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RESEARCH ARTICLE

Effect of Pulsed High Intensity Laser on Healing of Burn Wound: An Experimental Study

Anwar Abdelgayed Ebid PhD, PT*, Shamekh Mohamed El-Shamy PhD, PT, Ali Abd El Monsif Thabet PhD, PT.

Associate Professor Physical Therapy, Faculty of Applied Medical Science, Umm Al-Qura University, Kingdom of Saudi Arabia.

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*Corresponding Author

Anwar Abdelgayed Ebid
PhD, PT

Abstract

Background: wound healing is a complex biochemical and biologic process. **Purpose:** This study was carried out to investigate the influence of 1046-nm Pulsed High Intensity Nd: YAG Laser in the treatment of 3rd degree burn wound on rats. **Methods:** twenty clinical healthy male Sprague-Dawley (SD) rats with induced two full thickness burn wound measuring 5 cm² on the dorsal aspect, one wound act as control and the other as study. The control wound receive standard wound care without laser and study wound received 6 weeks pulsed high intensity laser 250 j/cm² in three phases (initial, intermediate and final phase) according to designed protocol. **Results:** The result revealed that there was statistical significant difference in wound surface area (WSA) and percentage of healing for study wound after 1,2,3,4,5 and 6 weeks as compared to control wound. **Conclusion:** pulsed high intensity Nd: YAG laser was an effective and a new trend physical therapy modality in the treatment of 3rd degree burn wound.

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INTRODUCTION

Thermal burns produce more severe physiological stresses than other forms of traumatic injuries **Barillo and McManus 1999**. Severe burns leads to extensive tissue damage and are complicated by injury in the cutaneous vasculature, loss of body fluids, and delayed wound healing **Stell et al., 2008**.

Third degree burns cause damage to all layers of the skin and some subcutaneous tissues which leads to lacks viable epithelial elements, thus skin grafting for wound closure is often required, the burned skin is characterized by painless, appears dry, cracked, white and leathery or charred and the underlying fat may be exposed **Wolf and Herndon 2001**.

Wound is a physical bodily injury characterized by disruption of the normal continuity of body structures and cause injury to superficial cutaneous structures and to the structures underlying the skin **Arun et al., 2009**.

Wound healing is a complex process that involves local and systemic responses and the wound healing process altered restitution of tissue structure and function and have the potential to cause dysfunction, lifelong disfigurement and death **Maurer et al., 2003**.

Lasers act as a unique tool for surgery and medical therapy and. One of the main basic mechanism of laser application is its monochromaticity, which allows efficient coupling to the peak absorption of chromophores, enabling stimulation of biological processes and maximal photoactivation **Conlan et al., 1996**.

Lasers used in the healing of different wound have been shown to speed up the healing process in burn wounds, leg ulcers and have been demonstrated to improve skin healing capabilities **Coulter 1994**. Laser stimulate the release of healing enzymes, which were required for optimal functioning, as well as the production of endorphins **Markolf 2003**.

Laser biostimulation of wound healing process was reported by several investigators as significant increases in re-epithelialization, stimulation of fibroblasts proliferation, macrophage stimulation, collagen synthesis, acceleration of wound closure, granulation tissue formation, and extracellular matrix production **Schindl et al., 2000, Reddy 2004**.

Many of previous studies was conducted in laser therapy have been carried using low and medium intensity laser devices (Low Level Laser Therapy: LLLT), with wavelengths ranged between 600 - 900 nm, in this spectrum laser beam is partially absorbed by the natural chromophores as melanin, which withhold part of the energy irradiated. In the other hand this study based on the use of Nd: YAG Pulsed High Intensity Laser Therapy (HILT), which characterized by a wavelength 1046 nm that allows it to spread and penetrate more easily through the tissue due to not having an endogenous chromophore **Parra et al., 1992, Parra 1990**.

High Intensity Laser Therapy has been used in many fields of physical therapy as sports, traumatology and pain therapy with high excellent results **Parra et al., 1992, Parra 1990, Pesare et al., 2000, Lubich et al., 1997**, so we decided to assess the possibility of transferring this method to the healing of burn wounds, therefore; the objective of our study was to evaluate the efficacy of Pulsed High Intensity Laser (HILT) on healing of 3rd degree burn wound.

