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OPTIMIZING LASER THERAPY

An Overview Of the Science
Optimizing Laser Phototherapy

Bryan J. Stephens, PhD

June 24, 2010

Therapeutic Laser Industry Overview

As has been the case with technology throughout history, this field was pioneered by engineers, not academics, and it's a good thing. After all, if we waited until we understood why something works before we used it, we would be in the stone-age, literally (people were eating vegetables before they knew why plants grew and warming their bodies with fire before they knew about combustion). This young field has therefore suffered from the zeal of its boosters; people had found a way to make money, and to their defense, improve patient quality of life, and so were much less interested in learning why this therapy worked. In this last decade, the research community has been catching up and we have ever-growing insight into the mechanisms of this highly successful field.

Differentiating What We “Know”

Because this field is so young, there are still huge gaps in our knowledge base, both in the physical properties of the individual lasers, as well as in the biological implications thereof. There is now, however, a well-established research community dedicated to the general study and optimization of the biological effects of laser phototherapy. Experimentation falls into two major categories: in vitro and in vivo studies, each of which is necessary, but extrapolating results from one to implications in the other is difficult and often misleading.

In Vitro Studies

Microscopic studies on laser interaction with biological material are invaluable. They give us the ability to precisely control the cellular environment and completely isolate a huge range of absorption mechanisms en route to a better understanding of not only the governing dynamics behind the macroscopic success of phototherapy, but also techniques to optimize its delivery and efficacy. Mountains of research have been done on individual bacteria and mammalian cells and on monolayers of such cells in petri dishes. From these, along with concurrent work on molecular biology, we have very clear picture of where light is absorbed in cells and which processes these interactions catalyze.
There is an important caveat, however, which resonates throughout the entire biological community: the reaction of a macroscopic matrix of cells that form tissue is **NOT** the sum of the reactions of each of the individual cells. One of the great mysteries of biology involves the complexity of cell-cell signaling and the ubiquity of bystander effects. A prime example of this intrinsic communication is in radiation oncology where researchers have used X-ray needles (microscopically narrow beams of x-rays) to irradiate individual cells growing in a monolayer. Amazingly, cells far away from the irradiated region somehow received information from the irradiated cells and underwent apoptosis (programmed cell death) in a way that is characteristic of cells that absorbed the ionizing radiation (even though they didn’t). Accordingly, we have to narrow the scope of individual cell and single cell monolayer studies to the search for absorption sites and the cellular functions affected by these sites, and stay away from making broader tissue-scale generalizations.

**In Vivo Studies**

Studies on human patients, and to a lesser extent laboratory animals, are often the most convincing to potential commercial users (i.e. chiropractors, podiatrists, veterinarians, dermatologists, etc.) of therapeutic lasers, and with good reason. Proven functionality on the proposed client base is a very marketable result. They are, by nature though, expensive and time consuming, and for that reason are usually biased to keep the number of possible outcomes narrow and manageable. Also, since they are typically carried out with commercial laser setups that require purchase, studies that compare multiple types (brands) of lasers to each other and to control groups are rare. Far more often, a study compares the effects of a single laser to a control group; the slightly more robust trials at least vary a parameter or two (e.g. power density, treatment length, pulse frequency, etc.).

Results from these types of studies yield conclusions that a particular treatment works, but not why or even if this particular treatment is most efficient. For example, a case study showing the elimination of a toe fungus with pictures before and after 6 weeks of therapy is encouraging in that it works, but does not tell you that the use of a different kind of laser could have provided the same results in 4 weeks.

Still, clinical trials are indeed necessary and continue to give important perspective into the macroscopic effects of phototherapy. The increasing popularity and success of this field is almost exclusively attributed to the number and breadth of clinical applications. To understand the mechanisms of action, though, we must combine our studies with a closer look into the cellular, and deeper still, molecular interactions of laser radiation with biological material. We can then use both micro- and macroscopic results to guide the search for the most efficient therapeutic techniques.

**The Well-Oiled Human Machine**

By far the most obvious and fortunate conclusion we have been able to extract from *in vivo* studies (not only with respect to laser phototherapy) is that our immune system is capable of handling an extraordinary range of pathologies. The time scale and degree to which our cells can react and combat these contaminants is the subject of much study,
but it is clear both that lasers do stimulate the immune system and that the restoration of healthy function continues well after the initial irradiation. The amount of healing done during the minutes of laser irradiation is minuscule compared to the time it takes to relieve the body of disease or infection. This leads to one very important piece of information: the body does most of the work itself and so the target for an effective laser treatment is NOT the pathology itself, but rather to stimulate the appropriate cell compartments that lead to the body’s natural repair mechanisms. Basically, we want to stimulate the cell’s metabolism (i.e. its ability to use oxygen to create energy).

**Bacteria, on the Other Hand**

There are about 1000 different types of bacteria commonly present in the human body most of which reside either on the skin, or in the digestive tract. Of these, only about 10% are maintainable in cell culture and able to be studied. Some are beneficial (e.g. those that aid in digestion of food) others pathological. With this wide variety of species, never-mind their different functions and chemical signatures, it is prohibitively difficult to target any individual candidate or even to make the generalization that these candidates are more abundant than any other with respect to a particular pathology. Instead we can capitalize on one common feature in most bacteria: they do not like oxygen. Most bacteria are anaerobes that proliferate and metabolize much better in the absence of oxygen. Fortunately, this is in direct contradiction with the way our cells flourish and so stimulating the oxygen intake and conversion process will simultaneously help our healthy cells and inhibit bacteria.

**Mechanisms**

**Identifying Targets**

The most fundamental thing to keep in mind is that the cell (and the body as a whole) is comprised of more than 80% water. The variation in water content between different kinds of cells (with the exception of bone cells) is negligible and so laser therapy as a whole is highly non-selective. Cells do, however, contain some heavier elements that can act as a contrast agent against water, and which can therefore be targeted with laser radiation; the most relevant examples are iron and copper. Not surprisingly, these elements are the ones that exist at the core of the two most important photoacceptors in the body: hemoglobin at the core of blood cells and cytochrome c oxidase in the mitochondria. By and large these complexes are the principle absorbers of mammalian tissue by light in the near infrared (NIR) range of the electromagnetic spectrum (other than melanin in the skin). As such, and before any attention to their function, the characterization of absorption of these complexes was of paramount importance, and the subject of much study. Action spectra (i.e. the dependence of wavelength on absorption) have been generated for these (and other) targets *in vitro* and the peaks have been isolated and correlated with the biologically state of these complexes (see section “Clinical Functionality”).
Metabolic Action

The action spectra tells us where in the spectrum and at what rate laser radiation is absorbed by these chromophores, but we must address the biology of the cell to understand the subsequent chain of events that lead to a beneficial, curative result. As discussed earlier, the central goal is to stimulate the cell (and ultimately, the body) to perform its natural functions, but at an enhanced rate. These natural functions are not only extremely numerous (ranging from protein synthesis to enzyme secretion, from cell signaling to physical movement) but also highly cell-type dependent. Any attempt to directly target one of the multitude and variety of these specific enzymes is difficult, and fundamentally unnecessary. If instead, the metabolism, specifically the respiratory chain, can be stimulated, the cell will enhance the functionality of all of its natural processes.

Fortunately, both hemoglobin and cytochrome c oxidase are involved in cell metabolism and their roles in the respiration chain are linked. Hemoglobin is the molecule, at the core of red blood cells, that transports oxygen through the body to the cells. When it reaches the cell it has to be de-oxygenated or “reduced”. The oxygen is then passed through the cell membranes and into the mitochondria where it is processed by a series of enzymes, the last of which is cytochrome c oxidase. Here the oxygen is again “reduced” as it is converted into water; this reaction is the stimulus for the enzyme ATP synthase to create ATP, the source of chemical energy in cells. This is the reason we need oxygen, slightly more in depth than “to breathe”.

Optimizing Efficiency

Zooming out to the big picture, hemoglobin carries the oxygen through the blood from the lungs to the cells. It has to be reduced and the oxygen flows through the respiratory chain to the terminal enzyme, cytochrome c oxidase, which then reduces again to create energy for the cell. Think of the hemoglobin as the faucet that governs the rate at which oxygen flows into the cell and cytochrome c oxidase as the drain that determines the rate at which oxygen can exit the cell in the form of ATP (energy). To optimize efficiency of the flow of oxygen through the respiratory process, the most appropriate course of action would be to open both the faucet and drain as wide as possible (opening one without the other would not increase the overall throughput); that is, stimulate the amount of hemoglobin that reaches the cell, the rate at which it reduces its oxygen, and then the rate at which the cell can process that oxygen and output energy. The goal then is to increase local blood circulation, stimulate the reduction of hemoglobin, then stimulate both the reduction and immediate re-oxygenation of cytochrome c oxidase so the process can start again.

Clinical Functionality

Circulation

Recall the first goal of an effective therapy was to increase the amount of oxygen available for the cell to process. This means increasing blood circulation since the
Hemoglobin in red blood cells are the transporters of oxygen from the lungs to the cells. On the macroscopic scale, this relies on increasing the heart rate, which in turn slightly increases body (and blood) temperature. This is why exercise is good therapy for almost any ailment; increasing blood flow increases metabolism and stimulates the immune system. Locally around a wound, however, topographical heating does very little, resulting in neither an increase in circulation nor metabolism. This type of thermal effect is not the mechanism for laser stimulation of circulation. Laser irradiation instead creates local temperature gradients; that is, temperature differences on the molecular level that create potentials along which blood cells are more likely to flow. The stronger and more numerous the gradients, the more local circulation of oxygen can be stimulated.

What is the most efficient way to cause these temperature fluctuation? Recall that the cell is more than 80% water. If you can target the absorption of water by a particular wavelength of radiation, you can cause local resonances that reinforce themselves. In the entire NIR region (i.e. from 700-1000 nm) the strongest and most distinct peak in absorption is at 965 nm; the right side of Figure 1 shows the absorption spectrum of brain tissue in the NIR.

Hemoglobin Deoxygenation

Once the increased circulation gets the blood to the cell, the hemoglobin that carry the oxygen in the blood have to drop off their oxygen supply. Oxygenated and deoxygenated hemoglobin have very distinct signatures in the NIR. We are not concerned with the process of re-oxygenating the hemoglobin, because this occurs in the lungs. Instead we are interested in the absorption spectrum of oxygenated hemoglobin ($HbO_2$) whose deoxygenation can be stimulated by the absorption of a photon of radiation. Figure 1 shows this rather broad peak that covers the higher end of the NIR.

Cytochrome c Oxidase Redox

As discussed earlier, the terminal enzyme in the respiratory chain of a cell, cytochrome c oxidase, is the principle absorber of radiation in the entire cell and governs the rate at which oxygen is processed into ATP. Unlike the one-way deoxygenation of hemoglobin, cytochrome receives and delivers its oxygen in cycles within the cell and so we need to stimulate both processes in order to maximize efficiency. It turns out that laser irradiation does both, depending on the oxidation state of the enzyme. When deoxygenated, laser irradiation will stimulate oxygenation, and vice versa [1]. This effect has resounding implications and is thought to be the universal validation of laser therapy. The different oxygenation states of this enzyme have peaks throughout the visible-NIR spectrum, which is why virtually all wavelengths used have shown to be useful.

Laser phototherapy with wavelengths throughout the NIR spectrum enhances cellular metabolism, but there exists a peak in the absorption spectrum that can maximize this effect. Figure 1 shows the difference spectrum in the absorption of oxygenated vs. deoxygenated cytochrome. Remember, when the enzyme is either fully oxygenated or fully deoxygenated, irradiation will push the cycle along in the right direction, so
we want to stimulate the process at both endpoints. The peak in the difference spectrum reflects the wavelength at which laser irradiation will have the greatest effect to change the oxygenation state, which will subsequently turn the wheels on the cellular metabolism most efficiently. This is analogous to firing the spark plugs at the exact time in the engine cycle to get the maximum effect.

![Figure 1: Absorption spectra of cytochrome (left axis), oxygenated hemoglobin (a.u.), and brain tissue (right axis). Data re-digitized from [2].](image)

**Take Home Message**

**FACT:** Laser phototherapy, if administered by someone trained in the art, is beneficial in almost all of its forms and has no adverse side effects.

The differences between commercially available laser units lie solely in the wavelength, power density, pulse modulation, and aesthetics. From these parameters, you can derive the penetration depth, dose distribution, treatment time, and the estimated biological effect. There is *NOT* a “magic” wavelength or setting that is the cure for a disease, and to claim otherwise (as many distributors or salesmen do) is irresponsible. There are, however, certain operating regimes that give better results than others and are more effective for particular symptoms. The select few modalities that have been specifically designed to isolate and capitalize on a fundamental therapeutic mechanism,
have continually proved successful in the clinic. And since the primary mechanism of action is the stimulation of the body’s natural anti-pathological immune system, the range of symptoms for which this treatment modality is useful knows no bound.

References


Laser-Accelerated
INFLAMMATION/PAIN REDUCTION AND HEALING

Low Level Laser Therapy (LLLT) precipitates a complex set of physiological interactions at the cellular level that reduces acute inflammation, reduces pain, and accelerates tissue healing.

by Richard Martin, BS, CLT

Compromised cells and tissues respond more readily than healthy cells or tissues to energy transfers that occur between LLLT-emitted photons and the receptive chromophores found in the various cells and sub-cellular organelles. Cells and tissues that are ischemic and poorly perfused as a result of inflammation, edema and injury have been shown to have a significantly higher response to LLLT irradiation than normal healthy structures. Cell membranes, mitochondria and damaged neurological structures exhibit less than optimal metabolism and stasis conditions. Multiple studies have demonstrated that under these compromised conditions, the introduction of energy transfers and the resultant enhancement of metabolic activity is most pronounced in biologically challenged components. While it may appear that LLLT is thus selectively targeting compromised cells, in reality, these cells exhibit a lowered reaction threshold to the effects of laser light and are more easily triggered to energy transfer responses. The result is that LLLT has a significant effect on damaged cells and tissues while normative biological constituents are appreciably less affected.

The cellular cascade effect — precipitated by the actions of enzymes and having a significant in the presence of LLLT — has a significant impact on cellular and tissue function. Since a considerable number of the reactive proteins that respond to laser stimulation are enzymes, laser light effects are amplified in the stimulation of beneficial enzymes and depression of deleterious enzymes.

At the cellular level, cytochromes can be defined as electron or proton-transfer proteins that act as energy producers for human biological functions. Both of the cytochrome enzymes, Cytochrome c Oxidase and Nitric Oxide Synthase (NOS) have been found to be particularly reactive to laser photon stimulation. The particular affinity of these and other photoreactive enzymes to accelerate their functions in the presence of LLLT provides critical increases in the molecule ATP and Nitric Oxide (NO) which enhances cellular metabolism, circulatory improvement and nerve function.

Although the various actions of LLLT in regards to inflammation, pain and healing have been separated categorically here for the purpose of process identification, their interactions are not so easily distinguished. In response to LLLT, the reduction in inflammation, pain and healing time all compliment each other and many of the processes are either simultaneous or overlapping.

Acute Inflammation Reduction
Immediately after an acute injury event, the body, in response to the disruption of the integrity of vascular, soft tissue, connective tissue and neurological processes, initiates a series of biological responses. The inflammatory reaction consists of both vascular and cellular events. Injury responsive components such as Mast cells, Bradykinins and Prostaglandins are activated along with the vascular responses and cellular membrane reactions. All of these combined processes and events are represented by the symptoms of edema, inflammation, pain and functional debility. LLLT can be effective in mediating both the symptoms and the underlying inflammatory process by the following actions:

1. Stabilization of cellular membrane — Ca++, Na+ and K+ concentrations as well as the proton gradient over the mitochondria membrane are positively influenced. This is accomplished in part by

2. Stimulation of cellular metabolism — Increased cellular energy production through the processes of ATP synthesis and NO cascades.

3. Reduction of pain — Increased endorphin release and decreased pain neurotransmitter activity.


5. Reduction of inflammation — Decreased inflammatory mediator release and activation of anti-inflammatory processes.

6. Reduction of edema — Increased capillary permeability and decreased tissue fluid accumulation.

These beneficial effects of LLLT are evident in a wide range of applications, including the treatment of acute and chronic pain, wound healing, tissue regeneration, and the management of inflammatory and degenerative diseases.
Cold or soft laser therapy, also known as low level laser therapy (LLLT), is being used for an increasing number of medical and rehabilitative applications including pain management. The nomenclature alludes to the athermic or non-heat producing characteristic of these FDA class 2 and 3 devices. Unlike hot lasers used to cauterize, vaporize, coagulate, or ablate tissue or tumors, cold lasers work through more subtle tissue effects that can result in the reduction of both pain and inflammation, devoid of tissue destruction. Consequently, cold lasers are finding a niche with soft tissue specialists of varying backgrounds including medicine, podiatry, dentistry and physical rehabilitation. Although a relatively new modality in the United States, cold lasers have been used in Canada, Europe and some parts of Asia for many years. Lasers fall under the general category of photomedicine, but this broader name often obscures the unique properties inherent with laser, properties which serve to distinguish this form of light therapy from other, perhaps less potent, forms of light energy.

In 2002, the FDA issued the first 510k premarket notification for a soft or cold laser device based largely on the strength of earlier large scale multi center clinical trials that had examined the effectiveness of cold lasers in the primary treatment of carpal tunnel syndrome. The GM study, as it has come to be known by, was arguably, the pivotal investigation that “tipped” the scale in favor of FDA approval for these devices. Since then, a number of laser manufacturers have followed suit with their versions of the ideal lasering device. To date, all these devices have been under a specified power level of 1 watt (considered to be threshold for thermal effect) and usually between 5 and 100mW. As a point of reference, a laser pointer is approximately 2-3mW in power. Recently, FDA class 4 devices have been introduced into the marketplace with much higher average power levels than their class 2 and 3 counterparts. Typically seen in veterinary medical use, time will tell how these devices will add clinical utility to the already growing number of lasers in the marketplace.

While numerous studies utilizing cold lasers have been performed to date, many do not provide precise test parameters such as power density, treatment duration, wavelength and site of application — all essential information needed to replicate findings. Despite the currently limited amount of quality research supporting cold laser use, the number of double blinded, randomized and controlled clinical trials is growing, as well as the amount of empirical evidence gathered from the non daily use of these instruments across the country.

**Laser-Tissue Interaction**

The two most important modes of light interaction with tissue during laser treatment is through absorption and scattering. This has been studied predominantly at the molecular and macro-molecular level. Absorption is considered to be a conversion of energy from light to another form. Tissue absorbing properties are dependent on their concentration of light accepting molecules such as amino acids, cytochrome, chromophores and water. Each of these interacts with light at specific wavelength ranges (bandwidths). Scattering also occurs during cold laser treatment and is considered to be a change in light propagation direction and thought to occur due to the varying shapes of biomolecules and varying tissue interface configurations. Depth of penetration...

Low energy laser therapy has been shown — at appropriate dosimetry, wavelength, duration, and site-specific application — to reduce tissue pain/tenderness, normalize circulation patterns in tissue trauma, and increase collagen formation in wounds.

by Tiziano Marovino, PT, DPT, MSc, BA, BHSc, BRLS, Dip.PT, FAAPM
Mechanisms of Low Level Light Therapy.

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ABSTRACT

The use of low levels of visible or near infrared light for reducing pain, inflammation and edema, promoting healing of wounds, deeper tissues and nerves, and preventing tissue damage has been known for almost forty years since the invention of lasers. Originally thought to be a peculiar property of laser light (soft or cold lasers), the subject has now broadened to include photobiomodulation and photobiostimulation using non-coherent light. Despite many reports of positive findings from experiments conducted in vitro, in animal models and in randomized controlled clinical trials, LLLT remains controversial. This likely is due to two main reasons; firstly the biochemical mechanisms underlying the positive effects are incompletely understood, and secondly the complexity of rationally choosing amongst a large number of illumination parameters such as wavelength, fluence, power density, pulse structure and treatment timing has led to the publication of a number of negative studies as well as many positive ones. In particular a biphasic dose response has been frequently observed where low levels of light have a much better effect than higher levels. This introductory review will cover some of the proposed cellular chromophores responsible for the effect of visible light on mammalian cells, including cytochrome c oxidase (with absorption peaks in the near infrared) and photoactive porphyrins. Mitochondria are thought to be a likely site for the initial effects of light, leading to increased ATP production, modulation of reactive oxygen species and induction of transcription factors. These effects in turn lead to increased cell proliferation and migration (particularly by fibroblasts), modulation in levels of cytokines, growth factors and inflammatory mediators, and increased tissue oxygenation. The results of these biochemical and cellular changes in animals and patients include such benefits as increased healing in chronic wounds, improvements in sports injuries and carpal tunnel syndrome, pain reduction in arthritis and neuropathies, and amelioration of damage after heart attacks, stroke, nerve injury and retinal toxicity.

Keywords: biostimulation, low level laser therapy, wound healing, biomodulation, cold laser, action spectra

1. HISTORY

In 1967 a few years after the first working laser was invented, Endre Mester in Semmelweis University, Budapest, Hungary wanted to test if laser radiation might cause cancer in mice [1]. He shaved the dorsal hair, divided them into two groups and gave a laser treatment with a low powered ruby laser (694-nm) to one group. They did not get cancer and to his surprise the hair on the treated group grew back more quickly than the untreated group. This was the first demonstration of “laser biostimulation”. Since then, medical treatment with coherent-light sources (lasers) or noncoherent light (light-emitting diodes, LEDs) has passed through its childhood and adolescence. Currently, low-level laser (or light) therapy (LLLT), also known as “cold laser”, “soft laser”, “biostimulation” or “photobiomodulation” is practiced as part of physical therapy in many parts of the world. In fact, light therapy is one of the oldest therapeutic methods used by humans (historically as solar therapy by Egyptians, later as UV therapy for which Nils Finsen won the Nobel prize in 1904 [2]). The use of lasers and LEDs as light sources was the next step in the technological development of light therapy, which is now applied to many thousands of people worldwide each day. In LLLT the question is no longer whether light has biological effects but rather how energy from therapeutic lasers and LEDs works at the cellular and organism levels and what are the optimal light parameters for different uses of these light sources.
POWER AND DOSAGE STUDIES
Synopsis of Articles on Power and Dosage

1-On the Necessity of Class IV
This paper explains that the biological effect stimulated by laser therapy is related to the number of photons that reach the affected area, not the number of photons incident on the surface. It discusses the how parameters of the Laser need to account for the absorbing and scattering properties of the tissue.

2-Class IV Laser in Non-invasive Laser Therapy
Practical experience of our clinic, which has been working as a supervisory and consultatory workplace for almost the whole of the country, allows us to state that the preponderance of clinical fiascos of LLLT is caused by underdose.

3-808 nm Wavelength Light Induces a Dose-Dependent Alteration in Microglial Polarization and Resultant Microglial Induced Neurite Growth
Concludes that the Arndt-Schulz Law and its relation to Laser dosage does not apply across all Photobiomodulation reactions.

4-Limb Blood Flow After Class 4 Laser Therapy
**Key Points:** Using a class 4 laser in a human clinical model, we found a protocol-response effect: a 3-W protocol at a 50% duty cycle applied to the biceps brachii muscle was the most effective for increasing blood flow to the distal forearm.
Laser therapy is an effective, noninvasive treatment modality to improve blood flow and perhaps tissue healing in the clinical setting.

5-Effects of Class IV Laser Therapy on Fibromyalgia Impact and Function in Women with Fibromyalgia
This study provides evidence that LHT may be a beneficial modality for women with FM in order to improve pain and upper body range of motion, ultimately reducing the impact of FM.

6-The Effectiveness of Therapeutic Class IV (10 W) Laser Treatment for Epicondylitis
This study’s findings suggest that laser therapy using the 10 W Class IV instrument is efficacious for the long-term relief of the symptoms associated with chronic epicondylitis.

7-Intricacies of Dose in Laser Phototherapy for Tissue Repair and Pain Relief
Looks at a wide range of patient related factors and Laser parameters that need to be considered when determining optimal dosage.
On the Necessity of Class IV

Bryan J. Stephens, PhD

July 7, 2010

The biological effect stimulated by laser therapy is related to the number of photons that reach the affected area, not the number of photons incident on the surface. Penetration is a complicated quantity that depends on wavelength of the radiation, power density of the beam delivered, and the absorbing/scattering properties of the particular tissue-type being treated. Far too often, laser companies make claims of treatment capabilities based on the amount of energy (number of Joules) their laser can produce. This is a completely meaningless quantity on its own. For example, a single fluorescent bulb will produce about 4 million Joules in a normal 12-hour work day, and you would be exposed to far more just by stepping outside for a couple hours. It is important to overcome this dangerous shortage of information in both deciding between lasers and developing treatment planning strategies. Starting from output power, we can step through the calculation that leads us to the meaningful quantity of dose.

