Photobiological fundamentals of low-power laser therapy

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PHOTOBIOLOGICAL FUNDAMENTALS OF LOW-POWER LASER THERAPY

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I. Basics of the action of monochromatic visible and near infrared radiation on cells

1.Irradiation with visible or near IR radiation at certain doses, intensities and wavelengths may stimulate the proliferation of mammalian cells, as well as the growth of prokaryotic and eukaryotic microorganisms.

2.The main regularities in irradiating cells with continuous wave light are as follows: (a) There is a bell-shaped fluence vs. biological effect curve characterized by a threshold, a distinct maximum, and a decline phase. However, there are exceptions from this rule.

(b) In most cases, the final photobiological effect only depends on the radiation dose and not on the radiation intensity and exposure time (the reciprocity rule holds true), but sometimes the reciprocity rule proves invalid.

(c) Though the biological responses of various cells may be qualitatively similar (e.g. characterized by bell-shaped dose dependences), they may have essential quantitative differences as it was established for various yeast organisms.

(d) The biological effects of irradiation do depend on wavelength (action spectra). The action spectra for both eukaryotic and prokaryotic cells are of the same type, having maxima in every visible light band.

3.The biological responses of the same cells to pulsed and continuous wave light of the same wavelength, average intensity, and dose, can be different.

4.The main regularities when irradiating cells with pulsed light are as follows:

(a) Dose dependences have more than one maximum. The reciprocity rule holds in all maxima of these curves.

(b) There is strong dependence on the pulse repetition rate, pulse duration, and quite probably, on the duty cycle.

II. Primary and secondary mechanisms of the action of monochromatic visible and near infrared radiation on cells

1. The terminal respiratory chain oxidases in eukaryotic cells (cytochrome c oxidase) and in the prokaryotic cells of the bacterium *Escherichia coli* (cyt bd and cyt bo complexes) are believed to be photoacceptor molecules for red-to-near-IR radiation. In the violet-to-blue spectral region, flavoproteins (e.g. NADH-dehydrogenase) are also among the photoacceptors as well as terminal oxidases.

2. It is suggested that the photoacceptors are not the fully reduced or oxidized enzymes, but one of their intermediate forms (a so-called mixed valence oxidase), which has not yet been identified.

3. At least four types of reactions can occur with the participation of a photoacceptor molecule after its electronic excitation: changes in redox properties and the acceleration of electron transfer, one-electron auto-oxidation (0_2.-formation), photodynamic action ('0 formation), and changes in biochemical activity induced by the local transient heating of the absorbing chromophores. It is unreasonable to believe that only one of these reactions occurs under irradiation. The question is which one is responsible for the specific cellular responses under study? Recent experimental results indicate that changes in redox properties of absorbing chromophores in photoacceptor molecule might have a great importance.

4. The primary physical and/or chemical changes induced by light in the photoacceptor molecules are followed by a cascade of biochemical reactions in the cell which require no further light activation and occur in the dark (photosignal transduction and amplification chains). These reactions are associated with the changes in the cellular homeostasis parameters. The crucial step here is thought to be the alteration of the cellular redox state.

III. Explanation of controversies and limitations of low-power laser effects on cellular level

1. The diversity of low-power laser effects on the cellular level can be explained by the similarity in principles of respiratory chain function.

2. Variations in the magnitude of low-power laser effects on the cellular level are explained by the overall redox state of the cells at the moment of irradiation. The cells with a lowered internal pH, pH. (whose redox state is shifted to the reduced side) respond more strongly than the cells with the normal pH value.

3. It is suggested that such pathological conditions as chronic inflammation and indolent wounds respond to irradiation because of their lowered pH value and hypoxia. Irradiation can also affect the stimulus-response-recovery cycle which naturally includes changes in step of redox state and pH_i.

4. Irradiation with low and high doses of light of the same wavelength causes different reaction channels to prevail, which results in different cellular responses: stimulation of the vital activity or its inhibition or even destruction.

