Biological Effects of Yellow Laser- Induced of Cell Survival: Structural DNA Damage Comparison is Undergoing Ultraviolet Radiation Photocoagulation

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Abstract— In this work, we have a tendency to study lymphocyte cells survive before irradiated with a yellow laser (578nm) , and its protecting of deoxyribonucleic acid (DNA) damage once being irradiated with ultraviolet radiation (UVB-320nm) to activate DNA damage. Total range of blood samples (200) is collected from healthy donors. Donor blood volume varied from 5ml to 7ml in heparin tubes. Samples dole out to check the radiation effect on cell viability by using the trypan blue exclusion. The experiments implemented during 1, 24, 48 and 72 hours prior UVB to achieve the repair development.DNA gel electrophoresis technique is performed on samples to check the effect of radiation on the separating DNA molecules of varying sizes extending. The results show a decrease of separating or breaking of DNA manifest on gel electrophoresis experiments as a result of the smear length is reduced significantly for UVB, alternative results for cell viability tests showed that yellow laser ought to increase the survival of cells before irradiated with UVB demonstrating (91%, 87% , 80% , and 71%) amelioration.Improvement of lymphocyte survival by yellow laser can be attributed to the induction of endogenous radiation protection and doubtless enzymes elicited by laser irradiation that may be scaled back the free radical either by scavenging concern or by improved cell repair, we have a tendency to may conclude that yellow irradiation shall shield cells from radiation damage.Abstract must be of Time New Roman Front of size 10 and must be justified alignment.

Keywords— Ultraviolet Radiation, Deoxyribonucleic Acid, Cell Survival, Viability, Yellow Laser.

INTRODUCTION

The form LASER stands for Light Amplification by Stimulated Emission of Radiation. Laser is a device that creates and amplifies electromagnetic wave of a particular frequency through the method of stimulating emission[1, 2]. Lasers work as a result of optical resonator[3].

In a basic laser, a chamber known as an optical cavity is purposed to internally Reflect Infrared, visible light or ultraviolet waves in order that they reinforce one another[4]. The cavity will contain gases, liquids, or solids. The monochromatic output of laser or laser with high frequency stability[5]. The laser might have high energy density, which might use as centered laser in surgery or Low Level laser therapy (LLLT) with power density (1-5)mW /cm^2[6].

LLLT is very safe and therefore the advantage is going down in all organs and tissues of the body for the creation of excellent cell perform[7] such as, treatment of each acute and/or chronic pain[8], stopping a tissue flow of fluids, disappearance of swelling reduction and heat[9].Additionally speeding up of bone repair from the stimulation offered by fibroblasts and osteoplastic proliferation increase blood circulation[10].

The laser will have an effect on the cell as a result of the light reacts with the cell and absorbed among the mitochondria and therefore the infrared absorbed on the plasma membrane, this leads to ever changing within the membrane permeability during a class cell and increased Adenosine triphosphate (ATP) levels[11].

Ultraviolet (UV) light is a type of electromagnetic radiation with a shorter wavelengths than visible light and longer than x-ray the spectrum of ultraviolet light varies from 100 to 400nm and energies from (3-124) eV and is split into UVA, UVB, UVC. The absorption of UV by DNA might cause cancer changes, kills cells by damaging their DNA[12, 13]. This happens as a result of

chemical reactions, prone to deteriorate the genetic code, is triggered by the surplus energy deposited by the UV absorption[14]. The excitation later on transfers to different components of the double helix, losing energy at every step[15]. It will manufacture many varieties of the DNA damage single strand, double strand break and thymine dimer[16]. The defective piece of DNA will be repaired by completely different mechanisms like excision repair and photoreactivation.[16] Up to now, it absolutely was not renowned however. This excess energy was distributed among the bases of the DNA. It absolutely was solely postulated that every base absorbs a photon separately.

MATERIAL AND METHOD:

This study is undertaken during the period from January 2013 to January 2014. It was mentioned in the pathology laboratory, Al-Hilla General Hospital. A study of the yellow laser effect on the cell damage and repair was performed on (200) blood samples that were obtained from adult healthy donors came to the hospital for a medical checkup. The amount of blood drawn varies from 5ml to 7ml in heparin tubes to prevent blood clotting and the samples were checked for diseases such as HIV, malaria, and viral hepatitis. Two types of irradiations (yellow laser and UVB) irradiated the cells.

Laser irradiation done by using 578nm yellow light with an output power of 10mW, operating in continuous wave and spot diameter 0.6mm was utilized to irradiate the cells for a period of 10 min at a distance of 2cm.The cells and the medium were rotated everyone min to ensure that all cells receive the same amount of laser irradiation.