From Power to Power Density

One quantity any laser company will be able to report is power output. This is the amount of energy per unit time emitted in the laser’s beam, usually reported in Watts, or for Class III or below, milliWatts (thousandths of a Watt). Even worse, some laser companies report this value as the power they are able to “produce”, determined by applied voltage and the current through the actual laser manifold. This is all that is necessary when classifying, for example, a table-top pumping laser used strictly for research in materials science or quantum optics. In lasers for which it is necessary to steer the beam by way of fiber optics, much of the power “produced” is lost in the coupling of even the most efficient fiber optic setups. A simple power meter placed along the beam-line can confirm that many lasers do not deliver all the power they can “produce”.

Beyond mere power output, the treatment area is of paramount importance to understanding the number of photons delivered to the affected area. For comparison, a long tube 100 Watt fluorescent bulb like those in any office building is bright enough to light up an entire room, and it does, in fact, spread 100 Joules of light per second across the entire breadth of a room. If instead, that bulb were only as big as your fist (like a normal incandescent bulb), those 100 Watts would be much “brighter”. Better still, if all of that light was collimated into a beam (as in a laser) the “brightness” would be extreme. The power output simply defines the number of total photons emitted, but it is...
exactly the concentration (density) of the photons that dictates the number of photons delivered to a target area. That target area, for the most part however, is not on the surface of the skin and so it is important to understand how treating a highly-scattering medium like human tissue will affect the dose delivered to non-superficial targets.

**From Surface Power Density to Power Density Delivered**

Power density is the only necessary intensity parameter for *in vitro* experimentation because there is no attenuation due to a monolayer of cells. From power density measurements, calculating the energy density (i.e. dose) is straightforward: power density in units of Watts/cm$^2$ multiplied by treatment time in seconds yields dose in units of Joules/cm$^2$. This is the energy deposited per area of irradiated tissue. *In vivo*, however, this parameter does not tell the whole story. Tissue is a highly scattering medium and there is non-trivial attenuation at depths in the human body. The power density simply refers to the intensity (number of photons) at the output of the laser. This intensity decays exponentially with depth in tissue, and the decay constant (related to the penetration depth) is determined by the wavelength of the laser and the optical properties of the tissue.

Furthermore, radiation will scatter laterally (radially, since the beam is cylindrical) and so there will be dose deposited beyond the spot size of the laser. It is important to remember that only a finite number of photons is emitted from the laser, so this spreading of the beam will also lead to a further decrease of photons along the central beam axis. Combating this spread is the flip-side of the scattering coin. Even the most coherent lasers are not completely collimated; that is, they slightly diverge, but scattering of the divergent parts of the beam will add more photons to the collimated path.

Again, these are complicated phenomena that have to be modelled and measured to give an accurate description of the dose deposition of any laser beam to be used *in vivo*. Comparing lasers to each other must therefore include more than just power density analysis. Figure 1 is an example of such analysis. From these profiles and a detailed analysis of the optical properties of the different types of tissue, we can calculate the necessary treatment distances and times for therapeutic regimens.

**From Power Density to Dose**

Now it is possible to understand the number of Joules to be delivered to a target area. Again it is not the total number of Joules delivered at a certain depth that is important since we can see that the beam is spread over an area and we are not treating infinitely small regions. Instead, the important parameter is the energy density; that is, energy per unit area, more commonly called dose with units of J/cm$^2$. From the depth dose profile, a distinct version of which is necessary for each wavelength, frequency, and power setting as well as for every type of material through which the laser beam will penetrate (skin, bone, soft tissue, fat, etc.), you can map out the intensity and determine...
Figure 1: Example of an actual measured 3-D dosimetric beam profile of the K-Laser K-1200 in a water phantom. For instance, a point (red arrow) that is 6.1 cm deep in water and 0.5 cm from the central beam axis will be exposed to radiation whose intensity is 29% of the full intensity at the surface of the skin. This type of information is crucial to determining the dose delivered to tissue at a distance inside the body.

the power density across a desired area. From the power density at a given depth, we can then revert to the same simple formula used \textit{in vitro}: power density in units of Watts/cm$^2$ multiplied by treatment time in seconds yields dose in units of Joules/cm$^2$.

**Sample Calculation**

If you wish to deliver 1000 Joules to a lower lumbar ailment at a depth of 3 inches (~8 cm), for example, you cannot simply take a 500 milliWatt Class III laser and treat for 2000 seconds (500 milliWatt = 0.5 Joule/sec x 2000 sec = 1000 Joule) because you would only actually be delivering 276 Joules to the affected area (0.5 Watt at surface x 27.6% intensity at 8 cm [from Figure 1 along the central beam axis] x 2000 seconds). In fact, you would have to irradiate for 7246 seconds (over 2 hours!!) to build up enough dose. If instead you used a 12 Watt laser, you could achieve 1000 Joules at 8 cm in 302 seconds (only about 5 minutes). Depending on the beam size of the laser and the scattering properties of the tissue involved, the energy density (dose) can be determined, but according to this first-order analysis, about 5-10% of the beam is scattered away from the 1 cm$^2$ surface beam and so these treatment times would have to be corrected to 105-110% of their values to achieve 1000 J/cm$^2$. 
Take Home Message

There is much more to consider when comparing lasers or predicting success of treatments than either a simple power output or surface energy value. The central thesis to this entire discussion, though, is the necessity of starting off with high power density since all contributing factors lead to severe attenuation of the beam. If only superficial dermatology concerns you, than less intricate and less expensive Class II or III lasers may be suitable for you. But for any subcutaneous, and especially deep muscle or joint ailments, if you wish to achieve any analgesic or biostimulatory effects, these lower power lasers simply cannot deliver sufficient dose at depths in the body in reasonable treatment times.
Class IV Laser in Non-invasive Laser Therapy
Miroslav Prochazka, M. D., Head Doctor of the Private Rehab Clinic Jarov, Prague, CZ
Mail
Abstract

The title must have awakened curiosity of every supporter of non-invasive laser therapy, or at least a bit of amusement. Since the very first small steps on the long and manifold path of laser medicine we have been aware that despite dynamic development of this technique, yet there is a couple of firm reference points. Laser will always mean radiation of light with perfect coherence and monochromaticity. We will never direct a laser in the eye and we will always mind also other contra-indications. Lasers in class IIIa and IIIb are intended for applications in terms of non-invasive laser therapy (LLLT), whilst class IV lasers are meant for use in surgical specialties ... Or perhaps, maybe it is not quite so?

Output power is one of the most important parameters of a laser, indirectly affecting also the spectrum of possible applications as well as time required to perform therapy. Years back, low level therapy (LLLT) manufacturers had provided devices fitting into class IIIa, often with output power not exceeding 3 or 5 mW, especially HeNe sources which were mainly used for superficial conditions, such as wound healing. Expansion of application spectrum of LLLT into pain management and therapy of locomotive apparatus clearly pointed to the need of higher output levels and, similarly, led to implementation of other wavelengths with deeper penetration in tissue (IR). Nowadays, therapists usually work with infrared laser probes with 300 and more milliwatt of power, and values of 450 - 500 mW represent an imaginary boundary for both manufacturers and therapists, behind which laser devices are classified in laser class IV, with all consequences in terms of hygienic rules and labour safety applicable. There is no need to remark that hand in hand with increasing output of non-invasive lasers, as well as due to long-term clinical experience, higher and higher dosages of energy are also being administered.

Arndt-Schultz Law stipulates that effect of therapeutic laser appears when a certain threshold limit of irradiated energy is exceeded, and is rising till a level called plateau of effect is reached. As soon as the plateau is reached, further increasing of energy dosage has allegedly no influence on desired result of therapy. On the contrary, from a certain level on (literature indicates ca 16 J/cm2) the effect of laser is purportedly decreasing. However, in my opinion this applies only (though not quite unequivocally) to in-vitro experiments with cellular cultures, not taking into consideration (as it is practically impossible) the whole complex of effects of laser on living organism within the frameworks of therapy of a defined syndrome or complaint, comprising systematic effect, analgesic and antalgic effect, antiphlogistic and vasodilatation effect, biostimulation etc. Vice versa, in clinical practice it is obviously necessary to declare that the higher dosage of energy is irradiated, the better therapeutic effect is achieved. Furthermore, in case of deep seated applications we must take into account substantial loss of energy in the course of penetration of laser beam through tissue structures (skin, subcutaneous fat, muscles, sinew, bone). That is why we have been meeting with significant increase of recommended dosages of energy in treating deeply located conditions. However, this is only a nominal increase; in fact the real irradiated dosage of energy into the target structure is lower just due to high absorption in tissue. Herat a certain sufficient real amount of energy in the target area must be accomplished, which is unfortunately something that can hardly be achieved working with lasers with too low output power. Apart from that we have to note that higher output of our device equals shorter therapy time, which is in terms of laser therapy more than a substantive factor. Lack of power is something that surgical high power lasers surely do not suffer from. In fact, any class IV surgical laser can be used for therapeutic irradiation. Thus we are well familiar with Nd:YAG laser inducing analgesic effects prior to a preparation in dentistry, or in treatment of arthritis in rheumatology. Argon and KTP lasers are routinely used for teeth whitening, though they have also been tested in dermatological applications, such as treatment of psoriasis. Ruby laser had been used by Endre Mester as the first laser for biostimulation and wound healing, furthermore proving to be useful in healing up bed sores, varicose ulcers and shingles. Excimer and Alexandrite lasers are capable of treating some superficial dermatologic conditions, whilst high power diode lasers can manage treatment of painful joint ailments. The advantage of all those lasers is their ability to irradiate high dosages of energy on large areas. However, it is necessary to defocus the beam or to scan it over the skin so that concentration of high dosages of energy to a little spot, leading to thermal damage of tissue, be eliminated. On the other hand, high price of those lasers is a serious disadvantage, almost disqualifying them economically for the use in laser therapy, because comparable results can be obtained with lasers the price of which is roughly 10 to 50 times lower.

Our clinic had also been using a CO2 surgical laser which was used in co-operation with a dermatologist and a surgeon to handle various minor excisions of superficial skin efflorescences. In several patients we had also tried the effect of therapeutic
808 nm Wavelength Light Induces a Dose-Dependent Alteration in Microglial Polarization and Resultant Microglial Induced Neurite Growth

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INTRODUCTION

Photobiomodulation (PBM), or low level light therapy, uses low power density light of specific wavelengths from light-emitting diodes or lasers to alter cellular function via several proposed mechanisms, including alteration of mitochondrial activity or reactive oxygen species (ROS) production [1]. PBM promotes tissue regeneration, reduces inflammation, and modulates pain after trauma [2]. It has also been used effectively to improve recovery after peripheral nerve injuries [3] and cutaneous wounds [4], and shows promise for use in central nervous system (CNS) injury [5–9].

PBM responses are tissue, wavelength and energy dose-dependent. In fact, the biological effects of PBM are currently theorized to follow the Arndt–Schulz law [2], in which low to moderate energy fluences induce activity while higher doses result in inhibition. Sharma et al. [10] found that this law was true for energy production in cultured neuronal cells, in which low fluences of 810 nm wavelength laser (with constant power density, 25 mW/cm², and varying administration time), resulted in induction of ATP production, while high fluence led to reduced responses. However, a triphasic response was also observed in this study, with an induction of reactive oxygen species and nitric oxide (NO) at both low and high fluence and a reduction at midlevels, which draws into question the applicability of this law.

As the primary mediators of immune and inflammatory responses in the CNS, microglia not only respond to...
Limb Blood Flow After Class 4 Laser Therapy

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Context: Laser therapy is purported to improve blood flow in soft tissues. Modulating circulation would promote healing by controlling postinjury ischemia, hypoxia, edema, and secondary tissue damage. However, no studies have quantified these responses to laser therapy.

Objective: To determine a therapeutic dose range for laser therapy for increasing blood flow to the forearm.

Setting: Controlled laboratory setting.

Patients or Other Participants: Ten healthy, college-aged men (age = 20.80 ± 2.16 years, height = 177.93 ± 3.38 cm, weight = 73.64 ± 9.10 kg) with no current history of injury to the upper extremity or cardiovascular conditions.

Intervention(s): A class 4 laser device was used to treat the biceps brachii muscle. Each grid point was treated for 3 to 4 seconds, for a total of 4 minutes. Each participant received 4 doses of laser therapy: sham, 1 W, 3 W, and 6 W.

Main Outcome Measure(s): The dependent variables were changes in blood flow, measured using venous occlusion plethysmography. We used a repeated-measures analysis of variance to analyze changes in blood flow for each dose at 2, 3, and 4 minutes and at 1, 2, 3, 4, and 5 minutes after treatment. The Huynh-Feldt test was conducted to examine differences over time.

Results: Compared with baseline, blood flow increased over time with the 3-W treatment (F3,12 = 5.468, p < .011) at minute 4 of treatment (2.417 ± 0.342 versus 2.794 ± 0.351 mL/min per 100 mL tissue, P = .032), and at 1 minute (2.767 ± 0.358 mL/min per 100 mL tissue, P < .01) and 2 minutes (2.657 ± 0.369 mL/min per 100 mL tissue, P = .022) after treatment. The sham, 1-W, and 6-W treatment doses did not change blood flow from baseline at any time point.

Conclusions: Laser therapy at the 3-W (360-J) dose level was an effective treatment modality to increase blood flow in the soft tissues.

Key Words: therapeutic modalities, circulation, musculoskeletal injuries

Key Points
- Using a class 4 laser in a human clinical model, we found a protocol-response effect: a 3-W protocol at a 50% duty cycle applied to the biceps brachii muscle was the most effective for increasing blood flow to the distal forearm.
- Laser therapy is an effective, noninvasive treatment modality to improve blood flow and perhaps tissue healing in the clinical setting.

The use of laser as a clinical modality has increased greatly over the past decade. Positive effects of laser therapy for the treatment of acute and chronic musculoskeletal disorders include pain control1,2 and improved tissue repair.3,4 However, the underlying mechanisms and clinical effectiveness of laser therapy remain poorly understood.

Lasers are classified by power level and their ability to produce eye injury. These power and beam characteristic ratings are established by the American National Standards Institute and the International Electrotechnical Commission. Most therapeutic lasers available for use in clinical practice are classified as 3B or 4. Class 3B lasers emit power of 5 to 500 mW, whereas class 4 lasers emit power of more than 500 mW. A few therapeutic laser manufacturers offer divergent-beam power outputs greater than 10000 mW. Class 3B level emitting lasers are known as low-level, low-intensity, and cold lasers because they generate no significant thermal effect in the superficial tissue during irradiation. Class 4 lasers are known as high-power and hot lasers because they can produce rapid increases in superficial tissue temperatures when maximum permissible exposure limits are exceeded. Recent trends in laser therapy show a preference for class 4 lasers in patient care settings.5 Class 4 lasers can emit greater photon energy in a shorter period of time than class 3B lasers without producing an appreciable rise in tissue temperature under normal treatment protocols.6 This higher power becomes important when treating injuries to deeper tissues such as ligaments, muscles, tendons, and cartilage.

Authors of most published clinical studies on laser therapy to treat musculoskeletal injuries have used class 3B low-power lasers. Several published reports7,8 have questioned the ability of low-power lasers to effectively transmit energy beyond the skin into deep musculoskeletal tissues. Excessive beam scattering and attenuation within the skin limit the potential biostimulative effects of laser in the deeper target tissues because of several factors related to dosimetry, such as subthreshold optical power, insufficient treatment durations, and varied treatment frequencies.9 Therefore, it is relevant and timely to study the dosimetric responses of specific infrared wavelengths of high-power class 4 lasers and their ability to modulate the physiologic effects that are conducive to healing.

Positive therapeutic effects of laser have been attributed to increased blood flow in soft tissues and, coincidentally, the
Effects of Class IV Laser Therapy on Fibromyalgia Impact and Function in Women with Fibromyalgia

Lynn Panton, PhD, FACSM, 1 Emily Simonavice, PhD, 2 Kristen Williams, MS, 1 Christopher Mojock, MS, 1 Jeong-Su Kim, PhD, 1 J. Derek Kingsley, PhD, 3 Victor McMillan, MD, 4 and Reed Mathis, DC 5

Abstract

Objectives: This study evaluated the effects of Class IV laser therapy on pain, Fibromyalgia (FM) impact, and physical function in women diagnosed with FM.

Design: The study was a double-blind, randomized control trial.

Setting: Testing was completed at the university and Rheumatologist office and treatment was completed at a chiropractic clinic.

Participants: Thirty-eight (38) women (52±11 years; mean±standard deviation) with FM were randomly assigned to one of two treatment groups, laser heat therapy (LHT; n=20) or sham heat therapy (SHT; n=18).

Intervention: Both groups received treatment twice a week for 4 weeks. Treatment consisted of application of LHT or SHT over seven tender points located across the neck, shoulders, and back. Treatment was blinded to women and was administered by a chiropractic physician for 7 minutes.

Outcome measures: Participants were evaluated before and after treatment for number and sensitivity of tender points, completed the FM Impact Questionnaire (FIQ) and the pain question of the FIQ, and were measured for function using the continuous scale physical functional performance (CS-PFP) test. Data were evaluated using repeated-measures analysis of variance with significance accepted at p≤0.05.

Results: There were significant interactions for pain measured by the FIQ (LHT: 7.1±2.3 to 6.2±2.1 units; SHT: 5.8±1.3 to 6.1±1.4 units) and for upper body flexibility measured by the CS-PFP (LHT: 71±17 to 78±12 units; SHT: 77±12 to 77±11 units) with the LHT improving significantly compared to SHT. There was a time effect for the measure of FM impact measured by the FIQ, indicating that FM impact significantly improved from pre- to post-treatment in LHT (63±20 to 57±18 units), while no change was observed in the SHT (57±11 to 55±12 units).

Conclusions: This study provides evidence that LHT may be a beneficial modality for women with FM in order to improve pain and upper body range of motion, ultimately reducing the impact of FM.

Introduction

Fibromyalgia (FM) is the second most common rheumatologic disorder in the United States, affecting approximately 4-6 million, the majority being women. 1-4 Fibromyalgia is characterized not only by chronic pain, but also by a wide array of symptoms. 2-3 Symptoms include sleep disturbance, chronic fatigue, morning stiffness, irritable bowel syndrome, anxiety, depression, mental fogginess, slowness of thought, decreased concentration, paresthesia, and pain upon moderate pressure at various tender points across the body. 3-6 According to the American College of Rheumatology, the diagnosis of FM is widespread in at least three of the four body quadrants for at least 3 months and localized pain on palpation in at least 11 of 18 selected muscle-tendon junctions or tender points. 1 See Figure 1 for tender-point locations. The pain associated with FM may lead to deconditioning, job loss, and the inability to perform activities of daily living (ADL). 7 Between 20% and 50% of people with FM work fewer days compared to the average population or do not work at all, 8 36% average two or more absences from work per month, 9 and 27%–55% receive disability or social security payments. 9 The etiology of FM is not known, and the prognosis for recovery in traditional medicine is generally poor.
The Effectiveness of Therapeutic Class IV (10 W) Laser Treatment for Epicondylitis

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Background and Objective: Photobiomodulation has been shown to modulate cellular protein production and stimulate tendon healing in a dose-dependent manner. Previous studies have used class IIIb lasers with power outputs of less than 0.5 W. Here we evaluate a dual wavelength (980/810 nm) class IV laser with a power output of 10 W for the purpose of determining the efficacy of class IV laser therapy in alleviating the pain and dysfunction associated with chronic epicondylitis.

Methods: Sixteen subjects volunteered for laser therapy, or an identically appearing sham instrument in a randomized, placebo-controlled, double-blinded clinical trial. Subjects underwent clinical examination (pain, function, strength, and ultrasonic imaging) to confirm chronic tendinopathy of the extensor carpi radialis brevis tendon, followed by eight treatments of 6.6 ± 1.3 J/cm² (laser), or sham over 18 days. Safety precautions to protect against retinal exposure to the laser were followed. The exam protocol was repeated at 0, 3, 6 and 12 months post-treatment.

Results: No initial differences were seen between the two groups. In the laser treated group handgrip strength improved by 17 ± 3%, 52 ± 7%, and 66 ± 6% at 3, 6, and 12 months respectively; function improved by 44 ± 1%, 71 ± 3%, and 82 ± 2%, and pain with resistance to extension of the middle finger was reduced by 50 ± 6%, 93 ± 4%, and 100 ± 1% at 3, 6 and 12 months, respectively. In contrast, no changes were seen until 12 months following sham treatment (12 months: strength improved by 13 ± 2%, function improved by 52 ± 3%, pain with resistance to extension of the middle finger reduced by 76 ± 2%). No adverse effects were reported at any time.

Conclusions: These findings suggest that laser therapy using the 10 W class IV instrument is efficacious for the long-term relief of the symptoms associated with chronic epicondylitis. The potential for a rapidly administered, safe and effective treatment warrants further investigation. Lasers Surg. Med. 45:311–317, 2013. © 2013 Wiley Periodicals, Inc.

Key words: epicondylalgia; photobiomodulation; tendinopathy; tendinosis; tenosynovitis

INTRODUCTION

Tendinopathy is a common and painful condition that occurs following damage to a tendon [1–3]. The onset of symptoms is associated with overuse, increased load, vibration and/or repetitive movements and while tendon injuries are sometimes acute, they are most often chronic in nature resulting in significant restriction of activity and lost work-time [3,4]. Characteristic findings include necrosis [3], abnormal neovascularization [5], edema, crepitis, and impaired function [4,6]; however, the etiology remains incompletely understood. Furthermore, while most cases resolve themselves within 12 months of rest, approximately 15–20% are persistent, with reoccurrence of symptoms when activity is resumed [6,7].

There is little consensus regarding effective treatments for tendinopathy [4,8]. Rest, ice, and analgesics are general guidelines used to provide pain relief. Orthotic devices [9], ultrasonography [10] and deep transverse friction massage [11] are often recommended, although there is no conclusive evidence as to the effectiveness of these treatments. Similarly, while eccentric exercises have been shown to be more effective than no treatment in relieving symptoms for some tendinopathies, compliance can be problematic and there is a great deal of heterogeneity in protocols [12]. Randomized controlled studies of epicondylitis have determined that oral non-steroidal anti-inflammatory treatment was not significantly better than placebo [13] and although early corticosteroid injection did provide symptom relief in some patients, studies that were extended to 3 [14] and 12 [13] months post-injection indicated that corticosteroid injection could even produce a detrimental outcome. Extracorporeal shock therapy for treatment of tendinopathy is also not supported by systematic reviews of the literature [15], except perhaps for cases resistant to conventional treatments [16].

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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Inaccurate measurement and incorrect reporting of dosages are major shortcomings of phototherapy articles. As many as 30% of published reports in the field either lack relevant information needed to determine a dosage or report dosages that are altogether inaccurate. The high prevalence of dosage-related mistakes in published reports suggests that dosage determination errors are common among clinicians and other end-users. This special article is designed to advance understanding of the relevant parameters used in phototherapy for tissue repair and pain relief, particularly among clinicians and others who may not be completely familiar with the technology. I define and discuss five key parameters that influence dosage, including 1) radiant power, 2) radiant energy, 3) power density, 4) energy density, and 5) wavelength, and use hypothetical cases to demonstrate how factors such as beam spot size, size of lesion, mode of treatment (contact, noncontact, or scanning), frequency of treatment, dose per treatment, and cumulative dose affect dosages and treatment outcomes. The potential effects of patient-related factors, such as etiology, pathology, tissue optical density, depth of target tissue, and skin pigmentation are discussed concurrently and strategies are suggested to improve dosage determination.

