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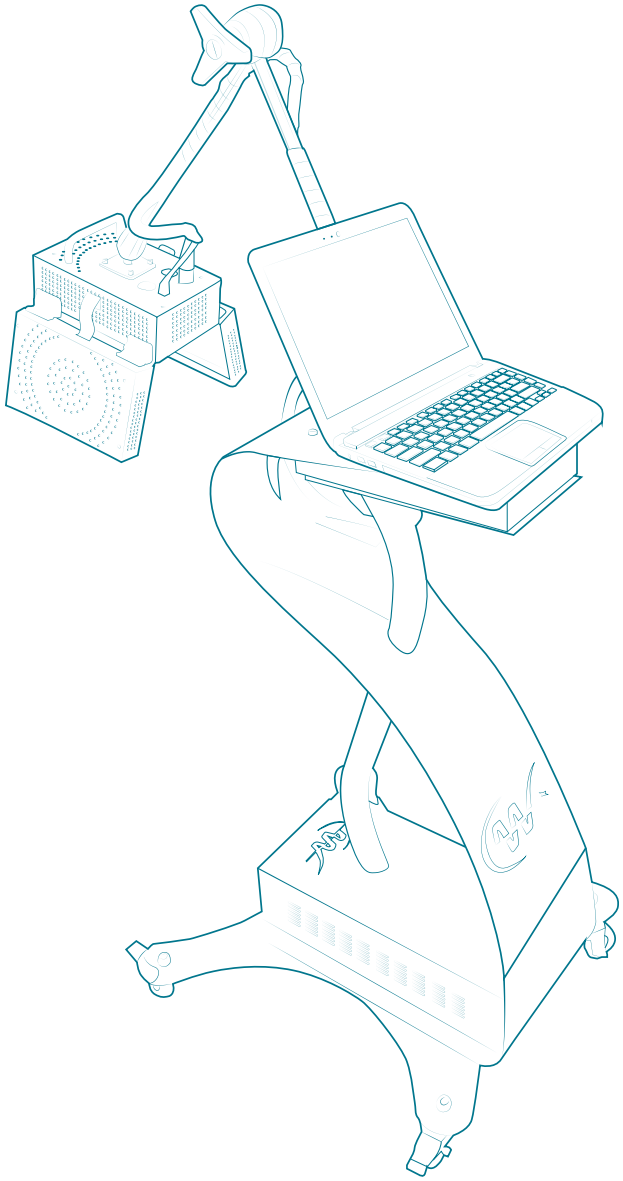

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A. EFFETS PRINCIPAUX DU LLLT

1. EFFET ANTALGIQUE

1.1 Douleur post-opératoire.

Marković AB, Todorović L – 2006

1.2 Antalgique dans la pathologie d'arthrose secondaire associée à la polyarthrite rhumatoïde.

Starodubtseva IA, Vasil'eva LV – 2015

But

Pour évaluer les indicateurs de la modification oxydative des protéines (OMP) pour les patients présentant une arthrose secondaire associée à la polyarthrite rhumatoïde (PR) et pour déterminer leur réaction sous l'effet du traitement combiné avec l'utilisation de l'irradiation photonique de faible intensité (LLLT).

Méthode

Un total de 50 patients atteints de PR associées à l'arthrose secondaire et 25 sujets sains ont été inclus dans cette étude. Les patients du sous-groupe étude une (n = 25) ont reçu une thérapie combinée avec l'utilisation de LLLT, ceux du deuxième sous-groupe (n = 25) ont reçu seulement un traitement médicamenteux. Nous avons utilisé les échelles VAS et DAS 28 pour estimer l'intensité de la douleur et des OMP sérique par rapport aux patients et aux sujets sains.

Résultat

Les analyses des données obtenues ont montré l'OMP accrue chez les patients atteints de PR par rapport aux sujets sains. Les patients du sous-groupe 1 ont connu une diminution significative des paramètres cliniques de la douleur sur la base des 28 échelles VAS DAS et accompagnés de la réduction marquée de l'OMP. Dans le sous-groupe 2, les patients présentaient également l'intensité statistiquement significative de ces indicateurs, mais elle était moins prononcée que dans le sous-groupe 1.

Conclusion

Les patients présentant une polyarthrite rhumatoïde sont caractérisés par une modification du taux élevé de protéine oxydative, un marqueur de stress oxydatif. La thérapie LLLT introduit dans le traitement combiné des patients atteints de PR, non seulement augmente les effets anti-inflammatoires et analgésiques, mais a également des propriétés anti-oxydantes.

Vopr Kurortol Fizioter Lech Fiz Kult. 2015 Jan-Feb;92(1):19-22. [The analysis of dynamics of oxidative modification of proteinsin the blood sera of the patients presenting with secondary osteoarthrosis associated with rheumatoid arthritis and treated by laser therapy] - Starodubtseva IA, Vasil'eva LV.

1.3 Low-level laser therapy as a treatment for chronic pain.

J. Derek Kinglsey, Timothy Demchak, Reed Mathis – 2014

Chronic pain is defined as pain that persists for greater than 12 weeks (Task-Force, 1994) and currently affects roughly 30% of the population in the United States (Johannes et al., 2010). The most common method for managing chronic pain has traditionally been pharmacological (Nalamachu, 2013). These treatments often include non-steroidal anti-inflammatory drugs (NSAIDS), opioids, acetaminophen, and

anticonvulsants (Nalamachu, 2013). Alternative medicine is now also being used more frequently to treat chronic pain and may consist of acupuncture (McKee et al., 2013), Tai Chi (Wang et al., 2010; Wang, 2012), and low-level laser therapy (LLLT) (Enwemeka et al., 2004; Ay et al., 2010). The focus of this manuscript is to highlight the physiological aspects of LLLT, and to discuss its application for those suffering from chronic pain, alone and in combination with exercise. It will also provide justification for the use of LLLT using specific data and case studies from the existing literature which have resulted in positive outcomes for those suffering from chronic pain.

The physiological mechanisms of LLLT are not well-understood and the mechanisms tend to be very broad (Yamamoto et al., 1988; Kudoh et al., 1989; Campana et al., 1993; Sakurai et al., 2000; Chow et al., 2007; Moriyama et al., 2009; Cidral-Filho et al., 2014). One hypothesis is that there may be an increase in nociceptive threshold after LLLT resulting in neural blockade, specifically an inhibition of A and C neural fibers (Kudoh et al., 1989; Chow et al., 2007). This inhibition may be mediated by altering the axonal flow (Chow et al., 2007) or by inhibiting neural enzymes (Kudoh et al., 1989). In addition, data suggests an increase in endorphin production (Yamamoto et al., 1988) and opioid-receptor binding via opioid-containing leukocytes with LLLT (Cidral-Filho et al., 2014). LLLT may also mimic the effects of anti-inflammatory drugs by attenuating levels of prostaglandin-2 (PGE2) (Campana et al., 1993) and inhibiting cyclooxygenase-2 (COX-2) (Sakurai et al., 2000). In addition, data have suggested that LLLT may augment levels of nitric oxide, a powerful vasodilator, which would in turn act to increase blood flow and assist with healing (Samoilova et al., 2008; Moriyama et al., 2009; Cidral-Filho et al., 2014; Mitchell and Mack, 2013). While the mechanisms have not been completely explained, it is clear that LLLT may have an analgesic effect.

Studies have demonstrated that LLLT may have positive effects on symptomology associated with chronic pain (Fulop et al., 2010; Hsieh and Lee, 2013); however this finding is not universal (Ay et al., 2010). A meta-analysis utilizing 52 effect sizes from 22 articles on LLLT and pain from Fulop et al. (2010) demonstrated an overall effect size of 0.84. This would be classified as a large effect size and suggests a strong inclination for the use of LLLT to reduce chronic pain. Twenty-two studies were utilized with doses ranging from 1 to 30 J/cm2. On the other hand, a meta-analysis from Gam et al. (1993) demonstrated no effect of LLLT on musculoskeletal pain but this study was published over 20 years ago when LLLT was just emerging. More recently data from Ay et al. (2010) have reported no difference in chronic pain compared to placebo using twice weekly treatment 5 days a week for 3 weeks. Treatment consisted of a total energy of 40 J/cm2 (850 nm, 100 mV, a treatment spot area of 0.07 cm2, 4 min over each of the four different points). Taken together, it is hard to assess whether LLLT is an effective modality. However, it is clear that LLLT may be effective in treating chronic pain in many individuals and should not be overlooked as a treatment modality.

A systematic review and meta-analysis from 16 randomized control studies on LLLT and neck pain (Chow et al., 2009) interpreted the analysis that LLLT caused an immediate decrease in pain for acute neck pain and up to 22 weeks post in chronic neck pain patients. Recently, in a double blinded placebo control study Leal et al. (2014) reported a decrease pain and increase in function in patients with knee pain.

