

Modulating Heat Shock Proteins 70 and 90 Expression by Low Power Laser Irradiation (635nm and 780nm) in Jurkat E6.1 T-lymphocyte Leukemia Cell Line

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Abstract:

Introduction: Heat shock proteins (HSPs) are molecular chaperones involved in protein folding, stability and turnover, and due to their role in cancer progression, the effect of low power laser irradiation (LPLI) on the expression of HSP70 and HSP90 in Jurkat E6.1 T-lymphocyte leukemia (JETL) cell line was investigated in vitro.

Methods: JETL cells were irradiated with LPLI at 635nm and 780nm wavelengths (energy density 9.174 J/cm²), and assessed for the expression of HSP70 and HSP90 by flow cytometry after 24, 48 and 72 incubation time periods (ITPs).

Results: At 24 hours ITP post-irradiation, control cultures showed that 10.7% of cells expressed HSP70, while LPLI cultures at 635nm and 780nm manifested a higher expression (32.1 and 21.3%, respectively), and the difference was significant ($P \leq 0.05$). However, at 48 hours ITP, the three means were decreased but approximated (5.6, 4.9 and 6.2%, respectively), while at 72 hours ITP, they were markedly increased (45.2, 76.5 and 66.7%, respectively). In contrast, HSP90 responded differently to LPLI. At 24 hours ITP, control cultures and 780nm cultures showed a similar expression (55.9 and 55.9%, respectively), but both means were significantly higher than that of 635nm cultures (24.0%). No such difference was observed at 48 hours ITP, and at 72 hours ITP, control cultures and 635nm cultures shared approximated means (31.7 and 35.6%, respectively); but both means were significantly higher than the observed mean in 780nm cultures (15.2%).

Conclusion: The results highlighted that HSP70 and HSP90 expression responded differently to LPLI in JETL cells; an observation that may pave the way for further investigations in malignant cells.

Keywords: laser Irradiation, low-Power; Jurkat cell; leukemia cell; Heat shock proteins.

Please cite this article as follows:

Ad'hiah AH, Al-Ameri LMH, Maki AM, Wang Q, ALQaisi MH. Modulating Heat Shock Proteins 70 and 90 Expression by Low Power Laser Irradiation (635nm and 780nm) in Jurkat E6.1 T-lymphocyte Leukemia Cell Line. *J Lasers Med Sci* 2015;6(1):17-21

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Introduction

Heat shock proteins (HSPs) are molecular chaperones involved in protein folding, stability and turnover. Many of their client proteins play critical roles in signal transduction and cell cycle progression, and their function

as protein chaperones aids cells to recover from thermal-, radio-, or chemical-induced injuries¹. In cancer, HSPs have been found to be over-expressed in a wide range of solid tumors and hematological malignancies. This may be an adaptive response by cancer cells to maintain protein homeostasis and promote cell survival in an

unfavorable environment, as well as to stimulate cell proliferation and inhibit cell death ².

Chaperones, such as HSP70 and HSP90, are known to be highly expressed in most tumor cells, and also acts as a biochemical buffer for genetic lesions found in cancer, allowing mutated proteins to perform their malignant functions while conferring cellular tolerance to the imbalanced signalling produced by these oncoproteins ³. In this regard, HSPs have been found to participate in the six essential alterations in cell physiology to define cancerous cellular growth (self-sufficiency in generating growth signals in cancer cells, insensitivity to anti-proliferative signals, avoidance of apoptosis, unlimited replicative capacity, angiogenesis and invasive and metastatic capability) ⁴. Given the many roles that HSPs play in tumorigenesis and cancer progression, these molecules are potentially ideal therapeutic targets for cancer treatment, and in this context, numerous reports have shown that inhibition of HSPs (for instance HSP70 and HSP90) may enhance radio-sensitivity of tumors or sensitizes them to other chemotherapeutic agents ^{5,6}.

Although HSPs have been a subject of recent investigations that targeted anti-cancer agents and their effect on the expression of these thermal proteins, little information has been available to assess the role of low power laser irradiation (LPLI) on the expression of HSPs in malignant cells. However, studies have discussed treatment planning models designed to predict the tissue response to laser irradiation, and Rylander et al. ⁷ have been the first to develop a mathematical treatment planning model to permit prediction of HSP expression in addition to temperature and injury associated with laser heating. Their model was based on measuring thermally induced HSP26, HSP60 and HSP70 expression and injury fraction in prostate cells and tumors of the prostate. The authors concluded that utilization of the treatment planning optimization model can permit more effective tumor destruction by mitigating tumor recurrence and resistance to chemo-therapy. Before that, Beckham et al. ⁸ employed HSP70 transcription to track cellular response to laser-induced injury. A stable cell line (NIH-3T3) was generated containing the firefly luciferase (luc) reporter gene attached to the HSP promoter (murine HSP70). After thermal injury with a pulsed holmium-YAG laser, luciferase was produced on HSP70 activation and emitted broad-spectrum bioluminescence over a range of 500-700nm, with a peak at 563nm. The onset of bioluminescence was seen as early as two hours after treatment and peaked at 8-12 hours depending on the

