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Research Article

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Ultraviolet-A Light Induces Micronucleated Erythrocytes in Newborn Rats

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Abstract

Background: Ultraviolet-A (UV-A) light induce DNA damage by creating pyrimidine dimers, or indirectly affects DNA by the formation of reactive oxygen species. The objective was to determine DNA damage by micronucleus test in neonatal rats exposed to UV-A light.

Methods: Rat neonates were exposed to light from a LED lamp (control group), to UV-C light 254 nm (control group to desquamation skin) or UV-A light 365 nm and in one group the dams were supplemented with folic acid (FA), to determine micro nucleated erythrocytes (MNE) and micro nucleated polychromatic erythrocytes (MNPCE) in peripheral blood of offspring.

Results: All the rat neonates exposed to UV-C lamp showed desquamation skin, while for UV-A lamp no desquamation was observed, and there was MNE differences in all sampling times ($P<0.02$) and for MNPCE in 9 min group ($P=0.001$). No differences between the groups with and without FA were observed.

Conclusion: Increased MNE frequencies without apparent damage to the skin could be induced with UV-A light exposure. Under these conditions, FA no protected against UV-A light exposure. This study shows a manner to quantify the genotoxic effects of UV-A light in peripheral blood erythrocytes of rat neonates.

Keywords

UV-A light; Folic acid; Micronucleated erythrocytes; DNA damage; Neonates

Introduction

Solar radiation may cause undesirable health effects primarily in the skin, due to the action of ultraviolet light (UV) [1]. UV is divided into three types, UV-A (400-315 nm), UV-B (315-280 nm) [2,3], and UV-C (280-100 nm); UV-C light is largely blocked by the atmosphere [4-6], but accidental exposure could occur from germicidal lamps, with symptoms such as erythema, skin irritation, burning, and skin desquamation [7,8]. UV-C light induced skin erythema faster, and oxygen radicals may be important in initiating the erythematic response [9]. UV-A, UV-B and UV-C light can damage the DNA of cells [4,7,10,11] through the formation of pyrimidine dimers between adjacent bases [1,6,12-14]. These dimers distort the conformation of the double helix and interfere with normal DNA replication, which can produce mutations [4,12,15,16] and chromosomal fragmentation [5]. UV-A light also indirectly affects DNA by promoting the formation of reactive species, mainly reactive oxygen species (ROS) [1,4,12,17]. These entities oxidize the bases within DNA, which can lead to the fragmentation of DNA strands [1,17,18] and may lead to the formation of micronuclei [5,10,19,20]. The UV-B in sunlight is a causative agent of skin cancer [21]. In addition, UV-B and UV-C stimulates melanogenesis and produces erythema [4,7,8,22,23] more rapidly than UV-A light [24]. Skin cancer can also start as a result of exposure to UV-A light. The DNA lesions resulting from UV-A exposure can be mistakenly repaired and may consequently lead to the formation of mutations [25]. However, as mentioned, UV-B and UV-C light produces erythema [4,23], but UV-A light may require much longer exposure to induce pigmentation. Accordingly, levels of UV-A radiation exposure cannot be adequately quantified. Accidental UV-A exposures can occur, and this exposure may be one reason for the increased incidence of melanoma in people who use sunscreens [1,22]. It is clear that DNA damage occurs independently of erythema, and the protection against erythema does not a guarantee that DNA is protected from UV-A light damage [26]. Recently, increases in micronucleated erythrocytes (MNE) and micronucleated polychromatic erythrocytes (MNPCE) were observed in the rat neonates born to mothers exposed to UV-A [27]. Since the damage caused by the exposure to UV light is DNA fragmentation, in this study, we used MNE formation to quantify the DNA damage in the peripheral blood of rat neonates. MNE formation in newborn rats was chosen for these examinations because micronucleation can be easily observed in erythrocytes [28,29], and neonatal skin is thinner and due to UV-A penetrates more deeply, neonates show greater sensitivity to genotoxic damage [29]. Furthermore, part of the damage from UV light is believed to be caused by ROS generation, and it has been proposed that antioxidants be added to sunscreens or be administered separately for damage protection [4,27,30-36]. In the present study, folic acid (FA) was administered as an antioxidant to protect against DNA damage and to scavenge ROS [29,37-39].