One issue with these meta-analyses is that participants were grouped together, under the heading of chronic pain. However, chronic pain has different manifestations which inhibit the ability to make general observations. Separate subheadings of chronic pain may include but are not limited to chronic neck pain and lower back pain, myofascial pain syndrome, and fibromyalgia. A meta-analysis by Gross et al. (2013) worked to separate out the effect of LLLT on a variety of different conditions. Based on their review, the effect of LLLT on chronic neck pain has a moderate level of evidence for effectiveness when using 830 or 940 nm but not 632.8 nm. However, it was mentioned that the trials investigating chronic neck pain and LLLT failed to blind participants which may limit the application of the data. The authors also included the

effect of LLLT on myofascial pain syndrome and reported that the data are mixed and evidence is lacking. In addition, LLLT treatments have been reported to be effective for decreasing pain and increasing function in other chronic pain pathologies including fibromyalgia syndrome (Gur et al., 2002a,b; Armagan et al., 2006; Moore and Demchak, 2012).

Studies that examine the use of LLLT combined with exercise seem to have merit, as exercise is a staple of rehabilitation. Interestingly, Djavid et al. (2007) and Gur et al. (2003) both combined LLLT with exercise and each reported no additional effect of exercise in patients with chronic lower back pain. Djavid et al. utilized 27 J/cm2 of total energy (810 nm, 50 mW with an aperture of 0.2211 cm2, 8 points total) while Gur et al. utilized 1 J/cm2 (10 W with an aperature of 10.1 cm2, 4 min per point) for each of the 8 points. Matsutani et al. (2007) combined stretching exercise with LLLT (830 nm, 30 mW with an intensity of 3 J/cm2 over 18 tender points) in 20 women with fibromyalgia. There was no additive effect of combining stretching with LLLT in this study. Both groups reported reductions in pain scores and fatigue. Ultimately, the data are scarce and more are needed to truly understand the implications of LLLT when combined with exercise.

What tends to plague research using LLLT as a treatment modality is that there is no standard of care. Studies differ in overall dosage and wavelength which limits the ability to accurately draw conclusions. Currently, there are also no long-term studies that have evaluated LLLT. Pain is a very complex condition that manifests itself in a variety of different forms. Perhaps there is no set standard of care that will encompass everyone's needs. However, it is clear that LLLT may be beneficial for many individuals suffering from pain, regardless of the condition that is causing it.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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2. CICATRISATION

2.1 La prevention de la cicatrice en utilisant la thérapie LLLT en chirurgie plastique.

Capon A, Iarmarcovai G, Gonnelli D, Degardin N, Magalon G, Mordon S – 2010

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Abstract

Background

The use of lasers has been proposed for scar revision. A recent pilot clinical study demonstrated that lasers could also be used immediately after surgery to reduce the appearance of scars. The LASH (Laser-Assisted Skin Healing) technique induces a temperature elevation in the skin which modifies the wound-healing process. We report a prospective comparative clinical trial aimed at evaluating an 810-nm diode-laser system to accelerate and improve the healing process in surgical scars immediately after skin closure.

Methods
Twenty-nine women and 1 man (mean age = 41.4 years; Fitzpatrick skin types I-IV) were included to evaluate the safety and performance of the laser system. The laser dose (or fluence in J/cm(2)) was selected as a function of phototype and skin thickness. Each surgical incision (e.g., abdominoplasty) was divided into two parts. An 8-cm segment was treated with the laser immediately after skin closure. A separate 8-cm segment was left untreated as a control. Clinical evaluations (overall appearance ratings, comparative scar scale) of all scars were conducted at 10 days, 3 months, and 12 months by both surgeon and patients. Profilometry analysis from silicone replicas of the skin was done at 12 months. Wilcoxon signed-rank test analyses were performed.

Results
Twenty-two patients were treated using a high dose (80-130 J/cm(2)) and 8 patients with a low dose (<80 J/cm(2)). At 12 months in the high-dose group, both surgeon and patients reported an improvement rate of the laser-treated segment over the control area of 72.73 and 59.10%, respectively. For these patients, profilometry results showed a decrease in scar height of 38.1% (p = 0.027) at 12 months for the laser-treated segment versus control. Three patients treated with higher doses (>115 J/cm(2)) experienced superficial burns on the laser-treated segment, which resolved in about 5-7 days. For the eight patients treated at low dosage (<80 J/cm(2)), there was no significant difference in the treated segment versus the control segment. No side effects were observed.

Conclusion
This prospective comparative trial demonstrates that an 810-nm diode laser treatment, performed immediately after surgery, can improve the appearance of a surgical scar. The dose plays a great role in scar improvement and must be well controlled. There is interest in LASH for hypertrophic scar revision. LASH can be used to prevent and reduce scars in plastic surgery.

2.2 Amélioration de la cicatrisation par la thérapie LLLT des fibroblastes gingivaux.

Basso FG, Pansani TN, Turrioni AP, Bagnato VS, Hebling J, de Souza Costa CA – 2012

Abstract
The aim of this study was to determine adequate energy doses using specific parameters of LLLT to produce biostimulatory effects on human gingival fibroblast culture. Cells (3 × 104 cells/cm2) were seeded on 24-well acrylic plates using plain DMEM supplemented with 10% fetal bovine serum. After 48-hour incubation with 5% CO2 at 37°C, cells were irradiated with a InGaAsP diode laser prototype (LASERTable; 780 ± 3 nm; 40 mW) with energy doses of 0.5, 1.5, 3, 5, and 7 J/cm2. Cells were irradiated every 24 h totalizing 3 applications. Twenty-four hours after the last irradiation, cell metabolism was evaluated by the MTT assay and the two most effective doses (0.5 and 3 J/cm2) were selected to evaluate the cell number (trypan blue assay) and the cell migration capacity (wound healing assay; transwell migration assay). Data were analyzed by the Kruskal-Wallis and Mann-Whitney nonparametric tests with statistical significance of 5%. Irradiation of the fibroblasts with 0.5 and 3 J/cm2 resulted in significant increase in cell metabolism compared with the nonrradiated group ($P < 0.05$). Both energy doses promoted significant increase in the cell number as well as in cell migration ($P < 0.05$). These results demonstrate that, under the tested conditions, LLLT promoted biostimulation of fibroblasts in vitro.

Introduction
Tissue healing involves an intense activity of diverse cell types, such as epithelial and endothelial cells, as well as fibroblasts which play a key role in this process [1]. Fibroblasts secrete multiple growth factors during wound reepitelialization and participate actively in the formation of granulation tissue and the synthesis of a complex extracellular matrix after reepitelialization [1]. All these processes directly involve the prolifera-

tion and migration capacity to these cells [1]. The use of low-level laser therapy (LLLT) has been proposed to promote biostimulation of fibroblasts and accelerate the healing process [2].

Previous studies have evaluated the effect of LLLT on the proliferation and migration of human gingival fibroblasts as well as other cellular effects and responses, such as protein production and growth factor expression [2–6]. Nevertheless, there is a shortage of studies investigating irradiation parameters capable of promoting biostimulatory effects on fibroblasts in order to establish an ideal irradiation protocol for these cells [7]. Therefore, the aim of this study was to determine the most adequate energy doses using specific parameters of LLLT to produce biostimulatory effects on human gingival fibroblast cultures in an in vitro wound healing model.

Material and Methods

Gingival Fibroblast Cell Culture

All experiments were performed using human gingival fibroblast cell culture (continuous cell line; Ethics Committee 64/99-Piracicaba Dental School, UNICAMP, Brazil). The fibroblast cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), with 100 IU/mL penicillin, 100 µg/mL streptomycin, and 2 mmol/L glutamine (Gibco, Grand Island, NY, USA) in an humidified incubator with 5% CO2 and 95% air at 37°C (Isotemp; Fisher Scientific, Pittsburgh, PA, USA) [8]. The cells were subcultured every 2 days in the incubator under the conditions described above until an adequate number of cells were obtained for the study. The cells (3×104 cells/cm^2) were then seeded on sterile 24-well acrylic plates using plain DMEM supplemented with 10% FBS for 48 h.

LLLT on Fibroblast Culture

The LLLT device used in this study was a near infrared indium gallium arsenide phosphide (InGaAsP) diode laser prototype (LASERTable; 780 ± 3 nm wavelength, 0.04 W maximum power output), which was specifically designed to provide a uniform irradiation of each well (2 cm^2) in which cultured cells are seeded [8, 9]. The power loss through the acrylic plate was calculated using a potentiometer (Coherent LM-2 VIS High-Sensitivity Optical Sensor, USA), which was placed inside the culture plate. After this measure, the power loss of the plate was determined as 5%. After that, the power of all diodes was checked and standardized. Therefore, a final power of 0.025 W reached the cultured cells. This standardization was performed as previously described in the literature [8, 9]. For the evaluation of cell metabolism, the radiation originated from the LASERTable was delivered on the base of each 24-well plate with energy doses of 0.5, 1.5, 3, 5, and 7 J/cm^2, and irradiation times of 40, 120, 240, 400, and 560 s, respectively. The laser light reached the cells on the bottom of each well with a final power of 0.025 W because of the loss of optical power in each well due to the interposition of the acrylic plate. The cells were irradiated every 24 h totalizing 3 applications during 3 consecutive days. The cells assigned to control groups received the same treatment as that of the experimental groups. The 24-well plates containing the control cells were maintained at the LASERTable for the same irradiation times used in the respective irradiated groups, though without activating the laser source (sham irradiation) [8, 9]. Twenty-four hours after the last irradiation (active or sham), the metabolic activity of the cells was evaluated using the MTT assay (described below). Based on cell metabolism results, the two most effective irradiation doses were selected to evaluate the cell number (trypan blue assay), cell migration capacity by using the wound healing assay (qualitative analysis) and the transwell migration assay (quantitative analysis), as described below.