5. There are biological limits in low-power laser effects: the proliferation of fast-growing cells can not be stimulated, or not all cellular functions can be activated. Also, not all species among yeast strains, *E. coli* mutants, and cells cultivated *in vitro* can be stimulated by irradiation.

6. Not all cells in tissues or cellular cultures will respond to irradiation in exactly the same way. The reason is the heterogeneous nature of the cell cultures and tissues (with regard to their proliferative activity, for example).

7. When complex systems like blood or spleen cell suspensions are irradiated, the irradiation effect (its magnitude or even the nature of the response, stimulation or inhibition of some parameter) depends on the physiological status of the host organism.

IV. Responses of neurons and lymphocytes to direct irradiation

1. Using individually identified nerve cells of *Helix pomatia,* it was shown that silent neurons were not excited by laser radiation (λ = 632.8 nm, maximum intensity $4x10^4$ W/m^2), while spontaneously active neurons responded to irradiation with membrane depolarization.

2. The rate of membrane depolarization, duration of the latent period, and probability of spike generation were dependent on the intensity of He-Ne laser radiation when the spontaneously active neurons of *Helix pomatia* were irradiated.

3. The direct measurement of ionic currents through the membranes of rat spinal cord neurons, rat hippocampus pyramidal neurons, Guinea pig cardiomyocytes and rat brain glial cells proved that He-Ne laser radiation influenced the background single-channel currents recorded in the cell-attached patch pipette configuration. It is thought that the channels sensitive to irradiation are the ATP-dependent K^+- channels or Ca^{2+} -dependent K^+- channels.

4. The irradiation of human lymphocytes with a He-Ne laser can activate some short-term reactions in these cells (increase in chromatin template activity, expression of r -genes, Ca^{2+} influx, increase in the c-myc RNA content, activation ofmitochondrial function concurrently with the formation of giant mitochondria), but full mitogenic activation and blast transformation do not occur. At the same time, irradiation has a boosting effect on the DNA synthesis in lymphocytes treated with phytohemagglutinin prior to irradiation (a higher number of cells were activated).

5. The absence of expression interleukin-2 receptors in irradiated lymphocytes is believed to be connected with the absence of blast transformation in irradiated lymphocytes.

V. Responses of blood and spleen cells to the irradiation

1. Laser radiation was found to increase or suppress the spontaneous chemiluminescence (CL) of splenocytes in suspension, the amplitude and the nature of the effect depending on the cellular composition of the samples. Direct correlations were established between the effect of laser radiation (percentage changes of CL when irradiated at 820 nm, $1.1x10^3$ J/m^2, 292 Hz) and percentage of plasmacytes ($r = 0.743$, $p < 0.001$) neutrophils ($r = 0.650$, $p < 0.001$), myelocytes and metamyelocytes ($r = 0.505$, $p < 0.01$). The correlation with the percentage of lymphocytes($r = -0.590$, $p < 0.001$) was found to be a reverse one.

2. The chemiluminescence of blood from 28 clinically healthy donors was found not to be influenced by laser radiation at 820 nm (292 Hz, 1xlO^4 J/m^2, 13 s).

3. The chemiluminescence (CL) of peripheral blood from the donors was recorded after irradiation with various lasers and superluminous diodes (660, 820 and 950 nm, pulse repetition rates 16, 292 and 5000 Hz) during two periods of acute viral respiratory illness and in the normal state of health. It was found that precise and statistically significant effects of laser radiation on CL (suppression of spontaneous CL) depend on the radiation wavelength, pulse repetition rate and dose, and could be recorded only in the periods of acute illness (i.e.

at a certain immunological status of the organism). There are practically no effects of laser radiation when the blood of a healthy donor is irradiated. The optimal irradiation parameters for the suppression of free radical processes in human blood were as follows: dose range 10^3-10^4 J/m, pulse repetition rate 292 and 5000 Hz (16 Hz was ineffective). All the wavelengths studied (660, 820, 880 and 950 nm) had an inhibitive effect but l = 660 nm was found to be the most effective (65% of CL was suppressed).