Introduction

Inaccurate measurement and incorrect reporting of dosages rank high among the shortcomings of phototherapy papers submitted for publication in professional journals. A recent review indicates that as many as 30% of published phototherapy reports lack details of the relevant information needed to determine dosage or report dosages that are altogether inaccurate.¹ The prevalence of dosage-related mistakes suggests that dosage determination errors are common among clinicians and other end-users. That phototherapy equipment is, at times, labeled with inaccurate parameters further complicates the situation. Yet successful treatment outcomes hinge directly on correct dosimetry and informed selection of treatment parameters. Just as a certain dose of medicine can relieve pain, a higher dose can be toxic, or a lower dose can be ineffective, so too can doses of light be beneficial, detrimental, or ineffectual.

The well-informed clinician can, within reason, control dosages, even though certain parameters are preset by equipment manufacturers. However, knowledge of the potential effects of various parameters (wavelength, pulse frequency, power, power density, energy, and energy density), their relationships to one another, and an understanding of certain patient-related characteristics and beam behavior, are essential to determining the right amount of energy needed to treat a particular condition. The purpose of this paper is to advance understanding of the relevant parameters used in phototherapy for tissue repair and pain relief. The specific aims are to 1) define and explain the relationships between key parameters, 2) use examples and hypothetical cases to demonstrate the potential effects of relevant parameters, and 3) offer information that enables researchers, clinicians, and other end-users to improve measurement and reporting of dosages.

Basic Treatment Parameters

The parameters that influence dosage include 1) radiant power, 2) radiant energy, 3) power density, 4) energy density, and 5) wavelength. As a foundation for further discussion of these parameters and their potential effects on dose, I will begin by defining them in the following segments.
Synopsis of Articles on Pulsing

1- Effects of Laser Pulsing on Cell Viability
Due to the effect of Pulsing on distinct cell types we can use pulsing to optimize Laser therapy and improve clinical outcomes.

2- Effect of Pulsing in Low-Level Light Therapy
These authors scoured the literature to find studies that compared Laser therapy using Pulsed parameters vs those that just delivered CW only Laser therapy. Their conclusion states the following: CW light is the gold standard and has been used for all LLLT applications. However, this review of the literature indicates that overall pulsed light may be superior to CW light with everything else being equal.

3- The Necessity for Increased Attention to Pulsed Low-Level Laser Therapy
Lasers with the ability to Pulse provide more parameters which can be used to achieve better outcomes.

4- Pulsing Influences Photoradiation Outcomes in Cell Culture
This investigation suggests that light pulsing may improve outcomes by mitigating the filtration effects of cutaneous melanin.

5- In Vivo Effects of Low Level Laser Therapy on Inducible Nitric Oxide Synthase
Found that the intensity and time course of inducible nitric oxide expression was found to not only depend on wavelength, but also on the mode of delivery, continuous or pulsed radiation.

6- Effects of Pulse Frequency of Low-Level Laser Therapy (LLLT) on Bone Nodule Formation in Rat Calvarial Cells
Concludes that low-frequency pulses significantly stimulate bone formation and is an important factor in biologic responses involving bone formation.

7- Near infrared Transcranial Laser Therapy applied at Various Modes to Mice Following Traumatic Brain Injury Significantly Reduces Long-Term Neurological Deficits
Our data suggest that non-invasive TLT of mice post-TBI provides a significant long-term functional neurological benefit, and that the pulsed laser mode at 100 Hz is the preferred mode for such treatment.
PHOTOBIOMODULATION

EFFECTS OF LASER PULSING ON CELL VIABILITY
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Background: Continuous wave photobiomodulation (cwPBM) has been shown to induce cell proliferation in many different cell lines both in vitro and in vivo. While pulsed wave photobiomodulation (pwPBM) has shown similar effects, the parameters for pwPBM are not as well characterized as cwPBM. Laser treatment can be manipulated by changing a variety of pulsing parameters including pulse interval, pulse duration, pulse train interval, and pulse train duration in addition to total irradiance and time providing more treatment variables than cwPBM. These parameters could facilitate better optimization of PBM for therapeutic benefit.

Study: This study investigates how various pulsing parameters affect cell viability and proliferation of oral keratinocyte and fibroblast cell lines. Cells were irradiated with an 810 nm diode using different pulsing parameters and following incubation for 24 hours, proliferation was quantified using an Alamar Blue assay and compared to untreated controls.

Results: We noted that keratinocytes were more responsive to the effects of the laser than fibroblasts, resulting in a discrete set of optimal pulsing parameters for each cell type. Pulsing allows significant control on therapeutic dosing as it allows the ability to distinct eliminate thermal damage while facilitating increased dosing. Keratinocytes were observed to respond differently to changes in pulse intervals, pulse durations and total irradiance than fibroblasts. We further characterized that the cell type responses were due to variations in their inherent redox potentials.

Conclusion: This study demonstrates the effects of laser pulsing on distinct cell types and suggests that optimization of laser treatments based on target cell types could improve clinical efficacy and therapeutic benefit for photobiomodulation.
Effect of Pulsing in Low-Level Light Therapy

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Background and Objective: Low level light (or laser) therapy (LLLT) is a rapidly growing modality used in physical therapy, chiropractic, sports medicine and increasingly in mainstream medicine. LLLT is used to increase wound healing and tissue regeneration, to relieve pain and inflammation, to prevent tissue death, to mitigate degeneration in many neurological indications. While some agreement has emerged on the best wavelengths of light and a range of acceptable dosages to be used (irradiance and fluence), there is no agreement on whether continuous wave or pulsed light is best and on what factors govern the pulse parameters to be chosen.

Study Design/Materials and Methods: The published peer-reviewed literature was reviewed between 1970 and 2010.

Results: The basic molecular and cellular mechanisms of LLLT are discussed. The type of pulsed light sources available and the parameters that govern their pulse structure are outlined. Studies that have compared continuous wave and pulsed light in both animals and patients are reviewed. Frequencies used in other pulsed modalities used in physical therapy and biomedicine are compared to those used in LLLT.

Conclusion: There is some evidence that pulsed light does have effects that are different from those of continuous wave light. However further work is needed to define these effects for different disease conditions and pulse structures.


Key words: low level light therapy; photobiomodulation; frequency; pulse duration; duty cycle; clinical trials

INTRODUCTION

Since the introduction of low-level laser (light) therapy in 1967, over two hundred randomized, double-blinded, and placebo-controlled phase III clinical trials have been published from over a dozen countries. Whereas there is some degree of consensus as to the best wavelengths of light and acceptable dosages to be used, there is no agreement on whether continuous wave (CW) or pulsed wave (PW) light is more suitable for the various applications of LLLT. This review will raise (but not necessarily answer) several questions. How does pulsed light differ from CW on the cellular and molecular level, and how is the outcome of LLLT affected? If pulsing is more efficacious, then at what pulse parameters is the optimal outcome achieved? In particular, what is the ideal pulse repetition rate or frequency to use?

PULSE PARAMETERS AND LIGHT SOURCES

There are five parameters that could be specified for pulsed light sources. The pulse width or duration or ON time (PD) and the pulse Interval or OFF time (PI) are measured in seconds. Pulse repetition rate or frequency (F) is measured in Hz. The duty cycle (DC) is a unitless fractional number or %. The peak power and average power are measured in Watts.

Pulse duration, pulse repetition rate, and duty cycle are related by the simple equation:

$$\text{DC} = \frac{F}{C_2} \times \text{PD}$$

Peak power is a measure of light intensity during the pulse duration, and related to the average power (measured in Watts) by:

$$\text{Average power} = \frac{\text{Peak power} \times F \times \text{PD}}{\text{DC}}$$

Alternatively,

$$\text{Peak power} = \frac{\text{Average power}}{\text{DC}}$$

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The Necessity for Increased Attention to Pulsed Low-Level Laser Therapy

Mohammad Bayat, PhD

The term low-level laser therapy (LLLT) is broadly defined as the therapeutic benefit of lasers. After Mester, in Hungary, first uncovered the therapeutic value of lasers, different wavelengths of continuous wave (CW) LLLT have been shown to promote healing in skin and musculoskeletal tissues. CW LLLT has been used in the treatment of serious medical conditions and for pain control. However, the benefits of CW LLLT in cell proliferation and wound healing are controversial; numerous other authors have failed to observe positive effects of CW LLLT on cell proliferation, wound healing models in vitro and in vivo, and the repair of fractures and osteochondral defects.

Both CW LLLT and pulsed wave (PW) LLLT devices are currently available. These devices provide medical practitioners with a wide range of therapeutic options. The PW LLLT device has more laser (illumination) parameters, such as peak and average power outputs, pulse frequency, and pulse duration, than CW LLLT, all of which add to the medical applicability of this technique.

It is assumed that by investigating different values of these parameters, researchers can select better protocols and achieve more satisfactory outcomes with PW LLLT devices than with CW LLLT devices. Barolet et al. have investigated the impact of various light delivery modes on collagen production in human primary fibroblasts cultured in monolayers. The fibroblasts underwent three treatments with a red light-emitting diode illumination at 630 nm, irradiance of 8 mW/cm², total fluence of 1–33 J/cm², time duration of 1000 sec, pulse duration (PD) of 500 μs, pulse interval (PI) of 150 μs, four pulses per pulse train (PPT), and pulse train interval (PTI) of 1550 μs. The remainder of the reference light parameters remained constant. In this research, they evaluated two PDs, three PIs, four PTTs, and three PPTs compared with a CW light. The results showed that the manner in which the light was delivered impacted the cellular response. Sequentially pulsed optical energy was reported to be more efficacious in stimulating collagen production than the CW mode in a suction blister model. Low PD (100 μs), PTI (750 μs), and four PPTs as well as high PI were the best pulsing parameter levels that enhanced collagen secretion in fibroblast cells.

Brondon et al. investigated the photoradiation outcome after delivery of 670 nm (10 mW/cm², 5 J/cm²) light through a 0.025% melanin via both the CW and PW delivery techniques at various frequencies. The PW photoirradiation had a significantly greater stimulating effect on cell proliferation and oxidative burst than did CW photoirradiation.

These results agree with recent work in my laboratory. CW LLLT did not accelerate the osteochondral defect healing process in rabbits according to biomechanical evaluation, nor did it accelerate the second and third degree burn healing process in rats. Our studies showed that PW LLLT significantly increased the stiffness of repaired osteochondral tissue at the defective site in rabbits, and accelerated the healing process in surgically induced open skin wounds, and in second and third degree burns in rats. Despite the failure of some earlier studies to show positive effects of PW LLLT on the healing of radiation-induced wounds in mouse models and in pressure ulcers in human patients, other studies have reported positive effects of PW LLLT on healing pressure ulcers in patients, and wounds in volunteers, as well as wounds in animal models.

In conclusion, the PW LLLT devices provide more laser (light) parameters than CW LLLT devices. It is assumed that by investigating different values of these parameters, research models can be more effectively studied in these devices compared with CW LLLT devices, with the purpose of achieving better outcomes.

References

Pulsing Influences Photoradiation Outcomes in Cell Culture

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Background and Objectives: Skin pigmentation can adversely affect phototherapy outcomes. Delivering pulsed light has been suggested as a means of enhancing efficacy. Suitable pulse frequencies remain indeterminate, often being selected empirically. This study was undertaken to determine whether pulsed light delivery mitigates the filtering effect of melanin pigment on photomodulation in vitro.

Study Design and Methods: Human HEP-2 cells were cultured in complete DMEM media. Photoradiation was delivered through 0.025% melanin filters at 670 nm (5.0 J/cm²/treatment/24 hours) for 72 hours at different pulse rates. Group A received no light treatment. Group B received treatments without pulsing. Groups C, D, E, F, and G received treatments at 6, 18, 36, 100, and 600 Hz. Cell proliferation was assessed by MTT assay and oxidative burst was measured using the 2.7 dichloro-fluorescein-diacetate assay.

Results: Cell proliferation was maximally stimulated at 100 Hz at 48 and 72 hours (n = 4, P ≤ 0.05). Oxidative burst was maximally stimulated at 600 Hz (n = 4, P ≤ 0.05). All frequencies were stimulatory at 48 and 72 hours (n = 4, P ≤ 0.05).

Conclusion: This investigation suggests that light pulsing may improve outcomes by mitigating the filtration effects of cutaneous melanin. Further studies to further define these effects are warranted. Lasers Surg. Med. 41:222–226, 2009. © 2009 Wiley-Liss, Inc.

Key words: cells; cell culture; photoradiation; melanin; pulsed light; photobiology; biostimulation; NIR light

INTRODUCTION

Low level laser therapy is being used currently to treat a wide variety of medical conditions [1,2]. Several in vitro and in vivo studies have shown that light therapy has a significant influence on cellular functions [1–8]. However, other studies have concluded that light therapy produces no measurable effects [8].

Light therapy is often administered transcutaneously to treat the skin and structures within it, as well as to treat deeper tissues. The epidermis contains the pigment melanin, which accounts for most of the differences in human skin color. Melanocytes located in the epidermis are responsible for producing melanin pigment. Melanin is a complex heterogeneous polymer, whose exact chemical structure has not been satisfactorily determined. There are two major classes of melanin: black-brown eu-melanin that is found in black human hair and the retina, and yellow-red pheo-melanin found in red human hair [9–18]. Skin in all races and ethnicities of humans are similar with regard to skin structure and function. Skin color varies between individuals and ethnicities even though the relative number of melanocytes remains constant between races [9,12,15,18]. The difference in skin color is accounted for by relative amount of melanin produced by the melanocytes present. The melanin pigment is a photobosorber at wavelengths in the ultraviolet and visible spectrum [9–18]. Light therapy is generally performed using wavelengths from the visible and near-infrared spectrum. Red wavelengths such as 670 nm are commonly used for phototherapy and are wavelengths absorbed by this pigment. The concentration of melanin also changes following exposure to sunlight. The concentration of melanin pigment observed in skin increases after extended exposure to ultraviolet rays from the sun, but this does not occur in a uniform fashion. Melanin does not typically exist in uniform concentrations over an individual’s skin surface. Melanin’s light absorptive properties have been implicated in poor outcomes of phototherapy, as a result of delivering a lower than intended light dose to the target tissue. Anecdotal evidence suggests that patients whose epidermis has greater concentrations of melanin respond less well to these regimes than do more fair skinned individuals. These concepts are supported by previous in vitro studies from our laboratory that demonstrated decreasing cellular response to 670 nm photoradiation delivered through gelatin filters with increasing concentrations of melanin [19].

Pulsed light delivery has been used as a treatment strategy for phototherapy. Certain frequencies of pulsed light have been shown to be effective for low level light therapy (LLLT) in various cellular and whole animal models [20–30]. These studies have evaluated lasers, superluminous diodes, and other light sources. The specific mechanism for these observations is unknown. However,
In Vivo Effects of Low Level Laser Therapy on Inducible Nitric Oxide Synthase

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Background and Objective: Low level laser therapy (LLLT) has been demonstrated to modulate inflammatory processes with evidence suggesting that treatment protocol, such as wavelength, total energy, and number of treatments determine the clinical efficacy. In this study, the effects of LLLT modulated by different wavelengths and continuous versus pulsed delivery mode were quantified in a transgenic murine model with the luciferase gene under control of the inducible nitric oxide synthase (iNOS) expression.

Study Design/Materials and Methods: LLLT modulated iNOS gene expression in the acute Zymosan-induced inflammation model is quantified using transgenic mice (FVB/N-Tg(iNOS-lacZ)). Here an energy density of 5 J cm⁻² at either 650, 660, 690, and 905 nm in continuous wave mode and at 905 nm for short pulse delivery were evaluated. Age of the animals was determined as additional modulating the inflammatory response and the LLLT efficacy for some treatment protocols.

Results: Animals younger than 15 weeks showed mostly reduction in iNOS expression, while older animals showed increased iNOS expression for some LLLT protocols. Intensity and time course of inducible nitric oxide expression was found to not only depend on wavelength, but also on the mode of delivery, continuous, or pulsed irradiation.

Conclusion: LLLT exhibit different effects in induced inflammatory process according to different wavelengths and wave mode. Upregulation of iNOS gene following 905 nm pulsed wave suggests a different mechanism in activating the inflammatory pathway response when compared to the continuous wave. Lasers Surg. Med. 41:227–231, 2009. © 2008 Wiley-Liss, Inc.

Key words: bioluminescence imaging; biostimulation; inflammation; pulsed laser; Zymosan A

INTRODUCTION

Low level laser therapy (LLLT) has been demonstrated to promote photobiomodulation, including stimulation and inhibition effects and is used clinically for various conditions, including treatment of wounds, chronic pain, inflammation, and infections [1–6]. One of the proposed mechanisms of laser photobiomodulation involves the absorption of photons by intracellular chromophores and the production of reactive oxygen species (ROS), which in concentrations below the cytotoxic level has positive stimulatory effects on the cell [7].

For the continuous mode of light delivery, the wavelength-dependent ability to alter cellular mechanisms in the absence of significant heating has been demonstrated through action spectra, suggesting a direct photochemical basis for the LLLT efficacy. A variety of potential photochemical targets have been suggested to give rise to the action spectra such as cytochromes within the red part of the optical spectrum, and temporary increase in the cell membranes to calcium ions [8]. Thus, the red and infrared portion of the optical spectrum opens possible therapeutic modulations in living tissues as their effects are dependent on the physiological state of the tissue at the moment of irradiation [9]. Studies show that stimulation and inhibition due to light irradiation can occur via the same photoreceptors, and therefore as the light dose increases, the photoreceptors are damaged and the effect decreases, showing a bi-phasic LLLT responses as function of irradiance (W cm⁻²) [10]. Similar studies have been executed at 50% duty cycle in frequency modulation up to the kHz range [11]. Pulse laser radiations with low duty cycle [12] suggested transient heating possibly inhibiting NADPH oxidase.

While previously LLLT or laser biostimulation research was predominantly based on subjective evaluation of clinical or semi-quantitative pre-clinical studies, recently quantifiable pre-clinical models have been exploited to access the molecular basis for LLLT efficacy [13]. In this study, transgenic animals carrying the reporter luciferase were used to quantify iNOS gene expression following induction of acute inflammation and immediate single LLLT irradiation mediated by various wavelengths in continuous and pulsed mode.

Zymosan A has been used in several studies as a model system for inflammation induction in vivo [3,5,6,14,15].

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**Objective:** The purpose of this study was to determine the effect of pulse frequencies of low-level laser therapy (LLLT) on bone nodule formation in rat calvarial cells in vitro. Background Data: Various photobiostimulatory effects of LLLT, including bone formation, were affected by some irradiation factors such as total energy dose, irradiation phase, laser spectrum, and power density. However, the effects of pulse frequencies used during laser irradiation on bone formation have not been elucidated. Materials and Methods: Osteoblast-like cells isolated from fetal rat calvariae were irradiated once with a low-energy Ga-Al-As laser (830 nm, 500 mW, 0.48-3.84 J/cm2) in four different irradiation modes: continuous irradiation (CI), and 1-, 2-, and 8-Hz pulsed irradiation (PI-1, PI-2, PI-8). We then investigated the effects on cellular proliferation, bone nodule formation, alkaline phosphatase (ALP) activity, and ALP gene expression. Results: Laser irradiation in all four groups significantly stimulated cellular proliferation, bone nodule formation, ALP activity, and ALP gene expression, as compared with the non-irradiation group. Notably, PI-1 and -2 irradiation markedly stimulated these factors, when compared with the CI and PI-8 groups, and PI-2 irradiation was the best approach for bone nodule formation in the present experimental conditions. Conclusion: Since low-frequency pulsed laser irradiation significantly stimulates bone formation in vitro, it is most likely that the pulse frequency of LLLT an important factor affecting biological responses in bone formation.

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Near infrared Transcranial Laser Therapy applied at Various Modes to Mice Following Traumatic Brain Injury Significantly Reduces Long-Term Neurological Deficits

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Near-infrared transcranial laser therapy (TLT) has been found to modulate various biological processes including traumatic brain injury (TBI). Following TBI in mice, in this study we assessed the possibility of various near-infrared TLT modes (pulsed vs. continuous) producing a beneficial effect on the long-term neurobehavioral outcome and brain lesions of these mice. TBI was induced by a weight-drop device, and neurobehavioral function was assessed from one hour and up to 56 days post-trauma using a neurological severity score (NSS). The extent of recovery is expressed as dNSS, the difference between the initial score, and that at any other, later, time point. An 808nm Ga-Al-As diode laser was employed transcranially 4, 6 or 8 hrs post-trauma to illuminate the entire cortex of the brain. Mice were divided into several groups of 6-8 mice: one control group that received a sham treatment and experimental groups that received either TLT continuous wave (CW) or pulsed wave (PW) mode transcranially. MRI was taken prior to sacrifice 56 days post-CHI. From 5 to 28 days post-TBI, the NSS of the laser-treated mice were significantly lower (p<0.05) than the non-laser-treated, control mice. The percentage of surviving mice that demonstrated full recovery 56 days post-CHI, namely NSS=0 (as in intact mice) was the highest (63%) in the group that had received TLT in the PW mode at 100 Hz. In addition, MRI analysis demonstrated significantly smaller infarct lesion volumes in laser treated mice as compared to control. Our data suggest that non-invasive TLT of mice post-TBI provides a significant long-term functional neurological benefit, and that the pulsed laser mode at 100 Hz is the preferred mode for such treatment. Key words: low-level laser therapy; mice; traumatic brain injury; pulsed laser; motor function, MRI.
SUPER PULSING STUDIES
Synopsis of Articles on SuperPulse

1-Superpulsed Laser Irradiation Increases Osteoblast Activity Via Modulation of Bone Morphogenetic Factors
Concluded that Superpulse delivery of Laser energy, like CW and Pulsed, possesses osteogenic properties.

2-Skin Penetration Time-Profiles for Continuous 810nm and Superpulsed 904nm Lasers in a Rat Model
The finding that superpulsed 904nm LLLT light energy penetrates 2–3 easier through the rat skin barrier than 810nm continuous wave LLLT, corresponds well with results of LLLT dose analyses in systematic reviews of LLLT in musculoskeletal disorders. This may explain why the differentiation between these laser types has been needed in the clinical dosage recommendations of World Association for Laser Therapy.
Superpulsed Laser Irradiation Increases Osteoblast Activity Via Modulation of Bone Morphogenetic Factors

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Background and Objective: Laser therapy is a new approach applicable in different medical fields when bone loss occurs, including orthopedics and dentistry. It has also been used to induce soft-tissue healing, for pain relief, bone, and nerve regeneration. With regard to bone synthesis, laser exposure has been shown to increase osteoblast activity and decrease osteoclast number, by inducing alkaline phosphatase (ALP), osteopontin, and bone sialoprotein expression. Studies have investigated the effects of continuous or pulsed laser irradiation, but no data are yet available on the properties of superpulsed laser irradiation. This study thus aimed to investigate the effect of superpulsed laser irradiation on osteogenic activity of human osteoblast-like cells, paying particular attention to investigating the molecular mechanisms underlying the effects of this type of laser radiation.

Study Design/Materials and Methods: Human osteoblast-like MG-63 cells were exposed to 3, 7, or 10 superpulsed laser irradiation (pulse width 200 nanoseconds, minimum peak power 45 W, frequency 30 kHz, total energy 60 J, exposure time 5 minutes). The following parameters were evaluated: cell growth and viability (light microscopy, lactate dehydrogenase release), calcium deposits (Alizarin Red S staining), expression of bone morphogenetic factors (real-time PCR).

Results: Superpulsed laser irradiation decreases cell growth, induces expression of TGF-β2, BMP-4, and BMP-7, type I collagen, ALP, and osteocalcin, and increases the size and the number of calcium deposits. The stimulatory effect is maximum on day 10, that is, after seven applications.

Conclusions: Reported results show that superpulsed laser irradiation, like the continuous and pulsed counterparts, possesses osteogenic properties, inducing the expression of molecules known to be important mediators of bone formation and, as a consequence, increasing calcium deposits in human MG-63 cells. Moreover, the data suggest a new potential role for PPARγ as a regulator of osteoblast proliferation.

INTRODUCTION

Laser therapy is a new approach in different medical fields, including orthopedics and dentistry, when bone loss occurs, that is, in cases of bone fracture and tooth extraction (1). Recent studies have reported the benefits of low level laser therapy (LLLT), which has been used to induce soft-tissue healing, for pain relief, bone, and nerve regeneration (2), although the molecular mechanisms triggered are not yet fully clear.

