Original Article

The effect of laser radiation on DNA damage and repair

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Summary:

Background: The effect of Helium Neon laser (He-Ne 632.8 nm) was reported to protect cells from damage. We studied lymphocyte cells pre irradiated with (UVC 260 nm) to induce DNA damage. Investigations were carried using gel electrophoresis and test for cell viability. It has also been reported that effect depends on incubation period after damage. The extent of damage to the cells depends on the period of irradiation with UVC also on its intensity.

Objective: In this work we studied the effect of UVC on DNA damage and cell survival
Also study of the effect of He-Ne laser on cell survival after all being pre irradiated with UVC light and its protective effect on DNA post UV damage.

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Method: This study was conducted in pathology department post graduate laboratory - College of medicine-Baghdad University. The total number of samples was (147). Blood samples were collected from healthy donors came to the blood bank, the amount of blood drown varies from 5ml to 7ml in heparin tubes .The work was carried out during the period between November 2010 to August 2011. In this experiments examination of samples was carried out to test the radiation effect on cell viability by using trypan blue dye, the experiments were preformed after 1, 24 and 72 hours post UVC irradiation to test the repair development. In other experiments Gel electrophoresis were carried out on samples to study the effect of radiation on the DNA fragmentation.

Result: Results reveal a reduced DNA fragmentation appeared on gel electrophoresis experiments as the smear length is reduced significantly for both UV10 and UV20 , other results for cell viability tests revelled that He-Ne can increase survival of cells pre irradiated with UVC irradiation giving (66%, 57%, 70%(improvement in UV exposure for10 min and (59%,56%,59%) improvement for UV exposure for 20 min respectively .

Conclusion: The effect of the laser in the improvement of cell survival may be attributed to the induction of endogenous radioprotectore and probably enzymes induced by laser irradiation which may be either reduce the free radical by scavenging effect or by improved cell repair, we may conclude that He-Ne laser can protect cells from radiation damage

Keywords: DNA damage, Low Level Laser Therapy (LLLT), laser and cell survival, UVC damage and cell survival.

Introduction:

Laser is a device that creates and amplifies electromagnetic radiation of a specific frequency through the process of stimulated emission (1). There are several types of laser such as Chemical lasers, Solid-state lasers, Semiconductor laser, Dye lasers, Excimer lasers, Gas lasers (2,3,4,5,6) . The laser may have high energy density which can he used as focused laser in surgery or Low Level Laser Therapy (LLLT) with power density (1mw -5mw /cm^2) the LLLT is completely safe and the advantage of LLLT is take place in all organs and tissues of the body for creation of good cellular function such as the treatment of both acute and chronic the pain and stopping a tissue influx of fluids the disappearance of swelling reduce and heat in addition the speeding up of bone repair from the stimulation offered by fibroblastic and osteoblastic proliferation increase blood circulation (7.8.9.10).

Laser can affect cell because the light reacts with the cell and absorbed within the mitochondria and the infrared absorbed at the cell membrane, this results
in changing in the membrane permeability in a mammalian cell and increased ATP levels.(11). The ultraviolet UV is electromagnetic radiation with a wavelength shorter than visible light and longer than x-ray the spectram of ultraviolet light range from 100 to 400 nm and energies from 3ev to 124 ev and is divided into UVA,UVB, UVC (12 ,13) . UV light kills cell by damage their DNA (14) it can produce many types of DNA damage single strand, double strand break and thymine dimer,(15,16). The defective piece of DNA can be repaired by different mechanisms such as excision repair and photo reactivation. (17, 18).

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Material and method:

The work was carried out during the period from November 2010 to August 2011.It was conducted at the pathology department post graduate laboratory - College of medicine-Baghdad University. A study of He-Ne laser effect on the cell damage and repair was preformed on (147) blood samples that were collected from adult healthy donors came to the blood bank .The amount of blood drown varies from 5ml to 7ml in heparin tubes to prevent blood clotting and the samples were tested for diseases such as HIV, malaria, and viral hepatitis. The cells were irradiated by two types of irradiations laser and UV. Laser irradiation The He-Ne laser (632.8 nm) operating in continuous wave with output power 1mw and spot diameter 2mm was used to irradiate the cells for a period of 10 min at a distance of 10 cm. The cells and the medium were swirled every two min to ensure that all cells receive the same amount of laser irradiation. UV irradiation After laser irradiation was completed cells were incubated for one hour prior to UV irradiation. The UVC (260nm) irradiation to the cells was operated in continuous wave with power 15 W and positioned at a distance of 10 cm from cell suspension with mixing the cells once every 2 minute to ensure homogenous irradiation of the cell suspension which was irradiated For two periods (10 and 20 min) in all experiments the cells were kept on ice this was done to retard cellular repair processes and thereby conserve DNA damage induced during irradiation. Gel electrophoresis Gel electrophoresis was carried out on DNA of lymphocyte post UVC irradiation in these experiment we assessed the DNA fragmentation after UVC irradiation. Other experiments were carried out for the assessment of the DNA fragmentation for pre irradiated cells with laser with 1h incubation then exposed to UV light. DNA was extracted from $5x10^6$ cells then it was tested for purity by using spectrophotometry (19). Cell culture procedure; before each experiment the cells concentration of lymphocytes were counted by microscopic examination using a neubauer haematocytometer. After irradiation the cells were grown in a suspension in 5ml RPMI 1640 medium with 10% fetal calf serum and 0.4 mg/ml of PHA grown at 37°C for 3 days without changing the nutrient medium and without CO_2 supply. This enables us to examine the effect of radiation during and after the cell division after 24 h and 72 h then cells was tested for viability (20). Viability test using trypan blue To check the viability of the cells with trypan blue cells were centrifuged to remove the medium and resuspended in (PBS PH =7.4) and checked by viewing under a microscope with phase contrast option the dead cell discriminated visually From live cells because of their darker appearance(21).