Analysis of Cell Metabolism (MTT Assay)

Cell metabolism was evaluated using the methyltetrazolium (MTT) assay [8–10]. This method determines the activity of succinic dehydrogenase (SDH) enzyme, which is a measure of cellular (mitochondrial) respiration and can be considered as the metabolic rate of cells.

Each well with the fibroblasts received 900 µL of DMEM plus 100 µL of MTT solution (5 mg/mL sterile PBS). The cells were incubated at 37°C for 4 h. Thereafter, the culture medium (DMEM; Sigma Chemical Co., St. Louis, MO, USA) with the MTT solution were aspirated and replaced by 700 µL of acidified isopropanol solution (0.04 N HCl) in each well to dissolve the violet formazan crystals resulting from the cleavage of the MTT salt ring by the SDH enzyme present in the mitochondria of viable cells, producing a homogenous bluish solution. Three 100 µL aliquots of each well were transferred to a 96-well plate (Costar Corp., Cambridge, MA, USA). Cell metabolism was evaluated by spectrophotometry as being proportional to the absorbance measured at 570 nm wavelength with an ELISA plate reader (Thermo Plate, Nanshan District, Shenzhen, China) [8, 9]. The values obtained from the three aliquots were averaged to provide a single value. The absorbance was expressed in numerical values, which were subjected to statistical analysis to determine the effect of LLLT on the mitochondrial activity of the cells.

Viable Cell Counting (Trypan Blue Assay)

Trypan blue assay was used to evaluate the number of cells in the culture after LLLT application. This test provides a direct assessment of the total number of viable cells in the samples as the trypan blue dye can penetrate only porous, permeable membranes of lethally damaged (dead) cells, which is clearly detectable under optical microscopy [11]. The LLLT protocol was undertaken as previously described using energy doses of 0.5 and 3 J/cm^2. Cell counting was performed in the experimental and control groups 24 h after the last irradiation (active or sham). The DMEM in contact with the cells was aspirated and replaced by 0.12% trypsin (Invitrogen, Carlsbad, CA, USA), which remained in contact with the cells for 10 min to promote their detachment from the acrylic substrate. Then, 50 µL aliquots of this cell suspension were added to 50 µL of 0.04% trypan blue dye (Sigma Aldrich Corp., St. Louis, MO, USA), and the resulting solution was maintained at room temperature for 2 min so that the trypan blue dye could pass through the cytoplasmic membrane of the nonviable cells, changing their color into blue. Ten microliters of the solution were taken to a hemocytometer and examined with an inverted light microscope (Nikon Eclipse TS 100, Nikon Corporation, Tokyo, Japan) to determine the number of total cells and nonviable cells. The number of viable cells was calculated by deducting the number of nonviable cells from the number of total cells [8]. The number of cells obtained in the counting corresponded to n × 104 cells per milliliter of suspension.

Cell Migration

Wound Healing Assay

The wound healing assay was used because it is a classic method of evaluation in vitro tissue healing assays [12, 13]. After 48 h of cell culture, a sterile 5 mL pipette tip was used to make a straight scratch on the monolayer of cells attached to the acrylic substrate, simulating a wound. Formation of the in vitro wound was confirmed under an inverted microscope (TS 100, Nikon, Tokyo, Japan). The LLLT protocol was undertaken as previously described using energy doses of 0.5 and 3 J/cm^2. Twenty-four hours after the last irradiation, the cells were fixed in 1.5% glutaraldehyde for 1 h, stained with 0.1% violet crystal for 15 min, and washed twice with distilled water. Wound repopulation was assessed with a light microscope (Olympus BX51, Miami, FL, USA) equipped with a digital camera (Olympus C5060, Miami, FL, USA).

Transwell Migration Assay

The capacity of human gingival fibroblasts to migrate through a cell permeable membrane was assessed using 6.5 mm-diameter transwell chambers (Corning Costar, Cambridge, MA, USA) with polycarbonate membrane inserts (8 µm pore size) [14]. The chambers were placed in 24-well plates containing 1 mL of plain DMEM per well. The cells were seeded onto the upper compartment of the chamber (1.5 × 104 cells/cm^2) and incubated at 37°C for 48 h. After this period, the LLLT protocol was undertaken as previously described using energy doses of 0.5 and 3 J/cm^2. Twenty-four hours after the last irradiation (active or sham), the cells that had migrated through the membrane to the lower compartment of the chamber were fixed in 1.5% glutaraldehyde for 1 h, incubated with 0.1% violet crystal dye for 15 min, and washed

twice with distilled water. After the last wash, the stained cells were viewed under a light microscope (Olympus BX51, Miami, FL, USA) equipped with a digital camera (Olympus C5060, Miami, FL, USA) and photomicrographs from three randomly chosen fields were taken at ×10 magnification for counting the number of migrated cells using the image-analysis J 1.45S software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). Two samples of each group were evaluated and the experiment was performed in triplicate.

Analysis of Migrated Cells by Scanning Electron Microscopy (SEM)

Part of the specimens used in the transwell migration assay was also used for the analysis of the cells by SEM. Twenty-four hours after the last irradiation (active or sham), the culture medium was aspirated and the transwell inserts were fixed in 1 mL of 2.5% glutaraldehyde in PBS for 2 h. Then, the glutaraldehyde solution was aspirated and the cells adhered to the transwell inserts were washed with PBS and distilled water two consecutive times (5 min each) and then dehydrated in a series of increasing ethanol concentrations (30, 50 and 70%, one time for 30 min each; 95 and 100%, two times for 60 min each) and covered 3 times with 200 µL of 1,1,1,3,3,3-hexamethyldisilazane (HMDS; Sigma Aldrich Corp., St. Louis, USA) [8]. The transwell inserts were stored in a desiccator for 24 h, sputter-coated with gold, and the morphology of the surface-adhered cells was examined with a scanning electron microscope (JMS-T33A scanning microscope, JEOL, Tokyo, Japan).

Statistical Analysis

Data from MTT, Trypan blue and Transwell assay had a nonnormal distribution (Kolmogorov-Smirnov, $P < 0.05$) and were analyzed by the Kruskal-Wallis and Mann-Whitney nonparametric tests. A significance level of 5% was set for all analyses.

Results

Analysis of Cell Metabolism (MTT Assay)

Data from SDH production by human gingival fibroblast cultures (MTT assay) after LLLT, according to the energy dose are presented in Table 1.

Table 1

Succinate dehydrogenase enzyme (SDH) production by human gingival fibroblasts detected by the MTT assay according to the energy dose used in the low-level laser therapy.

Regarding the energy dose of 5 J/cm² no statistically significant difference between the irradiated group and the nonirradiated control group was observed ($P > 0.05$). Conversely, irradiation of the fibroblast cultures with doses of 0.5 J/cm² and 3 J/cm² resulted in 11% and 17% increases in cell metabolism, respectively, differing significantly from the control group ($P < 0.05$). The cells irradiated with 1.5 J/cm² and 7 J/cm² presented the lowest metabolic rate compared with the nonirradiated control group (6% and 8% decrease, resp., $P < 0.05$).

Viable Cell Counting (Trypan Blue Assay)

The number of viable cells (%) after LLLT application, according to the energy dose, is presented in

Table 2

Number of viable cells (%) detected by the trypan blue assay, according to the energy doses used in the low-level laser therapy.

Comparison among the energy doses revealed that irradiation of the human gingival fibroblast cultures with 0.5 J/cm² and 3 J/cm² increased the number of viable cells by 31% and 66%, respectively, differing significantly from the control ($P < 0.05$), but without statistically significant difference between each other

(P > 0.05).

Fibroblast Migration

Wound Healing Assay

The analysis of the monolayer of human gingival fibroblasts after irradiation of the "in vitro wound" showed more intense cell migration, with consequent better coverage of the substrate (wound repopulation) (Figure 1).

Figure 1

Photomicrographs showing human gingival fibroblast cultures seeded in 24-well plates after LLLT. The control group exhibits a large cell-free area on acrylic surface. The group irradiated with 0.5 J/cm² exhibits cell proliferation and ...

3.3.2. Transwell Assay

Data from the transwell assay after LLLT, according to the energy dose are, presented in Table 3.

Table 3

Cell migration (%) by the transwell assay, according to the energy dose used in the low-level laser therapy. Comparison among the energy doses revealed that irradiation of the human gingival fibroblast cultures with 0.5 J/cm² and 3 J/cm² increased cell migration by 16% and 18%, respectively, differing significantly from the control (P < 0.05), but without statistically significant difference between each other (P > 0.05).

