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WALT

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Editors
E.-L. Laakso PhD and C. Young
Foreword

The WALT2012 biennial Congress was held at the QT Hotel in the heart of Australia’s magnificent Gold Coast. The Australian Medical Laser Association (AMLA) was pleased to host the 9th World Association for Laser Therapy (WALT) Congress in Australia, for the first time. WALT2012 was held in conjunction with the World Federation for Laser Dentistry (WFLD; Asia Pacific Division). The Congress was a resounding success in terms of delegate, sponsor and exhibitor numbers as well as financially. The Congress Co-Chairs (A/Prof Liisa Laakso and Dr Roberta Chow) wish to thank all who were involved in making the conference a success.

The conference theme was: “The spectrum of laser – translating basic research to clinical outcomes”. The Congress scientific program was diverse across a number of streams. Over 120 abstracts from across the globe were submitted for consideration for podium and poster presentations. All abstracts were subjected to a blinded, peer-review process by the international Scientific Committee. After initial review, some abstracts were returned to authors for revision and re-submission. Accepted abstracts were published in the conference handbook at the time of the congress. Where possible, only those abstracts whose authors registered for the congress were printed in the conference program. The Scientific Committee and delegates agreed the scientific rigour and quality of papers was strong overall.

The papers published in these conference Proceedings, were submitted by presenters who wished to have their papers published in full, thus not all of the papers presented at WALT2012 appear herein. Authors submitted their full papers to the publisher (Medimond International) who in turn, forwarded the papers to the Editors. Editing of papers occurred on the basis of ensuring basic consistency of language, grammar and typography according to accepted scientific standards, and where possible, a consistent approach to the reporting of laser parameters. In some cases, where time permitted, manuscripts were returned to authors for revision and re-submission. The approach taken to editing was one of ensuring quality wherever possible, and we strove to retain the accuracy of content and meaning. Responsibility for the accuracy and veracity of information however, lies with the authors, and in the main, the papers appear as they were submitted. It was the responsibility of submitting authors to follow the publishing guidelines supplied by the publisher. Agreement regarding ownership of intellectual property and copyright has been determined between the authors and the publisher. The Editors take no responsibility for errors or omissions.

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Associate Editor: Cath Young

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Global DNA Methylation Status of Colorectal Cancer Cells Exposed to Photodynamic Therapy

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Abstract

Introduction: DNA methylation and histone modifications are epigenetic mechanisms that allow heritable silencing of genes without introducing mutations or alterations to the coding sequences of the genes. Being an important regulator of gene transcription, DNA methylation and its role in carcinogenesis has received considerable attention recently. Although hypermethylation represses transcription of promoter regions of tumour suppressor genes has been extensively studied, global hypomethylation has also been identified as an oncogenic inducer. Photodynamic therapy (PDT) is the use of low intensity laser irradiation (LILI) in conjunction with a photosensitiser (PS), in this instance Zinc (II) Phthalocyanine (ZnPc), to treat cancer cells. A single wavelength is used to activate the PS, which in turn causes changes in cellular functions. DNA methylation is an epigenetic regulator. The methylation status of a gene determines if it is expressed or silenced. Aim: This study aimed to determine if DNA methylation has an effect on PDT. Method: Cancer cells, demethylated and normal, were exposed to PDT with different incubation times. Cell viability, proliferation and morphology were measured. Results: The results indicate that when cells are exposed to PDT, a statistical significant decrease (P< 0.001) in viability is achieved with an incubation time of 24 hours. The viability of cells treated with a high concentration of the demethylating agent showed a statistical significant decrease (P<0.01) in viability when incubated for 24 hours. Conclusion: DNA methylation does have an effect on the efficiency of PDT depending on the incubation time of the PS. 

Keywords: Photodynamic therapy, DNA methylation, Low intensity laser irradiation

Introduction

Low Intensity laser irradiation (LILI) or photostimulation has been shown to have anti-inflammatory and healing effects when used on damaged tissue. Although it does not have a direct healing effect, it stimulates a much more effective inflammatory response and acts as a pain relieving agent [1]. When LILI is used with a photosensitizer (PS), it is called photodynamic therapy (PDT) [2].

1.1 Photodynamic therapy

PDT can be defined as a process where LILI is used in conjunction with a non-toxic dye called a PS. The PS has a high affinity for tumour cells and has to localise in a short period of time. After an incubation time the light sensitive PS is activated by a light source with a wavelength specific to the PS [2,3]. This photodynamic effect takes place due to the energy transfer from the PS in its excited triplet state to an oxygen molecule in its triplet ground state to form excited singlet state oxygen. This excited oxygen molecule is highly reactive and so will react with other molecules in its immediate vicinity inducing oxidative damage and thus leading to cell death [2,4]. In clinical practice, PDT is considered as an ablative procedure directed at oncological diseases and their vascular supply. Oncological diseases are usually treated with surgery, chemotherapy, or radiation therapy, but often these treatment modalities have life-threatening effects. To overcome these life-threatening effects, PDT can be used as an alternative treatment modality or in conjunction with these treatments to improve the prognosis of patients [2,3].

The properties of ZnPcSmix as a potential photosensitizing agent has shown promise. Most second-generation PSs undergoing clinical trials are phthalocyanines, chlorins, and porphyrin derivatives. Photosensitizers that absorb light above 600 nm are usually targeted in PDT treatment. Phthalocyanines are among the most promising second-generation photosensitizers, exhibiting excellent photodynamic activity with a macrocyclic structure consisting of four benzopyrrolic subunits. Researchers have shown a lot of interest in phthalocyanines because of their wavelength of absorbance (650–680 nm) and optimal tissue penetration [3].
1.2 DNA methylation and cancer

Epigenetics can be defined as the study of mechanisms that control the expression of genes. Thus epigenetic modifications can lead to diseases such as cancer [5]. One such epigenetic modification is known as DNA methylation. DNA methylation can be defined as the covalent adding of a methyl group (CH₃) to the 5’ position of a cytosine (C). Methylation is found throughout the genome, but does occur in high frequency in cytosine-p-guanine di-nucleotides (CpG). These di-nucleotides are found in high concentration in the promoters of genes in so called CpG islands. Almost half the genes in the human genome have GpG islands and they are associated with housekeeping genes [6,7].

Genes can either be hypermethylated or hypomethylated. Genes that are hypermethylated are normally silenced and genes that are hypomethylated are normally over expressed. Both hyper- and hypomethylation have been associated with various types of cancer [7]. DNA hypomethylation are known to be caused by certain factors leading to significant consequences for subsequent genetic transcription (table 1).

<table>
<thead>
<tr>
<th>DNA HYPMETHYLATION</th>
<th>Causes</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered DNMT activity</td>
<td>Aberrant gene expression</td>
<td></td>
</tr>
<tr>
<td>Histone modifications; loss of trimethylation; increased acetylation</td>
<td>Loss of imprinting</td>
<td></td>
</tr>
<tr>
<td>Exogenous insults; diet; environment; infection</td>
<td>Microsatellite instability</td>
<td></td>
</tr>
<tr>
<td>Non-coding RNA</td>
<td>Activation of retrotransposons; insertional mutagenesis; recombination</td>
<td></td>
</tr>
<tr>
<td>Defective DNA repair</td>
<td>Chromosomal instability and anomalies</td>
<td></td>
</tr>
</tbody>
</table>

Methodology

Colorectal adenocarcinoma cells (CaCo2, #HTB-37) were cultured in minimum essential media eagle (Sigma M2279) until confluent. After confluence was achieved, cells were treated with aza-5-dc (Sigma A3656) to demethylate the DNA of the cells at 0.1 µM and 3 µM respectively. When seeded into small plates for two groups irradiated, were prepared for different incubation times of the PS, 3h and 24h. Cells were then irradiated with 5 J/cm² at 680 nm (43.0 mW; 5.03 mW/cm²; 16.5 min; 990 s).

Viability was determined using Trypan Blue staining in conjunction with the Countess Automated Cell Counter (Invitrogen C10227). ATP concentration was determined as a more sensitive method of analysing cell viability using luminescence. Cell proliferation was measured using the AlamarBlue stain and the Victor3 multi-label plate reader (PerkinElmer) (results not shown). Cell morphology was viewed using inverted light microscopy using the Hoechst stain.

Results and Discussion

1.1 Morphology

Morphological changes were observed as shown in figure 1 using brightfield inverted microscopy. The control groups (Control, PS and LILI, aza-0.1 and aza-3) showed almost no morphological changes. The group exposed to PDT showed, after 3h of incubation the cells became rounded and started to detach from the plate surface, while after 24h incubation the PDT treated group showed signs of severe structural damage.

An interesting observation is the size and shape of the vacuoles in the cells. Cells exposed to a high concentration of aza-5-dc (group aza-3 in figure 1) showed a decrease in these vacuoles.
1.2 Viability and proliferation

Cells exposed to PDT showed a significant statistical difference in viability when compared to the control ($P < 0.05$ for the 3h and $P < 0.001$ for the 24h) as shown in figure 2. In the 24h group, demethylated cells showed a significant difference when compared to the control, but less than the PDT group. As for the 3h group, it was the exact opposite. However when comparing the PDT group to the demethylated groups, no significant difference was found.

Figure 2: Percentage viability of cells exposed to LILI, PS, PDT, demethylating agent and a combination of demethylating agent and PDT after 3hr and 24hr incubation. Viability results for the different groups. Both 3hr and 24hr groups exposed to demethylating agent and PDT showed a marked decrease in viability although the 24hr exposure group showed a slight improvement in viability which may be explained by activation of repair mechanisms. $*P<0.05$, $**P<0.01$ and $P<0.001$ ($n = 4$).
The cells exposed to a high dosage of aza-5-dc and PDT showed a significant difference when compared to the demethylated cells not exposed to PDT, but viability was still reduced compared to the PDT group. The viability results show that PDT significantly decreased the viability of cells exposed to it when the PS is incubated for 24h. DNA methylation did not affect PDT when incubated for 24h. This can be due to the fact that the DNA repair mechanisms had time to be activated before PDT was administered. Another factor that can play a role in the effectiveness of the aza-5-dc and PDT is the stage of the cell cycle the cells were in.

**Conclusion**

Colorectal cancer cells are susceptible to PDT treatment with 20 μM ZnPc and irradiated with a wavelength of 680 nm and a fluence of 5 J/cm². DNA methylation does have an effect on PDT effectiveness, but this effect is subject to the incubation time of the PS. Demethylation prior to PDT treatment enhances cancer cell death induction. Future work will investigate the morphology of the nucleus and genetic material by fluorescent live cell imaging and the comet assay as well as genetic transcription of key genes using RT-PCR.

**References**

Application of computer modelling to predict the effect of melanin concentration on treatment time

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Abstract

The South African population consists of individuals from European, Asian and African descent who present a wide range of skin phototypes. This poses a problem when treating patients with lasers due to a difference in melanin content and the associated absorption of light in the epidermal layer for the different individuals. Suitably calibrated computer models can be used to predict the fluence rate transmitted through specified skin layers. A computer model (allowing for different skin layers) was developed in the ASAP software environment. The epidermal absorption coefficients for 28 individuals was measured with a diffuse reflectance probe. Epidermal absorption coefficients between 0.002 and 3 mm\(^{-1}\) were measured and used in the computer model. Photodynamic Therapy (PDT) is one of the treatments currently under investigation to treat skin cancer, specifically squamous cell carcinomas (SCC). The fluence rate reaching the tumour (at a depth of 200 \(\mu m\) below the surface) was calculated for different absorption coefficients (present in the South African population). For an epidermal thickness of 90 \(\mu m\) with an absorption coefficient of 3 mm\(^{-1}\), less than 30 % of incident light reached the tumour as opposed to the 54% for a very low absorption coefficient (0.002 mm\(^{-1}\)). For effective treatment a dose of 4.5J/cm\(^2\) is required. Therefore for a constant laser power of 50 mW (at the skin surface) the higher absorbing skin needs to be treated for 413 s and the low absorbing skin needs to be treated for only 222 s. This variation highlights the need for in-situ calibration and modelling during treatment planning.

Keywords: epidermal absorption modelling, South African skin phototypes, light transmission modelling, photodynamic therapy.

Introduction

The optimal use of lasers as a treatment modality requires an understanding of the parameters involved in the process. Some of the major parameters are the penetration depth of the laser (determined by the optical properties of the tissue), the laser power and the laser beam profile. In laser treatment, it is important to determine the fluence rate reaching a certain depth into the organ to be able to provide the correct laser irradiance dose (J/cm\(^2\)) at the intended treatment site. In vivo measurements to determine the fluence rate are impractical. Computer modelling of light interaction with the tissue is one of the methods that can be used to predict the fluence rate at any given depth in the tissue.

Australia and South Africa are countries with the highest incidence of skin cancer in the world. Photodynamic Therapy (PDT) is a cancer therapy currently under investigation [1]. In PDT a photosensitiser (PS) is administered to a patient. The PS accumulates in the tumour and after a period of time the cancerous tumour is irradiated with a laser [1]. In the process singlet oxygen is formed that leads to the destruction of the cancerous cells.

The South African population consists of individuals of different ethnicity with a wide range of skin colours or skin tones. The variation of skin tone is normally referred to as skin type or skin phototype and the standard classification used is the Fitzpatrick skin scale [2]. This scale classifies skin by its reaction to sun (UV) exposure and ranges from skin phototype I (the lightest skin that will always burn due to sun exposure and only turn red and not turn brown) to skin phototype VI (the darkest skin that does not appear to be affected by sun exposure). The range of absorption coefficients for the different skin phototypes normally present in the South African population is not available in the literature. Measurements were done on a small sample of the South African population to determine the range of absorption coefficient that may be expected in clinical settings in South Africa. The results of the measurements were used as input values for a computer model of the skin to predict the fluence rate inside the skin.
Materials and methods

1.1 Layered skin model

The ASAP (Advanced Systems Analysis Program) software from Breault Research Organization (Tucson, Arizona in the USA) was used to model the light interaction with skin. The software is able to perform non-sequential raytracing where rays (or photons) can interact with objects as they encounter them. The rays are not restricted to the order in which the objects were defined in the model as is the case in sequential raytracing [3], [4].

A model has been developed to mimic the layered structure of skin (See Fig. 1). Each layer is described as a rectangular slab with known dimensions and known optical properties, i.e. absorption coefficient ($\mu_a$), scattering coefficient ($\mu_s$), anisotropy ($g$) and refractive index ($n$). Each layer is considered to be homogenous hence the same (averaged) optical properties apply everywhere in the layer. The model used for this work consisted of two layers (epidermis and dermis) with a skin tumour (squamous cell carcinoma) embedded in the dermis. Three different epidermal thicknesses were used in the model to evaluate the influence of the epidermal thickness and fluence rate loss during treatment.

Absorption coefficients calculated with the probe in section 2.2 were used to determine the $\mu_a$ of the epidermis. The optical properties as well as the geometric dimensions of the model are given in Table 1. The value of the anisotropy ($g$) was kept constant at 0.8 for all the layers. The refractive index ($n$) for the epidermis was 1.5 and 1.4 for both the dermis and the tumour. A laser beam (wavelength 676 nm) with a beam diameter of 12 mm and an output power of 50 mW was modelled and traced through the layered skin. Two evaluation detectors were used to evaluate the back scattered light and the light transmitted through the model. The model was validated against physical phantoms and actual measurements performed with an integrating sphere as described elsewhere [5].

![Figure 1: Schematic of the computer model with the detectors and the different layers.](image)

Table 1: Geometrical dimensions and optical properties of the different layers in the skin model.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Width (mm)</th>
<th>Start depth (mm)</th>
<th>Thickness (mm)</th>
<th>$\mu_s$(mm$^{-1}$)</th>
<th>$\mu_a$(mm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>40</td>
<td>0</td>
<td>0.04, 0.06, 0.09</td>
<td>0.002, 1, 3</td>
<td>22.4</td>
</tr>
<tr>
<td>Dermis</td>
<td>40</td>
<td>0.04, 0.06, 0.09</td>
<td>3</td>
<td>0.15</td>
<td>13.9</td>
</tr>
<tr>
<td>Tumour</td>
<td>10</td>
<td>0.2</td>
<td>2</td>
<td>0.11</td>
<td>8.55</td>
</tr>
</tbody>
</table>

1.2 Absorption coefficient of the epidermis

The epidermal absorption coefficients required for the computer model were calculated from diffuse reflectance measurements. Zonios [6] developed a method to calculate the absorption and scattering coefficients of tissue from diffuse reflectance measurements. The measurement system consists of a fibre based diffuse reflectance probe (DRP), a white light source, a spectrometer (all from Ocean Optics Inc. Florida, USA) and a computer. The probe consists of seven 200 µm diameter fibres with the six outer fibres delivering light in a ring and one central collecting fibre (R200-7-UV-VIS). A halogen white light source (HL-2000) emitting between 450 and 900 nm was used as illumination source and the reflected light was collected in the central fibre and used as input to an USB-4000 spectrometer. More detail on the experimental setup and calibration procedure can be found in [7].
The dependence on wavelength of the diffuse reflectance \( R_p(\lambda) \) can be described by Eq. 1:

\[
R_p(\lambda) = \frac{\mu_s^I(\lambda)}{k_1 + k_2\mu_a(\lambda)}
\]

where \( k_1 \) and \( k_2 \) are parameters that depend on the probe geometry as well as the indices of reflection for the measurement sample and the surrounding medium (normally air); \( \mu_s^I \) is the reduced scattering coefficient; \( \mu_a \) is the absorption coefficient.

A set of 60 liquid skin simulating phantoms were prepared from a 1:1 mixture of Intralipid (IL) and black ink diluted in distilled water. The optical properties of the phantoms were known and the DRP measurements were done on the phantoms and these measurements were used to obtain the values of \( k_1 \) and \( k_2 \).

The absorption coefficient can be described through Eq. 2 [6]:

\[
\mu_a(\lambda) = c_{HbO_2}[\alpha_{HbO_2}(\lambda) + (1-\alpha)\epsilon_{Hb}(\lambda)] + c_{Hb}[\epsilon_{Hb}(\lambda)] + c_{Eum}[\epsilon_{Eum}(\lambda)]
\]

with \( c_{HbO_2} \) = oxyhaemoglobin concentration; \( \epsilon_{HbO_2}(\lambda) \) = extinction coefficient for oxyhaemoglobin [8]; \( \epsilon_{Hb}(\lambda) \) = extinction coefficient for deoxyhaemoglobin [8]; \( \alpha \) = oxygen saturation level of blood, defined as the ratio of the oxygenated blood to the total blood concentration \( \alpha = \frac{c_{HbO_2}}{c_{HbO_2} + c_{Hb}} \); \( c_{Eum} \) = eumelanin concentration; \( \epsilon_{Eum}(\lambda) \) = eumelanin extinction coefficient [9]; \( c_{Hb} \) = oxyhaemoglobin concentration.

The reduced scattering coefficient can be described by Eq.3 [6]:

\[
\mu_s^I(\lambda) = \left[1 - \left(\frac{d_s^2}{\lambda^2 - \lambda_1\lambda_2}\right)^{3/2}\right] \mu_s^I(\lambda_2)
\]

with \( d_s \) = constant = 0.0625 \( \mu m \) [6]; \( d_s \) = effective scatter size; \( \lambda_1 = \min(\lambda) = 450 \ nm; \lambda_2 = \max(\lambda) = 800 \ nm. \) The measured probe reflectance \( R_p(\lambda) \) can be described mathematically in terms of six coefficients \( c_{HbO_2}, c_{Hb}, c_{Eum}, \epsilon_{Eum}, \alpha, \mu_s^I(\lambda_{min}) \) that are optimised to best match the equation to the experimental data.

Diffuse reflectance probe measurements were done on 28 volunteers according to approved ethical procedures (CSIR: Ref 17/2011 and University of Pretoria: EC110830-060).

**Results and discussions**

The absorption coefficients extracted from the diffuse reflectance measurements at a wavelength of 676 nm, are presented in Fig. 2 and Fig. 3. The results are presented separately for lighter and darker skin phototypes to ensure that all the data are visible on the different scales.
The results show the expected trends (higher absorption for the sun exposed parts) for most of the measurements. The exposed $\mu_a$ (=0.06) value for volunteer 6 is not shown in Fig. 1 because it would reduce the resolution on the Y-axis for Fig. 2. In the computer model $\mu_a$ values of 0.002 mm$^{-1}$, 1 mm$^{-1}$ and 3 mm$^{-1}$ were used to present the expected absorption coefficients in the South African population.

The epidermal thicknesses of 40, 60 and 90 $\mu$m (typical of the sun exposed areas of the upper body) were evaluated. For ease of comparison, all the evaluations are reported at a depth of 200 $\mu$m into the model (just before the tumour). The fraction of the light that reached the tumour is reported in Table 2 for the different epidermal thicknesses and absorption coefficients. The laser beam diameter expanded from 1.2 cm to 1.3 cm at the tumour depth (200 $\mu$m into the model).

Table 2: Fraction of power reaching the tumour at a depth of 200 $\mu$m into the skin and the resulting treatment time to deliver a light dose of 4.5 J/cm$^2$ onto the tumour.

<table>
<thead>
<tr>
<th>Epidermal thickness (mm)</th>
<th>$\mu_a$ (mm$^{-1}$)</th>
<th>Power fraction transmitted</th>
<th>Required treatment time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.002</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Not exposed</td>
<td>0.54</td>
<td>0.48</td>
<td>0.40</td>
</tr>
<tr>
<td>Exposed</td>
<td>0.54</td>
<td>0.48</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Conclusions

Both the thickness of the epidermis and the absorption coefficient of the epidermis are important parameters for planning PDT treatment for embedded tumours. An increase in the epidermal thickness results in increased absorption of light in the epidermis. For the parameters used in this work, the effect of the absorption coefficient in the epidermis (due to the skin phototype) is more important than the effect of the epidermal thickness. The analysis for this work was aimed at typical sun exposed areas of the skin where the incidence of skin cancer is more likely. Other areas of the skin may have thicker epidermal layers which will increase the total absorption through the epidermis for the darker skin phototypes. The computer model may be used in these predictions.

The results reported above underline the importance of taking the epidermal absorption into account in skin based laser treatments. The DPR model still needs to be calibrated on a much larger sample of the population in order to use it in combination with the computer model in clinical settings.

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   http://omlc.ogi.edu/spectra/hemoglobin/index.html

Quantification of the Absorption of Low-Level 904 nm Super Pulsed Laser Light as a Function of Skin Colour

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Abstract

Low-level laser therapy is an effective treatment for relief of pain, tissue repair and inflammation and has been extensively used in clinical settings. The effects of laser are dose-dependent and a number of identified factors affect the laser dose received by the patient. To examine the relationship between laser transmission and skin pigmentation, a power meter was used to measure transmission of super pulsed 904 nm low-level laser therapy through the thenar web and cheek pad of participants with skin colour represented by a range of values on the Fitzpatrick scale of skin darkness. The slope of the function of laser transmission against skin darkness value was not significant at the cheek where the confounding effect of cheek pad thickness could not be quantified, however dorso-palmar depth of the thenar web was measured to control for the thickness factor. Laser transmission was found to significantly decrease with increasing skin darkness at the thenar web. Over the range of Fitzpatrick scale values employed, from 2 to 6, there was a 24% decrease in energy transmission at this site. We calculated that for each Fitzpatrick scale point of skin pigmentation after a value of 2, there was a 6% decrease in energy transmission due to absorption by progressively darker skin colours.

Keywords: skin pigmentation, 904 nm super pulsed low-level laser therapy, laser absorption, laser transmission

Introduction

Low level laser therapy (LLLT) at 630-910 nm has been used in clinical practice for the treatment of acute and chronic pain, inflammation, and wound healing for over 40 years. Since Mester (quoted by Ferreira [1]) made the first observation of light being absorbed in human tissue and causing chemical change there have been over 100 phase III randomised controlled trials (RCTs) on clinical use of laser and over 1000 laboratory studies that have investigated the primary therapeutic mechanisms of low level laser, as well as its secondary effects [2].

Research on the underlying mechanisms suggests that a therapeutic dose of laser has the potential to affect a variety of biochemical systems in the body, including cellular enzymes such as Cytochrome – C – Oxidase [3] and Na-K-ATPase [4]. A change in Na K-ATPase resulting from the doses used has been shown to affect the action potential of nerve impulses in the saphenous nerve in rats [4] LLLT has been shown to affect peripheral nerve function by decreasing fast axonal flow [5] and by the hyperpolarisation of pre-ganglionic sympathetic cells [6]. Specific wavelengths and doses of laser have inhibitory effects in acute and chronic pain conditions [7] and enhance tissue repair [8].