Statistical analysis

Results were expressed in mean ± SD Change percentage of mean has been calculated and used for comparison between results. Results were also tested for significance. Unpaired student test (t-test) was preformed for compression between the two groups, the difference was considered to be significant at 0.05 levels. ANOVA has also been carried out to test the changes between the mean value of total cells number, living cells, dead cells and percentage survival

Results:

Gel electrophoresis

Gel electrophoresis shows the difference in band length depending on time of irradiation. Samples which were irradiated by UVC for 10 min gave shorter smear than those irradiated for 20 min. If the cells were irradiated for 10 min with He-Ne laser prior to UV irradiation and incubated for 1 hour before UV irradiation the short smear became approximately a band similar to control and the long smear will be short. Other experiments were performed, in these experiments the cells were irradiated for 10 min by UV radiation prior to laser exposure tested for DNA fragmentation such experiments did not show a significant effect on DNA fragmentation. These results are shown in table 1

Table1: Frequency distribution of appearance of DNA on gel electrophoresis

Type of electrophoresis	control	UV 10	Laser+ UV10	UV 20	Laser+ UV20	UV10+ laser	laser
band	9	0	4	0	0	0	6
Thick band	0	0	5	0	0	0	3
Short smear	0	2	0	0	9	4	0
Long smear	0	7	0	9	0	5	0

Abbreviation as in table 1:-
UV10= Ultraviolet exposure for 10 min
UV20= Ultraviolet exposure for 20 min
B- Cell viability test Using Trypan Blue

Results for the survival test revealed that in cells exposed to laser only has a small effect was on cell survival compared with control appeared on the trypan blue test other results were appeared when laser irradiation administered Post UV10 irradiation which has given small change from cell survival exposed to UV only, table (3-3).A significant improvement was observed when laser irradiation administered one hour prior to UV10 irradiation (66%, 57% and70 %) for UV10 and for periods of 1, 24 and 72 hours incubation respectively. A significant improvement has also been observed for cells irradiated with laser and given one hour period prior to UV20 irradiation the improvement was (59%, 56% and 59%) for 1, 24 and 72 h respectively. In other experiments the cells were irradiated for 10 min by UV radiation prior to laser exposure tested for cell viability Such experiments did not show a significant effect on cell survival it has given insignificant change between cells irradiated with UV only or UV+laser

Table 2: Viable Cell Counts Using Trypan Blue

Type of radiation	Survival after 1h ± SD	Survival after 24h ± SD	Survival after 72h±SD with PHA
control	94% ± 1.7	89% ± 0.9	88% ± 0.5
UV10	45% ± 1.2	51% ± 4.9	44% ± 1.2
Las+UV10	66% ± 2.2	57% ± 4.0	70% ± 1.2
UV20	38% ± 2.2	35% ± 3.4	29% ± 7.6
Las+UV20	59% ± 1.0	56% ± 1.2	59% ± 0.9
UV10+las	50% ± 5.4	44% ± 4.5	43% ± 1.2
Laser10	87% ± 0.9	85% ± 0.8	76% ± 1.7

The effect of laser on cell survival test by trypan blue after exposure to UV10and UV20

Discussion:

The He-Ne laser has provided protection which appeared both on cell survival and DNA fragmentation against UVC light irradiation. These results can be observed on the cell survival of cells exposed to UV10 tested by trypan blue for the three post irradiation periods (1, 24 and 72 h) which resulted in (45%, 51% and44 %) Survival respectively compared with control (this is the effect of UV for 10 min on cell survival). The survival was increased to (66%, 57% and 70%) when cells exposed to laser first and left for 1h incubation at 37°C prior to UV10. A similar results was observed for cells exposed to UV20 minutes it has given survival of (38%, 35% and29 %) relative to control. When those cells were treated for 10min laser, given one 1h incubation time at 37°C then exposed to UV20 irradiation it has given cell survival (59%,56% and 59%) representing an increase of (21 %, 21%, and30 %) respectively. To investigate and confirm these results we have preformed gel electrophoresis. These experiments have shown an increased smear length for cells exposed to UV10 or UV 20 (2 short smears with 7 long smears for 10min UV exposure and 9 long smear for 20min UV exposure) . While those exposed to laser given 1h incubation prior to UV exposure have given shorter smears length almost a band for 10min exposure similar to control. And short smears for 20 min UV exposure. These results indicate that low power laser He-Ne can improve cell survival for cells damaged with UV radiation (20). The mechanism of the He-Ne laser induced protection appears to be a sort of the adaptive response and this follows because He-Ne laser irradiation has been reported to lead to generation of single oxygen(22), and also the observation that He-Ne laser has led to an increase in the activity of antioxidant enzymes(23,24)

Conclusion:

UV has a damaging effect on DNA and this damage is dose dependent, as UV20 has inflicted more damage than that of UV10. Laser can improve survival significantly when given1hr prior to UV irradiation. The survival improvement by using laser may be attributed to the induction of endogenous radio protectors and/or it may provide some protection.

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