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Effect of Low Level Laser on the Perineural Thickness of the Inferior Alveolar Nerve

Efecto del láser de Baja Potencia sobre el Grosor del Perineuro del Nervio Alveolar Inferior

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ABSTRACT: Of the different physiotherapy techniques used in the nervous process repair, the use of Low level laser therapy (LLLT) has generated results, due to the biostimulant effects that generates laser irradiation while interacting with tissues and cells. This study seeks to identify changes or morphological microscopic alterations of inferior alveolar nerve in healthy rats. We used 16 Sprague Dawley rats, which were exposed to Low level Laser in the side area of the mandibule. The left side was taken as a control on the right side was applied low-level laser so transcutaneous for 1 minute with a power density of 5 Joules/cm^2, 3 sessions per week, a total of 3 weeks. The sample unit and the histological analyses were obtained from the inferior alveolar nerve section and its surrounding tissue. The results show that there is a noticeable difference in the thickness of perineural tissue in irradiated side compared to the control side, the average thickness was 21.125 mm in irradiated group and 27.575 mm for the control group showing a statistically difference significant between them. In conclusion, the application of low-power laser at low dose produces a variation in the nervous morphology, increasing the density of its components.

KEY WORDS: Low level laser therapy, laser biostimulation, inferior alveolar nerve.

INTRODUCTION

Low-level laser produce a stimulating effect on cells, which manifests itself in biochemical, bioelectricity, bioenergy and microcirculation changes (Oltra-Arimon *et al*., 2004).

Of the different physiotherapy techniques used in the process of nerve repair, the use of Low level laser therapy (LLLT) has produced very interesting results in pilot studies. Since the eighties have shown that therapy with transcutaneous LLLT bioestimulant promotes posttraumatic/postoperative nerve fibers damaged regeneration (Gigo-Benato *et al.*, 2004).

Rodriguez *et al.* (1996) studied the effect of transcutaneous HeNe laser applying on rat isquiatic nerve, noting that the He-Ne laser has a therapeutic effect on the severed and sutured nerves regeneration, and that lasers use in the preoperative period is higher than its use in the postoperative period.

Shamir *et al*. (2001) conducted a similar pilot study, after the ischiatic nerve section and communication, using transcutaneus low level laser in the relevant segment of the spinal cord and nerve. Histologically there was an increase in the number of axons and greater diameter together with better quality in the process of regeneration. Matamala *et al.* (2001) described an increase in the epineuro thickness after the application of laser therapy. Similar results have been reported by Anders *et al.* (1993) and Lowe *et al.* (1993).

Garrigo & Valiente (1996), argue that the laser action in histic repair is based in the cellular proliferation increase, activation in the collagen and alkaline phosphatase production, vascular endothelial activation, nerve fibers and bone tissue regeneration, increase of angiogenesis (Garrigo & Valiente; Suazo *et al.*, 2007; Landaeta *et al.*, 2008). and induction from

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the epithelial cells adjacent to the injury to re-epithelization process, and a significant increase in fibroblasts, collagen and elastic fibers proliferation (Souza *et al*., 2003; Moreira *et al.,* 2006).

One possible explanation for the phototherapy effects on cell biology, is through an increase in expression of growth associated protein-43 (GAP 43) in initial statements of sciatic nerve regeneration in rats undergoing laser therapy (Shin *et al.* , 2003).

Milorad & Repasky (2000), showed that neurosensy recovery after bilateral sagittal split osteotomy surgery, could be improved a return of function in time and magnitude terms with the use of attachment laser therapy treatment. This irradiated laser type may be a non-invasive method in promoting healing of neural wounds of defects repaired with polytetrafluoroethylene tube (Milorad *et al*., 2002).

Therefore, when making treatment with low-level laser, irradiation not only could compromise the injured tissue, but also the healthy tissue that is in relation to the sick or injured area.

Pedemonte *et al*. (2008) conducted an extensive review of the use of LLLT in odontostomatology concluding that it can help damage nerve repair in the maxillofacial territory.

Based on this background, this paper attempts to quantify and analyze the morphological changes or microscopic alterations in healthy perineurium of inferior alveolar nerve of rat before the stimulation of low-level laser.

