

Effect of Helium-Neon Laser on the Lymphocyte Cells and their DNA

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Abstract

- Background** Laser light is widely used for a wide range of medical applications. He-Ne laser application in medicine as in any type of laser is based on the interaction of laser light with the biological system.
- Objective** To show the effect of helium-neon (He-Ne) laser (632.8 nm) irradiation on human lymphocyte blood cells and their DNA.
- Methods** This study involved 72 blood samples, taken from apparently healthy volunteers. The samples were divided into two groups; the 1st group consisted of 27 samples were processed only for lymphocyte blood cells separation, while the 2nd group, which consisted of 45 samples were employed to evaluate the influence of He-Ne laser irradiation on the extracted DNA from the lymphocyte blood cells.
- Results** At the used doses of He-Ne laser (18, 35, 52.5, and 69 J/m²), a significant difference was found ($P < 0.05$) in survival percentage of lymphocyte cells (99.8, 99.74, 99.68, and 99.59) in comparison with those cells untreated with He-Ne laser irradiation. Immediately after He-Ne laser irradiation alone, the following doses (18, 35, and 69 J/m²) were applied on the extracted DNA, the DNA demonstrated a significant damaging where the fraction of DNA survival percentage was (88.6, 87.7, 86.1) respectively, with significant difference ($P < 0.05$) between the DNA survival before and after He-Ne laser irradiation.
- Conclusion** The percentage of lymphocytes survival is decreasing with increasing dose of He-Ne laser and longer exposure time where time exposure (2.5, 5, 7.5, and 10 s). He-Ne laser irradiation causes a significant degree of DNA damaging independent on the irradiation doses.
- Keywords** Lymphocyte cells, He-Ne laser irradiation, DNA.
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List of abbreviation: Helium-neon laser = He-Ne laser, phr = Photoreactivation gene, RecA = Recombination gene, UV = Ultraviolet radiation

Introduction

Laser light is extensively used for a wide range of applications in medicine. However, the effect of laser light irradiation is debatable and the mechanisms of its exact action are still unclear. Laser in experimental medicine requires detailed information on the mechanism of their

biological effects ⁽¹⁾. Since laser light has the unique properties of polarized coherent, monochromaticity (single wavelength), and directionality, which may enhance laser absorption by different tissues ⁽²⁾. Tissue bio-stimulation is only possible if irradiated cells possess molecular photoacceptors or photosensitive capacity that absorb the light and enter into state of excitation, that trigger intracellular cascade of signals leading to measurable biological effect ⁽³⁾. The bio-

stimulatory effect of laser irradiation is determined by the magnitude of the absorbed light energy, which depends on many factors; wavelength of laser source, power, exposure time, and characteristics of absorption and scattering of tissue ⁽⁴⁾.

Helium-neon (He-Ne) laser application in medicine as in any type of laser is based on the interaction of laser light with biological system ⁽⁵⁾. He-Ne laser (632.8 nm wavelength) has low photon energy and output power that produces minimum biomolecular damage ⁽⁶⁾. Because it produces a temperature elevation of less than 0.5 °C in the irradiated cells ^(7,8), the irradiation of He-Ne laser causes photochemical interaction with the cells rather than thermal effect.

He-Ne laser (632.8 nm) irradiation of the lymphocytes may cause such photochemical interaction that may be useful in medicine ⁽⁹⁾. Laser irradiation, in this red spectral area, influences the proliferative activity of peripheral blood lymphocytes ⁽¹⁰⁾, promotes tissue repair ⁽¹¹⁾, and has a protective effect on lymphocyte cells by stimulation of cytokines production ⁽¹²⁾. Moreover, He-Ne laser irradiation (632.8 nm) of cells has been reported to result in a variety of effects on cell structure and function ⁽¹³⁾, such as remodeling of the cytoskeletal network ⁽¹⁴⁾. Cellular proliferation could be triggered by the interaction of a He-Ne laser with the mitochondrial photoacceptor-cytochrome oxidase, which is the enzyme that catalyzes the final step in the mitochondrial respiratory chain for transfer of electron from cytochrome c to molecular oxygen ⁽¹⁵⁾. Another effect is an increased content of ATP (adenosine triphosphate), growth of the electric potential across inner membranes ⁽¹⁶⁾, and formation of giant mitochondria ⁽¹⁷⁾. Low energy lasers (low-level laser) light in the red and far red regions of electromagnetic radiation spectrum are considered to have positive effects in wound healing ^(18,19). Since ionizing energy such as X and γ - rays, UV light and α - particles cause cell and tissue damaging, therefore a lot of works

