Mammalian cells exposed to ionizing radiation: structural and biochemical aspects

Myrna Sabanero[1], Juan Carlos Azorín-Vega[2], Lérida Liss Flores-Villavicencio[1], J. Pedro Castruita-Dominguez[3], Miguel Angel Vallejo[2], Gloria Barbosa-Sabanero[4], Teodoro Cordova-Fraga[2], Modesto Sosa-Aquino[2]

[1] Departamento de Biología, DCNE, Universidad de Guanajuato
Noria Alta s/n, Col. Noria Alta
36250 Guanajuato, Guanajuato, México

[2] Departamento de Ingeniería Física, DCI, Universidad de Guanajuato
Loma del Bosque 103, Lomas del Campestre
37150 León Guanajuato, Guanajuato, México

[3]Departamento de Ecología, CUCBA, Universidad de Guadalajara
Las Agujas, Zapopan, Jalisco, 45100, México

[4] Departamento de Ciencias Médicas, DCS, Universidad de Guanajuato
20 de Enero No. 929; Col. Obregón; C.P. 37000; León, Gto.

E-mail: myrna.sabanero@gmail.com, modesto@fisica.ugto.mx

Abstract

Acute or chronic exposure to ionizing radiation is a factor that may be hazardous to health. It has been reported that exposure to low doses of radiation (less than 50 mSv / year) and subsequently exposure to high doses have greater effects in people. However, it is unknown molecular and biochemical level alteration. This study, analyzes the susceptibility of a biological system (HeLa ATCC CCL-2 human cervix cancer cell line) to ionizing radiation (6 and 60mSv/90s). Our evaluate multiple variables such as: total protein profile, mitochondrial metabolic activity (XTT assay), cell viability (Trypan blue exclusion assay), cytoskeleton (actin microfilaments), nuclei (DAPI), genomic DNA. The results indicate, that cells exposed to ionizing radiation structurally show alterations in nuclear phenotype and aneuploidy, further disruption in the tight junctions and consequently on the distribution of actin microfilaments. Similar alterations were observed in cells treated with a genotoxic agent (200μM H_2O_2/1h). In conclusion, this multi-criteria assessment enables precise comparisons of the effects of radiation between any biological systems. However, it is necessary to determine stress markers for integration of the effects of ionizing radiation.

Keywords: ionized radiation, susceptibility cells, organelles structural, DNA.

1.- INTRODUCTION

Ionizing radiation (IR) generates both direct and indirect damage to biological molecules. Radiation damage to biological systems is determined by the type of radiation and the total dosage of exposure (Manesh et al., 2015).

In high linear energy transfer (LET) radiation, such as neutrons and alpha particles, most of the cellular damage results from the direct ionization of cellular macromolecules including DNA, RNA, lipids, and proteins (Azorín and Azorín, 2010).

In contrast, low LET radiation, such as X-rays and gamma rays, indirect damage to biological macromolecules occurs following the generation of reactive oxygen species (ROS). ROS, especially superoxide and hydroxide radicals from the radiolysis of intracellular H2O, can have many effects, including the oxidation of biological macromolecules and activation of intracellular signaling pathways affect the cellular physiological (Panganiban et al., 2013).

In this work, the models biological to analyzed the ionizing radiation is the culture cells i.e. Hela (human cervix cancer cell line). This cells present increase indices proliferative and can be applied different IR doses. We investigate the signaling mechanisms activated by radiation for the induction of toxicity in transformed cells. Understanding the molecular mechanisms of radiation toxicity is critical for the development of radiation countermeasures as well as for the improvement of clinical radiation in cancer treatment.

2.- MATERIALS AND METHODS

Cell culture

The human cervix cancer cell line HeLa ATCC® CCL-2™ was selected for this study. The cells were cultivated at subconfluence in DMEM (Dulbecco's Modified Eagle's Medium, GIBCO, USA) supplemented with fetal bovine serum 10% (GIBCO, USA) and incubated at 37 °C, 5% CO_2.

Exposure of HeLa cells to ionizing radiation

The HeLa cells were exposed to ionizing radiation to 6 and 60 mSv during 90seg (Figure 1). After to exposition were determinate some parameter such as: morphology, metabolic activity, genomic DNA integrity, cytoskeleton (actin) and nuclei.

