Influence of Argon Laser on some Hematological and Biochemical parameters in vitro

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ABSTRACT

The effect of low power laser light on biological system and mechanism of its action still unknown. The purpose of this study was to investigate the effects of low-level laser radiation (LLLR) on whole blood in vitro. Blood samples were exposed to Argon laser of wavelength 488 nm & power of 13mW. Different energies 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 J were used. The study comprised the determination of lipid peroxidation, superoxide dismutase activity, hemoglobin concentration, number of white and red blood cells, and platelets. The results showed an exponential enhancement when irradiating them with different laser energies. This work indicated that there is a probability to use our results for getting a new biological dosimeter after exposure to argon laser because low doses of laser irradiation ameliorated oxidative stress in normal blood cells.

Key words: argon laser, oxidative stress, blood cells, antioxidant enzymes.

INTRODUCTION

Laser has become an effective working instrument for physicians actually of all specialties during the second half of the last century, which passed from the time of developing the first optical quantum generator (¹). LLLT (low level laser therapy, including phototherapy and photostimulation) has been shown to modulate biological processes, depending on the power density, wavelength, and frequency, and have positive effects on wound healing, modulating angiogenesis, muscle regeneration and diabetic wounds repair (²,³). Moreover, the histological analysis of tissue indicates that laser irradiation shortens the inflammatory phase as well as accelerating the
proliferative and maturation phase, and positively stimulates the regeneration of injured epidermis and the reparation of injured striated muscle \(^4\). The pioneering work of Karu \(^5\) has defined critical parameters in rapidly growing area governing wavelengths, output power, continuous wave or pulsed operation modes, pulse parameters, coherence and polarization, and has also indicated possible biological light acceptors at organic, cellular, subcellular and molecular level. On the basis of these extensive studies it has been proposed that the terminal enzyme of the respiratory chain cytochrome oxidase located in mitochondria acts as photoacceptor for the red-to-near IR region in eukaryotic cells, and the modulation of the redox state of the mitochondria generates secondary reactions through cell signaling molecules \(^6\).

MATERIAL AND METHODS

Experimental animals

Blood samples were taken from heart puncture of anesthetized male rats weighting 100-120 g. For hematological measurements, 42 blood samples were collected on anticoagulant (EDTA) for biochemical analysis. Such samples were divided into 7 groups. Blood samples were centrifuged for 10 minutes at 5000 rpm and plasma was separated. Irradiated sample are immediately subjected to analysis.

Radiation facility

Argon laser source type (05-LHD-001) of wavelength 488 nm and of maximum power 13 mW is directed towards the blood inside the tube. The exposure to laser beam was at energies 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 J. The system is located at the National Center for Radiation Research and Technology.

Biochemical analysis

1-Malondialdehyde was estimated in plasma by thiobarbituic acid (TBA) reaction according to procedure of Yoshioka et al., \(^7\).

2-Superoxide dismutase activity was estimated in whole blood by the method that depends on detection superoxide anions by nitroblue tetrazolium formazan color development \(^8\).

Hematological analysis

Hemoglobin content, red blood cells, leukocytes, platelets and Lymphocytes counts determined according to method described by Dacie and Lewis\(^9\).
Statistical analysis:

Student t-test was used for the statistical analyses of results, according to Snedecor and Cochran\(^{(10)}\).

RESULTS

Biochemical analysis:

\(a\)-Lipid peroxidation (malondialdehyde (MDA)):

Blood exposed to argon laser light at energies 0.5 and 1.0 J induced non-significant increase in MDA level while irradiation with 1.5, 2.0, 2.5, and 3.0 J induced significant increases as compared to control level, with percentage differences +24.7, +29.2, +45.2 and +57.2\%, respectively. Variation of MDA with laser energy was plotted in Fig.1. MDA increased when laser energy increased.

