RESEARCH ARTICLE

Effect of diode pumping solid state laser with wavelength 589 nm on gene expression of interlukine-2 and interferon-γ in human T-lymphocytes

Marwa Akram Nafe'a, Nibras Hatim Khamees, Mustafa Salih Ali Almusawi

Abstract

Objective: To evaluate the impact of laser irradiation of human T-cell lymphocyte in culture to determine the photobiomodulatory effects of such irradiation on gene expression, and the release of growth factors and cytokines.

Method: The experimental study was conducted from November 2021 to April 2022 at the Postgraduate Anatomy Laboratory, Mustansiriyah University, Baghdad, Iraq, and comprised human blood samples T-cell lymphocytes were extracted and irradiated, followed by the extraction of ribonucleic acid to assess the effect of laser on gene expression of cytokines, interlukine-2 and interferon-gamma. A 589nm yellow light low-level laser beam from a diode pumping solid state laser was used at doses 30J/cm^2, 50J/cm^2 and 70J/cm^2 for an exposure time of 15 minutes. Reverse transcriptase-polymerase chain reaction was used to measure gene expression. Data was analysed using GraphPad Prism 9.

Result: Cellular gene expression of the mediators changed significantly in response to laser irradiation at a variety of dosage parameters, and these effects depended on the wavelength and radiation exposure ($p<0.05$).

Conclusion: The laser light may photobiomodulate the production of cytokines by human cells in vitro.

Key Words: Interferon, Lasers, Lymphocytes, Gene, Polymerase, Radiation

(JPMA 74: S352 (Supple-8); 2024) **DOI: https://doi.org/10.47391/JPMA-BAGH-16-80**

Introduction

It has been demonstrated that low-energy visible light stimulates cellular processes. Over the past three decades, a technique known as photobiostimulation has been employed to treat a variety of illnesses, including soft tissue injuries, severe wounds, chronic pain and others[1]. Low-level laser therapy (LLLT) is hypothesised to photobiomodulate cell activity through a number of mechanisms, including increased cell proliferation, growth factor response within cells and tissues, and adenosine triphosphate (ATP) and protein synthesis[2].

When an injury is in its inflammatory and reparative phases, cytokines play a crucial role. They are tiny, secreted proteins that have an impact not just on other cells, but also on immune cells. They comprise the interleukins (ILs), lymphokines and other related signalling molecules, such as interferons (IFNs) and tumour necrosis factor-alpha (TNF-α). Studies showed that laser treatment and mitogen stimulation significantly boosted the production of cytokines, such IL-1α, IL-2, IFN-gamma (γ) and TNF-α in cultured blood mononucleated cells. These findings suggest that laser can alter immune processes[3].

Department of Physiology and Medical Physics, Mustansiriyah University. Baghdad, Iraq.

Correspondence: Mustafa Salih Ali Almusawi

Email: dr.mustafa.salih@uomustansiriyah.edu.iq

IFN was formerly known as immune IFN, then type II IFN. Natural killer (NK) cells, T cells and NKT cells are the main providers of IFN, and its receptor is found on practically all cell types. The Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway is activated when IFN binds to its receptor. IFN may trigger both pro- and anti-inflammatory responses, which is important for a balanced immune response. T-cell development is also influenced by IFN signalling. Because of its pro-inflammatory qualities, IFN has been linked to the promotion of autoimmune disorders[4].

IL-2 was first classified as a T-cell growth factor[5]. Additionally, IL-2 transmits vital signals that help regulatory T-cells (T-reg) grow in the thymus and later support their homeostasis and function. Low levels of IL-2 signalling are sufficient for many important characteristics of T-reg cells, but different levels of IL-2 signalling are required for each of these effects on T-effector and T-reg cells[6].

It is self-evident that light must be absorbed by intracellular chromophores in order to interact with a living cell. Endogenous porphyrins, mitochondrial and membranal cytochromes, and flavoproteins were discovered to be suitable candidates in the search for photobiostimulation chromophores[1]. Experimentally, the biological function of certain gene products is frequently determined by stopping their expression in an organism

and monitoring the phenotype that results[7]. The accuracy of gene expression analysis and other ribonucleic acid (RNA)-based downstream applications is dependent on the quality and quantity of RNA. Only a few methodological investigations comparing sample storage and RNA extraction techniques for human cells have been done so far[8]. The underlying issues should have also made it clear that reverse transcriptase-polymerase chain reaction (RT-PCR) assay design and analysis require extreme caution[9].