have been carried on showing that the low-level laser irradiations modify the response of cells to ionization ^(18,20).

The present study was done to show the effect of He-Ne laser (632.8 nm) irradiation on human lymphocyte blood cells and their DNA.

Methods

This study was carried out at the Department of Physiology and Medical Physics, College of Medicine, Al-Nahrain University, during the period from November 2010 to June 2011.

In order to assess the effect of He-Ne laser on DNA of human blood lymphocyte cells, 72 blood samples were taken from healthy volunteers (47 females and 25 males), with age ranged from 19-45 year, with mean age of 32.4 ± 7.68 . The 72 blood samples were divided into two groups. The 1st group, consisted of 27 samples, was used to estimate the effect of He-Ne laser on lymphocyte cells; therefore, these samples were processed only for peripheral blood lymphocytes cell separation (PBL) using Boyum method ⁽²¹⁾. The 2nd group, consisted of 45 blood samples, were processed for DNA extraction to evaluate the influence of He-Ne pre-irradiation on the extracted DNA from the lymphocyte cells. The degree of damage by He-Ne laser on lymphocyte cells number, and DNA concentration measured by the haemocytometer, and the spectrophotometer respectively.

A continuous He-Ne laser beam, of 1 mm diameter and 632.8 nm wavelength was employed (Griffin and George, Britain). The laser maximum output power was 1 mW. To ensure uniform illumination on the sample, the He-Ne laser beam diameter was expanded to a spot of 1.3 cm (using a converging lens) which corresponded to the sample tube diameter of 1.3 cm. Irradiation of laser was done employing different exposure times (2.5, 5, 7.5, and 10 s), which equal to energy doses of (18.8, 37.6, 56.4, and 75 J/m²) respectively. Since at each lens surface about 4 % from the intensity reflected back, so about 8% will be lost at the

two lens surfaces, therefore, the final energy doses became (18, 35, 52.5, 69 J/m²).

Twenty-seven blood samples, each one undergoes lymphocyte cells isolation. Each lymphocyte cells suspension sample was divided into approximately five equal parts, one of them (untreated) was used as a standard. The trypan blue exclusion test was employed to assess lymphocyte viable cells number for the untreated sample part by a haemocytometer. The counts expressed as number of viable cells/ mm³ and the other four parts of the sample, each one was exposed to He-Ne laser beam for only one of the following doses (18, 35, 52.5, and 69 J/m²). After each irradiation, the fractional of cell survival % was evaluated relative to untreated (standard) cells viability.

Forty-five blood samples were used to study the effect of He-Ne laser irradiation on the DNA. The DNA was extracted from human blood lymphocyte cells using phenol-chloroform method ⁽²²⁾. A part from each extracted DNA sample was employed to evaluate the DNA purity. The optical density (OD) of DNA, which is measured by spectrophotometer at UV wavelength of 260 nm for the untreated sample part was used as a standard. The second part, firstly irradiated with He-Ne laser beam (632.8 nm), and then incubated for 45 min at room temperature. After each irradiation, the OD of DNA was measured. Three different He-Ne laser exposure time periods were used (2.5, 5, and

10 s). Therefore, the 45 samples in this group divided into three sub-groups. Each sub-group contains 15 samples, and each sub-group irradiated with He-Ne laser beam for one of the used exposure time 2.5, 5, or 10 s (doses 18, 35, 69 J/m²).

