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Effect of Ultraviolet Radiation on Hsp70 Protein Expression in HaCaT Cells

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Abstract

Ultraviolet radiation by its wavelength is divided into: UVA, UVB and UVC. Only UVA and UVB manage to penetrate the ozone layer, but due to anthropological activities, all of them are capable of interacting with humans to a greater or lesser extent, and can generate adverse effects such as cellular stress when interacting with intra- and extracellular biomolecules. The skin is the first organ in contact with UV radiation, and the stress it generates can be analyzed by the expression of a bioindicator of cellular damage such as Hsp70. Therefore, the objective of the project was: to determine the effect of UVA, UVB and UVC radiation on HaCaT epithelial cells, by analyzing the expression of Hsp70. Materials and methods: HaCaT cells were cultured *in vitro*, which were irradiated with UVA, UVB and UVC light at different doses, to subsequently determine the degree of Hsp70 expression by Immunodetection by PAGE-SDS and Western Blot. Results: Basal expression of Hsp70 was observed in no irradiated HaCaT cells. When HaCaT cells were irradiated with UVA, UVB, UVC, an increase in this Hsp70 protein was observed. With UVA, a higher degree of expression was observed at a time of 30 minutes of irradiation. With UVB the highest expression shifted to a time of 20 minutes. With UVC, overexpression was observed after 10 minutes. Conclusion: UV radiation generates cellular stress on HaCaT cells, evaluated by the stress bioindicator Hsp70. According to the wavelength of UV radiation, those that have a shorter wavelength have a greater potential for cellular damage, such as UVC.

Keywords

Ultraviolet A Light (UVA), Ultraviolet B Light (UVB), Ultraviolet C Light (UVC), Heat Shock Protein 70 (Hsp70), HaCaT

1. Introduction

The sun provides most of the energy that living beings require on the planet [1]. Solar radiation is a set of waves that oscillate at different frequencies (Hertz) and wavelength [2], which includes ultraviolet (UV) radiation with a wavelength of 100 - 420 nm [3], dividing into three subclasses: UVA, from 420 to 320, UVB from 320 - 280, and UVC from 280 - 100 nm [4].

The natural UV radiation that penetrates the ozone layer, and that is generated artificially, induces various biological effects [5], which vary depending on the wavelength and intensity with which they affect the cells. Prolonged exposure can produce chronic and acute effects on the skin (redness, which later turns into burns), eyes and immune system, not only in humans, but also in both wild and domestic animals, and can generate erythema, edema, hyperplasia, immunosuppression, photoaging and melanogenesis that can lead to the development of cancer [6] [7] [8].

The skin is the first barrier to UV radiation, consisting of a stratified squamous keratinized epithelium that is constantly growing, has an inner layer of living epithelial cells and an outer layer of dead cells rich in keratin [9] [10] [11]. When the effects of UV radiation exceed the natural protection offered by the skin, an inflammatory reaction occurs that results in the action cascade of arachidonic acid, mediated by the release of substances such as bradykinin, prostaglandins, histamine and serotonin, which they induce vascular permeability [12] [13].

UVA Radiation has the ability to penetrate the epidermis of the skin, generating premature photoaging, with the possibility of suppressing immune functions, triggering the production of reactive oxygen species (ROS) [14] [15] [16], and reactive nitrogen species (RNS) [14], which cause damage to DNA, proteins, lipids and carbohydrates, which over time produces necrosis of the endothelial cells, and dermal blood vessels [5] [11].

UVB Radiation, considered "burn radiation," penetrates the epidermis acting at the basal layer, damages the keratinocyte genome, stops the cell cycle, generates premature photoaging, photocarcinogenesis and melanogenesis due to the generation of free radicals in the irradiated area and by the decrease in antioxidant enzymes, which may be responsible for inducing squamous cell skin cancer and basal cell carcinoma [5] [14] [17].

