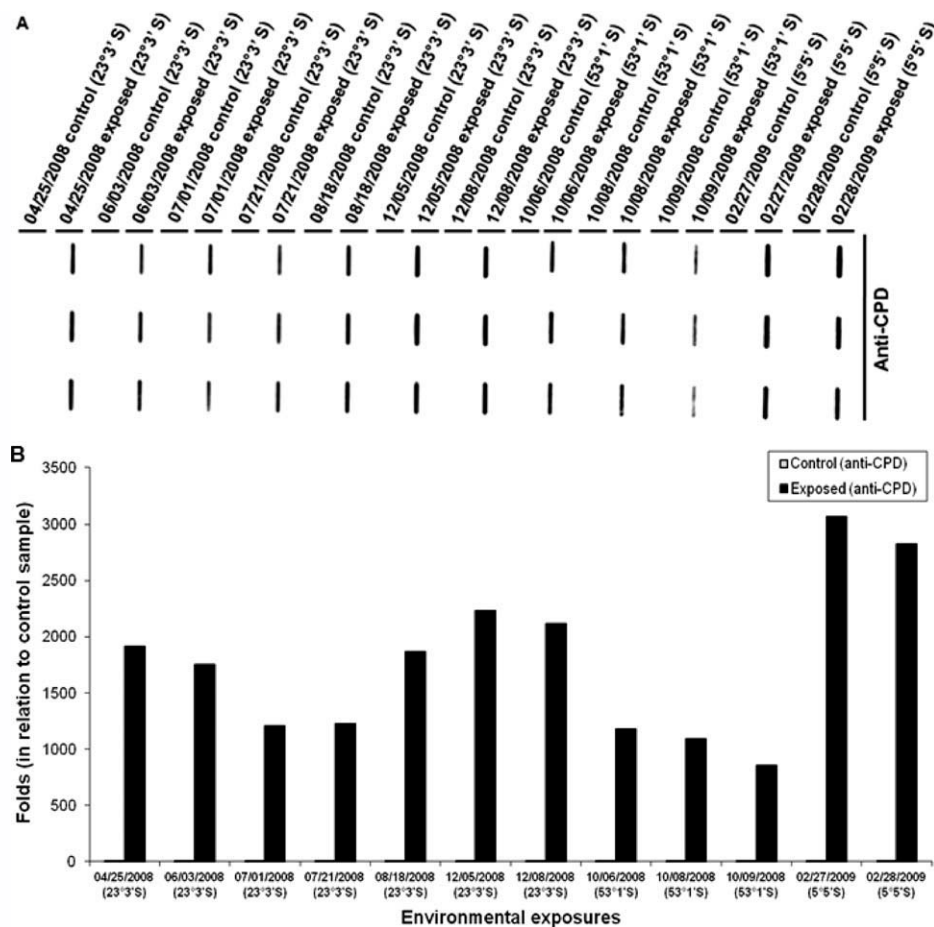
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Fig. 6. Immunological detection (**A**) and quantification of CPD antibody signals (**B**) in DNA samples exposed to sunlight in São Paulo (23°3′S), Punta Arenas (53°1′S), and Natal (5°5′S). The intensity of chemiluminescent signals were measured through densitometry analysis and quantified as folds in relation to unexposed control samples for each exposure to sunlight. Dates are indicated as month/day/year.

the environment. Furthermore, through the use of solar UVB/UVA radiometers, it becomes possible to compare biological responses with the physical data of solar UVB/UVA radiation incidence at various geographic locations.

In a daylong field-experiment carried out in São Paulo (July 8, 2010), the quantification of DNA damage indicated the effective induction of CPDs, 6-4PPs, and oxidized DNA bases, the three major types of DNA lesions caused by sunlight (Fig. 1). On comparing these biological data with physical UV doses provided by UVB/UVA radiometers, there were clear indications of the usefulness of this DNA-based dosimeter for evaluating variations in the daily genotoxic impact of solar UV radiation observed at a specific location (Fig. 2). Furthermore, so as to better demonstrate the environmental applicability of this biological system, following each daily short exposure, the profiles of DNA damage and UV incidence were defined and compared (Table I). It was clearly evident that both profiles undergo changes throughout the day, the induction of pyrimidine dimers (T4-endo V-SS and UVDE-SS) favorably occurring around midday (when UVB incidence is the

highest), whereas this is so with oxidized DNA bases (Fpg-SS) and single strand breaks (SSBs) in the first and last hours of sunlight (when UVB incidence is lower).

Environmental exposures of the DNA-dosimeter were extended to Punta Arenas (Chile), São Martinho da Serra (Brazil), and Natal (Brazil), in order to compare the profiles of DNA damage and UVB/UVA incidence with those observed in São Paulo (Brazil). Since exposure only took place on sunny days, this limited the number of experiments according to the possibility of remaining in each town (Table II). Importantly, although there was a strong variation in the environmental temperatures during the different exposures, DNA lesions were not significantly detected in control samples, indicating that there was no direct effect of temperature on the DNA damage profiles.