Materials and Methods

This report presents an experimental study performed in accordance with institutional and governmental regulations for ethical use and handling of experiment animals [40,41].

All procedures were completed in accordance with the institutional guidelines of the Centro de Investigación Biomédica de Occidente (Instituto Mexicano del Seguro Social), Guadalajara Jalisco, México, which are consistent with those approved by the national and international institutes of health, based on the NOM-062-ZOO-2001 and on the American Psychological Association guidelines for the humane treatment of research animals [40,41]. 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 (registry number: R-2010-1305-10 and R-2012-785-035).

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Animals

Thirty-two 3-month-old female Sprague Dawley (hairless) rats were used [42,43], four rats per group. The rats were mated with a male adult (4:1) and allowed to deliver normally. Upon confirmation of pregnancy by determining sperm presence in a smear of a vaginal wash with sterile water, the pregnant rats were housed in individual polycarbonate cages in windowless rooms with automatic temperature control (22 ± 2°C), light control (lights on at 07:00 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.

For MNE and desquamation skin induction, immediately after birth the neonates were exposed to UV light daily. The UV lamps used were UVP models (UVP-Ultraviolet Products, Upland, CA, USA): UVS-26 (Mineralight® lamp, 115 V, 60 Hz, 0.32 W) at 254 nm (UV-C radiation, to induce skin desquamation) using a lamp with 2 tubes of 6 W, or UVL-24 (EL Series UV lamp, 115 V, 60 Hz, 0.32 W) at 365 nm (UV-A radiation) using a lamp with 2 tubes of 4 W.

Hairless rats were used as they provide a suitable experimental model for photo dermatology studies [44,45]. The whole litter was exposed daily; we randomly sampled only 4 newborns at each time. All of the rats were supplied by the laboratory animal facility at the Centro de Investigación Biomédica de Occidente (Instituto Mexicano del Seguro Social), Guadalajara, Jalisco, México.

Study groups

The pups were exposed daily for five consecutive days using the following scheme. Group 1, the negative control group, was exposed to a conventional light lamp with 60 LED (SE-EM-60L, 125 V, 60 Hz, 2.8 W, Sanelec, Estado de México, México) for 3 min per day (every 24 h). Groups 2, 3 and 4 were exposed to UV-C light from a 254 nm lamp for 3, 6 and 9 min, respectively to demonstrate skin damage. Groups 5, 6 and 7 were exposed to UV-A light from a 365 nm lamp for 3, 6 and 9 min, respectively. Group 8 were exposed to UV light from a 365 nm lamp day for 9 min and were also supplemented daily with FA (0.25 mg/kg).

Sample preparation and micronuclei analysis

All neonates from each litter were exposed daily to UV light and 4 randomly chosen neonates from each litter were sampled each time. For the group supplemented with FA (5 mg tablets, Lab. Valdecasas, S.A., Reg. No. 82231, S.S.A., México), the compound was dissolved in the drinking water. The FA-water solution was placed in amber bottles and changed daily. FA supplementation started two days before the birth of the newborns (day 20 of gestation) and continued until the end of the experiment; the dosage of FA to the neonates was always administered through the mother's milk [46,47].

Lamps were placed 7cm from the newborns. Intensity of the UV light emitted was measured with a digital radiometer UVX [Sensor UVX-36 long-waves (335-380 nm) and sensor UVX-25 short-wave (250-290 nm), UVP-Ultraviolet Products, Upland, CA, USA). For the conventional light, an Extech photometer was used (Lightmeter, EIC401025, Extech Instruments Corporation, Nashua, NH, USA). Intensity of the led lamp light was 1647 lux, while the intensity emitted by the UV-C light (254 nm) was 2.26 mW/cm^2 and 1891 μW/cm^2 for the UV-A light (365 nm).

The newborns were sampled using one drop of blood obtained from the tip of the tail of each one of the offspring after zero hours (basal sample), after 48 hours and after 120 hours. Blood smears were made in duplicate 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 MNE in each sample were scored manually using a microscope equipped with epifluorescence (Olympus BX51) and a 100×objective. The MNE frequency was established from the number of micronuclei in 10,000 total erythrocytes (TEs) (TEs: normochromic and polychromatic erythrocytes). The number of MNPCE in 1,000 polychromatic erythrocytes (PCE) was also determined. Both parameters were used for determining genotoxicity. In addition, the proportion of PCE in 1,000 TEs was evaluated to determine cytotoxicity and as internal technique control. All slides were coded before microscopic analysis and were evaluated by the same reader, who was blinded to the animals' group assignments.

