

# Cellular Response to Low Gamma-ray Doses

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## Abstract

Lymphocytes, obtained from healthy donors, were exposed to a low strength gamma-ray field to determine heat shock protein expression in function of radiation dose. Protein identification was carried out using mAb raised against Hsp70 and Hsc70. Hsp70 protein was detected after lymphocyte irradiation. In all cases, an increasing trend of relative amounts of Hsp70 in function to irradiation time was observed. After 1.25 cGy gamma-ray dose, lymphocytes expressed Hsp70 protein, indicating a threshold response to gamma rays.

## 1.- INTRODUCTION

One of the Radiation Biology tasks is to develop tests to indicate exposure to natural or artificial, ionizing radiation fields. Some of these tests use biological indicators (biodosimeters), which correlate the exposure levels with the biological effect. Molecular chaperons, including the heat-shock proteins (Hsp) are a ubiquitous feature of eukaryotic and prokaryotic cells.<sup>[1]</sup> Hsp are diverse in size and oligomeric composition, yet several have a common functional theme-modulating the folding and unfolding of other proteins and facilitating assembly and disassembly of multisubunit complexes.<sup>[2]</sup>

Hsp are a family of proteins expressed in response to a wide range of biotic and abiotic stressors to protect the cell from the damage produced by the stress.<sup>[3]</sup> Due to their extraordinary high degree of identity at the amino acid sequence level and because this cellular stress response has been described in nearly all organism studied, make this group of proteins unique.<sup>[1]</sup>

Anoxia, heat, ethanol, hydrogen peroxide, heavy metals, arsenicals, UV radiation, low frequency electromagnetic fields and intensive gamma-ray radiation fields are known as cell stressors that promote Hsp expression.<sup>[4-7]</sup> This has led to the recognition that Hsp protect cells from many forms of stress through chaperoning effects on proteins.<sup>[8]</sup> Low doses of ionizing radiation, or chemical agents, could induce mechanisms whereby cells become better fit to cope with subsequent exposures to high doses.<sup>[6]</sup> It has been shown that Hsp70 plays a central role in stress preconditioning, where Hsp induction correlates with protection from subsequent injury.<sup>[9]</sup>

In most organisms, Hsp70 are among prominent proteins induced by heat.<sup>[10]</sup> There is a close correlation between the induction of these proteins and the induction of tolerance to high temperatures.<sup>[2, 11]</sup> Species thresholds for Hsp expression are correlated with levels of stress that they naturally undergo. Due to its responsiveness to diverse forms of stress, the heat-shock response has undergone widespread applications in biomonitoring and environmental toxicology.<sup>[7]</sup>

Cells of radiosensitive mammalian cell lines usually have a mean lethal dose of about 0.5 Gy, and the majority of them have a defined DNA repair defect. However, defects in DNA repair enzymes alone are not sufficient to explain the range of cellular responses to ionizing radiation. There are normal cells that are very radiosensitive, such as some peripheral body lymphocytes.<sup>[5]</sup>

Ionizing radiation produces denatured proteins via direct ionization as well as by reaction with radiolysis products, enhancing Hsp 70 synthesis in Chinese hamster ovary cells exposed to 400 or 1000 Gy.<sup>[12]</sup> However there is an interest to determine cell response at low doses of gamma rays,<sup>[13]</sup> and to search for a cellular indicator of ionizing radiation exposure.<sup>[15, 16]</sup>

The aim of this research was twofold: To determine Hsp level expression in human lymphocytes irradiated by low strength gamma-ray field, and to observe if there was a threshold response. Lymphocytes were collected from healthy donors blood samples. Lymphocytes were irradiated at seven irradiation doses to determine the Hsp expression. After this step we design another radiation treatment to found a threshold dose. Protein identification was carried out using mAb raised against Hsp70/Hsc70. We found that Hsp70 was expressed by three sets of lymphocytes, after radiation treatment.

An increasing trend of relative amounts of Hsp70 protein in function to irradiation doses was observed. In the second treatment doses the lymphocytes enhanced Hsp70 expression close than 1.25 cGy and upper doses, indicating a threshold doses.

## 2.- MATERIALS AND METHODS

Blood samples from four healthy male individuals were used to isolate their lymphocytes. The individuals have similar life style standards, the average age is 30 years old. All individuals do not smoke.

