RESEARCH ARTICLE

The effect of a diode-pumped solid-state laser with a wavelength of 589 nm on T-lymphocyte proliferation

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Abstract

Objective: To assess how low-level laser irradiation affects T cells.

Method: The case-control interventional study was conducted from November 2021 to April 2022 after approval by the ethics review committee of the College of Medicine, Mustansiriyah University, Baghdad, Iraq, and comprised healthy individuals. Phlebotomy was used to collect 4ml of blood. A portion of each sample was exposed to 589nm laser radiation, while the other portion was used as a control. The irradiated sample was divided into whole blood and in-plate subgroups. Phytohemagglutinin was used for mitotic stimulation of T-lymphocytes, and cell proliferation assay was used to stimulate T lymphocytes measured using MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). Optical density was measured using enzyme-linked immunosorbent assay. Data was analysed using GraphPad prism software.

Results: There were 35 subjects, 17(49%) males and 18 (51%) females, with an age range between 19-25 years, all without any disease or medication. Laser irradiation stimulated T-lymphocyte proliferation to levels that depended on the dose of laser used. At energy 30J/cm^2 there was a significant increase in the in-plate subgroup of irradiated samples compared to controls and between the two irradiated subgroups ($p<0.05$). At 50J/cm^2 dose, there was a significant increase in the whole blood subgroup compared to the controls ($p<0.05$). At 70J/cm^2 dose, there was a significant difference in the whole blood subgroup compared to the controls, and between the two irradiated subgroups ($p<0.05$).

Conclusion: The stimulation of T lymphocyte proliferation by the application of a 589nm laser could have medical applications in the field of immune system enhancement.

Key Words: Phytohemagglutinins, Phlebotomy, Lasers, Lymphocytes, Proliferation, Immunosorbent

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Introduction

Medical lasers are commonly utilised in the modern era. Cosmetology, diagnostics, surgery and a slew of other medical procedures use a wide range of laser beams with a variety of optical qualities[1]. Low-level laser irradiation (LLLI) refers to the use of red and near-infrared light to treat wounds and soft tissue lesions, as well as to provide pain relief in both acute and chronic pain. Literature has reported many laser wavelengths with varying powers and exposure times for therapeutic purposes[2]. The effects of LLLI on cultured human lymphocytes in vitro are well-documented, but nothing is known about the effects of the compound on whole blood[3]. Multiple biological processes, such as cell development, proliferation and differentiation, can be influenced by LLLI. Experimental studies in vitro have shown that LLLI has an influence on cell growth in many cell types, such as fibroids and endothelial cells. In spite of this, the molecular mechanism of LLLI's stimulatory actions has yet to be fully understood. Since cellular adenosine triphosphate (ATP) levels rise as a result of the laser energy being absorbed by intracellular chromophores, this is a well-established mechanism[4,5]. The possibility of tissue bio-stimulation exists only if irradiated cells possess molecular photoreceptors capable of absorbing light and entering an excitation state, generating an intracellular signal cascade that results in a quantifiable biological effect[1]. Cell proliferation is an important physiological effect of low-level laser therapy (LLLT), and increases the proliferation of numerous cells, mostly through the activation of the mitochondrial respiratory chain and the beginning of cellular signalling (particularly red and near-infrared light) and superoxide anion hypothesis. After helium-neon (He-Ne) laser irradiation, cellular signalling pathways are included in the secondary processes following light absorption, with mitochondrial retrograde signalling being nearly twice as easy to add[4].

The current study was planned to look into the proliferation of T lymphocyte blood cells caused by the use of the 589nm diode-pumped solid state (DPSS) laser.

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Materials and Methods

The case-control interventional study was conducted from November 2021 to April 2022 after approval by the ethics review committee of the College of Medicine, Mustansiriyah University, Baghdad, Iraq. The sample was raised using consecutive nonprobability sampling technique. Those included were healthy individuals.

Any subject with chronic disease and/or on medications were excluded from the study.

Phlebotomy was used to collect 4ml of blood from each individual. A portion of each sample was exposed to laser radiation, while the other portion was used as a control. The irradiated sample was further divided into whole blood subgroup and another subgroup which was irradiated after the cell cultured in plate at the same dose and time of irradiation.

The blood was collected using ethylenediaminetetraacetic (EDTA) acid. A 589nm yellow light LLL beam from a DPSS laser was used. The laser beam was given to the blood samples in tubes with a 5mm diameter irradiation spot. Both samples in tubes containing the same volume of blood were diluted with phosphate buffer saline (PBS), and then transferred to a new tube adding 3cc of lymphocyte separation before being centrifuged (Jouan c4i, manufactured by Thermo electronic corporation, USA) at G800 for 20 minutes.

After plasma was removed, peripheral mononuclear cells (PMC) were washed twice with PBS to avoid contamination. The media containing phytohemagglutinin (PHA) was added to the PMC. T lymphocyte proliferation was stimulated by PHA and the cells were cultured in plate (96 walls with flat surface). After 48 hours, cell proliferation assay was added, and optical density was measured after 3 hours using enzyme-linked immunosorbent assay (ELISA).

Results

There were 35 subjects, 17(49%) males and 18 (51%) females, with the age range between 19-25 years, all without any disease or medication. Laser irradiation stimulated T-lymphocytes proliferation to levels that depended on the dose of laser used. At energy 30J/cm^2 there was a significant increase in the in-plate subgroup of irradiated samples compared to controls, and between the two irradiated subgroups (Figure 1).

