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Genetic damage in human blood cells exposed to germicidal lamps and cytoprotection of ascorbic acid

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RESUMEN

Daño genético en células sanguíneas humanas expuestas a lámparas germicidas y citoprotección del ácido ascórbico
Introducción. Las lámparas germicidas tienen un rango de longitud de onda de 200-280 nm y pueden afectar la integridad del ADN de personas que manipulan erróneamente estos equipos. Los linfocitos humanos son excelentes biomonitores de daño genético y ampliamente utilizados con la prueba del cometa.
Objetivo. Evaluación de genotoxicidad en células sanguíneas humanas expuestas a radiación UV (254 nm) emitida por lámparas germicidas y el efecto citoprotector del ácido ascórbico usando la prueba del cometa.
Material y métodos. Laminillas conteniendo linfocitos inmersos en gel de agarosa fueron expuestos a radiación UV-C (254 nm) por periodos de 5, 10 y 15 minutos a una distancia de 70 cm. El efecto antigenotóxico se determinó en células expuestas a UV-C durante 5 minutos a una distancia de 70 cm, posteriormente las laminillas se sometieron a una solución de ácido ascórbico por periodos de 5, 10 y 15 mM durante dos horas. En ambos casos se cuantificó el daño genético mediante la prueba cometa con el uso de tres parámetros: longitud de la cola, momento de la cola y grupos de migración.
Resultados. Los tres parámetros detectaron actividad genotóxica significativa ($p<0.05$) en los tiempos de exposición a UV-C y efecto citoprotector del ácido ascórbico ($p<0.05$).
Conclusiones. El manejo de lámparas germicidas UV-C es frecuentemente erróneo y peligroso para personas u organismos expuestos. Estos datos sugieren que el ácido ascórbico aumenta la protección del ADN en las células expuestas a la radiación UV-C.

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ABSTRACT

Introduction. Germicidal lamps have a wavelength range of 200-280 nm and can affect the integrity of the DNA of people who handle this equipment. Human lymphocytes are excellent biomonitors of genetic damage and widely used with the comet assay.

Objective. Evaluation of genotoxicity in human blood cells exposed to UV-C radiation (254 nm) emitted by germicidal lamps and the cytoprotective effect of ascorbic acid, using the comet test.

Material and methods. Slides containing lymphocytes immersed in agarose gel were exposed to UV-C radiation (254 nm) for periods of 5, 10 and 15 minutes and 70 cm away. The antigenotoxic effect was determined in cells exposed to UV-C for 5 minutes and 70 cm away, subsequently the slides were subjected to an ascorbic acid solution for periods of 5, 10 and 15 mM for two hours. In both situations, genetic damage was quantified by the comet test using three parameters: tail length, tail moment, and migration groups.

Results. The three parameters detected significant genotoxic activity ($p<0.05$) in the times of exposure to UV-C and cytoprotective effect of ascorbic acid ($p<0.05$).

Conclusions. The handling of UV-C germicidal lamps is often wrong and dangerous to exposed people or organisms. These data suggest that ascorbic acid increases DNA protection in cells exposed to UV-C radiation.

INTRODUCTION

DNA damage in a somatic cell may lead to the development of cancer and in a germ cell has the potential to cause a heritable altered trait (1). Several decades ago, interest began in studying the mutagenic and carcinogenic effects of occupationally exposed people (2). The monitoring of genetic damage has become highly relevant, and lymphocytes are frequently used due to the ease of obtaining them and because they are considered reliable sensors of the effects caused by genotoxic agents (3). For this reason, they are also used to evaluate genetic damage in workers exposed to different types of radiations (2, 4), therefore, they can also be used as biomonitors of genetic damage in people exposed to UV radiation emitted by germicidal lamps. The ultraviolet light from these lamps has a wavelength range of 200-280 nm, which can be easily absorbed by DNA (5). These lamps are widely used as a disinfecting tool because they do not use heat, are inexpensive and do not generate toxic products (6). They are used to sterilize medical equipment (7), rooms in hospitals (8), households (9) and in facilities owned by the agricultural sector (10). Lamps containing UV light have been identified by the United States Environmental Protection Agency as an excellent technology for reducing the risks of diseases transmitted by microbial pathogens (11) and is now being used increasingly. Nevertheless, in recent years, various reports have mentioned skin and eye injuries caused by accidental exposure in people handling germicide equipment, due to the lack or breakage of the protector, malfunction of the system or the installation and incorrect use of lamps (8, 12). The 187 report of the International Lighting Commission on the Risks of Germicide Lamp Cancer reports that there is sufficient evidence on carcinogenicity in laboratory animals (13) and direct UV exposure induces genetic damage and disrupts the transcription, translation and replication of DNA (5,14).

