

Effectiveness of Laser Therapy (AlGaInP) on Cicatrization of Experimental Cutaneous Wound in Rats

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Received 14 January 2014; revised 13 February 2014; accepted 12 March 2014

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Abstract

The use of laser therapy is one of the most effective tools in the prevention and treatment of skin ulcers. It is an effective treatment choice because it potentiates the trophic regenerative, anti-inflammatory, and painkiller effects. This study aimed to assess the effectiveness of low level laser therapy in treating induced cutaneous ulcers in rats. An experimental study using 12 female Wistar rats (*Rattus norvegicus*), weighing between 150 and 250 g, from the room of animal husbandry at the Center of Health Sciences of UNIFOR. Rats underwent surgery to induce cutaneous ulcer. Subsequently, the animals were divided into two randomized groups: 1) Control group; and 2) Experimental group. They received nine applications of AlGaInP laser treatment (power of 30 mW) with an intensity of 4 J/cm², using the scan method, for 15 seconds over the entire ulcer. The cicatrization process was evaluated from the 1st to 19th postoperative (PO) day, but on alternate days. The results showed that animals treated with laser had less area of cutaneous wound than the control group from the 7th until the 17th postoperative day, highlighting the 7th and the 9th PO with a significant reduction in wound area of 28.8% (p = 0.00) and 23,5% (p = 0.01), respectively. Treatment with the AlGaInP laser was effective in the repair of cutaneous wound when compared to the control group—particularly on the 7th postoperative day.

Keywords

Laser Therapy, Rats, Wound Healing, Regeneration

1. Introduction

Skin wounds are tissue disorders that affect millions of people around the world and have major physical and emotional consequences which can make a person unable to move spontaneously [1].

Skin ulcers are characterized by integumentary loss, which can be limited or irregular. Among the most common types of ulcers are arterial vascular and venous ulcers. These ulcers are caused by pressure as well as peripheral neurological deficit, and may evolve over time [2].

The process of skin wound healing involves a series of factors that aim to restore the normal function of the affected region [3]. This process is divided into three phases: inflammation, proliferation and remodeling of the extracellular matrix [4].

The therapies used on skin ulcers aim to prevent and treat these wounds. The physiotherapy can contribute to recovery from these injuries by reducing the healing period and facilitating the return to functional activities more quickly.

Among the several existing physiotherapeutic treatments is the use of the low level laser (*Light Amplification by Stimulated Emission of Radiation*), which stimulates the healing process, and is considered more effective than other modalities used in treatment clinics [5].

Laser therapy is one of the most used techniques in the treatment of skin ulcer because it enhances the trophic-regenerative, anti-inflammatory and analgesic effects [6]. In addition, tissue regeneration is highlighted [7].

Some studies, correlated with the irradiation of low-level laser in the production of collagen, have shown very positive results [8]. They indicate an increase in the production of fibroblasts in induced and irradiated skin wounds, which supports the healing process [9]. Currently, a new type of laser is being used in the domestic market: the AlGaInP (Aluminium, Gallium, Indium, Phosphorus) laser, which has a wavelength similar to the HeNe (Helium Neon), but that needs a shorter time of irradiation because it presents a higher average power [10].

This study aimed to analyze the effectiveness of low level laser therapy on skin wound induced in rats and verify the application parameters in skin wound, since there are still some controversies regarding its standardization.

2. Method

2.1. Design

An experimental quantitative study was conducted in Laboratories H-08 and H-10 at the University of Fortaleza (UNIFOR).

2.2. Participants, Therapists, Centers

We used 12 female Wistar rats (*Rattus norvegicus*), weighing between 150 and 250 g, from the room of animal husbandry from the Center for Health Sciences at UNIFOR. The animals were housed in acclimatized cages containing until six animals, with natural day/night cycles, and were provided with water and *ad libitum* feeding, except during the period of intervention.

This study followed the ethical principles of animal research, adhering to the rules of the National Council for the Control of Animal Experimentation (CONCEA) and the incorporation of the “3Rs” (*reduction, replacement, refinement*), which is an experimental practice that leads to reducing the number of animals used, their replacements (when possible or relevant) and the refinement of the techniques used. It was submitted to the Ethics Committee on the Use of Animals (CEUA) at UNIFOR, with legal opinion No. 010/2011.

2.3. Intervention

On the day of surgery, after a fasting period of 8 hours, the animals were weighed and anesthetized intraperitoneally (i.p) with ketamine (70 mg/kg) and xylazine (10 mg/kg) in distilled water, with a dosage of 0.1 ml/30 g of the animal weight.