UVB irradiations after laser irradiation was completed cells were incubated for one hour prior to UVB irradiation. The UVB (320nm) irradiation to the cells was operated in continuous wave with power 25W and positioned at a distance of 10 cm from cell suspension with mixing the cells once every 2 minutes to ensure homogenous irradiation of the cell suspension, which was irradiated for period 10 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 was performed on DNA of lymphocyte post UVB irradiation in these experiments we assessed the DNA fragmentation after UVB irradiation. Further experiments were performed for the assessment of DNA fragmentation before irradiated cells with laser with 1h incubation then exposed to UVB light.

DNA was extracted from 6x104 cells then it was tested for purity by using spectrophotometry. Cell culture procedure; before each experiment the cell concentration of lymphocytes counted by microscopic examination using a Neubauer Chamber Cell Counting.

After irradiation, the cells were grown in a suspension in 5ml RPMI - Roswell Park Memorial Institute 1640 medium with 10% FBS - Fetal Bovine Serum and 0.4 mg/ ml of PHA- Phytohemagglutinins grown at 37oC for 3 days without changing. The difference was estimated significant at 0.05 levels. ANOVA has also been undertaken to test the changes between the mean value of total cell number, living cells, dead cells, and percentage survival.

RESULTS:

Gel electrophoresis indicate the difference in bond length. Samples, which were irradiated by UVB for 10 min, gave shorter smear. If the cells were irradiated for 10 min, with 578nm yellow laser beam prior to UVB irradiation and incubated for 1 hour before UVB 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 UVB radiation prior to laser exposure tested for DNA fragmentation such experiments did not demonstrate a significant effect on DNA fragmentation. These results are shown in figure 1.

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 appearing when laser irradiation administered post UVB irradiation which has given small change from cell survival nutrient medium. This allows us exposed to UVB only, Table 1.

A significant improvement to examine the effect of radiation during and after the cell division after 24 h, 48 h and 72 h when cells were tested for viability.

Viability test using trypan blue to check the viability of the cells with trypan blue cells were centrifuged to remove the medium and re suspended in PBS – Phosphate Buffered Salines - 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.

STATISTICAL ANALYSIS:

Results expressed in (mean ± SD). Change, a percentage of the mean has calculated and used for comparison between the results. The results were further analyzed for significance.

Unpaired student test (t-test) performed for compression between observed when laser irradiation administered one hour prior to (91%, 87%, 80%, and 71%) for periods of 1, 24, 48, 72 hours of incubation respectively.

Another experiment, cells irradiated for 10 min by UVB radiation prior to laser exposure tested for cell viability. Such experiments did not show a significant effect on cell survival, which, given an insignificant change between cells irradiated with UVB only or UVB +laser.

DISCUSSION:

The yellow laser has provided protection, which was both cell survival and DNA fragmentation[17, 18] against UVB light irradiation[13].

These results are observed on the cell survival of cells exposed to UVB tested by trypan blue for the four post irradiation periods (1, 24, 48, and 72 h) which resulted in (40%, 48%, 42%, and 46%). Survival extended to (70%, 66%, 69%, and 73%) when cells exposed to yellow laser and left for 1h incubation at 37oC prior UVB.

To investigate and confirm these results we have performed gel electrophoresis. These experiments have shown an increased smear length in cells exposed to UVB 3 short of smears with 11 long smears for 10 min UVB exposure.

While those exposed to laser given 1h incubation prior to UV exposure have bestowed shorter smears, length almost a band for 10 min exposure compared with control.

These results indicate that low power laser yellow laser can improve cell survival for cells damaged by UV radiation. The mechanism of the yellow laser induced protection appears sort of the adaptive response and this follows because yellow laser irradiation has reported leading to generation of singlet oxygen and the observation, that yellow laser has led to an increase in the activity of antioxidant enzymes.

CONCLUSION:

UVB is very a matter of damaging effect on DNA and this damage is dose dependent. These characteristics are derived from the UVB photon's energy to transform chemical bonds in molecules, even without having sufficient energy to ionize.

The yellow laser can improve cell survival significantly when gave a time about 1hr prior to UVB irradiation. The cell survival improvement by using yellow laser may be attributed to the induction of endogenous radio protectors or it may provide some protection.

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Table1: Trypan blue used to determine the number of viable cells after exposure with yellow laser, UVB, laser before UVB and UVB before laser

Type of Radiation	Cell Survival after 1h ± SD	Cell Survival after 24h ± SD	Cell Survival after 48h± SD	Cell Survival after 72h±SD
Control	92% ± 1.9	86% ± 0.6	83% ± 0.6	80% ± 0.5
Yellow Laser	91% ± 0.6	87% ± 0.3	80% ± 0.9	71% ± 1.2
UVB	40% ± 1.5	48% ± 3.1	42% ± 0.8	46% ± 1.4
Yellow Laser + UVB	70% ± 2.7	66% ± 5.0	69% ± 0.4	73% ± 1.7
UVB + Yellow Laser	59% ± 2.4	46% ± 2.5	48% ± 0.8	50% ± 1.6

Figure 1: Classification of manifestation of DNA in gel electrophoresis according to size.