Statistical analysis

The mean and the standard deviation for each group parts data were estimated employing Microsoft Excel program. A paired sample t-test was used comparing the data for pre-laser irradiation and then after UV-light irradiation. The difference was considered statistically significant, when the P value was less than 0.05 ⁽²³⁾.

Results

Lymphocyte cells results

The average percentage of lymphocytes viability of untreated part samples (27 blood samples; standard) was 99.9±0.06.

Table (1) shows the effect of different laser times of exposure (2.5, 5.0, 7.5, and 10 s), which correspond to the following doses of energy (18, 35, 52.5, and 69 J/m²) respectively, on lymphocytes viability percentage. There is a small difference in the cells viability after He-Ne laser irradiation in comparison to that before cells irradiation (untreated) (P < 0.0001).

It is clear from the results that the viability of lymphocyte cells is higher at the smallest time of exposure (2.5 s), as shown in figure 1.

Table 1. The mean of the lymphocyte blood cells viability % after different time exposure and He-Ne laser doses irradiation

Time exposure (s)	Dose (J/m ²)	Mean±SD	P value
0	0	*99.9±0.06	
2.5	18	99.8±0.14	< 0.0001
5.0	35	99.74±0.15	< 0.0001
7.5	52.5	99.68±0.17	< 0.0001
10	69	99.59±0.23	< 0.0001

*The comparison is done with lymphocyte viability before treatment

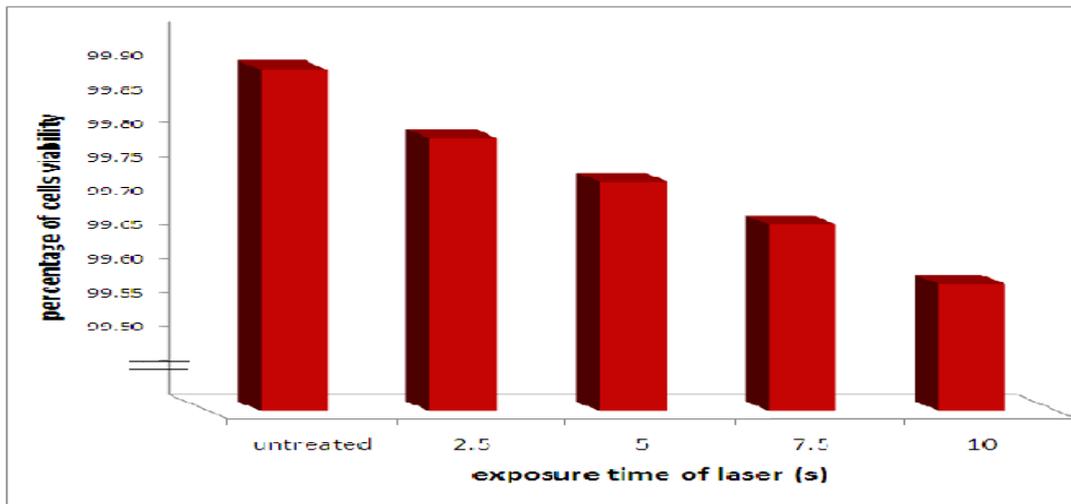


Figure 1. Histogram of the average percentage of lymphocyte cells viability for different He-Ne laser exposure times (doses)

