Formation of DNA Strand Breaks in Peripheral Lymphocytes of Rats After Exposure to Natural Sunlight

Dorival Mendes RODRIGUES-JUNIOR1,2,*, Ana Amélia de CARVALHO MELO1, Benedito Borges da SILVA2, and PEDRO Vitor LOPES-COSTA2

1. Department of Biology, Federal University of Piaui-Teresina, Piaui, Brazil; 2. Department of Gynecology, Federal University of Piaui-Teresina, Piaui, Brazil

Abstract

Objective This paper aims to evaluate the genotoxicity in peripheral blood lymphocytes of rats after exposure to sunlight at different time points of day in a tropical region of Brazil (5°S, 42°W).

Materials and Methods Thirty Wistar-Hannover rats, three months old, were randomly divided into three groups of 10 animals each: Group I [control, without exposure to ultraviolet (UV) radiation], Group II (exposed to sunlight during 08:00 a.m. to 10:00 a.m.), and Group III (exposed to sunlight during 10:00 a.m. to 12:00 a.m.). After a week of exposure, peripheral blood samples were taken from the tail of these animals to prepare smears on two slides per animal. In 24 h after exposure to sunlight in Group III, a new collection was obtained to observe the repair activity. The alkaline comet assay was used in this study to evaluate the genotoxic activity of sunlight (P<0.05).

Results There was no statistical difference between Group I and II (P=0.672). On the other hand, the exposure to sunlight in Group III showed genotoxic action in comparison to the other groups (P<0.0001). Also, there was no significant repair in Group III R (P=0.407).

Conclusion This study has shown a genotoxic potential of sunlight (UVA-B) in lymphocytes of mammals from 10:00 a.m. to 12:00 a.m., due to a higher intensity of UV in this tropical region.

Key words: Comet assay; DNA damage; Rats; Sunlight; Ultraviolet radiation

INTRODUCTION

Eleven hundred thousand cases of non-melanoma skin cancer (NMSC) are registered in Brazil each year and 5,900 people are diagnosed with cutaneous melanoma (CM)[1]. Although solar radiation is present on every part of the earth and supports life forms worldwide, the main risk factor for the said cancers is exposure to sunlight[2]. The increase in solar ultraviolet (UV) radiation at environmental levels, due to depletion of the stratospheric ozone layer, becomes a focus of social concern. The risk even turns more dramatic in tropical and subtropical regions where radiation-intensity is much higher[3].

UVA (320-340 nm) constitutes the large majority of solar UV radiation, but less effective than UVB (280-320 nm) at damaging DNA by presenting mutagenic and carcinogenic properties[4-5]. Although UVA has been implicated in photocarcinogenesis, its relevance to solar mutagenesis and the mechanisms by which it induces mutations remain a matter of debate[6]. On the other hand, the most effective wavelength for the induction of maximum DNA

*Correspondence should be addressed to: Dorival Mendes RODRIGUES-JUNIOR, Avenida Dom Severino, 2600 Apto 604, Bairro São Cristovão, 64049-375, Teresina, Piaui, Brazil. Tel: 55-86-88128658; Fax: 55-86-32150470; E-mail: derivalmrjr@gmail.com

Biographical note of the first author: Dorival Mendes RODRIGUES-JUNIOR, male biologist, M.D., experienced in Genetics, focusing on Human and Medical Genetics, especially Cancer Molecular Biology.