MATERIAL AND METHOD

We used 16 Sprague Dawley rats, adult females, healthy normal weight (250-280 g). Throughout the experimental period is housed in commonly used cages, maintained with 12-12 h. light-darkness cycles, with heating and with a regular diet of pellets and drinking water *ad libitum*. This study was conducted in the anatomy and pathology laboratories, Universidad de Talca. Rats were transcutaneously irradiated in the area of right side of the jaw, an area corresponding to the path of the inferior alveolar nerve, 5 mm below the posterior edge of the mandibular ramus and 5 mm to basilar edge (Fig. 1). We carried out a protocol for implementation of 5 Joules/ cm^2 in the low-level laser for clinical, use three times a week for three weeks, according to what has been described by Suazo *et al.* and Giga-Benatar *et al.* The left side of the jaw is not irradiated, regarded as a control. After the indicated time irradiation, all the mice were sacrificed. We obtained the block jaw in the irradiated and the control area, which contained respectively the left and right inferior alveolar nerve.

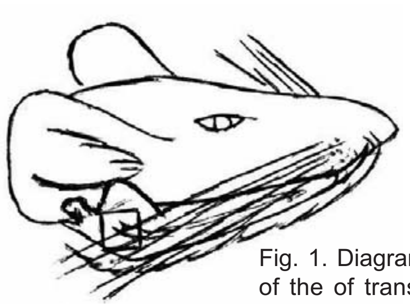

Fig. 1. Diagram shows the location of the of transcutaneous low level laser irradiation point on the inferior alveolar nerve in rat.

Nerves were fixed in 10% buffer formalin and processed for Hematoxilina & eosin, with transverse cross-section (5mm). Two plates were selected from each specimen, one on each side, which were observed in the optical microscope (025 ZEISS, Germany). In each histological slide, under an increase of 40x, the perineurium was measured at 4 points, according to the clock pointers corresponded to 12, at 3, at 6 and 9. These measurements were conducted with the Software Macromedia Freehand.MXa after digitalization of microscopic images (Fig. 2).

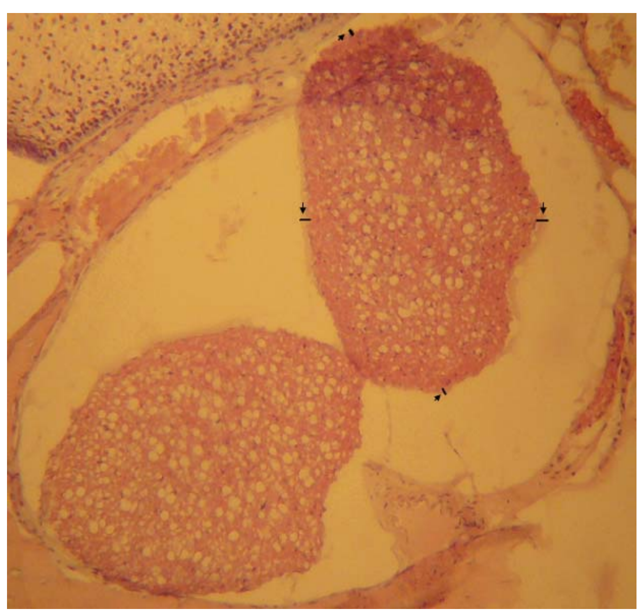

Fig. 2. Histological image shows the 4 points of rat perineurium inferior alveolar nerve measurement through software program.

Using the SPSS 15.0 statistical software for Window, descriptive statistics were calculated from the sample, the statistical significance of differences was established using the test with a t p <0.05%.

RESULTS

During dissection was possible to observe, to the naked eye under white light and magnifying glass, a larger amount of fatty and connective tissue around the nerve irradiated compared with the control nerve.

The highest average perineurium thicknesses was found in the irradiated group with an average of 27.575 mm, the control group, had a change in average thickness of 21.125 mm. The statistical indicators of perineurium thicknesses in both groups are in Table I.

In order to analyze the statistical significance of the difference in the average perineurium thicknesses in the irradiated and control groups t-test was applied for independent samples, resulting highly significant with p <0.01.

DISCUSSION

The low-level laser are widely used in the clinic for humans and animals for their antiinflammatory and analgesics effects, also represents an element in healing accelerates processes.

When applying LLLT photons enter the tissue and are absorbed in mitochondria and the cell membrane. The photonics energy is converted into chemical energy within the cell, in the form of ATP, which leads to the normalization of cellular function, pain relief and healing. The cell membrane alter its permeability, and then produce physiological changes. These physiological changes affecting macrophages, fibroblasts, endothelial cells, mast cells, bradykinin, and the nerve (Karu *et al*., 1995; Schindler *et al*., 2000; Hawkins & Abrahams, 2006).

