Original Article

Protection Properties of He-Ne Laser (632.8 nm) Against UV-light (253.7 nm) On the Lymphocytes Blood Cellsand Its DNA.

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Summary:

Background: He-Ne laser (632.8 nm) pre-irradiation may lead to modulate the damaging effects of ionizing radiation and decreasing in radiation damage on cells, by the induction of antioxidant defence mechanisms and accelerated the repair.

Objective: To study the protection properties of He-Ne laser (632.8 nm) pre-irradiation against UV light (253.7 nm) damage on human lymphocyte blood cells and its DNA.

Subject & Methods: 27 samples were processed only for lymphocyte blood cells separation, while a 45 samples for the extracted DNA from lymphocyte. The total of 72 sampleswere employed to evaluate the influence of He-Ne laser pre- irradiation against UV-light.

Results: The results showed that He-Ne laser (632.8 nm) pre-irradiation on human lymphocyte blood cells and its DNA leads to a decrease in the damage which caused by UV light (253.7 nm) irradiation.

Conclusion: The magnitude of protection depends on the He-Ne laser pre-exposure doses. A higher survival percentage of lymphocyte blood cells and its DNA occurred with low He-Ne laser dose.

Keywords: He-Ne laser (632.8 nm) irradiation, UV-light (253.7 nm) irradiation, lymphocyte blood cells and its DNA protection.

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Introduction:

The Biostimulatory or the damaging effects of laser irradiation on cells or tissues are determined by the magnitude of the absorbed energy, which depends on many factors: wavelength of laser source, power, exposure time, and characteristics of absorption and scattering of tissue. He-Ne laser (632.8 nm wavelength) has low photon energy and power output, which may produce minimum biomolecular damage. Because it elevate the irradiated cells temperature to less than 0.5 ºC (1), therefore the irradiation of He-Ne laser cause photochemical interaction with the cells rather than thermal effect. Laser irradiation in this red spectral area increases the proliferative activity of cells and promotes tissue repair (2) (3).Since ionizing energies such as X and γ- rays, UV light and α- particles cause cell and tissues damaging. Therefore a lot of works have been carried out, showing that the low- level laser irradiations modify the response of cells to ionization. Laser light when performed after the exposure to ionizing radiation causes an increase in mitotic activity of cells and tissue regeneration (4) (5). But the photoprotective effect of He-Ne laser light is more significant when cell irradiation is performed prior to the exposure of ionizing radiation, such as X-ray (4), and UV light (6) (7) and IR light (8). The toxic effect of UV light on cells is well known through the generation of the free radicals. However the UV light with a shorter wavelengths (UV) (280-200 nm) are the most potent inducer of DNA-damage and then cell death (9). Recently many workers showed that He-Ne laser pre-irradiation, on different types of cells, lead to increase DNA repair and then rising cells survival against subsequent UV-light exposure. They conclude that the protection properties of He-Ne laser depends on the He-Ne laser exposure time (dose), the period of the incubation between He-Ne laser exposure and subsequent UV-light irradiation, and the UV doses (6). Since most of the previous works show the pre-irradiation protection effect of He-Ne laser (632.8 nm) against UV exposure on different cultured cells such as E. coli cells, carcinoma cells, hela cells. The aim of this study is to investigate the He-Ne laser pre- irradiation protection ability on human blood lymphocyte cells and its DNA using different He-Ne laser doses against UV light of wavelength (253.7 nm).

Material & Method:

To assess the effect of He-Ne laser pre-irradiation against UV light on human blood lymphocyte cells and its DNA, a 72 blood samples were taken from healthy volunteers, their age ranging from 19-45 year, with mean of 32.4 ± 7.68 (47 female and 25 male). The 72 blood samples were divided into two groups. The first group, which consists of 27 samples, were used to estimate the pre-irradiation effect of He-Ne laser against UV light on lymphocyte cells. Therefore these samples were processed only for peripheral blood lymphocytes cell

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separation (PBL) using boyum method (10).The second group of 45 blood samples were employed to evaluate the influence of He-Ne pre-irradiation against UV light on the extracted DNA from the lymphocyte cells. So in this group the 45 samples were processed for DNA extraction. The degree of damage by He-Ne laser and UV light irradiation on lymphocyte cells number, and DNA concentration measured by the haemocytometer, and the spectrophotometer respectively. A continuons He-Ne laser beam, of 1 mm diameter and 632.8 nm wave length was employed (Griffin and George, Britain). The laser maximum out put powerwas 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 converginglens) 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/m^2) 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/m^2). UV light from Philips, of 253.7 nm wave length and a power of 20 W was used. The samples exposed to UV light for 5 min, which is equal to energy dose of 2.71 kJ/m^2.27 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/mm^3. Then this part exposed to the UV light of energy dose (2.71 kJ/m^2) and the viable cells number was checked again. To estimate the influence of the He-Ne laser pre-irradiation against UV light on lymphocyte cells viability, the others 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/m^2). Afterwards these parts were incubated at room temperature for 30 min before the UV light irradiation. Again the cells viability was counted. After each irradiation the fractional of cell survival % was evaluated relative to untreated (standard) cells viability.The protection % of cells induced by He-Ne laser pre-irradiation doses against UV light, were calculated using equation (1) [(N-$N_.$)/ $N_.$] x 100…….. (1). Where N is the number of the cells viable after He-Ne laser pre-irradiation against UV light, $N_.$ is the number of cells viable after UV light irradiation alone.45 blood samples were used to study the effect of He-Ne laser pre-irradiation against UV light (253.7 nm) on the DNA. The DNA was extracted from human blood lymphocyte cells using phenol–chloroform method (11). A part from each extracted DNA sample was employed to evaluate the DNA purity. The optical density (O.D.) of DNA which is measured by spectro- photometer at UV wavelength of 260 nm for the untreated sample part was used as a standard.The remained DNA sample divided into two parts. The first part exposed to UV light (253.7 nm) only for five min. The second part firstly irradiated with He-Ne laser beam (632.8 nm), and then incubated for 45 min at room temperature. After the incubation period, this part again exposed to UV light for 5 min.After each irradiation theO. D. 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 period 2.5, 5, or 10 s (doses 18, 35, 69 J/m^2).

