TOWARDS AN EXPLANATION OF VISIBLE AND INFRARED LASER INDUCED STIMULATION AND DAMAGE OF CELL CULTURES

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A general mechanism is proposed, capable of accounting for both the stimulating action of visible and infrared lasers on cell cultures, at low laser doses, and the damaging action at larger doses. Laser irradiation is assumed to intensify the formation of a trans-membrane electrochemical proton gradient in mitochondria. This enhances ATP production which activates the Ca^{2+} pumps, depleting the Ca^{2+} concentration in the cytoplasm and increasing the Ca^{2+} concentration gradient of the surrounding medium relative to the cytoplasm. This triggers enhanced Ca^{2+} 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 the 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 the pmf triggers mitosis and enhances cell proliferation. At higher laser doses, too much Ca^{2+} is released. This causes hyperactivity of Ca^{2+} -ATPase and exhausts the ATP reserves of the cell. The nature of the photoacceptors and possible ways in which the visible and infrared laser energy is converted by the photoacceptors are discussed.

KEY WORDS ATP Calcium pump Proton-motive force (pmf)

Laser-induced Ca^{2+} Release in the Cytoplasm Responsible for Cell Stimulation or Cell Damage

A general mechanism is proposed, capable of accounting for both the stimulating action of visible and infrared (far-red) lasers^{1,2} on cell cultures, at small laser doses, and the damaging action,¹ at larger doses. Laser irradiation is assumed to intensify the formation of a transmembrane electrochemical proton gradient in mitochondria. Thus, the efficiency of the proton-motive force (pmf)³⁻⁶ is increased and more calcium is released⁷ into the cytoplasm from the mitochondria by an 'antiport'⁸ process:

In addition, the laser-enhanced pmf produces more ATP in the membranes of the mitochondria. This ATP fuels the Ca^{2+} pumps expelling more Ca^{2+} into the surrounding medium and increases the Ca^{2+} concentration gradient of the medium relative to the cytoplasm. This triggers a rapid influx of Ca^{2+} from the medium into the cytoplasm via the ion channels.

At low laser doses, the additional Ca^{2+} transported into the cytoplasm triggers mitosis⁹ and enhances cell proliferation. At higher laser doses too much Ca^{2+} is released. This causes hyperactivity of the Ca^{2+} -ATPase calcium pumps and exhausts the ATP reserves of the cell. Without the ATP energy reserves, the cell-pump activity slows down and the ionic concentrations in the cells and the surrounding medium tend to equilibrate. Thus, due to intracellular proteins (when not compensated by macromolecules in the surrounding medium), the intramolecular osmotic pressure becomes larger than that of the medium. Water influx then produces blebs extruding from the cell walls.¹⁰ At still higher laser doses, more Ca^{2+} influx completely exhausts the cell energy and the intracellular osmotic pressure explodes the cell.¹⁰

Reaction 1 takes place in the indicated direction under the usual conditions when the concentration

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of cytoplasmic Ca^{2+} is very low ($\sim 10^{-7}$ M), relative to that in the mitochondria ($\sim 10^{-3}$ M). At higher cytoplasmic Ca^{2+} concentrations, the reverse reaction becomes predominant.⁷ Then Ca^{2+} flows from the cytoplasm to the mitochondria against the Ca^{2+} concentration gradient, exploiting the negative potential inside the mitochondria produced by the electrochemical proton gradient.⁴ This reverse reaction together with the Ca^{2+} -ATPase calcium pumps¹¹ are responsible for Ca^{2+} regulation in the cytoplasm.

Besides the mitogenic action of transient Ca^{2+} release into the cytoplasm by the pmf, a short-term rise of the intracellular pH by the creation of the electrochemical proton gradient triggers mitogenic signals in the cells.¹² Moreover, it is well known that the pmf increases ATP production which activates Na^+ , K^+ -ATPase and other ion carriers. Thus, the intracellular Na^+ level is decreased and K^+ concentration together with the membrane potential are increased. This triggers a transient rapid influx of Na^+ from the medium into the cytoplasm changing protein conformation and influencing cell proliferation.¹³

Primary Photoacceptors

A correlation has been found between the action spectrum of visible light on various growth stimulating and damaging effect of certain organisms and the absorption bands of respiratory chain components of these organisms such as the flavins and the cytochromes.¹⁴ The spectrum¹⁵ of cytochrome aa_3 , or cytochrome oxidase, extends a weak tail to the far red which may account for the absorption of infrared diode-laser light in the region between 700 nm and 900 nm. These diode lasers have been used in low-level laser therapy at an intensity of about 60 mwatts/cm², which is an order of magnitude larger than the intensity of continuous-wave lasers and in the visible region. Thus a partial compensation is obtained for the fact that the absorption coefficient of cytochrome oxidase is from one to two orders of magnitude smaller in the 700 nm–900 nm region than in the visible part of the spectrum. In tissue irradiation, another compensating element is the deeper penetration (by a factor 2 to 3) of the 700 nm–900 nm radiation.¹⁶ Recently, however, we have obtained strong optimum cell culture photobiostimulation of fibroblasts at 780 nm with intensity (10 mW/cm²) and fluence (7 J/cm²) similar to those yielding optimum stimulation with visible 630 nm irradiation. This indicates that a different mechanism is involved. We shall discuss this effect in the next section.