At 50J/cm^2 dose, there was a significant increase in the whole blood subgroup compared to the controls (Figure 2).

At 70J/cm^2 dose, there was a significant difference in

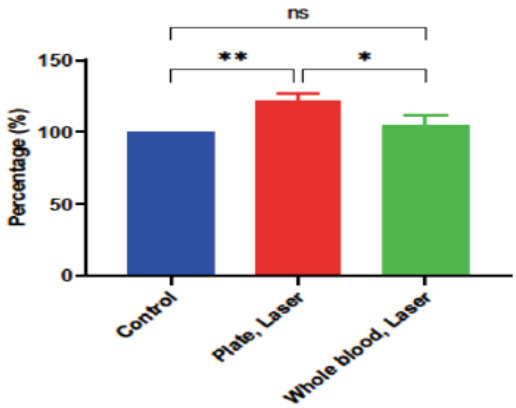

Figure-1: Comparison of T-lymphocytes proliferation as percentage of control following plate irradiation versus whole blood irradiation with laser dose 30J/cm^2 ns: p>0.05, *: p≤0.05, **: p≤ 0.01.

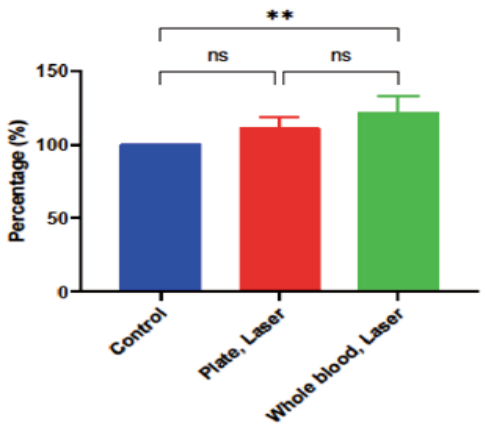

Figure-2: Comparison of T-lymphocytes proliferation as percentage of control following plate irradiation versus whole blood irradiation with laser dose 50J/cm^2. ns: p>0.05, **: p≤ 0.01.

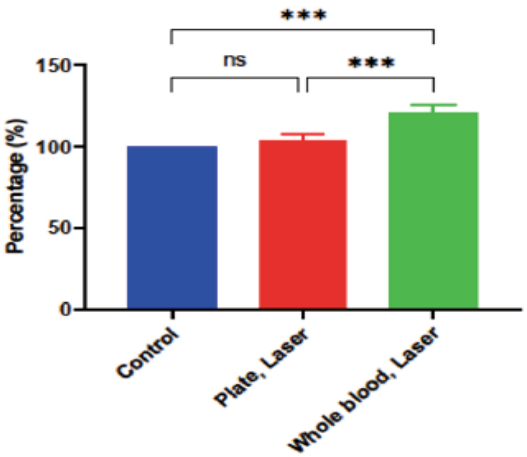

Figure-3: Comparison of T-lymphocytes proliferation as percentage of control following plate irradiation versus whole blood irradiation with laser dose 70J/cm^2. ns: p>0.05, ***: p≤ 0.001.

whole blood subgroup compared to the controls, and between the two irradiated subgroups (Figure).

Discussion

Previous research has shown that LLLs are capable of delivering such low-energy density that all biological effects are merely the product of direct irradiation, and not of the thermal processes[6]. Because LLLI can produce such low effects, the rise of temperature in the irradiated tissue is limited to <0.1-0.5°C(1). LLLI can prevent cell death and increase cell proliferation, migration and adhesion at low levels of visible light illumination[7]. The fluences in current study were in line with literature[8,9]. Regarding the assessment of T lymphocyte proliferation, 589nm wavelength with different dosages caused an increase in T lymphocyte proliferation. However, the basic mechanisms underlying LLLI's activities in tissues are still unknown. While some studies have shown that light increases cellular proliferation in a variety of cell types, the way light interacts with biological tissues is dependent on the qualities and parameters of light devices, particularly the fluence and wavelength, as well as the tissue's optical properties[10]. A study presented a chain of molecular events to describe the bio-stimulation impact of LLLI, beginning with light absorption by a photoreceptor, leading to photo-activation of enzymes in the mitochondria, including signal transduction and amplification activities, and ending with photo-response. It reported that laser light had an effect on cellular immunity, and laser treatment had an immune-modulating effect on T-lymphocytes[11]. A favourable impact of laser irradiation is the initiation of primary, free radical reactions, which are expressed in increased bactericidal activity, protein and cytokine synthesis, and cell proliferation. All of these events are necessary for laser treatment to have a therapeutic effect [6].

The current results indicated that the maximum increase in T-lymphocytes was observed when whole blood was laser-irradiated at a dose of 70J/cm^2. In contrast, when cells in the plate were laser-irradiated, the maximum increase in T-lymphocytes was observed at a dose of 30J/cm^2. This demonstrates the fundamental principle of the biphasic dosage response, also known as hormesis. Obtaining a reaction requires sufficient energy, while replacing bio-stimulation with bio-inhibition necessitates energy greater than the threshold energy[7,12].

Furthermore, the current findings can explain how exposure of whole blood to laser radiation in the laboratory can cause noticeable alterations in red blood cells (RBCs) and plasma proteins, resulting in a lower dose delivered to other cells[13-15] .

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

Conclusion

The stimulation of T lymphocyte proliferation by the application of 589nm laser could have medical application in the field of immune system enhancement.

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Conflict of Interest: None.

Source of Funding: None.

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