Analysis of Migrated Cells by Scanning Electron Microscopy (SEM)

The SEM analysis of the transwell inserts, which complemented the viable cell counting by the trypan blue assay, revealed that the fibroblasts were capable of migrating through the transwell membrane. The cells obtained from human gengiva did not change their morphology after been submitted to LLLT (Figure 2).

Figure 2

SEM micrograph showing cells with normal morphology that migrated through the transwell membrane. SEM ×500.

Go to:

4. Discussion

Different LLLT modalities have been used for diverse treatments in the health fields. In Dentistry, LLLT has been widely investigated and indicated for accelerating the healing process, especially in the treatment of ulcerative oral mucosa lesions [15, 16].

Several in vitro studies have evaluated the effect of LLLT on healing [7, 17]. Nevertheless, current research involving irradiation of cell cultures has not yet established the irradiation patterns specific for the different cell lines. Establishing the ideal irradiation parameters and techniques is mandatory for the development of sequential studies that can determine the potential biostimulatory effect of LLLT on oral mucosa cells, such as keratinocytes and fibroblasts, which are directly involved in the local healing process.

In the present study, the metabolic activity of human gingival fibroblast cultures after LLLT with different energy doses was evaluated to determine the adequate doses to produce biostimulatory effects on these cells in vitro. The results for SDH production showed that the 0.5 and 3 J/cm² doses increased cell metabolism. Therefore, these two most effective irradiation doses were selected to evaluate the number of viable cells as well as the cell migration capacity. The increase of SDH production after irradiation of

gingival fibroblasts has also been observed by Damante et al. [18], using a similar laser prototype to the one used in the present study. In the same way as in the present study, the SDH production results also served as guide for subsequent experiments that evaluated the expression of growth factors by cultured fibroblasts.

In the present study, a significant increase in the number of viable cells that presented normal morphological characteristics (SEM analysis) was observed after LLLT using doses of 0.5 and 3 J/cm2. These results confirm those of previous laboratory investigations in which LLLT with the same wavelength as that of the present study (780 nm) increased the proliferation of gingival fibroblasts [19, 20]. Kreisler et al. [2] also reported increase of fibroblast cell culture in vitro after direct and consecutive low level laser irradiations. The mechanism by which LLLT can promote biostimulation and induce proliferation of different cell types remains a controversial subject [20, 21]. Some authors [21, 22] claim that this mechanism is derived from light absorption by the enzyme cytochrome c oxidase in the cells, which participates in the cascade of oxidative respiration. Eells et al. [23] demonstrated the increase in the production of this enzyme after different LLLT application of cell cultures. It has also been suggested that the mechanism of cell proliferation induced by LLLT might be derived from the activation of singling pathways, such as the MAPK and PI3K/Akt pathways, which control both cell proliferation and regulation of gene expression [21, 24].

Fibroblast cell migration and proliferation are essential events for tissue healing and are directly related with its success [1, 3]. In the present study, the effect of LLLT on the capacity of gingival fibroblast migration, using two energy doses capable of increasing cell metabolism (0.5 and 3 J/cm^2), was evaluated qualitatively, by the wound healing assay, and quantitatively, by the transwell migration assay. Both methodologies demonstrated that LLLT was able to increase the migration capacity of fibroblasts and the quantitative analysis of the results revealed no significant difference between the energy doses. These results are in accordance with those of previous investigations [7, 17], but studies using the transwell migration method to evaluate the LLLT on cell cultures are still scarce. This methodology is relevant because it measures the number of cells that can pass through the transwell membrane inserts, demonstrating their migration capacity after stimulation by LLLT.

Diverse mechanisms are involved in cell migration during tissue healing, including expression and secretion of growth factors [1]. Previous studies demonstrated that LLLT may cause positive effects on cells by increasing growth factor expression, which could be a form of action of specific laser parameters on cell migration [2, 25]. A recent study of our research group demonstrated that LLLT had a biostimulatory effect on epithelial cells in vitro by increasing their metabolic activity, number of viable cells and expression of growth factors [8]. In the present paper, the biostimulation of human gingival fibroblast cultures by LLLT with consequent increase in the number of viable cells and cell migration capacity demonstrates the efficacy of specific laser parameters and irradiation technique on the healing process. In addition, the obtained results are supportive to those of previous in vivo studies in which acceleration of the healing process was observed after LLLT [15, 16, 26], but the limitations of an in vitro experiment should be considered.

In conclusion, the findings of the present study demonstrated that the preset laser parameters in combination with the sequential irradiation technique caused biostimulation, proliferation, and migration of human gingival fibroblast cultures. These encouraging laboratory outcomes should guide forthcoming studies involving tissue irradiation with laser and its effects on in vivo tissue healing.

Acknowledgments
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2.3 **La thérapie (LED-LLLT améliore la cicatrisation des plaies : une étude préliminaire.**
Mink PK, Goo BL – 2013

1) Regeneration Osseuse

a. **Evaluation de la thérapie LLLT en biomodulation pour la réparation osseuse dans les cavités faites dans le fémur de rats.**
Blaya DS, Guimarães MB, Pozza DH, Weber JB, de Oliveira MG – 2008

Conclusion
La thérapie LLLT dans le protocole de cette étude était efficace pour la réparation osseuse. L'utilisation de la technologie LLLT a été utilisée pour améliorer les résultats cliniques de la chirurgie osseuse et promouvoir une période postopératoire plus efficace et une guérison plus rapide.

Référence
J Contemp Dent Pract. 2008 Sep 1; 9(6):41-8.
Histologic study of the effect of laser therapy on bone repair.
Blaya DS[1], Guimarães MB, Pozza DH, Weber JB, de Oliveira MG.

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b. Efficacité thérapeutique de la thérapie LLLT et des Bio-Oss, les deux et séparément, sur le post traumatique de la régénération du tissu osseux chez les rats en utilisant la spectroscopie infrarouge comme une méthode de mesure informative et précise.
Rochkind S, Kogan G, Luger EG, Salame K, Karp E, Grafi M, Weiss J – 2004

Conclusion
Les résultats suggèrent que l'irradiation LLLT, seuls ou en combinaison avec le Bio-Oss améliorent la guérison osseuse et augmente la réparation osseuse.

Référence
Photomed Laser Surg. 2004 Jun; 22(3):249-53.
Molecular structure of the bony tissue after experimental trauma to the mandibular region followed by laser therapy.
Rochkind S1, Kogan G, Luger EG, Salame K, Karp E, Graif M, Weiss J.

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c. Evaluer sur le plan histologique l'effet de la thérapie LLLT 830nm sur la réparation des défauts osseux du fémur des rats Wistar alibinus greffé avec des bovins inorganiques et associés (ou pas) avec la membrane de l'os cortical bovine décalcifiées.
Pinheiro AL, Limeira Júnior Fde A, Gerbi ME, Ramalho LM, Marzola C, Ponzi EA, Soares AO, De Carvalho LC, Lima HC, Gonçalves To - 2003

Conclusion
Les résultats ont montré une réparation plus avancée des groupes irradiés par rapport à ceux non irradiées. La réparation du groupe irradié a été caractérisée par la formation de deux cavités osseuses accrues et une quantité de fibres de collagène autour de la prothèse dans la cavité, dès le 15e jour après la chirurgie, considérant la capacité ostéoconductrice du Gen-buffle et l'incrément de la réparation corticale chez les spécimens avec une membrane de Gen-derm. En conclusion, la thérapie LLLT a eu un effet positif sur la réparation osseuse par greffe associée ou non et de l'utilisation d'une membrane biologique.

Référence
J Clin Laser Med Surg. 2003 Oct; 21(5):301-6.
Effect of 830-nm laser light on the repair of bone defects grafted with inorganic bovine bone and decalcified cortical osseus membrane.
Pinheiro AL1, Limeira Júnior Fde A, Gerbi ME, Ramalho LM, Marzola C, Ponzi EA, Soares AO, De Carvalho LC, Lima HC, Gonçalves TO.

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d. Effect of low-level laser on bone defects treated with bovine or autogenous bone grafts: in vivo study in rat calcaria.

Mércia J.S Cunha, Luis A. Esper, Michyele C. Sbrana, Paula G.F.P. de Oliveira, Accácio L. do Valle, Ana Lúcia P.F. de Almeida – 2014

Abstract

Objective

The purpose of this study was to histologically evaluate the effect of low-level laser (LLL) on the healing of critical size defects (CSD) in rat calvaria, filled with autogenous or inorganic bovine bone grafts.

Methods

Sixty rats were divided into 6 groups (n = 10): C (control-filled with blood clot), LLL (low-level laser-GaAlAs, λ 780 nm, 100 mW, 210 J/cm(2), Φ 0.05 cm(2); 6 J/point), AB (autogenous bone), ABL (autogenous bone + low-level laser), OB (inorganic bovine bone), and OBL (inorganic bovine bone + LLL).

Material And Methods

The animals were killed after 30 days. Histological and histometric analyses were performed by light microscopy. Results. The groups irradiated with laser, LLL (47.67% ± 8.66%), ABL (39.15% ± 16.72%), and OBL (48.57% ± 28.22%), presented greater area of new bone formation than groups C (9.96% ± 4.50%), AB (30.98% ± 16.59%), and OB (11.36% ± 7.89%), which were not irradiated. Moreover, they were significantly better than group C (Kruskal-Wallis test followed by Dunn test, $P < 0.05$).