Received: March 29, 2011;  Accepted: June 9, 2011
photoproducts is UVC (100-280 nm)\textsuperscript{[7]}. However, ozone and oxygen completely absorb UVC radiation, thereby preventing these wavelengths from reaching the surface of the earth\textsuperscript{[8]}. UV is an environmental genotoxic agent and causes cellular DNA damage within skin cells\textsuperscript{[9]}. The UVA-B exposure could induce several DNA lesions including cyclobutane pyrimidine ine dimmers (CPD), (6-4) photoproducts (6-4 PP), dewater isomer of 6-4 PP, oxidized bases, protein-DNA crosslinks, and through the action of repair endonucleases, it can also generate double-strand breaks, which could be detected with the comet assay\textsuperscript{[10]}. DNA is the main UV target in living organisms; it is quite natural to employ DNA for damage detection with the comet assay that provides a rapid and visual tool for the quantitative assessment of DNA breakage in single cells\textsuperscript{[11-12]}. It is known that normal lymphocytes are highly sensitive to the damaging effect of UV-radiation and undergo cell death\textsuperscript{[13]}. Thus, there is a need to monitor the harmful effects of solar UV-radiation on the DNA molecule as a basis for assessing the risk to human health, biological productivity and ecosystems. In this study, due to ethical limitations in humans monitoring we exposed rats to sunlight (at different period of times) in Northeast Brazil, known for high incidence of sunlight, for subsequent monitoring of DNA damage.

MATERIALS AND METHODS

Animals

The entire study protocol was carried out in accordance with the ethical principles defined by the Brazilian College for Animal Experimentation (COBEA). Thirty Wistar-Hannover adult rats, obtained from the animal laboratory of the Federal University of Piaui (UFPI) were used in this study. The animals were kept under controlled temperature (25 °C) and artificial lighting conditions (7:00 a.m. to 7:00 p.m.) in individual cages with free access to water and standard laboratory rodent chow. The rats were randomly distributed into three groups of 10 animals each: Group I (control, without exposure to UV radiation), Group II (exposed to sunlight from 08:00 a.m. to 10:00 a.m.), and Group III (exposed to sunlight from 10:00 a.m. to 12:00 a.m.).

Exposure to sunlight was carried out for seven consecutive days. Shortly after exposure for the last day, animals had the peripheral blood collected from their caudal vein, in which the lymphocytes were purified for monitoring DNA damage, and then sacrificed. In 24 h after exposure to sunlight in Group III, a new collection of peripheral blood was obtained from the caudal vein to verify a possible repair in these cells (Group IIIR), and then the animals were sacrificed.

UV Sources

Environmental exposure of sunlight was carried out in Northeast Brazil (5°S, 42°W) for seven successive days (June 30 to July 5, 2007). The hourly incidence of solar UV dose in these seven days was collected from the records of the Brazilian National Institute of Meteorology and measured by intensity (Figure 1).

**Figure 1.** Monitoring of UV radiation in Northeast Brazil, collected from the records of the National Institute of Meteorology, Brazil.

Comet Assay

The comet assay was performed under alkaline conditions according to the procedure of Singh et al. (1988)\textsuperscript{[14]}, with slight modifications\textsuperscript{[15]}. The assay was carried out under yellow light to prevent any additional damage that could be induced by natural light.

The slides were immersed in cold lysis solution (2.5 mol/L NaCl, 100 mmol/L Na\textsubscript{2}EDTA, 10 mmol/L Tris-HCl, 1% N-lauryl-sarcosinate, pH 10-12, 1% Triton X-100, and 10% DMSO) for 90 min at 4 °C. The microscope slides were then placed in an electrophoresis tank, and DNA was allowed to unwind in freshly prepared alkaline electrophoresis buffer (30 mmol/L NaOH, 1 mmol/L EDTA, pH 13) for 20 min at room temperature. Electrophoresis was conducted for 20 min at 25 V and 300 mA.
were then washed three times with a cold neutralizing buffer (0.3 mol/L Tris-HCl, pH 7.5) for 5 min and dehydrated in pure methanol. The slides were kept in a dried-atmosphere chamber until assessment. To visualize and analyze DNA damage, slides were stained with ethidium bromide (2 μg/mL in deionized water).

