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THE PHOTOBIOLOGICAL BASIS OF LOW LEVEL LASER RADIATION THERAPY

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Low level laser radiation therapy is effective in a number of clinical situations (e.g. pain relief, wound healing, sports medicine), but the photobiological basis of this therapy is not well-understood. Since both visible and infrared radiations have been shown to be beneficial in such therapies, and since these two radiations differ dramatically in their photochemical and photophysical properties, how can they produce similar results clinically? I propose a modification of the model of Karu' to explain this. In her model, visible light produces photochemical changes in photoreceptors in the mitochondria, which alter metabolism, which leads to signal transduction to other parts of the cell (including membranes), which finally leads to the photoresponse (i.e. biostimulation). While visible light probably starts the cascade of metabolic events at the level of the respiratory chain of the mitochondria through photochemical events (probably the photoactivation of enzymes), I propose that because of the photochemical and photophysical properties of infrared radiation, infrared radiation starts the cascade of metabolic events by photophysical effects on the membranes (probably the Ca^{++} channels). Action spectra are needed to quantitate the relative effectiveness of the different wavelengths of radiation, since this can help to identify the photoreceptors for the photobiological response, and to establish the optimum conditions (i.e. wavelength, dose, and treatment schedule) for a particular therapy.

KEY WORDS Photobiological basis of LLLT Action spectra Quantum yield Absorption spectra First law of photochemistry True photochemical sensitivity Model to explain LLLT Photoactivation of enzymes Photomodulation of membranes Ca^{++} channels

Introduction

Low level laser radiation therapy is effective in a number of clinical situations (see below), but the photobiological basis of this therapy is not well understood. Since wavelengths both in the visible region (380–700 nm) and the infrared region (700 nm–1000 μm) of the electromagnetic spectrum are effective in such therapies, and since the radiation in these two wavelength regions differ so dramatically in their photochemical and photophysical properties, how can they produce similar clinical results? This paper proposes a model to answer this question.

Visible Radiation

In the visible region, when a photon is absorbed by a molecule, the electrons of that molecule are raised to a higher energy state. This excited molecule must then lose its extra energy, and it can do so either by re-emitting a photon of longer wavelength (i.e. less energy), as in fluorescence or phosphorescence, or it can lose energy by giving off heat, or it can lose energy by undergoing photochemistry. Photobiological responses are the result of photochemical and/or photophysical changes produced by the absorption of nonionizing electromagnetic radiation (see reference 2).

To gain a better understanding of the photobiological basis of low level laser radiation therapy, laser radiation therapists will have to become better photobiologists. Unfortunately, almost all photobiologists have been self taught, including me, and some of us have been both better teachers and better pupils than others. Even people who call themselves photobiologists sometimes make mistakes about the properties of light. One of these misconceptions is that visible light is 'safe'. The safety of light is not an intrinsic property of the light, rather, it is whether or not the light is absorbed that determines whether the light is safe or not (see reference 2).

The *first law of photochemistry* states that light must be absorbed before photochemistry can occur. This is a very simple but powerful law. If people would just remember this law, many mistakes about the properties of light (or more generally, of nonionizing radiation) would be avoided. The meaning of this law is that if radiation of a particular wavelength is *not absorbed by a system*, then it is *safe for that system*, since no photochemical or photophysical changes can occur. Visible light can be safe for one biological system, and be very

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damaging to another. For example, blue light is 'safe' for pure DNA since DNA does not absorb blue light, but blue light is *not safe* for bilirubin, since blue light is absorbed by bilirubin and produces a photochemical change in bilirubin. This photochemical change is the molecular basis of the phototherapy for jaundice in premature babies.

An *absorption spectrum* is a plot of the probability that light of a given wavelength will be absorbed by the system under investigation. Therefore, by simply running an absorption spectrum of the chemical or biological system that you are interested in, you can immediately assess the probability that light of a given wavelength will have an effect on that system.

The *quantum yield* (ϕ) of a photochemical reaction is the probability that photochemistry will occur when the energy of light is absorbed by the system.

Thus, the *true photochemical sensitivity* of a system is the product of these two probabilities, i.e., A, the probability that light of a given wavelength will be absorbed, and, ϕ, the probability that the absorbed light will cause a chemical change [true photochemical sensitivity = $A \cdot \phi$].

