Effect of pulsed high intensity Nd:YAG laser on healing of experimental burn wound

Anwar Abdelgayed Ebid*, Shamekh Mohamed El-Shamy and Ali Abd El Monsif Thabet

Faculty of Applied Medical Science, Umm Al-Qura University, Kingdom of Saudi Arabia.

*Correspondence: anwarandsafa@yahoo.com  Accepted: 29 May 2017 Published online: 07 June 2017

Wound healing is a complex and long lasting biochemical and biologic process. This study was conducted to investigate the influence of 1046-nm Pulsed High Intensity Nd: YAG Laser in the treatment of 3rd degree experimental burn wound on rats. Twenty four clinical healthy male Sprague-Dawley (SD) rats with induced two full thickness burn wound measuring 6 cm² on the dorsal aspect, one wound act as control and the other as study. The control wound receive only standard wound care without laser and the study wound received 8 weeks pulsed high intensity laser 350 J/cm² in three phases (initial, intermediate and final phase) according to designed protocol. The result revealed that there was statistical significant difference in wound surface area (WSA) and percentage of healing (%) for study wound after 2, 4, 6 and 8 weeks as compared to control wound. In conclusion pulsed high intensity Nd: YAG laser was an effective, non invasive and a new trend physical therapy modality in the healing of 3rd degree burn wound.

Key words: Pulsed Nd: YAG Laser, Wound Healing, Full Thickness Burn.

INTRODUCTION

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 and longtime process which involves local and systemic responses, including restoration of integrity to injured tissues by replacement of dead tissue with viable tissue; this starts immediately after an injury, may continue for months or years, and is essentially the same for all types of wounds (Maurer et al. 2003).

A laser is a medical device used to treat pain and speed up wound healing. It alters the healing process at a cellular level. One of the main basic mechanisms 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 including chronic ulcers as leg ulcers, acute infected wounds and surgical wound and burn wound through speeding up the healing process, and have been demonstrated to improve skin healing capabilities (Coulter. 1994). Laser stimulates the release of healing enzymes, which were required for optimal functioning, as well as the production of endorphins for healing without pain (Markolf. 2003).

Laser biostimulation has many physiological effects include 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).

This study based on the use of Nd: YAG Pulsed High Intensity Laser Therapy (HILT), which has wavelength 1046 nm that allows it to spread and penetrate more easily through the tissue (Parra et al. 1992; Parra. 1990).

High Intensity Laser Therapy has been used with high excellent results in many fields of physical therapy as pain management, sports, and traumatology (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 experimental burn wound.

MATERIALS AND METHODS

Animal preparation
Twenty four clinical healthy male Sprague-Dawley (SD) rats' weights ranging from 293–332 g (mean 307.9±10.03 g) and aged about 19 weeks were used in the study (supplied from Biochemistry Department, Faculty of Medicine, Umm Al-Qura University). 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 6 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 8 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, Laballasere-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. Animals were subjected to a preheated rounded metal probe weighing 300 g and measuring 6 cm² (3 x3 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 6 cm² were created aseptically on the back of each animal with 2 cm in-between.

Pulsed high intensity laser
Pulsed High Intensity Laser High intensity laser machine by ASAsrl Company, Hitlerapia, 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 8 weeks, 5 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
Table 1: Treatment protocol of Pulsed High Intensity Nd: YAG laser.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mode of application</th>
<th>Frequency (Hz)</th>
<th>joules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Fast Scanning (Vertical and horizontal on wound area)</td>
<td>25</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Applied at the periphery of the wound (Fixed points)</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td>Slow Scanning (Vertical and horizontal on wound area)</td>
<td>15</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 2: The mean values (Mean±SD) of WSA after 2, 4, 6 and 8 weeks and percentages of wound healing (%) of the study and control wound.

<table>
<thead>
<tr>
<th></th>
<th>Study wound</th>
<th>Control wound</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wound Surface Area (cm²)</strong> (Mean ±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
<td>3.99 ± 0.25</td>
<td>5.74 ± 0.08</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>2.50 ± 0.38</td>
<td>5.00 ± 0.13</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>1.00 ± 0.16</td>
<td>4.55 ± 0.17</td>
</tr>
<tr>
<td>8&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>0.10± 0.11</td>
<td>4.00 ± 0.14</td>
</tr>
</tbody>
</table>

| **Percentages of wound healing (%)** |             |               |
| 2<sup>nd</sup> week            | 40%         | 3.3%          |
| 4<sup>th</sup> week             | 64%         | 6.8 %         |
| 6<sup>th</sup> week             | 80%         | 8.5%          |
| 8<sup>th</sup> week             | 98%         | 12.8%         |
Figure 1: Mean values of wound surface area (WSA) for study wound and control wound from 2nd to 8th week

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, energy of 25 J/cm², and a total energy of 150 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.

The wound sizes of the rats were measured after 2, 4, 6 and 8 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.

Healing Percentage Calculation.

The healing percentage of the study and control wounds was calculated by comparing the wound size after 2, 4, 6 and 8 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.

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 2, 4, 6 and 8 weeks were shown in (table 2) and figure 1.
Percentage of healing
The percentages of healing of the study and control wound after 2, 4, 6 and 8 weeks were shown in (table 2). The wound healing percentage after 2, 4, 6 and 8 weeks which was obtained by comparing the wound size difference between first day after experimental burn wound and after 2, 4, 6 and 8 weeks was 40%, 64%, 80% and 98% for the study wound and 3.3%, 6.8%, 8.5%, and 12.8% for the control wound. Finally, after 8 weeks the wound healing percentage of the study wound reached 98% while the control wound attained 12.8%. There was significant difference in the wound healing percentage between the study and control wound in all time of evaluation 2, 4, 6 and 8 weeks as shown in table 2.

DISCUSSION
The results of this experimental study showed that pulsed high intensity laser enhanced wound healing due to 3rd degree burn wound in the study group greater than 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 the 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 reduces the intensity and duration of the inflammatory phase. For this reason, in our study the treatment was applied from the first day after induction of 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 meta-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 the 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 in humans. There were 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
In conclusion, we found that the pulsed high intensity and: YAG laser photo-stimulation has favorable, beneficial effects on healing of 3rd degree wound after thermal burn injury.

CONFLICT OF INTEREST
The present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT
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AUTHOR CONTRIBUTIONS
AAE, SME, AAT designed and performed the experiments and also wrote the manuscript. AE, SE, and AT performed animal treatments, wound size measurement, healing percentage calculations and data analysis. AE and SE designed experiments and reviewed the manuscript. All authors read and approved the final version.

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REFERENCES