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Cell Cycle Response to Low Power Laser Irradiation in Jurkat E6.1 T-lymphocyte Cell Line

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Abstract
Low-power laser irradiation (LPLI) effects on cell cycle progression in Jurkat E6.1 T-lymphocyte leukemia (JETL) cells were examined *in vitro* at 635nm (visible) and 780nm (near infrared) wavelengths. The cells were exposed to an energy density of 9.174 J/cm^2, and then examined 24, 48 and 72 hours post-irradiation. Cell cycle analysis by flow cytometry at 24 hours post-irradiation revealed that the three phases (G0/G1, S and G2/M) of cultured JETL cells showed different percentages in LPLI (635nm and 780nm) and unirradiated cultures, but S phase cells were observed with significant increased percentages (55.6 and 55.7%, respectively) compared to controls (37.3%). At 48 hours, again cells at S phase were observed with much higher percentages than control cells (48.2 and 51.5% *vs*. 29.9%, respectively), and the difference was significant ($P \leq 0.05$). At 72 hours, the S phase cells were also observed with much higher percentages than control cells (33.1 and 32.6% *vs*. 21.3%, respectively), and the difference was also significant ($P \leq 0.05$).
Keywords: Cell cycle, Jurkat E6.1 T-lymphocyte leukemia cell line, Low-power laser irradiation.

1. Introduction
low power laser irradiation (LPLI) was introduced more than 40 years ago to modulate biological processes, especially in the field of medicine, and since then medical treatment with coherent light sources (lasers) or incoherent light (Light Emitting Diodes; LEDs) has made pronounced advancements, and there is no doubt nowadays that low-intensity monochromatic light from lasers or LEDs acts directly on the organism at the cellular level, in which molecular changes have been observed (Karu, 2013). The specificity of cellular responses appear only during secondary reactions (cellular signaling), and interactions between various cell types on tissue level have also been demonstrated. Such interactions are complicated, and we are still far away from full understanding of LPLI action mechanisms on cellular, tissue or organism level (Abrahamse, 2012). However, it is well-known that LPLI can alter metabolic processes in mammalian cells, and can accelerate or inhibit cellular metabolism, depending on wavelength, dose and treatment protocol. *In vitro* studies have demonstrated several effects of LPLI that include changes in growth-factor production, ATP synthesis and phagocytic activity. Applications for this modality have been identified in wound healing and particularly in the treatment of venous, diabetic and pressure ulcers. The use of LPLI has also been extended to the treatment of inflammatory disease processes and to the alleviation of musculoskeletal pain (Doaga *et al*., 2009). Further investigations have also indicated that the photobiological effects of LPLI may involve changes in cell cycle progression, and several investigators have reported a reduction in cell proliferation following LPLI treatment, and furthermore, growing body of experimental and clinical studies have demonstrated that LPLI regulates cell survival, proliferation, and differentiation (Pislea *et al*., 2009; Yip, 2011; Gomes Henriques *et al*., 2014).
Such findings suggest that LPLI may have anti-cancer effects, but there is no direct evidence that can support such suggestion. Therefore the present investigation was planned to assess *in vitro* the effects of LPLI on cell cycle progression of cultured Jurkat E6.1 T-lymphocyte leukemia (JETL) cells.

2. Materials and Methods
2.1 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 in 1977 (Schneider *et al*., 1977). The cell line was purchased from Sigma-Aldrich (UK), and it was maintained at 37°C under humidified air supplemented with 5% CO_2 in RPMI- 1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 µg/ml streptomycin, and 100 units/ml penicillin (GIBCO, UK).
2.2 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 cm^2) 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 cm^2) 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.

2.3 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).

2.4 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 and 12.232 J/cm^2 and power density of 0.1529 W/cm^2. Each well in the tissue culture plate was irradiated with LPLI at a 635nm wavelength that had energy density 9.174 J/cm2. 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 cell cycle phases. 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.

2.5 Analysis of cell cycle phases

The Propidium Iodide Flow Cytometry Kit (Abcam, UK) was used for the analysis of cell cycle phases in cultured JETL cells after LPLI. The kit is designed for quantitative DNA content analysis in tissue culture cell lines using the nucleic acid stain propidium iodide (PI) followed by flow-cytometric analysis. Propidium iodide is a fluorescent molecule that binds nucleic acid with little or no sequence preference. The premise with this dye is that it is stoichiometric; i.e. it binds in proportion to the amount of DNA present in the cell. By this method, the distribution of cells in three major phases of the cell cycle (G0/G1, S and G2/M) was determined. Because PI binds RNA, as well as DNA, RNase A (ribonuclease A) was used to digest cellular RNA and thus decreasing background RNA staining from the experiment. The PI is also membrane impermeant; ethanol was therefore used to fix and permeabilize cells.

