RESEARCH ARTICLE

Evaluation of the effect of yellow laser (DPSS) light on human lymphocytes viability in vitro

Fatimah Saed Awad, Saba Fawzi Salih, Mustafa Salih Al Musawi

Abstract

Objective: To evaluate the lymphocyte apoptotic impact of yellow laser light in vitro.

Method: The experimental study was conducted from November 2021 to April 2022 at the Postgraduate Medical Physics Laboratory, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from healthy volunteers. The samples were subjected to 1:1 dilution in isotonic phosphate buffered saline, and each sample was divided into two equal aliquots; one for irradiation, and other as control.The percentage of apoptotic lymphocyte was estimated before and after radiation exposure with low-level laser 589nm at 30J/cm^2, 50J/cm^2 and 70J/cm^2 energy intensities. Post-exposure evaluation was done immediately, and 1 and 2 hours after radiation on the viability of normal human cells. The vitality of the cells was determined using the trypan blue exclusion test. Data was analysed using SPSS 24.

Results: There were 26 healthy volunteers (16 male, 10 females) with an age range of (20-40) years. After the exposure, the percentage of lymphocytes that apoptose increased significantly when cells were irradiated at 70J/cm^2 immediately compared to the controls ($p \leq 0.05$), but there was no significant differences with the 30J/cm^2 dose. After 1 and 2 hours, a significant reduction in the proportion of apoptotic lymphocytes was observed ($p<0.05$).

Conclusion: Yellow low-level laser showed a noticeable protective effect on lymphocytes by decreasing the percentage of dead cells.

Key Words: Trypan, Lymphocytes, Lasers, Radiation Exposure, Phosphates

(JPMA 74: S202 (Supple-8); 2024) **DOI: https://doi.org/10.47391/JPMA-BAGH-16-45**

Introduction

The use of low-level laser irradiation (LLLI) to treat a variety of pathological disorders, wound healing, pain relief and inflammation management, among other conditions, has been steadily increasing[1] since the early 1980s. The laser light that is released is both polarised and coherent, and it can be absorbed by a variety of tissues[2]. The three kinds of blood cells are erythrocytes (red blood cells [RBCs]), leukocytes (white blood cells [WBCs]), and platelets (PLTs)[3]. The main functions of WBCs are to combat infections, guard against alien organisms and to create, or at least transport and disperse, antibodies as part of the immune response. Individuals are at risk of infection due to a drop in WBC count, called leucopenia[4]. When photons from the laser reach the mitochondria and cell membranes of low-lying cells, like fibroblasts, the energy is absorbed by chromophores and converted to chemical kinetics, leading to the induction of initial reaction. Phototherapy is the only way to get primary results that are fairly predictable. Laser photons with wavelengths of 400-1100nm are absorbed by chromophores[5]. Within the cell, the photonic energy is transformed to chemical energy in the form of adenosine triphosphate (ATP), which improves cellular activities and cell proliferation rates. The permeability of cell membranes is altered, which leads to physiological alterations in the target cells. The size of the laser biostimulation effect is determined on the basis of the wavelength utilised as well as the cell's physiological state at the time of irradiation[6]. Components of the respiratory chain absorb light, causing changes in both the mitochondria and the cytoplasm. An antiport mechanism transports more calcium ions (Ca2+) into the cytoplasm at low laser dosages, triggering or stimulating biological processes, such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis, cell mitosis and cell proliferation. Too much Ca2+ is released at larger doses, resulting in hyperactivity as calcium pushes calcium adenosine-triphosphatase (ATPase) out of the cell's ATP pool, slowing cell metabolism[7].

Apoptosis, or programmed cell death, is a genetically controlled process that occurs when a cell dies, and it plays a vital role in tissue homeostasis and differentiation[8]. Apoptosis is a type of cell death that happens as a result of a cell's environment. Cell division and differentiation are important aspects of cellular and tissue physiology. The importance of this form of cell death is necessary for higher vertebrates' optimal

Department of Physiology, Mustansiriyah University, Baghdad, Iraq.

Correspondence: Fatimah Saed Awad

Email: mayona30y@gmail.com

embryonic development and tissue homeostasis[9]. Intracellular apoptotic signals, such as reactive oxygen species (ROS) or laser therapy, promote apoptosis at the mitochondrial membrane, causing the mitochondrial membrane transition pore to open, allowing cytochrome C to be released and the apoptotic process to begin[10]. Intracellularly and extracellularly, there are two phases: an initial commitment phase when the cell reacts to a signal that commits it to self-destruction, and an executional phase when cell death can not be stopped[11].