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 (12).

Results:

Lymphocyte blood cells results:The exposure of lymphocyte blood cells to the UV light irradiation of energy dose (2.71 kJ/m^2), indicate a significant reduction in the fraction of cells survival percentage 73.87 % ± 7.2 with a $P < 0.001$. This result it is significantly lower than that induced by the different laser light used doses (95.5 %, 90.6 %, 87 %, and 82.5 %), table (1).The table also show that the He-Ne laser pre-irradiation against UV light irradiation, give a higher survival percentage of lymphocyte cells (91, 86.1, 81.8, and 78) compared to the cells survival % after the UV light irradiation alone (73.87 %). But generally, the fraction of cells survival percentage for all doses used are still higher than the survival cells % exposed to UV light only, figure (1). The percentage of cells protection, which calculated according to equation (1), decreases with increasing the doses of laser, table (1).

Table (1): the fraction of the cells survival % after laser irradiation alone, pre-irradiation against UV light, and its protection %.

He-Ne laser	He-Ne laser		Laser + UV		Protection cells %
Dose (J/m^2)	Mean ± S.D.	P-value	Mean ± S.D.	P-value	
18	95.5 % ±2.7	<0.0001	91 % ± 4.3	<0.0001	22.7
35	90.6 % ±4.2	<0.0001	86.1 % ±5.5	<0.0001	17
52.5	87.0 % ±5.8	<0.0001	81.8 % ±7.1	<0.0001	11
69	82.5 % ±7.6	<0.0001	78 % ± 7.8	0.028	5.35

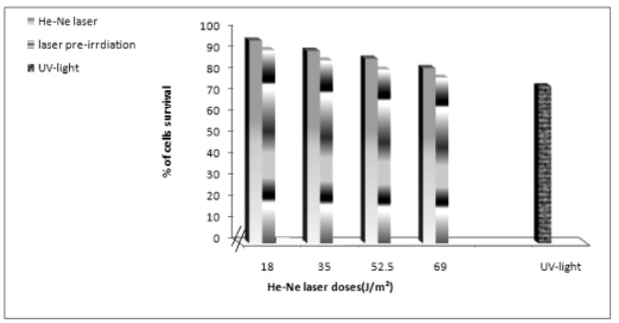

Figure (1): The fraction of cells survival % after UV light irradiation, The He-Ne laser irradiation, He-Ne laser pre-irradiation against UV light irradiation at different He-Ne laser doses.

DNA results:45 blood samples were used in this part of study divided into three groups according to the He-Ne laser irradiation doses used (18, 35, and 69 J/m^2). After the UV light irradiation alone, the data indicate a significant reduction in the DNA survival, where the average of DNA survival % for the total 45 samples is 64.5%, and the damaging % is 35.5%.The average percentage of DNA survival after each laser irradiation doses are (88.6 %, 87.7 %, and 86.1 %) respectively. These results demonstrate a significant DNA damage immediately after laser irradiation, and it is independent on the He-Ne laser doses, table (2).

Table (2): The percentage of DNA survival after He-Ne laser irradiation alone, He-Ne laser pre-irradiation, and the protection percentage of pre-irradiation laser for three used doses.

LaserDose (J/m^2)	Laser irradiation	Laser + UV light	p- Value	Protection%
18	88.6 % ± 6.8	82 % ± 7.6	< 0.0001	33.9
35	87.7 % ± 9.8	81.57 % ± 9	< 0.0001	32.7
69	86.1 % ± 7	80.97 % ± 12	0.005	27.4

The results of DNA survival percentage after the combined action of He-Ne laser pre-irradiated against UV light (82, 81.57, and 80.97) are higher compared to the UV light irradiation alone (64.5 %), table (2) and figure (2).