It has been proposed that LLLT increases cell growth both directly and indirectly through modulating the expression of genes involved in deoxyribonucleic acid (DNA) synthesis and repair, as well as cell metabolism[10].

The current study was planned to evaluate the impact of laser irradiation of human T-cell lymphocyte in culture to determine the photobiomodulatory effects of such irradiation on gene expression, and the release of growth factors and cytokines.

Materials and Methods

The experimental study was conducted from November 2021 to April 2022 at the Postgraduate Anatomy Laboratory, Mustansiriyah University, Baghdad, Iraq. A consecutive nonprobability sampling technique was adopted. Phlebotomy was used to collect 4ml of blood from healthy individuals. The study protocol was approved by the institutional ethics review committee, and all the volunteers furnished informed consent. A portion of each sample was exposed to laser radiation, while the other portion was used as a control. The blood was collected using ethylenediaminetetra acidic (EDTA) acid. A 589nm yellow light LLL beam from a diode pumping solid state (DPSS) laser was used. The laser doses were 30J/cm^2, 50J/cm^2 and 70J/cm^2 and the exposure time was 15 minutes. The laser beam was given to the blood samples in tubes with a 5mm diameter irradiation spot. Each blood sample was divided into two parts; one was the non-irradiated control, and the other was irradiated sample, which had samples irradiated directly before the procedure as whole blood, as well as another subgroup that was irradiated after the cell cultured in plate at the same dose and time of irradiation. Both tube samples were diluted with phosphate buffer saline (PBS) (the same volume of the blood), and then in a new tube with added 3cc of lymphocyte separation media before being centrifuged (ThermoScientific Jouan C4I Benchtop Centrifuge, USA) for 20 minutes at G800. After plasma removal, peripheral mononuclear cells (PMCs) were washed twice with PBS to avoid contamination, and then media containing phytohemagglutinin (PHA) was added to the PMC in order to stimulate T-lymphocyte proliferation. The cells were then cultured in a tissue culture plate having 96 wells with flat bottom.

RNA extraction was done according to manufacturer's protocol (step One RNA extraction kit, Qiagen, USA). Cultured T-lymphocytes were lysed, then cell lysate was collected from the wells and diluted with PBS and centrifuged. After that, buffer solution in specific volume was added, followed by washing and centrifugation for 30 seconds. The mixture was added into minicolumns, followed by ultracentrifugation. Finally, elution tubes and nuclease-free water was used to collect the extracted RNA. RNA concentration and purity were determined using Nanodrop (ThermoFisher Scientific, USA), and the RNA samples were stored at -80^{o}C.

One-step RT quantitative PCR (RT-qPCR) kit (Promega, GmbH, USA) was used for complementary DNA (cDNA) synthesis and PCR amplification, according to the manufacturer's protocol. In a labelled Eppendorf tube, reaction master mix, forward primer, reverse primer, RT mix, magnesium dichloride (MgCl2) and nuclease-free water were added, and then were mixed with the RNA sample in specific concentration. The mix was added into wells of 8-well PCR strips. The strips were covered and loaded into thermal cycler (3G Tower, Analytik Jena GmbH+Co. KG, Germany). The resultant CT (cycle threshold) values were extracted in the form of an Excel sheet for relative quantification analysis using ΔΔCT method.

Data was analysed using GraphPad Prism 9. Two-way analysis of variance (ANOVA) was used to determine statistical significance of genes' relative quantity.

Results

At 30J/cm^2, there was a 14.1-fold increase in IL-2 expression ($p>0.0001$) and a 9.65-fold increase in IFN-γ expression ($p>0.0001$) in case of in plate irradiated T-lymphocytes. On the other hand, T-lymphocytes from irradiated whole blood samples showed 4.05-fold increase in IL-2 expression ($p>0.001$), while there was no statistically significant increase in IFN-γ expression ($p>0.05$). In addition, there was a statistically significant ($p> 0.0001$) difference in IL-2 and IFN-γ expression between in plate irradiated T-lymphocytes and T-lymphocytes from irradiated whole blood samples (Figure 1).