Several testing systems are used to evaluate genetic damage (15) and among them is the comet test, which is a method to evaluate DNA damage and repair at the level of individual cells (16). The last decade witnessed an increase in the use of comet assay for DNA damage monitoring in cancer patients and controls (17). The comet test uses various parameters such as tail length and tail moment. Recently our group (18) used migration groups (MG) as an alternative parameter to evaluate genetic damage based on the heterogeneity of the migration of comet cells. Previous studies have reported great heterogeneity of damage in the DNA of comet cells due to variations in cell size, differences in sensitivity to genotoxic or differences in the type of genetic damage manifested (19-21).

The severity of cytotoxicity and mutagenicity caused by UV radiation in mammalian cells depends on the lesions produced mainly on the DNA (22). Because the detection and characterisation of genetic damage in human tissues provides clues to the aetiology of human cancer (4) therefore, it is necessary to know the degree of genetic damage produced in people exposed to UV radiation emitted by germicidal lamps. Simultaneously genoprotection of ascorbic acid (AA) in these people should be explored for its important role as a cytoprotective agent against medical exposure to radiation (23) and as a genoprotector agent against ionizing radiation injuries to protect individuals from unwanted effects (24).

In this work, the magnitude of genetic damage occurred in human lymphocytes exposed to UV lamps, as well as the cytoprotective capacity of AA, was investigated. Changes in DNA integrity were measured with three parameters: tail length, tail moment, and MG used in comet assay system.

MATERIALS AND METHODS

Chemical and physical agents

Ascorbic Acid (CAS 50-81-7) was obtained from Sigma Chemical Co. (GDL, Jal, México), while dimethyl sulfoxide (DMSO, CAS 67-68-5) and disodium salt EDTA (CAS 60-00-4) were obtained from J.T. Baker (CDMX, MX). UV-C radiation was generated from lamps Philips (G25T8) at 25W at λ=254, placed at 70 cm from the samples.

Obtaining of human blood cells (HBC)

Prior verbal consent, 300 µL of peripheral blood were obtained by annular puncture of 8 young people no older than 22 years of age, who have been not exposed to chemicals, environmental contaminants or medications (Information obtained by conducting a questionnaire in accordance with the ethical standards of human experimentation declared by Helsinki in 1975). Ethical approval was conferred from the institutional ethics committee for experimental treatments (DBCyM/200/2020/Hoja 13).

300 µL the total blood of each of the individuals to be studied were centrifuged at 3,000 rpm for 10 minutes with 3 mL of phosphate buffer (NaCl 160 mM, Na_2HPO_4 8 mM, Na_2HPO_4 4 mM, EDTA 50 mM). After the time the supernatant (which contains the lymphocytes) was removed, and the pellet was suspended in 1 mL of phosphate buffer and preserved at 4 ºC until the time of use.

Alkaline comet test

Preparation of gels on slides. The comet test was carried out using the Speit and Hartmann method (25). 8 slides for each individual studied were covered with 1% Normal Melting point (NMP) agarose, leaving it to solidify and then removed to have a completely clean surface. Next 100 µL of 0.6% Low Melting Point (LMP) agarose layer was then placed on the slide. Once the first layer has solidified, another second layer of agarose (5 µL of the suspension containing phosphate buffer and whole blood previously obtained and 95 µL of the 0.5% LMP agarose) was added; finally, a third layer of 0.5% LMP agarose was added.