With regard to bone synthesis, in vivo experiments on rat femur have shown that pulsed laser irradiation with high peak power stimulates bone formation by increasing osteoblast activity and decreasing osteoclast numbers (3). Beneficial properties have been ascribed to LLLT's anti-inflammatory effect, postulating that the treatment modulates transcription factors and regulates the expression of pro-inflammatory cytokines (4). A recent study on the subplantar tissue of rat's paw evidenced that LLLT decreased mRNA content of TNF-α, IL-1β, and IL-6 (5); it was suggested that an early target of radiation was TNF-α, which, in turn, activates other cytokines. These authors also reported a decreased expression of kinin receptors in the same experimental model (6).

It has also been reported that LLLT induces the formation of small amounts of reactive oxygen species (ROS), which can trigger cell stimulation via increased mitochondrial respiration and ATP formation (7).

The stimulatory effect of LLLT has also been confirmed in vitro in different cell lines. In osteoblast-like cells isolated from fetal rat calvariae, LLLT stimulated proliferation and differentiation, inducing alkaline phosphatase (ALP), osteopontin (OP), and bone sialoprotein expression (8). Similar results have been obtained in cultured human SaOS-2 cells, where early induction of ALP, type I collagen,

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Skin Penetration Time-Profiles for Continuous 810 nm and Superpulsed 904 nm Lasers in a Rat Model

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Abstract

Objective: The purpose of this study was to investigate the rat skin penetration abilities of two commercially available low-level laser therapy (LLLT) devices during 150 sec of irradiation. Background data: Effective LLLT irradiation typically lasts from 20 sec up to a few minutes, but the LLLT time-profiles for skin penetration of light energy have not yet been investigated. Materials and methods: Sixty-two skin flaps overlaying rat’s gastrocnemius muscles were harvested and immediately irradiated with LLLT devices. Irradiation was performed either with a 810 nm, 200 mW continuous wave laser, or with a 904 nm, 60 mW superpulsed laser, and the amount of penetrating light energy was measured by an optical power meter and registered at seven time points (range, 1–150 sec). Results: With the continuous wave 810 nm laser probe in skin contact, the amount of penetrating light energy was stable at ~20% (SEM ± 0.6) of the initial optical output during 150 sec irradiation. However, irradiation with the superpulsed 904 nm, 60 mW laser showed a linear increase in penetrating energy from 38% (SEM ± 1.4) to 58% (SEM ± 3.5) during 150 sec of exposure. The skin penetration abilities were significantly different (p < 0.01) between the two lasers at all measured time points. Conclusions: LLLT irradiation through rat skin leaves sufficient subdermal light energy to influence pathological processes and tissue repair. The finding that superpulsed 904 nm LLLT light energy penetrates 2–3 easier through the rat skin barrier than 810 nm continuous wave LLLT, corresponds well with results of LLLT dose analyses in systematic reviews of LLLT in musculoskeletal disorders. This may explain why the differentiation between these laser types has been needed in the clinical dosage recommendations of World Association for Laser Therapy.

Introduction

Lasers are used for a number of different purposes in medicine. Class 4 surgical lasers are used to cut and destroy biological tissue, whereas low-level laser therapy (LLLT) is administered with weaker class 3B lasers and mean output powers (MOP) < 500 mW.1 The most commonly used lasers in LLLT are gallium-aluminum-arsenide (GaAlAs) with a wavelength of 810 nm (± 50 nm) operating in continuous output mode, and gallium-arsenide (GaAs) with a wavelength of 904 nm superpulsed with high peak power pulses. LLLT emerged as a treatment option in clinical practice approximately three decades ago.2 After the initial upsurge, LLLT gained a poor reputation because of a string of negative research results being published. This picture has slowly changed again as an increasing amount of evidence is pointing toward the existence of specific therapeutic windows for LLLT. Several studies have found that anticipated optimal doses yield significantly better results in tendinopathies,3 osteoarthritis,4 and neck pain.5 The skin is the body’s external boundary in animals and humans. Among other things, the skin serves as a barrier against physical and chemical intrusions.5 The skin also represents a barrier to applied physical energy from...
905nm STUDIES
Synopsis of Articles on 905nm Wavelength

1t In Vivo Effects of Low Level Laser Therapy on Inducible Nitric Oxide Synthase

Studying multiple wavelengths including a 904 nm wavelength Laser this study found that the intensity and time course of inducible nitric oxide expression was found to not only depend on wavelength, but also on the mode of delivery, continuous or pulsed radiation.

2-Efficacy of 904 nm Gallium Arsenide Low Level Laser Therapy in the Management of Chronic Myofascial Pain in the Neck: A Double-Blind and Randomized Controlled Trial

Using a 904 nm wavelength this study revealed that short-period application of LLLT is effective in pain relief and in the improvement of functional ability and QoL in patients with MPS.

3-ATTENUATION AND PENETRATION OF VISIBLE 632.8nm AND INVISIBLE INFRA-RED 904nm LIGHT IN SOFT TISSUES

Compared to the 904nm wavelength, 632.8nm light is attenuated more by muscle tissue, suggesting that is absorbed more readily than the 904nm wavelength or conversely that the 904nm wavelength penetrates more. Thus, wavelength plays a critical role in depth of penetration of light.

4-Skin Penetration Time-Profiles for Continuous 810nm and Superpulsed 904nm Lasers in a Rat Model

The finding that superpulsed 904nm LLLT light energy penetrates 2–3 easier through the rat skin barrier than 810nm continuous wave LLLT, corresponds well with results of LLLT dose analyses in systematic reviews of LLLT in musculoskeletal disorders. This may explain why the differentiation between these laser types has been needed in the clinical dosage recommendations of World Association for Laser Therapy.

5-Investigation of the Effect of GaAs Laser Therapy on Lateral Epicondylitis

This study showed that patients receiving Laser therapy using a 905 wavelength Laser showed significant improvement in functional parameters in the long term.
In Vivo Effects of Low Level Laser Therapy on Inducible Nitric Oxide Synthase

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Background and Objective: Low level laser therapy (LLLT) has been demonstrated to modulate inflammatory processes with evidence suggesting that treatment protocols, such as wavelength, total energy, and number of treatments determine the clinical efficacy. In this study, the effects of LLLT mediated by different wavelengths and continuous versus pulsed delivery mode were quantified in a transgenic murine model with the luciferase gene under control of the inducible nitric oxide synthase (iNOS) expression.

Study Design/Materials and Methods: LLLT modulated iNOS gene expression in the acute Zymosan-induced inflammation model is quantified using transgenic mice (FVB/N-Tg(iNOS-luc)). Here an energy density of 5 J cm⁻² at either 955, 690, 690, and 905 nm in continuous wave mode and at 905 nm for short pulse delivery were evaluated. Age of the animals was determined as additional modulating the inflammatory response and the LLLT efficacy for some treatment protocols.

Results: Animals younger than 15 weeks showed mostly reduction of iNOS expression, while older animals showed increased iNOS expression for some LLLT protocols. Intensity and time course of inducible nitric oxide expression was found to not only depend on wavelength, but also on the mode of delivery, continuous, or pulsed irradiation.

Conclusion: LLLT exhibit different effects in induced inflammatory process according to different wavelengths and wave mode. Uptregulation of iNOS gene following 905 nm pulsed wave suggests a different mechanism in activating the inflammatory pathway response when compared to the continuous wave. Lasers Surg. Med. 41:227–231, 2009. © 2009 Wiley-Liss, Inc.

Key words: bioluminescence imaging; biostimulation; inflammation; pulsed laser; Zymosan A

INTRODUCTION

Low level laser therapy (LLLT) has been demonstrated to promote photobiomodulation, including stimulation and inhibition effects and is used clinically for various conditions, including treatment of wounds, chronic pain, inflammation, and infections [1–6]. One of the proposed mechanisms of laser photobiomodulation involves the absorption of photons by intracellular chromophores and the production of reactive oxygen species (ROS), which in concentrations below the cytotoxic level has positive stimulatory effects on the cell [7]. For the continuous mode of light delivery, the wavelength-dependent ability to alter cellular mechanism in the absence of significant heating has been demonstrated through action spectra, suggesting a direct photochemical basis for the LLLT efficacy. A variety of potential photochemical targets have been suggested to give rise to the action spectra such as cytochromes within the red part of the optical spectrum, and temporary increase in the cell membranes to calcium ions [8]. Thus, the red and infrared portion of the optical spectrum offers possible therapeutic modulations in living tissues so their effects are dependent on the physiological state of the tissue at the moment of irradiation [9]. Studies show that stimulation and inhibition due to light irradiation can occur via the same photoacceptors, and therefore as the light dose increases, the photosensors are damaged and the effect decreases, showing a bi-phasic LLLT responses as function of irradiance (W cm⁻²) [10]. Similar studies have been executed at 50% duty cycle in frequency modulation up to the kHz range [11]. Pulse laser radiation with low duty cycle [12] suggested transient heating possibly inhibiting NADPH oxidase.

While previously LLLT or laser biostimulation research was predominantly based on subjective evaluation of clinical or semi-quantitative pre-clinical studies, recently quantifiable pre-clinical models have been exploited to access the molecular basis for LLLT efficacy [13]. In this study, transgenic animals carrying the reporter luciferase were used to quantify iNOS gene expression following induction of acute inflammation and immediate single LLLT irradiation modulated by various wavelengths in continuous and pulsed mode.

Zymosan A has been used in several studies as a model system for inflammation induction in vivo [3,5,6,14,15].

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**Efficacy of 904 nm Gallium Arsenide Low Level Laser Therapy in the Management of Chronic Myofascial Pain in the Neck: A Double-Blind and Randomized Controlled Trial**

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**Background and Objectives:** A prospective, double blind, randomized, and controlled trial was conducted in patients with chronic myofascial pain syndrome (MPS) in the neck to evaluate the effects of infrared low level 904 nm Gallium-Arsenide (Ga-As) laser therapy (LLLT) on clinical and quality of life (QoL).

**Study Design/Patients and Methods:** The study group consisted of 60 MPS patients. Patients were randomly assigned to two treatment groups: Group I (actual laser; 30 patients) and Group II (placebo laser; 30 patients). LLLT continued daily for 2 weeks except weekends. Follow-up measures were evaluated at baseline, 2, 3, and 12 weeks. All patients were evaluated with respect to pain at rest, pain at movement, number of trigger points (TP), the Neck Pain and Disability Visual Analog Scale (NPAD), Beck depression Inventory (BDI), and the Nottingham Health Profile (NHP).

**Results:** In active laser group, statistically significant improvements were detected in all outcome measures compared with baseline (P < 0.01) while in the placebo laser group, significant improvements were detected in only pain score at rest at the 1 week later of the end of treatment. The score for self-assessed improvement of pain was significantly different between the active and placebo laser groups (63 vs. 19%) (p < 0.01).

**Conclusion:** This study revealed that short-period application of LLLT is effective in pain relief and in the improvement of functional ability and QoL in patients with MPS.

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**Action of 904 Nm Diode Laser In Orthopaedics and Traumatology**

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**Objective:** The semiconductor or laser diode (GaAs, 904 nm) is the most appropriate choice in pain reduction therapy.

**Summary Background Data:** Low power density laser acts on the Prostaglandins synthesis, increasing the change of PGG2 and PGH2 Periostidos into PG12 (also called Prostacilcyln or Endoprostol). The last one is the main product of the Arachidonic acid into the endothelial cells and into the smooth muscular cells of the vessel walls having a vasodilating and anti-inflammatory action.

**Methods:** Treatment was carried out on 447 cases and 435 patients (250 women and 185 men) in the period between 20.05.1987 and 31.12.1999. The patients, whose age ranged from 25 to 70, with a mean age of 45 years, were suffering from rheumatic and degenerative and traumatic pathologies as well as cutaneous ulcers. The majority of the patients had been seen by orthopaedists and rheumatologists and had undergone x-ray examination. All patients had received drug-based treatment and/or physiotherapy, with poor results. Two thirds were experiencing acute symptomatic pain, while the others presented a chronic pathology with recurrent crises. We used a pulsed diode laser, GaAs 904 nm wavelength. Frequency of treatment: 1 application per day for 5 consecutive days, followed by a 2-day interval. In the evaluation of the results the following parameters have been considered: disappearance of spontaneous and induced pain, anatomic and functional evaluation of the joints, muscular growth, verbal rating scales, hand dinamometer, patient’s pain diary.

**Results:** Very good results were achieved especially with cases of symptomatic osteoarthritis of the cervical vertebrae, with sport-related injuries, with epicondylitis, and with cutaneous ulcers; also, last but not of least importance, with cases of osteoarthritis of the coxa.

**Conclusions:** Treatment with 904 nm diode laser has substantially reduced the symptoms as well as improved the quality of life of the patient, thus postponing the need for surgery.
ATTENUATION AND PENETRATION OF VISIBLE 632.8nm AND INVISIBLE INFRA-RED 904nm LIGHT IN SOFT TISSUES

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We studied the depth of penetration and the magnitude of attenuation of 632.8nm and 904nm light in skin, muscle, tendon, and cartilagenous tissues of live anaesthetized rabbits. Tissue specimens were dissected, prepared, and their thicknesses measured. Then, each wavelength of light was applied. Simultaneously, a power meter was used to detect and measure the amount of light transmitted through each tissue. All measurements were made in the dark to minimize interference from extraneous light sources. To determine the influence of pulse rate on beam attenuation, the 632.8nm light was used at two predetermined settings of the machine; continuous mode and 100 pulses per second (pps), at an on/off ratio of 1:1. Similarly, the 904nm infra-red light was applied using two predetermined machine settings: 292 pps and 2,336 pps. Multiple regression analysis of the data obtained showed significant positive correlations between tissue thickness and light attenuation (p < .001). Student's t-tests revealed that beam attenuation was significantly affected by wavelength. Collectively, our findings warrant the conclusions that (1) The calf muscles of the New Zealand white rabbit attenuates light in direct proportion to its thickness. In this tissue, light attenuation is not significantly affected by the overlying skin, a finding which may be applicable to other muscles. (2) The depth of penetration of a 632.8nm and 904nm light is not related to the average power of the light source. The depth of penetration is the same notwithstanding the average power of the light source. (3) Compared to the 904nm wavelength, 632.8nm light is attenuated more by muscle tissue, suggesting that is is absorbed more readily than the 904nm wavelength or conversely that the 904nm wavelength penetrates more. Thus, wavelength plays a critical role in the depth of penetration of light.

key words: Laser Therapy, Light Attenuation, Light Absorption.

Introduction

As early as 1968, Mester and his associates [1-3] demonstrated that, at low power, red light promotes tissue repair. In the intervening period since then, the therapeutic value of phototherapy has been argued and debated by many, with several studies supporting the original hypothesis that they promote tissue repair processes in experimental animals [4-32] and human wounds and ulcers [8, 33-38], and others [39-46], suggesting the contrary.

A review of well controlled in vitro and in vivo laboratory experiments reveals a trend that suggests that low intensity lasers enhance wound healing by promoting cell proliferation [8, 28-30, 41-44], accelerating the formation of granulation tissue, promoting collagen synthesis [3-13, 47-61], fostering the formation of type I and type III procollagen specific pools of mRNA [62], increasing ATP synthesis within the mitochondria, activating lymphocytes, and increasing their abilities to bind pathogens [10, 52]. The trend is not as clear when clinical reports on tissue repair are examined. Rather, sufficient differences of opinion seem evident between studies showing beneficial effects and those reporting no effects whatsoever [10, 33-46].

Given the multitude of treatment parameters used in these studies, i.e., wavelength, pulsed versus continuous wave light, energy fluence, power density, exposure time, frequency and total duration of treatment, it is not surprising that results differ from one study to the next. A good understanding of the nature of light-tissue interaction could simplify and ease the choice of treatment parameters. In this regard, Welch et al [63], have provided a mathematical model that offers a fairly simple method of determining light distribution in tissues. However, their model seems significantly limited, as it applies to CO2, Excimer and Er:YAG lasers, situations involving high scattering of light and tissue depths that are less than 40 mm. Beer's Law which indicates that the incident light is exponentially attenuated as it passes through tissue, and Kubelka-Munk theory, a two-flux model used for uniformly diffused incident light can be used to compute quantitative laser
Skin Penetration Time-Profiles for Continuous 810 nm and Superpulsed 904 nm Lasers in a Rat Model

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Abstract

Objective: The purpose of this study was to investigate the rat skin penetration abilities of two commercially available low-level laser therapy (LLLT) devices during 150 sec of irradiation. Background data: Effective LLLT irradiation typically lasts from 20 sec up to a few minutes, but the LLLT time-profiles for skin penetration of light energy have not yet been investigated. Materials and methods: Sixty-two skin flaps overlaying rat’s gastrocnemius muscles were harvested and immediately irradiated with LLLT devices. Irradiation was performed either with a 810 nm, 200 mW continuous wave laser, or with a 904 nm, 60 mW superpulsed laser, and the amount of penetrating light energy was measured by an optical power meter and registered at seven time points (range, 1–150 sec). Results: With the continuous wave 810 nm laser probe in skin contact, the amount of penetrating light energy was stable at *20% (SEM – 0.6) of the initial optical output during 150 sec irradiation. However, irradiation with the superpulsed 904 nm, 60 mW laser showed a linear increase in penetrating energy from 38% (SEM – 1.4) to 58% (SEM – 3.5) during 150 sec of exposure. The skin penetration abilities were significantly different (*p < 0.01) between the two lasers at all measured time points. Conclusions: LLLT irradiation through rat skin leaves sufficient subdermal light energy to influence pathological processes and tissue repair. The finding that superpulsed 904 nm LLLT light energy penetrates 2–3 easier through the rat skin barrier than 810 nm continuous wave LLLT, corresponds well with results of LLLT dose analyses in systematic reviews of LLLT in musculoskeletal disorders. This may explain why the differentiation between these laser types has been needed in the clinical dosage recommendations of World Association for Laser Therapy.

Introduction

Lasers are used for a number of different purposes in medicine. Class 4 surgical lasers are used to cut and destroy biological tissue, whereas low-level laser therapy (LLLT) is administered with weaker class 3B lasers and mean output powers (MOP) < 500 mW.1 The most commonly used lasers in LLLT are gallium-aluminum-arsenide (GaAlAs) with a wavelength of 810 nm (±50 nm) operating in continuous output mode, and gallium-arsenide (GaAs) with a wavelength of 904 nm superpulsed with high peak power pulses. LLLT emerged as a treatment option in clinical practice approximately three decades ago.2 After the initial upsurge, LLLT gained a poor reputation because of a string of negative research results being published. This picture has slowly changed again as an increasing amount of evidence is pointing toward the existence of specific therapeutic windows for LLLT. Several studies have found that anticipated optimal doses yield significantly better results in tendinopathies, osteoarthritis,4 and neck pain.5 The skin is the body’s external boundary in animals and humans. Among other things, the skin serves as a barrier against physical and chemical intrusions.6 The skin also represents a barrier to applied physical energy from...
Investigation of the Effect of GaAs Laser Therapy on Lateral Epicondylitis

Saniye Konur Emaset, M.D., Lale İnceoğlu Altan, and Merih Yurtkuran

Abstract

**Background and Objective:** There are conflicting reports regarding the efficacy of low energy laser therapy in treatment of lateral epicondylitis (LE). Contradictory results are considered to be due to different joint treatment protocols regarding variables such as dose, duration, and frequency. The aim of this study was to investigate the efficacy of gallium-arsenide (GaAs) laser therapy, which was performed with the dose regimen recommended by the World Association for Laser Therapy, in relieving pain and improving functional activities in patients with LE. **Patients and Methods:** Forty-nine patients (50 elbows) evaluated in our outpatient clinic were included in the study. Elbows were randomized into two groups: laser (n = 25) and placebo laser (n = 25). Either laser or placebo laser therapy was applied to patients for 15 sessions (5 d per week for 3 weeks). Main outcome measures were visual analog scale, tenderness, Disability of the Arm Shoulder and Hand (DASH) questionnaire, the Patient-Related Lateral Epicondylitis Evaluation (PRTEE) test, pain-free grip strength, and the Nottingham Health Profile (NHP) questionnaire. Evaluations were performed before treatment, at the end of 3 weeks of treatment, and after the 12th week of treatment ended. **Results:** Upon post-treatment evaluation, a significant improvement in all parameters was observed for both groups (p < 0.05). No significant difference was found when the laser and placebo groups were compared. At the 12 week evaluation, a significant sustained improvement in all parameters was observed. On intergroup evaluation, a significant improvement was observed in favor of the active treatment group regarding pain with resisted extension of the wrist, tenderness with pressure, and for both the total and subgroup scores of the DASH questionnaire and PRTEE test, as well as for the pain subgroup of the NHP questionnaire (p < 0.05). **Conclusion:** Although low energy laser therapy had no advantage compared to placebo in patients with LE for the short term, a significant improvement, particularly in functional parameters, was achieved in the long term. Laser, which has relatively no side effects, might be included among long-term treatment options for LE.

Introduction

LATERAL EPICONDYLITIS (LE), or tennis elbow, is defined as pain around the lateral epicondyle of the humerus that is aggravated by activity of the wrist and elbow. The etiology of LE is due to damage caused by recurrent overuse of the extensor carpi radialis brevis and extensor digitorum communis muscles, specifically where they attach to the lateral epicondyle. It is most often encountered between the ages of 40 and 60 years, and the dominant arm is generally affected. Several months of conservative treatment is indicated before considering surgical intervention. The aim of conservative treatment is to reduce pain, control inflammation, accelerate healing, and enable patients to do their daily activities.

Conservative treatment methods include anti-inflammatory drugs, ice, LE bands, corticosteroid injections, and an exercise regimen, as well as various physical therapy methods such as massage therapy, laser, electrotherapy, and ultrasound. There are three Cochrane reviews on treatment of LE. In one of these reviews, the researchers could not find definitive conclusions concerning effectiveness of orthotic devices. Oral and topical nonsteroid anti-inflammatory drugs (NSAIDs) were found to be effective in one review, while in the other review, researchers failed to obtain sufficient evidence to support or refute effectiveness of acupuncture for LE treatment. In another review, a number of treatment modalities, including acupuncture, exercise therapy, manipulations and mobilizations, ultrasound, phonophoresis, Rebox, and ionization with diclofenac, were found to show positive effects in pain reduction or improved function for patients with LE based on at least level 2b evidence.
DERMATOLOGIC STUDIES
Synopsis of Articles on Dermatologic/Wounds

1-Low Level Laser Therapy of Serious Wounds of Dogs
The authors were amazed at the rapid regression of pain and the completeness of the healing with unexpected full regeneration and superb esthetic effects.

2-The Role of Laser Fluence in Cell Viability, Proliferation, and Membrane Integrity of Wounded Human Skin Fibroblasts Following Helium-Neon Laser Irradiation
Results show that 5 J/cm² stimulate mitochondrial activity, which leads to normalization of cell function and ultimately stimulates cell proliferation and migration of wounded fibroblasts to accelerate wound closure.

3-Low-Level Laser Therapy Enhances Wound Healing in Diabetic Rats: A Comparison of Different Lasers
The wound healing on control rats with diabetes was slower than on control rats without diabetes. LLLT at appropriate treatment parameters can enhance the wound healing on diabetic rats.

4-Design Development and Evaluation of Fiber Optic Probe Based He-Ne Low Level Laser Therapy System for Tissue Regeneration
Showed that Laser therapy at a dose of 2 J/cm² significantly reduced healing times compared to the controls.

5-Low Level Light Therapy by LED of Different Wavelength Induces Angiogenesis and Improves Ischemic Wound Healing
LED treatment of ischemia challenged tissue improved early wound healing by enhancing angiogenesis irrespective of the wavelength thus delineating this noninvasive means as a potential, cost effective tool in complicated wounds.

6-830-nm Irradiation Increases the Wound Tensile Strength in a Diabetic Murine Model
7-Phototherapy Promotes Healing of Chronic Diabetic Leg Ulcers That Failed to Respond to Other Therapies
Combined 660 and 890 nm light promotes rapid granulation and healing of diabetic ulcers that failed to respond to other forms of treatment.

Our clinical and histological data document promising effects of LLLT on hair regrowth in CNA.

9-Effects of Diode Laser Therapy on Blood Flow in Axial Pattern Flaps in the Rat Mode
We conclude that laser therapy increases the blood flow and perfusion of transferred flaps, and that this has significant effects on the survival of the flaps.
LOW LEVEL LASER THERAPY OF SERIOUS WOUNDS OF DOGS
Katalin Kovacs
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Background: Dogs often suffer serious injuries in fights or attacks by other dogs with behavioral problems. These injuries are often deep, torn and the wound itself is extensive in size. These injuries recover with difficulty, deteriorate the average state of the dog and often end up with death of the animal. Other dogs suffer such accidents which from the chance of recovery is minimal. Aim of the study is to give evidences of level laser therapy in deep, extended skin injuries and documentation of results of recovery.