2.6 Statistical analysis

Data were given as mean ± standard deviation (SD), and differences between means were assessed by ANOVA (Analysis of Variance), followed by the least significant difference (LSD), 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.

3. Results and Discussion

Twenty-four hours post-LPLI, the three phases (G0/G1, S and G2/M) of cultured JETL cells showed different percentages in LPLI and unirradiated cultures. For G0/G1 phase, control cells were observed with a frequency of 51.6±3.2%, which was significantly ($P \leq 0.05$) higher than the corresponding recorded percentages in LPLI cultures at 635nm and 780nm wavelengths (42.3±3.0 and 43.0±1.5%, respectively), but the latter two percentages showed no significant difference between them ($P > 0.05$). The S phase contradicted the results of G0/G1 phase, and LPLI at 635nm and 780nm wavelengths were associated with significant increased percentages of cells at such phase (55.6±2.0 and 55.7±2.1%, respectively) compared to controls (37.3±1.5%). Such findings had their effects on cells that reached G2/M phase, in which the irradiated cells were observed with percentages of 2.2±1.0 and 1.3±1.1%, respectively, which were significantly lower the recorded percentage (11.1±2.0%) in controls (Figure 1).

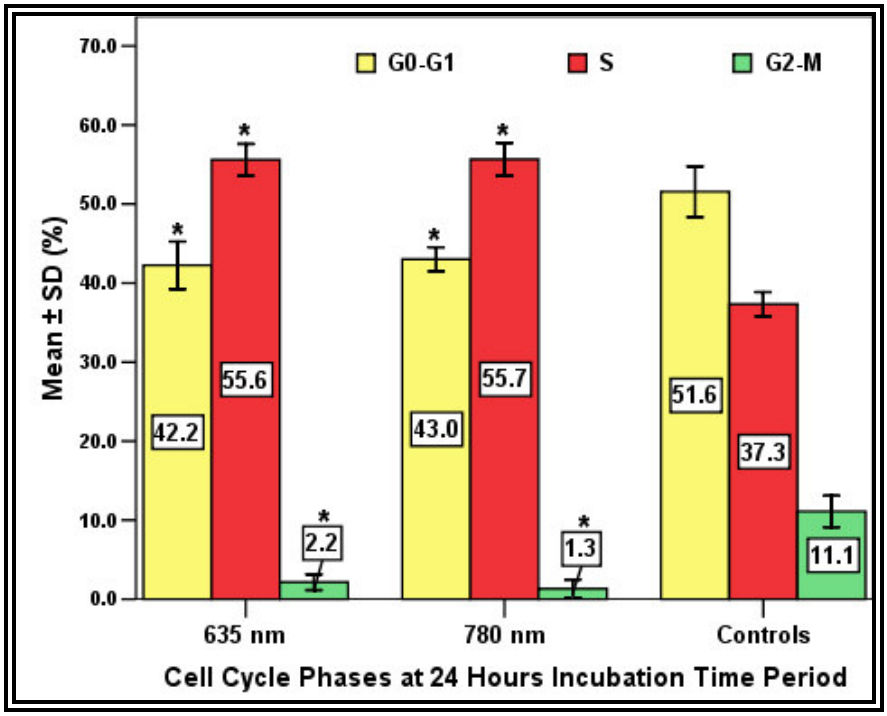

Figure 1. Flow-cytometric analysis for cell cycle progression of cultured Jurkat E6.1 T-lymphocyte cell line after low-power laser irradiation at 635nm and 780nm wavelengths (energy density 9.174 J/cm^2) for 24 hours incubation time period (*Significant difference at *p*-value ≤ 0.05 compared to corresponding control means)

At 48 hours ITP, cells at G0/G1 shared approximated percentages in cultures LPLI at 635nm and 780nm wavelengths (47.4±3.1 and 45.8±3.2%, respectively) or controls (47.2±1.5%), but again cells at S phase were observed with much higher percentages than the corresponding control cells (48.2±1.5 and 51.5±3.0 *vs*. 29.9±0.6%, respectively), and the difference was significant (P ≤ 0.05). However, the percentage of cells at G2/M phase was still significantly lower than the percentage of cells in control cultures (4.3±1.5 and 2.7±0.6 *vs*. 22.9±1.0%, respectively) (Figure 2).

At 72 hours ITP, cells at G0/G1 also shared approximated percentages in cultures LPLI with 635 and 780 nm wavelengths (46.7±2.0 and 51.1±2.5%, respectively) or controls (48.3±1.2%), but again cells at S phase were observed with much higher percentages than the corresponding control cells (33.1±3.1 and 32.6±2.3 *vs*. 21.3±1.7%, respectively), and the difference was significant (P ≤ 0.05). However, cells at G2/M phase recorded much higher percentages than the corresponding observed percentages in the two previous ITPs (24 and 48 hours), but they were still significantly lower than the percentage of cells in control cultures (20.2±1.2 and 16.2±1.0 *vs*. 30.4±1.5%, respectively) (Figure 3).