UV-C radiation exposure. 6 slides of everyone were exposed to UV-C radiation (254 nm wavelength) emitted by the germicide lamp at 70 cm for 5, 10 or 15 minutes. Two for each UV exposure time and two more were used as negative controls (unexposed lymphocytes).

AA cytoprotective effect evaluation. 10 slides of everyone were exposed to UV-C radiation, as before was mentioned, for 5 min, two for each concentration of AA used (5, 10 and 15 mM), two more were used as a positive control (exposed cells only to UV-C radiation) and two slides more were used as a negative control (unexposed and untreated cells). After UV exposure for 5 minutes, AA post-treatment was performed as follows: slides were individually immersed in AA solutions 5, 10 and 15 mM for two hours (this time is frequently used to detect interaction of substances with DNA in protocols that use the comet test) to then evaluate the radio-protective. Finally, the slides were washed three times with distilled water for five minutes to remove AA residues. The tests were

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done in duplicate. Comet assay procedure was continued; slides were immersed in lysis solution (NaCl 2.5 mM, Na_2EDTA 10 mM, Tris-HCl 10 mM, laurylsarcosinate 1%, Triton X-100 1% and DMSO 10%, pH 10) for 4 hours at 4 ºC and then placed in a horizontal electrophoresis system with electrophoresis buffer (NaOH 300 mM, Na_2EDTA 1 mM) for 45 min. The electrophoresis was then carried out for 15 minutes at 1.0 V/cm with an amperage of ~300 mA and at 10–15 ºC. Slides were washed immediately with distilled water and stained with 90 µL ethidium bromide. The washing was done by immersion in distilled water for minutes and then a new wash for 10 minutes was performed. The observation was performed with a fluorescence microscope with an excitation filter 515-560 nm.

The tail length and tail moment were measured with comet assay system II software. To take advantage of the heterogeneity of genetic damage observed in comet test, the grouping method proposed by Reynoso-Silva *et al.* (18) was used. Microsoft Excel 2019 software to form migration groups (GM) was used.

Data Analysis

Data was evaluated using Levene and Shapiro-Wilks test and Sigma Plot 12.0 and Stat graphics software. Since to the fact that the data of tail length and tail moment did not present normal distribution, the treatments were evaluated using a univariate variance analysis (ANOVA) constructed with a matrix of Euclidean distance. Statistical significance was subsequently tested using 10,000 permutations under a reduced type III square sum model, following the criteria of Anderson (26). GMs showed normal distribution, so treatments were evaluated by a single-pathway ANOVA from a matrix of Euclidean distances at 10,000 permutations. All ANOVA analyses were performed with PRIMER and the PERMANOVA complement.

RESULTS

Genetic damage in HBC from exposure to UV-C radiation from germicide lamps

To compare the basal genetic damage (negative control) and that induced by the UV-C lamps, three different parameters were used. Data analysis of each parameter showed a different degree of variation between treated and control groups. Table 1 shows the variation of genetic damage between the different treatments (basal genetic damage and induced by UV-C radiation 5, 10 and 15 minutes) with the parameters tail length, tail moment and GM. The treatments differed significantly in each the before mentioned parameters ($p<0.001$).

Table 1: Variation analysis between treatments: Treatment Group (TG) and Negative Control (NC).

Permutational ANOVA	Pseudo F	*p*-value	Variation components (%)
Tail moment			
Treatments	407.26	0.001	44.98
Residual			55.92
Tail length			
Treatments	497.97	0.001	45.52
Residual			54.48
Migration groups			
Treatments			
Residual	39.19	0.001	68.59
			31.41

The comparison between groups was statistically significant ($p<0.001$). Source: Own elaboration

The comparison of different times of irradiation assesses the genetic damage in cells from exposure to UV-C radiation from germicide lamp. Data show that the percentage of variation was 44.98 % for tail moment, 45.52 % for lead length and 68.59 % for GM.

