INFRARED (780 nm) LOW LEVEL LASER THERAPY FOR WOUND HEALING: IN VIVO AND IN VITRO STUDIES

Sima Halevy,1,2 Rachel Lubart,3 Haim Reuveni,2 and Nili Grossman2

1: Department of Dermatology and 2: Investigative Dermatology Laboratory, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva; and 3: Department of Physics, Bar Ilan University, Ramat Gan, Israel.

The potential therapeutic effect of 780 nm low power diode laser irradiation (LPDLI) was evaluated in vivo on wound healing, and in vitro on proliferation of cultured normal human fibroblasts (NHF) and keratinocytes (NHK). In five patients suffering from cutaneous fissures (3 patients) one fissure in each patient was irradiated along the length of the fissure every 2 or 3 days, using 780 nm LPDLI, 30 mW, 30 sec per point. Fissures located on the opposite side of the body served as controls. In vivo, NHF and NHK were irradiated with this diode laser, via a fiberoptic light guide (25 mW) for 0-20 sec. Complete closure occurred in 80% of irradiated fissures, and in 60% of controls. Initial healing (25% closure) occurred earlier, at 2.2±1.1 days in irradiated fissures vs. 3.5±0.9 days (p < 0.06) in the nonirradiated fissures, but the timing of complete healing did not differ significantly between the two groups. In vitro, a single laser exposure to NHK (2.7 sec) and NHF (6-10 sec) increased proliferation parameters compared with sham-irradiated controls: 3H-Thymidine incorporation at 6-24 hours by 2.29±0.31 (p<0.01); the percentage of dividing cells at 24 hours by 1.56±0.11 (p<0.05); and cell numbers at 48 hrs by 1.45±0.07 (p<0.01). These results suggest that 780 nm LPDLI irradiation promotes wound healing, presumably by enhancing proliferation of fibroblasts and keratinocytes.

Key words: Fibroblasts, skin fissures, keratinocytes, laser therapy, proliferation

Introduction

Low power lasers are emerging in recent years as a new therapeutic tool for wound healing. However, there is still controversy regarding the efficacy and the specific role of these lasers in wound healing.1-7 Some of these conflicts stem from the different types of lasers used, use of a wide variety of irradiation conditions, sometimes poorly specified, and utilization of different target cells and tissues for irradiation.

Low power lasers emitting at 780 nm are amongst the least studied lasers, although several lines of evidence imply that this wavelength might be a potent tool to promote wound healing: (a) 780 nm is absorbed by mitochondrial cytochrome oxidase,8 and thus may induce photoactivation of cellular functions; (b) 780 nm diode laser was shown to enhance mitosis in murine fibroblasts;9 (c) Gallium-aluminum-arsenide (GaAlAs) lasers emitting at 780 nm were shown to exert anti-inflammatory and analgesic responses in human rheumatoid arthritis10 and in a rat carrageenan inflammatory model.1-2 Finally, in a previous uncontrolled clinical study, GaAlAs 780 nm laser markedly reduced pain, and enhanced granulation tissue formation and to some extent epithelization of chronic venous ulcers.13

To determine the effects and the effective dose of 780 nm low power diode laser irradiation (LPDLI) on wound healing, two systems were studied. In the in vivo part, cutaneous fissures of various etiologies were irradiated, and clinical parameters of wound closure and

Address for Correspondence:
Prof. Sima Halevy, MD,
Department of Dermatology, Soroka Medical Center, P.O. Box 151, Beer Sheva, Israel 84101

Manuscript received: August, 1997
Accepted for publication: October, 1997

LASER THERAPY, 1997: 9: 159 - 164
healing were assessed and compared to nonirradiated parallel fissures. In the in vitro part, the proliferative response of cultured normal human fibroblasts and keratinocytes to irradiation was determined.

Patients, Materials and Methods

In Vivo Studies

Patients

Included in the present clinical study were five patients (3 males, 2 females, age range 48-65 yrs, mean age 58 yrs), suffering from cutaneous fissures due to various skin diseases, located on the hands and feet. In each patient, one fissure was irradiated, whereas a second similar fissure, located on the opposite side of the body, served as a control. The clinical data of the patients, including the initial dimensions (length, width, depth) of the five irradiated fissures and the five nonirradiated fissures (controls), are detailed in Table 1.

Irradiation source

Pocket low power diode laser, (model MED-140, Lasotronic AG Switzerland), emitting 780 nm at 35 mW, with a focal spot of 1 mm² has been used in this study. In all in vitro experiments, an optical fiber was attached, with its end positioned 2 cm above the tissue culture plates. Under these conditions, the irradiated area was 20 mm², and the measured power was 25 mW.

Irradiation conditions

Multiple irradiations were performed every 2-3 days. Fissures were irradiated along their length at points 2 mm apart. Each point (approx. 1 mm diameter) was irradiated for 30 sec. No other treatment modalities for wound healing were used.