These results demonstrated that the effects of LPLI were more pronounced in the S phase of cell cycle, at which an accumulation of cells was observed; especially at 24 and 48 hours ITPs (approximately 50.0%), while at 72 hours ITP, the percentage of cells at S phase was significantly lower than the observed corresponding frequencies at 24 and 48 hours ITPs, but it did not reach the value of controls; therefore the arrest was also suggested. The S phase of cell cycle is under the control of the CDK2, which complexes with cyclin A to promote the cell to enter into G2 phases. Mitosis is further regulated by cyclin B in complex with CDK2 (Caillava and Baron-Van Evercooren, 2012). Accordingly, LPLI might have exerted its effects on S phase of cell cycle through CDK2 and cyclin A pathway, and rendering it non-functional in controlling this part of cell cycle. In addition, the CDK2 is also under the control of a family of CDK inhibitors (p21, p27 and p57), which have been demonstrated to have an inhibitory effect on the CDK1-cyclin A complexes (Fuster *et al*., 2009). These inhibitors might have also been up-regulated by LPLI, and leading to arrest cells at S phase. There is no direct evidence that supports the effect of LPLI on the expression of CDKs and cyclins or their inhibitors, but Fukuhara *et al*. (2006) presented evidence that a G2/M arrest was demonstrated in rat osteoblasts *in vitro* after LPLI by the appearance of G2/M arrest marker 14-3-3-sigma or phospho-p53.

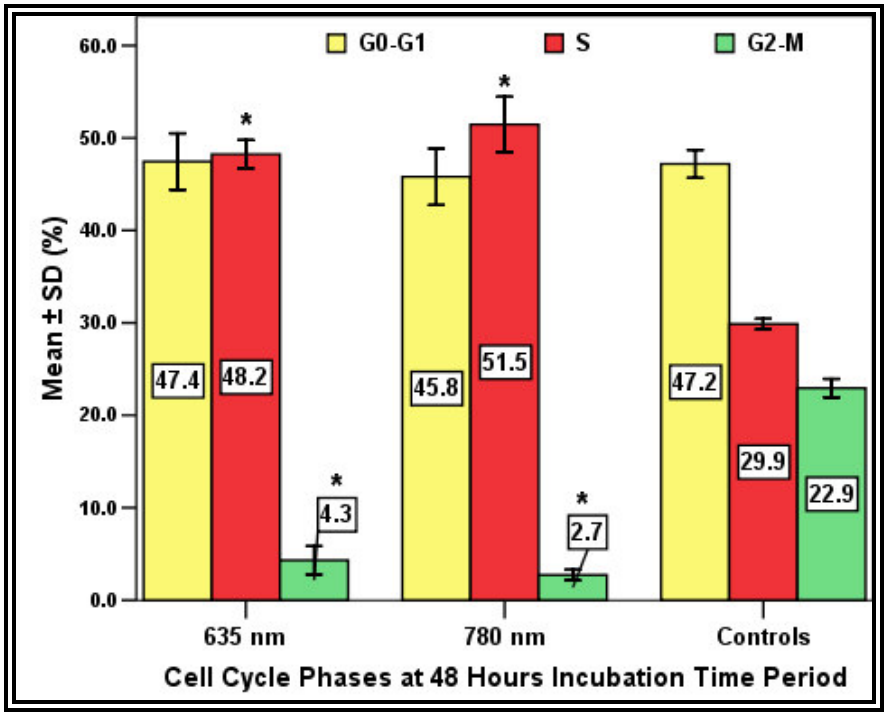

Figure 2. Flow-cytometric analysis for cell cycle progression of cultured Jurkat E6.1 T-lymphocyte cell line after low-power laser irradiation at 635nm and 780nm wavelengths (energy density 9.174 J/cm^2) for 48 hours incubation time period (*Significant difference at p-value ≤ 0.05 compared to corresponding control means)