The achievement of clinically desired outcomes with LLLT depends on selection of appropriate dose parameters [9]. Dosage is usually prescribed in terms of surface radiant exposure or energy density, measured in Joules per cm². Transmission of light through the skin is affected by reflection, absorption and scattering, which are in turn dependent on the characteristics of the skin and sub-cutaneous tissues, as well as on the nature of the radiation source [10]. Dose parameters are usually determined after consideration of skin type, skin thickness, propagating characteristics of different tissues [11] as well as the wavelength, power density and other characteristics of the laser dose [7, 12]. For example, there is a biphasic dose response [12, 13]. Hamblin [13] and Sharma [14] have both demonstrated an increase in mitochondrial membrane potential and ATP production at low irradiance and a decrease at high irradiance. In research on use of laser with animals after myocardial infarct, Oron [15] found that by keeping power constant and varying the irradiance, the beneficial effects were maximised at 5 mW/cm², reduced at power densities lower than 2.5 mW/cm², and also less at power densities higher than 25 mW/cm².
The pulsing parameters of laser are also important factors that must be taken into account in dose prescription. Bjordal [16] reported that an in vivo trial with 904 nm pulsed laser, the dose parameters for collagen stimulation were much lower when the laser was pulsed rather than continuous. Further, Barolet [12] showed that intermittent microsecond pulsing patterns in laser output showed enhanced photodisassociation of nitric oxide from cytochrome c-oxidase, compared with continuous, 50% duty cycle or millisecond pulsing laser application at comparative energy densities. Thus the cascade of respiration events might be enhanced by micropulsed laser application. Consistent with this, Hashmi [17] reported that pulsed lasers generate less heating of superficial tissues because of quench periods (pulse OFF times) and hence improved ability of the light to penetrate to deeper tissues, potentially achieving greater treatment affects from direct stimulation of the deeper structures.

LLLT has been found to be an effective treatment for a variety of musculoskeletal conditions. The Bone and Joint Task Force on Neck Pain and its Associated Disorders [18] concluded that LLLT outcomes showed similar levels of effectiveness to physiotherapy interventions such as mobilisation and exercises. In addition, Chow et al [19] in their meta-analysis of responses to neck pain treatments, found that LLLT was an effective treatment for chronic neck pain.

More than one biological mechanism may underpin laser treatment efficacy. The blocking of axonal flow in C fibres to create an analgesic effect of laser on the mitochondrial membrane potential using an 830 nm continuous laser has been described by Chow [5].

LLLT action as a ‘dose specific anti-inflammatory effect in the articulated joint capsule’ has been proposed as one explanation for the positive results in the reduction of pain in chronic joint disorders [16]. In his review of laser for joint-specific disorders, Bjordal concluded that there was strong evidence for LLLT effectiveness when the dosage was titrated for the physical and anatomical penetration characteristics of the target site [16]. Similarly, Tumulty [20] in a systematic review of laser treatment in tendinopathy concluded LLLT can be effective when current dosage recommendations are followed. In their review on the clinical effects of LLLT in tendinopathy, Steffens & Maher [21] noted that of the 13 trials from which the dosage could be calculated, four of the 6 positive trials met the World Association for Laser Therapy (WALT) guidelines for dosage, whereas none of the seven inconclusive or negative trials met these guidelines.

Laser dosage is thus seen as an important aspect of the effective application of laser. In clinical practice and research, dose adjustments allowing for the effect of skin type on laser transmission may be of critical importance. Although dose recommendations have been formulated (WALT guidelines) to inform prescription, as yet no studies elucidate suitable dose adjustments for the effect of skin colour on laser transmission.

Different skin types absorb photons differently due to variations in the quantity of melanin present. Battle [22] found that the absorption spectrum of melanin (250 – 1200 nm) allows for the absorption of visible light, ultraviolet light and infrared light. Melanin is an important chromophore (light sensitive molecule) in the epidermis, while chromophores in the dermis consist of haemoglobin and subcutaneous lipids, in addition to melanin [10]. A greater quantity of melanin may reduce the amount of energy reaching the intended subcutaneous chromophores during LLLT [22]. As a result, efficacy of laser in persons with darker skin colours may be reduced at equivalent power densities [22]. Some authors, for example Simunovic [23], arbitrarily recommend the use of 50% higher radiant exposure for persons with highly-pigmented skin, and 50% lower for those with lightly-pigmented skin, compared with ‘average white-skinned persons’.

Skin types vary widely in human communities. The Fitzpatrick Skin Typing scale was originally developed to enable clinicians to adjust the dose of ultraviolet A for the treatment of psoriasis with respect to client skin type [24]. One study to date has considered the role of skin colour in relation to efficacy of LLLT. Nussbaum [10] investigated the transmission from a Light Emitting Diode (LED) in the red and infrared spectra in people with increasing levels of skin pigmentation, finding that the transmission of red light was significantly diminished in those with darker skins. However, a commonly used clinical laser for treating deeper problems is the pulsed GaAs, 904 nm laser infrared diode laser [17]. There is therefore a need to study the transmission of super pulsed LLLT in order to inform treatment dose for individuals with differing amounts of skin pigmentation.

Aim

To measure the absorption of low power laser light through the skin of the thenar web and cheek in people with different skin pigment types.

Method

Volunteers with skin types representing a range of Fitzpatrick scale values were recruited by an advertisement placed on the counter in a suburban physiotherapy practice. Ten participants representing three
Fitzpatrick Scale skin types volunteered to participate; three had skin pigmentation rated at a Fitzpatrick Scale value of 2, four at Fitzpatrick Scale value 4, and three at Fitzpatrick Scale value 6.

To avoid the effects of concurrent sun tanning recruitment occurred during late Winter and early Spring. A one-second dose of an Irradia® (Irradia USA, Irvine, CA) 904 nm micropulsed laser (50% duty cycle) at an average output power of 60 milliwatts (peak power density = 1400 W/cm²) was delivered through two sites; the pouch region of the cheek and through the thenar web, midway between the thumb and the first finger, but close to the margin to minimize thickness. The dose was repeated three times at both sites. Transmission of energy from the pulsed laser was measured using a calibrated “New Focus” (2584 Junction Avenue, San Jose, CA 95134-1902 USA) output meter, with a purpose-designed jig constructed so as to maintain the alignment of the laser probe with the collection head of the output meter. A sterile plastic sleeve was placed over the collection head before insertion of the output meter into the mouth. Thenar web thickness was measured with a micrometer screw gauge, applied at a firm but not painful pressure. Ethics committee approval to conduct the study was obtained from the University of Canberra and all participants gave informed consent before measurement took place.

Statistical Analysis: The amount of light energy collected was converted to a percentage of the light transmitted without any intervening tissue, and plotted against the individual’s Fitzpatrick Scale value, both for the thenar web (Figure 1a) and the cheek (Figure 1b). Descriptive statistics were calculated and regression analyses conducted using SPSS (version17.0, SPSS Inc., Chicago, IL).

Results

Mean (SD) thenar web thickness measures for the 3 skin type groups at Fitzpatrick 2, 4 and 6 were 3.32mm (0.29), 3.16 (0.94) and 3.62 (0.72) respectively. The two factors relevant to laser absorption, thenar web thickness and Fitzpatrick Scale amount of skin pigmentation, were not significantly correlated (r = -0.26, p = 0.18). The mean percent of laser transmitted at the thenar web and the cheek is shown in Table 1. Regression analysis showed that the percent of laser transmitted was a significant linear declining function in relation to Fitzpatrick Scale score, (r = -0.846, p = 0.002). The power of the study to detect a correlation this large was 0.73. The percent of laser energy transmitted through the cheek was also a declining function in relation to Fitzpatrick Scale Score, however at this site the correlation of -0.344 was not statistically significant (p = 0.330).

Table 1. Mean percentage of laser transmission through the thenar web and the cheek for different skin types

<table>
<thead>
<tr>
<th>Fitzpatrick Scale skin type</th>
<th>Thenar web thickness (mm) Mean (SD)</th>
<th>Thenar Web transmission Mean (SD)</th>
<th>Cheek transmission Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.92 (0.29)</td>
<td>3.84 (0.79)</td>
<td>4.07 (0.15)</td>
</tr>
<tr>
<td>4</td>
<td>3.16 (0.94)</td>
<td>2.95 (0.56)</td>
<td>3.32 (0.84)</td>
</tr>
<tr>
<td>6</td>
<td>3.62 (0.72)</td>
<td>1.87 (0.54)</td>
<td>3.27 (0.72)</td>
</tr>
</tbody>
</table>
The absorption of photons in LLLT changes with tissue depth [11]. To control for the effect of variation in the thickness of the web space in this study, a partial correlation was computed. The relationship between Transmitted Energy and Fitzpatrick Scale score with the thenar web thickness held constant was $r = -0.841$. This partial correlation was only marginally less than the bivariate Pearson correlation between the two variables, indicating that tissue thickness did not affect the identified relationship between Transmitted Energy and Fitzpatrick Scale score.

Regression analysis with percent laser transmitted as the dependent variable showed a significant effect of degree of skin pigmentation. The resulting equation was significant ($F_{2,9} = 9.28, p = 0.011$), with Percent Transmitted $= (\text{constant}) \times 5.35 - (0.491 \times \text{Fitzpatrick Scale}) - (0.108 \times \text{Web Thickness})$. The weight for Fitzpatrick Scale value had an associated $p$-value of 0.005, and the weight for Web thickness had an associated $p$ of 0.61.

Because there is both a dorsal and a palmar surface involved in the thenar web measurement, it can be concluded that half of 0.491, or 0.245, is the reduction in percent of laser energy transmitted that results for each additional Fitzpatrick scale point of skin pigmentation.

Discussion

The data presented here show that skins of different Fitzpatrick scale values differentially absorb laser at 904 nm, with darker skin colours absorbing greater amounts of the laser. Because it has two pigmented skin surfaces and minimal intervening tissue thickness, the thenar web was an effective site at which to investigate the relationship between skin pigmentation and transmitted laser energy, due to minimum confounding from the tissue that the laser must pass through before being measured. We found significant differences between laser absorption in different Fitzpatrick skin pigmentation types with dual skin interfaces at the thenar web compared to the single layer through the cheek. The amount of laser absorption through the cheek pouch was possibly influenced by the Body Mass Index (BMI), in that the amount of body fat would have exerted a greater confounding effect at this site. Nussbaum [10] reported that transmission from both red light (660 nm) and infrared (840 nm) LED were declining functions of skinfold thicknesses in the 10 to 50 mm range, taken at the biceps, triceps and waist. In the present study, the mean value for web thickness, at 3.35 mm, was lower and thus caused less absorption of the laser.

The finding here of a relationship between skin pigmentation and laser absorption has implications for the reporting of laser research in RCTs involving people of different skin types. Melanin, the agent of skin pigmentation, is a sensitive chromophore and is also present in the nervous system in the form of neuromelanin. Neuromelanin is present in the human central and peripheral nervous system, brain stem nuclei, trigeminal ganglion, dorsal root ganglia and sympathetic ganglia [25]. A possible mechanism underlying the effectiveness of low-level laser may therefore be via absorption by neuromelanin.

Conclusion

People of different skin types display different dose responses to laser applied to the skin. Quantified knowledge of this dose response is important for parameter selection to obtain effective therapeutic dose windows. The quantification arising from our analysis indicates that the needed adjustment to maintain a fixed low-level laser treatment dose is 0.1 milliwatts of laser power per point, for each Fitzpatrick Scale point. For each additional Fitzpatrick scale point of skin pigmentation between 2 and 6, there is a 6% decrease in energy transmission.

Acknowledgements: Lars Hode, Stefan Jordison.

References

The helmet experiment in Parkinson’s disease: an observation of the mechanism of neuroprotection by near infra-red light

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Summary

A puzzling feature of reports of near infrared light (NIR) treatment of soft tissue wounds is the lack of laterality in the tissue response - it is typically bilateral after a unilateral exposure. This has led to the idea that NIR has an ‘indirect’ effect on non-irradiated tissues, mediated by circulating ‘factors’. We have recently reported that NIR protects midbrain dopaminergic cells of mice from parkinsonian insult. In those studies, NIR was directed to the head, on the assumption that it would penetrate the skull and brain to reach the midbrain; in practice the whole dorsum of the mouse was irradiated. In this study, we applied NIR to the body only, preventing the radiation reaching the head with a ‘helmet’ of aluminium foil. NIR radiation of the body only was effective in protecting these cells, although less protective than radiation of both body and head. The results suggest that the neuroprotective effect of NIR may be mediated at least partially by a systemic or indirect effect. The possibility of immune system involvement will be discussed.

Introduction

The mechanism by which near infrared light (NIR), from either lasers (low level laser therapy, LLLT) or from light emitting diodes (LED) (i.e., photobiomodulation, PBM) induces wound healing in damaged soft tissue and protects central nervous structures against degeneration remains elusive: although many groups have tested for, and identified, possible pathways. The idea that NIR acts directly is supported by evidence that a key enzyme in the oxidative phosphorylation pathways of the mitochondrion, cytochrome c oxidase, absorbs NIR. This understanding is encouraged by the ability of NIR wavelengths to penetrate deeply into tissue.

This present study reports a test of an alternative idea: that NIR acts indirectly, by activating factors that circulate around the body and can act at any site of tissue damage or stress. The evidence for this indirect action is limited, because only a few studies have tested for or considered the possibility. In essence, these few studies have noted remote, often bilateral, effects on tissues, after local exposure on skin wounds, gliomas (implanted on the dorsum of mice, and irradiating abdomen), skin abrasions and oral mucosa lesions. Further, recent studies have reported that critical-to-life tissues such as brain, heart and lung are protected from stress by remote ischaemic preconditioning. The stress involved in these conditioning regimes seems to elicit a protective response, and when the stress is limited to part of the body (say a limb), it becomes clear that remote organs are protected, supporting the idea of circulating factors.

As a first step to test whether the neuroprotective mechanism of NIR involves activation of a more global system, we undertook a series of experiments in which the NIR treatment of MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated mice was limited to the body. Our earlier studies achieved protection of midbrain dopaminergic cells in MPTP-treated mice by radiation directed at the head, but in fact reaching all of dorsum of the animal. We assumed in this work that NIR acted by directly impacting the mitochondria of protected cells, having penetrated the cranium and brain parenchyma. We have since wondered if we would achieve protection of midbrain dopaminergic cells if we applied NIR to just the body.

Materials & Methods

Male BALB/c (n=40) mice were housed on a 12hr light/dark cycle with unlimited access to food and water. Animal Ethics Committee of University of Sydney approved all experiments.

This study comprised two series of NIR treatment experiments, where NIR was applied to either the (i) head and body or (ii) body (“helmet”). There were four experimental groups, each of five mice, within each series. Mice received intraperitoneal injections of either MPTP or saline, combined with NIR treatment or not. The different groups were (1) Saline: saline injections with no NIR (2) Saline-NIR: saline injections with NIR (3) MPTP: MPTP injections with no NIR (4) MPTP-NIR: MPTP injections with NIR.
Following previous work\textsuperscript{8, 9}, we used an acute MPTP mouse model. We made two MPTP (total of 50mg/kg per mouse) or saline injections over a 30-hour period. Following each injection, mice in the MPTP-NIr and Saline-NIr groups were given NIr (670nm) treatment from a light-emitting device (LED; Quantum WARP 10, Quantum Devices, Inc, Barneveld, WI, USA), equating to ~0.5 Joule/cm\textsuperscript{2} to the brain\textsuperscript{9}. About 6 hours after each injection and first NIr treatment, mice in these groups received a second NIr treatment, but no MPTP or saline injection. For each NIr treatment, the LED was held 1-2 cm directly above either the head\textsuperscript{9, 10} or the body of the mouse. In the latter cases, the head region was protected by the aluminium foil helmet, which did not permit any penetration of NIr (after measurements using a calibrated sensor). For the Saline and MPTP groups, mice were held under the LED as described above, but the device was not turned on\textsuperscript{8-11}. After the last treatment, mice were allowed to survive for six days.

Following the survival period, mice were anaesthetised with an intraperitoneal injection of sodium pentobarbital (60mg/ml) and perfused transcardially with 4\% buffered paraformaldehyde. The brains were processed for routine tyrosine hydroxylase (TH) immunocytochemistry as described previously\textsuperscript{9}. Briefly, midbrain sections were incubated in anti-TH (Sigma-Aldrich), followed by biotinylated anti-rabbit IgG and streptavidin-peroxidase complex (Bioscientific Pty Ltd) and then reacted in a 3,3'- diaminobenzidine tetrahydrochloride (Sigma).

Following previous studies\textsuperscript{8-10}, TH\textsuperscript{+} cell number within the substantia nigra pars compacta (SNc) was estimated using the optical fractionator method (Stereoinvestigator, MBF Science). For comparisons between groups, a one-way ANOVA test was performed, in conjunction with a Tukey-Kramer multiple comparison test.

**Results**

Fig 1 shows the estimated TH\textsuperscript{+} cell number in the SNc of the four groups in the head and body (Fig 1A) and body only (Fig 1B) series. For the Saline and Saline-NIr groups of both series, TH\textsuperscript{+} cell number was similar; no significant differences were evident between these groups (p>0.05). For the MPTP groups in both series, TH\textsuperscript{+} cell number was significantly less than in the saline control groups (35-40\%; p<0.001). In the MPTP-NIr groups, TH\textsuperscript{+} cell number was higher than in the MPTP group of the head and body series (~30\%) and also, to a lesser extent, in the body only series (~20\%). These differences reached significance for both the head and body (p<0.001) and body only (p<0.05) series. Although TH\textsuperscript{+} cell number in the MPTP-NIr group was significantly higher than in the MPTP groups, it was slightly lower than the saline groups for both the head and body (~15\%) and body only (~15\%) series. This difference was not significant for the head and body series (p>0.05), but was significant for the body only (p<0.05) series.

![Graphs showing TH\textsuperscript{+} cell number in the SNc in the four groups, in either the head and body (A) or body (B) series. Columns show the mean ± standard error of the total number (of one side) in each group. The symbols in the MPTP groups represent levels of significant difference in number from the Saline groups in each series, while symbols in the MPTP-NIr groups represent those from the MPTP groups; † represents p<0.001 and * represents p<0.05.](Image)
Fig 2. Photomicrographs (A-D) and schematic diagrams (A1-D1) of TH+ cells in the SNC of the Saline (A, A1), Saline-NIr (B, B1), MPTP (C, C1) and MPTP-NIr (D, D1) groups. Similar patterns were seen in the same groups of the head and body series (not shown). Photomicrographs and schematics were from lateral SNC region. The inset (A1) shows the sections, across the rostrocaudal axis (from left to right), from where the SNCs were mapped. In the schematic maps of the SNCs, one black circle represents one cell. All figures are of coronal sections; dorsal to top and lateral to right. Scale bar = 500μm.

Fig 2 shows TH+ cells in each of the groups studied for the body only series, namely Saline (Fig 2A), Saline-NIr (Fig 2B), MPTP (Fig 2C) and MPTP-NIr (Fig 2D) groups. There were clearly fewer TH+ cells in the SNC of the MPTP group compared to the other groups. These trends are illustrated further in the schematics of this figure (Fig 2A1, B1, C1, D1), that show the distribution of the TH+ cells along the rostrocaudal axis of the SNC in the different groups. Note the lesser number of TH+ cells across the SNC in the MPTP group compared to the others.

Conclusions

Our working hypothesis in recent studies8-11 has been that NIR acts to protect midbrain dopaminergic cells by penetrating the cranium and the parenchyma of the brain, to reach the midbrain, there to be absorbed by photoreceptors in the mitochondria of the dopaminergic cells, upregulating protective pathways in stressed cells. Present results direct attention to an additional, perhaps alternative, idea, one that suggests that absorption of NIR by the skin or perhaps by lymph nodes near the skin upregulates a still-unknown response, which can circulate through the body, either as activated cells, or as cytokines released into the blood. It is possible that NIR exerts neuroprotective actions by both direct and indirect actions.

Much work will be needed to test, confirm and elaborate this indirect response. The work of Schwartz and colleagues12, 13 may provide a guide. Their studies adduce evidence of neuroprotection mediated by circulating monocytes and lymphocytes. Although Schwartz et al did not study wound healing, which is the most common focus of studies of LLLT or PBM, nor did they use LLLT or PBM, the concept of a cohort of protective immune-system cells is relevant in the present context.

Qualitatively, body-only and whole-body NIR produced the same effect on MPTP-stressed SNC cells – protection or rescue. Quantitatively, the lesser effect of body only radiation could reflect the loss of a direct, transcranial action or the reduction in skin area irradiated. This question – whether NIR acts both directly and indirectly – will be addressed in many future studies.
References

Modulation of Pain Pattern with Intravenous Laser Blood Irradiation and Enbrel in Juvenile Arthritis

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2 “Al. I. Cuza” University, Iasi, Romania
3 Laser Clinic, Iasi, Romania, laserail_mail@yahoo.com

Summary
The aim of this study was to investigate the effects of intravenous laser blood irradiation (ILBI) on the modulation of the chronic pain pattern in juvenile arthritis, moderate and severe forms, under pharmacological therapy with the biological agent etanercept (trade name Enbrel). Twenty patients (average age 12.4 yrs), diagnosed with active severe forms of arthritis, were included in a randomized placebo controlled study; 12 patients received 5 intravenous laser therapy sessions, repeated monthly for 12 weeks and 8 patients (control group) received only conventional therapy and placebo ILBI. At the end of the study the patients in group I who were treated with intravenous laser had significant improvements in neck pressure pain thresholds, thumb flexion strength and range of motion (p <0.05) compared to the control group.

Introduction
Juvenile idiopathic arthritis (JIA) is the most common chronic inflammatory disease in childhood and can lead to severe disability [1]. Chronic pain has a large and wholly negative impact on the physical and psychological well being of the children and their families. Childhood chronic pain is a modern public health disaster, which is only now coming to light [2]. The goals of any treatment program for juvenile arthritis are: to control inflammation, relieve pain, prevent or control joint damage and maximize joint and body function. Children with JIA have been helped with biological agents, such as alpha tumour necrosis factor (TNF) inhibitors such as etanercept (trade name Enbrel). Unfortunately, drugs such as Enbrel, which suppress the immune system (and thus inflammation related to arthritis), carry side effects including the increased risk of infection [3].

Study Participants
A total of 20 patients (average age 12.4 years) who were diagnosed with moderate and severe forms of JIA and who were receiving pharmacological treatment with the biological agent Enbrel were included in a 12 week randomized placebo-controlled trial. We excluded patients who did not meet the conditions of the JIA criteria (revised by the International League of Associations for Rheumatology (ILAR) 2004), who were: on steroid doses above 10 mg/day, non-steroidal anti-inflammatory drugs, or had a previous history of allergy or sensitivity to light. Participants were permitted maximum doses of methotrexate 0.6 mg/kg once a week with folic acid 5 mg and acetaminophen or opioid analgesics, when required.

Group I (12 patients) received intravenous laser therapy for 5 days consecutively, repeated monthly for 12 weeks. We used a laser device with three diodes that emitted light in the range from red (635 nm), green (532 nm) and blue (405 nm). Power measured at the end of each fibre was 5 mW in continuous mode. Each session lasted 30 minutes (10 minutes for each wavelength, in the following order: red, green and blue).

The remaining 8 patients acted as controls in Group II and received only conventional therapy with Enbrel and placebo ILBI.

Methods
The patients in both groups received treatment with Enbrel at a dose of 0.4mg/kg (maximum 25mg per dose) administered subcutaneously twice a week and individualised physical therapy. The pain pattern and the joint ranges of motion were assessed with two digital devices. The Commander™ Algometer (JTech Medical, Salt Lake City, USA) measured pressure pain thresholds in the cervical area at C2-C3 and the force of thumb
flexion of both hands. The Dualer IQ™ Inclinometer (JTech Medical, Salt Lake City, USA) measured cervical spine flexion, extension, rotation and side tilting. Local research ethics committee approval was obtained. All patients or their parents signed the informed consent.

Data were analysed statistically with the student t-test and Fisher's exact test with a confidence interval of 95%.

Results

Prior to the intervention there were no major differences between the two groups regarding the demographics, clinical and laboratory signs (Table 1).

