RESEARCH ARTICLE

A comparative study of 589 and 532 nm low level laser irradiation effects on normal human peripheral blood mononuclear cells viability

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

Abstract

Objective: To assess how laser light affects blood lymphocyte viability in vitro.

Method: The comparative study was conducted from November 2021 to April 2022 at the Department of Medical Physics, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples collected from healthy adults with no medical history of major illnesses or history of taking medications for major diseases.Low-level laser 589nm and 532nm was used at 30 J/cm^2, 50 J/cm^2 and 70J/cm^2 energy intensities and three different post-exposure time; immediately, 1h and 2h after radiation exposure. The viability of normal human lymphocytic cells of the blood was noted. Data was analysed using SPSS 24.

Result: The blood samples were drawn from 6 adult male voluteers. The percentage of cells that showed apoptosis post-exposure to 589nm laser was significantly lower than that following exposure to 532nm laser ($p<0.05$). However, the proportion of apoptotic cells was significantly higher following irradiation with 532nm at varying doses than after irradiation with 589nm ($p<0.05$).

Conclusion: A low-level laser could promote and prevent apoptosis in human peripheral blood cells in a dose- and time-dependent manner.

Key Words: Lasers, Apoptosis, Lymphocytes, Radiation

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Introduction

The oldest publication about low-level laser therapy (LLLT) was more than 30 years ago. Since then, the efficacy and usability of a wide range of light sources have been invesitigated both in vitro and in vivo in the treatment of a variety of medical problems[1]. It is possible to extract the effects of laser light on cells. Several investigations have revealed that the biological consequences of irradiation are dependent on the cell type being bombarded[2]. In addition, laser irradiation wavelength, fluence, laser output power, beam area, irradiance and polarisation are parameters of interest[3,4]. Low-power irradiation causes no harm to hydrogen bonds in tissues, and produces no changes other than photochemical effects by stimulating a cell through increased cellular metabolism[5]. Because LLLT has no thermal effects, it does not harm living cells[6]. Photons must be absorbed by electronic absorption bands belonging to a molecular photo acceptor in order to see LLL irradiation effects on living biological systems (chromophore)[7]. The mechanism of interaction between LLL radiation and blood is yet unknown, resulting in problems in LLLT for blood due to multiple responses that occur at the same time[8]. White blood cells (WBCs) are an important component of the immune system, and can be differentiated into five distinct categories. The differential WBC count at microscopic level is usually done by haematologists as diagnostic test on patients with a suspicion of malignancy[9]. The cellular membrane integrity would be destroyed as cells die, and the cellular fragments would be consumed by blood phagocytic cells. The contents of the cells can also be lost in the forms of cytosolic enzymes, like lactate dehydrogenase (LDH). When nuclear deoxyribonucleic acid (DNA) is exposed, a decrease in intracellular enzyme activity and a decrease in intracellular adenosine triphosphate (ATP) affect s cellular energy capacity and viability. Because of these outcomes, cell viability is determined through microscopic inspection of cell morphology[10]. Apoptosis, or programmed cell death, is linked to a variety of physiological and pathological situations, including immunological disorders, inflammation, neurodegenerative diseases and cancers[11]. Because it is regarded a sort of cell suicide based on a genetic basis, the historical idea of programmed cell death has been related with apoptosis, which is the most common type of cell death regarded as an "accidental cell death" as in the case of necrosis, which , in turn, has been referred to as an unintentional cell death rather than a result of defined pathways. The traditional meaning of the term 'necrosis' is not truly accurate because it does not always imply a

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certain type of cell death. The phrase is frequently used to describe alterations that occur as a result of cell death caused by any mechanism, including apoptosis. Many shocks can cause apoptosis at low doses, but necrosis happens only at high doses. Apoptosis and necrosis may exist on a continuum of cell death, depending on the given stimulus, and, therefore, the two death forms are not mutually exclusive and can coexist in many clinical circumstances[12].