Study: Infrared medical Laser device (wavelength: 810 nm Spectra Vet) was applied for laser therapy. Dose – area relationship, dose – depth relationship and dose – time relationship were calculated based on “target volume” of biological answer. Data are presented in tables case by case, concerning the healing process. For surface cleaning chlorhexidine solution was used. Narcosis was applied only at the first treatment for deep cleaning and removal of necrotized tissues. Post-treatments procedures as cleaning and laser therapy happened in awaken state of dogs. Follow up photos of most demonstrative 5 cases are presented.

Results: In every case the regression of pain at the injured area was evident soon after the first treatment. Effect of low level laser therapy resulted unexpected full regeneration of skin, gingival, and mucosal damages. Even aggressive animals turned to be cooperative during the treatments. The esthetic effects were also superb with regeneration along the residual scars was spectacular.
The Role of Laser Fluence in Cell Viability, Proliferation, and Membrane Integrity of Wounded Human Skin Fibroblasts Following Helium-Neon Laser Irradiation

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Background: In medicine, lasers have been used predominantly for applications, which are broadly termed low level laser therapy (LLLT), phototherapy or photobiomodulation. This study aimed to establish cellular responses to Helium-Neon (632.8 nm) laser irradiation using different laser fluences (0.5, 2.5, 5, 10, and 16 J/cm²) with a single exposure on 2 consecutive days on normal and wounded human skin fibroblasts.

Materials and Methods: Changes in normal and wounded fibroblast cell morphology were evaluated by light microscopy. Changes following laser irradiation were evaluated by assessing the mitochondrial activity using adenosine triphosphate (ATP) luminescence, cell proliferation using neutral red and an alkaline phosphatase (ALP) activity assay, membrane integrity using lactate dehydrogenase (LDH), and percentage cytotoxicity and DNA damage using the Comet assay.

Results: Morphologically, wounded cells exposed to 5 J/cm² migrate rapidly across the wound margin indicating a stimulatory or positive influence of phototherapy. A dose of 5 J/cm² has a stimulatory influence on wounded fibroblasts with an increase in cell proliferation and cell viability without adversely increasing the amount of cellular and molecular damage. Higher doses (10 and 16 J/cm²) were characterized by a decrease in cell viability and cell proliferation with a significant amount of damage to the cell membrane and DNA.

Conclusions: Results show that 5 J/cm² stimulates mitochondrial activity, which leads to normalization of cell function and ultimately stimulates cell proliferation and migration of wounded fibroblasts to accelerate wound closure. Laser irradiation can modify cellular processes in a dose or fluence (J/cm²) dependent manner. Lasers Surg. Med. 38:74-83, 2006. © 2006 Wiley-Liss, Inc.

Key words: laser therapy; cellular effect; phototherapy; photobiomodulation; inhibitory; stimulatory

INTRODUCTION

Phototherapy has been used for many years and is used in medical and dental professions; however, it is still not an established therapeutic modality [1]. In addition to accelerated wound healing, the main advantages of phototherapy include prevention of side effects of drugs and significantly accelerated functional recovery [3]. Low energy laser irradiation produces significant bioeffects, which are manifested in biochemical, physiological, and proliferative phenomena in various enzymes, cells, tissues, organs, and organisms [3]. Laser radiation has a wavelength-dependent capability to alter cellular behavior in the absence of significant heating [4]. Phototherapy includes wavelengths of between 500 and 1,000 nm and typically involves the delivery of 1-4 J/cm² to treatment sites. Red laser light (632.8 nm) appears to be the most effective frequency of laser at a cellular level. There is controversy about the results observed previously as visible laser light can cause stimulatory or inhibitory depending on factors such as the energy, wavelength, and irradiation time [5].

Beginning from the late 1980s, Endre Mester, a Hungarian physician, began a series of experiments with monochromatic light. Mester observed that in many cases, the skin incisions made to implant recalcitrant cells appeared to heal faster in treated animals compared to incisions of control animals that were not treated with light [6-8].

When wounded or scratched, cell monolayers respond to the disruption of cell–cell contacts with an increased concentration of growth factors at the wound margin and by healing the wound through a combination of proliferation and migration [9-11]. These processes reflect the behavior of individual cells as well as the properties of the cell sheet as a surrogacytomas. To perform a wound healing assay, a wound is typically introduced in a cell monolayer according to Cha et al. (1990) [12] using an object such as a pipette tip or syringe needle to create a cell-free zone. The monolayers recover and heal the wound in a process that can be observed over a time course of 3–24 hours. The wound heals in a stereotyped fashion—cells polarize toward the wound, initiate protrusion, migrate, and close the wound. In vitro wound closure can be monitored by manually imaging samples [13] or by the repopulation of

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Low-Level Laser Therapy Enhances Wound Healing in Diabetic Rats: A Comparison of Different Lasers

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Objective: The effects of wound healing acceleration on diabetic rats were determined and compared using different laser wavelengths and incident doses. Background Data: Many studies have demonstrated that low-level laser therapy (LLLT) can promote the wound healing on non-diabetic animals. Methods: Male Sprague-Dawley rats were used. Streptozotocin (70 mg/kg) was applied for diabetes induction. An oval full-thickness skin wound was created aseptically with a scalpel in 51 diabetic rats and six non-diabetic rats on the shaved back of the animals. The study was performed using 532, 633, 810, and 980 nm diode lasers. Incident doses of 5, 10, 20, and 30 J/cm² and treatment schedule of 3 times/week were used in the experiments. The area of wound on all rats was measured and plotted on a slope chart. The slope values (mm²/day), the percentage of relative wound healing, and the percentage of wound healing acceleration were computed in the study.

Results: Mean slope values were 6.0871 in non-diabetic control and 3.636 in diabetic control rats (p > 0.005). The percentages of wound healing acceleration were 15.23, 18.06, 19.54, and 20.39 with 532-nm laser, 33.53, 38.44, 32.05, and 16.45 with 633-nm laser, 15.72, 14.94, 9.62, and 7.76 with 810-nm laser, and 12.80, 16.32, 13.79, and 7.74 with 980-nm laser, using incident doses of 5, 10, 20, and 30 J/cm², respectively. There were significant differences (p > 0.001) in the mean slope value of wound healing on diabetic rats between control groups and treatment groups in 532, 633, 810, and 980 nm lasers. Conclusion: The wound healing on control rats with diabetes was slower than on control rats without diabetes. LLLT at appropriate treatment parameters can enhance the wound healing on diabetic rats. The optimum wavelength was 633 nm, and the optimum incident dose was 10 J/cm² in our study.
DESIGN, DEVELOPMENT AND EVALUATION OF FIBER OPTIC PROBE BASED HE-NE LOW LEVEL LASER THERAPY SYSTEM FOR TISSUE REGENERATION

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Background: The use of phototherapy in the field of wound healing has gained popularity over the years. In phototherapy, a suitable irradiation is applied on the wounds, accelerates healing process. In the current study our aim was to design and develop a fiber optic probe based low level laser therapy (LLLT) system using Helium-Neon (He-Ne) laser and to optimize the laser dose and treatment schedule for tissue regeneration on Swiss albino mice. For selecting optimum laser dose, the animal wounds were exposed with different doses (1, 2, 3, 4, 6, 8 and 10 J/cm²) separately except controls. The standardization of treatment schedule was carried out by irradiating wounds to 2 J/cm² at different post wounding time (0, 24 and 48 h).

Study: Auxiliary components were designed and fabricated for the development of the low level laser therapy system. Tissue regeneration potential of the system was evaluated by following the progression of wound contraction/area and mean wound healing time.

Results: Animals exposed to 2 J/cm² showed significant ($p < 0.01$) enhancement of wound contraction at day 5, day 9, day 12, day 14, day 16 and day 19 post irradiation compared to the controls. Treatment with laser at 2 J/cm² resulted a significant ($p < 0.01$) decrease in the healing time (19.33 ± 0.38 days, post irradiation) compared to controls (25.92 ± 0.8 days). The animals exposed to 2 J/cm² immediately (0 hr) after wounding showed enhanced wound contraction when compared to control and other treatment schedules.

Conclusion: The LLLT system designed and developed in this study has demonstrated optimum tissue regeneration for 2 J/cm² He-Ne laser irradiation, applied immediately after the wounding compared to other post wounding treatment schedules by promoting wound contraction and thereby reducing mean healing time.
Background and Objective: Low-level light therapy (LLLT) has been revealed as a potential means to improve wound healing. So far, most studies are being performed with irradiation in the red to near-infrared spectra. Recently, we showed that blue light (470 nm) can significantly influence biological systems such as nitric oxide (NO) metabolism and is able to release NO from nitrosyl-hemoglobin or mitochondrial protein complexes. Therefore, the aim of this study was to evaluate and compare the therapeutic value of blue or red light emitting diodes (LEDs) on wound healing in an ischemia disturbed rodent flap model.

Study Design/Materials and Methods: An abdominal flap was rendered ischemic by ligation of one epigastric bundle and subjected to LED illumination with a wavelength of 470 nm (blue, n = 8) or 629 nm (red, n = 8) each at 50 mW/cm² and compared to a non-treated control group (n = 8). Illumination was performed for 10 minutes on five consecutive days.

Results: LED therapy with both wavelengths significantly increased angiogenesis in the sub-epidermal layer and intramuscularly (panniculus carnosus muscle) which was associated with significantly improved tissue perfusion 7 days after the ischemic insult. Accordingly, tissue necrosis was significantly reduced and shrinkage significantly less pronounced in the LED-treated groups of both wavelengths.

Conclusions: LED treatment of ischemia challenged tissue improved early wound healing by enhancing angiogenesis irrespective of the wavelength thus delineating this noninvasive means as a potential, cost effective tool in complicated wounds.
830-nm Irradiation Increases the Wound Tensile Strength in a Diabetic Murine Model

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Background and Objective: The purpose of this study was to investigate the effects of low-power laser irradiation on wound healing in genetic diabetes.

Study Design/Materials and Methods: Female C57BL/KSj/db/db mice received 2 dorsal 1 cm full-thickness incisions and laser irradiation (830 nm, 78 mW/cm², 5.0 J/cm²/wound). Daily low-level laser therapy (LLLT) occurred over 0–4 days, 3–7 days, or nonirradiated. On sacrifice at 11 or 23 days, wounds were excised, and tensile strengths were measured and standardized.

Results: Untreated diabetic wound tensile strength was 0.77 ± 0.22 g/mm² and 1.51 ± 0.13 g/mm² at 11 and 23 days. After LLLT, over 0–4 days tensile strength was 1.15 ± 0.14 g/mm² and 2.45 ± 0.29 g/mm² (P = 0.0019). Higher tensile strength at 23 days occurred in the 3- to 7-day group (2.72 ± 0.56 g/mm²). LLLT vs. 1.51 ± 0.13 g/mm² non-treated; P ≤ 0.01.


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Key words: biostimulation; collagen; diabetes; murine fibroblast; impaired wound healing; laser; low-power laser therapy; wound; wound healing; wound tensile strength; experimental surgery; LLLT; type I diabetes

INTRODUCTION

Wound healing is a complex, well-coordinated response to injury characterized by inflammatory, proliferative, synthetic, and maturation phases. The inflammatory phase includes hemoisostasis, platelet degranulation, and activation of the complement and clotting cascades, which provide a scaffold for wound healing [1]. Platelet degranulation is responsible for the release of a series of potent cytokines [2–9]. Macrophages take the governing role during the inflammatory phase, being responsible for debridement of the injured area, matrix synthesis, angiogenesis, and the synthesis of nitric oxide, which regulates wound fibroblasts [7]. A reduction in the inflammatory response inhibits healing in clinical and experimental diabetes [8,9], whereas its prolongation results in the stigma of the fibroproliferative diseases [10].

The appearance of endothelial cells and fibroblasts marks the start of the proliferative phase [11]. Fibroblasts are transformed into “wound fibroblasts” [12] that migrate from the surrounding tissue, become proliferative, and produce collagen. Regulation of excess collagen deposition by collagenase activity signals the start of the maturation and remodeling phases. The main feature of the maturation phase is the deposition of collagen. This is the most important phase of wound repair because the rate, quality, and total amount of the deposited matrix determines the strength of the scar. Collagen I is the predominant type of collagen (90%) in the matured scar, whereas collagen III comprises up to 30% of the collagen in granulation tissue and does not contribute to wound strength [13]. Increased collagen synthesis persists for at least 3–5 weeks after wounding and is due to the increased number of fibroblasts and a net increase in collagen production per cell.

Newly synthesized collagen is biochemically and physically different and has a smaller fiber diameter than unwounded skin [14]. Unwounded skin shows a basket weave arrangement of collagen fibers, whereas the scar’s fibers are parallel to the skin. Fiber thickness and orientation with wound tensile strength are positively correlated [15]. The healed scar never becomes as organized as the unwounded dermis. The progressive increase in the tensile strength of a normally healing wound is 3% of unwounded tissue at 1 week after wounding, 20% at 2 weeks, and 80% at 4–6 weeks, with no subsequent increase thereafter [16,17].

Severely impaired wound repair occurs in diabetes mellitus, regardless of the type. Diabetics tend to heal much more slowly than normal individuals, and the resultant wounds are typically of poorer quality. The exact mechanism of impaired healing in diabetes is not clear, and the underlying causes may vary. The shorter duration of the inflammatory phase may contribute to impaired wound healing. Decreased collagen synthesis and deposition resulting from a poor nutritional supply to the fibroblasts are thought to be primary causes of the impairment observed. Healed skin incisions in an insulin-
Phototherapy Promotes Healing of Chronic Diabetic Leg Ulcers That Failed to Respond to Other Therapies

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Objective: We tested the hypothesis that combined 660 and 800 nm LED phototherapy will promote healing of diabetic ulcers that failed to respond to other forms of treatment.

Research Design and Methods: A double-blind randomized controlled design was used to study 28 diabetic leg ulcers in two groups of 14 patients. Group one ulcers were cleaned, dressed with 1% silver sulfadiazine cream and treated with “placebo” phototherapy (1.6 J cm⁻²) twice per week, using a Dynatron Solaris 75S device. Group two ulcers were treated similarly but received 9 J cm⁻² dose.

Results: At each of 15, 30, 45, 50, 75, and 90 days of healing, mean ulcer granulation and healing rates were significantly higher for group two than the “placebo” group (P < 0.02). While “placebo” treated ulcers worsened during the initial 30 days, group two ulcers healed rapidly; achieving 39% more granulation and 78% faster healing by day 30, and maintaining similarly higher rates of granulation and healing over the “placebo” group all through. By day 90, 58.3% of group two ulcers had healed fully and 75% had achieved 90–100% healing. In contrast, only one “placebo” treated ulcer healed fully by day 90; no other ulcer attained ≥90% healing.


Key words: diabetes; leg ulcers; phototherapy; biostimulation

INTRODUCTION

Evidence abounds that treatment with polychromatic or monochromatic light (phototherapy), in particular light and/or infrared radiation with less than 200 mW cm⁻² irradiance and 600–1,000 nm wavelength [1–2], promotes the repair processes of skin [3–12], ligament [13–16], tendon [17–20], bone [13,21–24], and cartilage [13,25–29] in experimental animal wounds and human ulcers of various etiologies [30–33]. There is evidence that phototherapy advances tissue repair by promoting fibroblast proliferation [34–44], synthesis of collagen and other components of tissue [45–48], and by enhancing the cellular and sub-cellular processes needed to enhance the formation of type I and type III procollagen pools of mRNA [49], ATP synthesis and lymphocyte action [50–51]. Other reports [52–58] suggest the contrary, prompting the prevailing skepticism about the value of phototherapy as a clinical armamentarium. For example, cultured human fibroblasts irradiated with 0.9 mW helium–neon laser over a 5-day period were neither stimulated nor inhibited in comparison with similarly handled control fibroblasts [58]. Likewise, rat skin incisions treated daily with 1, 2, or 4 J cm⁻² of “He-Ne laser” did not heal faster than control incisions when calipers were used to measure their rates of contraction [59]. And after seven or 14 days of treating the incisions, the tensile strength of treated wounds did not differ from the strength of an untreated control group [59]. Furthermore, a clinical study of 58 teeth extraction patients who had one of two gingival flaps lased with 0.34 J cm⁻² [57] nm “diode laser” and the other left as untreated control produced mixed results. Whereas, clinical and photographic evidence showed that 69% of irradiated incisions healed faster than controls, in contrast, no significant differences in healing were observed when the patients were compared by age, gender, race, and anatomical location of injury [60].

In general, clinical studies showing that phototherapy promotes ulcer repair lack experimental controls [5]. The dearth of well-controlled clinical studies and the wide range of variables that must be considered and controlled in clinical practice—such as wavelength, power, power density, energy, energy density, treatment duration, treatment intervention time post-injury and contact versus non-contact methods of treatment—complicate the scenario. Nonetheless, clinicians continue to embrace phototherapy as a non-invasive, safe and comparatively...

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BACKGROUND:
Canine noninflammatory alopecia (CNA) is a heterogeneous group of skin diseases with different underlying pathogenesis. The therapeutic approach is challenging, and new options for treatment are desirable.

HYPOTHESIS/OBJECTIVES:
To test the clinical efficacy of low-level laser therapy (LLLT) on hair regrowth in CNA.

ANIMALS:
Seven dogs of different ages, breeds and genders with a clinical and histopathological diagnosis of noninflammatory alopecia.

METHODS:
Each dog was treated twice weekly for a maximum of 2 months with a therapeutic laser producing the following three different wavelengths emerging simultaneously from 21 foci: $13 \times 16 \text{ mW, } 470 \text{ nm; } 4 \times 50 \text{ mW, } 685 \text{ nm; } $ and $4 \times 200 \text{ mW, } 830 \text{ nm.}$ The fluence given was $3 \text{ J/cm}^2$, frequency 5 Hz, amplitude of the irradiated area was 25 cm$^2$ and application time was 1.34 min. A predetermined alopecic area was left untreated and served as a control area. From one dog, post-treatment biopsies of treated and untreated sites were obtained for histological evaluation of hair density and the percentage of haired and nonhaired follicles.

RESULTS:
At the end of the study, coat regrowth was greatly improved in six of seven animals and improved in one of seven. By morphometry, the area occupied by hair follicles was 18% in the treated sample and 11% in the untreated one (11%); haired follicles were (per area) 93% in the treated sample and only 9% in the control sample.

CONCLUSIONS AND CLINICAL IMPORTANCE:
Our clinical and histological data document promising effects of LLLT on hair regrowth in CNA. Further studies investigating the biological mechanism underlying the effect of LLLT on hair follicle cycling are warranted.
Effects of Diode Laser Therapy on Blood Flow in Axial Pattern Flaps in the Rat Model

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Abstract. Axial pattern skin flaps are a very important reparative tool for the plastic and reconstructive surgeon in the reconstruction of tissue defects. From whatever unfortunate reason, part or all of such flaps occasionally suffers from irreversible ischaemia with loss of the flap. Infrared diode laser therapy has been shown to improve local and systemic circulation. The present study was designed to assess the effect of an 830 nm diode laser (power density, 18.5 W/cm², energy density 185 J/cm²) on the blood flow of axial pattern flaps in the rat model and their survival, compared with unirradiated controls. The flaps were raised in all animals (n=40), and blood flow assessed with laser speckle flowmetry (LSF). In the experimental groups (3 groups, n=10 per group), the flaps were irradiated either directly over the dominant feeder vessel (iliolumbar artery), at the proximal end or at the distal end of the flap itself and blood flow assessed during irradiation. Flowmetry was performed again in all animals at 5 and 10 min postirradiation, and the flaps sutured back in position. The unirradiated controls were handled in exactly the same way, but the laser was not activated. The survival rate of the flaps was assessed on the fifth postoperative day. LSF demonstrated significant increased blood flow in the flaps at 5 and 10 min postirradiation in all experimental groups compared with the control animals. At five days postirradiation, there was significantly better survival of the flaps in all the experimental groups compared with the controls (p<0.01), but no significant difference was seen between any of the experimental groups. We conclude that laser therapy increases the blood flow and perfusion of transferred flaps, and that this has significant effects on the survival of the flaps. One possible mechanism of modulation of the autonomic nervous system is discussed.

Keywords: Autonomic nervous system; Diode laser; Failing skin flaps; Low level laser therapy; Photobioactivation; Tissue ischaemia

INTRODUCTION

In the field of plastic and reconstructive surgery, a variety of flaps have been developed to repair tissue defects, and failure of flaps is a major problem for the plastic and reconstructive surgeon. Despite good intraoperative care, for whatever reason, irreversible ischaemia sometimes occurs in the distal portion of random pattern skin flaps or in random portions of axial pattern skin flaps. If the peripheral blood flow is sufficient in the distal portion of the skin flap then flap necrosis would become much less of a problem. Low incident levels of laser irradiation have been shown to improve circulation [1,2]. If, for example, application of a diode laser can improve the circulation in the distal portion of the flap, then this should, in theory, help to ensure a greater survival area.

Since 1989, the author and others have been reporting on enhanced blood flow following the application of low incident levels of laser energy, often referred to as laser therapy, in research with animal models [3–5]. Of particular interest have been the reports on the effects of 830 nm GaAlAs diode laser irradiation on random pattern skin flap survival in the rat model. It has been demonstrated that GaAlAs diode laser therapy produced (1) higher vascular perfusion, (2) greater fluorescent areas under fluorescein angiography and (3) significantly larger flap survival areas than either non-coherent LED-irradiated or un-irradiated control flaps. Laser therapy has been used in humans with significant success.
MUSCULOSKELETAL STUDIES
Synopsis of Articles on Musculoskeletal

1- Effects of Laser Treatment on the Expression of Cytosolic Proteins in the Synovium of Patients with Osteoarthritis
This study found that diode laser irradiation of the synovial samples of patients with osteoarthritis can statistically significantly alter the expression of some proteins in vitro. These findings provide some more evidence for biological efficacy of LLLT treatment, used for osteoarthritis.

2-Effect of Low Level Laser Therapy on Subchondral Bone Integrity on Knee Osteoarthritic Animal Model
Concluded that LLLT can improve subchondral bone mineral density and micro-architecture in ACLT induced knee osteoarthritis in rats.

3-Low-Energy Laser Irradiation Promotes Synovial Fibroblast Proliferation by Modulating p15 Subcellular Localization
This study gave further insight into the mechanisms of how Laser therapy produces biological effects and how these can contribute to the applications in regenerative medicine.

4-Low Level Light Effects on Inflammatory Cytokine Production by Rheumatoid Arthritis Synoviocytes
Showed how Laser therapy can relieve pain by reducing the levels of pro-inflammatory cytokines/chemokines and how this can relate to broad anti-inflammatory properties.

5-Musculoskeletal Disorders: Pain management thru Low Level Laser Therapy
It was shown that Laser therapy reduced pain and can have a positive benefit in work related musculoskeletal disorders.

6-Histological effects of 820 mm laser irradiation on the healthy growth plate of the rat
The results show that irradiation with low level laser light of wavelength 820 nm and energy density 5 J cm super(-2) had no significant effect on the healthy growth plates of the rat knee joint.

7-Effect of Laser Phototherapy (k660 nm) on Type I and III Collagen Expression During Wound Healing in Hypothyroid Rats: An Immunohistochemical Study in a Rodent Model
Laser light therapy performed with the parameters of this investigation increased immunoexpression of collagen type I during tissue repair, and improved the quality of newly formed tissue in the presence of hypothyroidism.

8-Effect of Low Level Laser Therapy (830) With Different Therapy Regimes on the Process of Tissue Repair in Partial Lesion Calcaneous Tendon
Low intensity Laser therapy was effective in the improvement of collagen fibers organization of the calcaneous tendon after undergoing a partial lesion.

9-Effectiveness of Class IV Therapy Lasers for Achilles Tendinopathy
This study showed that using Laser therapy twice weekly along with exercise showed superior results in improving the tendinopathy over exercise alone.
Effects of Laser Treatment on the Expression of Cytosolic Proteins in the Synovium of Patients With Osteoarthritis

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Background and Objective: Low level laser therapy (LLLT) has been developed for non-invasive treatment of joint diseases. We have previously shown that LLLT influenced synovial protein expression in rheumatoid arthritis (RA). The aim of this study was to assess the effects of laser irradiation on osteoarthritic (OA) synovial protein expression.