Clinical parameters

The dimensions of the treated and untreated fissures were measured before and after each irradiation session. To analyze the results and compare the kinetics of healing, the relative length of each fissure, at various stages of the study, was calculated as percentage of the initial length before treatment. Monitoring of other clinical manifestations (e.g., fine scaling) and subjective symptoms (e.g., decrease of pain) was performed in addition.

In Vitro Studies

Tissue culture media

Tissue culture media, fetal calf serum, trypsin and antibiotics were purchased from Biological Industries (Beth Haaeme, Israel). Growth factors and chemicals were purchased from the Sigma Chemical Co., St. Louis, MI, USA.

Cells, cultures and growth conditions

Normal human keratinocytes (NHK) were isolated from young foreskins (0 to 4 years old) as described by Reinwald and Green. Normal human fibroblasts (NHF) were initiated from explants of young foreskins. Growth media for NHK and NHF were prepared as described. Experiments were performed in 96 well clusters (Costar, Cambridge, MA, USA), seeded with 10,000 NHF or 13,000 NHK cells per well.

Irradiation conditions

Proliferating cells were irradiated 48 hrs after seeding while in phosphate buffered saline (PBS), for varying periods. The diode laser apparatus, with an optical fiber attached to the end, was kept 2 cm above the cultured cells. Following exposure, cells were re-fed with fresh growth medium and were further incubated. Sham-irradiated cells in PBS served as untreated controls. Experimental conditions were repeated in triplicate.

Proliferation parameters

Total cell number per well was determined 24 hrs or 48 hrs following irradiation, after removal of the cells from the wells by trypsinization (0.25% trypsin - 0.05% EDTA suspension) in a constant volume of 0.1 ml. Cells were counted microscopically using a counting chamber (Fuchs-Rosenthal, Germany). The fraction of cells at division was determined 24 hrs following irradiation as the number of unseparated daughter cells divided by the total number of cells in each well.

Table 1: Clinical data of patients suffering from cutaneous fissures.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Fissure Dimensions (mm) (length x width x depth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60 / M</td>
<td>Psoriasis vulgaris</td>
<td>4 x 1 x 2</td>
</tr>
<tr>
<td>2</td>
<td>58 / M</td>
<td>Infected tinea pedis</td>
<td>37 x 1 x 1</td>
</tr>
<tr>
<td>3</td>
<td>48 / F</td>
<td>Contact dermatitis</td>
<td>4 x 1 x 1</td>
</tr>
<tr>
<td>4</td>
<td>59 / F</td>
<td>Contact dermatitis</td>
<td>16 x 1 x 2</td>
</tr>
<tr>
<td>5</td>
<td>65 / M</td>
<td>Plantar keratoderma</td>
<td>16 x 1 x 1</td>
</tr>
</tbody>
</table>
Fig. 1: Effect of 780 nm diode laser irradiation on relative length of fissures in Patient 1. One fissure was irradiated (closed circles) and the other nonirradiated fissure served as control (open circles). The figure depicts the relative values of their initial lengths along the treatment. V = Irradiation; IR = irradiated; NIR = nonirradiated.

Fig. 2: Effect of 780 nm diode laser irradiation on relative length of fissures in Patient 2. Same as described for Fig. 1.

Fig. 3: Effect of 780 nm diode laser irradiation on relative length of fissures in patient 3. Same as described for Fig. 1.

Fig. 4: Effect of 780 nm diode laser irradiation on relative length of fissures in Patient 4. Same as described for Fig. 1.

Results

In Vivo Studies

Effect of 780 nm diode laser on healing of cutaneous fissures

Improvement in the length of cutaneous fissures was the principal parameter used to evaluate healing. In a preliminary phase of this open study, cutaneous fissures of 3 patients were irradiated by a 780 nm diode laser every 2 days for 1 sec per spot (4.4 J/cm²) along their length. During a period of 2 to 10 days, either none or a small improvement was observed in irradiated fissures (mean ± SD of 11 ± 12.7%; range 0% to 25%), which was similar or indistinguishable from their parallel nonirradiated controls (7.3 ± 7.0%; range 0% to 14%).

In the next stage of this study, 30 sec of 780 nm diode laser irradiation per spot were then applied (130 J/cm²) every 2 or 3 days. The following results were observed in the 5 irradiated fissures: A marked improvement occurred in all 5 (100%) irradiated fissures as seen in Figures 1-5, demonstrating the relative length of the fissure vs. a time scale in days. Complete closure was
observed in 4/5 (80%) fissures (Figures 1-4) and a decrease of about 40% in the length of 1/5 (20%) fissures (Fig. 5). Decrease in pain was reported in 3/5 (60%) of irradiated fissures (Nos. 1, 3, 4) on days 2, 3 and 2, respectively (2.3 ± 0.6 days after the first treatment). Fine scaling around the fissures was observed in 3/5 (60%) of the irradiated fissures (Nos. 2, 3, 4) on days 5, 5, and 2, respectively (4 ± 1.7 days after the first treatment).