At 50J/cm^2, in plate irradiated T-lymphocytes showed significant ($p>0.0001$) increase in IL-2 and IFN-γ expression at 7.05-fold and 5.6-fold, respectively. On the

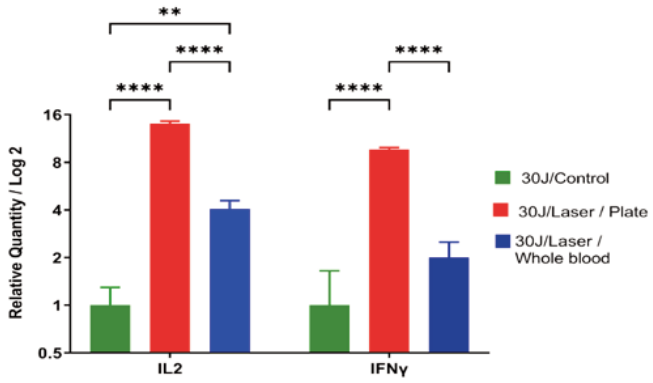

Figure-1: Impact of laser irradiation 30J/cm^2 on interleukin-2 (IL-2) & interferon-gamma (IFN-γ) gene expression. The samples were divided into 2 groups; T-lymphocytes obtained from irradiated whole blood, and in plate irradiated T-lymphocytes. Gene expression of IL-2 and IFN-γ was measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and expressed as Log2 of relative quantity to show fold change. ** = $p < 0.01$, **** = $p < 0.0001$

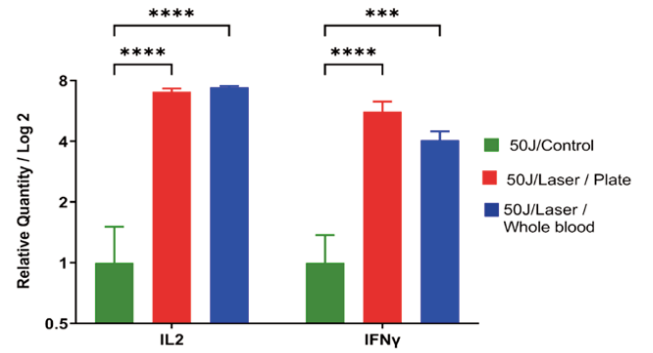

Figure-2: Impact of laser irradiation 50J/cm^2 on interleukin-2 (IL-2) and interferon-gamma (IFN-γ) gene expression. The samples were divided into 2 groups; T-lymphocytes obtained from irradiated whole blood, and in plate irradiated T-lymphocytes. Gene expression of IL-2 and IFN-γ was measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and expressed as Log2 of relative quantity to show fold change. *** = $p < 0.001$, **** = $p < 0.0001$

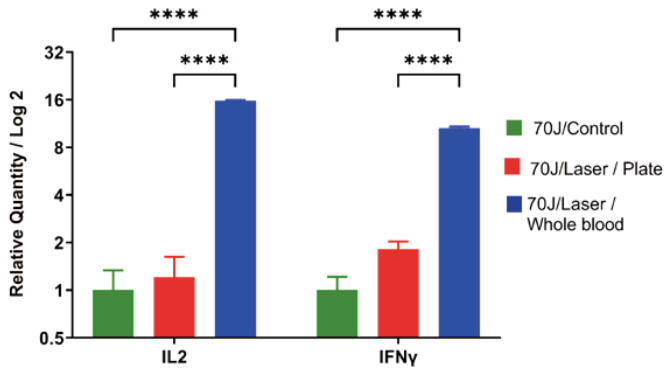

Figure-3: Impact of laser irradiation 70J/cm^2 on interleukin-2 (IL-2) and interferon-gamma (IFN-γ) gene expression. The samples were divided into 2 groups; T-lymphocytes obtained from irradiated whole blood, and in plate irradiated T-lymphocytes. Gene expression of IL-2 and IFN-γ was measured by reverse transcriptase-polymerase chain reaction (RT-PCR) and expressed as Log2 of relative quantity to show fold change. **** = $p < 0.0001$