Conclusion

The laser accelerated the healing of bone defects and the resorption of particles of the graft material.

e. Effect of low-level laser therapy irradiation and Bio-Oss material on the osteogenesis process in rabbit calcarium defects: a double blind experimental study.

Amir Alireza Rasouli Ghahroudi, Amir Reza Rokn, Katayoun A.M. Kalhori, Afshin Khorsand, Alireza Pournabi, A.L.B. Pinheiro, Reza Fekrazad – 2013

Abstract

This study aims to assess the effect of low-level laser therapy (LLLT) irradiation and Bio-Oss graft material on the osteogenesis process in the rabbit calvarium defects. Twelve white male New Zealand rabbits were included in this study. Four 8-mm diameter identical defects were prepared on each rabbit's calvarium. One site was left as an untreated control (C), the second site was filled with Bio-Oss (B), the third site was treated with laser irradiation (L), and the fourth site treated with Bio-Oss and laser irradiation (B + L). In the laser group, a diode laser (wavelength 810 nm, output power 300 mW, irradiation mode CW, energy density 4 J/cm2) was applied immediately after surgery and then one other day for the next 20 days. After 4 and 8 weeks, the animals were sacrificed and histological and histomorphometric examinations were performed and the data were subjected to Friedman and repeated measurements ANOVA tests. Significant differences were not found regarding inflammation severity, foreign body reactions, and vitality of newly formed bone on 4th and 8th week after operation. The mean amount of new bone was 15.83 and 18.5 % in the controls on the 4th and 8th week; 27.66 and 25.16 % in the laser-irradiated group; 35.0 and 41.83 % in Bio-Oss and 41.83 and 47.0 % in the laser + Bio-Oss treated specimens with significant statistical differences ($p < 0.05$). Application of LLLT in combination with Bio-Oss® can promote bone healing. Therefore, LLLT may be clinically beneficial in promoting bone formation in skeletal defects.

Keywords

Low-level laser therapyOsteogenesisBone graftInorganic bovine bone mineralAnimal study

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3. DOULEUR ARTICULATION TEMPORO-MANDIBULAIRE MAXILLOFACIALE

3.1 Evaluation of low-level laser therapy effectiveness on the pain and masticatory performance of patients with myofascial pain.
de Moraes Maia ML, Ribeiro MA, Maia LG, Stuginski-Barbosa J, Costa YM, Porporatti AL, Conti PC, Bonjardim LR – 2012

3.2 Management of myofascial pain: low-level laser therapy versus occlusal splints.
Oz S, Gokcen-Rohling B, Saruhanoglu A, Tuncer EB – 2010

3.3 Laser application effects on the bite strength of the masseter muscle, as an orofacial paintreatment.
De Medeiros JS, Vieira GF, Nishimura PY – 2005

3.4 A systematic review of low level laser therapy with location- specific doses.
Bjordal JM, Couppe C, Chow RT, Tuner J, Ljunggren EA – 2003

3.5 Treatment of persistent idiopathic facial pain (PIFP) with a low-level energy diode laser.
Yang HW, Huang YF – 2011

3.7 Can low reactive-level laser therapy be used in the treatment of neurogenic facial pain? A double-blind, placebo controlled investigation of patients with terminal neuralgia.
Arne Eckerdal, H Lehmann Bastian – 1996

3.8 Laser therapy for pain of trigeminal neuralgia.
J.B. Walker

2) Recherche sur les mécanismes d'action du LLLT (in vivo et in vitro)

4. ETUDE IN VITRO

4.1 Low level laser irradiation precondition to create friendly milieu of infracted myocardium and enhance early survival of transplanted bone marrow cells.
Zhang H, Hou JF, Wang W, Wei YJ, Hu S – 2009 J of Cell and Mol Med 2009 Sep 1.

Abstract We hypothesized that low-level laser irradiation (LLLI) precondition prior to cell transplantation might remodel the hostile milieu of infracted myocardium and subsequently enhance early survival and therapeutic potential of implanted bone-marrow mesenchymal stem cells (BMSCs). Therefore, in this study we wanted to address: (1) whether LLLI pretreatment change the local cardiac micro-environment after MI; and (2) whether the LLLI preconditions enhance early cell survival and thus improve therapeutic angiogenesis and heart function. Myocardial infarction was induced by left anterior descending artery ligation in female rats. A 635 nm, 5 mW diode laser was performed with energy density of 0.96 J/cm(2) for 150 seconds for the purpose of myocardial precondition. Three weeks later, qualified rats were randomly received with LLLI precondition (n=26) or without LLLI precondition (n=27) for LLLI precondition study. Rats received thoracotomy without coronary ligation was served as sham group (n=24). For the following cell survival study, rats were randomly received serum-free culture media

injection (n=8), LLLI precondition and culture media injection (n=8), 2 millions male BMSCs transplantation without LLLI pretreatment (n=26) and 2 millions male BMSCs transplantation with LLLI precondition (n=25). Vascular endothelial growth factor (VEGF), glucose-regulated protein 78 (GRP78), superoxide dismutase (SOD) and malondialdehyde (MDA) in the infarcted myocardium were evaluated by Western blotting, real-time polymerase chain reaction (real-time PCR) and colorimetry, respectively, at 1 hour, 1 day and 1 week after laser irradiation. Cell survival was assayed with quantitative real-time PCR to identify Y chromosome gene and apoptosis was assayed with TUNEL staining. Capillary density, myogenic differentiation and left ventricular function were tested by immunohistochemistry and echocardiography, respectively, at 1 week. After LLLI precondition, increased VEGF and
GRP78 expression, as well as the enhanced SOD activity and inhibited MDA production, was observed. Compared with BMSCs transplantation and culture media injection group, although there was no difference in the improved heart function and myogenic differentiation, LLLI precondition significantly enhanced early cell survival rate by 2-fold, decreased the apoptotic percentage of implanted BMSCs in infarcted myocardium and thus increased the number of newly formed capillaries. Taking together, LLLI precondition could be a novel non-invasive approach for intraoperative cell transplantation to enhance cell early survival and therapeutic potential.

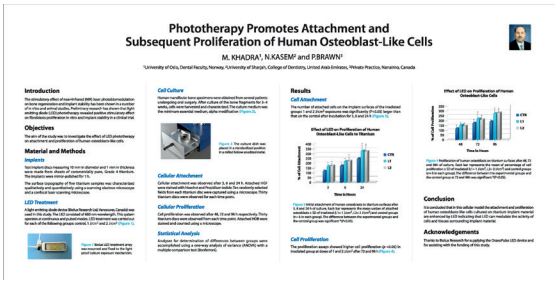
4.2 The effects of low-level laser irradiation on osteoblastic cells.

Coombe AR, Ho CT, Darendeliler MA, Hunter N, Philips JR, Chapple CC, Yum LW – Clin Orthod Res. 2001 Feb;4(1):3-14.

Abstract Low level laser therapy has been used in treating many conditions with reports of multiple clinical effects including promotion of healing of both hard and soft tissue lesions. Low level laser therapy as a treatment modality remains controversial, however. The effects of wavelength, beam type, energy output, energy level, energy intensity, and exposure regime of low level laser therapy remain unexplained. Moreover, no specific therapeutic window for dosimetry and mechanism of action has been determined at the level of individual cell types. The aim of this study was to investigate the effects of low level laser irradiation on the human osteosarcoma cell line, SAOS-2. The cells were irradiated as a single or daily dose for up to 10 days with a GaAlAs continuous wave diode laser (830 nm, net output of 90 mW, energy levels of 0.3, 0.5, 1, 2, and 4 Joules). Cell viability was not affected by laser irradiation, with the viability being greater than 90% for all experimental groups. Cellular proliferation or activation was not found to be significantly affected by any of the energy levels and varying exposure regimes investigated. Low level laser irradiation did result in a heat shock response at an energy level of 2 J. No significant early or late effects of laser irradiation on protein expression and alkaline phosphatase activity were found. Investigation of intracellular calcium concentration revealed a tendency of a transient positive change after irradiation. Low level laser irradiation was unable to stimulate the osteosarcoma cells utilized for this research at agross cell population level. The heat shock response and increased intracellular calcium indicate that the cells do respond to low level laser irradiation. Further research is required, utilizing different cell and animal models, to more specifically determine the effects of low level laser irradiation at a cellular level. These effects should be more thoroughly investigated before low level laser therapy can be considered as a potential accelerator stimulus for orthodontic tooth movement.