Therefore, it follows that, under ideal experimental conditions, an *action spectrum*, i.e., a plot of the relative effectiveness of different wavelengths of light in causing a particular biological response, should mimic the absorption spectrum of the molecule that is absorbing the light, and whose photochemical alteration causes the biological effect. Thus, by running an action spectrum one can often identify the biological molecule whose photochemical alteration results in the biological effect.

One simple example is the action spectrum for the killing of bacteria (Figure 1). This action spectrum mimics the absorption spectrum of DNA. This is an entirely understandable result based upon current knowledge of the unique importance of DNA to a cell. Other action spectra, however, have identified other chromophores (e.g. reference 2).

There is already a partial action spectrum for the treatment of postherpetic neuralgia by low level laser therapy. Walker *et al.*[3] reported that light at 633 nm was ineffective in reducing postherpetic neuralgia, but Moore *et al.*[4] reported that radiation at 830 nm was very effective in reducing such pain.

Figure 2 shows the absorption spectrum of the palm of a human hand. Note that radiation at 633 nm does not penetrate through tissues nearly as well as does radiation at 830 nm. It might be that the negative results at 633 nm for the treatment of postherpetic neuralgia was due to the lack of penetration of the light through the upper layer of skin cells, so that the light did not reach the target cells below the surface. To produce a proper action spectrum, one must run a dose response curve at each wavelength, then one usually plots the reciprocals of the doses to achieve a 50% response at each wavelength.

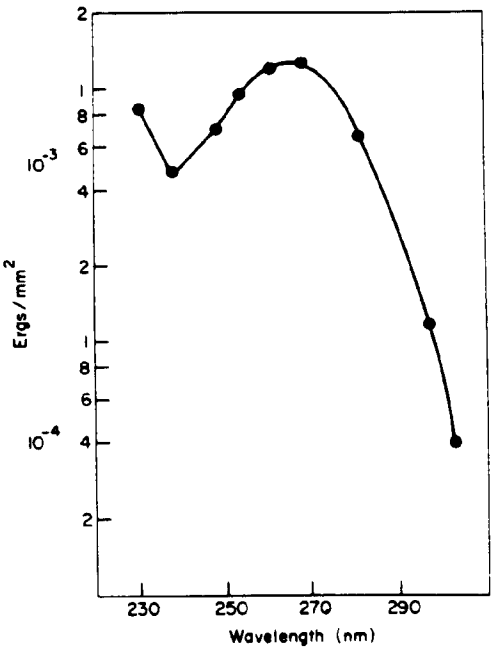

Figure 1. Action spectrum for the killing of bacteria; a plot of the reciprocals of the incident energies for 50% killing of *Escherichia coli* versus wavelength (adapted from reference 25). Note that the action spectrum mimics the absorption spectrum for DNA, suggesting that the photochemical inactivation of DNA is what kills the cells

Therefore, once a therapeutic benefit has been found for a given wavelength of light, one needs to determine the optimum dose and the optimum number of treatments to achieve that benefit. The next step is to find the optimum wavelength for that beneficial effect, i.e., determine an action spectrum.

Taking another example from the literature on the low level laser radiation therapy of several different types of pain, Walker[5] reported that those patients exposed to radiation at 633 nm showed a large increase in the urinary excretion of 5-hydroxyindoleacetic acid, which preceded pain relief by several days. 5-Hydroxyindoleacetic acid is a breakdown product of serotonin.[6]

Since the light therapy apparently stimulated excretion of 5-hydroxyindoleacetic acid, it is appropriate to inquire as to the photobiological basis of this excretion. The two most obvious mechanisms are: (1) the direct absorption of the light by serotonin results in the photochemical formation of 5-hydroxyindoleacetic acid, and (2) the absorption of light results in the photoactivation of the enzymes (i.e. monoamine oxidase and aldehyde dehydrogenase) that metabolize serotonin to form 5-hydroxyindoleacetic acid.

To answer the first question, it would be quite easy to irradiate a solution of serotonin with light at 633 nm, and see if 5-hydroxyindoleacetic acid is formed directly by the photochemical modification of serotonin.