### Table 1. Initial clinical and laboratory data

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>Group I (n=12)</th>
<th>Group II (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean± SD)</td>
<td>12.7±3.0</td>
<td>12.2±2.9 (NS)</td>
</tr>
<tr>
<td>Sex (%) female</td>
<td>58.3</td>
<td>50 (NS)</td>
</tr>
<tr>
<td>JIA Polyarthritis FR (%)</td>
<td>33.3</td>
<td>37.5 (NS)</td>
</tr>
<tr>
<td>JIA Oligoarthritis extensive (%)</td>
<td>33.3</td>
<td>25.0 (NS)</td>
</tr>
<tr>
<td>JIA Systemic arthritis (%)</td>
<td>25.0</td>
<td>12.5 (NS)</td>
</tr>
<tr>
<td>JIA Psoriatic arthritis (%)</td>
<td>8.3</td>
<td>25.0 (NS)</td>
</tr>
<tr>
<td>Duration of JIA(yrs; mean± SD)</td>
<td>1.7±1.0</td>
<td>1.6±0.5 (NS)</td>
</tr>
</tbody>
</table>

### DISEASE ACTIVITY CHARACTERISTICS

| The number of swollen joints            | 22.16±1.6     | 25.90±1.3 (NS) |
| Average neck pain (C3/C4)²              | 22.16±1.6     | 22.07±1.4 (NS) |
| Average force of flexion thumb²         | 34.25±1.2     | 32.68±1.6 (NS) |
| Average values (%) of muscle strength deficit of the thumb | 24.67 | 25.88 (NS) |
| Morning stiffness (minutes)             | 69.00±1.9     | 66.30±2.1 (NS) |
| CRP (normal < 6mg/dL)                   | 60.40±3.2     | 63.20±1.3 (NS) |

FR = rheumatoid factor; SD = standard deviation; NS = no statistically significant differences (p>0.05)²; Newton’s/1,5 seconds²

At the end of the trial the patients in Group I had significant reductions in the number of swollen joints, improvements in the neck pressure pain thresholds, average values (%) of muscle strength deficit of the thumb, the force of flexion of the thumb, and joint ranges, and of the biological parameters, compared to the control group (Table 2).

### Table 2. The initial characteristics in the end of study

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>Group I (n=12)</th>
<th>Group II (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of swollen joints</td>
<td>11</td>
<td>19 (p = 0.0058)</td>
</tr>
<tr>
<td>Average neck pain (C3/C4)²</td>
<td>45</td>
<td>35 (p = 0.0372)</td>
</tr>
<tr>
<td>Average force of flexion thumb²</td>
<td>48</td>
<td>36 (p = 0.0145)</td>
</tr>
<tr>
<td>Average value (%) of muscle strength deficit of the thumb</td>
<td>13</td>
<td>20 ( p = 0.0070)</td>
</tr>
<tr>
<td>Morning stiffness (minutes)</td>
<td>39</td>
<td>45 ( p = 0.0349)</td>
</tr>
<tr>
<td>CRP (normal &lt; 6mg/dL)</td>
<td>15</td>
<td>25 ( p = 0.0001)</td>
</tr>
</tbody>
</table>

FR = rheumatoid factor; SD = standard deviation; NS = no statistically significant differences (p>0.05)²; Newton’s/1,5 seconds²
Discussion

The goals of the laser blood irradiation treatment included minimizing clinical symptoms such as pain and swelling, preventing joint damage and deformity, and maintaining quality of life in terms of day-to-day activities [2]. Our study was performed on a small group of patients, and confirmed on one hand the results of other international studies of the beneficial effects of conventional therapy with biological agents in moderate and severe forms of juvenile arthritis [4]. We also showed an association between intravenous laser therapy in red, green and blue-violet domains and significant changes to the nociception pattern [5] that allowed earlier physical therapy and remission of the rheumatoid disease [6]. Improvement in pain was paralleled by a significant decrease of the laboratory data (CRP levels) in group II (Table 2). Enbrel and laser therapy were found to be safe and well tolerated in this group of patients. The ILBI may alter the nociception pattern by preferential stimulation of the A-alpha, delta and beta fibres, with the inhibition of the nociceptive C fibres. At the level of the spinothalamic and cortical tract, it may reduce substance P in the intermediate neuron, increase the secretion of enkephalins, block the receptor site of substance P and control the pain gate using hormonal positive feedback [6].

Conclusions

ILBI and Enbrel administered in combination was an effective method of complex therapy, opening a new field in the modulation of the chronic pain pattern in Juvenile Arthritis. Complex intravenous laser blood irradiation is a good option in the field of paediatrics, in the multidisciplinary management of chronic pain in JIA.

References

New Challenges in Treating Pediatric Rheumatic Diseases with Lasers in the Age of Biologic Therapy

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Summary

New advances in the management of pediatric rheumatic diseases include biologic therapy in an attempt to better control: daily persistent and nocturnal pain, abnormal laboratory findings, morning stiffness, systemic features and cortical destruction. Understanding the mechanisms through which laser photobiostimulation acts in pediatric immune–mediated inflammatory diseases (IMIDs) is essential for improving its efficiency, combined with biological therapy in severe forms. Successful treatments may combine the benefits of light at specific frequencies absorbed by the cells with biological therapy in IMIDs.

Introduction

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease of childhood. It is a heterogeneous disease group of unknown etiology with distinct presentation, clinical features, and genetic background [1]. JIA is a complex genetic disease caused by the effects of environmental factors and multiple genes that act in concert to predispose the host to the development of the condition and to determine the different disease phenotypes [2]. Common to all subgroups is the chronic inflammation within synovial joints [3]. Cytokines, a group of modulatory proteins or glycoproteins produced by a wide range of cells in response to a variety of stimuli, are important mediators and regulators of synovial inflammation [4]. Basal and cell-stimulated cytokine levels differ between individuals; both genetic and environmental influences have been shown to play a role in their variability [5]. T-cells, macrophages and B cells all have varying degrees of influence on the pathogenetic mechanisms. Cellular communication during inflammation occurs via direct cell to cell interaction or via soluble mediators. A number of cytokines have been detected over the years such as Interleukins (IL), Interferons (IF), Tumor necrosis factor (TNF), Growth factors (GF), Colony stimulating factors (CSF) and chemokines. These cytokines are of two kinds: pro inflammatory and anti-inflammatory and are responsible for regulating the magnitude, nature and duration of the inflammatory response. The pattern of rise of these cytokines may form an important indicator of disease activity and have future implications for monitoring of patients with IMIDs. The characterization of these cytokines also has an implication for therapy as newer anti-cytokine molecules have become available for treatment of JIA patients [6].

Materials and Methods

The treatment of JIA has been revolutionized by the use of novel biologic agents that have much improved patients’ short-term and, according to early evidence, long-term outcomes. Currently available biologic agents used to treat patients with JIA include TNF blockers, various agents that target IL-1 and IL-6 receptor, T-cell co-stimulation inhibitors and antibodies to B-lymphocyte antigen CD20. These agents are increasingly used early in the course of the disease (often in combination with other immunosuppressive medications) and often for long periods of time, as patients can be difficult to wean from their use. Safety concerns (especially the long-term effects of biologic therapy) are, therefore, being examined more closely. Children with severe forms of JIA could be significantly helped with biologic agents, such as TNF-alpha inhibitors (Enbrel). Unfortunately, side effects of these drugs, which suppress the immune system (and thus inflammation related to arthritis), can have an increased risk of infection. For instance, in 2009, the FDA issued a warning related to the development of malignancies in patients with JIA who had used anti-TNF medications for >2.5 years [7]. Due to recent years’ innovative developments in the pharmacotherapy of JIA with biological agents, the induction of remission has become a reachable goal. Remission will lead to less structural damage and fewer disabilities [8]. As a new biological treatment option, anti-TNF-alpha therapy has shown success in polyarticular JIA patients.
Results and Discussions

Biologic therapy refers to treatment that boosts or restores the ability of the immune system to fight inflammatory arthritis, cancer, and other diseases. The major biologic approaches in clinical use include agents that interfere with cytokine function and those that inhibit the “second signal” required for T cell activation as well as agents that deplete B cells [9].

Scientific studies published a few years ago have documented the effects of visible and infrared light on inflammatory cytokines (immune system messenger molecules) [10,11]. Biological effects of laser therapy on inflammation pathway are: stimulation of macrophages, reducing the number of polymorphonuclear neutrophils, decrease oxidative stress, increasing levels of anti-inflammatory cytokines and decrease the percentage of pro-inflammatory cytokines (Fig.1) [12,13]. Clinical effects are: stimulates tissue repair including collagen, myofibers and cartilage, prevents stalling in the inflammatory phase which can lead to chronic inflammation, decreases sensitivity to pain preventing hyperalgesia common in chronic inflammatory conditions, decreases vasodilation thereby reducing inflammatory edema and tissue swelling [13].

Laser therapy has been proposed as a physical therapy for musculoskeletal disorders and has attained popularity because no side effects have been reported after treatment. A very recent systematic review shows that laser therapy on joints reduces pain in patients [14]. The mechanism for relieving joint pain in rheumatoid arthritis 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 [11].

Conclusions

The goals of any treatment program for juvenile arthritis are: to control inflammation, relieve pain, prevent or control joint damage and maximize joint and body function. Biological agents together with LLLT can modulate inflammatory processes in a dose-dependent manner and can be titrated to significantly reduce acute and chronic inflammatory pain.
References

Effect of digital infrared thermal imaging and cold laser therapy on chronic lower back pain

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Summary

It is not always possible to identify the specific cause of pain and it is for this reason the treatment of any pain, including low back pain (LBP), using traditional methods may be ineffective. In this case series we make use of two technologies, digital infrared thermal imaging (DITI) and low level laser therapy (LLLT) in parallel for the treatment of patients with LBP. DITI was used to identify areas of symptoms and LLLT was used for treating the targeted areas. LLLT is the application of red or near-infrared radiation to an area of pain or injury. DITI was performed twice, once pre-treatment and another post-treatment to demonstrate change in the region of treatment. We recorded the patients’ perception of change in pain using a numerical pain scale (1 = least pain reduction, 10 = greatest pain reduction). Treatment was performed for a period of 4 months. Results from the case series indicate that the DITI and LLLT devices are complimentary to each other for diagnosing, treating and recording the progress of chronic lower back pain.

Introduction

The National Institute for Health and Clinical Excellence defines lower back pain as soreness with or without stiffness in the lower back area, associated with different structures of the back such as joints, disks and connective tissues. Three in 10 Australians lives with chronic pain on a daily basis and 20% of people suffering with chronic pain are suicidal (1). Access Economics (Australia) estimates that chronic pain is the third most costly health problem in most countries, and costs the economy 34.3 billion dollars annually thus having a significant impact not only on the sufferers but also on the broader community.

Low Level Lasers (LLL) have been used to treat inflammation, pain, to support rapid healing of deeper tissues, nerves and wounds and to prevent further tissue damage. Despite numerous positive findings from various in-vitro animal studies and randomized clinical trials, the efficacy of low level laser therapy (LLLT) remains controversial for LBP. This may be due to lack of studies on human subjects. This clinical case series was aimed at determining the combined effectiveness of digital infrared thermal imaging (DITI) and LLLT for the diagnosis and treatment of chronic low back pain patients.

Material

In this case series we used two different technologies: DITI and LLLT.

DITI is used as a diagnostic technique in medicine and rehabilitation sciences in particular for diagnosing latent or intractable forms of pain syndromes such as neck pain, back pain, limb pain and pain associated with cancerous states (2). Some conditions are accompanied by inflammation, which cause increases in vascular flow to the area leading to increases in temperature of the region (2). The highly sensitive thermal imaging cameras and computers capture and produce high-resolution images of these vascular and temperature changes. DITI allows the user to create a body map of temperature changes. We utilise this map for identifying the treatment area. For this purpose, we use an infrared camera manufactured by Meditherm Inc (Florida; USA).

Low level Laser therapy (LLLT) also known as “photobiomodulation” is an application of red or near-infrared radiation (3). The process stimulates a complex series of physiological interactions at the cellular level which accelerates tissue healing, reduces pain and inflammation (4). The process is entirely safe with no sensations or side effects (4). The principle of photobiomodulation by LLLT is similar to photosynthesis in plants. The correct application of the red and near-infrared light reduces oxidative stress, increasing the ATP synthesis in tissues which enhances the cell metabolism and reduces inflammation (4).
Methods

This clinical case series was conducted at the Your Healthy GP (Adelaide; Australia). The clinical method not only focused on treating patients with chronic LBP but also on educating them about their condition and on teaching them techniques to minimize further aggravation. That is, we take a whole body approach. As a part of the education, the patients were taught techniques to help them maintain good posture, which helps to reduce strain on existing structures and accelerate recovery.

The case series included 3 patients, aged between 30 – 75 years of age, who were randomly selected. The selection criterion for choosing patients was as follows:

- Patients experiencing LBP for more than 3 months.
- No medical history of epilepsy, heart condition, blood pressure or vascular diseases.
- No history of psychiatric illness.
- Non-pregnant patients.
- Patients available for the duration of the course of treatment.
- Patients not presently on anti-inflammatory or analgesic drugs.

Procedure:

The diagnosis of chronic LBP was confirmed, which included:

- GP examination, along with full medical history and physical examination focusing on the presenting symptom of LBP
- LLL counselling, where the treatment approach and requirements were explained and participant’s questions were answered or clarified.
- Pre-treatment DITI was performed.

After the diagnosis was confirmed, the second step was to expose the selected patients to a standardised LLL treatment protocol. During the therapeutic sessions LLLT probes of wavelength 830nm and 808nm were used. The total duration of treatment was 4 months; the initial sessions were 50 minutes twice weekly for the first 3 weeks followed by 25 minute sessions twice weekly until the conclusion of the treatment. On completion of the treatment a second thermal image was performed. Patients involved in the study where prohibited from undertaking any other form of treatments such as physiotherapy, massage, chiropractic treatment etc. Patients were also asked to avoid any form of exercise such as running, gym, yoga etc. The patient’s clinical outcomes were self-rated by numerical pain scale; and qualitative comparison of the pre- and post-treatment DITI images by the medical practitioner.

Results and Discussion:

Case 1:

A 74 year old male, with a history of LBP which was limiting his daily activity and movement. The patient suffered an injury whilst playing golf 4 years previously but he sought no treatment at the time. When seen in the clinic, the symptoms had become ‘excruciating’ and was limiting his movements. The patient was a farmer by occupation and his work was suffering as a result of his pain. He had received chiropractic treatment previously with no improvement in symptoms. The patient was prescribed the standardized LLLT for 4 months.

Fig 1a, is the pre-treatment DITI image of the patient, which clearly shows the area of “soft tissue injury” (STI - indicated by the white colour) surrounded by the area of increased temperature (in the hues of orange and red). Fig 1b is the post-treatment DITI image which indicates reduction in the size of the area of increased tissue temperature. The patient reported 70% reduction in the LBP and improvement in range of movement on completing the treatment.
Case B:
A 42 year old female, with a long history of LBP. She could not recollect the cause or beginning of the LBP however, she reported that the LBP worsened after pregnancy. The pain had become worse over a number of years. The pain was causing her grief and insomnia. She had tried different treatments prior to LLLT including physiotherapy and massage. These treatments gave her temporary relief only. The patient was put on the standardised LLLT treatment protocol for 4 months. Fig 2a, is the pre-treatment DITI image of the patient, which reveals 2 locations of symptoms, namely on the upper and mid-back surrounded by large area of increased tissue temperature. Fig 2b is the post-treatment DITI image of the same patient which shows reduction in the area of tissue temperature. On conclusion of the treatment, the patient reported 70-75% reduction in the LBP.

Case C:
A male patient, 51 years old (a fireman by occupation) was examined for chronic LBP. He injured his lower back 2 years ago while lifting heavy equipment at work. His work demands high physical activity further exacerbating the existing injury. Figure 3a is a pre-treatment image of the patient, showing a large area of increased temperature around the neck and the lower back. Fig 3b, which is a post-treatment image of the patient shows complete resolution around the neck and reduction in the temperature around the lower back area. The patient reported 85% reduction in LBP which helped him to improve his work related performance.

Conclusions
This case series has provided an insight into the combined effectiveness of using DITI and LLLT. The two modalities can be used in parallel for diagnosing and treating pain and inflammatory syndromes. Further research with large participant numbers and quantitative image analysis is being undertaken.

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The Role Of Channelopathies In Pain And The Implications For Laser Treatment

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1 Introduction

People affected by chronic pain conditions suffer the additional burden of loss of income and negative impact on daily life activities. Chronic pain patients are often unresponsive to currently available therapies and may benefit from a novel approach to treatment. For example, in cervicogenic headache 25% of patients are unresponsive to physiotherapy treatment [1]. Some chronic pain conditions can be viewed as channelopathies. Channelopathies are acquired or genetic dysfunctions of the sodium or potassium ion channels that underpin the nervous transmission, and several common pain conditions have been recently found to have channelopathies as either primary pathogenesis or as comorbidity, e.g. Headache including Migraine with Aura [2], Familial Hemiplegic Migraine Type I + II (calcium and sodium channelopathies)[3], Polymodal Pain Disorders (potassium channelopathy)[4] Irritable Bowel Syndrome and Erythermalgia (sodium channelopathy)[3].

These conditions often have poor clinical outcomes with current management. However an exogenous agent could potentially modulate the pathophysiology of channelopathy. Therapeutic laser is one such agent. The implications of current research into modulation of sensorimotor and second and third order neurons and its relevance to channelopathic pain are discussed here. We review the mechanisms of laser modulation of nerve excitability [5], neuroendocrine effects [6] and central nervous systems modulation via signalling pathways in the CNS [7]. An understanding of pain channelopathies and their relevance to pain pathways is important for both the treatment of chronic pain. Acquired ion channel dysfunction has also been linked to chronic pain evoked by physical insults. Accordingly, current research in humans and transgenic animals is reviewed here, with particular reference to 1) the role of potassium channels in the modulation of the somatosensory pain system and 2) the known and postulated mechanisms whereby laser can affect this system in channelopathies.

2 Aim

The purpose of this paper is to review recent basic research into the role of nerve channelopathies in chronic pain syndromes, because research over the past few years has pointed to a significant role of neuronal channelopathies in the genesis of chronic pain. Both the general public and researchers are fascinated by disease and detective stories, and channelopathy research is both of these [8].

Channelopathies exist in all neuronal channels in the body, however this paper will concentrate on the channelopathies encountered in patients who are unresponsive to treatment, and seen in primary care centres. The paper also considers previously researched and postulated mechanisms by which laser can modulate pain arising from impaired nerve channels.

3 Channelopathies

Common pain conditions - including chronic neck pain, chronic non-specific back pain, cervicogenic headache, migraine, temporomandibular joint (TMJ) dysfunction, radicular pain, painful arthritis, neuralgia, fibromyalgia and pain associated with hypermobility syndromes - account for a large portion of patients attending pain clinicians, neurologists, physiotherapists, general practitioners and other alternative health professionals. These conditions represent a considerable burden in terms of the associated loss of income and loss of ability to perform activities of daily living.
Increasingly, researchers have investigated the role of impaired ion channels in both the genesis of these conditions and as regards their comorbidity [3, 8].

Channelopathies are diseases caused by disturbed function of ion channel subunits or the proteins that regulate them. The diseases can be either congenital, resulting from a mutation or mutations to the encoding genes, or acquired, often resulting from an autoimmune attack on an ion channel. Although heritable, pain channelopathies associated with somatic mutations can occur.

Neuropathic pain may arise from misexpression of ion channels as a consequence of physical insults, viral infections or toxic drugs [9]. This misexpression of ion channels is potentially reversible, one example being glial cell derived growth factor (GDNF), which can normalize the regulation of Nav 3 expression and reverse neuropathic pain in arousal modes where neurons have been severed [10]. Kullman [11] found that small incremental compensatory actions by other ion channels can also normalize ion function. All ion channels can have the potential for dysfunction, and channelopathy can exist in various forms in peripheral ion channels, mitochondrial ion channels, skeletal muscle, smooth muscle, the spinal cord and in the cortical neurons. That is, all ion channels have the potential to become dysfunctional.

 Dysfunction of ion channels within the spinal cord can result in central sensitisation of pain [9]. The mechanism for this central sensitised pain includes the action of calcium-activated potassium channels which, when impaired by pro-inflammatory mediators cause ion channel hyperexcitability and induced hyperalgesia. If the neuropeptide tyrosine is also blocked from exerting its modifying effect on threshold excitation of the potassium channel, the result is neurogenic pain [12]. Dray also concludes that some inflammatory modifications can produce manifold and complex changes in afferent fibres [12], ranging from overt activation of ion channels to the sensitization of these channels to other stimuli. Examples of these include proinflammatory kinins such as prostanoids and cytokins. In addition, bradykinins can activate B2 kinin receptors and can be involved either directly, or indirectly through the release of prostaglandins from macrophages and leucocytes [12].

Disregulated neural ion channels, which have increased pain threshold sensitivity, are often unresponsive to treatment and have poor clinical outcomes given current management. Chronic pain associated with genetic mutations of the potassium 2 pore leak channels (K2P) include polymodal pain syndrome (K2P2 = TREK mutation) and migraneneurs (K2P18 = TRESK) and other hypothesized TRESK polymorphism headache conditions (such as cervicogenic headache), often do not respond to opioid drugs, physiotherapy [13, 14] radiofrequency neurotomy or to surgery [15]. Acquired channel sensitivity, for example lumbar disc dorsal root ganglion (DRG) sensitivity, or neural dysfunction in ion channels in Guillain-Barre Syndrome, both contribute to neuropathic pain. This is also present in other viral arthropathies such as Ross River Fever [9]. Central sensitivity in the case of chronic fatigue pain processing is mediated through a range of dysregulated channels and cytokine cascades in the DRG and insular and cingulate cortex [16].

Genetic channelopathies are involved in the neuropathic pain in migraine with aura and familial hemiplegic migraine (FHM). It is postulated that other headache types such as cervicogenic headache (CH) may also be a (TRESK) channelopathy. Bovim [17] found similar impairment in pressure threshold sensitivity over C4 unilaterally in CH compared to bilateral impairment in migraine with aura. This feature was absent in both migraine without aura and tension type headache. Other channelopathies associated with increased pain response include hypersensitive pain syndrome, sodium channelopathy [3], irritable bowel syndrome and erythromalgia. In erythromyalgia, congenital sensitivity can be experienced early or late depending on compensatory mechanisms [3].

Hall [14] and Niere [13] have both postulated that pain arising from neural structures does not respond to conventional physiotherapy that is aimed at mechanical or muscle structures. In a recent study of physiotherapist perceptions of unresponsive cervicogenic headache [18], clinicians proposed that neural sensitivity, genetic factors, severe trauma, comorbidities and latency of response to treatment were the main determinants of non responsiveness. Recent studies report the presence of neural sensitivity in chronic neck pain and CH (7-10%) [19, 20] and non-specific chronic low back pain [21]. Hall, Briffa and Hopper [14] suggested sub-classification of neural sensitivity into three categories: 1) peripheral neural sensitization, characterized as exhibiting increased nerve trunk mechanosensitivity; 2) denervation, characterized by signs of nerve conduction deficit; and 3) central sensitization, the mechanism of which has been explained as involving modulation of pain processing centres in the brain, including the insular cortex, cingulate cortex, hypothalamus and pre-frontal cortex [22, 23]. Importantly, abnormal ion channels can be involved in all of these conditions.

3 Mechanisms of Laser Modulation of Channelopathy Pain

Once laser is absorbed [23], the question can be asked as to how laser might modulate the dysregulation of Ion channels that result from congenital and acquired channelopathy? There are several possible actions associated with the bioelectrical and biochemical effects of laser.

Firstly, laser is absorbed by mitochondrial photoreceptors within the membrane, including cytochrome-C-oxidase. This can induce conformational change in enzymes, including Na-K ATPase and the subsequent
production of ATP in mitochondria. Depending on the dose, ATP can be increased or decreased to modulate ion function. A biphasic dose response has been noted [24]. The resultant increase in mitochondrial respiration can also increase reactive oxygen species (ROS) and modulate nitrogen oxide (NO) production, which can in turn regulate signal transduction in the nerves [7, 25].

Secondly, laser can directly block the axonal transport of mitochondria and influence mitochondrial membrane potential [26]. It also blocks retrograde flow of depleted mitochondria so that they are unable to produce ATP. This is accomplished by a change in neuronal architecture, varicosity formation, mitochondrial clustering and microtubule disarray that results in conduction block [27]. In this work, Chow also noted that 830 nm laser exerts pain-relieving effects via the PNS with nociceptive specific inhibition, and further proposed that absorption of laser energy and its transduction into electrochemical and/or electro-physical events triggers a secondary cascade of cell specific events, a sequence that has also been suggested by Karu and colleagues [28].

Thirdly, laser can affect neuro-endocrine modulation. Shimoyama [6] found that LLLT depresses sympathetic ganglionic transmission due to the hyperpolarisation of ganglionic cells, as part of its mechanism of action. In this work, there was a direct effect on the neurotransmitter release or its receptor uptake due to an unknown mechanism. He also noted a biphasic effect.