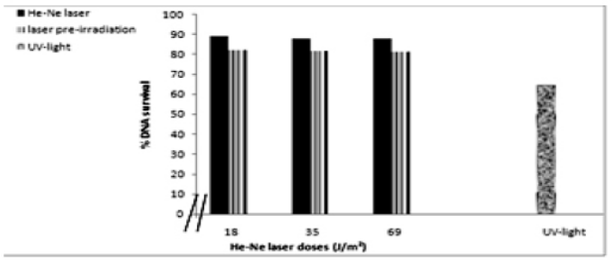

Figure (2): The histogram of DNA fraction survival % after He-Ne laser irradiation alone, He-Ne laser pre-irradiation against UV light irradiation UV irradiation alone.

Table (2) also demonstrate that He-Ne laser pre-irradiation has a significant protection property against UV light irradiation alone (33.9%, 31.7%, and 27.4%). The magnitude of protection decrease gradually with He-Ne laser doses and its better at the lower dose (18 J/m^2).

Discussion:

Immediately after UV light irradiation alone, the viability of the lymphocyte cells results showed a significant decrease in cells survival, so UV light can be triggered as a toxic radiation to lymphocyte blood cells. Since different wavelengths of UV light producing a variety of effects, either through direct DNA damage, or cell cycle control by genes encode protein, or via the reactive oxygen species (ROS) generation in nucleus, plasma membrane, cytoplasm, and mitochondria (13).The effect of He-Ne laser (632.8 nm) irradiation alone immediately shows a degree of cell death or sub-lethal damage, table (1), figure (1). These results are in agreement with El Batanouny and coworkers (14) study, they reported that low dose of He-Ne laser cause a decrease in cells damage percentage and promote the cell cycle of lymphocyte cells. Stadtman (15) observed a significant decrease in lipid peroxidation and proteins damage post He-Ne laser irradiation which cause a decrease in oxidative stress, this may becomes a threat to cells survival. Hu et al. (16) reported immediate rises in the growth factors such as cytochrome C oxidase enzyme, ATP gntent, and JNK phosphorylation in melanoma cell line A2058 after He-Ne irradiation of dose 1 J/cm^2 which leads to significant cell proliferation after 3 days of irradiation. Gulosoy et al. (17) reported that He-Ne laser increasing proliferation of blood mononuclear cells after 7 days of laser irradiation and suggested the optimum He-Ne dose of 2.5 J/cm^2. But Dube et al. (7) showed no significant effect of He-Ne laser of 1.5 kJ/m^2 doses on human B- lymphocyte line NC37 cells survival indicating that He-Ne laser has no cytoxic effect on these cells. Different studies reported the effect of the pre-irradiation by the He-Ne laser on the survival of different cells types against UV light irradiation. The results obtained in this study are in agreement with other works showing an increase in cells survival after exposure to UV light irradiation following pre-irradiation by He-Ne laser irradiation, even though they used different experimental conditions such as exposure time (different doses), and incubation period between the helium-neon laser exposure, and subsequent UV irradiation doses and wavelength, and types of cells (6) (8) (7). The processes of protection by pre-irradiation of He-Ne laser are not under stood. It may be a biological an adaptive response of lymphocyte cells to He-Ne laser irradiation making them more resistance to UV light irradiation through the stimulation of their antioxidant defence system. Urcinoli and coworker (18) reported that the He-Ne laser decrease the oxidative stress of UV light radiation from induced DNA damage by increase the activity of antioxidant enzymes. Kohli and coworker (19) revealed that the oxidative

stress due to He-Ne laser irradiation-induced generation of singlet oxygen leads to sub-lethal damage of cells, which may induce better cells repair. 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 (6) (7). So a further work required to investigate this situation. The results also revealed that the combined action of He-Ne laser and UV light irradiation with an incubation period of 45 min between the two irradiation increase the fraction of DNA survival % from 64.5% after UV light irradiation alone to (82 %, 81.57 %, and 80.97 %) relative to the He-Ne laser used doses, figure (2). The pre-irradiation of He-Ne laser indicate a significant decrease in DNA damage, and give a protection % of (33.9 %, 32.7 %, and 27.4 %) related to the He-Ne laser doses, table (2). Karu et al (20), Karu et al. (21), and Abvakhitova et al. (4) observed that the DNA protection against ionizing radiation, such as γ- ray, UV light by the pre-irradiation of He-Ne laser light on a number of cells types in conjunction with present findings depending on laser doses and dose rate, and also on the incubation period between the two irradiation (suggesting more than 30 min and the optimum time being one hour). Different mechanism may be involved in the protection phenomena. Since Manteifel et al. (22) demonstrated that the action of He-Ne laser irradiation excludes direct ruptures of covalent bond of DNA, because the DNA does not have absorption bands in the visible spectral region. So it is believed that the product of expressed genes are involved in the repair of DNA damage which caused by the ionizing radiation (23). This hypothesis further supported by Kohli et al. (24) they observed that He-Ne laser pre-irradiation on E. coli strain KY706.Ppl-1 leads to the induction of photolyase gene, phr. Ihara et al. (25), suggested the role of singlet oxygen in induction of phr gene, the magnitude of the gene induction depend on the laser fluence, fluence rate and post -irradiation incubation period. The photon energy of He-Ne laser irradiation may induce singlet oxygen which leads to sub-lethal damage of DNA.

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