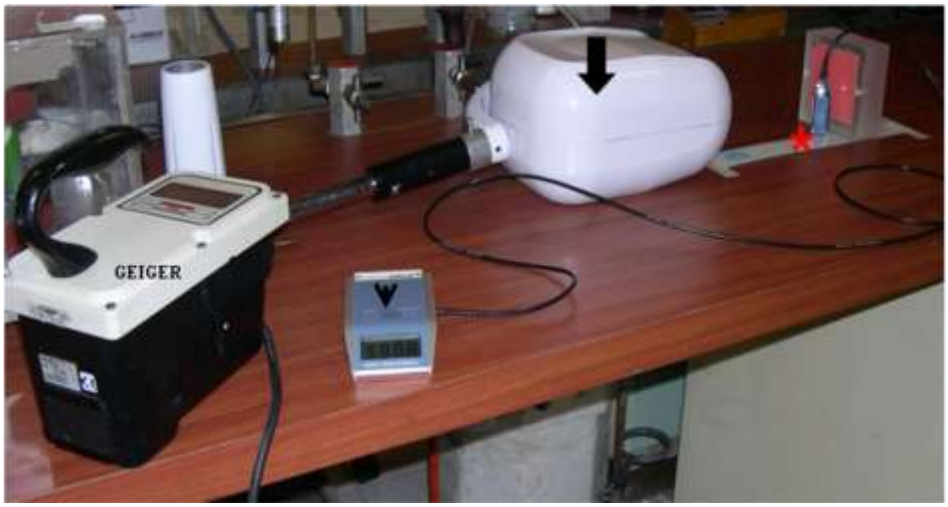

Figure 1. Radiation power.

It is show the radiation power (arrow), the space where the biological sample is placed (asterix) for IR exposing. Unform instruments (Unfors Mult-O-Meter) was used to determinate the radiation doses, the Geiger detector was used to detected no IR in the area.

Metabolic activity determination

The XTT assay is a colorimetric assay that detects the cellular metabolic activities. During the assay, the yellow tetrazolium salt XTT (2,3-bis (2-metoxi-4-nitro-5-sulfofenil)-2-h-tetrazolium-5-carboxanilide) is reduced to a highly colored formazan dye by dehydrogenase enzymes (mitochondria) in metabolically active cells [Silva et al., 2011]. The cells were exposed to IR as previously described. After exposure, 100µL of XTT detection solution (0.25mg/mL in 0.1mM Menadione, Sigma) were added to each well and the plate was returned to the incubator for 90min. The formazan dye formed in the assay was quantified by measuring the absorbance at 450nm in a spectrophotometer (Epoch Biotek).

SDS-PAGE

The separation of proteins from extract was performed in 10% gel of bisacrylamid/acrylamide, denaturalized conditions (SDS-PAGE), using the technique described by Laemmli (1970).

Genomic DNA integrity

Genomic DNA extraction was performed according to the AxiPrep™ Multisource Genomic DNA Miniprep kit (AXIGEN Biosciences). DNA samples of cells exposed to IR (6 and 60mSv) were analyzed on 0.8 % agarose gels and stained with Gel Red™ (BIOTUM). The size marker was HyperLadder™ 1kb (BIOLINE). The bands were visualized in ChemiDoc MP System (UV light at 260-280nm, BIORAD) and image acquisition with Image Lab™ software (BIORAD).

Cytoskeleton and nuclei analysis

Control cells and those exposed at IR, were fixed with 4% p-formaldehyde for 20 min, permeabilized with 0.5% Triton X-100 buffer containing 10 mM Tris, 5 mM KCl, 1 mM $MgCl_2$, and later exposed to FITC-phalloidin (Soto et al., 2014). The preparations were mounted using Vecta Shield-DAPI (4',6-diamidino-2-phenylindole) a fluorescent stain that binds strongly to A-T rich regions in DNA (Vector Laboratories, USA) and observed under a fluorescence microscope (Leica, DMLS) using a 450-490nm B filter. The images acquisition was done with camera AxioCam ICc1 (Carl Zeiss).

3.- RESULTS

The metabolic activity (Fig. 2), indicate that the ionizing radiation at 6 mSv is similar to control (100%), however, the cells expose to ROS (positive control) was 41%. At 60mSv the mitochondrial metabolic activity decrease to 86%.