Fourth, laser has the potential to modulate the nervous system more broadly. Evidence for more a systemic effect of laser has been demonstrated in studies by Tuby [29] and Oron [30], where effects on areas distal to the irradiated parts have been observed. Chow [27] hypothesised that there would be a reorganisation of neurons reflecting neuroplasticity and adaptive change in the dorsal root ganglion (DRG), which produces an altered response to nociceptive input. There would be a flow-on effect by this modulation to the second order neurons, and a further modulation of the ascending and descending pain pathways.

We need to ask: why might laser be effective here, in terms of these underlying mechanisms, when other pain relieving measures (opioid drugs, physiotherapy) are not working? One possibility is the view that laser has the potential to modulate the nervous system through an integrated mechanism that operates systemically through action on photo-receptors, such as cytochrome C oxidase, and indirectly through action of enzymes such as tyrosine hydroxylase, which are present in the peripheral, spinal and central nervous system ion channels [32]. These ion channels have their ionic gradients established by Na⁺ K⁺ ATPase and are regulated by calcineurin and thyroid hormones. Recent research has found a role for K⁺ ion channels to integrate a neural response to nociception [31]. Two pore K leak channels (K2P) have been found to be polymodal neural signal integrators, that is, they are important in responding to multiple stimuli.

### 3.1 Laser and the modulation of the somatosensory nociceptive and pain system in channelopathies

#### 3.1.1 The role of K⁺ channels in nociception

K2P are important in modulating neural sensitivity [31]. They act as both ion channels and receptors to sense mechanical, thermal and chemical stimuli via their terminals at nociceptive sensory nerve fibres, and they also determine nociceptor excitability and conductivity. K2P sensory receptors include: K2P ion channels, transient receptor potential channels (TRPC), acid sensory channels (ASIC), P2X receptors and voltage gated K⁺, Na⁺ and Ca²⁺ channels. Voltage-gated Potassium channels are regulated by ligand residues that are completely dependent on the availability of tyrosine. K2P leak channels contain a selectivity filter with tyrosine at its core.

All of these K2P channels operate through somatosensory neurons to determine excitability, synaptic function and neural responsiveness from distant sites in the body to higher processing centres of the brain [31]. Plant [31] concluded that K2P channels are important as polymodal signal integrators and respond to “neurogenic and immune inflammatory signalling pathways, feedback control of neurotransmitter release in the CNS” and are likely to regulate somatosensory neurons in both acute and chronic pain. These feedback control mechanisms may be important in neuronal homeostasis and possibly have a role in fine-tuning neural regulatory mechanisms.

#### 3.1.2 The role of laser in modulation of K2P channels

The author proposes that low level laser can modulate K2P channels via the photonic activation of tyrosine hydroxylase and the subsequent increased production of tyrosine [32, 33] as well as its secondary modulation of the melanocortins and their receptors [34]. Tyrosine(Tyr) is formed by melanosomes during the formation of pigment cells and differentiated sympathetic neurotransmitters. Tyrosine hydroxylase is also the rate-limiting enzyme for the receptor to open the ligand gate of voltage K⁺ channels. The modulation of the melanocortin pain system occurs through modulation of pro-opiomelanocortin (POMC) protein and its derivatives, adrenocorticotropic hormone (ACTH) and alpha melanocyte stimulating hormone (αMSH). These
hormones have been found to be affected by LLLT in a study by Laakso [34] on the modulation of myofascial pain.

Many molecules incorporating Tyr are present in the K2P neural system, from the periphery to the cortex, and include neuropeptides (such as neuropeptide tyrosine (NPY)), hormones (such as α-melanocyte stimulating hormone (αMSH), melanin concentrating hormone (MCH)), neurotransmitters (such as acetylcholine, epinephrine, norepinephrine, serotonin), melanocortin receptors (MCIR), signalling molecules (such as Tyr, TyrP1, MITF), selectivity filters (K2P selectivity filter) and ligand molecules (K voltage channels NPY).

These K2P channels are electrically silent [31] and laser may modulate their action by several mechanisms, such as
1. Direct change of membrane potential through the direct stimulation of ATPase
2. Modulating the K voltage channels including (NPY as ligand) [31, 35]
3. Transforming K2P channels by Tyr involvement in the sumoylation of the leak channel [31].
4. Changing mitochondrial fast axonal flow [27]
5. Modulating the TRPC receptors, [36, 37]
6. Modulating αMSH [34] and the Melanocortin and β opioid system, which prevents NF-KB activation [38]
7. Influencing the action of tyrosine hydroxylase [32]
8. Modulating the release of neurotransmitters containing Tyr (acetylcholine, epinephrine, norepinephrine)
9. Regulation of NPY as a signalling molecule acting on second order neurons and influencing ion channels in the brainstem, hypothalamus, insular and cingular cortex, including the release of BDNF [39]
10. Modulating the selective filtering of melanin specific receptors (Melanocortin MC3 and MC4 [38], Y1 + Y2 receptors the ligand responses to a single amino acid tyrosine) [40]

### 3.2 Further modulation of the somatosensory nociceptive and pain system in channelopathies

A further relevant mechanism is the potential regulation of ion channels by endogenously occurring photons [37]. The argument here is that the body cannot function fully by nerve electrical transmission alone, since this mechanism is simply not fast enough [37]. Recent research by Rahnama and colleagues has supported this idea: “synaptic transmission and axonal transfer of nerve impulses are too slow to organize co-ordinated activity in large areas of the central nervous system” [41]. Since modulation of the somatosensory system can occur with laser, it may be that an underlying endogenous photonic mechanism of self-regulation exists.

#### 3.2.1 Critical recent research papers informing the channelopathy modulation hypothesis

A more efficient neural transmission process is proposed wherein photonic quantum transference of information is present. The first person to consider photonic neural transmission was Cope, who postulated in 1973 that mitochondrial lipid membranes acted as resonance chambers and stored an IR standing wave [27, 42]. He postulated that the storage of infrared as standing waves is possible within mitochondrial lipid membranes, and that it involves redox potential and energy transduction.

This mechanism was further developed by Albrecht-Buehler in 1992, in his conceptual outline of cellular vision. In a study using hamster kidney cells, the cells were inoculated on one side of a glass film, the opposite side of which was covered with a 2-3 day old confluent layer of BHK cells. Seven hours after attaching and spreading in the absence of visible light, most of the cells had aligned their long axes in the direction of the whorls of the confluent cells opposite. A thin metal coating on the glass films inhibited the effect. In contrast, a thin coat of silicon on the glass did not inhibit the effect, suggesting that it was caused by red or near-infrared light. Albrecht-Buehler concluded that “biophotonic signals generated by light stimulation consist of two components: action and background biophotons. A possible explanation for this observation is that external light stimulation might generate action biophotons, being able to conduct along the neural fibers, and result in an increase in biophotonic activity”[42].

The next significant evidence was provided by Thar and Kuhl, in 2004 [43], who speculated that there is long range interaction between mitochondria mediated by electromagnetic radiation. They suggested that “Chemiluminescence from mitochondria originates generally from excited molecules generated by the oxidative metabolism of mitochondria.” The ability of these organelles to provide light guiding structures is made possible by the cellular network of microtubules and filamentous mitochondria. They hypothesize that the light generated internally within the mitochondria, would be “emitted at both ends of the mitochondria with high temporal coherence and high directivity i.e., mitochondria would act like lasers”.

The generation of endogenous photons has been noted in a number of biological systems, including bacteria, yeast, and protozoa, which produce weak electromagnetic radiation or light from exergonic chemical reaction [29, 44]. Interestingly, it has been well known for many years that mitochondria originated in cells from
bacterial symbiotes. Populations of bacterial cultures are able to influence growth parameters of other cultures, by photonic emissions and cell to cell communication through a clear glass window [45].

The concept of neural communication by photons was further elaborated by Bernroider in 2005 [46] arguing that quantum coherence may be sustained in ion channels long enough to be relevant for neural processes, and that the channels could be entangled with surrounding lipids and proteins and with other channels in the same membrane. Ion channels regulate with electrical potential across the axon membrane, and thus play a central role in the brain’s information processing [46]. This author has also proposed that K2P channels are the mechanism for this quantum coherence and the theory involves binding pockets where two K⁺ ions are trapped in the selection filter of the closed ion channel.

The theory has been investigated by Sun and colleagues in 2011 [47] who used LED light to stimulate biophoton production and demonstrated the conduction of the biophotons along nerves using “in situ biophoton autography”[47] with silver granules. These researchers speculate that these biophotons act as neural communication signals, proposing a protein to protein transmission. They also found that “different spectral light stimulation (infrared, red, yellow, blue, green and white) at one end of the spinal sensory or motor nerve roots resulted in a significant increase in the biophotonic activity at the other end”[47] with sensory nerves more sensitive to infrared and white light and motor nerves more sensitive to red and white light.

4 Conclusion

Current research indicates that a proportion of chronic pain can be associated with dysregulation of ion channels in various parts of the body- peripheral nerves, mitochondria, trigeminal nucleus, DRG and CNS- and that the modulation of these systems may be important in understanding the effect of laser on chronic pain. Research evidence supports the concept of channelopathies as a cause of chronic pain and suggests mechanisms by which laser may act to modulate pain pathways and achieve pain reduction for patients. Current research into the critical role of electrically silent K2P channels in the fine-tuning of excitation of sensory neurons, from the periphery to the cortex, indicates that they play a crucial role in integrating the function of the nervous system. It is thus possible that K2P, and therefore much of the nervous system function, is modulated by hormones and enzymes in the melacortin system as well as neuropeptide molecules. The agents of this modulation are as yet unknown but may possibly involve endogenously produced photons. The mapping outlined here represents a mechanism of laser success in the treatment of unresponsive conditions that exhibit neural dysregulation.

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Laser irradiation in the visible wavelength stimulates wound healing in vitro

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Summary

Low intensity laser irradiation (LILI) has been applied to stimulate healing in a variety of conditions, such as diabetes. This study aimed to determine the biochemical and molecular responses to LILI in the visible red spectrum. Various cell culture models, namely wounded, diabetic wounded and hypoxic/ischemic were used. Models were exposed to visible red laser light (632.8, 636 or 660 nm) at a fluence of 5 J/cm². Post-irradiation, the effect on cells was studied. Laser irradiation was shown to have a positive effect on stressed cells in vitro. There was an increase in migration, cell survival and proliferation, mitochondrial activity, secondary messengers and collagen. A decrease in cytotoxicity, DNA damage and pro-inflammatory cytokines were also seen. LLLT offers an alternative wound healing therapy. At a biochemical level there was a positive effect on cells, with stressed cells being pushed into cell survival pathways.

Introduction

Since the invention of the laser, its application in the health sector has been studied in an attempt to discover effective alternative treatments. Low intensity laser irradiation (LILI) has been applied to stimulate healing in a variety of conditions, such as diabetes. Although this therapy is in use worldwide, the full cellular and molecular mechanisms of action are not fully understood.

Mitochondria are involved in cellular respiration and the formation of adenosine triphosphate (ATP) via the electron transport chain (ETC). Mitochondria are also involved in programmed cell death. Light in the visible red spectrum (620-750 nm) is thought to be absorbed by mitochondria. When photon energy is absorbed by mitochondria, a cascade of events is set into action, including ATP synthesis, changes in membrane permeability and mitochondrial membrane potential (MMP), and release of nitric oxide (NO) and intracellular calcium ($Ca^{2+}$). It has been postulated that the photon energy is absorbed by the mitochondrial enzyme, cytochrome c oxidase (complex IV), which begins vibrating and alters the redox reactions resulting in an increase in electron transfer which in turn results in increased oxidative metabolism and ($Ca^{2+}$). Karu et al., showed that irradiation at 632.8 nm causes the reduction or oxidation of cytochrome c oxidase and this is dependent on the initial redox state of the enzyme at the time of irradiation. A number of studies have shown that LILI affects mitochondrial metabolism by measuring MMP, oxygen consumption, ATP and mitochondrial enzyme activity.

Secondary messengers are short-lived intracellular molecules which relay signals to intracellular targets often leading to rapid alterations in cellular enzyme activity. Adenosine 3',5'-cyclic monophosphate (cAMP) and ($Ca^{2+}$) are examples of such messengers. cAMP is generated from ATP and is involved in the phosphorylation of cellular proteins. ($Ca^{2+}$) regulates many processes and has a direct effect on cellular motility. Laser light is absorbed by photoacceptors which lead to changes in membrane permeability which in turn trigger the release of secondary messengers which initiate a wide variety of biological processes.

Materials and methods

Human skin fibroblast cells (WS1, ATCC CRL1502) were grown according to standard culture techniques. For experiments, $6 \times 10^5$ cells were seeded into 3.4 cm diameter culture plates. A wound model was achieved through the central scratch method, whereby a confluent monolayer of cells was scrapped with a sterile 1 ml pipette. A diabetic model was achieved by continuously growing cells in minimal essential media (MEM) containing an additional 17 mMol/L glucose. A state of hypoxia/ischemia was induced in vitro by culturing cells for 24 h in serum free media followed by a 4 h incubation in an anaerobic atmosphere. Cells were irradiated from above, with the culture dish lids off, and in the dark using a 632.8, 636 or 660 nm laser with a fluence of 5 J/cm² (Table 1). Unirradiated cells were sham-irradiated and used as controls. Cells
were incubated for variable periods post-irradiation, depending on the parameter being measured. The study design is summarized in Table 2.

**Results**

Irradiation of wounded and diabetic wounded cells at wavelengths in the visible red spectrum resulted in hastened cell migration and wound closure. Hypoxic/ischemic cells showed considerable cellular damage, with cells appearing irregular in shape, rounding off and detaching from the tissue culture plate. Post-irradiation, these cells began to regain their normal cellular morphology, with fewer cells detaching and less spaces seen between cells.

Table 1 Laser parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wavelength (nm)</th>
<th>Light source</th>
<th>Wave emission</th>
<th>Spot Size (cm²)</th>
<th>Power output (mW)</th>
<th>Power density (mW/cm²)</th>
<th>Irradiation time (s)</th>
<th>Energy density (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>632.8</td>
<td>Helium-Neon</td>
<td>Continuous wave</td>
<td>9.1</td>
<td>20</td>
<td>2.2</td>
<td>2,273</td>
<td>5</td>
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<tr>
<td></td>
<td>636</td>
<td>Diode laser</td>
<td>Continuous wave</td>
<td>9.1</td>
<td>95</td>
<td>10.44</td>
<td>479</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>660</td>
<td>Diode laser</td>
<td>Continuous wave</td>
<td>9.1</td>
<td>100</td>
<td>11</td>
<td>455</td>
<td>5</td>
</tr>
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</table>

Table 2 Study design (n=4).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Assay</th>
<th>Data Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Cellular migration</td>
<td>Light microscopy</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Caspase 3/7</td>
<td>Luminescence</td>
</tr>
<tr>
<td>Viability</td>
<td>Trypan blue staining</td>
<td>Light microscopy</td>
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<tr>
<td></td>
<td>ATP</td>
<td>Luminescence</td>
</tr>
<tr>
<td>Proliferation</td>
<td>XTT</td>
<td>Colorimetric (A₄₅₀ nm)</td>
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<tr>
<td></td>
<td>MTT</td>
<td>Colorimetric (A₅₅₀ nm)</td>
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<td></td>
<td>VisionBlue</td>
<td>Ex/Em 560/595 nm</td>
</tr>
<tr>
<td></td>
<td>Basic fibroblast growth factor</td>
<td>ELISA (A₄₅₀ nm)</td>
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<tr>
<td></td>
<td>(bFGF)</td>
<td></td>
</tr>
<tr>
<td>Cytokines</td>
<td>Alkaline phosphatase (ALP)</td>
<td>Colorimetric (A₄₀₅ nm)</td>
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<tr>
<td></td>
<td>Interleukin (IL)-6</td>
<td>ELISA (A₄₅₀ nm)</td>
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<tr>
<td></td>
<td>Interleukin (IL)-1-beta (β)</td>
<td>Flow Cytometry</td>
</tr>
<tr>
<td></td>
<td>Tumour Necrosis Factor-alpha</td>
<td>ELISA (A₄₅₀ nm)</td>
</tr>
<tr>
<td></td>
<td>(α)</td>
<td>Flow Cytometry</td>
</tr>
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<td>Mitochondria</td>
<td>Complex I-V activity</td>
<td>Enzyme kinetics</td>
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<td>Complex I-IV transcription</td>
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<td></td>
<td>Membrane potential</td>
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<td>cAMP</td>
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<td>Secondary messengers</td>
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<td>DNA damage</td>
<td>Comet assay</td>
<td>Fluorescent microscopy</td>
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<tr>
<td>Cytotoxicity</td>
<td>Lactate dehydrogenase (LDH)</td>
<td>Colorimetric (A₄₀₀ nm)</td>
</tr>
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When normal, wounded, diabetic wounded or hypoxic cells were irradiated at 636 nm with a fluence of 5 J/cm² there is an increase in cellular viability and proliferation, and a decrease in caspase 3/7 activity, as well as the inflammatory cytokines tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-1β. Hypoxic cells
showed a significant increase in IL-6. Wounded cells showed no significant change in DNA damage. Irradiated normal cells showed a significant increase in ATP. Quantitative real-time reverse transcriptase (RT) polymerase chain reaction (PCR) showed that COX6C, ATP5F1, NDUFA11 and NDUFS7 were significantly up-regulated in wounded fibroblast cells. COX6B2, COX6C and PPA1 were significantly up-regulated in diabetic wounded cells, while ATP4B and ATP5G2 were up-regulated in ischemic cells. When left to incubate for 48 h post laser irradiation, diabetic wounded cells showed an increase in viability and proliferation. The same cells showed a significant increase in collagen type I when left to incubate for 48 or 72 h. Exposure of cells to irradiation at 632.8 nm resulted in increased MMP in wounded and hypoxic cells. Wounded cells showed an increase in ATP and cAMP while wounded and hypoxic cells showed an increase in (Ca2+). There was no significant change in lactate dehydrogenase (LDH), and hence cytotoxicity, in wounded, hypoxic and diabetic wounded cells. Diabetic wounded cells showed a significant increase in cellular proliferation (bFGF, ALP, IL-6), and no change in LDH and DNA damage.

Conclusion

Irradiation of human skin fibroblasts with visible red laser light positively affects stressed cells. There was increased migration of cells and complete wound closure in irradiated wounded models, and normalisation of cellular features in stressed, ischemic/hypoxic models. This may be as a result of the increase in (Ca2+)i. Mitochondria are known as the powerhouse of the cell and are involved in a number of metabolic pathways, such as apoptosis, cellular differentiation, division, growth and signaling, and more importantly the production of ATP through the ETC. There is a definite influence on cellular mitochondria. LILI increases complex IV (cytochrome c oxidase) enzyme activity resulting in increased ATP. Irradiation not only affects mitochondrial enzymes directly, but also stimulates the up-regulation of genes involved in the ETC and oxidative phosphorylation. NDUFA11 and NDUFS7 are involved in complex I (NADH; ubiquinone oxidoreductase), COX6B2 and COX6C in complex IV (cytochrome c oxidase), PPA1 and ATP5F1 in oxidative phosphorylation, and ATP4B and ATP5G2 both encode subunits of ATP synthase. There is an increase in MMP, (Ca2+), ATP and cAMP.

Irradiation of stressed fibroblast cells to visible red laser light at a fluence of 5 J/cm2 does not produce any further cellular and nuclear damage, nor induce apoptosis, as seen by the non-significant changes in LDH, DNA damage and caspase 3/7 respectively. There is a decrease in inflammatory cytokines (TNF-α and IL-1β) and an increase in cellular viability and proliferation.

Collagen is a major structural protein in the extracellular matrix (ECM) and is essential in wound healing. There is a decrease in collagen in diabetic wound healing, and this is due to decreased production and or increased destruction. Irradiation of diabetic wounded cells to 660 nm stimulates collagen type I production. LILI at a fluence of 5 J/cm2 and a wavelength in the visible red spectrum positively effects wound healing in stressed models, normalises cellular function, and directs cells into cell survival pathways.

References


Managing Complex and Recalcitrant Wounds with Low-Intensity Laser Therapy

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Abstract

Background: Despite many advances in conventional wound care, the treatment of complex persistent lesions continues to be problematic with regard to effective healing. Whether they are aggravated by chronic illnesses, compliance issues or inappropriate wound care practices, many patients suffer from recalcitrant lesions. Laser Therapy has been proven to be highly effective in resolving these wounds and has been documented to accelerate wound healing and tissue repair.

Purpose: To evaluate and document the progress of patients with intractable wounds previously treated by traditional methods, including topical applications, antibiotics, dressings, etc. Measurements include circumference and depth of wound, rate of healing and visual pain analogue.

Methods: Patients with complex wounds including peripheral arterial occlusive disease, diabetes, post-surgical wounds, osteomyelitis, and infected post-surgical incisions were evaluated and photographed before, during and at the conclusion of Laser Therapy. The protocol consisted of the application of a red (660 nm) light-emitting diode (LED) array (750 mW, 10 mW/cm²), infrared (840 nm) LED array (1500 mW, 20 mW/cm²), red laser (660 nm, 75 mW, 750 mW/cm²) and infrared laser (830 nm, 180 mW, 1800 mW/cm²).

Results: Wounds that were enlarging or unresponsive to prolonged periods of conventional wound care demonstrated a significant response to Laser Therapy in terms of neovascularization, marginal epithelialization, granulation and collagen formation at an accelerated rate.

Conclusion: Laser Therapy is a specific approach leading to the resolution of the inflammatory process and thereby enhancing healing, even with the most problematic lesions.

Keywords: laser therapy, LILT, wounds, ulcers, diabetic neuropathy

Introduction

The management of wounds continues to challenge all medical disciplines involved in the process. Typical etiological factors causing ulceration include: atherosclerosis, systemic diseases such as diabetes mellitus, pressure induction, thrombophlebitis, venous stasis, post-surgical complications, burns and trauma often complicated by infection.

Current conventional approaches to wound healing involve daily visual inspection of the wound, strategies to off-load pressure, the improvement of venous and lymphatic drainage, debridement, regimens of antibiotics, vasodilators in problematic arterial circulation cases, frequent dressing changes and the utilization of a hyperbaric chamber⁴. The annual cost of providing products and services to patients with wounds is ~USD $200 billion per year worldwide², moreover conventional methods of wound management are frequently unsuccessful and may result in amputation.

In Canada, up to 2 million patients have diabetes and within the next year, ~7.2-10% (i.e. approximately 144,000 patients) will develop ulcers of the lower extremity⁵; 3500 will require amputation³. According to National Diabetes Statistics in the United States of America, over 82,000 amputations are reported on an annual basis⁶. In addition, within 3-5 years of the first amputation, the probability of a second can be up to 50%, with a mortality rate of 39-68%⁷.

Secondary to the lack of arterial circulation, dressings can compromise tissue oxygenation thereby causing wound dimensions to increase, regardless of the therapy applied. Tissue titer of antibiotics is invariably insufficient peripherally to destroy resistant bacteria⁸. This may also be a factor in the mutation and the proliferation of anaerobic organisms. The use of antibiotics may be helpful in the early stages, but wounds that persist and continue to enlarge requires the implementation of proven alternative solutions designed to improve the patient’s overall condition without causing further harm.
Low Intensity Laser Therapy (LILT) is not only non-toxic and non-invasive\(^7\), it is also clinically effective for an extensive number of pathologies including the healing of dermal ulcers\(^8\). Photon irradiation within a specific therapeutic window initiates a variety of positive physiological responses\(^9\). It has been suggested that LILT in the treatment of wounds has substantial effects on inflammatory infiltrates, thereby accelerating resolution. Studies of \textit{in vivo} wounds found that Laser Therapy not only healed wounds more rapidly\(^10\), but also formed thicker layers of epithelial cells than found in normal skin. This is also associated with the regeneration of rich networks of small blood vessels, in close proximity to the epithelial layer (i.e. angiogenesis)\(^11\). Laser Therapy has also been shown to stimulate the transcriptional level of collagen gene expression; consequently, increasing the collagen and procollagen mRNA levels in irradiated wounds. In particular, the infrared irradiation has been shown to enhance the quality and texture of the dermis\(^12\).

Overall, these morphological analyses suggest that the improvement of dermal repair, represented by the quantity and structural quality of connective tissue and cells, can be accomplished utilizing Laser Therapy.

**Methods**

1.1 \textbf{Laser Therapy Treatment for Wounds}

The objective was to determine the clinical outcomes of Laser Therapy applied to patients with intractable wounds previously treated with conventional methods i.e. the application of topical medications, antibiotics and dressings. Measures included wound dimensions, rate of healing and pain score.