4. The antitumor agents vinblastine and vincristine and laser radiation (820 nm, 292 Hz, $1x10^4$ J/m^2) are shown to inhibit the spontaneous chemiluminescence of blast cells in acute lymphoblastic leukemia patients.

5. Continuous-wave He-Ne laser radiation has practically no effect on the chemiluminescence ofsplenocytes of intact mice and mice with transplanted leukemia EL-4, nor on the blood of healthy people and patients suffering from cancer of the colon. In the same experimental conditions, pulsed He-Ne laser light in the same dose (5-l0^3 J/m^2) inhibited CL in all four model systems. The pulsed radiation (1-100 Hz, duty cycle 50 or 94%) had a weak inhibiting effect on samples from healthy organisms but inhibited markedly the chemiluminescence of samples from tumor-bearing organisms.

VI. Effects of visible and near infrared radiation on cultured cells

1. The proliferation of mammalian cells (measured by-3H-thymidine incorporation) increases after irradiation with various bands of visible and near infrared radiation: the effect depends on the radiation wavelength, dose, and intensity as well as on the cell cultivation conditions.

2. Increased 3H-thymidine incorporation is caused by the enhancement of DNA synthesis in S-phase cells and is due to an increased number of S-phase cells originating from that part of the G_i -phase population which is ready to pass to the S-phase. In other words, irradiation stimulates the progression of the cell cycle.

3. Irradiation increases the growth of relatively slowly proliferating subpopulations.

4. Irradiation increases the adhesive properties of cell membranes. The action spectrum of this phenomenon coincides with the action spectrum of proliferation increase measured by 3H-thymidine incorporation (peaks in the red-to-near IR region at 620, 680, 760, and 825 nm).

5. Irradiation can increase the cellular ATP level and increase or decrease the cellular cAMP level.

6. Preirradiation with a He-Ne laser decreases the cytotoxic response of cells to ionizing radiation.

VII. Activation of metabolism of nonphotosynthesizing microorganisms

1. The irradiation of bacteria *E. coli* WP2 with various bands of monochromatic visible and near IR radiation causes the shortening (or even) disappearance of the lag-period in the growth curves.

2. The experimental data evidences that the irradiation of *E. coli* cells causes a transient cell division acceleration (termed growth stimulation), reflecting a higher metabolic activity only

in those cells whose rate of growth is slow. The growth stimulation as well as injection of T4 phage DNA into host cell are both DpH-dependent processes and depend on wavelength and dose of light.

3. The irradiation of various strains of yeast organisms causes no changes in the length of the lag-period of the growth curves but increases growth in the log-phase (shortening of the generation time). The size of the cells and the amount of protein in a single cell do not differ between the exposed and unexposed cultures. Consequently, irradiation leads to the intensification of the protein synthesis and speeds up the preparation of the cells for division and budding.

4. The optimal dose for the stimulation of different cultures is in agreement with the degree of lability of their metabolism. The cultures with labile possibilities for accommodation (e.g. *T. sphaerica, S. ludwigii)* are most sensitive (the doses required are lower and the amount of protein synthesized under irradiation is higher). Cultures characterized by conservative type of metabolism *(E. magnussii, S. cerevisiae)* are rather insensitive.

5. Irradiation also stimulates the protein synthesis in *Saccharomycodes ludwigii* grown in anaerobic conditions. The growth curves of cultures grown in both aerobic or in anaerobic conditions are bell-shaped and have distinct maximal, but the magnitudes of these maximal are different (in the case of anaerobic cultures, they are approximately are order of magnitude higher). In anaerobically grown cultures, irradiation activates the NADH-dehydrogenase and in aerobically grown ones, the CO, production is increased in a dose-dependent manner. It is suggested that irradiation activates different metabolic pathways in aerobically and anaerobically grown cells.

6. Irradiation with a He-Ne laser increases the number of germinated and outgrown endospores of the bacterium Anaerobacter polyendosporus.