The nerves are very vulnerable to pressure, and the amount of damage depends on the area involved, the magnitude of the kind of pressure and time as far as the nerve was compressed. If the amount and duration of the pressure was low, most nerves will be recovered immediately or shortly after the trauma. The nerve regeneration is not a simple process of expanding the axons of extensions, but an integration of dynamic interactive processes, include soluble factors in the blood, high production of extracellular matrix, attraction molecules, cell adhesion, and secretion of trophic factors (Camargo *et al*., 2006).

According to Matamala *et al*. implementing low-level laser radiation on living tissues of animals or humans have a beneficial therapeutic effect in case of an injury, just as there is a disruption in healthy tissue and anatomy in normal tissues that are located in the treated region.

The analysis of these results and clearly information existing shows the effect of radiation on LLLT tissues, to be applied in a clinically acceptable range, is able to produce physiological effects on different tissues, which depends on the length of wave, energy and radiation laser time exposure (Greathouse *et al*., 1985).

In addition, this study was interesting to note that in both nerves, controls and irradiated nerves, perineurium thickness is not uniform because there are variations between 17 and 23 microns in nerve controls, whereas in the irradiated these variations ranging from 26 and 29 microns, values lower than those reported by Matamala *et al.*

The perineurium of alveolar nerve consists of a laminate and heavily intertwined tissue with thinner collagen fibers, which are grouped in compact form and mostly lined up lengthwise, which delivers resistance and resilience to system (Sunderland, 1985) observed a significant increase in the perineurium in bundles of irradiated nerves, which is a sign of increased collagen tissue, unlike unirradiated side. This can be correlated with other studies that showed that irradiation with low level laser energy crops on in vitro embryonic fibroblasts,

Table I. Descriptive statisticians perineurium thickness measured in the inferior alveolar nerve irradiated and control groups.

Group	N	Mean	DS	Std. Error Mean
Control	16	21.125	1.8660	0.4665
Irradiated	16	27.575	0.9176	0.2294

produced a significant proliferation of fibroblasts (Hrnjak *et al*., 1995). As for the absorption of this radiation by the tissues, Nissan *et al.* (1986) applied transcutaneous irradiation with HeNe laser on rat isquiatic nerve and found that there is an absorption of radiation in the overlapping nerve tissue, a fact also is found in this study.

McNally *et al*. (1999) in a study which was conducted welding solid proteins technique to repair nerve along with the use of LLLT, proteins are applied in epineuro which offers the advantage of not obstructing the neuron regeneration.

The inferior alveolar nerve damage can result from a variety of clinical circumstances, such as lower third molar extraction, odontosection, implants, tumor resection, preprosthetic surgery and local anesthetics procedures (Milorad *et al*.), including extensive procedures as sagittal osteotomy of the madibular ramus (Milorad & Repasky). Fortunately, cases of nerve damage are usually transient and spontaneous resolution with a minimum sequel (Upton *et al.,* 1987), but in cases of damage or more to accelerate its recovery use LLLT looming as an important therapeutic tool.

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RESUMEN: De las diferentes técnicas fisioterapéuticas utilizadas en el proceso de reparación nerviosa, la utilización de terapia con Láser de Baja Potencia (LLLT) ha generado resultados, debido a los efectos bioestimulantes que la irradiación láser genera al interactuar con los tejidos y células. Este estudio pretende determinar los cambios o alteraciones morfológicas microscópicas del nervio alveolar inferior sano en ratas. Se utilizaron 16 ratas Sprague Dawley, a las cuales se les aplicó Laser de Baja Potencia en la zona lateral de la mandíbula. El lado izquierdo se tomó como control; al lado derecho se le aplicó láser de baja potencia de manera transcutánea durante 1 minuto con una densidad de energía de 5 Joules /cm2 , 3 sesiones por semana, en un total de 3 semanas. La unidad de muestra y análisis fueron los cortes histológicos obtenidos de la sección del nervio alveolar inferior y su tejido circundante. Los resultados muestran que existe una notable diferencia en el espesor de los tejidos perineurales del lado irradiado en comparación con el lado control, el promedio de grosor fue de 21,125 mm en irradiados y 27,575 mm para el control, mostrando una diferencia estadísticamente significativa entre ellos. En conclusión la aplicación de láser de baja potencia en dosis baja produce una variación en la morfología nerviosa, aumentando la densidad de sus componentes.

PALABRAS CLAVE: Laserterapia de baja potencia, láser bioestimulación, nervio alveolar inferior.

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