severity of heat shock. The luminescence was quantified in live cells using bioluminescence imaging, and a minimum pulse energy (65 mJ/pulse) was needed to activate the HSP70 response, while an energy of 103 mJ/pulse was associated with a reduction in HSP70 response and cell death. In a further investigation, O'Connell-Rodwell et al. ⁹ created a transgenic reporter mouse where expression of luciferase gene is controlled by the regulatory region of the inducible 70 kDa HSP, and assessed activation of HSP70 transcription in live animals in response to rapid, high temperature stresses *in vivo* by using a 100-W Carbon Dioxide Laser (CO₂) laser. The HSP70 expression was affected, and the effect was time-dependent post-irradiation. Mackanos and Contag ¹⁰ extended the latter investigation and assessed the pulse duration dependence of the HSP70 expression after irradiation with a CO₂ laser, and found that HSP70 induction varied with changes in laser pulse durations and radiant exposures, which defined the ranges at which thermal activation of HSP70 can be used to protect cells from subsequent stress. In a more recent study, the biological effect of 810nm diode laser on the induction of HSP70 in choroid-retinal endothelial cells was investigated *in vitro*, and the results revealed that such irradiation induced hyper-expression of HSP70, especially at 12 to 18 hours post-irradiation in cultured cells ¹¹. In addition, Sajjadi et al. ¹² determined the spatiotemporal expression patterns of HSPs in order to understand the roles of HSPs in laser-induced tissue damage and repair, and to develop HSPs as tools to illustrate the extent of laser-induced damage and wound healing following irradiation.

The presented literature promoted for investigating the effects of low power laser irradiation (LPLI) (635nm and 780nm) on the expression of HSP70 and HSP90 in cultured Jurkat E6.1 T-lymphocyte leukemia (JELT) cells.

Methods

Cell line

Jurkat Clone E6.1 is a human T lymphoblastoid cell line, which was established from the peripheral blood of a 14-year-old boy with acute T cell leukemia by Schneider and co-workers ¹³. The cell line was purchased from Sigma-Aldrich (UK), and it was maintained at 37°C under humidified air supplemented with 5% CO₂ in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 µg/ml streptomycin, and 100 units/ml penicillin (GIBCO, UK).

Seeding of JETL cells

After assessing viability, the JETL cell suspension (1 ml) was made-up to 15 ml with RPMI-1640 medium and transferred to a tissue culture flask (25 cm²) and incubated at 37°C, 5% CO₂ and 80% relative humidity for three days. After that, the flask contents was mixed gently and transferred to two 10ml centrifuge tubes. The tubes were centrifuged (1200 rpm for 5 minutes) to pellet cells, and first, each cell pellet was suspended in 1 ml of culture medium to assess cell viability, and then, the cell suspension of each tube was made-up to 15 ml with culture medium and transferred to a tissue culture flask (25 cm²) and incubated at 37°C, 5% CO₂ and 80% relative humidity for three days to sub-culture cells. After incubation, the cells were cryopreserved in liquid nitrogen for a later use.

Setting-up cultures for LPLI

The cryotubes were obtained from the liquid nitrogen, and cell suspension was thawed and washed. The cell viability was assessed by a dye-exclusion (trypan blue) test, and cell count was adjusted to 4×10^5 cell/ml with culture medium. The cultures were set-up in 12-well flat-bottomed tissue culture plate (Sigma-Aldrich), and in each well, 2 ml of cell suspension were dispensed. The plate was then incubated overnight, and after incubation, the cells were ready for LPLI. For each treatment, there were hexa-replicates (6 wells).

LPLI of cultures

A continuous wave portable GaAlAs (Gallium, Aluminium, Arsenide) laser (Scientific Ltd., UK) with wavelengths of 635nm and 780nm were utilized for all experiments. Before starting the experiments, the GaAlAs laser equipment was calibrated in a laser power energy monitor (Scientific Ltd., UK). The laser parameters were: spot size, 5 mm; output power, 30 mW; exposure time, 60 seconds; energy density, 9.174 J/cm² and power density of 0.1529 W/cm².

Each well in the tissue culture plate was irradiated with LPLI at a 635nm wavelength. Further plates were similarly irradiated but at a wavelength of 780nm. After irradiation, the plate was incubated (37°C, 5% CO₂ and 80% relative humidity) for 24 hours, and after incubation, the cultured cells were assessed for HSP70 and HSP90 expression. Further plates were incubated for 48 hours and 72 hours; therefore the

laboratory assessments were carried out at the end of three incubation time periods (ITPs). Each type of irradiation and ITP was paralleled by a control culture plate, in which the cells were not exposed to LPLI.