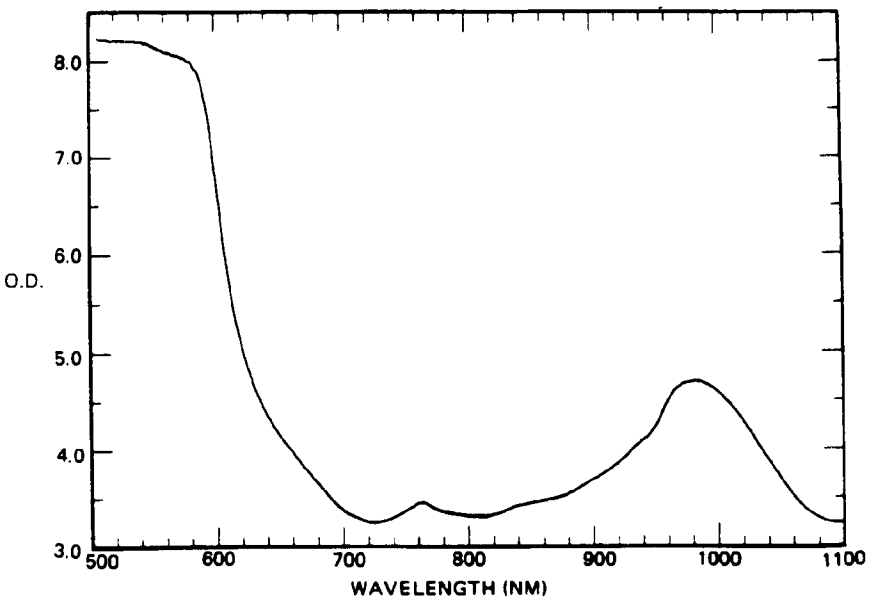

Figure 2. The absorption spectrum of a human hand. The spectrum was recorded with a very sensitive spectrophotometer with the palm of the hand in close juxtaposition to the photocathode (unpublished data of Karl H. Norris, from reference 26, p. 400)

It is more difficult to test if the radiation has activated the enzymes that produce 5-hydroxyindoleacetic acid from serotonin. However, one could contemplate testing the effect of light on an organ culture, a tissue mince, or a homogenate to see if this enhances the breakdown of serotonin.

Photoactivation of Enzymes

The light activation of enzymes is one of the fastest growing fields of photobiology, and several reviews on this subject have appeared.[7–9] The importance of the light activation of enzymes is that enzymes are catalysts. In principle, one photon can activate one enzyme molecule, which in turn can process many thousands of substrate molecules. This provides a huge amplification factor for initiating a biological response with light. This remarkable amplification factor may be the explanation for why low level laser radiation therapy is effective. If the effect of one photon can be amplified biologically, then one does not need a lot of photons to produce an effect. One just needs to find the proper wavelength of light to stimulate the proper enzyme, which in turn will stimulate the beneficial therapeutic effect.

There are a number of ways, both direct and indirect, that light can activate an enzyme.

Activate (Produce) the Substrate

For example, if one irradiates a cell with UV radiation, the photochemical damage that is produced in the DNA will be repaired by a set of DNA repair enzymes that have become active due to the presence of their substrates.

Activate the Enzyme–Substrate Complex

In another example taken from UV radiation photobiology, the photoreactivating enzyme (DNA photolyase) recognizes one type of DNA damage as its substrate, i.e., the cyclobutane-type pyrimidine dimer, and combines with these dimers in the dark. However, when the enzyme–substrate complex is exposed to visible light, the energy of the light is used by the enzyme to split the dimer to yield repaired DNA.

Activate the Enzyme Directly

This is generally accomplished by stimulating a conformational change in the enzyme molecule itself or in an attached photochromic inhibitor of the enzyme molecule, or by photochemically splitting an inhibitor from the enzyme. There are many examples of each of these mechanisms.[7–9]

Induce the Synthesis of the Enzyme

This would occur by gene activation. For example, when bacteria are UV irradiated, a whole group of DNA repair enzymes are induced. Some of these induced enzymes are not present in detectable amounts prior to induction, while other enzymes

are present in small amounts but are induced to higher amounts by UV irradiation (reference 2, p. 117). Laser radiation at 633 nm has been shown to stimulate collagen synthesis in cutaneous wounds by enhancing the synthesis of Type I and Type II procollagen mRNA levels.[10]

Therefore, the light activation of enzyme activity can occur by several diverse mechanisms. The first two mechanisms mentioned, i.e., the radiation production of the substrate, and the irradiation of the enzyme–substrate complex do not result in amplification, i.e., one absorbed photon is needed for each photochemical event. Therefore, a high level of radiation is required for these events.