The effect of LPLI on the cell cycle of dividing cells might be a matter of a controversy, and there have been no consistent observations. Some studies reported that LPLI enhances cell cycle progression (Reviewed and discussed by Gao and Xing, 2009), while others (including the present study) contradicted such theme, and several factors have been suggested to influence the LPLI effects on cell cycle. It has been augmented that each cell line may respond differently to LPLI of different wavelengths and doses and even within the same cell lineage, the feature-dependent response might be subjected to wavelength and dose utilized (Henriques et al., 2010). Moore *et al*. (2005) determined the effect of LPLI wavelength on proliferation of two types of cultured murine cells, and found that fibroblasts proliferated faster than endothelial cells in response to laser irradiation, and maximum cell proliferation occurred with 665nm and 675nm light, whereas 810 nm light was inhibitory to fibroblasts. In addition, Renno *et al*. (2007) investigated the effects of 670nm, 780nm, and 830nm laser irradiation on cell proliferation of normal primary osteoblast (MC3T3) and malignant osteosarcoma (MG63) cell lines *in vitro*. Based on the conditions of the study, the authors concluded that each cell line responded differently to specific wavelength and dose combinations. It has also been demonstrated that low dose near-infrared (830nm) irradiation promoted cell cycle progression, while at higher doses, an increase in percentage of cell subpopulations in G1 and G2 phases, and S-phase blockade with apoptosis promotion were observed in the near-infrared and Far-red (680nm) irradiated samples (Pislea *et al*., 2009).

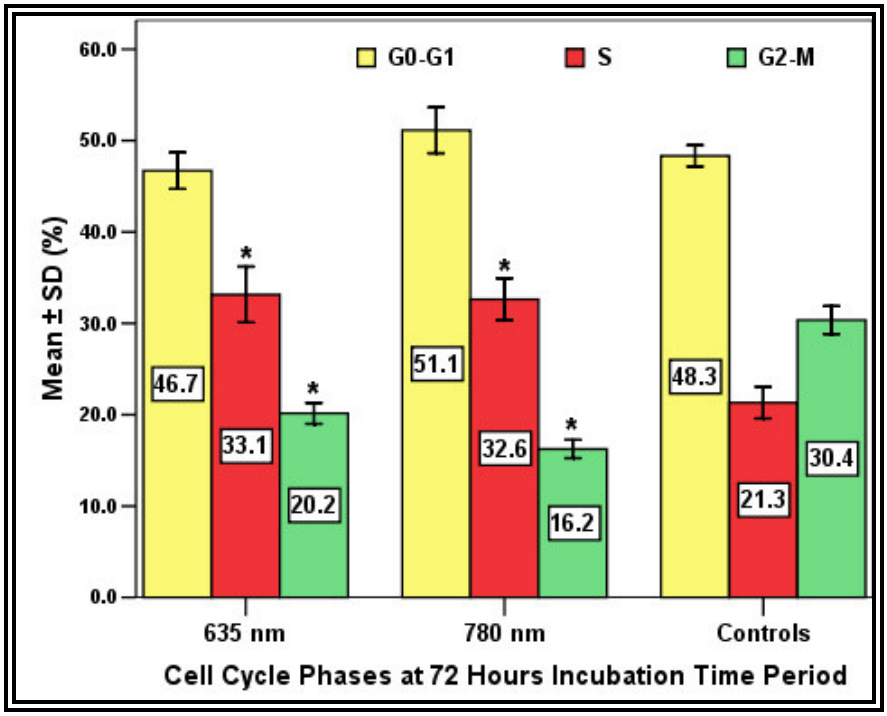

Figure 3. Flow-cytometric analysis for cell cycle progression of cultured Jurkat E6.1 T-lymphocyte cell line after low-power laser irradiation at 635nm and 780nm wavelengths (energy density 9.174 J/cm^2) for 72 hours incubation time period (*Significant difference at p-value ≤ 0.05 compared to corresponding control means)

In wound healing, morphologically wounded cells that were exposed to 5 J/cm^2 LPLI migrated rapidly across the wound margin indicating a stimulatory or positive influence of phototherapy, but higher doses (10 and 16 J/cm^2) were characterized by a decrease in cell viability and cell proliferation with a significant amount of damage to the cell membrane and DNA (Hawkins and Abrahamse, 2006). The same group of investigators extended their work and exposed wounded cells to 5 J/cm^2 using 632.8nm. Their results revealed that changes in cellular parameters (such as ATP viability, cytokine expression, alkaline phosphatase enzyme activity and DNA damage) can be observed directly after the laser irradiation, and the amount of DNA damage and cytotoxicity may be related to duration of the laser irradiation, which is dependent on the power density of each laser (Evans and Abrahamse, 2008).

4. Conclusions

It is concluded that cell cycle progression of JETL cells was affected by LPLI, but one has to remember that other investigators demonstrated that LPLI has neither enhancing nor inhibiting effects on cell cycle progression. In this regard, Frigo et al. (2009) reported that cell cycle analysis in B16F10 melanoma cells irradiated with LPLI at a wavelength of 660nm showed no statistically significant differences in the cell numbers in G0/G1, S, G2/M phases at 24, 48 and 72 hours among irradiated and control groups. Accordingly, LPLI can have different biological effects on cell cycle progression, and such effects are subjected to the type of cell, as well as wavelength and dose.

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