HSP70 and HSP90 Flow cytometry

The method of Dempsey et al.¹⁴ was followed to assess HSP70 and HSP90 expression in cultured JETL cells after LPLI by flow-cytometry. After the end of an ITP, the cells were washed three times of PBS, and their count was adjusted to 1×10^6 /ml. After centrifugation, the cell pellet was gently suspended in 0.1% FBS in PBS (500 µl), followed by further centrifugation, and the cell pellet was suspended 0.1% Triton X-100 (100 µl), and incubated on ice for 15 minutes. By then, the cells were washed and suspended in 50 µl of primary antibody (goat anti-human HSP70 or HSP90; Abcam, UK), and incubated on ice for 30 minutes. After that cells were washed and suspended in 50 µl of fluorescein isothiocyanate (FITC) conjugated secondary antibody (rabbit anti-goat IgG antibody; Abcam, UK), and incubated on ice for further 30 minutes. The cells were washed twice in 0.1% FBS in PBS (500 µl) and centrifuged for 5 minutes at 1700 rpm, and the supernatant was discarded. The cell was gently re-suspended in 0.1% FBS in PBS (300 µl) and filtered into Fluorescence-Activated Cell Sorting (FACS) tubes. The stained cells were analyzed with Becton-Dickinson's FACS Verse™ Flow Cytometer (FACS Calibur™, Becton Dickinson, USA).

Statistical analysis

Data were given as mean ± standard deviation (SD), and differences between means were assessed by ANOVA (Analysis of Variance), followed by Duncan test, in which the probability (P) was considered significant when it was ≤ 0.05. The analyses were carried out using the statistical package SPSS version 13.0.

Results

Heat Shock Protein 70

Flow cytometric analysis of HSP70 expression revealed that cultured cells responded differently to LPLI (Figure 1), and such differences were related to: whether the cells were irradiated or not; ITP and the wavelength of LPLI. At 24 hours ITP post-irradiation, control cultures showed that $10.7 \pm 2.1\%$ of cells expressed HSP70, while

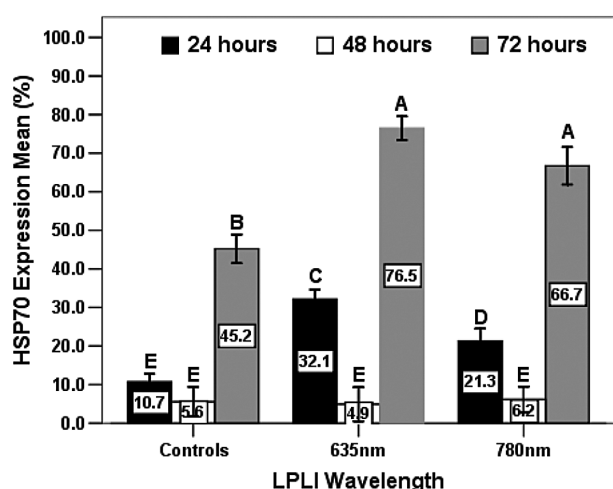


Figure 1. Flow-cytometric analysis of HSP70 expression in cultured Jurkat E6.1 T-lymphocyte cell line after LPLI at 635nm and 780nm wavelengths (energy density 9.174 J/cm²) for 24, 48 and 72 hours incubation time periods. Different upper case letters represent significant difference ($P \leq 0.05$) between means of bars (Duncan test).

LPLI cultures at 635nm and 780nm wavelengths (energy density 9.174 J/cm²) manifested a higher percentage of expression (32.1 ± 2.5 and $21.3 \pm 3.2\%$, respectively), and the difference between the three means was significant ($P \leq 0.05$). However, at 48 hours ITP, the three percentage means were decreased and approximated (5.6 ± 3.9 , 4.9 ± 4.4 and $6.2 \pm 3.3\%$, respectively), but at 72 hours ITP, these means were markedly increased (45.2 ± 3.7 , 76.5 ± 3.1 and $66.7 \pm 4.9\%$, respectively), especially in the irradiated cultures, which showed a significantly ($P \leq 0.05$) higher percentages than control cultures.

