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Stimulation of Phagocytic Activity of Human Polymorphonuclear Leukocytes in Vitro Using 10 mW He-Ne Laser.

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Abstract: Stimulative effect of 10 mW He-Ne laser on the phagocytic activity of human polymorphonuclear leukocytes(PMNs) has been studied in vitro. Normal polymorphonuclear leucocytes were isolated from the human peripheral blood. A mixture of 0.25 ml of Hanks solution, 0.25 ml of serum, 0.25 ml of Candida albicans suspension and 0.25 ml of PMNs suspension was prepared. The samples of mixture of PMNs and Candida were subdivided in 1 ml ependrof tubes and irradiated to He-Ne laser for 1, 3, 5, 10 and 20 min. The diameter of the irradiated area was 0.8 cm. For calculation of Phagocytic index before and after irradiation, the samples were incubated (37°C) at 5, 15, 30, 60 min. The slides of samples were prepared and stained using Giemsa stain. The results showed that the bio-stimulative effect of 10 mW He-Ne laser on the phagocytic activity of(PMNs) is more observable at 3 min exposure time with 5 mW/cm^2 power density. Many action mechanisms were reviewed and discussed in the term of possible photo- acceptor when cells are irradiated to laser.

Introduction

In laser- tissue interaction, the incidence laser light on the certain tissue may be undergoing reflection, scattering, transmission or absorption. The effects of lasers on biological tissue are complex processes resulting from two interaction mechanisms: wavelength dependent interaction mechanism that includes: photothermal and photochemical interactions. In photothermal interaction the elevation of temperature is the dominant feature leading to: coagulation, vaporization, and carbonization and photoablation due to thermal stress .In photochemical interaction, the main results are photodynamic action, biostimulation and photoablation due to volume stress then bond breaking (Niemz, 1996).

Phagocytosis by phagocytic cells is a crucial part of the host defense against invading microorganisms. The most important cell types involved are polymorphonuclear leukocytes. (PMNs) and mononuclear phagocytes (MNs; monocytes and macrophages). PMNs are specialized in ingestion and intracellular killing of a large array of different bacteria, yeast, fungi, mycoplasmas and viruses. The ingestion and destruction of living cells by phagocytes are divisible into the separate stages of: chemotaxis or attraction, attachment or opsonization, engulfment, intracellular killing and digestion (Verhof, 1998). Phagocytes kill or degrade engulfed microorganisms in at least three different ways; Oxygen-dependent mechanisms, oxygen indep-endent mechanisms and nitrogen dependent mechanisms.

Interest in biostimulation effect of low intensity laser light particularly He-Ne laser, has increased in the last few years and the stimulative effect of electromagnetic radiation in the form of low level laser light in biology and medicine is already used today (Baxter et al., 1997).

Many authors have suggested the biostimulation mechanism and many studies have been done with organisms of different complexity to prove the stimulating action of visible laser light at 632.8 nm, they have found

that low doses laser light can stimulate the vital activity of both prokaryotic and eukaryotic cells (Karu et al., 1984)

Quantitative studies have been performed to determine the action of low-intensity visible monochromatic laser light on various cells (E coli, yeast, Hela cells); also irradiation conditions (wavelength, dose) conductive to vital activity stimulation have been examined. Respiratory chain components are discussed as primary photo-acceptors. The possible ways for photo- signal transduction and amplification were discussed by many authors (Karu, 1983).

Materials and Methods

Isolation of normal polymorph nuclear leucocytes from human blood:

The blood samples were collected from healthy people of 22-40 yrs ages. The blood was mixed with heparin (20 IU/ml) and dextrin (5 ml/10 ml blood) in a sterile tube and the samples were incubated at 37°C for 45 min to obtain the blood plasma.