other hand, T-lymphocytes from irradiated whole blood samples showed 7.42-fold increase in IL-2 expression ($p>0.0001$), and a 4.5-fold increase in IFN-γ expression ($p>0.0001$). There was no significant difference in IL-2 and IFN-γ expression between in plate irradiated T-lymphocytes and T-lymphocytes from irradiated whole blood samples (Figure 2).

At 70J/cm^2 dose, in plate irradiated T-lymphocytes showed no significant difference in the expression of IL-2 and IFN-γ compared to the control samples ($p>0.05$). T-lymphocytes from irradiated whole blood samples showed 15.7-fold increase in IL-2 expression ($p>0.0001$), and a 10.5-fold increase in IFN-γ expression ($p>0.0001$) as compared to the control samples. There was a significant ($p> 0.0001$) difference in IL-2 and IFN-γ expression between in plate irradiated T-lymphocytes and T-lymphocytes from irradiated whole blood samples (Figure 3).

Discussion

The most significant discovery of the current study related to how well the laser therapy could control the release of growth factors and cytokines from lymphocytes, and how this depended on wavelength, energy density and other parameters[11]. The current study found a significant increase in the levels of IL-2 and IFN-γ after exposure to LLLL with a wavelength of 589nm, and there was a discrepancy in the case of exposing cells to the laser in whole blood and when exposed in the case of plate. Thus, it caused an increase in ATP and the regulation of cytokines and gene expression, and this corresponded to what other studies had found[12].

According to a study, laser irradiation causes an increase in mitochondrial membrane potential. LLL-induced cell proliferation and differentiation, as well as an increase in gene expression of cytokines and growth factors, were seen in the cells studied[13].

In the current study, three LLLs were used, 30J\cm^2, 50J\cm^2 and 70J\cm^2 that were applied to whole blood and in plate. There was highly significant increase of IL-2 and IFN-γ in the case of the plate at 30J\cm^2. The energy of 70J\cm^2 showed that the best increase was in the case of whole blood. Some research suggests that the electron transport mechanism of the cell appears to generate more reactive oxygen species (ROS), and in the body, mitochondria are the primary generators of free radicals [14]. According to Wasik et al., visible laser light stimulates the multiplication of B-lymphocytes while having only a small impact on T-lymphocytes. Laser irradiation initiates primary free radical processes

that activate cells, resulting in this effect[15]. Other investigators have found that laser irradiation stimulates B- and T-lymphocyte proliferation triggered by mitogens[16]. These findings encourage researchers to investigate the various mechanisms by which whole blood irradiation affects lymphocytes[17].

Additionally, the current study found the best dose after irradiating whole blood at 70J/cm^2 and the best dose after irradiating cells at 30J/cm^2. Biphasic dose response is also consistent with the findings. For maximum bio-stimulatory effect, it is important to identify the threshold dose, which is the amount of energy required to achieve this effect. When the dose exceeds the threshold by a large margin, bio stimulation is replaced by bio inhibition[18,19].

Limitation: The current study has limitations as the sample size was not calculated which could have affected the power of the study.