Statistical analysis

Results (‰) are expressed as mean ± standard deviation of MNE, MNPCE, and PCE number. All statistical tests were performed using the Statistical Package for Social Sciences (SPSS v.11.0, Chicago, IL) for Windows medical pack. All of the data were tested for normality using the Kolmogorov-Smirnov test and the micronuclei frequencies were analysed to determine the differences among groups behaviour during the treatments by means of a one-way ANOVA followed by a Dunnett t-test for multiple post hoc pairwise comparisons versus the appropriate control to correct the significance values for inter-group analysis. Intra-group comparisons were made between each treatment group and their respective basal value (0 h) by means of repeated measures ANOVA followed by a Bonferroni test for multiple post hoc pairwise comparisons. A P-value of less than 0.05 was considered statistically significant.

Results

The sixth day after start of UV light exposure, newborns exposed to UV-C lamp presented desquamation of the skin on the back of the body at the three different exposure times. This effect was not observed in any of the groups exposed to the UV-A lamp.

As shown in Tables 1 and 2, the UV-A lamp showed significant differences in MNE at 48 and 120 h (P=0.015 and, P<0.001) for the group exposed for 3 min; at 120 h, for the group exposed 6 min (P=0.001); for the group exposed 9 min (P=0.03, P<0.001) at 48 and 120 h, whereas in MNPCE differences were observed at 120 h (P<0.001); and 9 min with FA group showed significant differences in MNE at 48 and 120 h (P<0.001). In PCE frequencies differences were observed in the FA group at 0 h vs. control group (P=0.005).

Comparison between groups of the 365 nm/9 min with vs. without FA showed no significant differences for MNE or MNPCE parameters. The effect of FA on the basal values of MNE, MNPCE, and PCE is shown in Tables 1 and 2. In the case, of PCE an increment in the 365 nm/9min with FA was observed (P<0.001) compared to the group without FA.

Discussion

Induction of DNA damage by UV light

The UV-A light is capable of producing pyrimidine dimers [4], this meaning that the DNA molecule in these conditions are more susceptible to strand breakage [20] and can give rise to micronuclei. The UV-A lamp can also cause damage by induction of ROS

Table 1: MNE and MNPCE frequencies (‰) at different sampling times of each study group.

		MNE			MNPCE		
GROUP	n	0 h	48 h	120 h	0 h	48 h	120 h
Negative control	16	3.47 ± 0.58	4.04 ± 0.93	3.80 ± 0.66	4.43 ± 0.89	6.56 ± 1.99	5.56 ± 1.71
365 nm/3 min	16	3.38 ± 0.61 *NS	5.24 ± 0.77 **P*=0.015	5.95 ± 0.52 **P*<0.001	3.93 ± 1.84 *NS	7.62 ± 2.06 *NS	8.18 ± 3.18 *NS
365 nm/6 min	16	3.55 ± 0.81 *NS	4.93 ± 0.68 *NS	4.94 ± 0.68 **P*=0.001	4.00 ± 1.82 *NS	6.75 ± 2.20 *NS	6.75 ± 2.17 *NS
365 nm/9 min	16	3.26 ± 0.77 *NS	5.50 ± 1.26 **P*=0.03	6.36 ±1.39 **P*<0.001	4.12 ± 1.74 NS	9.25 ± 3.41 *NS	10.12 ± 2.24 **P*<0.001
365 nm/9 min + FA	16	3.81 0.85 *NS **NS	5.80 ±1.04 **P*=0.001 **NS	5.91 ± 0.98 **P*<0.001 **NS	5.00 ± 1.03 *NS **NS	9.31 ± 3.07 *NS **NS	8.25 ± 2.32 *NS **NS

Data (‰) are shown as mean ± standard deviation. MNE: Micro nucleated erythrocytes, MNPCE: Micro nucleated polychromatic erythrocytes, n: sample size, h: hours. min: minutes, nm: nanometres, FA: folic acid. NS: not significant. Shown significant differences in the groups exposed to UV light. The comparison was from each group versus the negative control (*), and comparisons between groups with and without FA (**).