Researchers have been investigating LLLT for decades, but LLLT's cellular mechanism is not yet fully known. Irradiating human lymphocytes with a helium–neon (He-Ne) laser, according to Karu et al.[13] can activate certain short-term responses in these cells. Irradiated lymphocytes, on the other hand, do not go through the S-phase of the cell cycle where S-phase is the synthesis phase during which DNA synthesis and replication takes place. In other words, neither mitogenic activation nor blast transformation have been accomplished. Irradiation also has a booting impact on DNA synthesis in cells that have been treated with phytohemagglutinin (PHA) before irradiation. The lack of interleukin-2 (IL-2) receptor expression in irradiated lymphocytes is hypothesised to be related to the cells' inability to perform blast transformation[14-16].

The current study was planned to compare the effects of LLL irradiation with 589nm and 532nm on the viability status of peripheral blood lymphocytic cells in vitro.

Materials and Methods

The comparative study was conducted from November 2021 to April 2022 at the Department of Medical Physics, Mustansiriyah University, Baghdad, Iraq. After approval form the institutional ethics review committee, blood samples were collected from randomly recruited healthy non-smoker adults aged 18-60 years with no history of major illnesses and had taken no medicines and antibiotics for at least the 7 preceding days. Informed consent was obtained from all the participants. The samples were collected by venipuncture. Using lymphocyte separation Ficoll-Hypaque (F-H) media, human peripheral blood lymphocytes were extracted, and phosphate-buffered saline (PBS) was used to dilute the blood. From each sample, 2ml was collected in an ethylenediaminetetraacetic (EDTA) acid tube, centrifuged after being gently covered with the same amount of F-H solution for 30 minutes at 3000rpm[17]. After centrifugation, the puffy coat of lymphocytes was removed using sterile pipettes, and the interphase between F-H and PBS was carefully collected and cleaned twice with PBS each time for 10 minutes in the centrifuge,

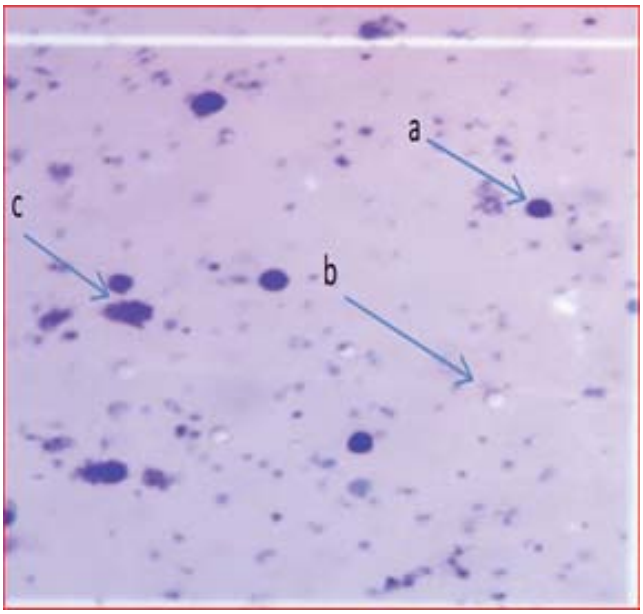

Figure-1: Under a light microscope, Trypan blue exclusion test (40X), showing apoptotic lymphocyte (a)m viable lymphocyte (b) and necrotic lymphocyte (c).

until a pure pellet of cells was obtained for suspension in PBS 0.5mL. The final suspension was divided into multiple sections, and the volume of samples and solutions was quadrupled. Trypan blue dye was used to detect cell viability as apoptotic cells accepted the dye and appeared blue in colour, whereas transparent live cells did not accept the dye, as seen by looking at the cells on the Neubauer chamber slide under a light microscope (Figure 1).

Lymphocytes suspension of one sample was subdivided into non-irradiated control, and three others that were irradiated for 15 minutes with 30 J/cm^2, 50 J/cm^2 and 70J/cm^2 energy intensity of 589nm and 532nm wavelengths. From an upside-down position, the laser beam was aimed straight to the middle of the blood sample tube. Through an irradiation point, a laser beam of about 4 mm^2 was delivered to the lymphocytes sample. The samples were irradiated at room temperature 24°C. Each sample was further subdivided to be examined immediately after preparation as well as 1 and 2 hours later with or without irradiation. Lymphocytes were counted using a haemocytometer counting chamber, and the count was expressed as cell/mm^3[18]. The vitality of the cells was determined using the trypan blue exclusion test. A known volume of lymphocyte suspension (100l) was combined with an equivalent volume of trypan blue dye (0.2% concentration) and was inspected under a light microscope right away. The remaining 1cc lymphocyte suspension was kept in a Westergren tube that was held immediately in front of the laser beam, allowing the beam

to pass straight through the opening end of the tube, exposing the entire suspension to the light for 15 minutes. Exposure time, cell count and viability were estimated.