In the separated tubes, the mixture of 2.5 ml histopaque 119 and 2.5 ml of lymphoprep were prepared and the plasma was added to this tubes. After centrifugation (700 g for 30 min) using cooled centrifuge, six layers were formed; plasma, monoclear layer, lymphoprep, granulocytes layer (nutrophile, Histopaque, RBCs) in their respective order from upper to lower part of the tube.

The granulocytes layer was washed by Hankes solution and the suspension of cells and Hanks solution containing 10% fetal calf serum was made for study phagocytosis. 0.2 ml of trypan blue was added to 0.2 ml of PMNs suspension. Total count and viability of PMNs were examined by microscope using hemocytometer.

Phagocytic index of Candida albicans:

Candida albicans was obtained from vaginal swab and was cultured on blood agar (48 hrs at 37°C). The Candida were collected and suspended in Hanks solution with total count about $3x10^7$ cell/ml.

A mixture of 0.25 ml of Hanks solution, 0.25 ml of serum, 0.25 ml of Candida albicans suspension and 0.25 ml of PMNs suspension was prepared.

To calculate the Phagocytic index before and after irradiation, the samples were incubated (37°C) at 5, 15, 30, 60 min (using 3 replicates) the slides of samples were made and stained using Giemsa stain (Desonottes, 1986).

Phagocytic index = 100 x Phagocytic cell / total count

The laser system:

The laser system that used in this study is CW Helium–Neon gas laser (Griffin and George, Britain) with a measured output power of 10 mW: it emits light at 632.8 nm in the visible region of the electromagnetic spectrum.

Irradiation setup:

Fig (1) illustrates the setup of irradiation. The laser beam was applied on the sample vertically using flat mirror. The beam was passed through a quartz lens to get certain diameter of the irradiated area equal to 0.8 cm.

The samples of mixture of PMNs and Candida were subdivided in 1 ml ependrof tubes and were irradiated with 10 mW He-Ne laser at 1, 3, 5, 10, 20 min exposure times, with power density equal to 5 mW/cm^2 .

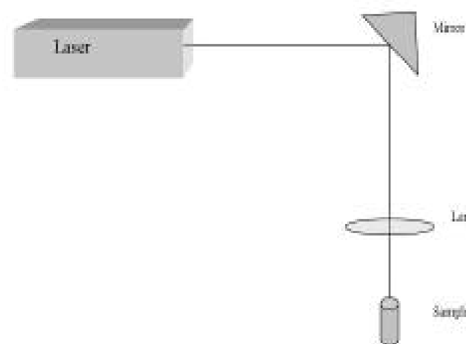

Fig. (1): Irradiation setup of bacterial samples with He-Ne laser.

Results and Discussion

Figures (2- 5) illustrate the effect of He-Ne laser with 5 mW/cm^2 power density on the phagocytosis indices of PMNs after 5, 15, 30 and 60 min of incubation periods in their respective order.

At 1 min exposure time, the values of phagocytic index increased slightly with all incubation periods except in 30 min of incubation, there was a slight inhibition in the

phagocytic index. At 3 min exposure time, high increasing in the values of phagocytic index was observed with all incubation periods in comparison with the control group and the biostimulative effect is more observable. Slight increasing in the values of phagocytic index was observed at 5 min exposure time with all incubation periods. At 10 min exposure time, increasing in the values of phagocytic index was observed after 5 min incubation period only, while a slight decreasing in phagocytic index was observed with the other incubation periods. Decreasing in phagocytic index was observed at 20 min exposure time with all of the incubation periods.