\(b\)-Superoxide dismutase (SOD):

SOD activity exhibited non-significant changes at energy 0.5 J, while it
was significantly increased (p<0.01), at energies 1.0, 1.5 and 2.0 of laser light, as compared to the control with percentage differences 40.5, +63.1 and +90.1%, respectively. Moreover, the results indicated that higher energies of 2.5 and 3.0 J were associated with highly significant increase p<0.001 in SOD activity with percentage differences +144.4 and +170.4 %, as compared to the control group. Variation of SOD with laser energy was plotted in Fig.2. SOD increased with laser energy.

**Hematological analysis:**

**a-Hemoglobin content:**

Hb content was non-significantly changed in blood irradiated with single dose 0.5 and 1.0 J, while exposure to the different laser energies 1.5, 2.0, 2.5 and 3 J caused significant increase (p<0.05) in Hb content with percentage changes +5.0, +7.5, +8.3 and 15.2%, respectively as compared to the control group. Variation of Hb with laser energy was plotted in Fig.3. Hb increased
with laser energy.

**b- RBCs count:**

The demonstrated data revealed that exposure of blood to single dose of 0.5J exhibited non-significant decrease in RBCs count. On the other hand, RBCs count showed a significant decrease (P<0.05) at 1.0, 1.5 and 2.0 J with percentage changes -8.1, -16.2 and -32.2%, respectively and (P<0.01) at 2.5, 3.0 J in irradiated groups with percentage changes -35.1 and -40.5 %, respectively as compared to the control group. Variation of RBC count with laser energy was plotted in Fig.4. RBC count decreased with laser energy.

**c- WBCs count:**

Data collected on WBCs count showed that exposure of blood to energies of 0.5 and1.0 J exhibited non-significant decrease compared to the control group. While WBCs count was significantly decreased p<0.05 and p<0.01 when blood irradiated with energies 1.5, 2.0 and 2.5 J with percentage changes -25.6, -25.6, -30.2 and -38.5%, as compared to the control group. Variation of RBC count with laser energy was plotted in Fig.5. WBCs count decreased with laser energy.
Data collected in Table (6) showed that Lymphocytes count on exposing blood to energies of 0.5 and 1.0 J exhibited non-significant decrease while Lymphocytes count was significantly decreased $p<0.05$, when blood irradiated with energies 1.5, 2.0, 2.5 and 3 J with percentage changes -20, -30, -35 and -45%, as compared to the control group. Variation of lymphocytes count with laser energy was plotted in Fig.6. Lymphocytes count decreased with laser energy.

**D- Lymphocytes count:**

**E-Platelets count:**

Figure (7) showed that platelets count exhibited non-significant change at energy 0.5 J, while significant increase $p<0.01$ was recorded at single dose of 1.0, 1.5 and 2.0 J of laser light with percentage of increase from control amounting +18.1, +26.2 and +28.2%, respectively. Moreover, the results indicated that higher energies of 2.5 and 3.0 J were associated with high
significant increase p<0.001 in platelets count with percentage changes +39.2 and +52.6 %, as compared to the control group.

The variation of platelets count with laser energy illustrates a linear relation with the equation:

\[ L = 17.6993 \times E + 111.627 \]

Where \( L \): platelets count
\( E \): laser energy

Therefore, by counting the platelet, laser energy illuminated the blood sample will be calculated according to the previous equation.

**DISCUSSION**

The progress of the clinical medicine is connected to a great extent with the development of physics, chemistry and biology. Achievements in these fields contributing to the perfection of diagnosis and healing of many diseases. This is true of the applications of the quantum electronic in medicine as well. No other branch in modern medicine has been developing so successfully and rapidly as laser therapy (11).

Malondialdehyde is one of the main end products of lipid peroxidation. The present study showed that the value of plasma level of malondialdehyde in the exposed group was significantly increased compared with the non-irradiated control. The significant increase in plasma MDA is explained by the overproduction of reactive oxygen species due to exposure, since non-ionizing radiation has been observed to increase the yields of some types of free radical intermediates and inhibits the recombination of free radicals (12). These results indicated that an altered oxidative pathway because increased production of MDA means that lipid peroxide formation is increased and led to interaction of oxygen free radical with cell membrane of blood. The results of MDA indicated that lipid peroxide formation in a cell free environmental to determine whether hemoglobin was actually able to production of lipid peroxides in the irradiated whole blood in the experiments. These data are agreement with those of Fedorova and Priezzhev (13).