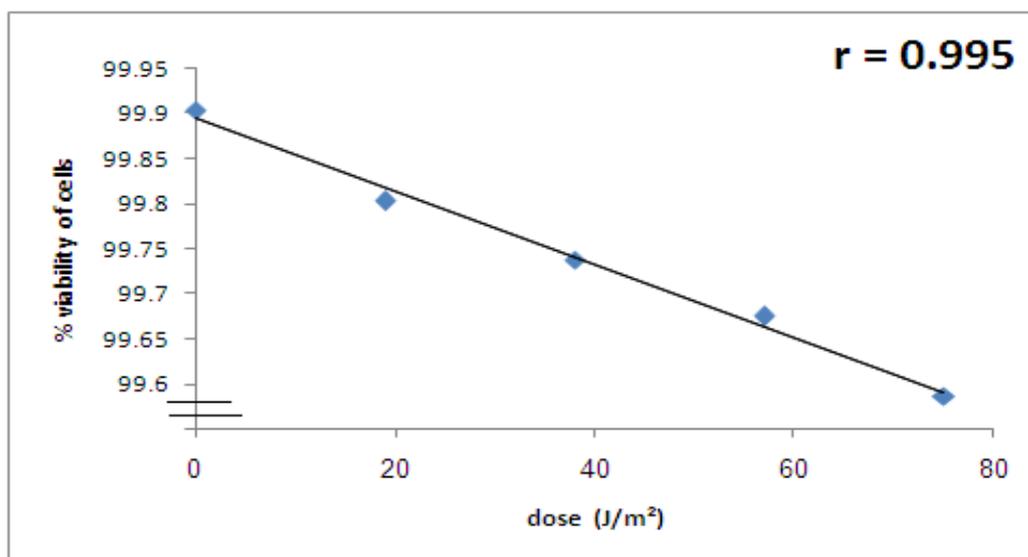


Figure 2. The relation between % viability of lymphocyte cells and the laser light energy doses

Table (2) represents the absorption optical density (OD) of DNA before and after He-Ne laser irradiation. These data showing that He-Ne (632.8 nm) irradiation alone employing the following three doses (18, 35, and 69 J/m²) causes a reduction in the DNA absorption (OD), which mean a reduction in DNA concentration survival compared with standard DNA (OD) results (untreated sample). The percentage of DNA survival after the three laser-irradiation doses are (88.7%, 87.4%, and 87.6%). These

results demonstrate a significant DNA damage immediately after laser irradiation, and it is independent on the He-Ne laser doses.

Discussion

The photobiological reaction in the cells to light in general and to He-Ne laser specifically is mainly related to the magnitude of the absorbed dose. He-Ne laser light (632.8 nm) induces many effects as result of a photobiological response, including increased

temperature as well as an electronical excitation of the photoacceptor molecules⁽²⁴⁾. The effect of the low dose used in this work of He-Ne laser is mainly due to electro-excited state rather than due to increase in the

temperature of the photoacceptor molecules, since the temperature elevation in the irradiated tissue is limited to less than 0.1→0.5 °C^(25,26).

Table 2. The optical density (OD) of DNA by spectrophotometer for before and after He-Ne laser irradiation, and the DNA survival % after laser irradiation

dose (J/m ²)	OD before laser	OD after laser	P value	DNA survival %
18	3.35±0.51	2.98±0.55	< 0.0001	88.6±6.8
35	3.41±0.38	2.98±0.44	0.0001	87.7±9.8
69	3.54±0.18	3.1±0.29	< 0.0001	86.1±7

In this work, the effect of low energy He-Ne laser (632.8 nm) irradiation alone immediately was examined on lymphocytes using different times of exposure (2.5, 5, 7.5, and 10 s) that give an energy values of (18, 35, 52.5, and 69 J/m²), respectively. These different energies or doses of He-Ne laser lead to a little degree of cell death or sub-lethal damage, since the mean viability percentage of lymphocyte cells are (99.8%, 99.74%, 99.68%, and 99.59%) respectively (Table 1) while figure (2) showed that the lymphocyte mean viability with doses of laser irradiated is highly correlated ($r= 0.99$). No cells protection was observed, because no incubation time was given after the irradiation. These results are in agreement with El-Batanouny and coworkers⁽²⁷⁾; they reported that low dose of He-Ne laser causes the lowest percentage in cells damage and promote the cell cycle of lymphocyte cells.