The measurement of the degree of genetic damage induced by UV-C at 5, 10 and 15 minutes in cells was conducted with the three mentioned parameters. Tail length and tail moment measured the degree of DNA migration and MG quantifies the amount of migration groups formed. Basal genetic damage and that induced by germicidal radiation at different times over cells and in the three parameters mentioned are shown in Figure 1A. The tail moment shows the increase of genetic damage with respect to the negative control ($p<0.05$) (Figure 1A). No dose-response relationship is observed. The treatment of UV-C over 5 minutes caused more genetic damage,

while the UV-C treatments of 10 and 15 minutes did not present significant differences between them. Similar results were observed for tail length and GM parameters.

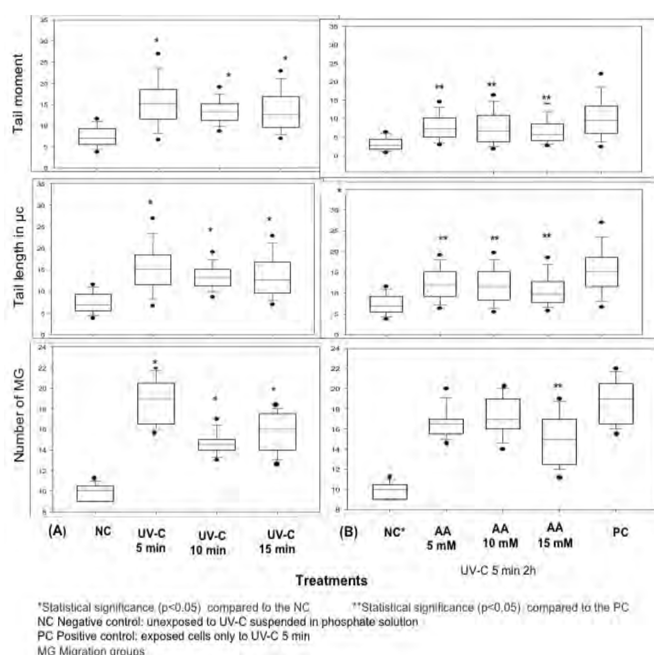

Figure 1. (A) Basal genetic damage (negative control) and induced genetic damage (UV-C 5, 10 and 15 minutes). Tail moment, tail length and MG show an increase of genetic damage with respect to the negative control (p<0.05) and statistical differences (p<0.05) *between different exposure times to UV-C*. (B) Post-treatment cytoprotective effect of AA. Tail moment, tail length shows a decrease of genetic damage respect to the positive control (p<0.05). In MG, only AA 15 mM show decrease of genetic damage respect to the positive control (p<0.05). Black line is SD.

Radio protective effect of post-treatment of AA in HBC

Comparison the genetic damage variation between the positive control (damage induced by UV-C lamps in cells) and the experimental groups (damage induced by UV-C lamps in cells and subsequent treatment with AA) observed in each parameter.

Table 2 shows the variation of genetic damage between different treatments (negative control, UV-C + AA 5,10 and 15 mM and positive control) in the parameters of tail moment, tail length and GM. The treatments differed significantly in each of the afore mentioned parameters (p<0.0001). The percentage of variation was 29.34 % for time of flow, 41.61 % for length of flow and 62.96 % for GM.

Table 2. Variation analysis between treatments: Negative control, UV-C 5 minutes + AA 5 mM, UV-C 5 minutes + AA 10 mM, UV-C 5 minutes + AA 15 mM and UV-C 5 minutes (positive control).

Permutational ANOVA	Pseudo F	p-value	Variation components (%)
Tail moment			
Treatments	333.24	0.001	29.34
Residual			70.66
Tail length			
Treatments	407.26	0.001	41.61
Residual			58.39
Migration groups			
Treatments			
Residual	24.12	0.001	62.96 %
			37.04 %

Source: Own elaboration

The protective effect of AA in the cells is shown in Figure 1B. The measurement of genetic damage induced by UV-C 5, 10 and 15 minutes in cells and subsequent treatment with AA was conducted with the parameters: tail length and tail moment were used to measure the degree of DNA migration and the amount of MG formed is indicated how MG in Figure 1B.