For decades, researchers have been looking into low-level laser therapy (LLLT), but the cellular mechanism of LLLT has remained a mystery. According to Karu et al,, irradiating human lymphocytes with a He-Ne laser can stimulate some short-term reactions in the cells, but irradiated lymphocytes do not enter the cell cycle's S-phase. In other words, there is no complete mitogenic activation or blast transformation. Irradiation also has a booting impact on DNA synthesis in cells that have been treated with phytohemagglutinin (PHA) prior to irradiation. The absence of interleukin-2 (IL-2) receptor expression in irradiated lymphocytes is thought to be linked to the absence of blast transformation in these cells[12-14].

The current study was planned to evaluate the lymphocyte apoptotic impact of yellow laser light in vitro.

Materials and Methods

The experimental study was conducted from November 2021 to April 2022 at the Postgraduate Medical Physics Laboratory, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from healthy volunteers. Approval was obtained from the institutional ethics review committee. The blood samples were collected from healthy adults with no medical history of major illnesses or history of taking medications for major diseases. The blood samples were drawn in a sterile ethylenediaminetetraacetic acid (EDTA) tube, and were subjected to 1:1 dilution in isotonic phosphate buffered saline, PBS (potential of hydrogen [pH][7.4]). Each sample was divided into two equal aliquots; one for irradiation, and other as control. Blood sample mixed with PBS was carefully laid on the top of 2ml of ficoll (lymphocyte separation medium). Lymphocyte separation was performed according to Boyum's method in a 10ml siliconized glass centrifuge tube. The compound was centrifuged for 30 minutes at 24°C in a refrigerated centrifuge at 3000rpm. After centrifugation, lymphocytes created a white buffy coat at the blood plasma interface, which was aspirated with a Pasteur pipette and put into a 10ml siliconised tube, which was cleaned 3 times with phosphate-buffered saline (PBS) for storage 20min each time. The lymphocyte pellet was re-suspended in 0.5ml of PBS until it formed a pellet[15].

The lymphocyte suspension was then exposed to laser beam 589nm for 15 minutes. The diode-pumped solid-state laser (DPSSL) (Changchun Dragon Lasers Co, Model F series' China) was used with a 50mW output power. The percentage of apoptotic lymphocytes were estimated immediately, and again after 1 hour and 2 hours of irradiation with doses of 30J/cm^2, 50J/cm^2 and 70J/cm^2 for both control and irradiated samples. The amount of lymphocytes in each sample was counted using a heamocytometer counting chamber, and the findings were represented as cell/mm^3 [16].

Cell viability was determined using the trypan blue exclusion assay. A known volume of lymphocyte suspension (100:l) was combined with an equivalent volume of trypan blue dye (0.2%) and seen under a light microscope right away (Figure 1). The volume the lymphocyte suspension was divided into 4 equal parts; one was the non-irradiated control, while the other 3 parts for the three laser intensities. The laser beam was pointed in one direction directly to the centre of the tube containing the cell sample upside down. Approximately 4mm^2 of the laser beam was applied to the lymphocytes through a spot of irradiation at room temperature 24°C. The lymphocyte sample was attracted to a Westergren tube fastened by holding it directly in front of the laser beam such that the beam passed straight through the opening end of the tube, and so the whole suspension was exposed to the light for 15 minutes. The viability and number of cells in each sample were calculated. This procedure was applied for each laser intensity and at 3 different times.

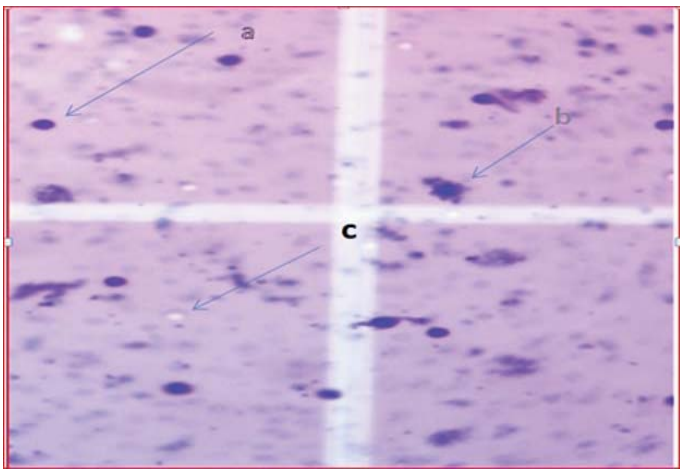

Figure-1: Trypan blue exclusion test under light microscope (40X).
(a) lymphocyte apoptosis, (b) lymphocyte necrosis, (c) lymphocyte viability.

Data was analysed using SPSS 24. The significance of differences between various independent means was investigated by using a students´ t-test, while paired t-test was employed for the mean values of dependent variables. P<0.05 was considered significant.

Results

There were 26 healthy volunteers (16 male, 10 females) with an age range of (20-40) years. After initial irradiation, the percentage of apoptotic cells increased significantly with 70J/cm^2 and 50J/cm^2 compared to non-irradiated samples, but there was no significant differences with the 30J/cm^2 dose (Figure 2).