The results revealed that, if the oxidative metabolism of the cells is enhanced by free radical formation from the interaction of low-level laser irradiation at with hemoglobin serving as a chromophore the intact organism should respond with defense mechanism to eliminate the damaging oxidative
metabolites. In this study, the increase of SOD formation enzyme activity is up regulated and higher concentration of enzyme can be detected.

Laser irradiation in therapeutic doses has an antioxidant effect in blood irradiation in vitro as shown by activation of superoxide dismutase (SOD) which is a key enzyme of the antioxidant system (AOS) and suppression of lipid peroxidation. This result agrees with Volotovskaia et al.\textsuperscript{(14)}.

The deleterious effects of non-ionizing radiation of low energy transfer in biological systems are mainly mediated through the generation of oxygen derived free radical intermediates. Besides the beneficial role of these free radicals in signal transduction, phagocytosis and apoptosis, it is established that excess generation of ROS is involved in structural alteration of cellular molecules leading to cytotoxicity and cell death. Oxidative damage to cellular components has serious effects leading to various diseases and ageing. This result in a variety of biological phenomena such as mutation, carcinogenesis, inflammation, diabetes mellitus and neuron-degenerative disorders\textsuperscript{(15)}. Hence in recent years much attention has been given to the subject especially in the field of clinical medicine. Oxidative damage to proteins as assessed by carbonyl and hydro peroxide formation has been implicated in etiology of many physiological disorders and diseases\textsuperscript{(16)}.

These results are in agreement with many previous finding of Nechifor and Nechifor, and Mester\textsuperscript{(17)}. A hypothesis of free radical mechanisms of stimulatory and inhibitory actions of low energy laser irradiation (LELI), used for therapy of a variety of inflammatory diseases, is formulated. Light absorption induces the production of initiating radicals that are involved in subsequent free radical reactions and subsequent leukocytes stimulation of the greater production of pro-oxidants and other biologically active products. These products include nitric oxide intermediates (NOI), reactive oxygen intermediates (ROI), which are dependent on their induced concentrations, can alter expression (increase or decrease) of proteins involved in multiple signal transduction pathways and induce the expression of inducible proteins, whose genes are highly influenced by external stimuli and could finally lead to apoptosis. Some of these proteins represent protective mechanisms against external stresses, while others amplify adaptation to the induced redox effect\textsuperscript{(18)}.

White blood cells are very sensitive to radiation representing the correlation between the white blood cells and laser irradiation dose. The relation illustrated an exponential decrease of white blood cell with irradiation energy.
The study revealed that the white blood cells are sensitive to the incident laser which absorbs it. The absorption is the main reason for the white cells and their derivative to be destructed by laser. These results are in agreement with Germann (19).

The present data showed non-significant difference in the values of lymphocytic and WBCs count in the low energy compared to the high energies. These findings are in agreement with those of Goldoni (20) who reported that high frequency of non-ionizing radiation had no effect on lymphocytes. However, the data of Zakharian et al., (21) are matched with our conclusion lymphocytic count and disagree the data of leukocytic count in group exposed to non-ionizing which was significantly decreased than control.

The present studies proved that hemoglobin absorbed the light of laser during photo-irradiation at different energies. The results are in agreement with previous results of John and Jennifer (22) in the presence of hemoglobin amplifies the effect of low power laser light on lymphocytes, and laser hemoglobin concentration. Hemoglobin absorbed the energy of laser irradiation to catalyzed biological reaction, formation of peroxides to induce the free radical reaction.