Table 2: PCE frequencies (‰) at different sampling times of each study group.

GROUP	n	0 h	48 h	120 h
Negative control	16	585.37 77.85	274.31 ± 52.77	368.37 ± 54.45
365 nm/3 min	16	638.43 ± 89.41 *NS	273.50 ± 32.53 *NS	386.12 ± 62.36 *NS
365 nm/6 min	16	606.75 ± 89.90 *NS	261.62 ± 47.25 *NS	414.00 ± 54.73 *NS
365 nm/9 min	16	618.50 ± 67.57 *NS	270.56 ± 32.38 *NS	387.56 ± 80.97 *NS
365 nm/9 min + FA	16	702.87 93.23 **P*=0.005 ***P*<0.001	299.12 ± 43.94 *NS **NS	425.87 ± 41.62 *NS **NS

Data (‰) are shown as mean ± standard deviation. PCE: Polychromatic erythrocytes, n: sample size, h: hours. min: minutes, nm: nanometres, FA: folic acid. NS: not significant. Shown significant differences in the groups exposed to UV light. The comparison was from each group *versus* the negative control (*), and comparisons between groups with and without FA (**).

[1,4,12,17,27,48], which may also produce chromosome breaks [10,19] and micronuclei, as was observed in the present.

The exposure time for this work was determined based on a previous study in skin cultures of hairless mice, where it was considered that the time chosen was not so large that could cause heating in their cultures [48]. So in the present study, even with an exposure time of 9 min, heating was not observed in neonates for the effect of the lamps.

In a previous study, increases in MNE and MNPCE were observed in the rat neonates born to mothers exposed to UV-A [27], possibly due to the production of long-lived protein radicals and protein hydro peroxides [49-53] which might pass through the placenta [49,54]. In the present, no measurements of any type of ROS were performed, so to try to explain MNE increase, we hypothesize that DNA damage in newborns could be done in these two ways: In a direct way, this is that the UV-A light could reach the erythropoietin organs of the newborn rat, induce the formation of ROS and pyrimidine dimers between adjacent bases locally, and thus, cause damage to the progenitor cells, this is feasible because newborn rats have very thin skin, which enables the light to penetrate. In an indirect way, by forming of long-lived radicals, which might form in the skin cells or blood proteins [49-51,54] and it is known that they can be formed by the effect of UV-A [55,56]. This can be possible because UV-A reaches the hypodermis, which is highly vascularized, so that this can give. So, long-lived reactive protein species [49] or extracellular genomic DNA fragments [57], they can reach erythropoietin organs and produce ROS for extended periods of time that react with DNA progenitor cells and, can produce breaks and thus the formation of micronuclei [51].

Skin damage (desquamation)

Lamps were selected for the type of damage caused by UV light on DNA and skin. Lamp of 254 nm (UV-C) produces evident desquamation skin as expected, whereas, with UV-A this does not happen. The UV-C light damaged the superficial layer of the skin of newborns [6]; this effect was observed on the sixth day of treatment. While all of the offspring of the three groups exhibited desquamation, this phenomenon was not observed with the 365 nm lamp [6,58]. In previous studies it was observed that with overexposure to UV-C radiation can cause skin erythema followed by skin exfoliation [7,8,59]. This information confirms how difficult it is to identify the skin damage produced by UV-A light (365 nm) in short exposure times, because it takes more time the appearance of the damage or skin darkening in response to UV-A. Thus, UV-A induced melanogenesis less efficient than UV-B [24]. So while all rats exposed to UV-C lamp exhibited desquamation on the sixth day, rats exposed to UV-A lamp, increased their MNE and MNPCE, with no apparent damage to the skin.

For proper analysis, we took into consideration the gradual increase in MNE values that arose in the days after birth. These changes were the outcome of physiological changes that naturally occurs in the neonate [60]. The resulting differences can sometimes reach a statistically significant level, and the statistical analysis should consider this naturally occurring increase.

365 nm UV light lamps

The harmful effects of UV light on genetic material are well-known [1,4,6,10,12,14,17], and the present studies demonstrated the induction of increased MNE frequencies by UV-A light. The 365 nm lamp (UV-A) 9 min exposure showed the highest increase of MNE and MNPCE.