While the degree of cell damaging after He-Ne laser may be explained by other worker results, such as Stadtman 1992 who observed a significant decrease in lipid peroxidation and proteins damage post He-Ne laser irradiation, which cause a decrease in oxidative stress, this may become a threat to cells survival⁽²⁸⁾.

Other authors showed that the irradiation of human lymphocyte cells with a He-Ne laser can activate some short-term reactions in these cells, increase in chromatin template activity, lead to increase activation of mitochondrial

function concurrently with the formation of giant mitochondria⁽²⁹⁾.

Gulosooy et al. in 2006⁽³⁰⁾ reported that He-Ne laser caused increasing proliferation of blood mononuclear cells after 7 days of laser irradiation and suggested that the optimum He-Ne dose of 2.5 J/cm². But Dube et al. in 2001⁽¹⁴⁾ showed no significant effect of He-Ne laser of 1.5 kJ/m² doses on human B-lymphocyte line NC37 cells survival indicating that He-Ne laser has no cytotoxic effect on these cells.

Hu et al. in 2007 reported immediate rises in the growth factors such as cytochrome C oxidase enzyme, Adenosine Triphosphate (ATP) content, and JNK phosphorylation in melanoma cell line A2058 after He-Ne irradiation of dose 1.0 J/cm², which leads to significant cell proliferation after 3 days of irradiation⁽³¹⁾.

Gao and Xing in 2009⁽³²⁾ reported that low level laser (red and near infra-red light) is absorbed by mitochondrial respiratory chain resulting an increased reactive oxygen species and ATP/cAMP which initiates signaling cascade promoting cellular proliferation and cytoprotection.

In general, He-Ne laser can stimulate the intracellular or extracellular effects, which pass in the initial commitment phase since the cell responds to signal that commit the cell to undergo self-destruction^(30,33) showing that the irradiation of mononuclear cells with He-Ne laser can stimulate short term reactions and

irradiated cells did not enter S phase of the cell cycle. The intracellular effect such as the generation of singlet oxygen in the different cell type, which can stimulate a redox control over the parameters cellular homeostasis ^(19,34-36). The extracellular signals include receptor ligand, proteins, and activate calcium channels ⁽³⁷⁾.

In the current study, the results of the fraction DNA survival %, which measured immediately after the He-Ne laser irradiation alone, showed a significant degree of DNA damaging independent on the irradiation doses, (Table 2), no such DNA damaging was reported previously. This may be attributed, that most of the workers studied the effect of He-Ne laser irradiation on DNA within the cells and not extracted one ^(14,38). So, a further work required to investigate this situation.

Different mechanism may be involved in the protection phenomena. Since Manteifel et al. in 1999 ⁽³⁹⁾ demonstrated that the action of He-Ne laser irradiation excludes direct ruptures of covalent bond of DNA. Because the DNA do not have absorption bands in the visible spectral region, therefore, it is believed that the products of expressed genes are involved in the repair of DNA damage caused by the ionizing radiation ⁽⁴⁰⁾. This hypothesis further supported by Kohli et al. in 2001 ⁽⁴¹⁾, they observed that He-Ne laser pre- irradiation on E. coli strain KY706.pPL-1 leads to the induction of photolysis gene "phr". Ihara et al. in 1987 ⁽⁴²⁾ suggested the role of singlet oxygen in induction of phr gene, the magnitude of the gene induction depend on the laser fluence, the photon energy of He-Ne laser irradiation may induce singlet oxygen which leads to sub-lethal damage of DNA. The singlet oxygen species response to the transcription of UVrA, UVrB, recA, and UmuDC genes, which trigger the DNA repair processes ^(43,44).

In conclusion, the percentage of lymphocytes survival is decreasing with increasing dose of He-Ne laser and longer exposure time. He-Ne laser irradiation causes a significant degree of

DNA damaging independent on the irradiation doses.

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Author contributions:

Dr. Abdullah and Taha did the sample collection, procedure and writing of the manuscript, Dr. Ahmed participated in medical consultation and final revision of the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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