Cytosolic Hsp70/Hsc70 Protein Expression in Lymphocytes Exposed to Low Gamma-Ray Dose

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Abstract

The purpose of this study was to evaluate the effect of low gamma ray intensity upon Hsp70 expression in human lymphocytes. The heat shock proteins (Hsp) family, are a group of proteins present in all living organism, therefore there are highly conserved and are related to adaptation and evolution. At cellular level these proteins acts as chaperones correcting denatured proteins. When a stress agent, such heavy metals, UV, heat, etc. is affecting a cell a response to this aggression is triggered only through over expression of Hsp. Several studies has been carried out in which the cellular effect are observed, mostly of these studies uses large doses, but very few studies are related with low doses. Blood of healthy volunteers was obtained and the lymphocytes were isolated by ficoll-histopaque gradient. Experimental lots were irradiated in a ^{137}Cs gamma-ray. Hsp70 expression was found since 0.5 cGy, indicating a threshold to very low doses of 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 (biodosimeter), 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 [1]. Hsp are diverse in size and oligomeric composition, yet several have a common functional theme-modulating the folding and unfolding of other proteins and faciliting assembly and disassembly of multisubunit complexes [2].

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 [3]. 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 [1].

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 [4-7]. This has led to the recognition that Hsp protect cells from many forms of stress through chaperoning effects on proteins [8]. Low doses of ionizing radiation, or chemical agents, could induce mechanisms whereby cells become better fit to cope with subsequent exposures to high doses [6]. It has been shown that Hsp70 plays a central role in stress preconditioning, where Hsp induction correlates with protection from subsequent injury [9].

In most organisms, Hsp70 are among prominent proteins induced by heat [10]. There is a close correlation between the induction of these proteins and the induction of tolerance to high temperatures [2, 11]. 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 [7].

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 [5].

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 [12]. However there is an interest to determine cell response at low doses of gamma rays [13], and to search for a cellular indicator of ionizing radiation exposure [15, 16].

The aim of this research was twofold: To determine cytosolic Hsp70/Hsc70 level expression in human lymphocytes irradiated by low strength gamma-ray field, and to observe if there was a threshold response.

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.[16] From each subject, lymphocytes were cultured in DMEM medium Methyonine 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 ^{o}C, 95% air, 5% CO_2), another aliquot were heat shocked (41 ^{o}C, 30 min) in circulating water bath and incubate (37 ^{o}C, 240 min, 95% air, 5% CO_2), and protein labeled with a mixture of 20 µCi/ml ^{35}S-methionine ^{35}S-Cysteine per ml medium (Amersham Laboratories, Buckinghamshire, England, UB2688). When were exposed to gamma radiation field produced by a 30 mCi ^{137}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^{-1}. The total doses exposure was 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 ^{o}C, 95% air, 5% CO_2.

After irradiation, lymphocytes from all aliquot were centrifuged at 10,000 rpm for 10 sec at 4 ^{o}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 ^{o}C/10 min, centrifuge (10,000 x g, 4 ^{o}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 ^{o}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 ^{o}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%) [18].

The gels was fixed in Methanol 45 % and acetic acid 35%; enhanced by ENH^{3}ANCE (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 ^{o}C for 48 hours. Autoradiography was read using optic densitometry (Eagle Eye II (Stratagene), Mitsubishi) to determine the amount of newly synthesized cytosolic Hsp70/Hsc 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 ^{35}S-methionine as label and irradiated by a low strength gamma-ray field at 37 ^{o}C.
In Figure 1 the protein expression in function of irradiation dose are shown for three 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.

