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Micronucleated erythrocytes in newborns rats exposed to three different types of ultraviolet-A (UVA) lamps from commonly uses devices

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ABSTRACT

Exposure to ultraviolet-A (UVA) light can accidentally cause adverse effects in the skin and eyes. UVA induces DNA damage directly by creating pyrimidine dimers or by the formation of reactive oxygen species that can indirectly affect DNA integrity. UVA radiation is emitted by lamps from everyday devices. In adult rats, micronucleated erythrocytes (MNE) are removed from the circulation by the spleen. However, in newborn rats, MNE have been observed in peripheral blood erythrocytes. The objective of this study was to use micronucleus tests to evaluate the DNA damage caused in newborn rats exposed to UVA light from three different types of UVA lamps obtained from commonly used devices: counterfeit detectors, insecticide devices, and equipment used to harden resins for artificial nails. Rat neonates were exposed to UVA lamps for 20 min daily for 6 days. The neonates were sampled every third day, and the numbers of MNE and micronucleated polychromatic erythrocytes (MNPCE) in the peripheral blood were determined. The rat neonates exposed to the three types of UVA lamps showed increased numbers of MNE and MNPCE from 48 h to 144 h ($P < 0.05$ and $P < 0.001$ respectively). However, no relationship was observed between the number of MNE and the wattage of the lamps. In conclusion, under these conditions, UVA light exposure induced an increase in MNE without causing any apparent damage to the skin.

1. Introduction

There is currently a lack of information available regarding human activities that are harmful to the skin, such as exposure to tanning beds, which are associated with the occurrence of skin cancer [1,2]. Humans perform unsafe procedures during daily life activities, and we are generally ignorant of the possible effects of continual exposure on our health [1,3,4]. The use of ultraviolet-A (UVA) lamps is a common practice in equipment such as counterfeit detectors, insecticide devices (which use this light to attract insects), nail lamps used during cosmetic nail treatment to cure gel nails [2,5], aquarium lamps, lamps in night clubs, or equipment used in clinical practices, such as dermatology clinics [6]. As a consequence, accidental exposure to UV radiation has become increasingly likely [7–9] to cause DNA damage and damage to the eyes of individuals [8,10–13]. Accidental exposure can occur when using germicidal lamps, resulting in symptoms such as erythema, skin irritation, burning, and skin desquamation [8,9]. UVC induced skin erythema faster, and oxygen radicals may be important in initiating the erythematic response [14]. UVA, UVB and UVC light can damage cellular DNA [8,15–17] by causing the formation of pyrimidine dimers between adjacent bases [18–22]. These dimers distort the conformation of the double helix and interfere with normal DNA replication, which can result in mutations [12,15,18,23] and chromosomal fragmentation [24]. UVA light has higher skin penetrance and could produce more damage than UVB or UVC light. UVA also indirectly affects DNA by promoting the

Abbreviations: MNE, micronucleated erythrocytes; MNPCE, micronucleated polychromatic erythrocytes; PCE, polychromatic erythrocytes; ROS, reactive oxygen species; UV, ultraviolet; UVA, ultraviolet-A light; UVB, ultraviolet-B light; UVC, ultraviolet-C light.

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formation of reactive species, mainly including reactive oxygen species (ROS) [15,18,20,25]. These entities oxidize the bases within DNA, which can lead to DNA strand fragmentation [20,25,26] and may lead to the formation of micronuclei (MN) [16,24,27,28]. The UVB in sunlight is a causative agent of skin cancer [29]. In addition, UVB and UVC stimulate melanogenesis and produce erythema [8,9,15,30,31] more rapidly than UVA [24]. Skin cancer can also be induced by exposure to UVA light [2]. The DNA lesions resulting from UVA exposure can be erroneously repaired, which can lead to the formation of mutations [32]. However, as mentioned, while UVB and UVC light produce erythema [15,31], UVA light may require much longer exposure to induce pigmentation. Accordingly, the level of damage caused by exposure to UVA radiation has not been adequately quantified. Accidental UVA exposure can occur, and this exposure may be one reason for the increased incidence of melanoma in people who use sunscreens [20,30]. It is clear that DNA damage occurs independently of erythema, and protecting against erythema does not a guarantee that DNA is protected from UVA light damage [33].

Micronucleation can be easily observed in erythrocytes obtained from rat neonates [34,35] because of their immaturity and the hypofunctionality of the neonatal spleen [34,36,37] and because neonatal skin is thinner, so they show greater sensitivity to genotoxic damage than older rats [35].

Recently, increases in micronucleated erythrocytes (MNE) and micronucleated polychromatic erythrocytes (MNPCE) were observed in rat neonates that were directly exposed to UVA light and neonatal rats that were indirectly exposed during gestation by exposing pregnant rats. These effects occurred because exposure to UV light may cause DNA fragmentation or chromatin loss [38,39]. As it has been previously shown this damage, in this study we select three similar lamps from equipment that are commonly and daily used, which emit in the range of UVA, to evaluate their genotoxic effect, with the intention to have more information about the risks of exposure to them.

In this study, we analyzed the frequency of MNE in the peripheral blood of rat neonates to quantify the DNA damage that was induced by three different types of UVA lamps in commonly used devices.