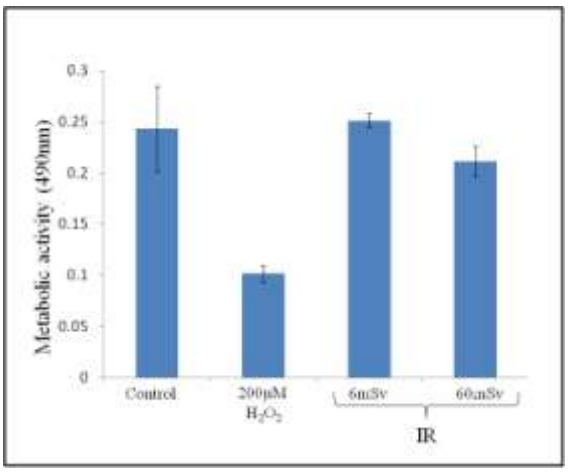

Figure 2. Metabolic activity of HeLa cells expose to ionizing radiation.

In other hands, morphologic changes were observed (Fig. 3I) in the cells expose to IR (Fig. 3B, B' and C, C'), these alterations are evident at 60mSv. The protein profile of cells expose to IR is similar to control cells, however, in proteins of Mr ≥ 60-40 kDa occur changes with ROS (Fig. 3II lane 2).

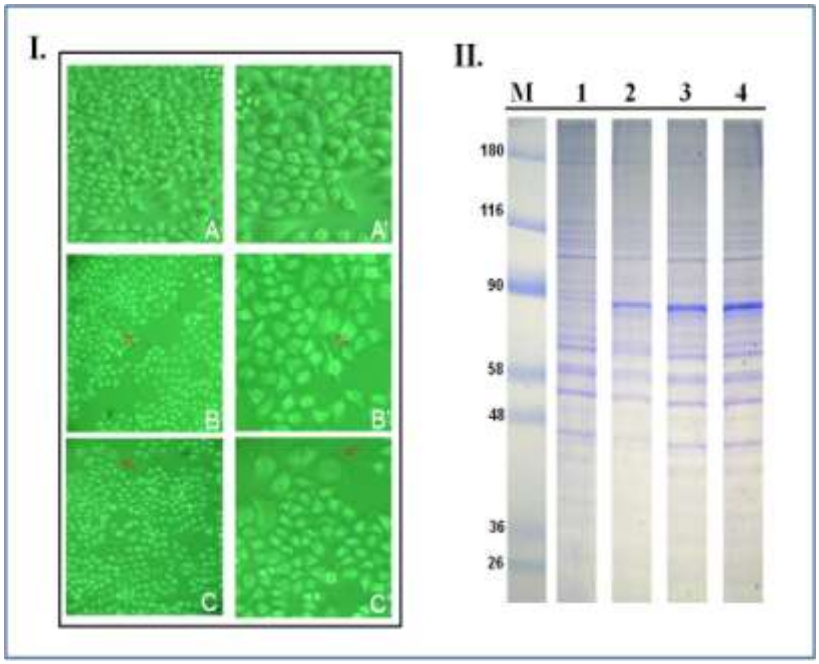

Figure 3. Morphological analysis (I) and protein profile (II) of the HeLa cells expose to ionizing radiation. Panel I. A,A'. Control cells, B, B' and C, C'. HeLa cells expose to 6mSv and 60mSv respectively. Panel II. M. Molecular weight marker, Lane 1, control

cells; Lane2, cells expose to 200µM H_2O_2; Lane 3 and 4, cells expose to 6mSv and 60mSv respectively.

Regarding organelles such as citoskeleton, there are alteration in the arrangement of actin microfilaments and detachment in junctions of cells exposed to ROS and IR (Fig. 4B, C and D).

The heterochromatin nuclei, was observed homogeneously distributed and some cells in division process. Particularly, genomic DNA shown the integrity in all conditions (Fig. 4II).