In addition to the pigments of the respiratory chain, there is also spectroscopic evidence that minute amounts of porphyrins, naturally present in the cells,¹⁷ are among the primary photoacceptors.¹⁸

Support in favour of the assumption that the primary photoacceptors are pigments, located in the mitochondrion is provided by the observation of enhanced ATP synthesis in isolated mitochondria at appropriate wavelengths.¹⁹

Possible Ways by which Visible and Infrared Laser Energy is Converted by the Photoacceptors

How is the energy absorbed by the mitochondrial pigments converted to the electrochemical energy of the pmf? As a first possible mechanism, we consider photosensitization in which energy of the photoexcited porphyrin (to be referred to as porphyrin*) is transferred to molecular oxygen in the ground state $\text{O}_2(^3\Sigma)$ (referred to as O_2) transforming it to singlet oxygen $\text{O}_2(^1\Delta_g)$ (referred to as $^1\text{O}_2$).²⁰

Replacing O_2 by $^1\text{O}_2$, a potent oxidizer,²⁰ stimulates the redox activity of the respiratory chain. Electron transport of the redox reaction pumps H^+ from the mitochondrial matrix to the exterior according to Mitchell's chemiosmotic hypothesis.²¹ The cell-growth stimulation by $^1\text{O}_2$, proposed here, is similar to that obtained by adding in the dark methylene blue, another potent oxidizer, to the cell cultures.¹

It is well known, however, that when porphyrins or other photosensitizers are introduced artificially into the tissues, as in photodynamic therapy, destruction of the (malignant tumour) cells by $^1\text{O}_2$ is obtained.²² This would seem to indicate, as proposed by Karu,¹ that laser stimulation and damage of cell cultures result from qualitatively different causes, cell damage being due to photosensitized $^1\text{O}_2$ production and cell stimulation resulting from the photoactivation of the redox reactions in the respiratory chain. Here, as pointed out earlier a different approach is envisaged: the action of light is assumed to cause stimulation of the cells and damage results when this stimulation becomes too strong, exhausting the energy reserves of the cell. The fact that $^1\text{O}_2$ is a more potent oxidizer than O_2 explains the enhanced oxygenation in the presence of light.²³

It is possible, as assumed by Karu¹ that, in addition to the production of the long-lived $^1\text{O}_2$ molecules, laser light also activates the redox reactions of the respiratory chain by exciting the mitochondrial flavins and cytochromes. Electronic excitation will probably be converted to vibrational energy by radiative and radiationless transitions from the excited electronic state. The vibrationally excited 'hot' molecules will react faster than the

'cold', vibrationally unexcited molecules. It is also possible that the weak absorption of cytochrome aa₃ in the 700 nm–900 nm region corresponds, not to electronic excitation, but to excitation of vibrational overtones. Thus the far-red infrared radiation produces directly the more reactive vibrationally 'hot' molecules which are obtained indirectly by electronic excitation. Note that the excitation of higher overtones is, in general, a process characterized by a very low transition probability. The fact that the far-red infrared spectrum of cytochrome oxidase is only one to two orders less intense than its visible spectrum can be ascribed to 'intensity borrowing'. The overtone then acquires additional oscillator strength because the high, vibrationally excited states in the electronic ground state interact strongly with the low-lying electronically excited state. This phenomenon of significant intensity borrowing by the high vibrational states in the electronic ground state should be the rule for chromophores with absorption peaks in the red region of the spectrum.

Instead of photoactivation via vibrationally hot molecules of the redox reactions of the respiratory chain, another possible mechanism can be envisaged. Instead of activating the redox reactions, the absorption of the laser photon can itself produce a photochemical reaction capable of causing proton transmembrane transport. A similar mechanism occurs when bacteriorhodopsin of the halophilic bacterium *Halobacterium halobium* is irradiated by visible light and produces a proton gradient.²⁴ Here we assume that the cytochrome oxidase plays the role of bacteriorhodopsin and acts as a proton pump as has been stated in the literature.²⁵ The absorbed photon excites the enzyme electronically and the excited electronic state decays in part, radiatively or non radiatively, to a chemically different configuration of the enzyme. Overtone excitation by far-red infrared absorption, facilitated by intensity borrowing, may produce a similar effect when the vibrationally hot molecule has sufficient energy to cross the potential barrier towards chemical transformation. Together with the photochemical reaction, conformational changes may also be induced by the photoexcitation process. Now, suppose that the overtone excitation by far-red infrared light has a larger probability of reaching those vibrational states that lead to a chemically different configuration than the indirect process where visible light excites an electronically excited state which only partly decays to the useful vibrationally excited states. Then of course, the weak absorption probability of the far-red infrared laser light relative to that of visible light will be compensated by the efficiency of the chemical change produced by the infrared laser-induced excitation.

Finally, we suggest that the action of the laser light on the pigments of the respiratory chain may be similar to the photophysical and photochemical

processes triggered in photosynthesis. As in the thylakoid membrane, there may be one or two photochemical reaction systems.²⁶ It is well known that the electron transport chain in the thylakoid membrane is similar to the electron flow in the respiratory chain in mitochondrial membranes and also produces the electrochemical proton gradient.

Conclusion

We suggest that laser-enhancement of the production of trans-membrane electrochemical proton gradient plays a major role in the understanding of both laser-induced stimulation and damage of cell cultures. We assume that the primary photoacceptors are porphyrins, naturally present in the cells, or pigments of the respiratory chain. We further suggest that the mechanism by which the photoacceptors convert visible or far-red infrared laser light to the pmf is analogous to one or a combination of the mechanisms presented in the biochemical literature to account for trans-membrane proton gradient formation. Mitosis is triggered mainly by Ca²⁺ influx, caused by the proton gradient.

Finally, we attribute the effect of far-red infrared laser light to overtone excitation in the presence of intensity borrowing. The overtone excitation leads to vibrationally hot molecules which either accelerate intermolecular redox reactions or produce intramolecular rearrangement causing proton translocations.

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