After the anesthetic procedure, each animal was placed on a surgical board in ventral decubitus position and immobilized by adhesive tape. Then, trichotomy by manual traction of part of the dorsal thoracic region was applied, and povidone iodine 1% was used to provide asepsis.

After the hair was removed, the same surgical technique was standardized for the animals from all groups.

Sterilized surgical drapes were placed on the animal and the skin to be excised was marked with a demographic pen and cast plastic molding, to define an area of $2 \times 2 \text{ cm}^2$. The skin was incised using a No. 21 scalpel blade, removing the layer of the dermis and epidermis up to the exposure of the muscle fascia. Hemostasis was achieved by digital compression with gauze.

After the described surgical procedure, the animals were randomized into two groups ($n = 6/\text{group}$) according to the type of treatment:

1) Control group: used only saline in the wound;

2) Experimental group: used the AlGaInP laser, Laserpulse Ibramed[®] model, with visible beam, in pulsatile mode, 30 mW power, with a 660 nm wavelength pen, an intensity of 4 J/cm^2 , and using the scan method for 15 seconds throughout the length of the wound.

The control and the experimental groups were submitted to the treatment on the following postoperative days (PO): 1st, 5th, 3rd, 7th, 9th, 12th, 15th, 17th and 19th. After inducing skin wound, the animals were assessed at the same times.

The wound areas were analyzed and the horizontal and vertical diameters were determined using calipers (Digital Caliper within 300 mm), with the calculation of the area obtained using the equation [11]. $A = R \times r$, in which A represents the area, R the larger radius, and r the smaller radius of the wound.

The area of lesion was calculated using the equation [12]. $\Delta A = 100 \times (W_o - W_i)/W_o$, in which W_o = initial wound area; W_i = final wound area.

2.4. Outcome Measures

Primary outcome: The aid of the therapy with the AlGaInP laser in reducing skin wound in the treated group as compared to the control group.

Secondary outcome: Period of greater neovascularization and re-epithelization and the consequent increased speed in the healing process of the skin wound.

2.5. Data Analysis

After data collection, the results were presented through the average \pm standard error, expressed in the form of figure and table. The t test for independent samples was conducted, following the normality test by Komogorov-Smirnov (KS), and only the analyses that presented a significance level of 5% ($p \leq 0.05$), by the *Statistical Package for the Social Sciences* (SPSS) program version 17.0, were considered statistically different.

3. Results

Results showed that the animals treated with laser presented a smaller area of skin wound than the control group from the 7th to the 17th postoperative days (**Figure 1**). In addition, it was observed that the group treated with laser showed better healing when compared to the control group, with significant reduction of the wound area of 28.8% ($p = 0.00$) and 23.5% ($p = 0.01$) on the 7th and 9th postoperative days, respectively (**Table 1**).

4. Discussion

As highlighted by epidemiological data, skin wound is the most common result of external physical forces [1]. Furthermore, the residual repair is a highly complex mechanism that involves local and systemic factors that can compromise wound healing [3].

The healing process of wounds may benefit from factors such as the nutrition of tissues, cell proliferation and inflammation control [13]. Among the therapeutic resources that promote the healing process, electrotherapy is one of the most used techniques in the treatment of skin wound because the application of the AlGaInP low level laser produces trophic-regenerative, anti-inflammatory and analgesic effects [13].

This type of laser produces light with a wavelength between 635 and 670 nm, occurring within the visible red color range of the light spectrum, with potential variation of 1 to 30 Mw, which gives it a biostimulant action [14].

The results of this study showed that AlGaInP laser therapy accelerated the healing process, possibly because it assists tissue regeneration. This finding is in agreement with previous study with low-level laser that used a wavelength of 670 nm and a dose of 30 J/cm^2 , which contributed to the repair of the dermis and epidermis, and