Study Design/Materials and methods: The synovial membrane samples removed from the knees of 6 OA patients were irradiated ex vivo using near infrared diode laser (807–811 nm; 25 J/cm²). An untreated sample taken from the same patient served as control. Synovial protein separation and identification were performed by two-dimensional differential gel electrophoresis and mass spectrometry, respectively.

Results: Eleven proteins showing altered expression due to laser irradiation were identified. There were three patients whose tissue samples demonstrated a significant increase (P < 0.05) in mitochondrial heat shock 60 kD protein 1 variant 1. The expression of the other proteins (calpain small subunit 1, tubulin alpha-1C and beta 2, vimentin variant 3, annexin A1, annexin A5, collagen 1, and collagen type VI alpha 2 chain precursor) significantly decreased (P < 0.05) compared to the control samples.


Key words: ex vivo; laser therapy; proteomics; synovial membrane

INTRODUCTION

Studies of the expression of proteins (proteomics) play a more and more important role in the investigation of various clinical entities [1]. This technique has been successfully used in studying the expression of cytosolic proteins of the synovial membranes in rheumatoid arthritis (RA), osteoarthritis (OA), and ankylosing spondylitis (AS). Differences were found in the protein expression of calgranulin A, vimentin, α-enolase, fructose biphosphate aldolase A in the synovial membrane of patients with RA and AS compared to OA [2]. The identification of new biological and clinical markers of inflammatory rheumatic diseases (RA, AS) opened new possibilities of further dissecting the synovial effects of low-level laser therapy (LLLT) [3]. Bjordal et al. [4] proved the anti-inflammatory and pain-relieving effects of LLLT in the infrared wavelength range in Achilles tendinitis in a randomized, placebo controlled study. Several authors have reported the beneficial clinical results of LLLT in OA and tendinopathy [5]. In in vitro studies LLLT decreased inflammation by diminishing the production of PGE2 and by inhibiting cyclo-oxigenase 2 [6–13]. Rizzi et al. [14] showed that LLLT reduced the inflammatory response induced by trauma in animals by blocking the effects of reactive oxygen species (ROS) and the activation of nuclear
EFFECT OF LOW LEVEL LASER THERAPY ON SUBCHONDRAL BONE INTEGRITY OF KNEE OSTEOARTHRITIC ANIMAL MODEL
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Background: Knee osteoarthritis (OA) is characterized by articular cartilage damage. Alteration of subchondral bone with aberrant loading has been proposed as one mechanism for the initiation and progression of OA. Previous in vitro studies demonstrated the osteogenic effect of low level laser therapy (LLLT) through stimulating gene expression in osteoblast. This study aims to evaluate LLLT on restoration of the subchondral bone integrity in knee OA.

Study: Forty rats were randomly allocated: intact control (CG), placebo LLLT (PG), low dose LLLT (LLG) and high dose LLLT (HLG). Knee OA was induced in right knee joints using anterior cruciate ligament transection (ACL-T) except CG. Only LLG and HLG were given ten sessions of 80- and 800-second LLLT at 820nm on right knee joint, respectively at 10 weeks post-ACL-T. Subchondral bone status of femorotibial joint was evaluated by micro-computed tomography.

Results: In proximal tibial epiphysis, trabecular material bone mineral density (tBMD), apparent trabecular bone mineral density (atBMD), bone volume fraction (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th) were significantly higher (p<0.05) in CG while connectivity, structure model index (SMI) and trabecular spacing (Tb.Sp) were significantly lower (p<0.05) in CG when compared to PG. After 800-second LLLT in HLG, tBMD, atBMD, BV/TV, SMI and Tb.Th were significantly restored compared to PG (p<0.05). Improvement of tBMD was also observed in LLG compared to PG. In distal femoral epiphysis, atBMD, BV/TV, Tb.Th were significantly higher (p<0.05) in CG while SMI and Tb.Sp were significantly lowered (p<0.05) in CG when compared to PG. High dose LLLT exerted significant improvement in atBMD, BV/TV and Tb.Th compared to PG (p<0.05). No treatment effect was observed in low dose LLLT comparing to PG.

Conclusion: LLLT can improve subchondral bone mineral density and micro-architecture in ACL-T induced knee OA rat. Further investigation on the mechanism of this improvement should be conducted.
Low-Energy Laser Irradiation Promotes Synovial Fibroblast Proliferation by Modulating p15 Subcellular Localization

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INTRODUCTION

Low-energy laser irradiation (low-level laser therapy) (LELI/LLLT/photobiomodulation), of which output power is in the range of mW, modulates various biological effects. In animal models, LELI promotes wound healing [1] and angiogenesis [2], and reduces infarct size following myocardial infarction [3]. In vitro, LELI has effects promoting collagen synthesis [4], stimulating release of growth factors [5], inducing anti-inflammatory effect [6], and promoting proliferation of such cells as muscle satellite cells [7], melanomas [8], skin fibroblasts [9], endothelial cells [10], mesenchymal bone marrow cells and cardiac stem cells [11], and chondrocytes [12].

The cell proliferation and progression of the cell cycle are regulated by the sequential activities of various cyclin-dependent kinases (CDKs). CDK activity is dependent on physical interaction with one of the cyclins, which are the regulatory subunits of these complexes. In addition, this activity is negatively regulated by a group of proteins, cell cycle regulators, collectively termed CDK inhibitors (CKIs) and consisting of two groups, the INK4 family and CIP/KIP family, classified according to structure and function [13,14]. An INK4 family member, p15 INK4B/CDKN2B, regulates cell cycle arrest at G1 by inhibiting CDK4 and CDK6 to inactivate the retinoblastoma family of tumor suppressor proteins [13,14]. On the other hand, CDK activity has a variety of effects on cell proliferation, that is, on inhibiting (15–18) or stimulating (19,20) cell growth. It is known that LELI brings about increased [8,21] or decreased [22,23] CAMP levels, which influence the cell cycle by inducing CKIs [15–18].

Synovial fibroblasts, a constituent of the synovial capsule, are important in maintaining the homeostasis of...
Low Level Light Effects on Inflammatory Cytokine Production by Rheumatoid Arthritis Synoviocytes

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Background and Objective: Low level light therapy (LLLT) is being evaluated for treating chronic and acute pain associated with rheumatoid arthritis (RA) and other inflammatory diseases. The mechanisms underlying the effectiveness of LLLT for pain relief in RA are not clear. The objectives of this study were to determine whether LLLT decreased production of pro-inflammatory cytokines by cells from RA joints, and, if so, to identify cellular mechanisms.

Study Design/Materials and Methods: Synoviocytes from RA patients were treated with 810 nm radiation before or after addition of tumor necrosis factor-α (TNF-α). mRNA for TNF-α, interleukin (IL)-1β, IL-6, and IL-8 was measured after 30, 60, and 180 minutes using RT-PCR. Intracellular and extracellular protein levels for 12 cytokines/chemokines were measured at 4, 8, and 24 hours using multiplexed ELISA. NF-κB activation was detected using Western blotting to follow degradation of IkBα and nuclear localization of the p65 subunit of NF-κB.

Results: Radiation at 810 nm (5 J/cm²) given before or after TNF-α decreases the mRNA level of TNF-α and IL-1β in RA synoviocytes. This treatment using 25 J/cm² also decreases the intracellular levels of TNF-α, IL-1β, and IL-8 protein but did not affect the levels of seven other cytokines/chemokines. TNF-α-induced activation of NF-κB is not altered by 810 nm radiation using 25 J/cm².

Conclusions: The mechanism for relieving joint pain in RA by LLLT may involve reducing the level of pro-inflammatory cytokines/chemokines produced by synoviocytes. This mechanism may be more general and underlie the beneficial effects of LLLT on other inflammatory conditions. Lasers Surg Med 41:282–290, 2009.

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Keywords: inflammation; IL-1β; low level light therapy; synoviocytes; rheumatoid arthritis; TNF-α

INTRODUCTION

Low level light therapy (LLLT) is being evaluated and introduced into clinical practice for treating both chronic and acute pain, reducing wound healing, decreasing the effects of stroke and for other applications [1–5]. LLLT utilizes wavelengths in the red and near infrared (NIR) region of the spectrum (630–1,000 nm). Low irradiances are used that typically do not produce thermal effects on tissue (i.e., 1–2°C temperature change) suggesting that the observed benefits are due to photochemical rather than thermal effects. However, many aspects of the mechanisms for the therapeutic effects of far red and NIR radiation are not understood. The pathology of many of the conditions treated by LLLT involves inflammation, and LLLT may be effective because it reduces inflammation. For example, LLLT has been used for many years for relief of the chronic pain in rheumatoid arthritis (RA), which is caused by sensitization of neurons by inflammatory mediators [6].

RA is a complex inflammatory autoimmune disease of synovial joints, especially the small joints of the hands and feet, that leads to joint destruction and disability. The major symptoms are chronic pain, swelling, and heat in multiple joints [7]. Inflammation of the synovial lining of joint results in increased expression of inflammatory cytokines, chemokine-mediated recruitment of additional inflammatory cells, as well as activation of B cells with autoantibody production. A vicious cycle of altered cytokine and signal transduction pathways and inhibition of programmed cell death contribute to cartilage and bone destruction by human fibroblast-like synoviocytes (HLF) [8, 9]. The major mediators of chronic inflammation in RA include tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, and prostaglandin E2 (PGE2). These mediators exist in a network that perpetuates the inflammation by forming positive feedback loops between the mediators from activated synoviocytes and infiltrating macrophages [9]. These inflammatory mediators sensitize the peripheral termini of primary afferent nociceptors to cause a decrease in the pain threshold, resulting in increased sensitivity to pain [6].

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MUSCULOSKELETAL DISORDERS: PAIN MANAGEMENT THROUGH LOW LEVEL LASER THERAPY

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Background: Musculoskeletal Disorders (MSDs) can affect the body’s muscles, joints, tendons, ligaments and nerves. Most work-related MSDs develop over time and are caused either by the work itself or by the employees’ working environment. Typically, MSDs affect the back, neck, shoulders and upper limbs. Despite of scientific progress there still exist many barriers in an adequate treatment of pain including the lack of knowledge of many drugs and pain management techniques. The application of Low Level Laser Therapy (LLLT) aims to restore the normal biological function of injured or stressed cells. We evaluated Low Level Laser Therapy (LLLT) on pain reduction in individuals with musculoskeletal disorders.

Study: It was analyzed 8 volunteers that presented chronic pains musculoskeletal, divided: in Group 1 (G1)- 5 individuals a single Laser application (λ = 904 nm, P = 40 mW, Dose = 4 J/cm²) and Group 2 (G2) - 3 individuals receiving Laser application for 8–10 times. It was used Visual Analogue Scale (VAS) to pain’s assessment. T-Test and Pearson Correlation Coefficient was employed.

Results: Both group patients presented relieve of their symptoms after laser therapy. In G1, pain index showed statistical significance (p = 0.0475) with average values of 6.8 (± 1.65) before LLLT and 4.4 (± 1.52) after LLLT. In G2, pain index was 6.85 (± 1.53125) before LLLT and 4.25 (± 1.86025) after LLLT, also revealing statistical significance (p = 0.00022).

Conclusion: We observed that LLLT reduces pain in these kinds of patients using just one application as well as 8–10 sessions, thus it can help several work-related MSDs disorders.
Histological effects of 820 mm laser irradiation on the healthy growth plate of the rat

Cheetham, MJ | Young, SR | Dyson, M


Low level laser therapy (LLLT) has been in clinical use in the United Kingdom for over 15 years. Recently, clinicians have expressed concern that if LLLT is used to treat a lesion adjacent to an active growth plate in a child, they may compromise the normal growth and development of that bone. The aim of this study was to examine the effect of 820 nm wavelength low level laser light on the healthy growth plate of the rat. Twenty-four female Wistar rats (aged 32 to 60 days) were used in the study. One knee joint of each animal in the experimental group was irradiated three times a week at an energy density of 5 J cm super(-2). Animals were examined histologically after six and 12 treatments. The irradiated growth plates were compared histomorphometrically with the untreated contralateral growth plates and also with the sham-irradiated growth plates of control animals. The results show that irradiation with low level laser light of wavelength 820 nm and energy density 5 J cm super(-2) had no significant effect on the healthy growth plates of the rat knee joint.

Descriptors: Article Subject Terms bone | growth plate | histology | laser radiation
Effect of Laser Phototherapy (λ660 nm) on Type I and III Collagen Expression During Wound Healing in Hypothyroid Rats: An Immunohistochemical Study in a Rodent Model

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Abstract

Objective: The aim of this study was to assess, immunohistochemically, the impact of hypothyroidism and the effect of laser phototherapy on the expression of type I and III collagen during wound healing. Background data: Hypothyroidism has been associated with the disruption of the body’s metabolism, including the healing process. Laser phototherapy has been shown to be effective in improving wound healing, but its usefulness on enhancing wound healing under hypothyroid condition remains unknown. Materials and methods: Using general anesthesia, a standard surgical wound (1 cm²) was created on the dorsa of 48 Wistar rats divided into four groups of 12 animals each: control euthyroid (EC), euthyroid plus laser (EL), control hypothyroid (HC), and hypothyroid plus laser (HL). The irradiation with laser GaAlAs (λ660 nm, 40 mW, 1 W/cm², continuous wave [CW], \( \phi = 0.04 \text{ cm}^2 \)) started immediately after surgery and was repeated every other day until end-point of study was reached, and animals were euthanized (i.e., 7 and 14 days). Laser light was applied on four different points (6 J, 150 sec and 150 J/cm² per point). Hypothyroidism was induced in rats with propylthiouracil (0.05 g/100 mL) administered orally for 4 weeks and maintained until the end of the experiment. Immunohistochemistry for collagen I and III was performed with EnVision TM in the specimens removed. Results: Seven days after the surgery EC, EL, and HL groups showed higher immunoexpression of collagen I and lower immunoexpression of collagen III in the newly formed tissue. There was increased immunoexpression of collagen I in EC when compared with HC (\( p = 0.019 \)). The immunoexpression of collagen III was significantly lower in EL than in EC (\( p = 0.047 \)) and HL (\( p = 0.019 \)). No significant difference was found in the experimental period of 14 days among the groups. Conclusions: Laser light therapy performed with the parameters of this investigation increased immunoexpression of collagen type I during tissue repair, and improved the quality of newly formed tissue in the presence of hypothyroidism.

Introduction

Tissue repair is a dynamic and complex process that comprises three continuous and overlapping phases: an inflammatory phase, a proliferative process leading to tissue restoration, and tissue remodeling.1-6 Collagen fiber is the most abundant protein in the human body and is the main component of the extracellular matrix of tissues.7 Its synthesis by fibroblasts is a fast and harmonic process that starts with the interstitial lesion and extends up to the end of the healing phase, when the tissue remodeling occurs,8 which requires interaction among a variety of cells, biochemical mediators, and extracellular matrix molecules.9 Thyroid hormones regulate the body’s metabolism and, among other actions, stimulate cellular differentiation and proliferation.10,11 When these hormones are below normal

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Effect of Low Level Laser Therapy (830 nm) With Different Therapy Regimes on the Process of Tissue Repair in Partial Lesion Calcaneous Tendon

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Background and Objective: Calcaneous tendon is one of the most damaged tendons, and its healing may last from weeks to months to be completed. In the search after speeding tendon repair, low intensity laser therapy has shown favorable effect. To assess the effect of low intensity laser therapy on the process of tissue repair in calcaneous tendon after undergoing a partial lesion.

Study Design/Materials and Methods: Experimentally controlled randomized single blind study. Sixty male rats were used randomly and were assigned to five groups containing 12 animals each one: 42 out of 60 underwent lesion caused by dropping a 156 g weight over their Achilles tendon from a 20 cm height. In Group 1 (standard control), animals did not suffer the lesion nor underwent laser therapy; in Group 2 (control), animals suffered the lesion but did not undergo laser therapy; in Groups 3, 4, and 5, animals suffered lesion and underwent laser therapy for 3, 5, and 7 days, respectively. Animals which suffered lesion were sacrificed on the 8th day after the lesion and assessed by polarization microscopy to analyze the degree of collagen fibers organization.

Results: Both experimental and standard control Groups presented significant values when compared with the control Groups, and there was no significant difference when Groups 1 and 4 were compared; the same occurred between Groups 3 and 5.


Key words: calcaneous tendon; diode laser; lesion tendon; low level laser therapy; physical therapy; repair tissue

INTRODUCTION

The calcaneous tendon is one of the most frequently injured tendons in human beings, followed by digital flexors, due to overuse, trauma caused by firearm wounds, and sharp objects [1].

Owing to the slow pace of healing, the rupture of the calcaneous tendon is considered a serious injury, and it has drawn the attention of several researchers [2].

Spontaneous rupture of the calcaneous tendon occurs between 2 and 8 cm of its insertion into the calcaneous bone. Histological examination has suggested that such tendons had already undergone primary degeneration [3] and showed important alterations in the type of collagen fibers [4].

In order to observe blood supply to the calcaneous tendon, CARR & NORRIS (1989) [5] verified that the number of blood vessels varies along the length of the tendon and their highest concentration occurs in the calcaneous insertion and up to 4 cm above it, considering that neangiogenesis is a vital part of the healing process, as it restores normal circulation and carries more cells and nutrients to the injured location, thus limiting ischemic necrosis and allowing tissue repair [6].

Due to its low blood supply, the calcaneous tendon is a structure that can take weeks or even months to heal completely [2,7].

During the period of the lesion, it is customary for the patient to remain immobilized in order to prevent a new rupture, which could generate countless functional complications, including ultra-structural and biomechanical alterations in the tendon [8,9].

Such complications, caused by prolonged immobilization, can be minimized by shortening the duration of the tendon repair [3].

Trying to accelerate tenon repair, several physical agents such as ultrasound [10], electrical stimulation [11], and low level laser therapy [12] have shown beneficial effects.

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EFFECTIVENESS OF CLASS 4 LASERS FOR ACHILLES TENDINOPATHY

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Background: The combination of eccentric exercise and low-level laser therapy (LLLT) may be beneficial in treating Achilles tendinopathy. Controversy exists over LLLT parameters and dose, especially irradiance and the 100 mW/cm² limit set for Achilles tendons. The optimum dose has yet to be defined. The aim of this work was to assess the effectiveness of a class 4 laser device delivering an irradiance above 100 mW/cm² as an adjunct to an eccentric exercise regime for Achilles tendinopathy.

Study: A double blind randomized controlled trial utilizing 2 groups; 1 (Exercise + placebo LLLT), 2 (Exercise + active LLLT). The primary end-point was at 12 weeks, and the main outcome measure was the Victorian Institute of Sports Assessment-Achilles Questionnaire (VISA-A). Forty participants 18–65 years of age with a diagnosis of Achilles tendinopathy and who had not had treatment for the condition within the last 3 months, were randomized into the two groups. LLLT or placebo was administered twice per week for the first 4 weeks prior to a supervised exercise session with a physiotherapist. The laser parameters used for this application were; power output 10 W; pulsed 100 Hz; time 30s; energy 150J, for a total time of 1:30 min and total energy delivered of 450J. The exercise regime was continued unsupervised for a further 8 weeks. Data was analysed using ANCOVA with baseline scores as the covariate on an intention to treat basis. Missing data was replaced using the multiple imputation method.

Results: There was no difference between groups at baseline, and both groups significantly improved from baseline to 12 weeks. The between group difference on VISA-A at 12 weeks was statistically significant in favor of the LLLT group; (11.34; 95%CI, 3.03–19.64; p = 0.002).

Conclusion: Four weeks (8 treatments) of LLLT as an adjunct to an eccentric exercise regime of two sessions per week provide superior results compared to exercise alone.
Anti-Inflammatory Effects of Low-Level Light Emitting Diode Therapy on Achilles Tendinitis in Rats

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Background and Objectives: The present study investigated the effects of low-level light emitting diode (LED) therapy (880 ± 10 nm) on inflammatory process in a model of Achilles tendinitis induced by collagenase.

Study Design/Materials and Methods: Fifty-six male Wistar were separated into seven groups (n = 8), three groups in the experimental period of 7 days and four groups in the experimental period of 14 days, the control group (CONT), tendinitis group (TEND), LED therapy group (LEDT) for both experimental periods, and LED therapy group 7th to 14th day (LEDT delay) for 14 days experimental period. The LED parameters was 22 mW CW of optical output power, distributed in an irradiation area of 0.5 cm², with an irradiation time of 170 seconds, the applied energy density was 7.5 J/cm² in contact. The therapy was initiated 12 hours after the tendinitis induction, with a 48-hour interval between the irradiations. The histological analysis and inflammatory mediators were quantified.

Results: Our results showed that LED decreases the inflammatory cells infux and mRNA expression to IL-1β, IL-6, tumor necrosis factor-α (TNF-α) in both phases, and cyclooxygenase-2 (COX-2) just in initial phase (P < 0.05).

Conclusion: Our results suggest that the anti-inflammatory therapy with low-power LED (880 nm) enhanced the tissue response in all groups. We can conclude that the LED was able to reduce signs of inflammation in collagenase-induced tendinitis in rats by reducing the number of inflammatory cells and decrease mRNA expression of cytokines. Lasers Surg. Med. 42:553–558, 2010. © 2010 Wiley-Liss, Inc.

Key words: calcaneal tendon; histological analysis; mRNA; RT-PCR; inflammatory mediators

INTRODUCTION

Calcaneal tendinitis (CT) is a common cause of disability and is clinically characterized by pain and swelling in and around the tendon, mainly arising from overuse and repetitive stretching triggered the release of pro-inflammatory mediators [1]. Over the past few years, various new therapeutic options have been proposed for the management of CT but the prescription of anti-inflammatory drugs (e.g., non-steroidal anti-inflammatory and corticosteroids) remains the therapy of choice. However, since pro-inflammatory mediators affect various cellular activities related to tendon healing, it is possible that anti-inflammatory agents might negatively affect tendon healing [2]. In addition, the CT is associated with disruption of collagen fibers, increase in non-collagenous matrix, haphazard proliferation of tenocytes, and subsequent decrease in biomechanical properties of tendon [3].

Thus, it is then reasonable that any modality that can enhance cell proliferation and not adversely affect inflammatory response may aid in the repair or recovery of the tendon. Most studies suggest that coherent light

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Muscular Trauma Treated with a Ga-Al-As Diode Laser: In Vivo Experimental Study

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Abstract. The aim of the study was to verify in an experimental model the effects of laser therapy performed with Ga-Al-As diode lasers (780 nm, 2500 mW) on traumatised muscles. Forty adult New Zealand male rabbits were divided into four groups (A, B, C and D) of ten animals each. Each group of animals was further divided into two subgroups of five animals each. The animals were submitted to muscular trauma for 7 min by clamping the posterior muscles of the left thigh under general anaesthesia. Four days later, the rabbits in the B1, B2, C1, C2, D1 and D2 subgroups started daily laser therapy. The parameters utilised were: 150 J/cm² energy density, 3 W, 50 Hz in group B; 250 J/cm², 3 W, 100 Hz in group C; and 800 J/cm², 3 W, 0 Hz (continuous output) in group D. The animals in subgroups A1 and A2 were used as untreated controls and allowed to heal spontaneously. In order to prepare samples for histological, histochemical and histomorphometrical studies, dissection of the posterior muscle of the thigh was performed under general anaesthesia and before sacrifice, after five days of laser therapy in the subgroups B1, C1 and D1 and after ten days of laser therapy in subgroups B2, C2 and D2. The samples of untreated subgroups A1 and A2 were subjected to the same procedure and at the same times as the corresponding laser-treated groups. The following parameters were analysed on muscular samples: qualitative histological aspect (lactate dehydrogenase (LDH), cytochrome oxidase, acid phosphatase and alkaline phosphatase concentration with histoenzymatic methods) and quantitative histomorphometrical evaluation of muscular damage and tissue repair. Blood samples were drawn from each subgroup before the trauma and again before sacrifice to measure the creatine phosphokinase (CK) and LDH levels. The results obtained in the tables are shown. Analysis of the results showed a better qualitative and quantitative healing process in traumatised muscles treated with Ga-Al-As diode laser therapy than in spontaneously healed ones. The results obtained with laser therapy were confirmed as haematic, histoenzymatic and histomorphometric values. According to these results, there is a positive relationship between the biostimulation properties of the laser and the healing of traumatised muscular tissue.