4.3 Phototherapy promotes attachment and subsequent proliferation of human osteoblast-like cells.

M. Khadran N. Kasem, P. Brawn


Phototherapy Promotes Attachment and
Subsequent Proliferation of Human Osteoblast-Like Cells
M. KHADRA[1], N.KASEM[2] and P.BRAWN[3]
Introduction
Objectives
Material and Methods
Implants
LED Treatment
Cell Culture
Cellular Attachment
Cellular Proliferation
Statistical Analysis
Results
Cell Attachment
Cell Proliferation
Conclusion
Acknowledgements

5. OSTEOBLASTES HUMAINES

5.1 Phototherapy promotes attachement and subsequent proliferation of human osteoblast-like cells

M. Khadra, N. Kasemn, P. Brawn


Phototherapy Promotes Attachment and Subsequent Proliferation of Human Osteoblast-Like Cells

6. CICATRISATION OSSEUSE

6.1 Increase of bone volume by a nanosecond pulsed laser irradiation is caused by a decreased osteoclast number and an activated osteoblasts.

Tadashi Ninomiya, Akihiro Hosoya, Hiroaki Nakamura, Kazuo Sano, Tsuyoshi Nishisaka, Hidehiro Ozawa – 2007


BONE

Increase of bone volume by a nanosecond pulsed laser irradiation is caused by a decreased osteoclast number and an activated osteoblasts

6.2 Effect of low-level laser therapy on proliferation and differentiation of the cells contributing in bone regeneration.

Reza Amid, Mahdi Kadkhodazadeh, Mitra Ghazizadeh Ahsaie, Arian Hakakzadeh – 2014


Effect of Low Level Laser Therapy on Proliferation and Differentiation of the Cells Contributing in Bone Regeneration

6.3 Enhancement of bone formation in rat calcarial bone defects using low-level laser therapy.

Maawan Khadra, Nesrin Kasem, Hans R. Haanæs, Jan Ellingsen, Ståle P. Lyngstadaas – 2004

Enhancement of bone formation in rat calvarial bone defects using low-level laser therapy

Maawan Khadra, DDS, MSc,[a] Nesrin Kasem, DDS, MSc,[b] Hans R. Haanæs, DMD, MD, PhD,[c] Jan E. Ellingsen, DDS, PhD,[d] and Ståle P. Lyngstadaas, DDS, PhD,[d] Oslo, Norway
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Objective. To evaluate the effect of low-level laser therapy (LLLT), using a GaAlAs diode laser device, on bone healing and growth in rat calvarial bone defects.
Study design. An animal trial of 4 weeks' duration was conducted using a randomized blind, placebo-controlled design. Standardized round osseous defects of 2.7 mm diameter were made in each parietal bone of 20 rats (n = 40 defects). The animals were randomly divided into an experimental and a control group of 10 animals each. In the experimental group, a GaAlAs diode laser was applied immediately after surgery and then daily for 6 consecutive days. The control group received the same handling and treatment, but with the laser turned off. Five rats from each group were killed on day 14 and the remainder on day 28 postoperatively. From each animal, tissue samples from one defect were prepared for histochemistry and samples from the contralateral defect for histology. Levels of calcium, phosphorus, and protein were determined by using atomic absorption spectrometry, colorimetry, and photometry, respectively. Student *t*-test and Mann-Whitney were used for statistical analyses.
Results. At both time points the tissue samples from the experimental animals contained significantly more calcium, phosphorus, and protein than the controls. Similarly, histological analyses disclosed more pronounced angiogenesis and connective tissue formation, and more advanced bone formation in the experimental group than in the controls.
Conclusion. LLLT may enhance bone formation in rat calvarial bone defects.
(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:693-700)

Defects in bone are not infrequent sequelae to pathological conditions such as tumors, cysts, or infections, or to trauma or surgery. The slow regeneration of bone in such defects can pose therapeutic problems.

Various strategies to stimulate healing of bone defects have been tested. These include autografting and allografting of cancellous bone, application of growth factors (mainly bone morphogenic proteins and OP-1), polymeric membranes, enamel matrix derivative, and others.[1-6] Autogenous bone grafts require lengthy surgery and are associated with significant donor site morbidity, including infection, pain, and hematoma formation. Furthermore, in large defects grafts are often resorbed before osteogenesis is completed.[7,8] Membrane application is also an expensive, lengthy process requiring surgical intervention. Although membranes like Emdogain have proved promising in healing of lesions in alveolar bone, its field of application to date is limited and it is indicated only for intrabony periodontal defects associated with periodontitis and trauma. Although the above methods are now established modes of treatment, failures still occur. The technical difficulties and unpredictable outcomes associated with surgical grafting techniques motivate further investigation of alternative, less technically demanding procedures to stimulate bone formation.

In this context, the concept of biostimulation of wounds by low-level laser therapy (LLLT) is attracting considerable attention. Although its effect on whole tissues has been studied quite extensively, the biological and cellular mechanisms underlying LLLT have not been clarified. It has been proposed that LLLT stimulates wound healing,[9-11] collagen synthesis,[12,13] and nerve regeneration.[14] In an experimental radius fracture in rabbits, Tang and Chai[15] reported that LLLT enhanced the activity of red blood cells, macrophages, fibroblasts, chondrocytes, and osteoclasts within the fracture area. LLLT may also increase DNA and RNA synthesis,[16,17] indicating an effect on cell growth and protein synthesis. It is suggested that low-level laser irradiation results in a small amount of singlet oxygen, which acts as a free radical that influences the formation of adenosine triphosphate (ATP), which has an important role in metabolism.[18]

Furthermore, laser irradiation is assumed to intensify the formation of transmembrane electrochemical proton gradients in mitochondria. Thus, the efficiency of the proton-motive force is increased and more calcium is

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6.4 Etude sur le contrôle de la douleur chez les patients atteints d'ostéonécrose induite par bisphosphonate en utilisant la thérapie LLLT : résultats préliminaires.

Romeo U, Galanakis A, Marias C, Vecchio AD, Tenore G, Palaia G, Vescovi P, Polimeni A – 2010

Conclusion

Cette étude pilote suggère que la thérapie LLLT peut être une technique valable pour soutenir le traitement de la douleur chez les patients atteints d'ostéonécrose induite par bisphosphonate.

Référence

Photomed Laser Surg. 2011 Jul;29(7):447-52. doi: 10.1089/pho.2010.2835. Epub 2011 Jan 16.
Observation of pain control in patients with bisphosphonate-induced osteonecrosis using low level laser therapy: preliminary results.
Romeo U1, Galanakis A, Marias C, Vecchio AD, Tenore G, Palaia G, Vescovi P, Polimeni A.

Author information

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6.5 L'influence de la thérapie LLLT sur la cicatrisation osseuse.

T. Ebrahimi, N. Moslemi, A.R. Rokn, M. Heidari, N. Nokhbatolfoghahaie, R. Fekrazad – 2012

7. PHYSIO

7.1 A new treatment protocol using photobiomodulation and muscle/bone/joint recovery techniques having a dramatic effect on a stroke patient's recovery: a new weapon for clinicians

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Europe

Summary
The subject of this case study is a 29-year-old woman who suffered a brainstem stroke. She remained severely dizzy, had a non-functional left hand secondary to weakness, severe spasticity in the right hand, a right lateral sixth nerve palsy and was unable to ambulate on presentation. The stroke occurred 2 years before presentation. The subject had been treated for 21 months at two different stroke rehabilitation centres before presentation. Our stroke protocol includes photobiomodulation administered with the XR3T-1 device and 'muscle/bone/joint/soft tissue' recovery techniques. The patient was seen once a week for 8 weeks and treatment sessions lasted approximately 60 min. The results were dramatic: after 8 weeks of implementation of our protocol, the patient demonstrated positive change in every area of her deficits as determined by improvements in physical examination findings. The gains achieved at 8 weeks have been maintained to this day and she continues to be treated once every 4 weeks.

BACKGROUND

The American Heart Association Statistics Committee and Stroke Statistics Subcommittee data reveal that someone has a stroke in the USA every 45 s. This means that roughly 750 000 people a year suffer a stroke in the USA. Of these cases, 87% are ischaemic strokes and most people survive. The American Heart Association (AHA) states that there are about 5.4 million stroke survivors in the USA alive today. Rehabilitation is thus a major issue. We believe that this case will be very important for publication because of these numbers and because of the dramatic improvement experienced by the patient. We believe that this is a very important report and adds an interesting element to the discussion of stroke rehabilitation. Its full potential and usefulness will be determined by its reproducibility. This case report introduces muscle/bone/joint/soft tissue recovery (MR) techniques. The techniques are most easily described as a combination of myofascial release, intense physical therapy, physiotherapy, cranio-sacral therapy and neuromuscular therapy. The MR techniques are important and a necessary part of the protocol because in stroke patients the involved muscles and joints have been immobile (and the muscles usually either spastic or flaccid) for such an extended period of time that they are extremely tight, having lost a significant range of motion and flexibility.Toxins have been allowed to build up in the soft tissue as well. Improvement in this setting will be severely limited. The MR techniques help us take these issues out of the recovery equation and set the stage for an optimal recovery. Photobiomodulation, also known as low-level light (or laser) therapy (LLLT), is a rapidly growing therapeutic approach for diseases requiring healing, tissue regeneration and reduction of pain and inflammation. However, many clinicians are not aware of this technology and its beneficial effects. Furthermore, a proper strategy of use is critical to its successful implementation. Over a decade ago, Naeser et al published an article reporting the use of laser acupuncture in the treatment of paralysis in stroke patients.1 We believe that our report can build upon this and add a new and potent weapon to the armamentarium of clinicians who are involved in helping stroke victims recover.