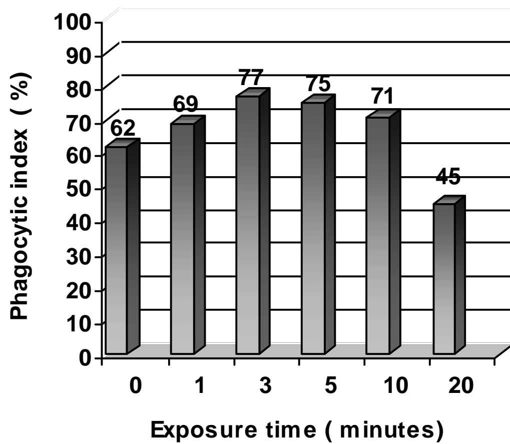

Fig. (2): Effect of He–Ne laser on phagocytic index of human PMNs after 5 min of incubation

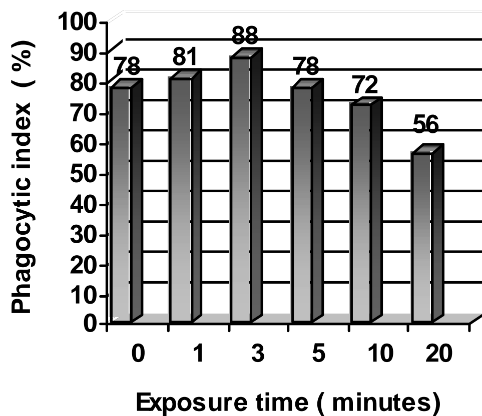

Fig. (3): Effect of He–Ne laser on phagocytic index of human PMNs after 15 min of incubation

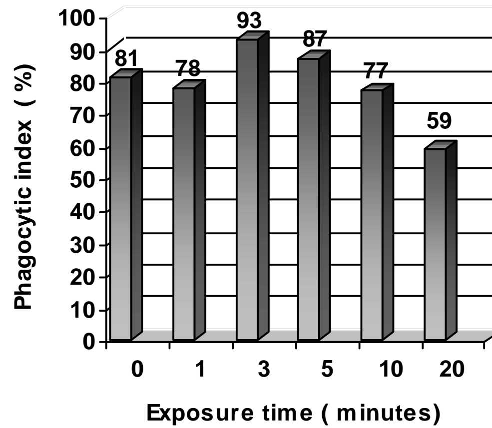

Fig. (4): Effect of He–Ne laser on phagocytic index of human PMNs after 30 min of incubation

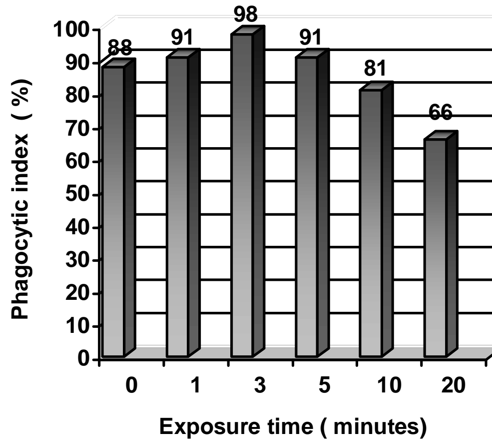

Fig. (5): Effect of He–Ne laser on phagocytic index of human PMNs after 60 min of incubation

It is clear from the results that laser light at low doses (5 mW/cm^2 power density at 3 and 5 min exposure times) leads to stimulate the phagocytic cells. On the other hand, at high doses (5 mW/cm^2 power density at 20 min exposure time), inhibition of the phagocytic activity was observed.

To explain this phenomenon, general mechanism can be proposed, capable of accounting for both the stimulating action of visible and infrared lasers on cell cultures at low laser doses, and damaging action on large doses. Laser irradiation may lead to formation of trans-membrane electrochemical proton gradient in

mitochondria. This enhances ATP production which activates Ca^{2+} pump depleting the Ca^{2+} concentration gradients of the surrounding medium relative to cytoplasm. This trigger enhances $Ca2^{+}$ influx into the cells via the Ca^{2+} ion channels of the cell membrane. In addition with sufficient irradiation, the proton motive force (pmf), due to proton gradient, causes more Ca^{2+} to be released from the mitochondria by an antiport process. The additional calcium transported into the cytoplasm, together with other factors controlled by pmf triggers mitosis and enhances cell proliferation. At high laser doses, too much Ca^{2+} is released. This causes hyperactivity of Ca^{2+}- ATPase and exhausts the ATP reserves of the cell.