Quantification

A single observer who was blinded to the grouping of the rats did the quantification. A light microscope (Nikon Eclipse E-400, optical microscope, Tokyo, Japan) connected to a color video camera (Samsung digital camera CHC-370N, Seoul, Korea) was used. An image analysis was performed by using the Fenestra Komet software (Linetics Imaging, Liverpool, UK, version 3.1) on 100 randomly selected cells from duplicate slides. DNA damage was quantified by the increase of tail. The cells were assessed visually and received scores from 0 (undamaged) to 4 (maximally damaged) according to the tail intensity (size and shape). The total score for 50 comets was obtained by multiplying the number of cells in each class by the damage class, according the formula: Total score = (0 x n0)+(1 x n1)+(2 x n2)+(3 x n3)+(4 x n4), where n = number of cells in each class analyzed (Miyaji et al., 2004). Thus, the total score for 50 cells ranged from 0 (all undamaged) to 200 (all maximally damaged)\cite{16}.

Statistical Analyses

With the ANOVA test, the mean scores were obtained between groups. Pairwise comparisons between means in groups were performed by using the Tukey method to maintain the overall significance level at 5%\cite{17}.

RESULTS

Table 1 and Figure 2 show the results of the comet assay after exposure to sunlight at different periods of time for Group II (08:00 a.m. to 10:00 a.m.) and Group III (10:00 a.m. to 12:00 a.m.). The exposure in Group II showed no genotoxic activity, since the mean score was not statistically different from Group I (control) (P=0.672). However, the exposure in Group III showed a significant genotoxic activity in comparison to that in Group I and Group II (P<0.0001). Moreover, our data showed no significant repair activity in Group III after 24 h' exposure to sunlight (P=0.407).

Table 1. Comet Assay Scores for Exposure to Sunlight, at Different Periods of Time, to Monitor Genotoxic Activity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Score (mean±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15.60±1.05*</td>
</tr>
<tr>
<td>II</td>
<td>21.20±1.21*</td>
</tr>
<tr>
<td>III</td>
<td>91.80±4.39</td>
</tr>
<tr>
<td>III R</td>
<td>83.40±8.85</td>
</tr>
</tbody>
</table>

Note. Group I (control); Group II (exposed from 8:00 a.m. to 10:00 a.m.), Group III (exposed from 10:00 a.m. to 12:00), and Group III R (24 h after sunlight exposure). Two replicates were made for each individual and 50 nuclei were scored per repetition (n=100 cells/group). Means with the same letter do not differ statistically at the level of 5%. s.d.: standard deviation. III and III R=1 and II, P<0.0001; I=II, P=0.672; III=III R, P=0.407.

Figure 2. Evaluation of genotoxicity by the comet assay after exposure to sunlight at different time points of day, Group I (control); Group II (exposed from 8:00 a.m. to 10:00 a.m.), Group III (exposed from 10:00 a.m. to 12:00), and Group III R (24 h after sunlight exposure). Data represent mean scores of the comet assay. *Genotoxicity, P<0.0001

DISCUSSION

Global UV studies have been faced with a great challenge that the majority of the monitoring stations are situated at high and middle latitudes, and consequently, measurements in tropical environments have rarely been performed despite the expected high doses due to high solar altitude and low ozone amounts. The monitoring of UV doses and column ozone amounts in the tropics is critically
important with regard to human health, productivity and conservation because of the large human populations in countries of this tropical region and because of the biodiversity and range of ecosystems located there\textsuperscript{[18]}. To the best of our knowledge, this is the first result of monitoring sunlight genotoxicity performed in Northeast Brazil in parallel with middle and high latitude places, where the UV incidence is constant during the year.

In this study, UV did not induce strand breaks directly, but could still generate them through the action of repairing endonucleases at sites of pyrimidine dimmers or oxidative DNA damage, which was detected with the comet assay after direct UV exposure of cells\textsuperscript{[9-10]}. It is therefore conceivable that lymphocytes from peripheral blood of rats were exposed to UV while transiting through the skin during exposure, and the comets observed in the present study were the results of DNA repair taking place at sites of pyrimidine dimmers and/or oxidative DNA damage, confirming that normal lymphocytes are highly sensitive to the damaging effect of UV-radiation\textsuperscript{[13]}. However, we observed that the damage identified by the comet assay had no significant repair after 24 h without exposure to sunlight in Group III. Despite exposure to sunlight for seven consecutive days which was considered an exhaustive effort to rats, a kind of nocturnal animals, the formation of sunburn was not noted in them.