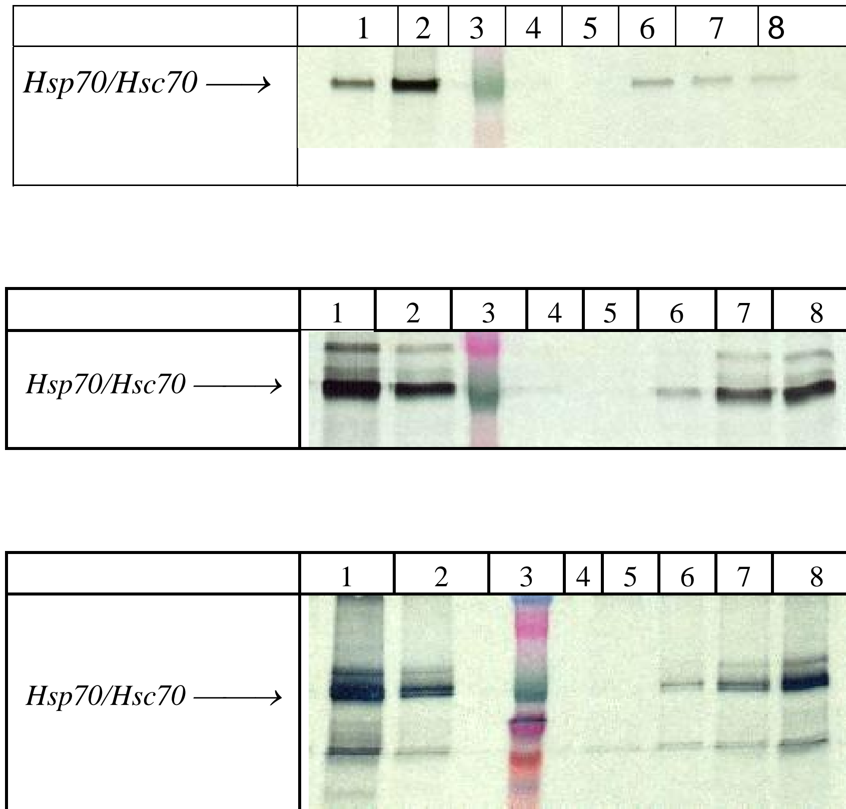

Figure 1. Expression of cytosolic Hsp70/Hsc70 in lymphocytes irradiated with a low strength gamma-ray field. Lane (1) is the positive control, obtained by exposing lymphocytes at 10 cGy, lane (2) is the positive control obtained using heat as stressor, and lane (3) is the molecular weight indicator. 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 5.0 cGy.

Autoradiographies were evaluated by densitometry to determine the amount of newly synthesized Cytosolic Hsp70/Hsc70 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.

4. DISCUSSION AND CONCLUSIONS

The aim of this research was twofold: To determine cytosolic Hsp70/Hsc70 expression in human lymphocytes irradiated by low strength gamma-ray field, and to observe in there is a threshold dose required to produce the Hsp70 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, and other stressors, as heat and hydrogen peroxide [7], that leads to the rapid and transient activation of genes encoding heat shock proteins [20].

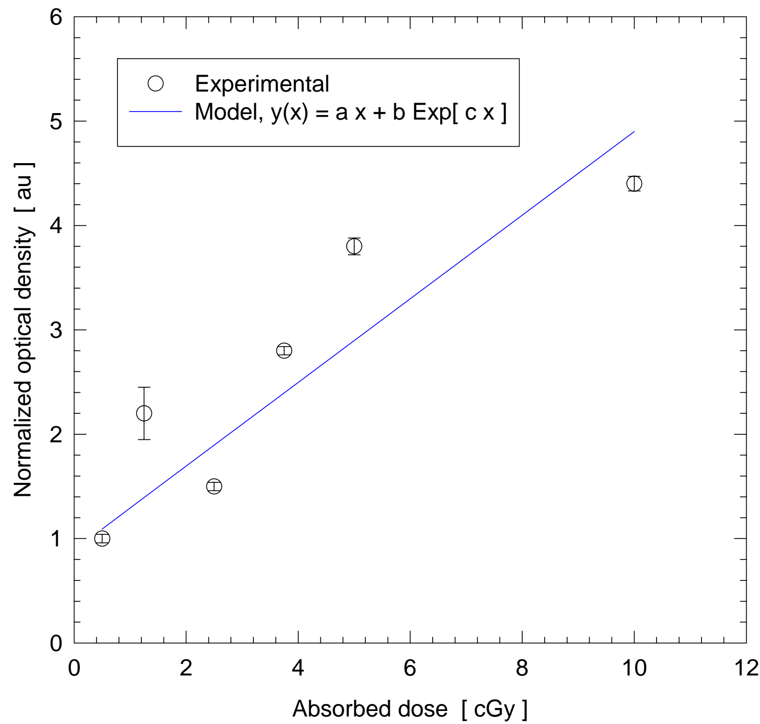

Figure 2. Newly Hsp70 expression in human lymphocytes in Function of radiation doses and tendency model.

Lymphocytes response was evaluated only through Hsp70 expression were irradiated in a low strength gamma ray field. The released Hsp70 protein bounds to aberrant polypeptides/proteins, due to the central role that it plays in the folding and unfolding of other proteins [2, 22]. All groups 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 [23]. However, further and extensive investigations are required before Hsp70 expression is used as biodosimeter. In search for ionizing radiation biodosimeter, Benderitter *et al*. [15] investigated structural membrane modifications in lymphocytes and erythrocytes and Doltchinkonva *et al*. [14] looked for alterations on electrokinetic's properties of purple membranes on *Halobacterium halobium* cells.

The low doses could be generate certain degree of tolerance to gamma rays (gamma tolerance) 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 [24-27]. Lymphocytes behavior indicates a gamma-ray threshold before human lymphocytes are stressed with ionizing radiation.

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