The BioFlex Laser Therapy system was used on all patients treated (Meditech Laser Rehabilitation and Wound Care Clinic, Toronto, Canada). The treatment regimen utilized in this study consisted of a four step approach. The steps were delivered using the superluminous diode (SLD) arrays and laser probes as indicated below:

1) Red light (660 nm) using a flexible GaAlAs 180 diode array of SLDs (750 mW)
2) Infrared light (840 nm) using a flexible GaAlAs 180 diode array of SLDs (1500 mW)
3) Red laser probe (660 nm) using a single AlGaInP laser source focused on the basic pathology (75 mW)
4) Infrared laser probe (830 nm) using a single GaAlAs laser source focused on the basic pathology (75 - 200 mW)

As a general rule, as much therapeutic value as possible is obtained with each protocol setting. Parameter settings including frequency, duty cycle and duration were gradually increased, based on the clinical response. The treatment program also stresses the following measures:

1) Leave wounds open to the atmosphere. Dressings often act as irritants and prevent moisture from escaping. In addition they reduce oxygenation.
2) Discontinue all medications except analgesics p.r.n. and those specific for systemic disease (i.e. insulin).
3) Elevate the limb as much of the time as possible, particularly if edema is present.
4) Protection of the ulcer bed using a device to prevent contact (off-loading and drainage strategies).
5) In the early phase, when devitalized tissue and bacteria are still present, cleanse gently using Q-tips soaked in hydrogen peroxide qid.
6) Compress with warm saline as much of the time as possible.
7) Laser Therapy daily.

**Results**

Three patients with complex wounds including vascular/diabetic ulcers, post-surgical wound with osteomyelitis and infected post-surgical incisions were evaluated before, during and at the conclusion of treatment. For each patient, the diagnosis, medical history, physical examination, treatment and progress photographs at each stage depict dimensions and describe progress.

1.1 \textbf{Case Profile 1}

\textbf{Diagnosis:} Non-healing dermal ulcer (osteomyelitis of the fibula)

\textbf{Medical History:} The patient is a 32 year old police officer who sustained significant trauma to the left ankle in a motorcycle accident 8 months prior to presentation. Following the initial trauma, he underwent surgical reduction and internal fixation of the fractures. The plate and screws utilized for stabilization were removed 5 months subsequent to the initial injury. A dermal ulcer overlying the injury site did not heal, despite prolonged immobilization and multiple therapies including vacuum dressings, antibiotics, analgesics, etc.
Physical Examination:
An ulcer extended over the lateral aspect of the left ankle. Moderate edema of the ankle and foot were noted. Pain restricted the range of motion of the ankle to a minimum and precluded weight bearing. With regard to the ulcer, there was no evidence of epithelialization or granulation tissue formation in the ulcer base. Two sinuses present in the ulcer bed revealed the discharge of purulent material (Fig. 1).

Discussion:
The patient received 30 Laser Therapy sessions over the course of 12 weeks. All medications except analgesics were discontinued at the initiation of Laser Therapy. Following 10 therapy sessions, the sinuses draining into the ulcer bed were covered by granulation tissue and discharge ceased. Epithelialization centrally directed from the wound margins progressed slowly. At the termination of treatment, the left ankle presented a relatively normal range of motion accompanied by a normal gait. The patient was asymptomatic and the ulcer had healed completely.

Fig 1. Non-healing Dermal Ulcer with accompanying osteomyelitis of the fibula.

1.2 Case Profile 2

Diagnosis: Pre-gangrenous foot – multiple ulcers secondary to Diabetes Mellitus

Medical History: The patient is a 50-year-old female who developed gangrene after several months of conventional wound care including antibiotics, topical ointments and dressings. She was seen by a vascular surgeon who referred her to a physician using hyperbaric oxygen therapy. She was advised that she would have to wait 6 months for hyperbaric treatment at a local hospital. She was also seen by an infection specialist and was prescribed 8 weeks of IV Ertapenem 1000 mg. Amputation of the lower extremity was recommended by an orthopedic surgeon.

Physical Examination: Gangrene was noted over the distal phalanx of the first toe. Edema, cyanosis, tenderness were noted to be affecting all the toes. The entire foot was cold and the joints immobile. There was no sensation in the toes. Pain was present 24/7, excruciating and intolerable.

Discussion: A course of Laser Therapy was administered over several months. Complete healing of the forefoot pathology was achieved and amputation of the lower extremity was avoided. Colour, temperature and the range of motion of all joints was returned to normal. The patient was able to resume her job. Arterial circulation was substantially improved and resulted in enhanced sensation of the foot. She continues to do well 4 years post therapy.
1.3 Case Profile 3

**Diagnosis:** Infected post-surgical incision of the right foot subsequent to open reduction and internal fixation of a right calcaneal fracture

**Medical History:** The patient is a 41-year-old male who sustained a comminuted fracture of the right calcaneum while vacationing in Europe. He was placed in a short leg cast. Post-cast removal, his fracture was re-evaluated and he underwent open reduction and internal fixation of the fracture, in addition to a peroneal tendon release. He smoked over 1 pack of cigarettes a day and wound healing was exceedingly slow. He was restricted to a non weight bearing state for almost 12 weeks post surgery. Despite this, the wound failed to heal. The patient presented at our clinic 4 weeks post surgery; however, still had significant pain, edema and non-healing of the wound.

**Physical Examination:** An L-shaped incision was noted accompanied by non-healing ulcer which was infected. Arterial circulation was inadequate.

**Discussion:** The patient had 12 Laser Therapy treatments over a period of 3 months. Although his compliance was irregular, the wound healed completely with an excellent functional result. Granulation tissue from the edges of the wound was visible within 2 weeks of initiating treatment. The wound reduced in size from 7.27 cm² upon initial assessment to 1.85 cm² after 7 treatments.
Conclusion

It has been well documented that Laser Therapy accelerates and resolves the inflammatory process and is therefore instrumental in cellular proliferation. The process involves increased proliferation of collagen, elastin, fibroblasts and keratinocytes. In addition, Laser Therapy generates new blood vessel formation, thereby improving microcirculation which enhances the oxygenation levels in the wound and surrounding tissue. Chronic ulcers are able to transition to an acute inflammatory state and permit the resolution of the ulcer through re-epithelialization of the wound margins.

Laser Therapy is a therapeutic technology that not only expedites the inflammatory process but also enhances tissue healing, even with the most challenging wounds. The utilization of the Meditech Wound Care Programme has resulted in the resolution of chronic wounds that did not respond to conventional methods.

References


Modifying laser induced shock waves for use in clinical endodontics

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Abstract

Background: Explosions and implosions generated by lasers in fluid-filled root canals can generate shock waves that can create a shear stress along the root canal walls, which can enhance removal of smear layer and biofilm. When used with sodium hypochlorite and EDTA, lasers can increase the efficiency of debridement and disinfection of the complex root canal anatomy. However, the use of conventional tips with these irrigating solutions poses a risk of driving the fluid past the root apex and causing postoperative complications. The purpose of this study was to evaluate the shockwave patterns of a new side-firing honeycomb tip. 

Methodology: Clear plastic replicas of single tooth root canals were used to study fluid movements. 200-micron diameter plain tips, tube etched conical tips and honeycomb tips were used with a 980nm diode laser at power settings of 1, 1.25, 2 and 3W in continuous mode for 15 seconds. To record the fluid movement, a digital camera was attached to a microscope and the resin replica was backlit to achieve adequate image quality in terms of exposure. 

Results: The modified honeycomb tips generated shockwaves directed at the walls of the root canal. Both the conventional plain fibres and the conical tips created shockwaves that were directed largely in a forward direction; however shockwaves from the conical tip were also directed onto the walls of the root canal apical to the tip.

Conclusion: The use of modified honeycomb tips may help enhance laser-assisted disinfection and debridement with lower risk of extrusion of fluids past the apex.

Key words: Modified laser fibers, endodontics, lasers, shockwaves, cavitations

Introduction

The primary goal of endodontic therapy is to ensure the elimination of microorganisms from the complex root canal system. Smear layer, a layer of debris on the surface of dental tissues following the use of cutting instruments (1) has been a subject of considerable debate and controversy, both in endodontics and in restorative dentistry. Overall, the removal of smear layer is strongly recommended by most authorities (2, 3). Presently, a number of instrumentation techniques and irrigants are used to achieve not only bacterial kill but also adequate removal of smear layer and debris (4-6).

Ideally, the agents used to remove smear layer should target both its organic and inorganic components (7). The most widely used agents are preparations based on EDTA (4, 5). Ultrasound, in the form of endosonics has also been used in combination with various irrigants (6). It is believed that endosonics enhance smear layer removal through its induction of cavitation-related shock waves in a fluid environment. Cavitation has also been reported as a possible mechanism that enhances the removal of smear layer and debris in root canals treated with lasers. Previously, it has been shown that erbium (Er:YAG and Er,Cr:YSGG) laser induced shockwaves in endodontic irrigants such as EDTA could enhance the removal of smear layer and debris (8). It has also been shown that the lateral dispersion of energy is more effective when using modified tips in the root canal (9, 10). Laser energy that is directed on canal walls can cause effective ablation by photomechanical effects (11-13). Previous work (14) has shown that the transfer of pulsed mid-infrared laser energy with modified conical tips influences the configuration of shockwaves in aqueous solutions in root canals. These conical tips have been shown to significantly improve smear layer removal when compared to the conventional fibers with bare or plain ends (8, 15). It was hypothesized that hydraulic stresses created in the EDTA solution by lasing, agitate it,
increase its ambient temperature, and increase its penetration into the smear layer and into any open tubules (8, 15).

Recently, there has been an interest in the use of near infrared lasers for disinfection of root canals. It is now apparent that these lasers may also be used to produce shockwaves in irrigating fluids to assist in removal of smear layer and debris. Near infrared lasers are known to absorb substantially less water than mid-infrared lasers, however recent research has shown that cavitation and pressure waves can be induced in water or fluids with both 940 and 980 nm wavelength diode laser systems. These longer diode laser wavelengths are much more strongly absorbed than shorter near-infrared wavelengths such as 810 and 830 nm (16).

In previous studies, we showed that erbium lasers could generate shockwaves in fluids, travelling at speeds of 90-120 km/hr. When emitted from conventional plain or modified conical tips, erbium lasers could propel fluids past the apex (14). The near infrared laser wavelengths do not produce as intense shockwaves but they have better penetration into dentin, and can exert disinfecting actions against bacteria in the root canal to a depth of 500 microns (17).

The present study examined the characteristics of shockwaves produced by a 980 nm diode laser when delivered into fluids using either a plain conventional laser fiber, or one of two modified laser tips (conical or honeycomb).

Material and methods

Laser Systems

The Sirolaser diode laser system (Sirona, Bensheim, Germany), of wavelength 980 nm, with a maximum output power of 7 W, was used for this study. A series of 200-micron plain-ended fibres suitable for endodontic applications was used. The fibres had a either conventional plain tips, honeycomb (1mm in length) or conical tip designs (9, 10). The laser parameters used for the study were 1.25, 2, and 3 W in continuous mode.

Capillary Tube Model

A glass capillary tube model allowed direct viewing of cavitations in aqueous media. Cavitation bubbles were generated in capillary tubes with a length of 15mm and an internal diameter of 1mm or 2mm in distilled water at different settings. One end of the tube was securely sealed with adhesive (Blu-Tac; Bostic, Sydney, Australia) and mounted on a template that included a measuring ruler to achieve a standardized position of the laser fibre. A stereo microscope (Olympus, Tokyo, Japan) fitted with a digital camera (CoolPix 4500; Nikon, Tokyo, Japan) and set at 20X optical magnification recorded each experiment. All studies were performed at an ambient room temperature of 25 degrees Celsius.

Before each experimental trial, distilled water from a 25-gauge needle attached to a 10 mL syringe was introduced into the capillary tube. The tube was overfilled with a flushing action to ensure that the entire volume of the tube had been filled and no air bubbles were present. The laser fibre was then inserted into the capillary tube to a preset position 5 mm from the opening. The cavitation formation and characteristics were recorded for a maximum of 15 seconds. The entire study was repeated eight times for reliability.

Data analysis

All raw video was converted into avi file format and the data was analysed using Image J software (version 1.45, © Wayne Rasband, National Institute of Health, USA) to determine the time needed for the formation of the initial and peak cavitation bubble. The size of the largest and smallest bubble was recorded, as well as bubble formation behind and in front of the various tips. The pattern of bubble formation around each tip was recorded. All data were tested for normality and then analysed using a t test or ANOVA, with post hoc tests.

Results

Negligible or no shock waves were observed in tubes with an internal diameter of 2 mm and hence all results and discussion will focus on data from cavitations produced in a 1mm tube.

The modified honeycomb tips generated shockwaves directed onto the walls of the root canal. Both the conventional plain fibres and the conical tips created shockwaves that were directed largely in a forward direction, however shock waves with the conical tip were also directed onto the walls of the root canal behind the tip (Fig.1). The conical fibres generated cavitations in a flame shaped pattern with the base at the tip and apex a few millimetres ahead of the tip. An oval shaped cavitation pattern was seen behind the conical tip. The honeycomb tip, on the other hand, created cavitations parallel to the length of the modified tip. Shockwaves were not observed for power levels of 1 or 1.25 W.
**Surface area of cavitation bubbles**

Conical and honeycomb fibres produced a number of micro-bubbles at 2 and 3 W, with only the occasional large cavitation bubble being seen. However, with plain tips, larger bubbles were seen consistently at 2 and 3 W (Fig. 2). There was a significant difference in the surface area of the major bubbles formed with plain tips when the power was increased from 2 to 3 W. A similar observation was not noted for conical fibres.

![Distance of bubble in front and behind the Fiber tip](image)

**Figure 1.** Distance (in mm) of cavitation bubbles in relation to the tip in a capillary tube with an internal diameter of 1mm.

![Area of Bubble](image)

**Figure 2.** Area of cavitation bubbles (in square mm) in relation to the different combinations of power and tip design in a capillary tube with an internal diameter of 1mm. Major bubbles (MjB) and minor bubbles (MnB) are seen with all groups.

**Time for formation of cavitation**

The time needed to initiate the first cavitation bubble was directly proportional to the power for plain, conical and honeycomb tips, and it took longer to observe the initial bubble with the plain tips than with the conical tips or honeycomb tips (Fig. 3).
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Fig. 3. Time to peak bubble formation (in seconds) in a capillary tube with an internal diameter of 1mm.

Speed of shock waves

The speed of shock waves with a straight fibre at 2W was 4.1 mm/sec (±/2.24) and at 3W was 5.3 mm/sec (±/2.23). With the conical fibres, the majority of the bubbles were small and had speeds of 4.1 mm/sec (±/2.55) at 2W and 4.9 mm/sec (±/1.53) at 3W. It should be noted that there was no significant difference in speed between the conical and straight fibres.

The larger area of dispersion of energy with the honeycomb tips meant that speeds of shockwaves were significantly less than with both the conical and plain fiber tips at both power settings. The speed of shock waves generated with honeycomb tips was 1.1 mm/sec (±/0.73) at a power setting of 2W and 1.48 mm/sec (±/0.86) at 3W.

Discussion

Shockwaves may assist root canal therapy, however, for the shockwaves to be effective a number of factors are important. The speed of the shockwaves, the shape of the cavitation bubble, and the time for cavitations to occur are important parameters. In the present study, we have shown that the 980 nm lasers can generate shock waves when powers are above 2 W within a capillary tube with an internal diameter of 1mm; however there are variations in the pattern of cavitations. This study observed that little or no cavitation was observed in groups with an internal diameter of 2mm. This indicates that cavitations in fluids are dependent on the overall volume of fluid irradiated and hence it is expected that in root canals with larger volumes, a larger energy level would be necessary to generate cavitations and shock waves.

Plain fibres form cavitations only in a forward direction, and hence when used in the root canal the fibre tip should always be behind the area of interest. With conical fibres and honeycomb fibres, a large proportion of energy is directed laterally onto the walls of the root canal. Hence, these fibres can be placed and activated at the point of interest. This may be an advantage when the same energy source is used not only for debriding the canals but also for photothermal disinfection of the root canal.

As the speed of shockwaves generated by 980nm lasers is substantially less than mid-infrared lasers, the possibility of extrusion of fluids past the apex is lessened. The bubble formation and the heat generated from these lasers may be sufficient to agitate and activate root canal irrigants such as EDTA and NaOCl thus enhancing their action. Similar to findings reported in a study by George et al (2008), this study did not see significant differences in speed of shock waves generated by either the conical or straight tips (14). Considerably higher energy settings may be needed to increase the velocity of shock waves that may assist in debriding the canal and increase the penetration of fluids into all regions of the root canal.

The times taken to initiate cavitation and to reach maximal activity are important. Plain fibres require a longer time for the initiation of a cavitation bubble, however, when the bubble does form it is large, which may be sufficient to place shear stress onto the smear layer. On the other hand, conical fibres take less time to initiate cavitation. This lag could be directly related to the effect the conical design has on converging laser energy at the tip. The time to peak bubble formation is longer, probably because the energy build up at the tip region is more dispersed. As well as the formation of smaller bubbles, there is a larger lateral component. The bubbles formed with the honeycomb tips were largely targeted onto the walls of the root canal with only fewer cavitations formed in front of the tips.

One limitation of this study is the power settings selected. A study by Hmud et al. (2010) showed that power settings could influence the time for cavitation to occur (16). With higher energies and the use of modified tips there could be changes in shape and form of cavitation. Hmud et al. (2010) reported that when lasing into the root canal at energy settings of 4W and 2.5 W (10 Hz and 20 Hz respectively) for 5 seconds there was little danger of elevating the external root temperature (18). Further work is needed to examine the relationships between time to peak cavitations and elevations in external root temperature.
Another important consideration is the influence of water itself. While water is important for ablation, it also can act as an attenuator (19, 20). In real time root canal laser procedures, variable surface wetting conditions of irrigants, canal size and shape and the rate of water or water/air spray delivered to the tip (21) could influence the cavitational affect and hence the removal of debris and smear layer. The effect gained with the modified fiber designs should allow a more even irradiation of the walls and direct cavitational hydraulic forces on the walls of the root canal.

Conclusion

Modifying fluid dynamics in root canals is important in achieving effective removal of smear layer and debris from the walls of the complex root canal anatomy. Modifying fiber optics may be helpful in achieving these goals however, for generation of shockwaves in fluids in the root canal there is a need to carefully select the appropriate power setting as the volume of fluids in the canals may vary.

References

Low intensity laser therapy for temporomandibular disorders and oral soft tissue lesion: development and efficacy

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Abstract

It has been clear that low intensity laser (LILT) has had a biomodulation effect. Therefore, this therapy is widely used for pain control and wound healing in oral and maxillofacial regions. However, there are still some controversies around the clinical efficacy and repeatability in terms of clinical application. This article comprises techniques and regimes based on basic studies, clinical trials and practices as follows: treating temporomandibular disorders using 820nm at 107J/cm², immediate pain relief of aphthous stomatitis using defocused CO₂ laser irradiating to energy absorption media, and promoting healing of erosive type lichen planus using a welding technique by 830nm at 2 W. These result in clinical efficacies and patients’ satisfactions.

Keywords: Novel technique, oral ulcer, synthesized research

Introduction

Low intensity laser therapy (LILT) has been accepted to have a biomodulation effect [1]. The main clinical applications in oral and maxillofacial regions are pain relief and promotion of wound healing [1-2]. This article presents the regimes and techniques using LILT for temporomandibular disorders and soft tissue oral lesions based on related in vitro studies, clinical trials and routine practices conducted by the Lasers in Dentistry Research Group, Khon Kaen University, Thailand.

LILT for temporomandibular disorders

There are a variety of applications and regimes using LILT for TMD. We have used LILT with the protocol which was explored the mechanism by the in vitro experiment and clinical efficacy by the clinical trial. The in vitro study in a model of interleukin 1 stimulated myoblasts showed that 820 nm gallium aluminum arsenide (GaAlAs) laser at 19J/cm² partially inhibited prostaglandin E2 production [3]. It should be emphasized that the energy density used in this in vitro experiment was based on a measurement of the laser energy under the skin flap above jaw joint area irradiated with 820 nm GaAlAs 300 mW continuous wave/20J/1.07 minutes/107J/cm². This regime was also be proved in double randomized placebo-controlled clinical trial to provide statistically significant increases in pressure pain threshold and voluntary clenching electromyography of the masticatory muscles of the unilateral myofascial pain and temporomandibular joint (TMJ) arthralgia patients. Regarding the irradiation methods, there were 3 areas around TMJ (Fig. 1) and on the trigger points of masticatory muscles for 3 episodes per week [4]. It was noticed that in the patient with dark brownish skin, the burning or tingling sensation could occur due to melanin absorbing the laser energy of this wavelength. In this case, we recommended the regime of 820nm/100 mW/4-6J/40-60 seconds/40-60J/cm² once a week for 3 episodes. This was able to gain favorable clinical efficacy based on the case-review of the laser clinic, faculty of dentistry, Khon Kaen University, Thailand.
Novel technique of LILT for immediate pain relief of aphthous stomatitis

Although LILT has been reported to alleviate the pain of ulceration such as aphthous stomatitis [1], the therapy needs repeating and it still has no immediate effect for pain relief. According to the hypothesis that delivery of a high amount of energy may gain abrupt pain alleviation and decrease the number of therapy sessions, we set up the in vitro study to invent the novel technique of using CO₂ laser irradiation of energy absorption media; high water containing gel. This experiment in the fresh tissue blocks found that defocused CO₂ laser at 2 Watt and 3 Watt continuous mode for 5 seconds irradiation of the transparent gel delivered the actual power of 15.5 Watt and 118 Watt, respectively, on the tissue surface without altering the tissues by photothermal effect [5]. The results of the experiment are shown in Fig 2.

The double blind randomized placebo controlled trial was conducted to explore the efficacy of using defocused CO₂ laser continuous mode irradiation for 5 seconds to absorption media on pain relief in the patients with aphthous stomatitis [6]. It was found that there was immediate pain alleviation in the control group and the treatment laser group. This should be from the effect of the media gel to relieve pain. However, the laser treatment group had statistically significant less pain score than the control group on day 3 after treatment (Fig 3.). Therefore, this novel technique can be benefit in case aiming for improved pain relief after treatment and numbers of treatment as conventional LILT could not meet this goal of therapy.
Laser soft tissue welding technique

Treating the chronic ulceration and erosive lichen planus particularly in refractory or complicated cases has been in a potential area of research. Although using LILT could be a choice of alternative treatment, the incomplete recovery was reported [7]. We invented the novel technique of using diode laser (830 nm) at 1 to 2 Watt light touching on the ulcer under topical anesthesia (Fig 4.), namely “laser soft tissue welding”. This technique was reported to provide improvement to recovery in the steroid refractory case of erosive type lichen planus and chronic ulceration or chronic inflammation in elderly patients after 3-6 treatments (Fig 5.) [7-8]. Recently, we conducted the in vitro study in tissue blocks to explore the histological changes and ablation properties of this technique. It was found that the histological changes were in the range of no alteration to narrow border of affected width not more than 560 microns [9]. This technique has been routinely used in the laser clinic, faculty of dentistry, Khon Kaen University for treating erosive type lichen planus. The patients were treated once a week to monthly depending on the severity of the lesion and availability of the patients.
Fig 5. The chronic inflammation of the palate (A) treated by diode laser soft tissue welding resulted immediately after therapy (B), a month after 1st treatment (C) and 3 months after 3 treatments (D)

Conclusion

The LILT for TMD and the novel technique of CO₂ laser irradiation of absorption media for immediate pain relief and diode soft tissue laser welding were the therapies developed by the basic hypothesis of laser-tissue biomodulation and aiming for improving quality of life of the patient. These also had correlated in vitro studies to explain the clinical efficacy of that which was proven by clinical trial or clinical practice review.

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Lymphoedema and low level laser

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Summary

Low level laser has been used for almost 20 years for the treatment of lymphoedema. While not a miracle treatment and one which seems best used in conjunction with other treatments, over a number of trials it has been shown to provide significant subjective and objective benefit for those with lymphoedema who have some level of fibrotic induration either through the surgical or radiotherapeutic scarring or just the progression of lymphoedema. Fibrosis is a key inhibitor of lymphatic regeneration and lymphatic function and its removal can help improve these processes. While the exact mode of action remains to be clarified there appears to be both local and systemic effects. The major outcomes of lymphoedema treatment with laser include reduction in the size of the affected limb, reduction in extra-cellular fluids, softening of fibrous tissues, as well as a range of subjective improvements such as pain, tightness, heaviness, cramps, and paraesthesia (sensation of pins and needles). As with most other studies of LLLT there is still the need for more research especially regarding the potential synergy between LLL and other common lymphoedema treatments/management strategies such as lymphatic drainage, massage, bandaging and compression garments.

Introduction

On many occasions a slight swelling in the arm (or trunk or breast) associated with surgery and / or radiotherapy for cancer treatment, fails to resolve and continues to worsen.