Keywords: Diode laser; Experimental model; Laser biostimulation; Low energy laser therapy; Muscle trauma
Low-Level Laser Therapy for Protection Against Skeletal Muscle Damage After Ischemia–Reperfusion Injury in Rat Hindlimbs

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INTRODUCTION

Prolonged ischemia followed by reperfusion (I/R) of muscle causes significant tissue injury that may have local as well as systemic consequences, including loss of function and viability of the perfused organ [1]. This damage can occur during clinical operations, especially cardiovascular and plastic and reconstructive surgeries.

Skeletal muscle is relatively resistant to ischemia/reperfusion injury [2], but 95% of the metabolic activity in the lower extremities occurs in this muscle, and its response to ischemia over a period determines the fate of the lower extremity [3]. Ultrastructural damage to the skeletal muscle is normally observed after 2 hours of ischemia and 1 hour of reperfusion, which is similar to the conditions during the process of membrane lipid peroxidation by excessive formation of oxygen-free radicals [4]. Reperfusion in ischemic tissue induces an acute inflammatory response that can result in necrosis and irreversible cell injury to both the local vascular endothelium and tissue, characterized by vascular leakage and permeability edema [5]. It is a known fact that the postreperfusion edema that occurs as a result of late revascularization may cause compartment syndrome, a reperfusion trauma, which results in loss of the extremity (52%) or death (48%) [6].

Many experimental techniques are not clinically applicable in surgery for protecting patients from I/R injury, and researchers are not yet able to utilize these methods in clinical practice [7]. Therefore, it is necessary to identify other methods to protect tissue against the catastrophic consequences of muscle damage. The effect of low-level laser therapy...
Use of Low-Level Laser Therapy (808 nm) to Muscle Fatigue Resistance: A Randomized Double-Blind Crossover Trial

Wouber Hérickson de Brito Vieira, PhD; Raphael Machado Bezerra; Renata Alencar Saldanha Queiroz; Nicia Farias Braga Maciel; Nivaldo Antonio Parizotto, PhD; and Cleber Ferraresi, PhD

Abstract

Objective: The purpose of this study was to investigate whether low-level laser (light) therapy (LLLT) can provide fatigue resistance via maximum repetitions (RM) with an isokinetic dynamometer, and decrease electromyography fatigue index (EFI). Background data: LLLT has been used to increase muscle performance when applied before or after intense exercises. Materials and methods: This study was a randomized, double-blind, crossover trial with placebo. Seven young men (21–3 years of age) who were clinically healthy, were allocated into two groups: active laser (LLLT) and placebo laser (Placebo). Both groups were assessed at baseline, at one training session, and at the end of this study. Baseline and final assessments recorded the number of RM of knee flexion-extensions using an isokinetic dynamometer at 60 degrees/sec in conjunction with EFI recorded by median frequency. The training sessions consisted of three sets of 20 RM of knee flexion-extensions using an isokinetic dynamometer at 60 degrees/sec plus LLLT (808 nm, 100 mW, 4 J), or placebo, applied to quadriceps femoris muscles between sets, and after the last series of this exercise. After 1 week (washout period), all volunteers were exchanged among groups and then all assessments were repeated. Results: LLLT group increased RM (52%; \( p = 0.002 \)) with a small EFI for the vastus medialis (\( p = 0.004 \)) and rectus femoris (\( p = 0.004 \)). Conclusions: These results suggest an increased muscle fatigue resistance when LLLT is applied during rest intervals, and after the last series of intense exercises.

Introduction

Muscle fatigue can be defined as the inability of skeletal muscles to maintain contraction at the same level over time, becoming a dangerous phenomenon that can predispose athletes to injuries. This phenomenon is a very common experience especially in athletes, but its mechanisms of action are not fully understood.\(^1,2\)

Muscle fatigue, or decline in performance, will be dependent upon several factors, including type of muscle fibers recruited during exercise, and intensity and duration of the activity.\(^3\) The most common explanation for muscle fatigue is an accumulation of hydrogen ions that impairs contractile function. Alternative explanations have considered effects of ionic modifications on muscle action potential, such as the presence of inorganic phosphate (Pi) and magnesium ion, lack of Ca\(^{2+}\) or its releasing from different mechanisms, and effects of reactive oxygen species (ROS).\(^1\) Furthermore, some strategies have identified the intensity of the muscle fatigue process through lactate levels in blood,\(^2\) functional performance, and electromyography (EMG) activity.\(^4,5\) These assessments can provide overall responses about muscle recruitment and contraction. In this context, the root mean square (RMS) and median frequency (MF) are two EMG parameters commonly used to infer muscle fatigue.\(^4,5\)

Low-level laser (light) therapy (LLLT) interacts with biological tissues\(^6\) and has been used to treat pain and promote tissue healing.\(^7,8\) Moreover, recently, LLLT has been used successfully to decrease muscle fatigue in experimental models and clinical trials.\(^9,10\) Muscle fatigue resistance can be increased with LLLT if applied before (muscular preconditioning) or after intense exercises (muscle recovery), increasing tetanic contractions and maximum repetitions.

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Pre-conditioning with low-level laser (light) therapy: light before the storm.
Agrawal T1, Gupta GK2, Rai V3, Carroll JD4, Hamblin MR5.

Abstract

Pre-conditioning by ischemia, hyperthermia, hypothermia, hyperbaric oxygen (and numerous other modalities) is a rapidly growing area of investigation that is used in pathological conditions where tissue damage may be expected. The damage caused by surgery, heart attack, or stroke can be mitigated by pre-treating the local or distant tissue with low levels of a stress-inducing stimulus, that can induce a protective response against subsequent major damage. Low-level laser (light) therapy (LLLT) has been used for nearly 50 years to enhance tissue healing and to relieve pain, inflammation and swelling. The photons are absorbed in cytochrome(c) oxidase (unit four in the mitochondrial respiratory chain), and this enzyme activation increases electron transport, respiration, oxygen consumption and ATP production. A complex signaling cascade is initiated leading to activation of transcription factors and up- and down-regulation of numerous genes. Recently it has become apparent that LLLT can also be effective if delivered to normal cells or tissue before the actual insult or trauma, in a pre-conditioning mode. Muscles are protected, nerves feel less pain, and LLLT can protect against a subsequent heart attack. These examples point the way to wider use of LLLT as a pre-conditioning modality to prevent pain and increase healing after surgical/medical procedures and possibly to increase athletic performance.
Effectiveness and Acceleration of Bone Repair in Critical-Sized Rat Calvarial Defects Using Low-Level Laser Therapy

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Background and Objective: Tissue regeneration remains a challenge for orthopedic and craniomaxillofacial surgery to treat bone loss. The use of low-level laser therapy suggests a promise on this road with positive results for narrow defects. However, temporal and quantitative evaluations are required to understand the healing process of large injuries. The aim of this study was to investigate the repair of critical-size bone defects in rat calvaria using a GaAlAs laser.

Study Design/Materials and Methods: Bone defects (9 mm in diameter) were created on the skull of 30 Wistar rats separated in control or irradiated group. GaAlAs laser (λ = 830 nm, energy density = 2.5 J/cm² and output power = 50 mW) was applied after surgery and six times more at 48 hours intervals. The animals were euthanized after 2, 4, and 8 weeks. Digital radiographs, descriptive histological and histomorphometric analyses were carried out.

Results: Radiographic analysis showed greater bone formation in the irradiated group than control at 8 weeks, covering 45% and 28% of the defect, respectively (P < 0.05). Histological analysis showed in the irradiated groups a higher amount of bone neoformation and greater maturity at 4 and 8 weeks. Histomorphometric analysis showed that the volume density of bone tissue at 4 weeks in the irradiated group was two times higher than the control (P < 0.01).

Conclusion: The biomodulation of low-level laser therapy using 830 nm wavelength light was effective in promoting bone healing in critical defects despite the unfavorable prognosis as well as it accelerated the maturation of bone tissue. Lasers Surg. Med. 46:61–67, 2014. © 2013 Wiley Periodicals, Inc.

Key words: low-level laser therapy; bone repair; critical-size defects

INTRODUCTION

According to the World Health Organization there are more of 150 diseases and syndromes related to skeletal and joint problems. Despite the great progress in development of surgical approaches and bone substitutes with their osteopromotive, osteoconductive or osteoinductive properties, there still is not established “gold standard” clinical technique to mimic the performance of autogenous cells. Tissue regeneration remains a challenge for orthopedic and craniomaxillofacial surgery to treat severe bone loss and to improve the quality of life for these patients [1].

In response to this demand, photobiomodulation or low-level laser therapy (LLLT) needs to be considered for promoting the bone repair process without thermal damage or tissue injury [2–6]. It is believed that the main structures responsible for absorption of light are proteins and their photobiomodulation involve reactions in cell membrane (by regulation of signal-transduction pathways) and organelles (e.g., mitochondria), which would generate acceleration of cellular metabolism. A short-term evaluation...
Infrared Laser Light Further Improves Bone Healing When Associated with Bone Morphogenic Proteins: An in Vivo Study in a Rodent Model


ABSTRACT

Objective: This study assessed histologically the effect of laser photobiomodulation (LPBM) on the repair of surgical defects created in the femurs of Wistar rats treated or not treated with bone morphogenic proteins (BMPs) and organic bovine bone graft. Background Data: This paper is part of an ongoing series of works in which biomaterials are used in association with LPBM. Several previous reports by our group have shown that the use of laser photobiomodulation improves the treatment of bone defects. Materials and Methods: Forty-eight adult male Wistar rats were divided into four randomized groups: group I (control, n = 12); group II (LPBM, n = 12); group III (BMPs + organic bovine bone graft, n = 12); and group IV (BMPs + organic bovine bone graft + LPBM, n = 12). The irradiated groups received seven irradiations every 48 h, beginning immediately after the surgical procedure. The laser therapy (λ = 830 nm, 40 mW CW, φ = ~0.6 mm) consisted of 16 J/cm² per session divided equally over four points (4 J/cm² each) around the defect. The subjects were sacrificed after 15, 21, and 30 d, and the specimens were routinely embedded in wax, stained with hematoxylin and cosin and sirius red, and analyzed under light microscopy. Results: The results showed histological evidence of increased deposition of collagen fibers (at 15 and 21 d), as well as an increased amount of well-organized bone trabeculae at the end of the experimental period (30 d) in the irradiated animals versus the non-irradiated controls. Conclusion: The use of LPBM with BMPs and organic bovine bone grafts increases the positive biomodulating effects of laser light.

INTRODUCTION

MODERN DENTISTRY is challenged daily by the need to recover bone loss due to several etiologic factors. Several autologous and xenographs have been used to provide a framework or stimulate new bone formation, and many times these grafts respond positively to the use of certain wavelengths of laser energy.1 The use of bone morphogenic proteins (BMPs) is not new,2-4 and they have been widely used in the reconstruction of the alveolar ridge,3 for the recovery of bone loss, and on several types of bone defects.6-13 Despite the growing successful application of laser photobiomodulation (LPBM) in bone repair, there are few studies assessing the association of laser light with biomaterials.1,8-10,12 Thus there is a need for further studies to determine the most effective ways to apply LPBM combined with different biomaterials for this new type of treatment.

MATERIALS AND METHODS

This study was approved by the Animal Ethics Committee of the School of Dentistry of the Federal University of Bahia.

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Effect of 830-nm laser light on the repair of bone defects grafted with inorganic bovine bone and decalcified cortical osseous membrane.


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Abstract

OBJECTIVE:

The aim of this study was to assess histologically the effect of LLLT (lambda830 nm) on the repair of standardized bone defects on the femur of Wistar albinus rats grafted with inorganic bovine bone and associated or not to decalcified bovine cortical bone membrane.

BACKGROUND DATA:

Bone loss may be a result of several pathologies, trauma or a consequence of surgical procedures. This led to extensive studies on the process of bone repair and development of techniques for the correction of bone defects, including the use of several types of grafts, membranes and the association of both techniques. There is evidence in the literature of the positive effect of LLLT on the healing of soft tissue wounds. However, its effect on bone is not completely understood.

MATERIALS AND METHODS:

Five randomized groups were studied: Group I (Control); Group IIA (Gen-ox); Group IIB (Gen-ox + LLLT); Group IIIA (Gen-ox + Gen-derm) and Group IIIB (Gen-ox + Gen-derm + LLLT). Bone defects were created at the femur of the animals and were treated according to the group. The animals of the irradiated groups were irradiated every 48 h during 15 days; the first irradiation was performed immediately after the surgical procedure. The animals were irradiated transcutaneously in four points around the defect. At each point a dose of 4 J/cm² was given (phi approximately 0.6 mm, 40 mW) and the total dose per session was 16 J/cm². The animals were humanely killed 15, 21, and 30 days after surgery. The specimens were routinely processed to wax, serially cut, and stained with H&E and Picrosirius stains and analyzed under light microscopy.
Photomedicine and Laser Surgery

Infrared Laser Photobiomodulation (λ 830 nm) on Bone Tissue Around Dental Implants: A Raman Spectroscopy and Scanning Electronic Microscopy Study in Rabbits

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Objective: The aim of this study was to assess, through Raman spectroscopy, the incorporation of calcium hydroxyapatite (CHA; ~960 cm⁻¹), and scanning electron microscopy (SEM), the bone quality on the healing bone around dental implants after laser photobiomodulation (λ830 nm).

Background Data: Laser photobiomodulation has been successfully used to improve bone quality around dental implants, allowing early wearing of prostheses. Methods: Fourteen rabbits received a titanium implant on the tibia; eight of them were irradiated with λ830 nm laser (seven sessions at 48-h intervals, 21.5 J/cm² per point, 10 mW, φ ~0.0028 cm², 86 J per session), and six acted as control. The animals were sacrificed 15, 30, and 45 days after surgery. Specimens were routinely prepared for Raman spectroscopy and SEM. Eight readings were taken on the bone around the implant. Results: The results showed significant differences on the concentration of CHA on irradiated and control specimens at both 30 and 45 days after surgery (p < 0.001). Conclusion: It is concluded that infrared laser photobiomodulation does improve bone healing, and this may be safely assessed by Raman spectroscopy or SEM.
NEUROLOGIC STUDIES
Synopsis of Articles on Neurologic

1-Near infrared Transcranial Laser Therapy applied at Various Modes to Mice Following Traumatic Brain Injury Significantly Reduces Long-Term Neurological Deficits
The percentage of surviving mice that demonstrated full recovery was the highest in the group that had received TLT in the PW mode at 100 Hz. In addition, MRI analysis demonstrated significantly smaller infarct lesion volumes in laser treated mice as compared to control. The data suggests that non-invasive TLT of mice post-TBI provides a significant long-term functional neurological benefit, and that the pulsed laser mode at 100 Hz is the preferred mode for such treatment.

2-Transcranial Application of Low-Energy Laser Irradiation Improves Neurological Deficits in Rats Following Acute Stroke
These observations suggest that LLLT applied at different locations in the skull post stroke effectively improves neurological function after acute stroke in rats.

3-Low-Level Laser Therapy for Closed-Head Traumatic Brain Injury in Mice: Effect of Different Wavelengths
This study not only demonstrated effectiveness but also highlights the importance of proper Laser parameters to improve results. The effectiveness of 810 nm agrees with previous publications, and together with the effectiveness of 660 nm and non-effectiveness of 730 and 980 nm can be explained by the absorption spectrum of cytochrome oxidase, the candidate mitochondrial chromophore in transcranial LLLT.

4-Non-Surgical Laser Therapy of Brain and Spinal Cord Injury
Showed that Laser therapy was very useful with spinal cord and brain injuries and has the potential to help patients untreatable with other therapies.

5-Increase of Neuronal Sprouting and Migration using 780nm Laser Phototherapy as Procedure for Cell Therapy
This study demonstrated migration and fiber sprouting of neuronal cells aggregates and therefore can be considered as a potential procedure for cell therapy of neuronal injury and disease.

6-Light Therapy Improves Functional Outcome in an Autograph Repaired Peripheral Nerve
They concluded that this Laser based non-invasive treatment has the potential to revolutionize post-operative repair of severe peripheral nerve injury.

7-Low-Level Laser therapy reduces time to ambulation in dogs after hemilaminectomy
Showed that the time to achieve a modified Frankel score of 4 was significantly lower in the Laser group (median 3.5 days) vs the control group (median 14 days).

8-Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials
Interpretation: We show that LLLT reduces pain immediately after treatment in acute neck pain and up to 22 weeks after completion of treatment in patients with chronic neck pain.
9-Effect of Diode Laser in the Treatment of Patients with Nonspecific Chronic Low Back Pain: A Randomized Controlled Trial
This study demonstrated that the use of diode laser (980 nm) with large diameter spot size, in association with exercise therapy, appears to be effective. Such treatment might be considered a valid therapeutic option within rehabilitation programs for nonspecific CLBP.

10-Post-Traumatic Neuropathic Pain Non-Responsive to Conventional Treatment:
Showed that Laser therapy relieved all neuropathic symptoms with no side effects. It is proving to be a helpful tool in pain armamentarium, especially when all other measures have failed.

11-The Brain-Derived Neurotrophic Factor, Nerve Growth Factor, Neurotrophin-3, and Induced Nitric Oxide Synthase Expressions After Low-Level Laser Therapy in an Axonotmesis Experimental Model
The reported data could have a relevant practical value because LLLT is a noninvasive procedure, and have revealed significant increase in neurotrophic factor expressions and inflammatory process reduction, opening the possibility of using LLLT as an important aid to nerve regeneration process.

12-TRANSCRANIAL LOW-LEVEL LASER (LIGHT) THERAPY IN MICE: TRAUMATIC BRAIN INJURY AND BEYOND
The beneficial effects in stimulating neurogenesis, synaptogenesis and BDNF after transcranial LLLT suggest it may have wider applications beyond TBI to neurodegenerative diseases such as Alzheimer’s and psychiatric diseases such as major depression.
Near infrared Transcranial Laser Therapy applied at Various Modes to Mice Following Traumatic Brain Injury Significantly Reduces Long-Term Neurological Deficits

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Near-infrared transcranial laser therapy (TLT) has been found to modulate various biological processes including traumatic brain injury (TBI). Following TBI in mice, in this study we assessed the possibility of various near-infrared TLT modes (pulsed vs. continuous) producing a beneficial effect on the long-term neurobehavioral outcome and brain lesions of these mice. TBI was induced by a weight-drop device, and neurobehavioral function was assessed from one hour and up to 56 days post-trauma using a neurological severity score (NSS). The extent of recovery is expressed as dNSS, the difference between the initial score, and that at any other, later, time point. An 808nm Ga-Al-As diode laser was employed transcranially 4, 6 or 8 hrs post-trauma to illuminate the entire cortex of the brain. Mice were divided into several groups of 6-8 mice: one control group that received a sham treatment and experimental groups that received either TLT continuous wave (CW) or pulsed wave (PW) mode transcranially. MRI was taken prior to sacrifice 56 days post-CHI. From 5 to 28 days post-TBI, the NSS of the laser-treated mice were significantly lower (p<0.05) than the non-laser-treated, control mice. The percentage of surviving mice that demonstrated full recovery 56 days post-CHI, namely NSS=0 (as in intact mice) was the highest (63%) in the group that had received TLT in the PW mode at 100 Hz. In addition, MRI analysis demonstrated significantly smaller infarct lesion volumes in laser treated mice as compared to control. Our data suggest that non-invasive TLT of mice post-TBI provides a significant long-term functional neurological benefit, and that the pulsed laser mode at 100 Hz is the preferred mode for such treatment.

Key words: low-level laser therapy; mice; traumatic brain injury; pulsed laser; motor function, MRI.
Background and Objectives: Low-level laser therapy (LLLT) has been shown to have beneficial effects on ischemic skeletal and heart muscles tissues. The aim of the present study was to approve the effectiveness of LLLT treatment at different locations on the brain in acute stroked rats.

Study Design/Materials and Methods: Stroke was induced in 169 rats that were divided into four groups: control non-laser and three laser-treated groups where laser was employed ipsilateral, contralateral, and both to the side of the induced stroke. Rats were tested for neurological function.

Results: In all three laser-treated groups, a marked and significant improvement in neurological deficits was evident at 14, 21, and 28 days post stroke relative to the non-treated group.


Key words: biostimulation; stroke; rats; acute; laser

INTRODUCTION

Treatment of active stroke in humans is still a major medical problem despite the extensive research studies [1]. The only therapy used currently in clinic is the thrombolytic one up to 3 hours post stroke. However, only about 5% of stroked patients in the US get this therapy due to side effects and late arrival to the hospital [1]. Several attempts to attenuate the ischemic process and improve functional outcome have been made recently in experimental animals. Erythropoietin (EPO) has been shown to increase angiogenesis, neurogenesis, and functional recovery in stroked rats [2]. Injection of bone marrow cells (MSCs) has also been shown to contribute to better functional outcome and to attenuate the response of reactive astrocyte to ischemia [3]. EPO has been shown to increase angiogenesis, neurogenesis, and functional recovery in stroked rats [2]. Injection of bone marrow cells (MSCs) has also been shown to contribute to better functional outcome and to attenuate the response of reactive astrocyte to ischemia [3].

Low-level laser therapy (LLLT) has been found to modulate various biological processes [4,5]. In an experimental model of the infarcted heart it was previously demonstrated that LLLT had a profound cardioprotective effect, resulting in a 50–70% reduction in infarct size 4–6 weeks post left descending coronary artery chronic occlusion. This phenomenon was partially attributed to a significant increase in the number of undamaged mitochondria and ATP content, as well as inducible heat shock proteins and catalase (in the serum) in infarcted laser-irradiated rats and dogs as compared to non-irradiated ones [5–9]. LLLT has also been shown to biomodulate processes in the nervous system. Anders et al. [9] recently reviewed the beneficial effects of LLLT on functional recovery of injured peripheral nerves. The effect of LLLT on stroke has been investigated to a limited extent. Leung et al. [10] have shown that LLLT causes suppression of nitric oxide synthase activity and upregulation of TGF-β1, in stroked rats. It was also demonstrated that transcranial infrared laser therapy applied 6 hours post embolic stroke improved clinical rating scores in rabbits [11].

In the present study we explored the effect of LLLT applied to various regions of the brain on the neurological outcome post acute stroke in rats.

MATERIALS AND METHODS

Experimental Procedure

We used 169 mature Sprague–Dawley rats (295–315 g body weight) supplied by Zivic Laboratories (Zelienople, PA) that passed the screening phase (see below). All rats underwent induction (under halothane inhalation) of acute stroke using a filament that was introduced into the middle cerebral artery to create a permanent occlusion (MCAO). The experimental protocol was approved by the animal care committee of Zivic laboratories. Rats were examined at 24 hours (screening phase) for their minimal neurological deficit according to the modified neurological score (MCAO). The experimental protocol was approved by the animal care committee of Zivic laboratories. Rats were examined at 24 hours (screening phase) for their minimal neurological deficit according to the modified neurological score (MNS) modified from Chen et al. [12]. Rats that were scored above five on the MNS (comprised to marked neurological deficit) were included in the study and divided into four groups...
Low-Level Laser Therapy for Closed-Head Traumatic Brain Injury in Mice: Effect of Different Wavelengths

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Background and Objectives: Traumatic brain injury (TBI) affects millions worldwide and is without effective treatment. One area that is attracting growing interest is the use of transcranial low-level laser therapy (LLLT) to treat TBI. The fact that near-infrared light can penetrate into the brain would allow non-invasive treatment to be carried out with a low likelihood of treatment-related adverse events. LLLT may treat TBI by increasing respiration in the mitochondria, causing activation of transcription factors, reducing inflammatory mediators and oxidative stress, and inhibiting apoptosis.

Study Design/Materials and Methods: We tested LLLT in a mouse model of closed-head TBI produced by a controlled weight drop onto the skull. Mice received a single treatment with continuous-wave 665, 730, 810, or 980 nm lasers (36 J/cm² delivered at 150 mW/cm²) 4-hour post-TBI and were followed up by neurological performance testing for 4 weeks.

Results: Mice with moderate-to-severe TBI treated with 665 and 810 nm laser (but not with 730 or 980 nm) had a significant improvement in Neurological Severity Score that increased over the course of the follow-up compared to sham-treated controls. Morphometry of brain sections showed a reduction in small deficits in 665 and 810 nm laser treated mouse brains at 28 days.