2. Materials and Methods

All procedures were conducted according to the institutional guidelines of the *Centro de Investigación Biomédica de Occidente (Instituto Mexicano del Seguro Social)*, Guadalajara Jalisco, México, which are in compliance with the guidelines approved by the National and International Institutes of Health for the humane treatment of research animals [40–42]. This project was approved by the Local and National Committee on Health Research and by the Institutional Committee for the Care and Use of Laboratory Animals (R-2010-1305-10 and R-2012-785-035).

2.1. Animals

Sprague Dawley Hairless rats were used because they provide a suitable experimental model for photodermatology studies [43–45]. All rats were supplied by the laboratory animal facility at the *Centro de Investigación Biomédica de Occidente* (*Instituto Mexicano del Seguro Social*), Guadalajara, Jalisco, México.

A total of 80 neonatal rats that were born from 20 female adult Sprague Dawley (*hairless*) rats were used [44,45], with 16 pups per group (four pups from each one of the four adult rats assigned per group) [46,47]. The female adult rats were mated with male rats (4:1). Each female rat was flushed daily with a vaginal wash of 0.1 ml of water with a pipettor and the wash product obtained was smeared onto clean slides. The presence of sperm in the smear indicated that mating had occurred and was defined as the first day of pregnancy, and allowed to deliver normally [35]. Upon confirmation of a pregnancy, the rats were individually housed in polycarbonate cages in windowless rooms with automatic temperature control (22 ± 2 °C), light control (lights on at 07:00 h and off at 19:00 h) and relative humidity maintenance (50 ± 10%). The animals received standard laboratory pelleted food (Purina, St. Louis, MO, USA) and tap water *ad libitum*.

2.2. Study Groups

For MNE induction, immediately after birth, all the neonates from the offspring were exposed to UVA light. The lamps were placed 7 cm from the newborn rats. The pups in the experimental groups were exposed daily for 20 min once per day for six consecutive days because this an exposure time that is known to increase the likelihood of developing melanoma in people who use tanning beds [1]. The following scheme was used: Group 1, the negative control group, was exposed to ambient light (conventional-light lamp, 32 W, 125 V, 60 Hz, and 4100 K); Group 2 was exposed to light from two lamps (F6T5BL of 6 W with a peak at 365 nm and an intensity of 6.34 mW/cm^2) in a piece of equipment used to harden the resins of artificial nails; Group 3 was exposed to light from one lamp in an insecticide device (GLEECON F6 T5/BL of 6 W with a peak at 352 nm and an intensity of 0.5 mW/cm^2); Group 4 was exposed to the light of two lamps in a counterfeit detection device (T5F6BLB of 6 W each with a peak at 365 nm and an intensity of 1.02 mW/cm^2); and Group 5, the positive control, was exposed to UVA radiation for 9 min daily [38]. The UV lamp used in Group 5 was a UVP model (UVP-Ultraviolet Products, Upland, CA, USA): UVL-24 (EL Series UV lamp, 115 V, 60 Hz, 0.32 W and P/N 95-0267-01) at 365 nm using two lamps of 4 W each with an emitted intensity of 1.87 mW/cm^2. The intensity of the UVA light emitted was measured using a digital UVX radiometer with a long-wave UVX-36 Sensor (335–380 nm, UVP-Ultraviolet Products, Upland, CA, USA).

2.3. Sample Preparation and MN Analysis

The whole litter was exposed daily to UVA light or conventional light and we randomly sampled the effects in 4 newborns from each rat per time-point. To sample the rats, one drop of blood was obtained from the tip of the tail of each of the offspring at the basal time-point (0 h) and after 48, 96 and 144 h. Blood smears were produced on pre-cleaned microscope slides. The smears were air-dried, fixed in absolute ethanol for 10 min, and stained with acridine orange (Sigma; CAS No. 10127023). The number of MNE in each sample was scored manually using a microscope equipped for epifluorescence (Olympus BX51) and with a 100× objective. The MNE frequency was established by determining the number of MN in 10,000 total erythrocytes (TE) (TE: normochromatic and polychromatic erythrocytes). The number of MNPCE in 1000 polychromatic erythrocytes (PCE) was also determined. Both parameters were used to determine genotoxicity. In addition, the proportion of PCE in 1000 TE was evaluated to determine cytotoxicity and to serve as an internal control. All slides were coded before the microscopic analysis.

2.4. Statistical Analysis

The data (‰) are expressed as the mean ± standard deviation of MNE, MNPCE and PCE frequencies. All statistical tests were performed using the Statistical Package for Social Sciences (SPSS v. 11.0, IBM Co., Armonk, NY, USA). All of the data were tested for normality using the Kolmogorov-Smirnov test, and the frequencies were analyzed to determine differences in group behavior during the treatments using one-way ANOVA followed by a Dunnett's *t*-test for multiple *post hoc* pairwise comparisons. Results were compared to the appropriate control to correct the significance values in the inter-group analysis. Intra-group comparisons were made between each treatment group and their respective basal value (0 h) using repeated measures ANOVA followed by a Bonferroni test for multiple *post hoc* pairwise comparisons. A *P*-value of <0.05 was considered to indicate statistical significance.