Irradiation of cells at certain wavelengths can also activate some of the native components. In this way, specific biochemical reactions as well as whole cellular metabolism can be altered. This type of reaction is believed to form the basis for low power laser effects. Several evidences show that mitochondria are sensitive to irradiation with monochromatic visible and near infrared light (Karu, 1999).

Friedman and Lubart (1998) proposed a general mechanism for the stimulating effect action of visible and infrared lasers on cell cultures at low laser doses due to enhancing of ATP production and increasing cells.

Walsh (1997) proposed that irradiation of human peripheral blood lymphocytes in vitro at low doses of laser light induces changes in nuclear chromatin similar to those found after stimulation with the mitogen Phytohaemagg-lutinin (PHA).

In a study of the effect of 670 nm laser light on laryngal carcinoma cells in vitro. Barbosa found that the best cell proliferation is obtained with doses of 0.04 to 0.48 J/cm^2 (Barbosa, 2002).

The wavelength between 600 and 700 nm (red region) seems to be especially effective (Istomin et al., 1995). Low level laser light from the red region and the near infrared region corresponds definitely with the characteristic energy and absorption levels relevant for the respiratory chain this hence at the reaction center of Low level laser light in this way that the electromagnetic energy stimulates the components of the so called antenna pigments of the respiratory chain and thus vitalizes the cell by increasing the mitochondrial ATP production (Mito, 2000).

Cytochrome C oxidase is discussed as a possible photo acceptor when cells are irradiated with monochromatic red to near –IR radiation. Changes in the redox properties of the respiratory chain components following photo excitation of their electronic states, generation of singlet oxygen, localized transient heating of absorbing chromopheres, and increased super oxide anion production with subsequent increase in concentration of the product of its dismutation, H_2O_2. A cascade of reactions connected with alteration in cellular homeostasis parameters (PH, Ca cAMP, [ATP]) is considered as photo signal transduction and amplification chain in a cell.

It can be concluded that 10 mW He-Ne laser with 5 mW/cm^2 power density lead to increase the phagocytic activity of human polymorphonuclear leukocytes (PMNs) in vitro at short exposure times, 3 and 5 min. At long exposure time, 20 min, laser light lead to decrease the activity of these cells.

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تحفيز فعالية البلعمة لخلايا الدم البيضاء متعددة اشكال الانوية في الأنسان خارج الجسم الحي بأستخدام ليزر الهليوم نيون ذي قدرة 10 ملي واط

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الخلاصة تمت دراسة التاثير المحفز لليزر الهليوم نيون بقدرة 10 ملي واط على فعالية البلعمة لخلايا الدم البيضاء متعددة اشكال الانوية في الانسان خارج الجسم الحي . عزلت الخلايا من الدم المحيطي للانسان و استخدم خليط من محلول هانكس و مصل الدم و المبيضات و خلايا الدم البيضاء بنسبة 0.25 مل . تم تقسيم النماذج في انابيب اختبار سعة 1 مل شععت باستخدام ليزر الهليوم نيون للفترات 1، 3، 5، 10 و20 دقيقة بقطر منطقة تشعيع 0.8 سم . لحساب معامل البلعمة قبل و بعد التشعيع ، تم حضن النماذج قبل وبعد التشعيع بدرجة حرارة 37 م للفترات 5، 15، 30، 60 دقيقة تم بعدها تحضير شرائح صبغت بصبغة كيمزا . أظهرت النتائج وجود تأثير تحفيزي ملحوظ على فعالية البلعمة عند زمن التشعيع 3 دقائق و كثافة قدرة 5 ملي واط/سم . كما تم مناقشة الآليات المحتملة لتفاعل اشعة الليزر مع الخلايا نتيجة لوجود متحسسات للضوء .