CASE PRESENTATION

The subject of this case study is a 29-year-old woman with no significant medical history, no family history of stroke and no social factors that predispose to stroke, who suffered a brainstem stroke in May 2008. The stroke was secondary to non-occlusive dissection of the vertebral arteries bilaterally; she suffered acute infarctions involving the medulla, superior-most aspect of the cervical spinal cord and cerebellar hemispheres bilaterally. She was treated for 3 months at an inpatient stroke rehabilitation centre and then for 18 months at an outpatient centre (where she was seen 3x/week). She was told that she had likely reached the pinnacle of her recovery. The patient presented to our institution with the following neurological examination Mental status: Alert, relaxed and cooperative. Mood was melancholic, anxious and fearful (mostly of falling) and emotional (easily moved to tears about issues such as being late for the appointment). No significant attention difficulties. No perceptual (environmental awareness, hallucinations, strange dreams, thought content) abnormalities. Long-term memory intact; however, there were deficits in short-term memory. For example, the patient had difficulty recalling information she had read in the morning newspaper. Thought processes coherent. Good insight into her condition. Oriented to person, place and time. Detailed cognitive testing was deferred. Cranial nerves: I—intact; II—visual acuity intact; visual

fields full; positive for double vision intermittently; III, IV, VI—right sixth nerve palsy—with the right eye fixed in the medial position; left eye without deficits; V, VII, VIII, IX, X, XII—intact; XI—weak trapezii and sterno-cleidomastoid muscles bilaterally (3/5 strength).
Motor: Good muscle bulk in all four extremities. Spasticity was assessed using a Modified Ashworth Scale. Right upper extremity muscle strength 1/5. Right upper extremity spasticity/rigidity as follows: shoulder mild, elbow moderate, wrist moderate to severe and the hand severe. Right shoulder, forearm, elbow, wrist and hand without voluntary movement; positive involuntary movements of the right upper extremity at the shoulder. Left upper extremity 4/5 strength (biceps, triceps and wrist); fingers of the left hand slightly flexed and hand grip strength 2/5; unable to grasp objects with the left hand. Right lower extremity 0/5 strength, good tone (not flaccid) and no spasticity/rigidity; left lower extremity 3/5 strength, good tone (not flaccid), no spasticity or rigidity. All four extremities without cyanosis or oedema. Core muscle strength severely compromised as evidenced by slumping in the wheelchair with the inability to straighten up.
Cerebellar: No ability to do rapid alternating movements, finger-to-nose, or heel-to-shin tests. Not able to stand. Not able to ambulate. Severe dizziness present when supine. Sensory: Light touch and proprioception intact.

TREATMENT

The treatment protocol requires the patient to be in the supine position. Our patient, however, became excessively dizzy when placed in this position and so this issue needed to be addressed first. The XR3T-1 device was placed on top of her head (main cranial), on each side of her head (fronto-parietal lobe), behind the ear on each side of the head (cerebellum) and in the centre of the back of her head (brainstem) for six positions in total, for 1 min per position, while the patient sat in her wheelchair. After 3 weeks she was able to lie supine on the treatment bed without dizziness. We were then able to continue with the treatment protocol. The treatment protocol has not yet been published in the world literature so it is described here briefly. It consists of techniques termed as MR and the XR3T-1 device. MR begins with a thorough evaluation and physical examination of the area in question to assess for muscle/bone/joint/soft tissue inflammation, oedema, weakness, stiffness, nerve injury and the presence of scar tissue. This is followed by a combination of myofacial release/neuromuscular therapy/cranio-sacral therapy and intense physical therapy. The techniques were originally created by two of the authors (MC and AB) and the exact implementation is dependent on the initial evaluation and physical exam. The XR3T-1 device uses light-emitting diodes at a wavelength combination of 660 nm visible red and 850 nm nearinfrared.
The energy is non-ionising and the device delivers 1400 mWof power. The spot size (area) is 0.196 cm2. The patient in this case study had a number of areas of her body affected by the stroke as detailed in the Case presentation section. The treatment protocol for her was as follows:
1. Begin each treatment section with application of the XR3T-1 device to cover a total of 32 locations, including the cerebral cortices, brainstem, cervical spine (8 locations) and the core musculature and lymphatics (24 locations). The XR3T-1 probe was placed over each site for 1 min. The total energy delivered to the patient was 2016 J and the total fluence was 2.95 J/ cm2 delivered to 32 areas of the body (figure 1).
2. Follow up with MR to the affected areas of the body: neck, shoulders, upper extremities, hands, chest, back, flanks, core musculature, hips, lower extremities, feet and ankles (figure 2). The major muscles targeted by MR were the sternocleidomastoid, trapezius, rhomboids, latissimus dorsi, biceps, triceps, brachioradialis, teres major, teres minor, pectoralis, quadriceps, hamstrings, psoas, gluteals, piriformis, gastrocnemius, soleus, IT band and the muscles of the wrist, hands, ankles and feet.
3. The effect on the body was then assessed with regard to muscle spasticity, flexibility and pain. Furthermore, the patient was evaluated to assess her tolerance of the treatment session. If she felt excessively tired or fatigued, the session was stopped. If she did not feel excessively tired or fatigued, then further application of the XR3T-1 device was employed along with more MR— targeted to a particularly problematic area of the body. It is vital to note that constant evaluation and discussion with the patient was instrumental in administering the protocol. The patient's condition and response to therapy that day would dictate how quickly we could proceed. Treatment on the right eye for the sixth nerve palsy consisted of the

XR3T-1 device being centred over the outside corner of the right eye. The sixth nerve palsy was resolved in three treatment sessions over a 3-week period. In summary, the treatment duration in this case report was a total of 8 weeks. The first 3 weeks of this were used to address the patient's dizziness.

OUTCOME AND FOLLOW-UP

The results were dramatic. After 8 weeks of implementation of our protocol:
1. Her mood became much less melancholic and she had become much less anxious and fearful. She engaged much more with us during the treatment sessions and she would often smile during the sessions. She reported to us being much more alert and aware of her environment and surroundings. She reported being able to read the newspaper again and retain information she was reading.
2. She was cured of her right sixth nerve palsy after three 1 min treatments.
3. Her double vision was eliminated.
4. She was cured of her dizziness and could thus lie supine on the treatment bed for her treatments.
5. Her right upper extremity strength improved to 2/5 and her right hand spasticity decreased; she could now move the fingers of the right hand.
6. Her left upper extremity strength improved to 4+/5 and she could now use her left hand to grasp objects (such as a cup, fork, etc).
7. Her right lower extremity strength improved to 4/5.
8. Her left lower extremity strength improved to 4/5.
9. She can stand with minimal to no assistance.
10. She is able to ambulate with a walker. The gains achieved at 8 weeks have been maintained to this day (12 months) and she continues to be treated once every 4 weeks. Further progress will be detailed in a subsequent report.

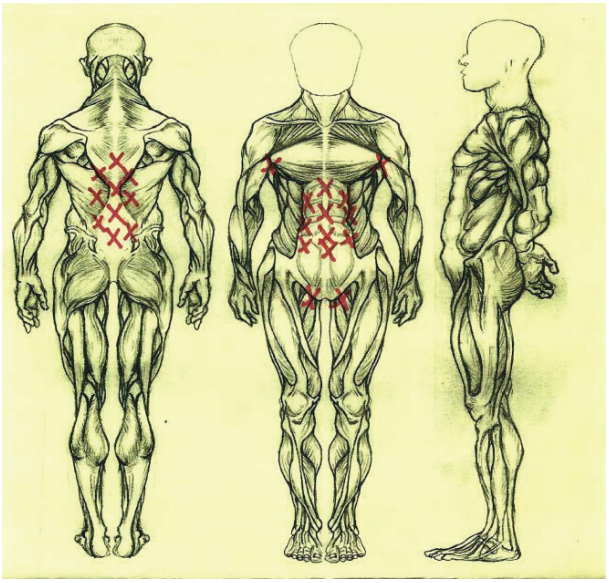

Figure 1 The red X's mark the 24 locations where the XR3T-1 device was administered. The locations include the core musculature (front and back) and the lymphatic drainage (armpits and groins bilaterally).

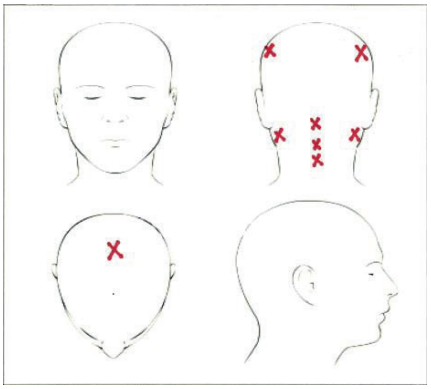

Figure 2 The red X's mark the eight spots on the head and neck where the XR3T-1 device was administered. This includes the cerebral cortex, cerebellar hemispheres, brainstem and cervical spinal cord.