Materials and Methods

Animal preparation

Twenty clinical healthy male Sprague-Dawley (SD) rats' weights ranging from 290–330 g (mean 305.8±11.05 g) and aged about 18 weeks were used in the study (supplied from Biochemistry Department, Faculty of Medicine). The animals were caged individually in a controlled environment at 25-27°C and 50% humidity with a 12 hours artificial light cycle. Animals were housed in solid bottomed cages; food and water were allowed on an ad libitum schedule. In the dorsal aspect of all animals there was an induced two full thickness burn wound measuring 5 cm² on the dorsal aspect, one wound act as control and the other as study. The control wound receive standard wound care without laser and study wound received 6 weeks pulsed high intensity laser according to designed protocol. Plastic jelly cups with a hole of 2.6 cm in diameter cut at the bottom were put around the neck of the rats as collars to prevent them from licking their wounds. The general anesthesia (Xylazine, Sanofi; Sante Nutrition, Laballarsrere-3301, Libonne Codex, France; Ketamine, Rotex-medicr GMBH, Germany) was used by using a mixture of Xylazine and ketamine at ratio of 1:0.5 which was injected IM (intramuscularly). The site for experiment prepped with 10% betadine solution, after anesthesia area of the back to which the burn would be inflicted were prepared (fixed part for all rats) using an electric hair shaver .

Experiment protocol

The model of the burn wound was produced according to Hoekstra standard [16]. Animals were subjected to a preheated rounded metal probe weighing 300 g and measuring 5 cm² (2.5 x2.5 cm), an electric soldering iron, was set at 180 °C. The metal probe applied in contact with the shaved flank of the rat for 20 s, there was no additional pressure applied to the rod while in contact with the skin except the natural gravity. Two round burn wound one in each side of the column measuring 5 cm² were created aseptically on the back of each animal with 4 cm in-between.

Pulsed high intensity laser

Pulsed High Intensity Laser High intensity laser machine by ASAsrl Company, Hilterapia, HIRO 3.0, Italy with high peak power produced 3 KW, wavelength 1064 nm, brief duration 120-150 μ. Sec, energy content 150-350 mJ, frequency 10-30 HZ, duty cycle 0.1%, , irradiated spot diameter 0.5 cm, energy density 360-710 J/cm² .

Laser protocol

In each rat, the control wound (untreated) was irrigated with normal saline and left without treatment, while the wound in study group (treated group) was exposed for 6 weeks, 6 days/week consecutively to a Pulsed High

Intensity Laser High intensity laser machine. The laser irradiation process was performed by keeping the head of the laser device 0.5 cm perpendicular to the wound and at a 1 cm distance from the wound surface. During laser application the control wound was covered. The irradiation was performed without contact with the wound. The irradiation was performed in three different phases: the initial, intermediate, and final phases. In the initial phase, fast scanning in the vertical and horizontal directions were applied to the wound area with an energy density of 510 mJ/cm², a frequency of 25 Hz, an energy of 25 J/cm², and a total energy of 100 J. In the intermediate phase, the probe was held at the periphery of the wound on fixed points at a distance of 1 cm away from the wound margin. The probe was in contact with the skin surface at the wound margin and fixed for 7 sec at each point providing energy of 10 J/point for at least 5 points (i.e., a total of 50 J). In the final phase, the same laser parameters were used as in the initial phase, except that the scanning was performed at a slower pace (table 1).

Wound Surface Area Measurement and Healing Percentage Calculation.

The wound sizes of the rats were measured after 1, 2,3,4,5 and 6 weeks .Rats was transferred to a transparent, air tight plastic box with diethyl ether soaked cotton wools for general anesthetization by inhalation. The small volume of the box limited the movements of the rat and saved the drug; yet there was sufficient air space to prevent suffocation when the lid was closed. A 75% alcohol cleaned transparency was laid on the wound surface while the wound size on the transparency was outlined with a permanent marker. The wound size was measured by counting the number of squares on a standard graph paper that were included by the wound outlines on the transparency. The healing percentage of the wounds was calculated by comparing the wound size after 6 weeks to that of first day of burn.

Results

Data were collected and statistically analyzed using repeated measures ANOVA to test hypothesis and to control both within and between variabilities at level of significance of 0.05 by using SPSS version 16.0.

Table 1. Treatment protocol of Pulsed High Intensity Nd: YAG laser.

Phase	Mode of application	Frequency (Hz)	joules
Initial	Fast Scanning (Vertical and horizontal on wound area)	25	100
Intermediate	Applied at the periphery of the wound (Fixed points)	15	50
Final	Slow Scanning (Vertical and horizontal on wound area)	15	100

Table 2: The mean values (Mean±SD) of WSA after 1, 2,3,4,5 and 6 weeks and percentages of wound healing (%) of the study and control wound.