UVC Radiation is extremely harmful to living beings due to its high energy capacity; it is stopped by the ozone layer [5]. However, when it is produced artificially and interacts with organisms, it induces damage through the formation of ROS, decreasing the concentrations of antioxidant enzymes and the repair of oxidative processes in biomolecules, which, thanks to the transfer of excited endogenous chromophores, single and double strand breaks can occur in DNA (SSBs and DSBs), alterations in melanin and in the aromatic amino acids tyrosine and tryptophan [18] [19], as well as damage in lipids and proteins, which causes serious aging cell damage, cancer and more serious inflammatory processes, with cellular homeostatic deterioration [1] [3] [19].

UV radiation not only affects humans, it is known that some animals exposed for long periods to solar radiation, that live at high altitudes, in tropical places, that lack pigment in the epidermis and have little hair, are more prone to skin diseases, since UV radiation damages its DNA, increasing the appearance of mutations, cell cycle arrest and cell death. One of the conditions that is related to these factors is squamous cell carcinoma. These tumors occur mainly in cattle of the Hereford, Simmental, and Holstein breeds, causing eye cancer, whose origin is genetic, but is also related to exposure to UV radiation, and also affects felines and canines. In horses, the most sensitive breeds are Belgian, Clydesdale, Shire and Appaloosa, which generate lesions mainly in muco-cutaneous regions (conjunctiva, vulva, perineum). In canids the lesions are located on the trunk, extremities, scrotum, lips, and nail bed, in felines, on the face, ears, mainly when there is white hair. UV radiation also induces melanocytomas, 80% to 90% of these tumors are benign in cattle, located mainly in the skin; In the rest of the animals, these tumors are usually malignant, called melanomas, being common in canines and horses, uncommon in cats and rare in other species [8].

The most common melanomas in dogs are located in the mouth, lips, skin, fingers and eye. Cutaneous melanomas occur on the head and scrotum. Other conditions caused by UV radiation are hemangiosarcomas, which most commonly affect dogs. There are also hemangiomas, it is a relatively benign neoplasm of the canine capillaries in the skin, trunk, extremities and soft tissues, they are frequently precursors of hemangiosarcomas. Thus, UV radiation affects some species significantly both in health aspects and in aspects of economic significance [8].

To counteract the effects of cellular stress, including those caused by UV radiation, there is a group of proteins known as heat shock proteins (Hsp), which were discovered in 1962 [20] [21] [22].

Hsp proteins are highly conserved in eukaryotic and prokaryotic cells, fulfilling the function of cytoprotectors and molecular chaperones, they participate in the regulation of protein assembly, folding, and export. They can be induced by various stress agents, such as heat shock, oxidative stress, oncogenic stress, due to UV radiation, low frequency electromagnetic radiation, due to gamma radiation, X-rays, among others. Their molecular size ranges between 10,000 to 110,000 Daltons, they are divided into 6 subfamilies: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, small HSPs [21] [23] [24] [25] [26].

The overexpression of these proteins minimizes the damage caused by stress [22], and can block apoptosis pathways by activating factors that degrade the cell matrix. However, if stress increases, the protective function of Hsp, stop their production and induce apoptosis along with other proteins such as p53 [21] [22].

The Hsp70 family is the most sensitive to thermal stress, being able to differentiate various molecules: Hsp70, Hsp72, Hsp73 or Hsp70, Hsp75 or GRP75 and Hsp79 or GRP78, which can be located in the cytosol, nucleus, endoplasmic reticulum and mitochondria [20]. It acts as chaperones, it is characterized by a rapid activation of its protective mechanisms together with Hsp27 and Hsp90 against aggressive stressors such as UVC radiation and temperatures above 40˚C, maintaining intracellular protective mechanisms, both cellular and nuclear. stable, limiting DNA damage, stimulating base excision repair through interaction with apurinic/apyrimidinic endonuclease and by stimulating the filling of gaps in a DNA strand thanks to DNA polymerase [21] [27] [28] [29]. It also participates in the repair of ion channels, the suppression of pro-inflammatory cytokines, preservation of mitochondria, in the prevention of cell apoptosis [25] [30]. Due to the above, its expression in the face of certain attacks can be used as a bioindicator of stress [31] [32].

When cell damage is great, whether due to physical, chemical, or endogenous stressors, cell death occurs through apoptosis. The term apoptosis or program cell death was used in 1972 to describe a morphologically distinct form of cell death, whose process is highly ordered and conserved [33]. Apoptosis aims to maintain genomic stability, controlling cell development and growth through a series of events programmed for the elimination of defective cells that can be generated through cellular signals, through genetic control or when induced by factors harmful to the cell, such as exposure to UV radiation [33]-[38].