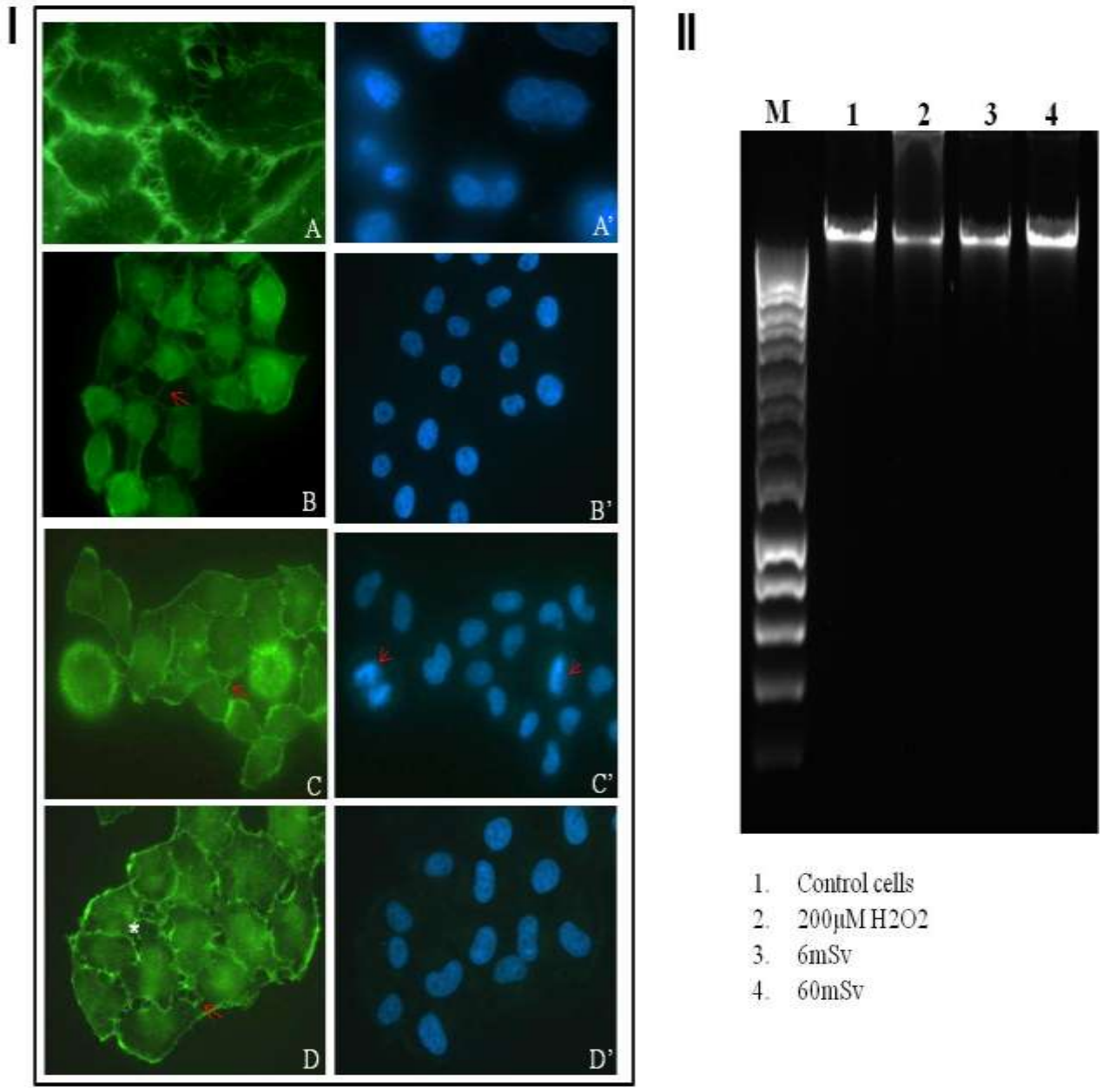

Figure 4. Structural of cytoskeleton, nuclei and genomic DNA integrity.

Panel I. Cytoskeleton control cells (A), cells expose to: 200µM H_2O_2 (B), ionizing radiation 6 and 60mSv (C and D respectively). Note alterate fibrilar actin cystoskeleton (B-D), detachment junctions cells (arrow) and cellular division (arrow head). Panel II. Note the DNA integrity in all the samples (Lanes 1-4).

4.- DISCUSSION

Exposures to IR of different dose rates either naturally or occupationally are inevitable scenarios in human life. The mechanisms of damage in biological systems are of relevance for radiation protection as well as for the clinical applications of radiation in medicine.

In response to IR, the cell are potentially involved in cell ageing as well as in preconditioning cells to neoplastic transformation (Hanahan and Weinberg, 2011; Manesh et al. 2015).