Heat Shock Protein 90

In contrast to HSP70, HSP90 in cultured JETL cells responded differently to LPLI (Figure 2). At 24 hours ITP, control cultures and cultures irradiated with the wavelength 780nm showed a similar percentage of expression (55.9 ± 5.2 and $55.9 \pm 10.2\%$, respectively), but both means were significantly higher than the corresponding mean in cultures irradiated with the wavelength 635nm ($24.0 \pm 3.6\%$). However, no such differences were observed at 48 hours ITP, and the three means showed no significant difference between them, although the cultures irradiated with the wavelength 635nm showed a less percentage of expression (56.1 ± 3.4 and 50.4 ± 5.6 vs. $24.0 \pm 3.6\%$, respectively). At 72 hours ITP, control cultures and cultures irradiated with the wavelength 635nm shared means without significant difference between them (31.7 ± 3.8 and $35.6 \pm 5.9\%$, respectively), but both of means were significantly higher

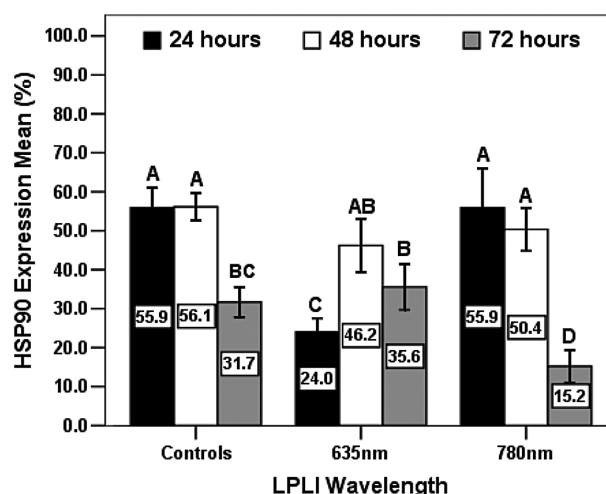


Figure 2. Flow-cytometric analysis of HSP90 expression in cultured Jurkat E6.1 T-lymphocyte cell line after LPLI at 635nm and 780nm wavelengths (energy density 9.174 J/cm²) for 24, 48 and 72 hours incubation time periods. Different upper case letters represent significant difference ($P \leq 0.05$) between means of bars (Duncan test).

than the observed mean in cultures irradiated with the wavelength 780nm ($15.2 \pm 4.2\%$).

Discussion

The two investigated HSPs responded differently to LPLI; HSP70 showed an increased expression, while HSP90 manifested a decreased expression in general, especially at the wavelength 780nm and after 72 hours ITP. It is difficult to explain such findings, especially for HSP90, because for the best knowledge of the investigator, the effect of LPLI on HSP90 expression has not been investigated. For HSP70, the studies almost agree that such HSP is affected positively by laser irradiation, although the obtained results were subjected to the protocols of irradiations employed, which were different^{8,9,10,11,12}.

It has been suggested that the HSP70 response behaves as many other proteins do in that it would be upregulated after an initial stimulus and then decline after the signal from the stimulus had stopped in a classic feedback inhibition manner. The presence of denatured proteins (i.e. a product of thermal stress generated by LPLI) within the cell may act as a stimulus for the transcription of HSP70, which is then recruited to repair the newly denatured proteins⁸. However, such manner might be affected by time post-irradiation as in the present study; the expression of HSP70 was gradually increased and reached the highest level 72 hours post irradiation. In agreement with such findings, Yamasaki et al.¹⁵ demonstrated that one hour post-irradiation with low-energy pulsed CO₂ laser coagulation mode irradiation, the epithelial

keratinocytes facing the laser wound exhibited an over-expression of HSP70 in their nucleus. The connective tissue cells facing the laser wound, which included fibroblasts and capillary endothelial cells, showed de novo expression of HSP70 at three hours post-irradiation, and the level of which peaked at 24 hours. In this study, HSP25 was also determined in addition to HSP70, and there was a temporospatial difference in the expression pattern between HSP70 and HSP25. In the present study, there was also a difference between HSP70 and HSP90 expression in their response to LPLI.

Although the effect of LPLI on HSP90 expression has not been investigated, its low expression after LPLI may be considered important in the control of malignant cells, because HSP90 mediates the maturation and stabilization of various cellular proteins. It influences the activity of many client proteins that function as critical regulators of cellular growth, differentiation, and apoptotic pathways^{16,17}. Client proteins of HSP90 include oncogenic proteins in human malignancies acting via multiple signal transduction pathways: steroid receptors, epidermal growth factor receptor family members, mutant p53, and many other molecules^{18,19}. Accordingly, inhibition of HSP90 can cause the simultaneous degradation of multiple oncogenic proteins and thereby affects signal transduction pathways that are important for cancer cell proliferation and survival; therefore, HSP90 inhibitors have attracted a great deal of attention as promising anticancer drugs¹⁹. These understandings of HSP90 strength and enhance the necessitation to subject such protein for further in vitro and in vivo analyses after LPLI, which may hold a promise in cancer therapy, especially if it is investigated at the molecular level together with other HSPs; for instance HSP27 in addition to HSP70²⁰. However, the mechanisms of HSP70 and HSP90 induction or inhibition following LPLI and the biological action of them are still far from been fully understood, and certainly merit further investigations.

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