John and Jennifer (22) recorded that, treatment of coetaneous vascular lesions (port wine stains etc.) using lasers has been guided by theories based on the “cold” or room-temperature optical properties of the hemoglobin target chromophore. We have recently presented evidence showing that under the influence of laser irradiation, the optical properties of blood in vitro are time and temperature dependent. Such complications are not currently subsumed into the in vivo theory. Here, Vijayalaxmi et al., (23) recorded that, the time-domain optical properties of blood undergoing photocoagulation in vitro using two newly developed time-resolved techniques.

Red blood cells (RBC) are the major scattering particles in whole blood due to their predominant volumetric concentration and high scattering cross-section (24). The cortically, the angular distribution of light intensity scattered by an individual particle is determined by a scattering phase function (SPF), which can be defined either by a phenomenological formula, or calculated on the basis of certain approximations, accounting for the shape of RBC (25). Recent results in measurement and calculation of light scattering phase functions of large optically soft particles of different sizes, shapes, and orientations, modeling the single non deformed, shear-deformed and aggregated RBC are discussed.
Results are in good agreement with Thattaliyath (26) simulations of the similar process.

A decrease in RBC, as a result of the increase of laser energy, is attributed to the membrane damage of the red blood cells or a complete disintegration of the red cell membrane, in addition, hemoglobin increases. This result agrees with Frank et al. (27).

Platelets, enucleated cells that originate from bone marrow megakaryocytic, circulate in the blood, surveying the integrity of the vascular system (28). As a response to vascular injury, platelets rapidly adhere to tissue and to one another to form a platelet plug, which, in combination with the coagulation system, allows the reestablishment of normal blood flow in the disrupted vasculature (29). Neither platelets nor other components of the hemostatic process can distinguish between traumatic wounds and lesions that occur in diseased vessels, eg, on rupture of an atherosclerotic plaque (30).

CONCLUSION

Such work had indicated that there is a probability of using the obtained results of platelet count as biological dosimeter when studying the effect of environmental parameters (like, humidity, storing in light or dark) on the platelet count and laser energy relation.

Photoactivation of blood, in vitro with an increasing energy of argon laser may be modulating immunity in patients. This is attributed to the inhibition of the inflammatory and induction of anti-inflammatory.

The mechanism of photobiostimulation effect after exposure of laser light with blood, lead to the production of free radical. A non significant change was observed at low energies of laser irradiation, but laser at higher energies may ameliorate oxidative stress in normal blood cells.

REFERENCES


تأثير ضوء ليزر منخفض القدرة على بعض متغيرات الدم معملياً

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تم استخدام ضوء ليزر منخفض القدرة لتشعيع دم الجرذان معملياً حتى لا يؤثر على تركيب ووظيفة الخلية. وتتم ترطيب عينات الدم للجرذان ليزر ذات الطول الموجي 848 نانومتر وبقوة قدرها 13 ميللي وات ببطاقات مختلطة 0.5، 1.0، 1.5، 2.0، 2.5 و 3.0 جول. استُخلِلت الدراسة على تحديد نشاط تركيز الهيموجلوبين، عدد خلايا الدم البيضاء والحمراء والصفائح الدموية. أوضحت الدراسة زيادة نسبة تشبعها بالطقاطات المختلفة من أرجون الليزر.

أظهرت النتائج زيادة مطرودة ذو دلالته إحصائية بزيادة جرعة الليزر في عدد الصفائح الدموية وتركيز المالونداي الدهيد الهيموجلوبين ونشاط إنزيم السوبروأكسيد ديزمونتاز في الدم المشع. كما أظهرت النتائج نقصاً معنويًا في عدد خلايا الدم الحمراء والبيضاء والليمفاوية في الدم المشع بالطقاطات المختلفة من الأرجون ليزر.

دلت الدراسة عن احتمال استخدام النتائج التي حصلنا عليها كدواعي بيولوجية كنتيجة لتحسين جهد الأكسدة في خلايا الدم العادية بعد تعرضها لجرعات من أشعة أرجون ليزر واستخدامها في النواحي العلاجية وكذلك حالات نقل الدم في بعض الأمراض.