Exposure to UV radiation induces a chronic state of constant wakefulness and repair in the human body, since as the years go by the protective response of these cells tends to decrease, generating mutations that can lead to the appearance of chronic and degenerative diseases such as cancer. Therefore, it is important to study cellular stress and the harmful effects produced by UV radiation in an appropriate biological model, such as human HaCaT cells, since they are non-tumorigenic immortalized transformed keratinocytes [39] [40].

It is of great importance to understand the maximum tolerance point to ultraviolet radiation, evaluated by quantifying a stress bioindicator such as the Hsp70 protein. For this, it is postulated that irradiation of HaCaT cells with UVA, UVB and UVC radiation generates cellular stress, which compromises cellular homeostasis, generating overexpression of the Hsp70 protein depending on cellular damage. Therefore, the objective of the present study was to determine the effect of UVA, UVB and UVC radiation on HaCaT cells, evaluated by the expression of the Hsp70 protein.

2. Material and Methods

Study model: HaCaT cells.

Cell culture: the HaCaT cell line was donated by the microRNA laboratory, Universidad Autónoma de Zacatecas, Mexico, and was acquired from the American Type Culture Collection. The cells were cultured at 37˚C in disposable plastic bottles (Costar 3151, Cambridge, Ma) with an atmosphere of 95% air and 5% carbon dioxide (SteriCult 200, Forma Scientific, Ohio) in 20 ml of medium Dulbecco's Modified Eagle basal (DMEM; D1152, Sigma Chemical Co. St. Louis, Mo.) supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL; *In vitro*, Mexico), insulin (0.08 U/mL; Eli Lilly, Mexico), and 10% certified fetal bovine serum (FBS) based on the methodology proposed by Huseynova *et al.* in 2021 [41] (Gibco BRL, 16000-028, Grand Island, N.Y.). The cells were cultured with trypsin EDTA (*In Vitro*, Mexico) and seeded at confluency (5×10^6/ml) in polystyrene dishes (Costar), **Figure 1**.

Exposure to ultraviolet radiation: 64 cell cultures were carried out at confluence, of which 24 experimental units were irradiated with UVA, 20 with UVB and 20 with UVC. A Handelheld® UV radiation emitting lamp (P/N 95-0343-01) with an exposure potential of 8 W/m^2 was used, which was placed inside an incubator at 37˚C and at a distance of 10 cm to irradiate samples, the emission wavelength for UVA was 365 nm, for UVB 302 nm and for UVC 254 nm, the irradiation periods were 10, 20 and 30 minutes, which corresponds to an exposure dose of 212.4, 424.8 and 637.2 J/cm^2. Non-irradiated cell cultures were taken as controls. During irradiation, cultures were maintained in cDMEM medium. After irradiation, the cells were kept for 40 minutes at 37˚C to allow the activation of cellular repair mechanisms. The cell cultures were observed in a LABOMED inverted optical microscope pre and post irradiation to denote morphological alterations.

Cell lysis: After irradiation, the cells were washed with 1 ml of cold phosphate buffer pH 7.2 (Gibco BRL, Grand Island NY, USA, 21300-58), to subsequently be lysed with 500 μl of lysis buffer (1% Triton X-100, 140 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl pH 7.6, and protease inhibitor 11697498001, Roche Diagnostics) with the help of scraping using a cell culture spatula. The cell extracts were collected in 1.5 mL microtubes, homogenized and centrifuged at 14,000 rpm for 10 min at 4˚C, in order to obtain soluble proteins from the supernatants.

Protein quantification, PAGE-SDS and Western blot: The analysis of Hsp70 protein expression was carried out for each of the experimental units, initially the protein concentrations were determined using the technique described by Bradford in 1976 [42]. For each experimental condition, the amount of volume necessary to run and characterize 20 μg of protein in polyacrylamide gels (7.5% PAGE-SDS) was calculated according to the technique described by Laemmli in 1970 [43]. Once the electrophoretic runs were performed, the proteins contained in polyacrylamide gels were transferred to nitrocellulose membranes (Hybond-C RPN 303 C, Amersham, Little Chalfont, Buckinghamshire), according to the method described by Towbin *et al.* in 1979 [44].