The swelling is due to the inability of the lymphatic system to remove fluids and their contents (which include cytokines and inflammatory mediators) from the tissues. The reasons for this dysfunction are wide and various but one key can be the presence of fibrous tissue along or near the major lymphatic drainage pathways. In the early stages of lymphoedema, the swelling can be associated with the surgery (especially when there is axillary or groin lymph node clearance) or with the radiotherapy. In the later stages, the fibrous build up may be part of the progression of lymphoedema, as low grade often sub-clinical inflammation occurs along with increases in the adipocyte size and numbers in the epi-fascial compartment due to the higher than normal concentration of adipogenic factors in the tissues seemingly associated with a failing lymphatic system.

Often (and especially when there is diffuse or concentrated fibrotic induration), the swelling is unlikely to resolve without some significant attention involving treatment (health professional based) and management (self/partner based) programs. Once clinically manifest (that is, one can see that the limb is swollen - generally this means at least a 2 cm circumference difference, or a 10% increase in volume compared to the unaffected limb), at best, the swelling may disappear. Most likely, the swelling can be reduced but at worst, it progresses insidiously. Sometimes the swelling resolves for a varying period of time but then returns after an event such as infection through a cut or scratch, or by weight gain.

Why? It’s all to do with the balance between lymphatic load and the ability of the lymphatic system to transport that load (fluids and its contents) away from the tissues. This ability can be significantly affected by many factors (and that is not within the scope of this article but details are available from Földi and Földi, 2012). One key factor can often be a build-up of fibrous tissue. The reasons for this build-up are also not within the scope of this article, but their presence can mean firstly a difficulty or inability of new lymphatic capillaries to regenerate through fibrous areas, and secondly an inability of the major lymphatic collectors to pulsate freely (they all generally pulsate between 6-10 beats per minute) (Avraham, et al 2009). The outcome is a reduced lymphatic transport ability and an increased tendency to accumulate oedema fluids (and their contents) leading to an increased risk of lymphoedema.

In these situations, what is important and crucial is the early detection of the often subtle levels of fluid in the tissues (Ward, 2006) leading to early treatment of any early stage lymphoedema (Piller 2006).

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1 This paper was awarded the WALT2012 prize for Best Presentation by an Established Researcher. The award was judged on the following criteria: content, structure and development, research base and rationale, and overall presentation.
Even for those without lymphoedema (but whose lymphatic system has the potential for failure), its prevention by accurate assessment, recognition and targeted response to the early pre-clinical signs of its appearance by education and some relatively simple management options, is crucial (Piller 2007).

What are the risk factors?

Some risk factors will be within a patient’s control and others outside of it. Those under their control are body mass, skin integrity, activity levels (with inactivity being the worst) and constrictive clothing (particularly underwear that has elastic across the line of the groin and brassieres which are under-wired and have narrow straps). Factors that are well established and out of a patient’s immediate control are the extent (level) of axillary or groin lymph node clearance and radiotherapy to the root of the extremity. Others also outside of their control (but less well established) are age, whether the intervention was on the dominant limb, seroma and its duration, the number of post-operative drains and wound infection.

Signs

If there is no swelling which can be measured objectively by a tape measure, it’s often worth testing if any part of the limb (particularly the distal part) shows signs of ‘pitting’ particularly the lateral/medial maleoli, dorsum and tibial areas. Pitting may be observable before any circumference change is detected. However bio-impedance spectroscopy can objectively detect fluids often months before it is clinically obvious (Ward et al 2006).

There may also be signs of tissue changes associated with the build-up of fatty tissue and fibrotic induration. These changes can be detected by conducting a pinch and roll test by holding the affected tissues between the thumb and forefinger and gently rolling the tissues between them or by a technique called tonometry which is now commonly called Indurometry (Piller, 2007).

When lymphoedema is detected or the patient is at high risk

As an expert in laser therapy, often there is little that one can do to become actively involved in the mainstay of lymphoedema treatments (which are bandaging, compression and a range of special light massage techniques going under the heading of Lymphatic drainage massage) (Foeldi et al 2012). However, a laser therapist (and laser treatment) can play a significant role in the effective treatment of lymphoedema, if the reasons for the swelling are understood and if the treatments are properly targeted and sequenced. The role of laser therapy is early on in the sequence of treatments, but to ensure it occurs at an early time point, there is a need for an accurate diagnosis and of course some objective information about the location and extent of fibrotic induration (Piller 2007).

Treatments

There are a plethora of treatments and many reviews of their efficacy (e.g., Badger, et al 2004; Földi et al 2012; Moseley, Carati and Piller, 2006). However, not all treatments have been thoroughly evaluated and so it’s hard to make strong recommendations about some of them, but if the big picture is kept in mind (that is to either increase the lymphatic transport load capacity or reduce the lymph load, and reasonable strategies undertaken to act either one or the other or both) then reasonable results are likely.

Low Level Laser

While we are not totally clear about the mode of action of laser some hypotheses have been presented in Cariati et al (2004). However, experimental evidence indicates increased lymphangiomotority, some bacteriocidal action, macrophage stimulation (at some wave lengths) and improved wound healing. Clinical evidence from our own and other studies indicates a reduction in limb size and circumference, a softening of hardened tissues and an improvement in a range of subjective symptoms is possible.

There seems to be local and systemic effects of LLLT. Locally, it is at the treated sites of fibrotic induration, surgical or radio-therapeutical scarring, around poor or non-healing wounds, but sometimes more generally if lymphoedema has progressed. Systemically, as is common with other laser treatments, there is an apparent cascade of outcomes, these in the main being enhanced general lymphangio-motoricity.

What Dose should be used?

It is general knowledge that lower dose levels of LLLT are stimulatory while higher ones are often inhibitory. Doses in the region of 1-4 J/cm² per treatment, gain best results (at least in our clinical trials and for those using our protocols).
However, it takes time for any treatment effect to become evident, so an apparent non-reaction can, the day following treatment, turn out to be a positive one. Generally, it would seem best to allow at least one day between treatments.

**How many treatments?**

This will depend on the initial assessment of the problem and on its nature and severity. Most clinical trials indicate multiple treatments spread over weeks or months are best, while some anecdotal reports suggest effects after one treatment. Some trials suggest an intensive phase followed by a less frequent maintenance phase.

**Which Laser?**

The major choices are basically hand-held or scanning type units, the wavelength, and whether single or dual beam. The choice depends on the needs and costs and area/region of the body to be treated, the clinic location, whether administered by patient or practitioner, depth of penetration required etc. Scanning laser is most suited (in fact, mandated) to larger clinics and large tissue areas, while the hand-held units are better for patients, small clinics and when equipment is to be loaned to patients. Again, no matter what the choice when treating lymphoedema (and its associated fibrosis), the dose need not be high (1-4 J/cm² per treatment) (high doses may inhibit some biological processes). The wavelength determines penetration: if > 800 nm then the deeper acupuncture/acupressure points, trigger points, and deep tissue injury become accessible while if the wavelength is < 800 nm then accessibility is limited to more superficial tissue problems.

**Early studies**

Some of the early studies of lymphoedema suggested LLLT to be relatively ineffective as a treatment of conditions where some tissue changes might have occurred, however in the main they were based on patients with acute disease (Piller and Thelander; 1998). The first study to attempt to redress this situation was by Piller and Thelander (1995) and had as a key focus the cost effectiveness of LLLT on a per percentage point reduction of the lymphoedema in chronic post-mastectomy lymphoedema. In summary, 16 patients were treated for 10 weeks at 2-4 J/cm² per treatment, with two beams at 628 and 904 nm. Outcomes were measured by tape (limb circumference), plethysmography (limb volume), tonometry and bio-impedance spectroscopy (BIS). Outcomes were a 19% (2cm) reduction in limb circumference, oedema, reduction (extracellular fluid (ECF) measured by BIS), progressive tissue softening (measured by tonometry) and subjective improvement, at a cost of $AUD16 per percentage point of oedema reduction/oedema.

Piller and Thelander (1998) followed these patients for 2.5 years as their treatment progressed using similar treatments and measurement tools. While there were some potential confounding factors, there was an improved 29% reduction in oedema, ECF returned to pre-treatment level, tissue fibrotic induration in the areas of forearm and chest lessened (and in fact, returned to near normal) but there was a hardening in the upper arm. Subjective improvements were maintained over the longer time period. While there were some longer term benefits of initial laser treatment, there was some loss of treatment effect after 30 months, but LLLT remained cost effective per percentage point reduction in oedema.

One of the more convincing studies of the efficacy of LLLT in the treatment of lymphoedema was that of Carati et al (2003) in that it was a double-blind, placebo controlled, cross-over trial. In this study, participants received placebo, or one or two cycles of LLLT to the axillary region of their affected arm. The researchers objectively measured limb parameters including fibrosis (tonometry), limb volume and circumference (perometry) and fluid levels (BIS) as well as evaluating LLLT effects on a range of qualitative parameters. The study found significant improvements in ECF levels after the 2 cycles of LLLT in the affected arm (maintained at 1 - 3 months), the trunk (maintained at 1 - 3 months) and (surprisingly) the unaffected untreated arm. Further perometry showed a significant reduction in overall limb volume.

Further, there were significant improvements in tissue fibrosis (hardness) after the second group of laser treatments, but only in the upper arm at 1-3 months, and the posterior thorax at the same time periods – the changes were maintained at follow-up. The trial also showed significant subjective improvements in pain, tightness, heaviness and muscle cramping as well as limb temperature difference, perceived size difference, and sensation of pins and needles. Full trial outcomes are reported in Carati et al (2003, and 2004).

The Carati study also compared limb volume changes in the limbs of patients with breast cancer-related lymphoedema (BCRL) where limb volume measurement data was available by perometry. The results showed longer term (2 cycles of LLLT) reductions were comparable with other treatment modalities (Figure 2). Due to
the range of criteria for entry into the other studies, the comparison is only indicative rather than definitive, and additional more rigorous comparative studies need to be conducted.

Treatment times are always an issue with patients and practitioners alike and a comparison of average treatment times for a range of standard and commonly used treatments shows the amount of time required for LLLT over an average week was less than other treatments, although again further well-controlled and definitive studies are required.

Figure 1: Reduction in limb volume measured by perometry after the two grouped treatment sessions for the forearm, upper arm and whole limb (Cariati et al 2003).

Figure 2: Comparison of limb volume reductions in a range of other treatments (millilitres) using perometry.
Since the trials described above, there have been a range of other studies with good outcomes measured objectively, albeit usually on small sample sizes. The magnitude of the impact of laser treatment on the measured parameters has however often been large enough to show statistical as well as practical and biological significance in most cases.

For example, Maiya (2008) studied 20 patients with BCRL, and utilised pre- and post-treatment measurements in a group who had completed adjuvant radiation. The treatment was 2.63/cm² to points in the axilla for 10 days resulting in significant improvement in pain (p< 0.001) and arm circumference (p<0.05) compared to the control group. The authors concluded LLLT was effective in treatment of post-mastectomy lymphoedema.

Wigg (2009) in a study of seven patients with BCRL who received laser (904 nm, 1.5 J/cm²) and Manual Lymphatic Drainage (MLD), only those in the laser treated group experienced a 70% improvement in movement, a 40% improvement in the area of the radiotherapy scar, and a 70% subjective improvement in symptoms. In the group receiving Laser and MLD there was 100% softening of the tissues and in joint range of movement. Forty percent thought MLD gave better outcomes after laser treatment while overall there was 100% softening, 85% improvement in movement, and 40% improvement in the area of the radiotherapy scar. Objectively however, only 10% showed a reduction in limb volumes. Wigg (2009) concluded that LLLT has a role in the therapeutic management of lymphoedema and that its use with other treatments such as MLD, intermittent compression pumps and kinesio-taping appears exciting but requires further research.

A recent review of the literature (Tilley 2009) concluded that LLLT is a viable treatment option in the management of lymphoedema and that the extensive case studies and limited research (in lymphoedema) shows positive and potentially long term effects; but indicated that further research is needed to define optimal parameters to maximise effect. A systematic review of the literature by Omar et al (2012) of the effect of LLLT in the management of BCRL concluded “there is moderate to strong evidence for the effectiveness of LLLT in the management of BCRL”.

**Conclusion**

LLLT has an important role to play in the treatment of BCRL and most likely other lymphoedemas. The mode of action of laser that is pertinent to BCRL would seem to be an effect on tissue fibrosis (induration). LLLT may have an important role in the targeting and sequencing of the treatment of BCRL and other lymphoedemas, with indications being that it should perhaps be considered as a first line treatment, as the softening of the indurated tissues (originating from the surgical or radiotherapeutical scarring) and through the progression of the lymphoedema is critical to ensure the optimal functioning of the lymphatic collector system, which is significantly constrained by any form of induration in terms of its transport (flow) capacity. Loosening
and softening of tissues may help other treatments gain improved outcomes as has been shown in some of the indicated studies. Since good lymphatic drainage is related to not only tissue and cellular health in terms of the physiochemical properties of the extra-cellular environment, but also to the tendency to deposit epi-fascial fat, and the immune response, any action which improves lymphatic function will have more than a local treatment area benefit. Much more remains to be discovered about optimal dose, timing and wavelengths but we are well on our way and already have been able to show significant objective and subjective improvements in BCRL and some other lymphoedemas.

Table 1 Summary of major outcomes of Low Level Laser Therapy

- Has a role in lymphoedema management
- Softens fibrous tissues
- Leads to good subjective outcomes
- Takes time to show objective effects
- Effects are of moderate duration that is, they continue after cessation of treatment
- Other treatment modalities may work better when appropriately sequenced with LLLT
- Treatment time is often less that that of other treatment modalities

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Application of Laser Therapy for Dermatological Conditions

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Abstract

Background: Dermatological conditions including Eczema, Non-Specific Dermatitis, Herpes Zoster, Psoriasis, etc. are frequently resistant to treatment using pharmaceuticals. These may consist of cortisone, topical applications, antibiotics, etc. Along with the irritation and discomfort generated by these conditions, patients are often sensitive to the social stigma associated with these problems.

Purpose: To evaluate and document the progress of patients with a number of these conditions both chronic and acute. Criteria denoting progress include discomfort/pain levels and the physical appearance of the lesions.

Methods: Patients suffering from Herpes Zoster (12 patients), Eczema (6 patients), Psoriasis (5 patients) and Dermatitis (3 patients) were evaluated and photographed before, during and after the final course of Laser Therapy. The protocol consisted of the application of a red (660 nm) light-emitting diode (LED) array (750 mW, 10 mW/cm²), infrared (840 nm) LED array (1500 mW, 20 mW/cm²), red laser (660 nm, 75 mW, 750 mW/cm²) and infrared laser (830 nm, 180 mW, 1800 mW/cm²). Patients were initially treated on consecutive days varying from two to six days. Subsequently they were treated two to three times per week until the condition was completely resolved.

Results: The number of treatments required ranged from two to fourteen with an average of eight treatments to completely resolve the disease processes encountered. Significant improvement in the physical appearance of these along with the degree of discomfort was achieved in as few as two treatments in several cases. Others required a more extensive number of therapy sessions.

Conclusion: Laser Therapy is a highly effective technology for the treatment of dermatological conditions, particularly those that have not responded to conventional therapies.

Keywords: Dermatology, Herpes Zoster, Psoriasis, Eczema, Dermatitis, Laser Therapy

Introduction

The skin is the body’s largest organ and therefore reflects many influences. These may be environmental, nutritional, viral, bacterial or fungal. The list is inexhaustive. Aging and trauma may be additional factors and in many instances dermatologists are at a loss to establish a specific diagnosis. The dermis may often be described as a reflection of the overall health and status of the patient. At times, the etiological factors may be readily apparent, however not infrequently, they are beyond the scope of diagnosticians even when biopsies are performed. The latter may be interpreted as “non-specific inflammatory change” or simply “dermatitis”.

At the Meditech Laser Rehabilitation Centre, we see an increasing number of patients with dermatological conditions that have been undergoing therapy under the supervision of individual specialists including allergists, dermatologists, rheumatologists and family physicians. The therapeutic approach utilized may incorporate oral and topical medications, including the preponderant overuse of steroids, antibiotics and antifungals. Often these serve only to make the condition more severe.

While Herpes Zoster affects only 500,000 people, psoriasis affects nearly 7.5 million people in the US alone. Total direct and indirect costs of the treatment of dermatological conditions are estimated at over $10 billion dollars per annum. Along with the economic burden of conventional therapies in managing these conditions, there is a psychological component that affects the patient’s quality of life.

Low Intensity Laser Therapy (LILT) is not only non-toxic and non-invasive1, it is also clinically effective for an extensive number of pathologies including the healing of dermal ulcers2. Photon irradiation within a specific therapeutic window initiates a variety of positive physiological responses3. It has been suggested that LILT in the treatment of wounds has substantial effects on inflammatory infiltrates, thereby accelerating resolution. Studies of in vivo wounds found that Laser Therapy not only healed wounds more
rapidly, but also formed thicker layers of epithelial cells than found in normal skin. This is also associated with the regeneration of rich networks of small blood vessels, in close proximity to the epithelial layer (i.e. angiogenesis). Laser Therapy has also been shown to stimulate the transcriptional level of collagen gene expression; consequently, increasing the collagen and procollagen mRNA levels in irradiated wounds. In particular, the infrared irradiation has been shown to enhance the quality and texture of the dermis.

**Methods**

**1.1 Laser Therapy Treatment for Dermatological Conditions**

The objective of this study was to assess the progress of patients affected with dermatological conditions over the course of the administration of Laser Therapy. The BioFlex System was used on all these patients who were treated at the Meditech Laser and Wound Care Clinic, Toronto, Canada. The treatment regimen consisted of a four step approach. The sequence of application combining superluminous diode arrays (SLD) and laser probes as indicated below:

1) Red light (660 nm) using a flexible GaAlAs 180 diode array of SLDs (750 mW)
2) Infrared light (840 nm) using a flexible GaAlAs 180 diode array of SLDs (1500 mW)
3) Red laser probe (660 nm) using a single AlGaInP laser source focused on the basic pathology (75 mW)
4) Infrared laser probe (830 nm) using a single GaAlAs laser source focused on the basic pathology (75 - 200 mW)

As a general rule, as much therapeutic value as possible was obtained with each protocol setting. Parameter settings including frequency, duty cycle, waveform and duration were altered based on the clinical response.

For geographical reasons, some patients were instructed to continue treatments with a BioFlex Home Unit. This encompassed the following:

1) Red light (660 nm) using a flexible GaAlAs 180 diode array of SLDs (500 mW)
2) Infrared light (840 nm) using a flexible GaAlAs 180 diode array of SLDs (1000 mW)

**Results**

Four patients are presented along with a specific diagnosis: Herpes Zoster, Psoriasis, Eczema and Dermatitis. For each patient, the diagnosis, medical history, physical examination and progress are described.

**1.1 Case Profile 1**

**Diagnosis:** Herpes Zoster (Shingles)

**Medical History:** The patient is a 51-year-old female complaining of pain and dermatological manifestations over the right lower back, thigh and inguinal regions. These are acutely inflamed and demonstrate vesicular lesions extensive in their distribution. Ten days prior to her initial presentation, the patient awoke with severe right lower back pain radiating to the inguinal and thigh areas. The acute dermal lesions developed over the subsequent 3 days. The pain was extreme in degree and required 10 or more Tylenol tablets each 24 hour period. These provided only minimal relief. The patient also recounted a recent history of “cold sores” covering the lower lip.

**Physical Examination:** The patient demonstrated an extensive variety of elevated dermal lesions that included weeping, vesiculo-papular formations and rough scaly plaques along the pathways of the L2-L5 dermatomes. All lesions were acutely inflamed and significant amounts of clear discharge were noted. Ideally, the patient should have been hospitalized for custodial care, including pain medication, rehydration, etc. (Fig. 1).

**Discussion:** The patient initially had 3 Laser Therapy sessions at the Meditech Clinic on consecutive days followed by a significant reduction in pain and marked improvement in the objective findings. She was then instructed to continue Laser Therapy using a Home Unit for geographic reasons. At the end of the first week of treatment, symptoms had largely subsided; the patient was pain free and did not require any medication. After 3 weeks of home treatment, all symptoms were completely resolved.
1.2 Case Profile 2

Diagnosis: Psoriasis

Medical History: The patient is a 55-year-old engineer who has a 25 year history of generalized psoriasis. Symptoms vary from chronic to acute. There was no family history of the disease. Diagnosis was established at biopsy in 1993. He had multiple therapies over the years without permanent relief. He had been subjected to a course of injections 2 years prior to presenting at the Meditech Clinic, which provided only temporary relief at a cost in excess of $20,000. Symptoms were frequently intolerable both locally and on a systemic basis (i.e. pain, fatigue, etc.).

Physical Examination: The patient presented with extensive dermal lesions primarily over the thorax and lumbar spine regions. Lesions displayed both circular and elongated patches of erythema and dry scaly areas, some with central ulceration (Fig. 2).

Discussion: A course of Laser Therapy treatment was initiated. Substantial improvement was noted almost at once and after 4 weeks of treatment, the patient continued using a home system. Following 2 months of home treatment, the patient returned in completely asymptomatic condition and no longer required any medication. Utilizing the Home Unit on an intermittent basis, 2 years post cessation of treatment at our clinic, the patient remains asymptomatic.
1.3 Case Profile 3

**Diagnosis:** Eczema of undetermined origin of the hands

**Medical History:** The patient is a 55-year-old male who had a “rash” of both hands present for 4 years somewhat progressive in nature as characterized by itching and general discomfort. Moreover, he finds it painful to use the hands in a normal fashion. He uses moisturizers with limited benefit.

**Physical Examination:** There are erythematous, purplish, scaling lesions covering both hands including the digits and, to a lesser degree, the dorsum. It is most pronounced in the distal palmar area and the proximal phalanges of the hands. There is no limitation of range of motion of the wrists and small joints of the hands (Fig. 3).

**Discussion:** The patient received 12 Laser Therapy treatments over the course of 6 weeks. Within one week of initiating treatment, the patient showed substantial improvement. At the conclusion of treatment the patient was asymptomatic and only minimal erythema was present.

1.4 Case Profile 4

**Diagnosis:** Generalized dermatitis of unknown etiology

**Medical History:** This 32-year-old office manager, who is 21 weeks pregnant, complains of recurrent episodes of acute dermatitis over the past 5 years. Symptoms are present at all times and in recent weeks have increased significantly with regard to the degree of severity. She is unable to sleep or function normally and involuntarily scratches the areas while sleeping. Recently symptoms have appeared in the facial area and are
expanding in extent. The lesions cover the extremities and trunk both anteriorly and posteriorly down to the S1 level. They do not involve the lower extremities. She has undergone allergic testing without significant findings. She is currently using hydrocortisone cream over the multiple areas without significant benefit. She has also used salt compresses, vinegar, etc. again without benefit.

**Physical Examination:** There are relatively flat chronic/acutely inflamed lesions covering her upper extremities, posterior trunk and the chest wall down to the inguinal areas. They are most severe over the upper chest wall, posterior trunk and flexor aspects of both upper extremities. There are varying degrees of erythema in these regions. There is some elevation, edema and tenderness. There are many abrasions scattered over the lesions, characterized by excoriations and punctate central hemorrhages (Fig. 4).

**Discussion:** The patient received 10 treatments over 2.5 weeks. Substantial improvement was noted almost immediately; this included reduced pruritus, erythema and elevation of the lesions. Upon completion of the treatment course the patient was asymptomatic.

![Initial vs After 5 Treatments](initial-after-5treatments.png)

**Conclusion**

These case profiles document the effectiveness of Laser Therapy in resolving challenging inflammatory conditions of the dermis that may be chronic or acute. The treatment is particularly effective in the resolution of the inflammatory process and cellular regeneration, thereby relieving the symptoms. Mechanisms of action include increased proliferation of collagen, elastin, fibroblasts and keratinocytes. In addition, Laser Therapy generates new blood vessels, thereby improving microcirculation; this enhances the oxygen levels in the tissues, which again accelerates the healing process.

Laser Therapy is a therapeutic technology that enhances tissue healing, even with the most challenging dermatological conditions. The utilization of the Laser Therapy, invariably results in the resolution of dermatological conditions that do not respond to conventional methods.
References


Transcranial low-level laser (light) therapy: mechanisms and application to traumatic brain injury and beyond.

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Abstract

Low-level laser (or light) therapy (LLLT) is attracting growing interest to treat both stroke and traumatic brain injury (TBI). The fact that near-infrared (NIR) light can penetrate through the scalp and skull into the brain, allows non-invasive treatment to be carried out with a low likelihood of treatment-related adverse events. It is proposed that red and NIR light is absorbed by chromophores in the mitochondria of cells (which are particularly abundant in cortical neurons) leading to changes in gene transcription and upregulation of proteins involved in cell survival, antioxidant production, collagen synthesis, reduction of chronic inflammation and cell migration and proliferation.