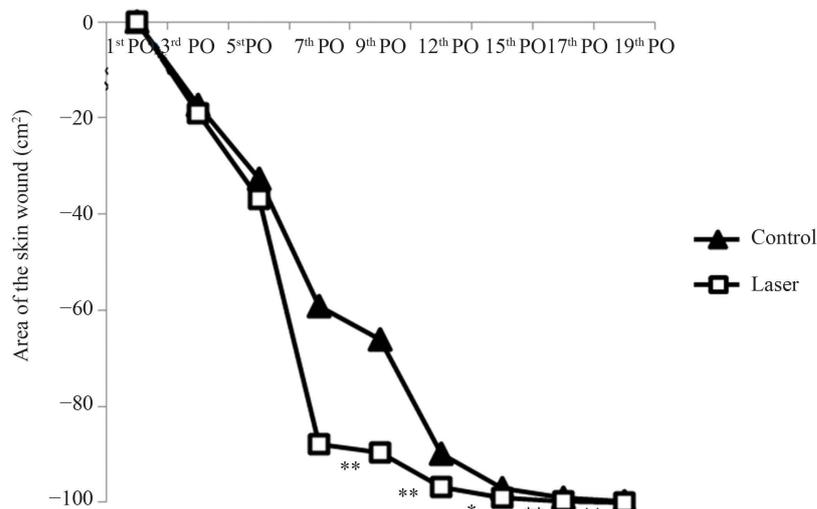


Figure 1. Analysis of the AlGaInP laser effect on the healing process of the skin wound in rats. The healing process was measured through the area of the skin wound in cm² through the \pm standard error average. The ordinate axis: averages of wound areas. Abscissas axis: postoperative day. There was a statistically significant difference between groups ($^*p < 0.05$ and $^{**}p < 0.01$).

Table 1. Analysis of the evolution of the healing process of the skin wound in animals treated with the AlGaInP laser and the control.

Postoperative period. (PO)	Area of skin wound			P
	Control	Experimental	Difference between groups	
3 rd PO	17.2 \pm 7.41	19.0 \pm 8.72	1.8	0.88
5 th PO	32.5 \pm 5.00	36.7 \pm 6.86	4.2	0.63
7 th PO	59.1 \pm 6.00	87.9 \pm 3.17	28.8	0.00
9 th PO	66.1 \pm 6.39	89.6 \pm 2.53	23.5	0.01
12 th PO	89.9 \pm 2.49	98.7 \pm 0.80	6.8	0.03
15 th PO	96.9 \pm 0.39	99.0 \pm 0.48	2.1	0.01
17 th PO	98.9 \pm 0.18	99.8 \pm 0.14	0.9	0.00
19 th PO	99.8 \pm 0.18	99.9 \pm 0.09	0.1	0.65

potentiated the healing of induced wounds on the backs of mice [15].

This research showed that the use of the AlGaInP laser therapy was effective in reducing the wound earlier, as this type of laser increases the secretion of growth factors (FGF) and cell replication, thereby increasing cell proliferation. It also reduces inflammation and promotes neovascularization that, regardless of the dose used, may be the determining factor in the tissue repair [5].

In another study, the therapy using the 785 nm AlGaInP laser, with a dose of 2.7 J/cm², showed positive effects in reducing the inflammatory infiltrate and in accelerating the healing process because the laser increased fibroblast proliferation, thus creating conditions for the alignment and organization of the muscle fiber [16].

In this study, we observed a significant reduction in the diameter of the wound areas in the group treated with laser, primarily on the 7th postoperative day, when compared to the control group. This improvement in the healing process may be attributed to the decreased inflammatory infiltrate and the early re-epithelization that usually occurs at this stage of tissue repair [17].

The extensive reduction of the ulcers on the 7th postoperative day is linked to the occurrence of hyperplasia and epidemic hypertrophy as well as an increase in neovascularization. These results are associated with fibrop-

lasia in the presence of fibroblasts and type III collagen, which aid in the healing process [18] [19].

All the effects achieved with the use of the AlGaInP laser may be related to its potent biostimulant action and because it has a wavelength that requires less irradiation to produce desirable effects. Recent studies show that this new type of laser may be effective when used at a dose of 4 J/cm² in the scan mode, on third degree burns [20].

The scan method with a dose of 4 J/cm², when used on rats that had received burns, proves more effective because it presents a faster healing process than the group that used the laser with the punctual method, showing the positive effects of laser irradiation [21].

In this study, we used the AlGaInP laser at a dose of 4 J/cm² in the scan mode for a period of 15 seconds. The authors report that the dose used in this research is more efficient in increasing the number of fibroblasts, achieving greater vascular proliferation, and in re-epithelization. Laser therapy with the use of 4 J/cm² is more efficient than with the use of 8 J/cm² because a smaller dose helps in reducing the diameter of the wound, edema and the number of inflammatory cells [20] [21].

Despite the positive results of the research, more studies are still needed with this type of laser to find more evidence of the effects, and there are still some controversy regarding the standardization that need to be clarified.

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Abbreviations

postoperative (PO)

Laser (Light Amplification by Stimulated Emission of Radiation)

AlGaInP (Aluminium, Gallium, Indium, Phosphorus)

HeNe (Helium Neon)

Control of Animal Experimentation (CONCEA)

Committee on the Use of Animals (CEUA)

University of Fortaleza (UNIFOR)

Wo = initial wound area

Wi = final wound area