Comparing the group exposed to 365 nm light and supplemented with FA against its corresponding group without FA supplementation showed no significant decrease in MNE and MNPCE as expected by being the FA an antioxidant, decrement previously observed with vitamin-C [27]. Since FA was given to the offspring via the mother milk, possibly the concentration of compound that was ingested by the pups was not enough to show any effect. Another possibility is that the observed increase in MNE, it is not produced by ROS, but rather as a direct effect of UV-A, in erythropoietin organs in neonates.

One point to note is the slight increase in PCE frequencies associated with FA ingestion and observed in the baseline sample. This increase may be due to the mitogenic effect of FA [61]. Previous studies have used erythropoiesis inductors with the intention of making the micronucleus test more sensitive due to increased cell division in bone marrow [62]. Moreover, in the present work, a similar effect can be attributed to FA. This finding raises a possible restriction on the use of FA, as this compound increased cell division in bone marrow of the newborn rats. However, this effect was not observed in adult organisms because they have less active erythropoiesis. Erythropoiesis in human newborns is different from that observed in rats, and the proportion of circulating PCE is much lower in human neonates than was observed in the rat neonates (a human neonate presents one-tenth or less PCE values than a newborn rat) [60,63].

The different values of PCE at different sampling times that were observed in all groups, are due to physiological changes experienced by the newborn during birth when they are changing to an environment with more oxygen, and are not due to exposure to UV-A lamps, as it was previously described [43].

UV-A light source was used at three different exposure times, and no dose-response effects were observed. This finding may be because the exposure times intervals (3,6 or 9 min) were very close to one another. Therefore, possibly a longer and separate exposure times are needed to observe dose-response effects. A similar result was observed in an earlier study conducted with caimans [5], which showed no dose-response effect. So in future studies of this type, it should extend the ranges between the different exposure times.

UV-A light measurement

The association between the occurrence of melanoma and exposure to tanning beds demonstrates a risk process in daily life [64]. The use of UV-A lamps is common (for example, counterfeit detectors, insecticides devices, equipment for artificial nails resins harden). In addition, UV-A light therapy is commonly used in dermatology treatments [65,66] and other clinical disciplines [67]. UV-A light exposure sources are found in many places.

This study shows a simple way to test UV-A exposure and to assess damage to genetic material induced by UV-A light. This study may be useful in assessing the effectiveness of in vivo sunscreens and may avoid testing in human volunteers [1,23]. The assessing the sun protection factors are easily performed when tested the effect of UV-B light [36] or UV-C because both produce erythema. However, erythema is not seen with UV-A light exposure [64].

The study of UV-A light is important, because it has a potential role in the increase in melanoma incidence in people using sunscreens and exposed to the sun [4]. It is difficult to determine UV-A light exposure, and some researchers are interested in this topic. It has been proposed that the effects of UV-A light be assessed based on their ability to induce pigmentation, as also occurs with UV-B light [68]. Another study, an in vivo micronuclei assay using rat skin for photogenotoxicity study (target organ for photo carcinogenicity) has recently been presented; this method is a promising alternative tool for photoclastogenicity studies [69], however, our procedure provides an easy alternative. UV-A light damage is observed in peripheral blood erythrocytes, and it requires only a drop of blood. Thus, it is also possible to take samples at different time points. Another advantage for this method is that newborn rats have very thin skin, which enables the light to penetrate and in addition, our methodology has greater sensitivity due to the use of immature organisms [28,29].

The exposure of newborn rats to 365 nm light increases MNE and MNPCE, and the results are quantitative. Eventually, degrees of damage induced by this exposure could be assessed by the number of MNE or MNPCE. Newborn rats could also be useful in verifying the effects of UV-B or UV-C, as their damage at the skin's surface leads to desquamation in a few days.

In conclusion, increased MNE frequencies without apparent damage to the skin could be induced with UV-A light exposure. Under these conditions, FA was not able to decrease MNE and MNPCE formation induced by UV-A light exposure. This study shows an alternative manner to quantify the genotoxic effects of UV-A light in peripheral blood erythrocytes of rat neonates, although more studies are needed to demonstrate the mechanism by which the damage occurs.

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