The tail moment parameter showed that post treatment with AA in concentrations of 5, 10 and 15 mM and two hours significantly reduced (p<0.05) genetic damage with respect to the positive control without reaching the level of basal genetic damage (Figure 1B). AA 5- and 10-mM treatments showed no significant differences. AA 15 mM caused the greatest reduction in genetic damage. Similar results were observed in tail length (Figure 1B). The GM number shows that only the treatment of AA 15 mM significantly reduced the genetic damage with respect to the positive control (p<0.05), without reaching the level of basal genetic damage (negative control), while treatments of AA 5 and 10 mM did not show differences with the positive control (p>0.05).

DISCUSSION

Concerns over highly infectious microbial and viral diseases have increased the use of germicide technology, even without proper supervision (9). The boom in the use of these lamps leads to an increased risk of exposure, so it is important to assess genetic damage. The detection and characterization of genetic damage in human tissues provides clues to the etiology of human cancer (27). The data obtained in this study confirmed the genotoxicity of UV-C light as reported in previous studies (5, 28). The evaluation with a third parameter allowed detecting not only the occurrence of genetic damage, as tail length and tail moment do, it also allows the determination of the most frequent type of genetic damage; the most frequent migration induced by UV-C (most frequent migration group) (18).

This increase in germicide technology makes it necessary to investigate DNA-protecting agents. It is known that some radio-protective substances as quinolone sulphonamide, 6-palmitoyl ascorbic acid-2-glucoside, and natural extracts as *Gingko biloba*, *Centella asiatica*, *Hippophae rhamnoides*, can both protect and improve the repair of genetic material, for this reason, these substances have been used in treatments to reduce morbidity, mortality or genotoxicity produced by ionizing radiation (29). Ascorbic acid is a powerful antioxidant capable of protecting DNA from genotoxic agents (30), however, the doses, times and distances of exposure to UV-C vary widely as well as the types of cells or organisms studied, so here is provided information on the UV-C exposure times we used in cells that are reliable monitors of genetic damage. It has been considered that AA's role in blood cells is still not entirely clear (31). Data collected in this study suggest that AA is a powerful cytoprotective agent in cells when added immediately after exposure to UV-C light and depends on the concentration, which is in accordance with what is reported by previous studies (32, 33). This highlights the importance of eating foods rich in AA for reducing genetic risks in people exposed to UV-C radiation, particularly in people dedicated to radiotherapy, food preservation, agriculture, industry and power generation, where there is a need to develop an effective and non-toxic cytoprotective.

Its cytoprotective effect is because it protects cells from genetic damage and cell death induced by ionizing radiation (32) as it acts as an electron donor, thus counteracting oxidative stress, and participates in the regulation of genes involved in DNA repair (34) specifically nucleotide excision repair.

The formation of migration groups or GM showed similar results to the parameters tail length and tail moment. This coincides with what was reported by Reynoso-Silva *et al*. (18) and indicates that GM is an efficient parameter for detecting basal and UV-C-induced genetic damage as well as for detecting AA antigenotoxic activity. The large amount of GM observed is related to the wide range of appearances of comets which is associated with the number of strand breaks in individual cells. Although the tail moment parameter is currently considered the most appropriate to accurately describe the damage (35) when the objective is to assess heterogeneity in response to DNA repair or damage, data analysis methods should be modified (29) and GM could be a tool to achieve this.

CONCLUSION

UV-C germicidal lamps are used in many different conditions and frequently in an erroneous way, which represents a risk to the genome of the people or organisms exposed. Data from this study shows the genotoxic activity of the UV-C radiation emitted by the germicide lamps in HBC in all times evaluated as well as cytoprotective effect of AA. This substance contributes to the maintenance of the integrity of the DNA of cells exposed to radiation. This information may be relevant for people exposed to UV-C radiation emitted by germicidal lamps to carry out prevention actions and consumption of foods containing ascorbic acid.

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