In Punta Arenas, on the first day, there was a strong reduction of concentration in the ozone layer (October 6, 2008), on the second, a lesser reduction (October 8, 2008), and on the third, normal ozone concentration (October 9, 2008). Although UV doses were slightly higher on days with less ozone, no expressive increase in
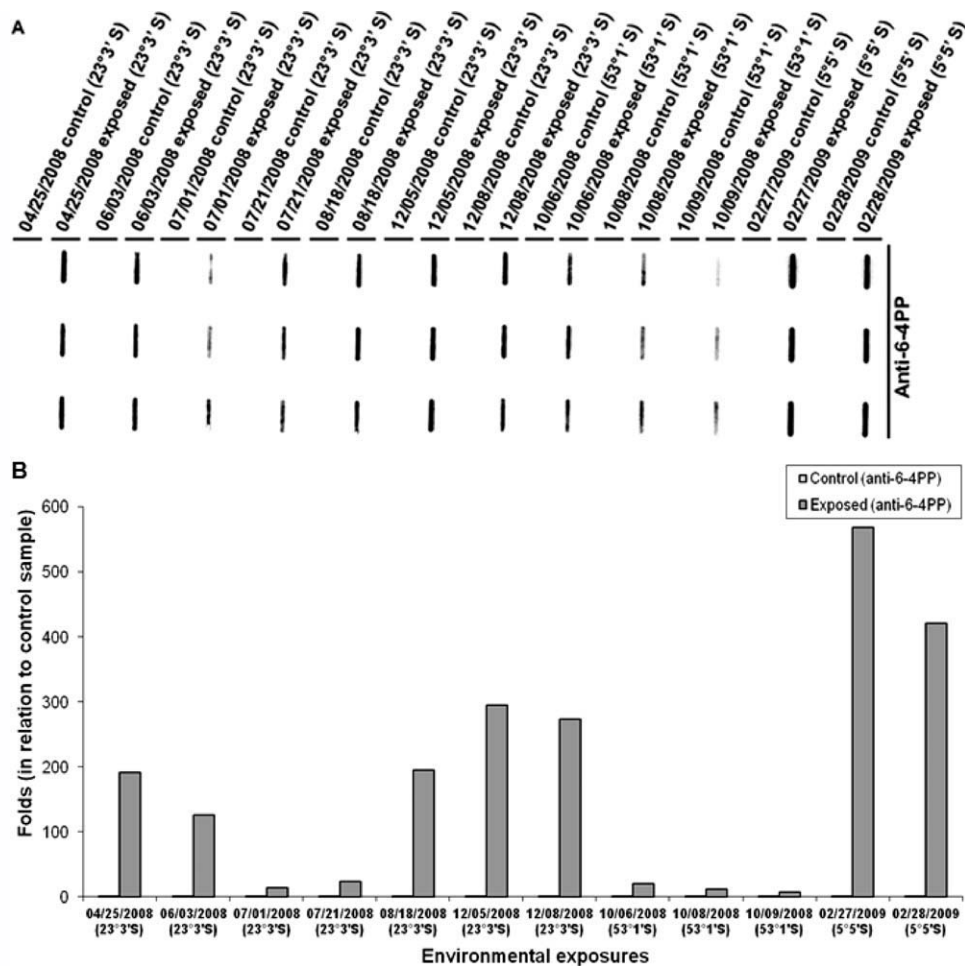
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Fig. 7. Immunological detection (**A**) and quantification of 6-4PP antibody signals (**B**) in DNA samples exposed to sunlight in São Paulo (23°3′S), Punta Arenas (53°1′S), and Natal (5°5′S). The intensity of chemioluminescent signals were measured through densitometry analysis and quantified as folds in relation to unexposed control samples for each exposure to sunlight. Dates are indicated as month/day/year.

UVB levels was detected on the first day, compared with the others, probably due to the naturally low incidence of UVB wavelengths at this latitude. On the other hand, there was no large difference among the levels of ozone concentration at the Brazilian locations (Table II).

On comparing the averages of total UVB/UVA doses measured at each location in relation to Punta Arenas, it was clear that, although UVB doses inversely increased dramatically with decreases in latitude, this was not so for UVA doses, where increases were only slight (Fig. 3). Thus, given the totally different incidence of UV light in each region, it is inferred that sunlight exerts a variable genotoxic pressure on the DNA molecule. With the approach used here, it was clearly demonstrated that sunlight induced different patterns of DNA damage in Punta Arenas (Fpg-SS ≈ T4-endo V-SS ≈ UVDE-SS), when compared with those in São Paulo (Fpg-SS < T4-endo V-SS < UVDE-SS) and Natal (Fpg-SS ≪ T4-endo V-SS < UVDE-SS) (Fig. 4).

A better qualification of these environmental experiments is given by comparing the averages of the profiles of DNA damage among all the studied latitudes (Fig. 5). These results indicate a certain tendency, when the induction of oxidized DNA bases and SSBs is directly dependent on an increase in latitude, whereas the induction of 6-4PPs is inversely dependent. Moreover, the profiles of DNA damage and UV incidence verified in Punta Arenas (Fig. 5) were very similar to those observed in early morning and late afternoon exposures to sunlight, which took place in São Paulo on July 8, 2010 (Table I).

Finally, the detection of both CPDs and 6-4PPs by immunoblot assays was efficient, and basically confirmed the formation of these DNA lesions after exposure to natural sunlight (Figs. 6 and 7). Furthermore, these results demonstrate the induction of 6-4PPs was much higher near the Equator compared to the other latitudes, whereas the induction of CPDs was less heterogeneous throughout the different locations. Therefore, it is inferred that 6-4PP

is a form of biomolecular marker of local incident UVB radiation.

Thus, the DNA-dosimeter has proved to be a very useful system for evaluating the amount and chemical nature of various types of sunlight-induced DNA lesions. The further application of this biosensor in combination with other physical and meteorological tools should help in improving knowledge regarding interactions between climate change and solar UV radiation, in order to reveal the importance of the influence of sunlight on DNA molecule and living organisms.

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