We developed two different models of TBI in mice: a closed head weight drop and an open skull controlled cortical impact (CCI). Transcranial laser therapy consisting of a single exposure 4 hours post-TBI to 36 J/cm² of various lasers was delivered to the closed head model. 810 nm or 660 nm laser significantly improved neurological severity score in TBI up to 4-weeks post-TBI. Laser therapy at 730 nm or 980 nm was ineffective. We then examined the effect of 0, 1, 3, and 14 daily 810 nm laser treatments in the CCI model. A single laser exposure gave a significant improvement while 3 laser exposures were better still. Our data suggest that transcranial LLLT is a promising treatment for acute (and chronic) TBI and may have much wider applications to neurodegenerative and psychiatric diseases. The lack of side-effects and paucity of alternative treatments for brain diseases should encourage more early clinical trials.

Keywords: low-level laser therapy, NIR laser, photobiomodulation, traumatic brain injury, neurogenesis, controlled cortical impact, neurological severity score.

Introduction

Traumatic brain injury (TBI), be it severe or moderate, accidental or inflicted, includes skull fractures, intracranial hemorrhages, elevated intracranial pressure, and cerebral contusion. It is a major health and socio-economic problem throughout the world that affecting mainly the young. In the United States alone, approximately 2 million injuries occur annually resulting in 56,000 deaths. Approximately 18,000 survivors suffer from various neurological impairments, some of them permanent [1-3]. The consequent (direct and indirect) annual costs in the USA are estimated to be at $56 billion [4]. The World Health Organization (WHO) has projected that by 2020, road traffic accidents, being the major cause for TBI, will rank third as a cause of global burden of disease and disablement, surpassed only by ischemic heart disease and unipolar major depression [5]. The pathophysiology of TBI is very complex and poorly understood. Despite advances in our understanding of the pathophysiological changes occurring after brain injury, current treatments are limited both in their efficacy and utility [6].

Immediately after skull impact, several different physiological pathways activate, resulting in secondary brain injury. Some of these processes include inflammation, oxidative stress, ionic imbalance, increased vascular permeability, mitochondrial dysfunction, and excitotoxic damage [7]. The result is brain edema, increased intracranial pressure and impaired cerebral perfusion. Combinations of cellular and physiologic disturbances cause increased neuronal cell death, enlargement of infarct size and sensory, motor and cognitive impairments. Efforts to improve the treatment and outcomes of TBI must therefore involve multiple approaches to the problem both for clinicians and researchers [8]. In an effort to elucidate its mechanisms of action and to ameliorate some of the adverse effects of TBI our group studies the effects of transcranial LLLT.
Mechanisms of Low-level laser therapy

In LLLT photo-thermal effects are believed to be insignificant and photo-biological processes in cells are photochemically induced via absorption of light energy [9]. When chromophores within the cell absorb photons of light their energy production becomes elevated (increased ATP), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) production rises, nitric oxide (NO) is released, cytochrome c oxidase activity enhanced, more reactive oxygen species (ROS) are produced, and modifications occur to intracellular organelle membrane activity particularly in mitochondria, calcium flux and stress proteins [18-22]. The use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-1100 nm) [10] because the “optical window” for effective tissue penetration of light is maximized between 600 nm and 1200 nm.

Mitochondria are thought to be the single most important organelle governing the LLLT response. In addition to acting as the cellular energy supply, mitochondria are also involved in signaling, cellular differentiation and proliferation, apoptosis, and control of cellular metabolism. An action spectral study showed that complex IV in the mitochondrial inner membrane, also known as cytochrome c oxidase (CCO) is the crucial chromophore in the cellular response to LLLT [10]. CCO is a large transmembrane protein complex consisting of two copper centers and two heme-iron centers, and is a component of the respiratory electron transport chain [11]. The precise manner in which laser light affects CCO is not yet known. However, it is expected that LLLT affects both cells and tissues in complex pathway cascades with altered intracellular signaling and changes to redox states. Mitochondrial ROS may act as a modulable redox signal, reversibly altering the activity of a range of functions in the mitochondria, cytosol and nucleus. LLLT was reported to shift the overall cell redox potential in the direction of greater oxidation [12] and increased ROS generation and cell redox activity [13]. It is known that several transcription factors are regulated by changes in the cellular redox state, including redox factor-1 (Ref-1), activator protein-1 (AP-1) (a heterodimer of c-Fos and c-Jun), nuclear factor kappa B (NF-kB), p53, activating transcription factor/cAMP-response element-binding protein (ATF/CREB), hypoxia-inducible factor (HIF)-1, and HIF-like factor. Also, LLLT induced ROS have been proposed to be involved in regulation of activation of redox-sensitive early/intermediate genes and related transcription factors (including NF-κB) [14].

One important feature of LLLT is its intrinsic biphasic dose–response curve [15, 16]; that is, where a moderate amount of light is helpful, more will lose the beneficial effects, and even more will be harmful. This effect could be explained by the “Janus” effect of two of the proposed mediators of LLLT signaling; ROS [17] and NO [18]. Both these species have been proposed to be beneficial in low concentrations but harmful in higher doses.

Nitric oxide (NO) is another possible regulator of cell signaling. NO has been observed to be released from cells during LLLT. Some authors argue that LLLT may cause photo-dissociation of NO from CCO [19, 20]. Cellular respiration is down-regulated by the production of NO by mitochondrial NO synthase (mtNOS, a NOS isoform specific to mitochondria), which binds to and inhibits CCO. Moreover, NO displaces oxygen from CCO, inhibiting cellular respiration and decreasing ATP production [21]. By dissociating NO from CCO, LLLT prevents this process from taking place and results in increased ATP production [22, 23]. Therefore, at the tissue level light can influence blood flow, following release of the vasodilator, NO [21]. As a result enhanced perfusion will facilitate improved oxygenation and recruitment of inflammatory cells to the areas undergoing repair as well as further re-vascularization and proliferation of cells to achieve systemic effect. Figure 1 graphically illustrates some of the proposed intracellular signaling pathways that are activated as a result of LLLT.

When considering the transcranial effects of LLLT, more specific mechanisms should be discussed regarding disorders of the brain. Figure 2 illustrates some of the possible brain-specific mechanisms of transcranial LLLT in TBI. It has been proposed that cortical neurons in the injured/damaged brain are prevented from dying by the cytoprotective effects of LLLT; this type of reduction of neuronal cell death has been reported as being associated with the effects of cyanide [24], tetrodotoxin [25], and also methanol [26]. Mediators of this type of protective effect may include inducible proteins, such as survivin [27], Bcl2, heat shock proteins [28] and superoxide dismutase [29]. Other processes that are associated with LLLT and that may be beneficial in TBI include a) the anti-inflammatory effect of LLLT (thought to include down-regulation of pro-inflammatory mediators from dendritic cells [30]); b) elevation of suppressor cell secretion of anti-inflammatory mediators such as IL10 and TGF-beta [31]; c) a pro-angiogenic effect that has been well documented in wound healing and similar studies [32-34]; d) neurogenesis or neuroplasticity (synaptogenesis) as an additional mechanism of action contributing toward improved outcomes after transcranial LLLT to the brain [35] and the process may be stimulated by the increased expression of neurotrophins such as BDNF and NGF.

Studies of Transcranial LLLT for TBI in mice

Oron et al. [39] evaluated the effects of LLLT for TBI in mice where a weight-drop device was used to induce the closed-head injury. A 808 nm GaAs diode laser with two different power densities (10 and 20 mW/cm²) applied for 2 minutes, giving energy densities of 1.2 and 2.4 J/cm² respectively, was used to irradiate
the brain 4hrs after TBI induction. The neurological severity score (NSS) was used to assess neurobehavioral function. Oron et al [39] showed that there was no statistical difference in NSS between the power densities of 10 and 20mW/cm². Also, there was no significant difference between control/non-laser-treated group and laser-irradiated group at 24 and 48hrs post-irradiation. However, there was a significant improvement in neurobehavioral function in the laser-irradiated groups from day 5 up to day 28, where the NSS were 26-27% lower in the laser-irradiated group. The laser-treated group showed less loss (1.4%) of cortical tissue at the injured site compared to the sham control group (12.1%) (p<0.001). The study clearly suggests that transcranial LLLT significantly reduces the long-term neurological damage in mice [36].

Moreira et al. reported the effect of LLLT on local and systemic immunomodulation in rats following cryogenic brain injury. The specimens were irradiated with 780 nm and 660 nm laser using 3J/cm² and 5J/cm². The authors concluded that LLLT could modulate TNF-alpha, IL-6 and IL-beta concentrations in the brain and blood of rats with cryogenic brain injury [37].

Khum et al. showed that treatment with LLLT could improve cognitive deficits after controlled cortical impact (CCI) in mice. The animals’ cognitive functions were evaluated in a Morris water maze (MWM); the motor function by wire grip test; nitrosative stress by nitrotyrosine ELISA; also brain edema and lesion volume were assessed. Mice with CCI treated with 60J/cm² (500mW/cm² x 2min) showed significant improvement of the latency to the hidden platform. The anti-inflammatory effect was noted with significant reduction of microgliosis at 48hr [38].

Oron et al [39] also examined the long-term effect of various transcranial laser therapy modes (pulsed-PW versus continuous-CW) and at different treatment time points in mild to moderate closed-head injury mice and studied the NSS effect. Their results showed that mice receiving transcranial LLLT with PW at 100 Hz 4h post injury had the highest full recovery (NSS 0) percentage (67%) at day 56. Lesion sizes were significantly smaller in both CW and PW laser treated groups than control group, seen by MRI [39].

1.1 Effect of different laser wavelengths in transcranial LLLT in closed head TBI model in mice

The following sections summarize studies from our laboratory that have explored the use of transcranial LLLT to treat TBI in mice models. All animal procedures used in our studies were approved by the Subcommittee on Research Animal Care (IACUC) of the Massachusetts General Hospital (protocol # 2010N000202) and met the guidelines of the National Institutes of Health.

In this set of studies the closed-head injury was induced by using a weight drop apparatus. Four hours post contusion mice sustaining a moderate to severe TBI (i.e., severity assessed via neurological severity score (NSS) to be 6-8) randomly received a single irradiation from a 665, 730, 810, or 980 nm laser with 36J/cm² (150mW/cm² over 4min). The 665 nm and 810 nm groups showed significant improvements on the NSS compared with the sham-treated control group after day 5 to day 28 as shown in Figure 3. The mean fractional areas of the brain (determined by morphometric analyses) for 665nm and 810nm LLLT groups was significantly decreased compared to the fractional areas for the sham-treated control group at day 28. The principal tissue chromophore proposed to be responsible for photo-biomodulation effects induced by LLLT is cytochrome c oxidase (CCO). CCO has distinct absorption bands in the red (~665 nm) and in the NRI (~810 nm), and there is a minimum CCO absorption spectrum at 730 nm [40].

1.2 Effect of pulsing in LLLT for controlled cortical impact (CCI)-TBI in mice

There is consensus about the laser wavelengths and the range of accepted dosages (irradiance and fluence) for treatment of brain disorders. However, there is no known consensus on whether continuous wave or pulsed light is the better choice for this purpose. To that end, Ando and colleagues [41] compared a single exposure from the same 810 nm Ga-Al-As diode laser delivered in different modes in a mouse TBI model. The parameters were either a pulsed 10 Hz or 100 Hz with 50% duty cycle or a continuous wave (CW) laser. The average power density was 50mW/cm² for 12 min giving a total fluence of 36J/cm². At 48hrs to 28 days after TBI, the neurological severity score (NSS) was significantly decreased in all laser treated groups. The improvement in the 10 Hz group became greater than in the PW 100 Hz and CW groups after day 7 as shown in Figure 4. The PW 10 Hz group showed a significant decrease of immobility time in the forced swim test for depression and anxiety compared to the untreated TBI group at day 28. There was a significant decrease of the immobility periods of the tail suspension test (another measure of depression and anxiety) in the PW 10 Hz group compared with untreated TBI group at day 1 and 28. These results suggest the antidepressant effect of LLLT. The significant decrease of lesion size in brain tissue around the traumatized site was noted in the PW 10 Hz group at day 15 and 28. The results imply that LLLT has a neuroprotective effect at the early stage of TBI. The therapeutic effects on the severity of injury, the antidepressant effects and protection of brain tissue of LLLT for TBI with 810 nm laser were more effective at 10 Hz pulse frequency than the 100 Hz and continuous wave. Ando et al. also hypothesized that a possible reason why laser irradiation at 10 Hz pulsed mode was most
effective for improving neurological outcome was that the frequency affects the whole brain. Resonance between the frequency of the pulsed light and that of the brain waves may occur. Particularly relevant is the fact that oscillation of theta waves that have a prominent 4-10 Hz rhythm in the hippocampal region of all mammals [42].

1.3. Effects of transcranial LLLT-repetition regimen in CCI-TBI in mice

The efficacy of LLLT on TBI has been previously investigated, though to a limited extent. As such there are many questions to be answered, for instance, what is the best regimen of treatment repetition? It is well established during the 40 years of LLLT studies that a pervasive biphasic dose response applies not only in cell culture studies, but also in preclinical animal studies and even in clinical cases [43]. It has been found that there is generally an optimum level of energy density (J/cm²), power density (mW/cm²) and/or treatment repetition required to give the best therapeutic effects. A less than optimal choice of parameters can result in reduced effectiveness of the treatment, or even a negative therapeutic outcome [44].

We (F. Vatansever, W. Xuan, L. Huang, Q. Wu, Y. Xuan, T. Dai, T. Ando, T. Xu, Y-Y. Huang, and M.R. Hamblin, 2012, submitted for publication) used CCI mouse model of severe TBI, and studied the effects of different treatment repetitions of 810 nm LLLT on neurobehavioral and vestibulomotor functioning, histomorphological analysis and histological evidence of neuroprotection and neurogenesis. The animals of the TBI treatment groups received transcranial LLLT (continuous wave 810 nm laser, 25 mW/cm², 18 J/cm²) either once at 4 hrs post-TBI, 3 treatments (once a day for 3 days) or 14 treatments (once a day for 14 days). We found that LLLT may have beneficial effects in the acute treatment of TBI, demonstrating that mice with severe TBI undergoing the laser treatment once (and to a greater extent 3 daily laser treatments) had significant improvements in NSS, and wire-grip and motion tests. However 14 daily laser treatments provided no benefit.Furthermore, the study results showed that LLLT for TBI in mice could significantly improve neural function, decrease lesion volume, augment cell proliferation, and even protect the brain against neuronal damage to some degree. The neurological behavior, evaluated by NSS, is shown in Figure 5. The deficits in neurobehavioral function in all groups of mice with severe TBI gradually but steadily improved with time after TBI induction. However, there were greater improvements in group 1 (single laser exposure given at 4 hours post-TBI; Fig 5A) compared to group 7 (TBI-sham, one immobilization and no laser) that became especially noticeable as time progressed up to 28 days post-TBI. The improvement seen in group 2 that received three laser treatments on days 1-3 post-TBI was even more pronounced and statistically significant when compared to control group 8 (TBI 3-sham exposures; Fig 5B). Group 3 that received 14 daily laser treatments (Fig 5C) showed marked improvement NSS until day 5 at which point the mice had received 5 daily laser treatments. However the improvement then ceased, as more laser treatments were given and at day 14 the advantage over group 9 (TBI 14 sham treatment) had disappeared. At day 28 there was no difference compared to TBI-sham group 9.

Conclusion

Our study has shown marked improvement in several parameters related to neurological, motor and lesion size in mice subjected to severe CCI-TBI and treated with transcranial LLLT. The mechanism appears to involve both prevention of tissue damage, in the short term, and increased brain repair, in the longer term, due to neurogenesis. LLLT appears to be effective based on many different biological mechanisms: neuroprotection or prevention of spreading the brain cell death (occurring in the hours and days after the brain injury); anti-inflammatory, anti-edema and pro-angiogenic effects. The most exciting possible beneficial effect of LLLT is that it may stimulate neurogenesis or improve the ability of the brain to repair itself. Not only might new brain cells be formed after LLLT but also the existing brain cells may be encouraged to form new synaptic connections in a process known as synaptogenesis or synaptic plasticity.

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Figure 1. Molecular mechanisms of LLLT - Light passing through the scalp and the skull is absorbed by cytochrome c oxidase and activates the mitochondrial respiratory chain of the cortical neurons. As a result, cell signaling and messenger molecules (such as reactive oxygen species (ROS), nitric oxide (NO), and ATP are upregulated. This signaling cascade then activates transcription factors (such as AP-1, NF-κB) that trigger the transcription of a range of new gene products.

Figure 2. Functional mechanism of LLLT - Facilitated and upregulated gene transcription (as seen in Figure 1) then leads to declines in neuronal apoptosis, lessening the inflammation and edema. This process also leads to escalating angiogenesis and expression of neurotrophins, which cause activation of neural progenitor cells and elevated synaptogenesis (all being factors contributing to the brain repair process).

Figure 3. Effect of laser wavelengths in transcranial LLLT in closed head TBI in mice. Time lapse of NSS scores of sham and laser-treated mice. Laser wavelengths used (A) 665nm, (B) 730nm, (C) 810nm, (D) 980nm versus their respective sham-treated controls. Points are means of 8-12 mice and bars are SD. *p<0.05; **p<0.01; ***p<0.001 (one-way ANOVA).
Figure 4. Effect of pulsing laser transcranial LLLT in CCI-TBI in mice. (A) Time course of NSS with 810nm laser in CW, PW 10Hz, PW 100Hz laser treated, and untreated results. ***p<0.001 vs. the other conditions. (B) Mean areas under the NSS-time curves over 28-day study.

Figure 5. NSS scores measured over 4 weeks of transcranial LLLT in sham TBI mice, 810nm laser treated TBI mice, and sham control mice. (A) One laser treatment/One sham treatment @ 4hrs after TBI; (B) 3 daily laser treatments/3 sham treatments; (C) 14 daily laser treatments/14 sham treatments. *p<0.05; **p<0.01; ***p<0.001 (one-way ANOVA). (D) Mean areas under the curve of NSS time courses.

References


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Treatment of dry Age-related Macular Degeneration with Photobiomodulation

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Abstract

Objective: To evaluate if LED Photobiomodulation (PBM) can affect vision in patients with dry Age-Related Macular Degeneration (AMD).

Methods: Prospective interventional case series. Near Infra Red (NIR) and yellow wavelengths of low powered LED light were applied to eyes with AMD in serial consecutive treatments. Included were patients with dry AMD, 50 years or older and with visual acuity between 20/20 - 20/200. Primary outcome measures selected were change in visual acuity, contrast sensitivity and fixation stability.

Results: The treatment protocol was completed in 18 eyes (9 patients). Changes in visual acuity (p<0.0001) and contrast sensitivity (p<0.0001 at 3 cycles/degree and p<0.0032 at 1.5 cycles/degree) were positive and significant. There were no significant changes in fixation stability parameters.

Conclusions: LED PBM proves to be beneficial for improvement of vision and contrast sensitivity as well as a safe treatment for dry AMD in this pilot study. Larger studies are warranted to validate the findings from this study.

Introduction:

AMD is a retinal degenerative disease that causes irreversible, profound vision loss in people over the age of 60 years. AMD occurs in two major forms: exudative (wet) and atrophic (dry) AMD. These two forms of AMD are both part of the same disease process continuum and share similar risk factors for their development. Exudative AMD is characterized by choroidal neovascularization (CNV). In contrast, atrophic AMD is characterized by retinal pigment epithelial (RPE) cell atrophy and subjacent photoreceptor degeneration. Factors involved in causing RPE cell injury and dysfunction have been shown to include oxidative stress, inflammation and genetic disposition.

Damage caused by oxidative stress and inflammation leads to progressive loss of cell function and thus contributes to the development of atrophic AMD.

There are no proven treatments for the dry or atrophic form of AMD to date. Potential therapeutic approaches for atrophic AMD include inhibiting inflammatory responses as well as reducing oxidative stress secondary to anoxia.

Photobiomodulation when used in the red to near-IR range (630–1,000 nm) using low-energy lasers or light-emitting diode (LED) arrays has been shown to accelerate wound healing, improve recovery from ischemic injury in the heart and attenuate degeneration in the injured optic nerve. LED treatment significantly improved rod- and M-cone- mediated ERG responses in methanol-intoxicated rats.

Ivandic and Ivandic have shown that LLLT with a laser diode aimed at the macular area (trans sclerally) in human subjects significantly improved visual acuity in a case series of both dry and wet AMD. Visual acuity in the control group remained unchanged. No adverse effects were observed in those undergoing therapy.

We designed this study as the first globally to look at the effect of PBM with low powered LED (non coherent) devices shone through the pupil in patients with dry AMD.
Methods:

The study was designed as a prospective non-randomized interventional case series. Patients were identified prospectively as they presented to clinics run by two of us (GM and RD). We selected subjects with previously diagnosed dry age-related macular degeneration (AMD).

Inclusion criteria were documented dry AMD, best corrected visual acuity (BCVA) of 20/20 to 20/200 and older than 50 years of age. Excluded from the study were subjects with previous or active wet AMD, with a previous history of epilepsy, with cognitive impairment, other retinal disease, previous retinal surgery, significant media opacity or contraindications to dilation drops.

The non-presence of neovascularization was ascertained prior to enrollment by examination with Ocular Coherence Tomography (OCT) and Intra-venous Fluorescein Angiography (IVFA) and confirmed by a retina specialist. All subjects were assessed for Visual Acuity with ETDRS charts at 4 meter distance (Precision Vision, USA) recorded in log MAR units, contrast sensitivity at 1.5 and 3 cycles per degree (Stereo Vision Optec 6500, USA) recorded as log contrast sensitivity and for fixation stability with the Nidek MP1 micro perimeter (Nidek Technologies, Padova, Italy). Accurate estimates of fixation stability could be obtained from raw data provided by the instrument by calculation of a Bi-Curve Ellipse Area (BCEA) \( i \). Calculations are based on the minor and major axes of an ellipse area covering fixational eye movements and takes into account two standard deviation measures of each recorded eye movement. The results are expressed in square degrees. Measurements took place prior to treatment, immediately following the treatment protocol (6 weeks), 4, 6 and 12 months after.

The intervention consisted of using LLLT in the yellow and red to near-IR range using low-energy delivery with the Warp10 (Quantum Devices) and the Gentlewaves (Light Bioscience) instruments. The instruments used are commercially available and have been approved for use in other conditions by the FDA and Health Canada. The FDA considers one of the devices a non-significant risk device for using on the eye. The other device is of even lower power.

The treatment parameters followed for the Warp10 delivery system were 670nm +/- 15nm at 50-80 mW/cm\(^2\), 4-7.68 J/cm\(^2\), for 88 +/- 8 seconds.

The treatment parameters followed for the Gentlewaves delivery system were 590nm +/- 8nm at 4mW, 790nm +/- 60nm at 0.6mW, for 35 seconds, pulsed at 2.5 Hz (250 milliseconds on, 150 milliseconds off) while delivering 0.1J/cm\(^2\)/treatment.

All subjects were treated with the two devices used sequentially at each treatment visit for a total of 18 treatments over a six-week period (3 times per week for 6 weeks).

The primary outcome measures selected for analysis were visual acuity, contrast sensitivity and fixation stability estimates. Data analysis was based on descriptive statistics that include frequency distributions, a measure of central tendency (mean) and a measure of dispersion (standard deviation). A statistical comparison of means between populations was made by t-test and repeated measures analysis of variance (repeated measures ANOVA). Differences were considered to be statistically significant at p values of less than 0.05. The study was performed in adherence to the guidelines of the Declaration of Helsinki. The study protocol was approved by an independent Research Ethics Committee (IRB Services, Aurora, Canada). Informed consent was obtained from all participants.

Results:

Over a span of 12 months 18 AMD study eyes (6 males and 12 females) were recruited and treated; aged 61 to 90 years old (mean 74.3 years/ SD 7.7).

Average ETDRS BCVA for the AMD group was measured at 0.25 log Mar units before the treatment and at 0.13 log Mar units 12 months after the treatment (p<0.0001).

Repeated Measures ANOVA yielded \( F (4,68) = 18.86, p < 0.0001 \).

Contrast sensitivity:

Repeated measures ANOVA for Contrast sensitivity (3cycles/degree): \( F (4,68) = 11.44, p < 0.0001 \).

Repeated measures ANOVA for Contrast Sensitivity (1.5 cycles/degree): \( F (4,68) = 4.39, p < 0.0032 \).

Fixation stability:

Repeated measures ANOVA for Fixation Stability (BCEA): \( F (4,68) = 0.90, p < 0.4661 \).