The comet assay proves to be a rapid, inexpensive and visual method for quantitative assessment of DNA breakage and repair in single cells\textsuperscript{[21]}. The DNA damage is visualized at the individual cell level as an increased migration of genetic material from the nucleus\textsuperscript{[16]}. Singh et al. (1988)\textsuperscript{[13]}, with slight modifications\textsuperscript{[15]}, enabled this technique to permit an evaluation of DNA damage and repair in single cells under alkaline conditions, which optimizes DNA denaturation and makes it possible to carry out an evaluation of single-strand breaks and alkali-labile sites (region in which the polynucleotide chain is broken when the DNA is incubated at a high pH).

The UV portion of sunlight is approximately 95% UVA, and in contrast to UVC and UVB, damage and mutations induced by UVA is still a matter of debate\textsuperscript{[19]}. The physics dose rate could be determined under natural sunlight within several minutes of exposure. However, in order to determine cumulative natural UV doses in longer time spans, two problems need to be circumvented: one is to reduce the dose and the other is to protect the samples from various environmental assaults including rain and snow\textsuperscript{[18]}. Thus, in the present study which was designed to be free of interruption from these environmental problems and the UV physics dose, we could observe after exposure to sunlight (10:00 a.m. to 12:00 a.m.) the induction of lesions in DNA by the comet assay, and statistical difference was found between the group exposed to 08:00 a.m. to 10:00 a.m. and the control group, due to higher incidence of UV radiation in this period of day.

The UVA penetrates deeper into the skin than UVB. However, UVB is not completely absorbed or reflected in the epidermis and some parts of it also can reach the dermis with its blood vessels, producing erythema, burns and eventually skin cancer\textsuperscript{[9]}. There is evidence that the genotoxicity of UVA has most commonly been attributed to endogenous photosynthesizing, causing reactive oxygen species mediated induction of DNA damage\textsuperscript{[19]} and for this reason it is implicated in the etiology of human skin cancer\textsuperscript{[20]}. It has been reported that UVA and UVB should induce CPDs in mammalian cells\textsuperscript{[21]}. UVA induced mutations significantly increasing G-to-T transversions and small tandem base deletions relative to spontaneously derived mutations, mediated through oxidative DNA damage\textsuperscript{[22]}. As shown by data, physiologically relevant UVA doses cause DNA strand breaks, a prerequisite for chromosomal aberration that is most likely to contribute to tumorigenic conversion of the HaCaT cells, a human keratinocytes cell line\textsuperscript{[23]}.

There is no consistent evidence for a separate UVA-signature mutation\textsuperscript{[6]}. Furthermore, Schuch and Menck (2010)\textsuperscript{[12]} clearly demonstrated a direct relationship between the efficiency to generate CPDs by different UV sources and the induction of genotoxic effects. Also, these authors indicated that CPDs were the lesions responsible for the biological effects observed, independent of the wavelength of radiation, including sunlight. Thus, they concluded that the genotoxicity induced by UVA suggested that a direct link between UVA-induced damage and mutagenesis had not been clearly established as yet.

UV radiation is like two sides of the same coin-on one side, it has detrimental effects, and on the other side, it has beneficial effects\textsuperscript{[9]}. Our data highlight the possible role that sunlight might play in the induction of DNA lesions by UVA and UVB radiation, especially because the effects of UVA are still open for discussion\textsuperscript{[6]}. Therefore, our study will provide a wider perspective for further studies aiming at elucidating and monitoring the mechanisms by
which DNA damage can be harmful to human health, particularly in tropical regions.

REFERENCES