The last two mechanisms, i.e., the direct activation of an enzyme and the induction of the synthesis of an enzyme, result in more chemical changes than the number of photons absorbed, and are produced by lower levels of radiation than the two processes mentioned above. Therefore, these last two mechanisms of enzyme activation are strong candidates for the photobiological basis of low level laser radiation therapy in the *visible region of the spectrum.*

Infrared Radiation

The absorption of radiation in the *infrared region* results in molecular rotations (rotation of the whole molecule about some axis) and molecular vibrations (the stretching or bending of bonds resulting in the displacement of atomic nuclei relative to each other, but not affecting the equilibrium positions of nuclei). Thus, infrared radiation would not be expected to cause chemical changes in molecules, although reaction rates might be increased due to heating (reference 11, p. 4).

If the biological effect of low level visible light therapy is through photochemistry (probably the photoactivation of enzymes), and the biological effect of infrared radiation is through molecular rotations and vibrations, *how can laser radiation therapy produce similar clinical responses when either visible radiation or infrared radiation is used?* For example, Abergel and coworkers[12, 13] found that the irradiation of fibroblasts in culture either at 633 nm or at 904 nm stimulated the synthesis of collagen. In separate studies, both 633 nm radiation[14] and 1060 nm radiation[15] were beneficial in reducing the pain of rheumatoid arthritis.

Biostimulation

To explain the biostimulation effect of low level radiation at 633 nm, Karu[1] proposed a chain of molecular events starting with the absorption of light by a photoreceptor, which leads to signal transduction and amplification, and finally results in the photoresponse. In her model (Figure 3), light is absorbed by components of the respiratory chain (i.e. flavine dehydrogenases, cytochromes and cytochrome oxidase), which causes an activation of the respiratory chain and the oxidation of the NAD pool, which leads to changes in the redox status of both the mitochondria and the cytoplasm. This in turn has an effect on membrane permeability/transport, with changes in the Na^+/H^+ ratio and increases in Na^+/K^+-ATPase activity, which in turn has an effect on the Ca^{++} flux. The Ca^{++} flux affects the levels of cyclic nucleotides, which modulates DNA and RNA synthesis, which modulates cell proliferation (i.e. biostimulation).

This model also suggests an explanation for why radiation at 904 nm can produce biological effects similar to those produced by radiation at 633 nm. In the model of Karu,[1] radiation at 633 nm initiates, probably by photoactivating enzymes in the mitochondria, a cascade of molecular events leading to the photoresponse (Figure 3). I suggest that radiation at 904 nm produces the same final response, but initiates the respone at the membrane level (probably through photophysical effects on Ca^{++} channels) at about halfway through the total cascade of molecular events that leads to biostimulation (Figure 3).

Calcium ions are intracellular messengers in many signal-transducing systems. The intracellular level of Ca^{++} must be kept low because phosphate esters are prevalent and calcium phosphates are very insoluble. The cytosolic level of Ca^{++} in unexcited cells (~0.1 μM) is several orders of magnitude less than the extracellular concentration. Thus, the cytosolic Ca^{++} concentration can be abruptly raised for signaling purposes by transiently opening calcium channels in the plasma membrane or in an intracellular membrane. There is not room here to adequately discuss all the ways in which calcium functions in signal transduction and the multiple pathways for the control of metabolism. The reader is referred to recent reviews,[16–21] including discussions of the effect of low level electric fields on Ca^{++} concentrations in tissues.[22, 23]

Theoretically, the potential for rotation and vibration by the action of infrared radiation on the membrane molecules that make up the calcium channels could alter the functionality of these channels, and thereby provide a mechanism for the therapeutic effect of low level infrared radiation. An important requirement for any theory is that it be testable. The techniques of molecular biology lend themselves very nicely to the testing of this theory.

In a recent paper, Karu[24] makes the interesting statement that 'the magnitude of the laser biostimulation effect depends on the physiological state of the cell at the moment of irradiation. This explains why the effect is not always detectable, as well as the variability of the results reported in the literature.