To obtain enough suspended lymphocytes, the blood sample volume and solutions were doubled.

Data was analysed using SPSS 24. Students' t-test was used to determine the importance of discrepancies between different independent means. For the dependent means, a paired t-test was utilised. $P<0.05$ was considered statistically significant.

Results

The blood samples were drawn from 6 adult male voluteers. Lymphocyte apoptosis before and after exposure to 589nm and 532 nm laser for 70J/cm^2, 50J/cm^2 and 30 J/cm^2 doses) were significanly different at all time points except for 2hrs post-irradiation with 50J/cm^2 dose (Figure 2).

murine embryonic fibroblasts by LLLT (810nm) along with the activation of Nuclear factor kappa B (NF-κB).

Although these investigations revealed that LLLT had an effect on numerous mitochondrial dynamics, the clinical consequences are unknown, and they are only used as LLLT activity markers.

Conclusion

LLL could promote and prevent apoptosis in human peripheral blood cells in a dose- and time-dependent manner.

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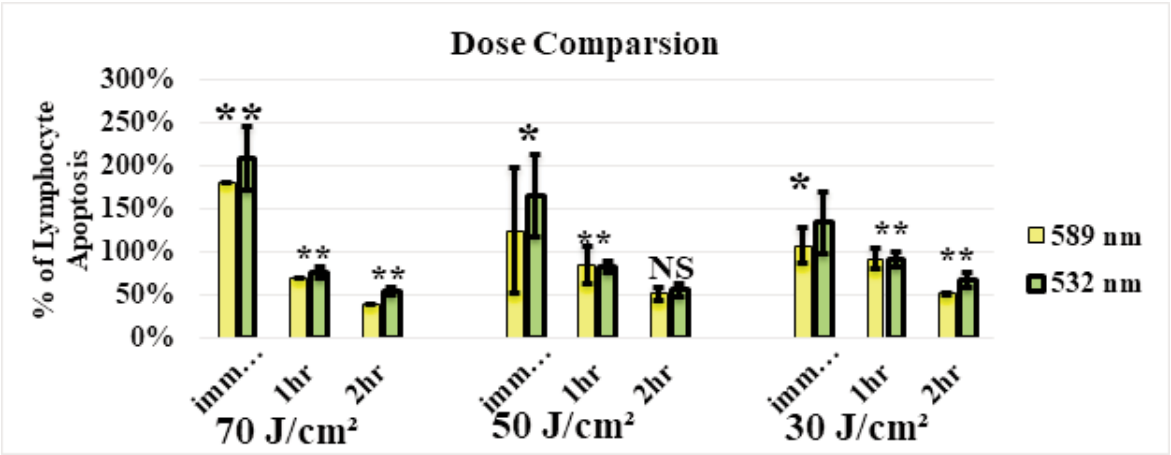

Figure-2: Lymphocyte apoptosis before and after the exposure to 589nm and 532nm laser for 70 J/cm^2, 50 J/cm^2 and 30J/cm^2 doses measured immediately, 1hr and 2hr post-irradiation by trypan blue exclusion test under light microscope..

Discussion

The current study focussed on the effects of LLLT modulation on healing and recovery, which is a mechanism still not fully known. Further research is needed to increase the knowledge of LLLT-induced cell death and cell viability changes. LLLT has been shown to affect the mitochondrial membrane potential status, as well as alterations in the levels of reactive oxygen species (ROS), nitrogen oxides (NO), and intracellular calcium in several laboratory-based investigations[19] LLLT was also observed to induce a change in the mitochondrial shape from filamentous appearance to a granular one, which was linked to an increase in proliferation of some cell types[20]. In a study[21], ROS and ATP were produced in

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