From each subject, blood samples were taken in heparinized tubes. Lymphocytes were isolated using gradient of Ficoll-Histopaque (Sigma Chemical Co, St Louis MO, USA, 1077-1), as described by Boyum.<sup>[16]</sup> From each subject, lymphocytes were cultured in DMEM medium Methionine and Cysteine free (Gibco BRL, Grand Island NY, USA), supplemented with 100 U/ml of penicillin, 100 ng/ml of streptomycin (In Vitro, Mexico city, Mexico), 0.08 U/ml of insulin (Eli Lilly Mexico city, Mexico), and 5% fetal bovine serum (Gibco BRL, Grand Island, NY, USA, 16000-044).

Following harvesting and assessment of number and viability using Trypan blue (Sigma Chemical Co, St Louis MO, USA, T-6146). From each subject, lymphocytes were divided in nine aliquots. One aliquot was taken as control to determine the basal levels of Hsp70 (37 °C, 95% air, 5% CO<sub>2</sub>), another aliquot were heat shocked (41 °C, 30 min) in circulating water bath and incubate (37 °C, 240 min, 95% air, 5% CO<sub>2</sub>), and protein labeled with a mixture of 20 µCi/ml <sup>35</sup>S-methionine <sup>35</sup>S-Cysteine per ml medium (Amersham Laboratories, Buckinghamshire, England, UB2688). Then were exposed to gamma radiation field produced by a 30 mCi <sup>137</sup>Cs gamma ray source (DuPont Radiopharmaceutical Div. N. Billerica, MA, USA). At the irradiation site the source and background radiation produce a dose rate in air of 3.04 cGy·h<sup>-1</sup>. The total doses exposure was 0.05, 0.5, 1.25, 2.5, 3.75 and 5.0 cGy, respectively. After each irradiation treatment, lymphocyte viability was measured. All samples were incubated at 37 °C, 95% air, 5% CO<sub>2</sub>.

After irradiation, lymphocytes from all aliquot were centrifuged at 10,000 rpm for 10 sec at 4 °C, and washed 2 X PBS (pH 7.2) (Gibco BRL, Grand Island NY, USA, 21300-058). Lymphocytes were homogenized with lysis buffer containing 10 µl AEBSF (Alexis Biochemicals, USA) and 1 ml PBSTDS, incubate 4 °C/10 min, centrifuge (10,000 x g, 4 °C, 10 min), lysate (1 ml) was immunoprecipitated add 10 µl AEBSF (stock 100 mM) and 10 µl Hsp72/73 (AB-1, Calbiochem) to each tube sample, rotate to - 4 °C overnight. Add 100 µl Protein G Plus/Protein A Agarose, incubate 2 hs, wash with PBSTDS (2 X) and centrifuge (10,000 x g, 4 °C, 10 min). The pellets were resuspended in sample buffer (0.1 M TRIS, SDS 4%, β-Mercaptoethanol 10% and 2 µl Bromophenol blue 0.1%), heating for 5 min. Samples was resolved by SDS polyacrylamide gel electrophoresis (SDS-PAGE) (12%).<sup>[18]</sup>

The gels was fixed in Methanol 45 % and acetic acid 35%; enhanced by ENHANCE (Perkin Elmer) and rinsed by distilled water (each step by 30 min). The gel was dry and exposed to a radiographic film BioMax MR (Eastman Kodak Co, Rochester, NY, USA, 8736936) -70 °C for

48 hours. Controls autoradiographies were read using optic densitometry (Eagle Eye II (Stratagene), Mitsubishi) to determine the amount of newly synthesized Hsp70 protein.

### 3.- RESULTS

Before and after lymphocytes irradiation, viability was larger than 90%, this implies no cell death due gamma rays was observed. The expression of Hsp70 in lymphocytes was analyzed using <sup>35</sup>S-methionine as label and irradiated by a low strength gamma-ray field at 37 °C.

In Figure 1 the protein expression in function of irradiation dose are shown for two different experiments. Here, lanes (1) and (2) are the positive controls used and lane (3) is the molecular weight indicator. From lane (4) to (8) are the protein expression in function of radiation doses.