Conclusions: The effectiveness of 810 nm agrees with previous publications, and together with the effectiveness of 660 nm and non-effectiveness of 730 and 980 nm can be explained by the absorption spectrum of cytochrome oxidase, the candidate mitochondrial chromophore in transcranial LLLT.

Key words: photobiomodulation; low-level laser therapy; traumatic brain injury; mouse model; Neurological Severity Score

INTRODUCTION

Head injury in humans is the most common neurological disorder under the age of 50. Victims of traumatic brain injury (TBI) suffer short- and long-term physical, cognitive, behavioral, and emotional impairments that depend on the severity of the injury [1,2]. Moderate and severe TBI, accidental or inflicted, is a major health and socio-economic problem throughout the world. In the United States alone, approximately 2 million injuries occur each year resulting in 56,000 deaths and 18,000 survivors suffering from permanent neurological impairment [3–5]. The consequent direct and indirect annual costs in the United States are estimated at $56 billion [6].
NON SURGICAL LASER THERAPY OF BRAIN AND SPINAL CORD INJURIES

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Background: Some years ago the effects of non surgical laser and light on nerve cells in culture have been demonstrated. Aim of our study is to verify the antinflammatory and regenerative effects of non surgical laser therapy on patients with spinal cord injuries, and to verify if same effects are obtainable on brain injuries.

Study: We enrolled patients both sex, age middle-old, with traumatic spinal cord and traumatic brain injuries occurred since one year minimum before laser treatment. Standardization of these patients is difficult because each patient is totally different in term of lesion, loss of sensibility, degree of inflammation/ degeneration. Since the trauma all patients had total-subtotal paralysis sensitive and motorial under lesion level. EEG, NMR, neurophysiologic tests and electromyograms documented spinal cord and brain lesions. We used a diode laser 808 nm, with fluence 12 Joule/cm² in average, with a first cycle of 15 sessions, three for day. We repeated in average half cycle for month, until today. We don’t know how many cycles do these patients need and what is the end point of their improvement. In addition, we associated olistic massage with laser treatment. At first drug therapy prescribed before laser treatment was confirmed, then gradually stopped following the results obtained.

Results: After first cycle all the patients had different degree of improvement sensorial, motorial and voluntary command and important improvement of the EEG, RMN and electromyogram features. Further improvement added after each cycle.

Conclusion: Non surgical laser therapy was very useful in these first patients with spinal cord and brain injuries. Number of sessions and time of improvement are variable for each patient and years of treatment could be required. If this result will confirmed on a larger number of spinal cord injuries, we could help patients untreatable with other therapies.
Increase of Neuronal Sprouting and Migration Using 780 nm Laser Phototherapy as Procedure for Cell Therapy

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Background and Objectives: The present study focuses on the effect of 780 nm laser irradiation on the growth of embryonic rat brain cultures embedded in NVR-Gel (cross-linked hyaluronic acid with adhesive molecules laminin and several growth factors). Dissociated neuronal cells were first grown in suspension attached to cylindrical microcarriers (MCs). The formed floating cell-MCs aggregates were subsequently transferred into stationary cultures in gel and then laser treated. The response of neuronal growth following laser irradiation was investigated.

Materials and Methods: Whole brains were dissected from 16 day Sprague–Dawley rat embryos. Cells were mechanically dissociated, using narrow pipettes, and seeded on positively charged cylindrical MCs. After 4–14 days in suspension, the formed floating cell-MCs aggregates were seeded as stationary cultures in NVR-Gel. Single cell-MCs aggregates were either irradiated with near-infrared 780 nm laser beam for 1, 4, or 7 minutes, or cultured without irradiation. Laser powers were 10, 30, 50, 110, 160, 200, and 250 mW.

Results: 780 nm laser irradiation accelerated fibresprouting and neuronal cell migration from the aggregates. Furthermore, unlike control cultures, the irradiated cultures (mainly after 1 minute irradiation of 30 mW) were already established after a short time of cultivation. They contained a much higher number of large size neurons (P<0.01), which formed dense branched interconnected networks of thick neuronal fibers.


Key words: axonal sprouting; cross-linked hyaluronic acid with laminin gel; embryonic nerve cells; low power laser irradiation; microcarriers

INTRODUCTION

The therapeutic effect of low power laser irradiation (LPLI) was detected on peripheral and central nervous systems [1–6]. Previous studies, which evaluated the effects of LPLI on crushed injured peripheral nerves of rats, discovered protective immediate effects which increase the functional activity of the injured peripheral nerve [7]; maintenance of functional activity of the injured nerve over time [8]; decrease or prevention of scar tissue formation at the injured site [9]; prevention or decreased degeneration in corresponding motor neurons of the spinal cord [10]; increase in the rate of axon growth and myelination, thus accelerating and improving the regeneration of the injured nerve. LPLI was found to increase migration and neurite sprouting of cultured embryonic nerve cells [11], as well as cultured adult brain microexplants [12], and to alter gene expression of olfactory ensheathing cells [13]. Our previous studies found that LPLI accelerated axonal growth into injured rat’s spinal cord after an implantation of a composite implant, which was based on embryonic spinal cord nerve cells and cultured on biodegradable microcarriers (MCs) that were embedded in hyaluronic acid [8].

In this in vitro study we investigated the effect of 780 nm laser phototherapy on growth and development of embryonic brain neurons and their fibers in culture.

MATERIALS AND METHODS

Cell Culture

Sixteen-day-old rat embryos (Sprague–Dawley) whole brains were dissected. After mechanical dissociation with narrow pipettes, 5×10⁶ cells were suspended in medium attached to DE-53 positively charged cylindrical MCs in 60 mm bacteriological plastic dishes as previously described [14]. After 4–14 days in suspension, the formed floating cell-MCs aggregates were collected and seeded in NVR-Gel (in 12 wells or 35 mm plastic dishes) as stationary cultures. Each single cell-MCs aggregate was either treated with LPLI within 1 hour after seeding, or cultured without irradiation.
LIGHT THERAPY IMPROVES FUNCTIONAL OUTCOME IN AN AUTOGRAFT REPAIRED PERIPHERAL NERVE

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Background: Loss of large segments of peripheral nerves results in chronic loss of sensation and paralysis. For this type of severe injury, the defect can be bridged by nerve grafts, but even with state-of-the-art microsurgical techniques, there is minimal recovery of sensation and motor function. Light therapy (LT) has been shown to improve functional outcome after surgical intervention to repair injured nerves using different techniques. We hypothesize that light applied non-invasively will improve nerve regeneration and function after severe peripheral nerve injury and autograft repair.

Study: A 6–7 mm segment of the rat median nerve was excised. Sural nerve segments from the same animal were used to bridge the gap. There were four experimental groups: Sham, Injured (I), sural nerve autograft (S), sural nerve autograft + LT (SL). SL group received LT at the surgery site for 14 consecutive days using an 810 nm laser (beam area 7 cm², dose 25 J/cm², intensity 21.4 mW/cm²). Functional recovery was assessed weekly by the grasping test. Compound muscle action potential (CMAP) measurements were taken pre-injury as baseline and at 16 weeks post surgery. Flexor muscles innervated by the median nerve were removed bilaterally and weighed.

Results: SL group had better functional outcome when compared to S group with a statistically significant difference at weeks 5, 8, 9 and 11. SL and S had significantly higher flexor muscle weights than I group but there was no significant difference between the two. There was a significant reduction in the CMAP latency of SL group compared to the S group. CMAP amplitude showed a statistically significant increase only in the SL group compared to the I group.

Conclusion: In this study, LT improved function after autograft repair of the severely injured median nerve. This laser based non-invasive treatment has the potential to revolutionize post-operative repair of severe peripheral nerve injury.
Low-level laser therapy reduces time to ambulation in dogs after hemilaminectomy: a preliminary study

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Objectives: A prospective study to determine if low-level laser therapy and surgery for intervertebral disk herniation encourage ambulation faster than surgery alone.

Methods: Thirty-six dogs with acute paraparesis/paraplegia due to acute intervertebral disk herniation were evaluated and given a modified Frankel score. Dogs with scores 0 to 3 were included in the study. Dogs were assigned to the control group (1) or the laser treatment group (2) based on alternating order of presentation. All dogs underwent surgery for their herniated disk. Dogs in group 2 were treated postoperatively with low-level laser therapy daily for five days, or until they achieved a modified Frankel score of 4. A 5x200-mW 810-nm cluster array was used to deliver 25 W/cm² to the skin. All dogs were scored daily by the investigators using the modified Frankel scoring system.

Results: The time to achieve a modified Frankel score of 4 was significantly lower (P=0.0016) in the low-level laser therapy group (median 3-5 days) than the control group (median 14 days).

Clinical Significance: Low-level laser therapy in combination with surgery decreases the time to ambulation in dogs with T3-L3 myelopathy secondary to intervertebral disk herniation.

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INTRODUCTION

Intervertebral disk disease is the most common cause of endogenous acute spinal cord injury in dogs and typically results from mechanical failure of a degenerated intervertebral disk (Hoerlein 1987, Davis and Brown 2002). The thoracolumbar region is the most common region for intervertebral disk herniation (Griffin and others 2009). Dogs that are non-ambulatory as a result of disk herniation are commonly treated by surgical decompression, and 83 to 95% of these dogs will regain voluntary ambulation after decompression if they had entire pedal deep pain sensation before surgery (Gambardella 1980, Muir and others 1995, Duval and others 1996), while 58 to 69% of dogs will regain voluntary ambulation after decompression if they lacked pedal deep pain sensation (Duval and others 1996, Scott and McKee 1999, Olby and others 2003, Ito and others 2005, Ruddle and others 2006). A previous study showed that dogs with absent deep pain sensation before surgery were 1.7 times less likely to regain voluntary ambulation than dogs with entire deep pain sensation (Ruddle and others 2006). The mean time to ambulation after surgery can vary. On the basis of two studies, small breed dogs with entire deep pain sensation before surgery have a mean time to recover voluntary ambulation in 10 or 13 days (Davis and Brown 2002, Ferreira and others 2002). Large breed dogs had a mean recovery time to ambulation of seven weeks, though the majority recovered in four weeks, and the time to ambulation increased with increasing weight and age (Cudia and Duval 1997).

Low-level laser therapy (LLLT) has been used to treat injuries of various portions of the body in human medicine. The theory behind this is called photobiomodulation. This is the application of a particular wavelength of light at a certain energy density to a cell (or cells) in the body. These cells will react to that light in a predetermined fashion depending on the absorption spectrum of the cells. It has been shown that energy density between 0.2 and 10 J/cm² applied directly to central nervous system (CNS) tissue enhances neuronal cell metabolism (Roehl and Ouaknine 1992, Byrnes and others 2005), while 632- or 780-nm wavelength light at an energy density of 60 J/cm² projected directly on fibroblast cells decreases cell mitoses (Roehl and Ouaknine 1992). There are a multitude of studies, ranging from in vitro cell culture experiments to clinical trials, indicating that
Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials

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Summary

Background Neck pain is a common and costly condition for which pharmacological management has limited evidence of efficacy and side-effects. Low-level laser therapy (LLLT) is a relatively uncommon, non-invasive treatment for neck pain, in which non-thermal laser irradiation is applied to sites of pain. We did a systematic review and meta-analysis of randomised controlled trials to assess the efficacy of LLLT in neck pain.

Methods We searched computerised databases comparing efficacy of LLLT using any wavelength with placebo or with active control in acute or chronic neck pain. Effect size for the primary outcome, pain intensity, was defined as a pooled estimate of mean difference in change in mm on 100 mm visual analogue scale.

Findings We identified 16 randomised controlled trials including a total of 820 patients. In acute neck pain, results of two trials showed a relative risk (RR) of 1.69 (95% CI 1.22–2.33) for pain improvement of LLLT versus placebo. Five trials of chronic neck pain reporting categorical data showed an RR for pain improvement of 4.05 (2.74–5.98) of LLLT, with a minimal difference in effect size for the primary outcome, pain intensity, was defined as a pooled estimate of mean difference in change in mm on 100 mm visual analogue scale.

Interpretation We show that LLLT reduces pain immediately after treatment in acute neck pain and up to 22 weeks after completion of treatment in patients with chronic neck pain.

Funding None.

Introduction

Chronic pain is predicted to reach epidemic proportions in developed countries with ageing populations in the next 30 years. Chronic neck pain is a highly prevalent condition, affecting 10–24% of the population. Economic costs of this condition are estimated at hundreds of millions of dollars, creating an imperative for evidence-based, cost-effective treatments. Low-level laser therapy (LLLT) uses laser to aid tissue repair, relieve pain, and stimulate acupuncture points. LLLT is light that is generated by high-intensity electrical stimulation of a medium, which can be a gas, liquid, crystal, dye, or semiconductor. The light produced consists of coherent beams of single wavelengths in the visible to infrared spectrum, which can be emitted in a continuous wave or pulsed mode. Surgical applications of laser ablate tissue by intense heat and are different from LLLT, which uses light energy to modulate cell and tissue physiology to achieve therapeutic benefit without a macroscopic thermal effect (sometimes termed cold laser). LLLT is non-invasive, painless, and can be easily administered in primary-care settings. Incidence of adverse effects is low and similar to that of placebo, with no reports of serious events.

Research into the use of LLLT for pain reduction and tissue repair spans more than 30 years. However, reports do not identify this therapy as a potential treatment option, possibly because of scepticism about its mechanism of action and effectiveness. Research from the past decade suggests that LLLT produces anti-inflammatory effects, contributing to pain relief. Cochrane reviews of the efficacy of LLLT in low-back pain and rheumatoid arthritis have been unable to make firm conclusions because of insufficient data or conflicting findings. However, effectiveness depends on factors such as wavelength, site, duration, and dose of LLLT treatment. Adequate dose and appropriate procedural technique are rarely considered in systematic reviews of electrophysiological agents. Research into the dose-response profile of LLLT suggests that different wavelengths have specific penetration abilities through human skin. Thus, clinical effects could vary with depth of target tissue. We have shown the importance of accounting for dose and technique in systematic reviews of transcutaneous electrical nerve stimulation and LLLT, and our approach is an acknowledged means of establishing efficacy.

The only systematic review focusing solely on LLLT in treatment of neck pain included four randomised controlled trials, and concluded that there was evidence of short-term benefit of LLLT at infrared wavelengths of 780, 810–830, and 904 nm. A Cochrane review of physical medicine for mechanical neck disorders, since...
Effect of Diode Laser in the Treatment of Patients with Nonspecific Chronic Low Back Pain: A Randomized Controlled Trial

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Abstract

**Background data:** Low back pain is a common, highly debilitating condition, whose severity is variable. This study evaluated the efficacy of treatment with Ga-Al-As diode laser (980 nm) with a large diameter spot (32 cm²), in association with exercise therapy, in reducing pain. **Objective:** The present study aimed to evaluate the pain reduction efficacy of treatment with the Ga-Al-As diode laser (980 nm) in combination with exercise therapy, in patients with chronic low back pain (CLBP). **Methods:** This study evaluated 100 patients with CLBP (mean age 60 years) who were randomly assigned to two groups. The laser plus exercises group (Laser+EX: 50 patients) received low-level laser therapy (LLLT) with a diode laser, 980 nm, with a specific handpiece [32 cm² irradiation spot size, power 20 W in continuous wave (CW), fluence 37.5J/cm², total energy per point 1200 J] thrice weekly, and followed a daily exercise schedule for 3 weeks (5 days/week). The exercises group (EX: 50 patients) received placebo laser therapy plus daily exercises. The outcome was evaluated on the visual analogue pain scale (VAS), before and after treatment. **Results:** At the end of the 3 week period, the Laser+EX group showed a significantly greater decrease in pain than did the EX group. There was a significant difference between the two groups, with average Δ VAS scores of 3.96 (Laser+EX group) and 2.23 (EX group). The Student’s t test demonstrated a statistically significant difference between the two groups, at \( p < 0.001 \). **Conclusions:** This study demonstrated that the use of diode laser (980 nm) with large diameter spot size, in association with exercise therapy, appears to be effective. Such treatment might be considered a valid therapeutic option within rehabilitation programs for nonspecific CLBP.

Introduction

**Chronic low back pain** (CLBP) was defined by van Tulder in 1998 as pain in the lumbosacral area of the spine of >12 weeks’ duration, which may or not have the characteristics of limiting the patient’s range of movements. The etiopathology of this form of pain is not specific; however, it is often related to disc degeneration or other spinal disorders. It is a major cause of morbidity and affects 80–85% of people at some time during their lifetime. The severity of symptoms is variable; some are self-limiting, others require therapy, and others again require emergency room treatment.

The main goal of CLBP therapy is rarely the complete eradication of pain. Because of the etiopathology of this disorder, there may be many underlying causes, and often no specific cause can be found. Management of CLBP can choose from a range of different strategies, including surgery and drug therapy, together with nonmedical interventions including exercise therapy, manipulation, acupuncture, electrical treatments, and cognitive-behavioral interventions. During recent years, a large number of randomized controlled trials have been published. It currently appears that the ideal treatment for CLBP is a multidisciplinary intervention with a stepwise approach; studies examining the effectiveness of this approach are now numerous.

In a systematic review, Marienke et al. analyzed 83 clinical trials on physical therapy and rehabilitation for CLBP; they suggest that the only treatments that are effective in reducing CLBP are multidisciplinary treatment and behavioral therapy. Treatment with low-laser level therapy (LLLT) has given contrasting results; Jang et al. conducted a meta-analysis on the pain relief effects of laser irradiation, and...
POST TRAUMATIC NEUROPATHIC PAIN NON-RESPONSIVE TO CONVENTIONAL TREATMENT: A CLINICAL TRIAL APPLYING LOW LEVEL LASER THERAPY

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Background: Post-traumatic chronic pain is a result of peripheral or central nervous systems' damage. Even slightly non-noxious stimuli are detected and converted into electric potential by nociceptors, causing awkward symptoms. Its clinical management is tough to control, and drug interactions may cause intense systemic side effects. Patient limitation is common acknowledged with consequences to life quality. A therapeutic approach, applying Low Level Laser Therapy (LLLT), may diminish the need of drug administration. Analgesic effects of LLLT have been poorly reported either in clinical protocols or experimental studies. LLLT acts on Ca-channel and Na-K pump of cellular membranes, also enhancing the ATP intracellular production, reducing edema and inflammation. Besides, it promotes augment of circulating endorphin and serotonin leading to successful analgesic effect.

Objective: To evaluate the response of LLLT application in patients with neuropathic pain refractory to conventional treatment, and looking forward to optimizing the dose.

Study: Pilot study, 5 patients relating spontaneous pin pain for long lasting period, that also complaining of hypalgesia, burning and shock sensation without anticonvulsant therapy response were included. Pain in the scar area above the stump indicated a high score in Visual Analogue Scale (VAS), causing severe problems in Activities of Daily Living (ADLs) (Barthel, Lawton’s Scales). Patients were evaluated before and after each diode laser point application, infrared wavelength, once a week.

Results: In all cases there was expressive remission of hypalgesia and severe pain, from day-one, and even a total pain relief in two patients that had been discharged with follow up of 12 months.

Conclusion: Our study signalizes that LLLT was able to relieve all neuropathic symptoms without side effects. It is a non invasive method, that can improve patients’ quality of life, proving to be a helpful tool in pain armamentarium, especially when all other measures have failed.
TRANSCRANIAL LOW-LEVEL LASER (LIGHT) THERAPY IN MICE: TRAUMATIC BRAIN INJURY AND BEYOND
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Background: Traumatic brain injury (TBI) has no good treatment at present. Transcranial low-level laser (light) therapy at near-infrared wavelengths (810 nm) penetrates the scalp and skull and provides many beneficial effects to the brain. These include neuroprotection, anti-apoptosis, anti-inflammation, angiogenesis, neurogenesis and synaptogenesis. These effects could also be beneficial in numerous brain disorders.

Study: Mice were subjected to two different types of TBI (closed head and controlled cortical impact (CCI)) and treated with LLLT to the head starting at 4 hour post-injury. The wavelength, fluence, power density, pulse structure and treatment repetition were varied. Mice were followed with neurological severity score, wire grip test, forced swim test, tail suspension test, Morris water maze, and numerous immunofluorescence studies on brain sections removed at sacrifice.

Results: In the closed head model a single treatment 4-hours post-TBI with CW lasers at 660nm and 810nm were effective, while 730nm and 980nm were not. In the CCI model 810-nm laser pulsed at 10Hz was superior to 810nm laser at CW or pulsed at 100 Hz. In another study we compared a single treatment 4 hours post TBI with three daily treatments and with fourteen daily treatments. Three daily treatments gave best results while 14 treatments gave no benefit. This result was explained by the lack of neurogenesis after 14 treatments that was apparent after 3 treatments. Upregulation of a neurotrophin (BDNF) and markers for synaptogenesis was also seen.

Conclusion: The beneficial effects in stimulating neurogenesis, synaptogenesis and BDNF after transcranial LLLT suggest it may have wider applications beyond TBI to neurodegenerative diseases such as Alzheimer’s and psychiatric diseases such as major depression.
The Brain-Derived Neurotrophic Factor, Nerve Growth Factor, Neurotrophin-3, and Induced Nitric Oxide Synthase Expressions After Low-Level Laser Therapy in an Axonotmesis Experimental Model

Abstract

Background data: A robust body of evidence has shown that low-level laser therapy (LLLT) improves peripheral nerve regeneration. However, the biochemical background triggered in this process is not yet fully understood.

Objective: The purpose of this study was to evaluate the mRNA expression of neurotrophic factors (brain-derived neurotrophic factor [BDNF], nerve growth factor [NGF], and neurotrophin-3, [NT-3]) and also an inflammatory marker (induced nitric oxide synthase [iNOS]) in an axonotmesis experimental model after low-level laser therapy.

Methods: Thirty-six adult male Wistar rats (250–350 g) were subjected to right sciatic nerve crush injury, and 24 h later, the animals in the three different experimental groups (n = 18) were irradiated on a daily basis with helium-neon laser (collimated HeNe laser, continuous emission, wavelength: 632.8 nm, power density: 0.5 mW/cm², irradiation time: 20 sec, energy density: 10 J/cm²) during 7, 14, and 21 consecutive days, respectively. The control group (n = 18) underwent the same procedures, but with the equipment turned off. At the end of the experiments, animals were killed with an overdose of anesthesia to remove samples from the sciatic nerve lesion epicenter to determine the mRNA expression of BDNF, NGF, NT-3 and iNOS enzyme.

Results: Comparisons between groups showed that HeNe laser increased the mRNA expression of both BDNF and NGF factors after 14 days of LLLT, with peak expression at the 21st day. Increase in NT-3 mRNA expression was not observed. In addition, HeNe laser produced iNOS expression reduction, which played an important role in the inflammatory process.

Conclusions: The reported data could have a relevant practical value because LLLT is a noninvasive procedure, and have revealed significant increase in neurotrophic factor expressions and inflammatory process reduction, opening the possibility of using LLLT as an important aid to nerve regeneration process.

Introduction

Peripheral nerves are frequent targets of traumatic injuries ranging from minor injuries to nerve transaction, lacerations, or avulsions.1 Peripheral nerve injuries can be classified as neuropraxia: a lesion with mild motor and sensory loss without structural change; axonotmesis: in which there is loss of continuity and subsequent axonal Wallerian degeneration of the distal segment without losing Schwann cells, and the recovery will depend upon the nerve disorganization degree and also the organ distance target; and neurotmesis: nerve section with complete axon disorganization caused by tissue fibrosis, proximal and distal degeneration, and consequent axonal growth disruption.2 After axonotmesis, the inflammatory process is triggered; the proximal stump axons degenerate and distal fibers from the lesion undergo Wallerian degeneration between 48 and 96 h after nerve transaction. Particularly, but not exclusively, after axonotmesis, pro-inflammatory cytokine mobilization and increased activity of induced nitric oxide synthase (iNOS), which results in inflammatory cell recruitments to the affected site, occurs, leading to increased production and releasing of several pro-inflammatory mediators, which ultimately reduces significantly the nerve recovery process. In addition, there are also myelin debris degradation and other structural proteins that finally inhibit axonal growth.3,4 Simultaneously, substances known as neurotrophic factors (e.g., nerve growth factor [NGF], brain-derived neurotrophic factors [BDNF], and nerve growth factor [NGF]) play a key role in the recovery process.