At cellular level, hydroxyl radicals generated in the mitochondria by ionizing radiation either directly by radiolysis of water, or indirectly by the formation of ROS (Reactive Species Oxygen), induce a oxidative stress (Riley, 1994). In this work, the metabolic activity was reduced at 41% in conditions oxidative stress (200µM H_2O_2), however, the cell expose IR presented a 86% of mitochondrial metabolic activity at 60mSv. Furthermore, when the concentration of intracellular free radicals is high, DNA double strand breaks (DSB) is produced by interaction of radiation with DNA or by radiation induced free radicals resulting from radiolysis of cellular water (Pomerantseva et al., 1984). However, it is generally believed that the initial event for radioadaptive response (Manesh et al. 2015), involved trigger activation of DNA repair genes leading to repair of DNA damage, such proteins involved in DNA repair as DNA-PK, ATM, TP53and poly (ADP-ribose) polymerase (PARP) have been proposed to participate in adaptive response. The change in chromatin conformation could protect DNA against indirect effects of challenging dose in two ways; either through folding of the chromatin into a higher ordered structure that makes it less accessible to damaging factors or by increasing the accessibility of damaged sites to repair enzymes (Manesh et al., 2014).

In according to our results, the chromatin of nuclei and genomic DNA was observed the integrity. Although, organelles such as cytoskeleton that controls essential cellular functions as proliferation, adhesion, motility, etc. exhibiting despolymerized stress actin fibers, detachment of cell junctions by exposed IR. The effects of radiation on stress fiber formation are somewhat poorly understood in tumor cells. Hence, we characterized these effects with immunofluorescence experiments by labeling F-actin in HeLa cells indicating damage cellular.

The pathways action IR on the macromolecules, should be explored in future studies to reach a better understanding of the functional and biological connections between the resistance to radiation in human models of cancer cells and normal cells.

5.- CONCLUSIONS

The transformed cells show a high resistance to ionizing radiation, the results indicate that HeLa cells can be exposed to a higher dose of ionizing radiation to induce cellular damage or death. Furthermore, is possible the reversibility of phenomena in according to the mitochondrial metabolic activity.

Our investigation, reflects the molecular mechanisms of radiation toxicity and it provides the basis for the improvement of clinical radiation in cancer treatment.

Acknowledgments

The authors acknowledge financial support from Universidad de Guanajuato -DAIP- to MSA.

REFERENCES

Azorín Nieto J;Azorín Vega JC. *PROTECCIÓN RADIOLÓGICA*. México, Universidad Autónoma Metropolitana (First edition, 2010).

Hanahan D; Weinberg RA. (2011). *Hallmarks of Cancer: The Next Generation*. Cell **144**:649-674

Laemmli UK. (1970). *Cleavage of structural proteins during the assembly of the head of bacteriophage T4.* Nature **227**: 680–685

Manesh SS.; Deperas-Kaminska M; Fotouhi A; Sangsuwan T; Harms-Ringdahl M; Wojcik A; Haghdoost S. (2014). *Mutations and chromosomalaberrations in hMTH1-transfected and non-transfected TK6 cells afterexposure to low dose rates of gamma radiation*. Radiation Environ Biophys **53**: 417–425.

Manesh SS; Sangsuwan T; Wojcik A; Haghdoost S. (2015) *Studies of adaptive response and mutation induction in MCF-10A cells following exposure to chronic or acute ionizing radiation.* Mutat Res 780:55-59

Panganiban RAM.; Snow AL; Day RM. (2013) *Mechanisms of Radiation Toxicity in Transformed and Non-Transformed Cells.* Int. J. Mol. Sci. **14**: 15931-15958.

Pomerantseva MD.; Goloshchapov PV; Vilkina GA; Shevchenko VA. (1984). *Genetic effect of chronic exposure of male mice to gamma-rays*. Mutation Research **141**:195–200.

Riley PA. (1994). *Free radicals in biology: oxidative stress and the effects of ionizing radiation.* Int J Radiat Biol. **65**:27-33.

Soto Arredondo KJ. Flores Villavicencio LL, Serrano Luna JJ, Shibayama M, Sabanero López M. (2014) *Biochemical and cellular mechanisms regulating Acanthamoeba castellanii adherence to host cells* J. Parasitology **141**: 531–541.