Conclusion

There was unequivocal evidence that laser irradiation could control gene expression and the release of growth factors and cytokines from cells in culture. To clarify the putative therapeutic implications of these cellular-level changes, additional clinical research is needed.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Lubart R, Eichler M, Lavi R, Friedman H, Shainberg A. Low-energy laser irradiation promotes cellular redox activity. Photomed Laser Surg 2005;23:3-9. doi: 10.1089/pho.2005.23.3.
2. Peplow PV, Chung TY, Ryan B, Baxter GD. Laser photobiomodulation of gene expression and release of growth factors and cytokines from cells in culture: a review of human and animal studies. Photomed Laser Surg 2011;29:285-304. doi: 10.1089/pho.2010.2846.
3. Cronshaw M, Parker S, Anagnostaki E, Mylona V, Lynch E, Grootveld M. Photobiomodulation Dose Parameters in Dentistry: A Systematic Review and Meta-Analysis. Dent J (Basel) 2020;8:114. doi: 10.3390/dj8040114.
4. Lin FC, Young HA. The talented interferon-gamma. Adv Biosci Biotechnol 2013;4:6-13. doi: 10.4236/abb.2013.47A3002.
5. Törnroos H, Hägerstrand H, Lindqvist C. Culturing the Human Natural Killer Cell Line NK-92 in Interleukin-2 and Interleukin-15 - Implications for Clinical Trials. Anticancer Res 2019;39:107-12. doi: 10.21873/anticanres.13085.
6. Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity 2010;33:153-65. doi: 10.1016/j.immuni.2010.08.004.
7. Al Musawi MS, Al-Gailani BT. In Vitro Biostimulation of Low-Power Diode Pumping Solid State Laser Irradiation on Human Serum Proteins. Photobiomodul Photomed Laser Surg 2020;38:667-72. doi: 10.1089/photob.2020.4873.
8. Rodríguez A, Duyvejonck H, Van Belleghem JD, Gryp T, Van Simaey L, Vermeulen S, et al. Comparison of procedures for RNA-extraction from peripheral blood mononuclear cells. PLoS One 2020;15:e0229423. doi: 10.1371/journal.pone.0229423.
9. Bustin SA, Nolan T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. J Biomol Tech 2004;15:155-66.
10. Guo Y, Qu Q, Chen J, Miao Y, Hu Z. Proposed mechanisms of low-level light therapy in the treatment of androgenetic alopecia. Lasers Med Sci 2021;36:703-13. doi: 10.1007/s10103-020-03159-z.
11. Al Musawi MS, Jaafar MS, Al-Gailani B, Ahmed NM, Suhaimi FM, Suardi N. Effects of low-level laser irradiation on human blood lymphocytes in vitro. Lasers Med Sci 2017;32:405-11. doi: 10.1007/s10103-016-2134-1.
12. Al Musawi MS, Al-Gailani BT. ATP level in red blood cells improves by altering the low-level DPSS laser irradiation condition. Appl Nanosc 2021;10. doi: 10.1007/s13204-021-01848-x.
13. Peplow PV, Chung TY, Ryan B, Baxter GD. Laser photobiomodulation of gene expression and release of growth factors and cytokines from cells in culture: a review of human and animal studies. Photomed Laser Surg 2011;29:285-304. doi: 10.1089/pho.2010.2846.
14. DiMauro S, Hirano M, Schon EA. The mitochondrial respiratory chain and its disorders. Mitochondrial Medicine, 1st ed. Florida, United States: CRC Press, 2006.
15. Wasik M, Gorska E, Modzelewska M, Nowicki K, Jakubczak B, Demkow U. The influence of low-power helium-neon laser irradiation on function of selected peripheral blood cells. J Physiol Pharmacol 2007;58 (Suppl 5): s729-37.
16. Al-Musawi MS, Jaafar MS, Al-Gailani TB, Ahmed NM, Suhaimi FM. In vitro effects of low level yellow laser irradiation on human red blood cells. In: IEEE 6th International Conference on Photonics(ICP). Kuching, Malaysia, 2016; pp 1-3, doi: 10.1109/ICP.2016.7510024.
17. Yahia MJ, Hasan JA, Musawi MS. Influence of DPSS laser radiation with different power densities and exposure times on erythrocyte sedimentation rate–an in-vitro investigation. AIP Conf Proc 2020;2213,020028. doi: 10.1063/5.0000436.
18. Yahia MJ, Hasan JA, Musawi MS. Biostimulation effect of DPSS laser irradiation with different power densities and radiation times on blood viscosity in vitro. AIP Conf Proc 2020;2213,020118. doi: 10.1063/5.0000438.
19. Huang YY, Chen AC, Carroll JD, Hamblin MR. Biphasic dose response in low level light therapy. Dose-response. 2009;7. doi:10.2203/dose-response.09-027.Hamblin.