		Study wound	Control wound
Wound Surface Area (cm ²) (Mean ±SD)	1 st week	3.88 ± 0.28	4.84 ± 0.08
	2 nd week	2.90 ± 0.34	4.74 ± 0.14
	3 rd week	2.10 ± 0.19	4.55 ± 0.12
	4 th week	1.29 ± 0.25	4.42 ± 0.14
	5 th week	1.0 ± 0.16	4.08 ± 0.11
	6 th week	0.12± 0.12	4.00 ± 0.21

Percentages of wound healing (%)	1 st week	26%	2.2%
	2 nd week	45%	4.9 %
	3 rd week	59%	8.4%
	4 th week	77%	10.4%
	5 th week	85%	12.6%
	6 th week	98%	16.8%

Wound surface area

There was significant difference in the WSA between the study and control wound in all time of evaluation. The WSA (mean±SD) after 1, 2,3,4,5 and 6 weeks were shown in (table 2).

Percentage of healing

The percentages of healing of the study and control wound after 1,2,3,4,5 and 6 weeks were shown in (table 2).The wound healing percentage after 1,2,3,4,5 and 6 week which was obtained by comparing the wound size difference between first day after experimental burn wound and after 1,2,3,4,5 and 6 week was 26%,45%, 59%,77% and 85% for the study wound and 2.2%,4.9 %, 8.4%, 10.4% and 12.6% for the control wound. Finally; after 6 weeks the wound healing percentage of the study wound reached 98% while the control wound attained 16.8%. There was significant difference in the wound healing percentage between the study and control wound in all time of evaluation (1,2,3,4,5 and 6 weeks) (table 2).

Discussion

The results of this study showed clear promotion of healing in the study wound in comparison with the control one during the whole period of the study.

Lasers currently enjoy wide application in physiotherapy practice and there was many previous studies that explain the mechanisms that the laser can accelerate the healing of wounds as stimulate release of growth factors, increased epithelialization, improved tensile strength of scars **Farouk et al.,2005**, decrease period of inflammatory phase and the proliferation phase of healing begins earlier, facilitate fibroplasia during the repair phase of tissue healing **Mary 2003,Ribeiro et al.,2009** and stimulates fibroblasts on wound regeneration by maintenance of a high mitotic activity of the fibroblast in the later healing period **John and Michael 2003**.

Also laser enhance collagen syntheses that may be due to light energy was observed by endogenous chromophores in the mitochondria and used to synthesize adenosine triphosphatase (ATP), the resulting ATP was used to power metabolic processes, synthesize RNA, DNA, enzymes, proteins, and other biological materials which needed to repair or regenerate cell and tissue components, cell proliferation or rapid mitosis and restore homeostasis **Mary 2003,Ribeiro et al.,2009**.

The stimulatory effects of laser appear to be related to specific events during the inflammatory and the proliferative phase, indicating that the period of intervention may be critical **Reddy 2004**.According to **Medrado and collaborators.,2003** laser treatment reduce the intensity and duration of the inflammatory phase. For this reason, in our study the treatment was applied from the first day after induction pf the experimental wound, 5 times/week aiming to reduce the period of the inflammatory and proliferative phase.

Previous preliminary studies have found that pulsed high intensity laser seems to be more effective than low intensity laser because of its higher intensity and to the increased depth reached by the laser **Zati and Valent 2006, Fortuna et al.,2006**.

Also previous meta-analysis study showed the positive influence of laser therapy on reduction in wound size, wound tensile strength, total collagen content and healing time **Woodruff et al.,2004**, another met-analysis study on the effect of laser therapy on tissue repair in clinical cases showed a small to moderate positive effect size **Fulop et al.,2009**.

In our study we observe clear differences between the study and control wound from first week, when wounds would have been well into the repair phase of soft tissue healing but this may be differ according to specific laser irradiation settings, such as wavelength, duration, power, and intensity.

The pulsed high intensity laser is an effective treatment for enhancing wound contraction of full thickness burn wound. Our data focused on enhanced healing of full thickness burn wounds on rats, we believe this is the first step to use pulsed high intensity laser for application on humans.

Limitations of This Study

There was some limitations in this study First, this study examined relatively small areas of third degrees burn wounds and the results would differ when more extensive wound was selected. Second, the rat model was used to simulate the human model.

Conclusion

Pulsed high intensity Nd: YAG laser photostimulation have a favorable beneficial effects on healing of 3rd degree burn after thermal burn injury

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