Visible Lasers Were Better Than Invisible Lasers in Accelerating Burn Healing on Diabetic Rats

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Abstract

Objective: This study was designed to assess and compare the efficacy of accelerating burn healing in diabetic rats using low-power visible and invisible lasers. Background Data: Low-level laser therapy (LLLT) has been used in a number of diabetic animal and human studies, with both positive and no effects. Materials and Methods: Male Sprague-Dawley rats were used in the study. Streptozotocin (70 mg/kg) was given for diabetes induction. A burn wound was created on the shaved back of the animals using a metal rod heated to 600°C. The study was performed using 532-, 633-, 670-, 810-, and 980-nm diode lasers. Incident doses of 5, 10, 20, and 30 J/cm² and a treatment schedule of three times per week were used in the experiments. The burned areas on all rats were measured and plotted on a chart, and the slope values (mm²/d) and the percentages of burn healing were compared. Results: The percentage of burn healing on diabetic rats after LLLT was 78.37% for the visible lasers and 50.68% for the invisible lasers. There was a significant difference (p < 0.005) between visible lasers and invisible lasers in the percentage of burn healing on diabetic rats after laser therapy. Conclusion: LLLT at the appropriate treatment parameters can accelerate burn healing on diabetic rats using both visible and invisible lasers. The effects of visible lasers were better than those of invisible lasers in accelerating burn healing on diabetic rats in this study.

Introduction

Diabetes mellitus is a metabolic condition resulting in increased blood glucose, leading to a variety of complications, including delayed wound healing. Burn injuries in diabetic patients may have negative effects, such as increased length of stay and increased number of operations required. Low-level laser therapy (LLLT) has been used in a number of diabetic animal and human studies, with both positive and no effects. Many in vitro studies comparing the effects of lasers using visible and near-infrared wavelengths have shown better results with visible wavelengths. The purpose of this study was to assess and compare the effects on burn healing in diabetic rats using visible and invisible lasers.

Materials and Methods

Animals

A number of male Sprague-Dawley rats were used in this study. The animals weighed 410 ± 36 g and were 21–23 wk old at the start of the experiment. The rats were originally imported from Charles River Co. (Margate, Kent, U.K.) in 1984, and now they are bred at the animal facility of King Faisal Specialist Hospital and Research Centre (KFSHRC). Animal protocols were reviewed and approved by the Animal Care and Use Committee. During the study, the rats were housed one per cage, and maintained under controlled environmental conditions (12-h light/dark cycle and temperature ~23°C), and provided with standard laboratory food and water ad libitum.

Chemical induction of diabetes

Diabetes was induced in the rats by an IP injection of the pancreatic β-cell toxin streptozotocin (freshly dissolved in 0.9% sterile saline; Sigma, St. Louis, MO, USA) at a dose of 70 mg/kg body weight. Diabetes was assessed by estimating hyperglycemia and glycosuria. Animals were rejected from the study if blood and urine glucose did not reach 200 mg/dl and four pluses (++++) on dipstick, respectively, 24 h post-induction, and if their body weight did not increase consistently during the first 3 d of induction. Hyperglycemia, glycosuria, and rat weight were determined and monitored on a schedule as described previously.
<table>
<thead>
<tr>
<th>Laser (nm)</th>
<th>Power (mW)</th>
<th>Spot size (cm²)</th>
<th>Power density (mW/cm²)</th>
<th>Irradiation time (min)</th>
<th>Incident dose (J/cm²)</th>
<th>Treatment schedule (times/wk)</th>
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**Table 1. Laser Treatment Parameters**

**FIG. 1.** The percentage of relative burn healing in diabetic rats after irradiation using different laser wavelengths and incident doses.
**Visible Lasers Were Better at Accelerating Burn Healing**

Diabetic rats were anesthetized with 35 mg/kg ketamine and 15 mg/kg xylocaine (70% the dosage given to non-diabetic rats). The surgical site was shaved using an electric clipper, excess hair was removed by a lotion, and the skin was disinfected with an isopropyl alcohol swab. Burn wounds 1.5 ± 0.3 cm² in size were created using a metal rod heated to 600°C that was applied for 2 sec. All diabetic rats with burn wounds were divided randomly into control (n = 600°C that was applied for 2 sec. All diabetic rats with burn wounds were divided randomly into control (n = 15) and treatment groups (n = 60) on the basis of the designated treatment they received.

**Laser System and Treatment Parameters**

The study was performed using a 532-nm diode laser (Green Laser System; Intellite, Inc., Menden, NV, USA), a 633-nm diode laser (Biophoton, St. Alban, France), a 670-nm diode laser (B&W Tek Inc., Newark, DE, USA), and 810-nm and 980-nm diode lasers (Biophoton). The laser beam was delivered through an optical fiber and projected to cover the entire burned area including the borders. The output power was measured continuously using a Moletron MAX5200 power meter (Moletron Detector, Inc., Portland, OR, USA). Laser treatment parameters are listed in Table 1. While undergoing treatment each rat was restrained in an acrylic glass cage without anesthesia during the laser irradiation period. The control group carefully received the same manipulations, except for laser exposure.