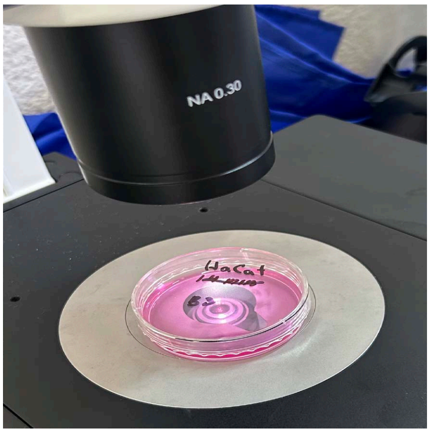


Figure 1. Example of HaCaT cells seeded at confluence in Petri dishes.

Immunodetection: Once the nitrocellulose paper retained the proteins, the nonspecific sites of the membranes were blocked with a 3% PBS-Casein solution overnight at 4˚C. After this time, the primary anti monoclonal antibody was added. Hsp70 (SC-24 Santa Cruz Biotechnology®, USA) in 1:1000 dilution over a period of one hour at room temperature and stirring (25 rpm), followed by 7 washes with PBS and PBS-TWEEN solution. alternately (5 min, with constant stirring of 45 rpm), next, the peroxidized anti-mouse IgG secondary antibody (anti-mouse IgG-HRP conjugate SC-2005 Lot F0412 Santa Cruz Biotechnology®, USA) was added in a 1:1000 dilution for one hour, and then washed again 8 times. Finally, to reveal, an ECL solution (GERPN2232 - ECL™ Prime Western Blotting Detection Reagent System, Solution A Luminol and Solution B Peroxide) was added to the membranes, which interacted with the peroxidized antibodies and emitted a photoluminous signal, which was evaluated by the photodocumenter. Image Lab Bio-Rad® Laboratories obtained the optical densitometry analysis to determine the degree of Hsp70 protein expression (Cornejo *et al*., 2014) [45].

Statistical Analysis: For the experiments with UVA, 6 repetitions were done and for UVB and UVC 5. The statistical analyzes were carried out using the Microsoft Excel® and GraphPad Prism 8.0.1 programs. Hsp70 expression was quantified by calculating arbitrary units of the optical density of protein expression using Image Lab software version 2.0.1 build 18 (Copyright © 2009 by Bio-Rad ® Laboratories). Differences between experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by student's t tests. Graphs comparing differences between experimental groups were plotted using GraphPad Prism and expressed as mean ± standard error. A value of $p < 0.05$ was considered statistically significant.

3. Results

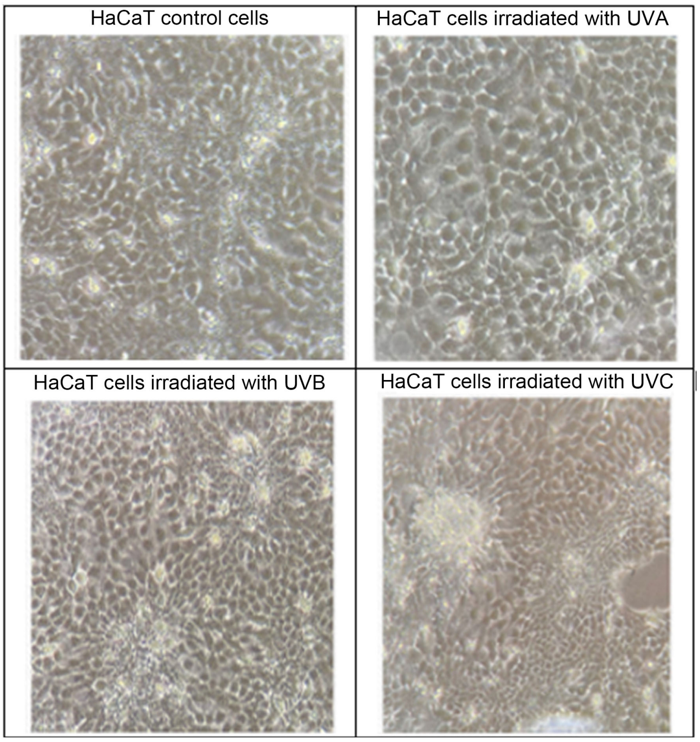
HaCaT cell culture and ultraviolet irradiation