Correlation analysis between Fixation Stability and ETDRS VA:

Pearson R value of 0.6776, p less than 0.001.
Discussion:

Potential therapeutic approaches for atrophic AMD include neutralizing ROS, promoting cell survival, and inhibiting inflammatory responses that could potentially delay the progression of this disease and even improve cellular function.

Photobiomodulation (also known as LLLT) has both primary and secondary effects on cellular responses to disease including anti inflammatory, anti oxidant and anti apoptosis effects.

The precise biochemical mechanisms underlying the therapeutic effects of PBM are not yet well established. At the most basic level, LLLT acts by inducing a photochemical reaction in the cell, a process referred to as biostimulation or photobiomodulation. When a photon of light is absorbed by a chromophore in the treated cells, an electron in the chromophore can become excited and jump from a low-energy orbit to a higher-energy orbit. This stored energy can then be used by the system to perform various cellular tasks.

The influence of LLLT on the electron transport chain extends far beyond simply increasing the levels of ATP produced by a cell. Oxygen acts as the final electron acceptor in the electron transport chain and is, in the process, converted to water. Part of the oxygen that is metabolized produces reactive oxygen species (ROS) as a natural by-product. ROS are chemically active molecules that play an important role in cell signaling, regulation of cell cycle progression, enzyme activation, and nucleic acid and protein synthesis.

Within the cell, there is strong evidence to suggest that LLLT acts on the mitochondria to increase adenosine triphosphate (ATP) production, modulation of reactive oxygen species (ROS), and the induction of transcription factors.

These transcription factors then cause protein synthesis that triggers further effects downstream, such as increased cell proliferation and migration, modulation in the levels of cytokines, growth factors and inflammatory mediators, and increased tissue oxygenation.

In vitro studies, animal experiments and clinical studies have all tended to indicate that LLLT with fluences of red or NIR as low as 3 to 5 J/cm² will be beneficial in vivo, but a large dose like 50 to 100 J/cm² will lose the beneficial effect and may even become detrimental.

The RPE is the major local source of Complement Factor H (CFH) at the retina/choroid interface. Mutations or down regulation of CFH may increase the chance of RPE cells being attacked by activated complement systems. Damage caused by oxidative stress and inflammation lead to progressive loss of cell function and thus contributes to the development of atrophic AMD. Genes in different pathways influence progression to different stages of AMD. The genes CFH, C3, CFB, and ARMS2/HTRA1 have been associated with progression from intermediate drusen to large drusen, and from large drusen to GA or NV. By altering gene expression PBM can influence factors involved in progression of AMD such as VEGF and inflammatory cytokinins.

Recently there has been interest in tissue sparing or sub threshold laser at 577nm and 810 nm to produce therapeutic effects without clinical evidence of intra retinal damage. It has been proposed that the benefits might be due to the up- and down-regulation of angiogenic growth factors (e.g., VEGF) mediated by the biological reaction of RPE cells that have been only sub lethally injured. We feel that the same benefits to cellular function can occur with PBM and that there is no damage to any cells with the low powered LED light sources used in our study.

We used fixation stability, a novel testing parameter as one of our primary outcome measures and although there were changes both in the BCEA and PRL location after the treatment these were not statistically significant, however correlation analysis between visual acuity and fixation stability was improved after the treatment – further evidence of a treatment effect.

Average ETDRS visual acuity was statistically significantly improved immediately following the treatment and this improvement remained at statistically significant levels at 12 months although some decline in the ETDRS log MAR score is evident after 4 months. This would suggest that some patients would benefit from re-treatment somewhere after the 4-month interval.

Contrast sensitivity was statistically significantly improved with the treatment with a peak response at the 6-month stage and again like ETDRS VA, remaining at significant levels of improvement at 12 months.

LED PBM is extremely well tolerated, there is no discomfort and the individual treatments are easily dispensed taking less than 5 minutes per eye.

There were no significant adverse events noted during the course of the study, one subject with a history of migraine headaches felt that she was more susceptible to getting a migraine after one treatment session however it did not occur and this sensation lasted less than one hour.

We believe the results obtained warrant further evaluation of LED PBM as an important, safe and effective treatment in this potentially devastating disease where there are no proven treatments to date.


x Low-Level Laser Therapy Improves Vision in Patients with Age-Related Macular Degeneration
Boris T. Ivandic, M.D., and Tomislav Ivandic, M.D.

xi Tarita-Nistor L, González EG, Markowitz SN, Steinbach MJ. Fixation characteristics of patients with macular degeneration recorded with the MP-1 micro perimeter. Retina. 2008;28:125–133.


xx Sub threshold Micropulse Laser Therapy for Retinal Disorders
CHRISTINE KIIRE, MD; SOBHA SIVAPRASAD, MD; AND VICTOR CHONG, MD Retina Today Jan/Feb 2011


Induction of autologous mesenchymal stem cells at the bone marrow by low level laser therapy has beneficial effects on the ischemic heart and kidney

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Abstract

Multiple clinical trials were performed recently on the use of various stem cells to the ischemic heart. The general outcome of these trials was that the procedures are safe but improvement in the functional performance of the heart was marginal. Acute renal failure has a 50-80% mortality rate. Currently, treatment options for this life-threatening disease are limited. The aim of the present study was to demonstrate that low level laser therapy (LLLT) application to stem cells at the bone marrow (BM) may have beneficial effects on the infarcted rat heart post myocardial infarction, as well as on the ischemic kidney. LLLT applied to the infarcted area in the heart caused a significant reduction of 39% in the infarct size compared to control infracted, non-laser treated rats. LLLT applied directly to stem cells in the BM caused significant (p<0.001) reduction of 79% in the infarct size compared to control. Ventricular dilatation measurements also showed a marked reduction (74%, p<0.001) in the laser treated rats compared to control. In the group of rats in which LLLT was applied to the BM a significant (p=0.05) elevation of 27-fold in the density of c-kit immunopositive cells (a marker of MSCs) in the infarcted area as compared to control was noticed. Electron microscopy indicated newly formed cardiomyocytes at the hearts of the laser treated rats. Quantitative histomorphometric analysis of the histological sections revealed that dilatation of the renal tubules had been reduced, structural integrity of the renal tubules restored, and there was reduced necrosis in the laser-treated rats as compared to the control non-laser-irradiated group. In conclusion, the present study demonstrates a novel approach of applying LLLT to autologous BM of rats with ischemic heart or kidney in order to induce stem cells that are consequently recruited to the ischemic organs, leading to a marked beneficial effect to the heart post-myocardial infarction. The possibility that this approach can also be applied to other ischemic/injured organs, or organs undergoing degenerative processes, with consequent beneficial effects there too, cannot be ruled out.

Keywords: Heart, Kidney, Ischemia, Rat, Low level lasers.

Scientific Background

The mammalian heart has a very limited capacity to regenerate following an acute ischemic event like myocardial infarction (MI), due to the very low level of cardiomyocyte proliferation and the limited number of cells expressing stem-cell marker proteins. Stem-cells-based therapy was suggested as a potential solution to the above situation. Cell therapy for cardiac repair and remodeling has been the focus of intense research. In most animals that were used for these experiments, it has been shown to improve cardiac function after MI (1). In the last decade cell-based therapy for cardiac repair has undergone a rapid transition from basic science research to clinical reality (1-3). Multiple clinical studies have been performed in recent years on the use of various stem cells to repair the ischemic heart. Most clinical trials used certain cell types isolated from the autologous bone marrow (BM) of patient's post-MI or in acute heart failure. The general outcome of these trials was that the procedures and long-term outcome post-stem-cell implantation to the heart via the coronary arteries were safe. However, improvement in long-term functional performance of the heart was either not achieved or was marginal (1-3).

Acute renal failure has a 50-80% mortality rate. Currently, treatment options for this life-threatening disease are limited. Stem cells offer an exciting potential for kidney regeneration. In both mice and humans there is evidence that extra renal cells of bone marrow origin take part in tubular epithelium regeneration (4). Recent studies have demonstrated that hematopoietic stem cells were mobilized following ischemia/reperfusion (IR) to the kidney, engrafted onto the kidney, and differentiated into tubular epithelium in the areas of damage. The
evidence that mesenchymal stem cells, by virtue of their renoprotective property, restore renal tubular structure and also ameliorate renal function during experimental acute renal failure provides opportunities for therapeutic intervention (4).

There are several central issues pertaining to the use of cell implantation in stem-cell therapy for cardiac repair: the number of implanted stem cells has to be high (at least several millions) since there is massive cell death following implantation or injection of cells into the heart or the blood circulation. Also injected cells may have to migrate from the circulating blood to the heart or within the heart to an appropriate area in the heart.

We were trying to overcome some of the above mentioned issues of stem cell therapy for cardiac repair by using light stimulation therapy (low level laser therapy) to stem cells in the BM. Low-level laser therapy (LLLT) has been found to modulate various biological processes (5), such as increasing mitochondrial respiration and ATP synthesis, facilitating wound healing, and promoting the process of skeletal muscle regeneration and angiogenesis (6). In an experimental model of the infarcted heart in rats and dogs, it was demonstrated that LLLT application directly to the infarcted area in the heart at optimal power parameters significantly reduced infarct size (scar tissue formation) (6,7).

The effect of photobiostimulation on stem cells or progenitor cells has not been extensively studied. A remarkable increase in stem cell counts was observed on the fourth day of regeneration in Dugesia tigrina (worms) stimulated by laser irradiation (8). LLLT application to normal human neural progenitor cells significantly increased ATP production in these cells (9). LLLT delivery to MSCs and cardiac stem cells in vitro caused a significant enhancement in their proliferation rate (10).

Recently, based on our previous studies that showed an increase in cytoprotective effect on the ischemic heart following LLLT, we took a new approach to the application of laser irradiation to stem cells prior into their implantation to the infarcted heart as a cell therapy for heart repair (11) and demonstrated significant reduction in infarct size. In the present study we addressed the possibility of recruiting autologous stem cells stimulated by LLLT in the BM to the infarcted heart and the ischemic kidney.

**Experimental Procedure**

For the experiment of the infarcted heart a total of 72 Charles River male rats, that underwent ligation of the left anterior descending (LAD) artery to induce MI, were used as described by us previously (10). After induction of MI rats were randomly assigned to a laser-treated or control non-laser-treated group. A diode (Ga-Al-As) laser, wavelength 804 nm with a tunable power output of maximum of 400 mW (Lasotronic Inc., Zug, Switzerland) for application to the heart or the BM was used. Histology, infarct size determination and immunohistochemistry for c-kit cells were carried out as described previously (10). For ischemic kidney a total of 42 Wistar male rats that were divided into 2 groups (control and laser treated) were used. Ischemia-reperfusion (IR) injury was performed by clamping of the renal artery about 5 mm distant from its entry into the kidney. The contra-lateral kidney in each rat remained intact. Laser was performed as above. Two weeks post-IR injury to the kidney the rats were sacrificed and IR-injured and intact kidneys were removed. The kidneys were separated by longitudinal sectioning of the mid plane of each kidney into two identical halves. One half was taken for histology (cold fixation in 4% Neutral buffered formalin for 48 hours) and immunohistochemistry.

**Results and Discussion**

LLLT to the BM (at about 20 minute's post-MI) caused a marked and significant decreased (79%) in infarct size 3 weeks post-MI. This extent of infarct size reduction was even more effective in reducing scarring than that of laser application directly to the infarcted heart, as also found in our previous studies with infarcted rat and dog hearts (7). Concomitant with the reduction in infarct size, a decrease in ventricular dilatation (VD) was noticed in the laser-treated (to the BM) rats relative to control non-laser-treated rats. Even when laser was applied 4 hours post-MI to the BM of infarcted rats, a marked (52% and 42%) and significant (P<0.01) reduction in infarct size. In the present study we addressed the possibility of recruiting autologous stem cells stimulated by LLLT in the BM to the infarcted heart and the ischemic kidney.

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model, using organotypic cultures of endomyocardial biopsies in the presence or absence of MSC feeder layers, that there was stimulation of cardiac stem cells by those MSCs that reached the infarcted area. Another finding of our recent studies is that of the preferred homing of the recruited or endogenous c-kit+ cells in on the infarcted area, rather than their random deposition throughout the LV. This could be observed at both the 3 and 6 week time intervals post-MI in the rats whose BM had been laser-treated. This indicates that c-kit+ cells have the capacity to migrate to the uninjured/ischemic zone and most probably attenuate the scarring process. Indeed, at 3-weeks post-MI the density of c-kit+ cells in the infarcted area was 27-fold higher in the rats whose BM had been treated with LLLT as compared to control rats. Similarly, Hatzistergos et al. (12) found that endogenous c-kit+ cardiac stem cells increased by 20-fold in the porcine infarcted heart as compared to control following transcardial injection of BM-derived MSCs. Our recent study also has direct clinical relevance. The laser can be applied non-invasively (or invasively by inserting a fiber optic probe to the iliac crest in obese patients) to the bone marrow of the pelvic girdle, tibia or other parts of the skeleton containing BM up to 4 hours post-MI. This time interval post-MI is a reasonable therapeutic window for the laser treatment. Thus it can be postulated that the novel approach presented in our present study, of the stimulation of stem cells in order to increase their number in the blood, and eventually in the infarcted heart, may also attenuate the scarring process in human patients post-MI. C-kit+ cell density in the laser-treated group of 2-3 month old rats that were IR injured for 15 min was significantly (p=0.015) 3.2-fold higher than in the control group (Fig.1a). In the intact kidney of these rats there was no significant elevation in the c-kit density over blank-control immunostained sections (data not shown). C-kit density in the laser-treated group (2-3 month old) and IR injured for 30 min was significantly (p=0.02) 2.5-fold higher compared to control group. Histopathological changes which were visible in the control, non-laser-treated, histological sections, consist mainly of tubular dilatation, loss of contour of those tubules, necrosis as well as some blood cells in the interstitial tissue of the kidney. However, in those rats that received LLLT to the BM post-IR injury to the kidney, the contour of renal tubules was clear and almost no tubular dilatation was observed. In addition necrosis is almost not visible. Overall, necrotic (cell infiltration) areas tubular and dilatation and degenerative epithelium was much more prominent in the control IR kidneys as compared to the IR-kidneys that were laser treated. The present study indicates that LLLT has beneficial effects on the kidneys post-IR injury. This phenomenon corroborates also the beneficial effects of induction of stem cells in the BM by LLLT in the rat post-myocardial infarction (MI).

In conclusion, we have demonstrated here a novel approach, of applying LLLT to autologous bone marrow of infarcted rats in order to induce the proliferation of stem cells that are consequently recruited to the ischemic heart and kidney, leading to a marked beneficial effect. The possibility that this approach can also be applied to other ischemic/injured organs or organs undergoing degenerative processes (i.e. neurodegenerative diseases), with consequent beneficial effects, cannot be ruled out.

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Reference


Low-level laser therapy is a safe and effective method of body contouring

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Abstract

Background: Low-level laser therapy (LLLT) is a non-invasive treatment for a wide-assortment of medical ailments. A recent application is for non-invasive body slimming. A Level 1 clinical study was completed and recorded a significant reduction in circumferential measurements across waist, hips, and thighs compared to placebo subjects. Questions remain unanswered to whether the result observed was based upon simple fluid redistribution. The purpose of this retrospective study was to evaluate the efficacy of low-level laser therapy for non-invasive body slimming and determine if the loss was attributable to fluid or fat relocation. Methods: Data from 689 participants were obtained to evaluate the circumferential reduction demonstrated across the treatment site of the waist, hips, and thighs as well as non-treated systemic regions. Patient data were not pre-selected; all reports provided by clinics using LLLT for body contouring were used to evaluate the efficacy of this treatment. Participants received a total of six LLLT treatments across two-weeks having baseline and post-procedure circumferential measurements recorded. Measurement sites included waist, hips, thighs, arms, knees, neck, and chest. Results: The mean circumferential reduction reported for the waist, hips, and thighs one week after the treatment regimen was 3.27 inches (p<0.0001). Furthermore, participants demonstrated an overall mean reduction of 5.17 inches across all measurement points 5.17 inches (p<0.0001). Each anatomical region measured exhibited a significant circumferential reduction. Conclusion: These data reveal that the circumferential reduction exhibited following LLLT is not attributable to fluid or fat relocation as all measurement points, including non-treated regions, reported an inch loss.

Keywords: Low-level laser therapy, noninvasive body contouring, cosmetic aesthetics, 635 nm laser light, efficacy, safety

Introduction

Body contouring is a long-established part of aesthetic dermatology; however, older methods such as lipoplasty are associated with numerous clinical disadvantages including the need for anesthetics, pain, and slow recover times. A recent review of more 100,000 liposuction procedures under tumescent anesthesia revealed no serious adverse effects; however, liposuction under general anesthesia was associated with complications including deep venous thrombosis or pulmonary embolus, abdominal or organ perforation, infection, and bleeding [1]. A review of the European literature revealed 72 cases of severe complications from liposuction over a 5-year period including 23 fatalities. Causes of death included bacterial infections, gas gangrene, sepsis, hemorrhages, perforations and pulmonary embolism [1].

Although these adverse effects are rare, there is a growing demand for less invasive procedures. The application of low-level laser therapy (LLLT) has been shown to induce intracellular, photochemical reactions through a process referred to as biostimulation or photobiomodulation [2]. Based on the properties of photobiomodulation, LLLT has grown into an important medical procedure with many clinical applications including the treatment of chronic inflammatory joint disorders [3], improved healing of wound and injured nervous tissue [4, 5], and alleviating chronic pain [6].

Mechanism of action

When a beam of laser light is directed toward tissue, approximately 3% is reflected and the remaining light enters the tissue where it is absorbed and scattered [2]. The extent of light absorption and scattering is a function of the physical characteristics of the tissue and the color and wavelength of the light [7]. When the absorptive capacity of the tissue is very high relative to its scattering ability, the laser beam remains strongly
collinear and the depth of penetration becomes a function of the wavelength-dependent "absorption coefficient."

The study described below was performed using a red 635 nm laser device. The photobiomodulation process begins when a suitable chromophore becomes stimulated by absorbing a photon of laser light. In adipose cells, the chromophore is the copper-containing mitochondrial cytochrome C oxidase [2]. Activation of cytochrome C oxidase triggers a number of cellular events including an increase in adenosine triphosphate synthesis with subsequent up-regulation of cAMP and activation of cytoplasmic lipase. The activated lipase breaks down intracellular triglycerides into fatty acids and glycerol [8, 9].

An additional effect of cytochrome C oxidase activation is the transient formation of pores in the cell membrane of adipocytes which allows the newly formed fatty acids and glycerol to pass through the membrane into the extra-cellular space [10]. Images obtained using electron microscopy have demonstrated pore formation in the cell membranes of adipose cells exposed to low-level laser light. In one study, 80% of intracellular fat was released from adipose cells after 4 minutes of laser exposure and almost all of the fat was released after 6 minutes of exposure [11], resulting in the complete collapse but not death of treated adipocytes.

Upon entering the extra-cellular space, lipids released following LLLT are transported to lymph nodes where lysosomal acid lipase hydrolyzes the released triglycerides to generate nonesterified free fatty acids [11, 12]. Alternatively, released lipids may be transported via the lymphatic system to the liver where they undergo normal fatty acid oxidation. Although the metabolic fate is not known with certainty, it is known that the release of intracellular lipids does not contribute to elevated plasma lipids. Clinically, the use of LLLT has been associated with decreased plasma triglycerides and cholesterol [13, 14]. Importantly, LLLT does not result in tissue necrosis, thereby preserving the endocrine functions of adipose tissue [15] and preventing the inflammatory response associated with the use high-intensity focused ultrasound (HIFU) [16, 17] and cryolipolysis [18] for body contouring.

Methods

With respect to body sculpting, LLLT was first used to minimize tissue trauma and inflammation and promote wound healing after lipoplasty-assisted liposuction [19, 20]. Subsequently, LLLT has been shown to be an effective stand-alone method for body contouring. The results of two randomized, double-blind, sham-controlled studies demonstrated significant reductions in waist, hip, thigh [21] and upper arm circumference [22] following LLLT. This retrospective study was designed to evaluate the efficacy of LLLT for noninvasive body slimming and determine if the change in body circumference was due to fluid or fat relocation.

Data was gathered from patients with voluminous subcutaneous fat who had undergone LLLT in 50 independent private practice clinics throughout the USA [23]. The circumference of the treated area (waist, hips, bilateral thigh, bilateral arm across the bicep muscle, bilateral knee, chest, and neck) was recorded prior to treatment and 2 weeks after treatment. All circumferential measurements were made by enclosing the greatest area of subcutaneous fat volume and were identified based on anatomical landmarks in order to maintain proper repositioning at subsequent time-points. Treatment consisted of a LLLT device consisting of five independent diode laser heads emitting 635 nm (red) laser light, and generating a power output of 17-mW per diode in the constant wave mode (Zerona™, Erchonia Medical Inc., McKinney, TX).

The treatment phase consisted of three 40-minute LLLT treatments per week for 2 consecutive weeks. Each treatment session was separated by one day. The center diode was positioned 4 to 6 inches above the abdomen, centered along the body’s midline, with the four remaining diodes positioned above the lateral abdomen and thighs; each diode mechanically scanned an area of 182cm² through energy dispersing optics creating a circular pattern and delivering 20.4 joules per area. After 20 minutes, participants assumed a prone position and the diodes were repositioned in a similar fashion above the anterior abdomen and thighs for 20 additional minutes of treatment. The primary outcome measure was the change in the total combined circumferential measurements (bilateral arms and knees, neck, and chest). The change in total combined circumferential measurement for the waist, hips, and thighs was also evaluated. Significant changes were determined using paired t-tests and repeated-measure analysis of variance.

Results

Data was gathered for 689 participants of whom 666 reported data for the treatment of waist, hips, and thighs. Their mean age was 48.67 years (range, 20–84 years) and their mean weight was 159.75 lbs. Women comprised the majority of patients (92.3%). The mean baseline circumferential measurement for the waist, hips, and thighs was 121.41 inches decreasing by 3.27 inches following six LLLT treatments (p<0.0001). Changes in individual body areas are shown in Table 1.
### TABLE 1. Mean Circumferential Change for Waist, Hips, and Thighs

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline (inches)</th>
<th>Post-procedure (inches)</th>
<th>Difference (inches)</th>
<th>Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist</td>
<td>689</td>
<td>35.87</td>
<td>34.73</td>
<td>-1.14</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Hip</td>
<td>677</td>
<td>39.68</td>
<td>38.73</td>
<td>-0.95</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Right thigh</td>
<td>678</td>
<td>23.80</td>
<td>22.51</td>
<td>-0.57</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Left thigh</td>
<td>679</td>
<td>22.96</td>
<td>22.35</td>
<td>-0.61</td>
<td>$p&lt;0.0001$</td>
</tr>
</tbody>
</table>

Circumferential measurements of the non-treated areas also showed significant reductions in baseline circumference (Table 2).

### TABLE 2. Circumferential Changes for Non-Treated Anatomical Areas

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline Mean (SD)</th>
<th>Post-procedure Mean (SD)</th>
<th>Difference Mean (SD)</th>
<th>Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck</td>
<td>592</td>
<td>13.62 (1.516)</td>
<td>13.36 (1.463)</td>
<td>-0.26 (0.403)</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Right Arm</td>
<td>631</td>
<td>12.30 (2.65)</td>
<td>11.98 (2.56)</td>
<td>-0.32 (0.47)</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Left Arm</td>
<td>632</td>
<td>12.29 (2.43)</td>
<td>11.97 (2.34)</td>
<td>-0.32 (0.50)</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Chest</td>
<td>630</td>
<td>34.99 (4.66)</td>
<td>34.25 (4.49)</td>
<td>-0.74 (0.97)</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Right Knee</td>
<td>614</td>
<td>15.29 (1.81)</td>
<td>15.00 (1.73)</td>
<td>-0.29 (0.53)</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>Left Knee</td>
<td>611</td>
<td>15.63 (9.41)</td>
<td>15.34 (9.37)</td>
<td>-0.29 (0.53)</td>
<td>$p&lt;0.0001$</td>
</tr>
</tbody>
</table>

When the total circumferential reduction of the waist, hips, and thighs was combined with the decrease in circumference of non-treated areas, the mean total circumferential loss ($N=556$) was 5.17 inches ($p<0.0001$). The reduction in circumference of non-treated areas supports the hypothesis that fluid redistribution is not responsible for the reduction in the waist, hips, and thigh measurements. There were no reports of adverse events.

### Conclusion

Two randomized, sham-controlled trials demonstrated LLLT can significantly reduce waist, hip, thigh and upper-arm circumference. Data from this retrospective study indicate the decreased circumference of treated body areas following LLLT treatment is not due to fluid or fat relocation as all circumferential measurements including non-treated areas showed a significant decrease.

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