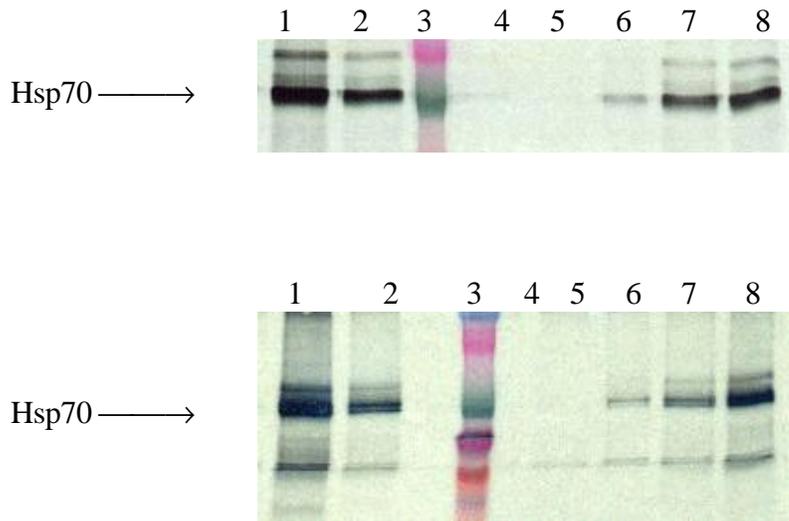


Figure 1.- Expression of Hsp70 in lymphocytes irradiated with a low strength gamma-ray field. Lane (1) is the positive control, obtained by exposing lymphocytes at 10 cGy, lane (2) is the positive control obtained using heat as stressor, and lane (3) is the molecular weight indicator. From lane (4) to (8) are the protein expression for different doses: lane (4) is 0.5 cGy, lane (5) is 1.25 cGy, lane (6) is 2.50 cGy, lane (7) is 3.75 cGy and lane (8) is 5. cGy.

The autoradiographies were evaluated by densitometry to determine the amount of newly synthesized Hsp70 in lymphocytes exposed to low strength gamma-ray field. For each irradiation dose, the ratios of optical densitometry readings were calculated to determine the new synthesized Hsp70 protein.

Figure 2.- Newly Hsp70 expression in lymphocytes in function of irradiation doses, and tendency model.

#### **4.- DISCUSSION AND CONCLUSIONS**

The aim of this research was twofold: To determine Hsp expression in human lymphocytes

irradiated by low strength gamma-ray field, and to observe in there is a threshold dose required to produce the Hsp expression.

When human lymphocytes are irradiated with low strength gamma ray field Hsp70 protein expression is enhanced. This response has been observed in other types of cells exposed to more intensive gamma-ray radiation fields,<sup>[7]</sup> and other stressors, as heat and hydrogen peroxide,<sup>[7]</sup> that leads to the rapid and transient activation of genes encoding heat shock proteins.<sup>[20]</sup>

Low intensity gamma-ray radiation field stress human lymphocytes, the probable explanation is the following: During gamma ray interaction with water, free radicals such as hydrated electron ( $e_{ag}^-$ ),  $OH^\bullet$  and  $H^\bullet$  are produced. Hydroxyl radical is the most effective for producing protein damage, contributing to protein denaturalization, which has been pointed out as the trigger of cell stress.<sup>[21]</sup>

Lymphocytes response is only through Hsp70 expression, we found no evidence of expression of another Hsp when human lymphocytes were irradiated in a low strength gamma ray field. The released Hsp70 protein binds to aberrant polypeptides/proteins, due to the central role that it plays in the folding and unfolding of other proteins.<sup>[2, 22]</sup> All sets of lymphocytes shown approximately the same relative level of Hsp70 expression for 2.5 and upper gamma-ray dose treatments, differences could be attributed to individual response. After 2.5 cGy gamma dose, the relative amount of Hsp70 protein depends upon gamma rays dose. This, makes the Hsp70 protein quantification a possible biodosimeter, sensitive to low intensity gamma-ray radiation field, in the same sense as it is used in biomonitoring and environmental toxicology.<sup>[23]</sup> However, further and extensive investigations are required before Hsp70 expression is used as biodosimeter. In search for ionizing radiation biodosimeter Benderitter *et al.*<sup>[15]</sup> investigated structural membrane modifications in lymphocytes and erythrocytes and Doltchinkonva *et al.*<sup>[14]</sup> looked for alterations on electrokinetic properties of purple membranes on *Halobacterium halobium* cells.

The low doses could be generate certain degree of tolerance to gamma rays (gammatolerance) in comparison with medium and high doses, this could be the probable explanation to differences on Hsp70 expression observed in lymphocytes after 2.5 cGy gamma dose. This is in agreement with similar situations found in other types of cells and stressors.<sup>[2, 7, 9, 11, 24-27]</sup> Lymphocytes behavior indicates a gamma-ray threshold before human lymphocytes are stressed with ionizing radiation.

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