HaCaT cells, cultured to confluency in Petri dishes, were subjected to ultraviolet radiation A, B, C. Once the cells were irradiated, they were observed in the optical microscope, distinguishing a uniform layer of cells in the dishes not subjected to radiation (control). and with ultraviolet light A and B, while in the dishes irradiated with ultraviolet light C, a discrete detachment of this monolayer was observed, which are possibly regions where cell death occurred, **Figure 2**.

Hsp70 protein expression

After irradiation, Hsp70 expression was assessed by Western-blot-ECL. With the help of a photodocumenter, images of the different protein expressions were obtained, **Figure 3**.

Subsequently, for each signal obtained, optical densitometry was performed, finding that for UVA irradiation the maximum peak of expression gradually increased up to 30 minutes of exposure, for UVB in a similar way, but shifting to 20 minutes with a subsequent drop at 30 minutes, and for UVC the maximum peak of expression now shifted to 10 minutes with a subsequent drop at 20 and 30 minutes, **Figure 4**.


HaCaT control cells

HaCaT cells irradiated with UVA

HaCaT cells irradiated with UVB

HaCaT cells irradiated with UVC

Figure 2. Cultures of control HaCaT cells and irradiated with ultraviolet light. Inverted optical microscope at 40x.

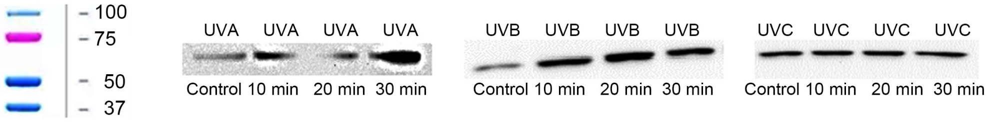


Figure 3. Example of the characteristic banding of Hsp70 expression in control HaCaT cells and irradiated at different times with UVA, UVB and UVC, using the Western-blot-ECL methodology, revealing by the Photo documenter Image Lab Bio-Rad® Laboratories.

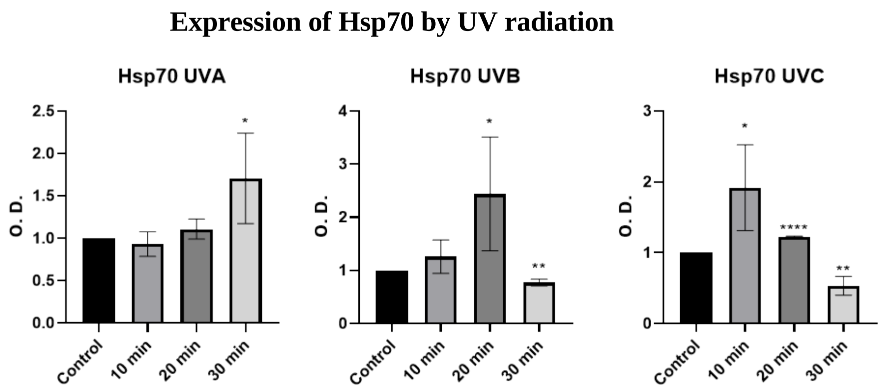


Figure 4. Mean optical densities (±SD) of the Hsp70 protein in control HaCaT cells and irradiated with UVA, UVB and UVC. Photo documenter Image Lab Bio-Rad® Laboratories, obtaining the optical densitometry analysis.

4. Discussion

In the present study, HaCaT cell cultures were used, which were irradiated with the three wavelengths of ultraviolet radiation, to subsequently determine the degree of expression of the Hsp70 protein as a bioindicator of cellular stress.