Re-examination of irradiated samples after 1 hour showed a decrease in the percentage of apoptotic cells at 70J/cm2 and 50J/cm^2. Highly significant decrease in the percentage of apoptosis was observed 2 hours after irradiation with 70J/cm^2 and 30J/cm^2 (Figure 2).

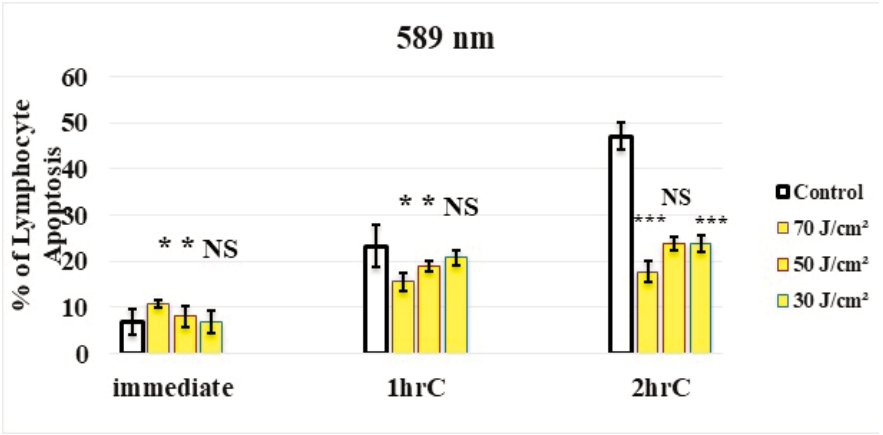

Figure-2: : Fraction of lymphocytes that apoptosed before and after laser exposure (589 nm) for 70J/cm^2, 50J/cm^2 and 30 J/cm^2 measured immediately and at 1h and 2h post-raditaion.

Discussion

LLLI can provide low fluency caused by direct irradiation rather than thermal processes, and the irradiation tissue's temperature rise is controlled to less than 0.1-0.5°C with this technique[17].

The current study noticed increase in the percentage of apoptotic lymphocytes immediately after being irradiated with high -energy density of yellow laser probably due to its ability to produce a substantial amount of singlet oxygen when a range of biological components were oxidised at the start of the apoptotic cascade and nuclear DNA damage[18,19]. Studies have reported the involvement of the glycon synthase kinase 3 which is a signal transducer of Akt protein (Akt/GSK3) pathway, and the expression of pro-apoptotic Bax protein in the cells soon after cellular exposure to LLLT[20,21].

The paradoxical decrease in the percentage of apoptotic lymphocytes with time post-irradiation might have been due to the upregulation of the signaling cascade of proliferating cell nuclear antigen (PCNA) and Ki-67 protein (nuclear proteins associated with proliferation of tumour cells), both of which were important in the activation of p15 gene which encodes a protein that induces a G1-phase cell cycle arrest during photo biostimulation[22]. Photo biomodulation enhances respiratory chain mitochondrial enzymes, influencing electron transfer and increasing intracellular calcium level, and, as a result, cell proliferation is boosted[23-25]. The mitochondrial cytochrome C oxidase, which is one of the respiratory chain components, is thought to absorb red to near-infrared light, resulting in the formation of ROS and ATP[25].

Limitations. The current study has limitations as the sample size was not calculated which could influence the power of the study.

Conclusion

Low-level laser had a significant protective effect on lymphocytes by reducing the percentage of dead cells after they had been irradiated by yellow laser, and the effect was influenced by doses and the duration of exposure.

Acknowledgement: We are grateful to the Medical Physics Department of the University of Mustansiriyah, Baghdad, Iraq, for facilitating the study.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

Bihari I, Mester A. The biostimulative effect of low level laser therapy of long standing crural ulcers using HeNe laser, HeNe plus IR laser, and incoherent light: A preliminary report of a randomized double blind comparative study. Laser Ther 1989;1:97–102.
2. Vladimirov YA, Osipov AN, Klebanov GI. Photobiological principles of therapeutic applications of laser radiation. Biochemistry (Mosc) 2004;69:81-90. doi: 10.1023/b:biry.0000016356.93968.7e.
3. Zhou P, Meng Z, Liu M, Ren X, Zhu M, He Q, et al. The associations between leukocyte, erythrocyte or platelet, and metabolic