DISCUSSION

Photobiomodulation and LLLT are emerging technologies in the field of science. Their beginnings can be traced back to the discovery of laser biostimulation by Endre Mester of Semmelweis University in Budapest in 1967. Since then, there have been over 200 clinical trials and over 2000 laboratory studies reported in the world literature covering a wide range of applications. However, its use in clinical medicine is not yet widespread and many clinicians are unaware of the science and its bene-

fits. We believe that this is due to a number of reasons: first, the biochemical mechanisms underlying the effects have only been partially elucidated and not yet completely understood;second, the complexity of choosing among a large number of illumination parameters, such as wavelength, power density, fluence, pulse structure and treatment timing, has led to the publication of negative results2; and, third, many clinicians were not taught about this technology in medical school or residency. LLLT acts by inducing a photochemical reaction in the cell, a process known as photobiomodulation. The principle is that when light hits certain molecules called chromophores (examples of chromophores are chlorophyll in plants and haemoglobin in red blood cells), present in the protein components of the mitochondrial respiratory chain, the photon energy causes electrons to 'jump' from low-energy orbits to higher-energy orbits. This stored energy is used by the cell to perform various tasks (such as photosynthesis in plants).3 The mitochondria are where ATP production occurs, through the oxidative phosphorylation process and the electron transport chain. It has been shown that visible light and near-infrared light are absorbed by the mitochondria with a resultant upregulation of cellular respiration. When the energy is absorbed (the chromophore is cytochrome c oxidase, or CCO) there is photodissociation of nitric oxide (NO) from CCO which prevents the displacement of oxygen from CCO. The result is unhindered cellular respiration and an increase in ATP production. Increased ATP production will raise cellular energy levels and also result in upregulation of the cyclic AMP molecule, which is involved in many signalling pathways.3 LLLT also promotes the metabolism of oxygen, so there is an increase in reactive oxygen species (ROS). These ROS are chemically active molecules that are involved in cell signalling, regulation of the cell cycle, enzyme activation, nucleic acid synthesis and protein synthesis.4 An increase in ROS leads to the upregulation of various stimulatory and protective genes. Other mechanisms of action of LLLT include the following:

1. The photodissociation of NO from CCO leads to vasodilatation as NO is a potent vasodilator through its effect on cyclic guanine monophosphate (GMP) production. Cyclic GMP is involved in other signalling pathways.
2. LLLT promotes the synthesis of DNA and RNA1 and increases the production of proteins.6
3. LLLT also modulates enzymatic activity, affects intracellular and extracellular pH and accelerates cellular metabolism.3 6
4. LLLT has been shown to stimulate the expression of multiple genes related to cellular proliferation, migration and the production of cytokines and growth factors.7

One can see that there are a myriad of cellular and molecular effects induced by LLLT. These photobiomodulation treatments have been reported to be effective in a variety of clinical applications, but the use of LLLT in the treatment of chronic stroke patients has never been studied. Naeser et al in 19951 used LLLT-laser acupuncture to stimulate acupuncture points on the body in chronic stroke patients with paralysis. Their findings suggested that some recovery of motor function can occur. Three patients had increases in the active range of motion for shoulder abduction, knee flexion and knee extension. One patient with hand paresis had slight increases in grip strength, lateral pinch and tip pinch. Since 1995, however, the only report of photobiomodulation and its use in stroke patients was by the (NeuroThera Effectiveness and Safety Trial) NEST-18 and NEST-29 investigators. They used a similar (although not identical) LLLT technology for the treatment of ACUTE stroke (within 24 h of the event). The authors document the safety and effectiveness of this technology and showed a trend towards statistical significance. They believe that further studies will clarify the subsets of stroke patients who will truly benefit from this treatment. Our case report demonstrates the benefits of the treatment protocol in helping a stroke patient rehabilitate. The protocol includes two components—photobiomodulation (using the XR3T-1) and MR techniques. Both parts are integral for achieving a successful rehabilitation outcome. Photobiomodulation and LLLT have been described above. A new term (MR) was coined to describe the second component of the protocol because the techniques are novel—they were created by two of the authors (MC and AB) and have been refined over the course of 15 years. The techniques are most easily described as a combination of physical therapy, physiotherapy, cranio-sacral therapy and neuromuscular therapy. The MR techniques played an essential role in allowing the muscle groups affected by the stroke to obtain full benefit from the LLLT treatments. The techniques were instrumental in helping prevent the patient's muscles from

becoming overly stiff and inflexible secondary to decreased use. If the pain and the stiffness and inflexibility had not been addressed, optimal muscular recovery and subsequent motor function recovery would not have been achieved. Implementation of the protocol begins with a knowledgeable and thorough initial evaluation to direct treatment. This is followed by an evaluation of the patient's response (to the XR3T-1 and MR), assessment of the gap between the actual response and the desired response, and then further treatment to bridge that gap. Analogous to different physical therapists employing different strategies to achieve a desired goal, or different cardio-thoracic surgeons employing different strategies to go on bypass, arrest the heart or expose the mitral valve, an MR therapist employing our protocol will implement different strategies for the recovery of a stroke patient as dictated by the clinical situation. One can become an MR therapist and proficient in MR techniques with proper training from our institute.

The patient in this case report experienced a dramatic recovery/rehabilitation outcome after being treated with the treatment protocol. We believe that our protocol was directly responsible for the dramatic outcome because the clinical impact was seen after the treatment protocol had been used in her recovery for 2 months and after she had been treated for the previous 2 years at two different stroke recovery institutions with only minimal benefit. Nothing changed in the daily routine or in the activities of the patient other than treatment at our institute. In summary, we believe that all of the above-listed mechanisms of action could have contributed to the improvement of our patient's motor function. Research suggests that the underlying mechanisms for the functional benefit are possible induction of neurogenesis,10 prevention of apoptosis and exertion of a neuroprotective effect11 and increased mitochondrial function.12 As Lampl states, '… although the mechanism of action of infrared laser therapy for stroke is not completely understood … infrared laser therapy is a physical process that can produce biochemical change at the tissue level. The putative mechanism … involves the stimulation of ATP formation by mitochondria and may also involve prevention of apoptosis in the ischaemic penumbra and enhancement of neurorecovery mechanisms'.8 The cognitive and psychological improvements brought about via the photobiomodulation treatments have been described in case reports.13 A few mechanisms are likely involved:

1. Increased ATP production would provide beneficial effects, including an increase in cellular respiration and oxygenation. This will help restore function in damaged cortical cells.
2. Oxidative stress plays a role in the damage after stroke. It is believed that photobiomodulation technology produces low levels of ROS in mitochondria of illuminated cells and that these ROS cause nuclear factor kappa B (NF-κB) activation via the redox-sensitive sensor enzyme protein kinase D1, which results in upregulation of the mitochondrial superoxide dismustase.

In the short term, NF-κB is increased, but in the long term it is decreased, leading to a decrease in inflammation (less NF-κB) and an upregulation of gene products that are cytoprotective, such as superoxide dismutase, glutathione peroxidase and heat shock protein 70.13

3. Transcranial application of LLLT may irradiate the blood via the emissary veins located on the scalp surface, which interconnect with veins in the superior sagittal sinus. Thus, it is possible that the transcranial application of LLLT may have affected local, intracerebral blood and circulation.13 There is no study or report confirming this suggestion; further research is indicated.
4. There may be an increase in regional cerebral blood flow to the frontal lobes as evidenced by her improvements in memory and in her ability to read the newspaper.

There may have also been increased regional cerebral blood flow to frontal pole areas, as observed in a recent study to treat major depression14; this may be responsible for the patient feeling less anxious, less melancholic, less fearful and more engaged during her sessions.

Until more basic science and clinical research is done, the exact mechanisms responsible for the observed benefits will be only partly understood. What is clear, however, is that in our patient a dramatic change did occur in both motor and cognitive areas. These changes occurred after implementation of our protocol and these dramatic changes were noted by our staff as well as by the patient and the patient's spouse. The stroke recovery literature is adamant that rehabilitation should begin as soon as possible after the stroke and that only intense training brings about the best results. We began treatment in our patient almost2 years after the stroke. We look forward to studying the possibilities and potential of the treatment

protocol
in the case when it is implemented sooner. We also look forward to implementing this strategy on future patients and reporting our results. We understand that the efficacy and applicability will depend on its reproducibility.

Learning points

- The technology of photobiomodulation can have an impact in the field of stroke rehabilitation. The wavelengths of light chosen and the other illumination parameters employed are critical elements of a successful outcome. However, muscle/bone/joint/soft tissue recovery (MR) techniques as described are also essential—the MR techniques prime the muscles and tissues to obtain full benefit from the low-level light therapy-induced photobiomodulation.

- There is potential for stroke victims to benefit from photobiomodulation when it is applied with a proper protocol in the setting of stroke rehabilitation and recovery. These benefits may be seen in the realm of cognitive improvement and motor function (such as walking).

- The benefits to our patient occurred almost 2 years after the stroke. There is a potential for our protocol to help even when instituted years after the insult. However, the possibility of a greater impact if instituted sooner needs to be explored.

Competing interests

The protocol was created by Dr. Boonswang and Ms. Chicchi.
Patient consent Obtained.

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