Before and after irradiation, the HaCaT cells were analyzed under a microscope to see the integrity of the monolayer, observed normally in the control, with UVA and UVB, but not with UVC, where discrete empty spaces were observed in the monolayer. Indicating detachment due to cell death, since these cells are alive when they are attached to a surface. The above coincides with other reported works, where UV radiation induces cell death by apoptosis [33]-[38]. Wang *et al.*, in 2014 [25] irradiated HaCaT cells treated with the PCF polypeptide from Chlamys farreri with UVA, which is used as a therapeutic agent against sunburn and UV damage, since it functions as an antioxidant and cellular antiapoptotic, finding that the samples treated with PCF had lower cell apoptosis. It is worth mentioning that in this work UVC induced greater cell detachment and/or death, since it has a shorter wavelength and is more energetic, therefore it induces greater damage [1] [3] [5].

In HaCaT cells, changes in the expression of the Hsp70 protein were evident in response to stress caused by exposure to ultraviolet radiation. Hsp70 protein was expressed basally in HaCaT cells (control). This expression is not surprising, since this protein is highly conserved in both prokaryotic and eukaryotic cells [21] [24] [25] [26] [27]. During irradiation, HaCaT cells overexpress the Hsp70 protein at all times (each time equals one dose) and with different types of ultraviolet light. For UVA, the maximum expression peak appeared at the 30-minute dose, for UVB the highest expression was at 20 minutes, and for UVC the highest expression shifted to 10 minutes. The above suggests that as the wave size of the radiation decreases, its potential energy increases and with it the interaction with cellular macromolecules, triggering greater damage in irradiated cultures, where Hsp70 is expressed as a cytoprotective protein.

Hsp70 has been reported by Yuspa, *et al.*, (1988); Wilson (2014) [40] [46], as well as by Ortíz Letechepia, (2019) [31] as a suitable bioindicator of cellular stress, which can be induced by both physical and chemical stressors. The results of this project can be correlated with those of other authors, for example, Roh *et al.*, (2008) [47] demonstrated that when skin cells are exposed to UV radiation, the Hsp70 protein is overexpressed, denoting cellular damage and functioning as a radio protective protein; In the same way but in leukocytes, Félix *et al.* in 2006 [24] found similar results; Regarding other stress factors such as thermal and oxidative stress, Mayer in 2005 [48] determined that Hsp70 is an excellent bioindicator of cellular stress, which is supported by the reports of Mayer in 2013 and Multhoff *et al.*, in 2015 [49] [50].

In the study carried out by Park, *et al.*, in 2000 [51], we sought to determine whether apoptosis of human melanoma cells can be induced by UVB radiation. This was done by transfecting the human melanoma cell line G361, with the plasmid Pure MFG.hsp70, which promotes the expression of the Hsp70 protein, demonstrating that the increase in this protein inhibited procaspase-3 and thereby decreased cell apoptosis, which would implicate the Hsp70 protein as radioprotective. Similarly in the project of Yoshihisa, *et al.*, from 2012 [52] when using Alkannin, an active component of the root of Alkanna tinctoria, as an inducer of the Hsp70 protein in HaCaT cells irradiated with UVB radiation, where the treatment inhibited the Caspase-3 which prevented the induction of apoptosis, apparently proving to be a beneficial compound for the photoprotection of the skin by the induction of the Hsp70 protein.

The Hsp70 protein is overexpressed and functions as a cytoprotector against various stress agents, including ultraviolet light [22] [23]. In this study, greater expression of this protein is observed in a shorter time due to UVC radiation, whose wavelength is short and very energetic and causes greater damage to cells.

5. Conclusions

The present study showed that UV radiation generates stress on HaCaT cells (keratinocytes), which, in relation to a normal control, expressed a greater increase in the thermal stress bioindicator Hsp70. Its expression was longer with UVA and UVB, and shorter with UVC, whose wavelength is short. Thus, it is concluded that UVC radiation, with a shorter wavelength, has a greater power for cell damage.

This project managed to identify the differences in cellular damage caused by different wavelengths of UV radiation. Suggesting that UVC radiation was the most damaging to the HaCaT cellular model. It is proposed as future work to complement the analysis of the expression of stress bioindicators induced by this factor, analyzing other molecules such as ROS, p53, proteins related to cell apoptosis, such as caspase 8 and 3.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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