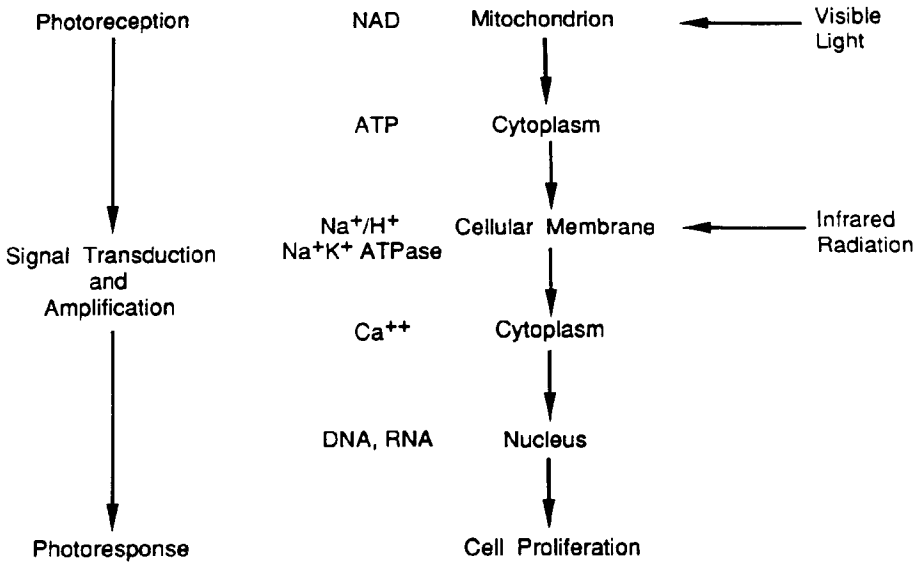

Figure 3. A model for the low level laser radiation stimulation of biological systems (modified from Karu[1]). Biostimulation at 633 nm results from the absorption of light by components of the respiratory chain in the mitochondria (see text), which results in increased metabolism, which leads to signal transduction to other parts of the cell, and ultimately to the photoresponse. Because infrared radiation is expected to exert its photophysical effect on the molecules that make up cellular membranes (see text), an arrow has been added to suggest that infrared radiation produces the same final biological result as does 633 nm radiation, but the two radiations start the cascade of metabolic events at different levels

For example, it has been established that irradiation accelerated the proliferation of slowly growing HeLa subpopulations. In medicine, laser treatment appears to work in cases of severe damage (e.g. trophic ulcers), and the effect of light on normally regenerating wounds may be insignificant (if there is any)'. Note that she emphasizes that light only stimulates cell proliferation if the cells are growing poorly at the time of the irradiation. Thus, if a cell is fully functional, there is nothing for laser radiation to stimulate, and no therapeutic benefit will be observed. A similar analogy is that patients will not show a beneficial effect of vitamin therapy if they already receive an adequate supply of vitamins in their daily diets.

Summary

In this paper I have tried to mention the photobiological principles that should govern the use of low level laser radiation in therapy, and to suggest a mechanism by which both visible light and infrared radiation, which produce quite different photochemical and photophysical effects, can produce the same clinical response.

(1) Lasers are just convenient machines that produce radiation.
(2) It is the radiation that produces the photobiological and/or photophysical effects and therapeutic gains, not the machine.
(3) Radiation must be absorbed to produce a chemical or physical change, which results in a biological response.
(4) The absorption spectrum of a biological system will show what wavelengths of radiation will be absorbed, and therefore what wavelengths have a chance of producing an effect.
(5) The true radiation sensitivity of a system at a given wavelength is the product of the absorbance, A, i.e. the probability that radiation will be absorbed, times the quantum yield, ϕ, i.e. the probability that the absorbed radiation will produce a chemical or physical change in the system.
(6) Once a photobiological response is observed, the next step is to determine the optimum dose of radiation at a given wavelength, and the optimum number of treatments required to produce the effect.
(7) Then, determine the optimum wavelength of radiation to produce the effect (the action spectrum).
(8) Consider that the biological effect of low level visible radiation may be due to the photoactivation of enzymes.
(9) Consider that the biological effect of low level infrared radiation may be due to the photophysical modification of membranes.
(10) Use proper controls for each clinical or laboratory protocol.
(11) Consider that low level laser radiation therapy

may not be successful if the cells are already functioning at the maximum normal rate.

The first era of low level laser radiation therapy and photobioactivation occurred in the 1960s and 1970s. During this period, every laser that was available was tried to see if it had a biological effect. These were largely anecdotal observations made by people with little knowledge of photobiology, or an understanding of the need for controls.

The second era began in the 1980s. In this period, proper controls were set up to eliminate the chance that the response observed was just a placebo effect. People became interested in the wavelengths of the radiation produced by the lasers, and began to investigate the photobiological basis of the therapeutic use of laser radiation.

The third era will probably not begin until the dramatic data of 2001. When this new era begins, people will know enough about the photobiological basis of existing phototherapies, and will know enough about the photoactivation of enzymes and membranes, and about metabolism in general so that they will be able to predict what enzyme or gene needs to be activated to promote a therapeutic benefit, and can then select the best wavelength of radiation to accomplish this therapeutic benefit.

The future for Low Level Laser Radiation Therapy looks very promising!

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