syndrome in different genders of Chinese. Medicine (Baltimore) 2016;95:e5189. doi: 10.1097/MD.0000000000005189.
4. Luntsi G, Daniel VS, Paul BT, Nwobi IC, Abdullahi AM, Ahmadu MS, et al. Evaluation of low dose diagnostic X-rays induced effect on the white blood cells count in Guinea pigs. Int J Radiat Res 2018;16:129-32. DOI: 10.18869/acadpub.ijrr.16.1.129.
5. 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.
6. Al Musawi MS, Al-Gailani BT. Retracted Article: ATP level in red blood cells improves by altering the low-level DPSS laser irradiation condition. Appl Nanosci 2023;13:1751. doi: 10.1007/s13204-021-01848-x
7. Schindl A, Schindl M, Pernerstorfer-Schön H, Schindl L. Low-intensity laser therapy: a review. J Investig Med 2000;48:312-26.
8. Wyllie AH. The genetic regulation of apoptosis. Curr Opin Genet Dev 1995;5:97-104. doi: 10.1016/s0959-437x(95)90060-8.
9. Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ 2015;22:58-73. doi: 10.1038/cdd.2014.137.
10. Al-khazragi RA, Al-Samaraee IF. Effect of Laser light ou lymphocyte Apoptosis. Iraqi J Med Sci 2008;6:45-51.
11. Alhamdi AA. Facial Skin Lines. Iraqi J Med Sci 2015;13:103-7.
12. Al-Kaabi AAK, Al-Musawi MS, Hasan AA. In Vitro Effect of Low-Level Lasers on Total Bilirubin Concentration in Human Blood Plasma Using 375 and 650 nm Lasers. Photobiomodul Photomed Laser Surg 2024;42:49-53. doi: 10.1089/photob.2023.0141.
13. Maldaner DR, Azzolin VF, Barbisan F, Mastela MH, Teixeira CF, Dihel A, et al. In vitro effect of low-level laser therapy on the proliferative, apoptosis modulation, and oxi-inflammatory markers of premature-senescent hydrogen peroxide-induced dermal fibroblasts. Lasers Med Sci 2019;34:1333-43. doi: 10.1007/s10103-019-02728-1.
14. Zamani ARN, Mashayekhi MR, Jadid MFS, Faridvand Y, Tajalli H, Rahbarghazi R. Photo-modulation of zinc phthalocyanine-treated breast cancer cell line ZR-75-1 inhibited the normal tumor activity in vitro. Lasers Med Sci 2018;33:1969-78. doi: 10.1007/s10103-018-2563-0.
15. Boyum A. Separation of lymphocytes, lymphocyte subgroups and monocytes: a review. Lymphology 1977;10:71-6.
16. Doyle A, Griffiths JB, eds. Haemocytometer cell count and viability studies: Cell and Tissue Culture for Medical Research. New York, USA: Wiley, 2000; pp 150-65.
17. 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.
18. Morkunas V, Urbonaite G, Gabryte-Butkiene E, Sobutas S, Vengris M, Danielius R, et al. DNA-Damaging Effect of Different Wavelength (206 and 257 nm) Femtosecond Laser Pulses. Photobiomodul Photomed Laser Surg 2019;37:254-61. doi: 10.1089/photob.2018.4540.
19. Al Musawi MS, Jaafar MS, Al-Gailani BT, Ahmed NM, Suhaimi FM. In vitro effects of low level yellow laser irradiation on human red blood cells. In: 2016 IEEE 6th International Conference on Photonics (ICP). Kuching, Malaysia: IEEE; 2016. doi: 10.1109/ICP.2016.7510024.
20. Huang L, Wu S, Xing D. High fluence low-power laser irradiation induces apoptosis via inactivation of Akt/GSK3β signaling pathway. J Cell Physiol 2011;226:588-601. doi: 10.1002/jcp.22367.
21. Jere SW, Houreld NN, Abrahamse H. Role of the PI3K/AKT (mTOR and GSK3β) signalling pathway and photobiomodulation in diabetic wound healing. Cytokine Growth Factor Rev 2019;50:52-9. doi: 10.1016/j.cytogfr.2019.03.001.
22. Taniguchi D, Dai P, Hojo T, Yamaoka Y, Kubo T, Takamatsu T. Low-energy laser irradiation promotes synovial fibroblast proliferation by modulating p15 subcellular localization. Lasers Surg Med 2009;41:232-9. doi: 10.1002/lsm.20750.
23. Lima PLV, Pereira CV, Nissanka N, Arguello T, Gavini G, Maranduba CMDC, et al. Photobiomodulation enhancement of cell proliferation at 660 nm does not require cytochrome c oxidase. J Photochem Photobiol B 2019;194:71-5. doi: 10.1016/j.jphotobiol.2019.03.015.
24. Paschalidou M, Athanasiadou E, Arapostathis K, Kotsanos N, Koidis PT, Bakopoulou A, et al. Biological effects of low-level laser irradiation (LLLI) on stem cells from human exfoliated deciduous teeth (SHED). Clin Oral Investig 2020;24:167-80. doi: 10.1007/s00784-019-02874-4.
25. 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..