**Data and Statistical Analyses**

The burned areas on all rats were measured daily 5 d/wk using a caliper and the sizes were plotted on a slope chart and the slope value (mm²/d) of burn healing in all rats was computed using the linear type and set intercept option. The mean slope value of burn healing was counted in every group. The percentage of relative burn healing (RBH) was calculated using the following formula: percentage of RBH = (slope value in treatment groups – slope value in controls) / slope value in controls × 100. Statistical analyses were performed using the Student’s t-test for comparison of data from the study. Differences were considered statistically significant when the p value was <0.05.

![Graph showing percentage of relative burn healing in diabetic rats after irradiation using visible and invisible lasers.](image)

**FIG. 2.** The percentage of relative burn healing in diabetic rats after irradiation using visible and invisible lasers.

**Results**

Seventy-five diabetic rats were induced successfully by streptozotocin injection and used in the study. The percentage of RBH in diabetic rats after irradiation using different laser wavelengths and incident doses are shown in Fig. 1. The percentage of RBH in diabetic rats after irradiation using visible and invisible lasers are shown in Fig. 2. The results of statistical analysis using the Student’s t-test on mean percentage of RBH between visible and invisible lasers are listed in Table 2.

**Discussion**

This study indicates that LLLT using visible and invisible lasers at the appropriate treatment parameters can accelerate burn healing in diabetic rats. Our results showed that visible lasers were better than invisible lasers in improving burn healing on animals with diabetes. It is unclear, however, why the irradiation by visible lasers had better effects. Hartmann found that a photo-biological response involves the absorption of a specific wavelength of light by some unknown photoreceptor. It is hypothesized that light activation of this unknown photoreceptor may occur in tissues irradiated by visible laser energy. Diode lasers with different wavelengths were used in our experiment. Currently, diode lasers are finding more and more applications in medicine, as they have several advantages over other types of lasers. They are among the most efficient converters of electric energy into coherent radiation. Diode lasers employ semiconductor crystals as active media, which, after excitation, emit coherent radiation in the visible or infrared region, which can easily be transmitted via optical waveguides to the patient. In addition, the treatment schedule also influences the effects of burn healing in LLLT. Most animal and clinical studies irradiate 4–5 d/wk until there is wound closure. Turner and Hode suggested that it is better to use 3–4 treatments per week with moderate doses, rather than higher doses and fewer treatments. Three treatments per week were used in our present study because our previous experiments showed that this schedule was most appropriate.

The exact mechanism by which LLLT facilitates burn and wound healing is largely unknown. However, some in vitro studies have shown an increase in fibroblast proliferation after irradiation, suggesting that LLLT therapy may facilitate fibroplasia during the repair phase of tissue healing. Literature indicates that LLLT accelerates inflammation, modulates the level of prostaglandin, enhances the action of macrophages, promotes fibroblast proliferation, facilitates...
collagen synthesis, fosters immunity, and even accelerates the healing process.\textsuperscript{16} Growth factors are classified as cytokines, and are essential to wound healing. Growth factors attract useful cells and proteins to the wound, including immune cells to fight infection and other cells to form connective tissue. They stimulate and increase production of connective tissue, create a new supply of blood vessels to nourish the site, and promote remodeling.\textsuperscript{17} An increase in cytokine expression is characteristic of accelerating the reparative process.\textsuperscript{18} The function and variation of growth factors in burn and wound healing after LLLT will be our next project and direction of research.

**Conclusions**

Diabetic patients are usually complicated patients, owing to the high prevalence of systemic microvascular damage affecting multiple organs. Also, peripheral neurological impairments are known to be predisposing factors for burn injury, because of the decrease in protective sensation and tissue vascularity.\textsuperscript{19–23} Furthermore, diabetes mellitus is well known to be associated with decreased healing ability and increased susceptibility to infection. Diabetes in burn patients may therefore have implications for an increased number of surgical procedures required, as well as higher complications rate and longer length of stay. With the emphasis today on cost-effective treatment, it will be also a useful treatment option for improving and accelerating burn healing in diabetic patients using LLLT. The results of this study will be beneficial for the clinical application of treating the burn patients with diabetes.

**Acknowledgment**

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**Disclosure Statement**

No conflicting financial interests exist.

**References**


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3. Adenilson Souza Fonseca, Mauro Geller, Mario Bernardo Filho, Samuel Santos Valença, Flavia Paoli. 2011. Low-level infrared laser effect on plasmid DNA. *Lasers in Medical Science*. [CrossRef]