The following results were observed in the 5 nonirradiated (control) fissures. Improvement occurred in 4/5 (80%) of the control fissures: complete closure in 3/5 (60%) fissures and a 55% decrease in the length of 1/5 (20%). One control fissure (1/5) did not show any detectable improvement (Figure 5). Decrease in pain was reported only in 1/5 (20%) of nonirradiated fissures (No. 1, on day 2); and fine scaling was observed in 1/5 (20%) fissures (No. 2, on day 3).

The time for complete healing observed in patients Nos. 1, 2, 3 did not differ significantly between irradiated fissures (4.7 ± 2.5 days) and their controls (6.3 ± 1.1 days). However, the rate of healing was enhanced in 4/5 (80%) irradiated fissures (Figs. 1, 2, 4, 5), or was identical in 1/5 (20%) (Figure 3), compared to their controls. In the irradiated fissures that completely healed (Nos. 1-4), an initial decrease by 25% of fissure length was observed earlier, at 2.2 ± 1.1 days after the first treatment, than in their controls, 3.5 ± 0.9 days (p < 0.06). This enhanced rate of healing was observed after the fissures were irradiated cumulatively for 48 ± 16 sec per spot (one or two treatments, as detailed in Figures 1-5). Apparently, the initial length of the irradiated fissures did not play a role in this response.

**In Vitro Studies**

**Effect of 780 nm diode laser on proliferation of cultured skin cells**

780 nm laser irradiation promoted cultured NHK proliferation in an energy density-dependent manner, expressed in an increased number of cells 48 hrs following exposure (Figure 6). The maximal response followed a dose of 2 seconds of irradiation (0.25 J/cm²) and yielded a significant 1.6-fold increase in cell number (64,300 ± 2,600 cells in irradiated vs. 40,600 ± 4,800 in sham irradiated wells; p < 0.001). To analyze this proliferative response further, cultured NHK were irradiated and three proliferation parameters were assayed at the end of the first 24 hrs following exposure: (a) ³H-thymidine incorporation into TCA precipitable material, as a measure of DNA synthesis between 6 to 24 hrs following exposure; (b) the fraction of cells at division; and (c) the number of cells at the end of 24 hrs.

As demonstrated in Figure 7, all three parameters were enhanced significantly by exposure to 780 nm for 2 to 7 sec of irradiation vs. sham-irradiated controls: (a) thymidine incorporation was increased by an average factor of 2.29 ± 0.31 (p < 0.001); (b) the fraction of dividing cells increased by an average factor of 1.55 ± 0.11 (p < 0.05); and (c) the average cell number increased by an average factor of 1.45 ± 0.07 (p < 0.01). It is assumed that the difference in optimal exposure period between Figure 6 and Figure 7 was related to the natural variability among normal primary cell cultures, while the difference in maximal increase in the number of cells was related to the longer incubation period before analysis. Similarly to NHK, NHP responded to 780 nm diode laser irradiation in an energy density-dependent manner (Figure 8). The maximal response was measured 48 hrs following a dose of 8 to 10 seconds of irradiation, yielding a significant 2-fold increase in cell number (80,000 ± 10,000 cells in irradiated vs. 80,000 ± 10,000 cells in sham-irradiated wells).
matrix formation and remodeling phase. Enhanced proliferation of fibroblasts and basal keratinocytes is thus an important component of dermal repair and of reepithelization, respectively. In an attempt to analyze the interaction of the 780 nm low power diode laser with the tissue, we have examined its effect in vivo on wound closure, and in vitro on the proliferation of each cell type in culture. The present controlled clinical study shows that multiple exposures to 780 nm low power diode laser, has a significant effect on the enhancement of wound healing in humans, as manifested by the accelerated dynamics of the response (enhanced initial healing rate), and the end result (number of closed or improved fissures). Healing of irradiated fissures was accompanied with a marked decrease in pain, as described previously for the effect of similar lasers in other human models and with fine scaling and hyperkeratosis around the fissures, similar to the phenomenon observed during enhanced healing of cutaneous ulcers following application of cultured keratinocyte grafts (unpublished data).

The observed enhancement of proliferation parameters of cultured skin cells (fibroblasts and keratinocytes) following exposure to 780 nm diode laser, suggested that this proliferative response might be involved in the beneficial effect of 780 nm irradiation on the treated patients. The optimal 780 nm dose for cultured cells was, however, much smaller than that applied in vivo, reflecting differences in interaction of light with a monolayer of cultured cells lacking a stratum corneum compared with laser interaction with living tissue, as well as differences attributed to the difference in parameters studied.

The exact mechanism responsible for the beneficial effect of 780 nm low power diode laser or low power lasers in general, has not been yet clarified. It is apparent, from in vitro and in vivo studies reported to date, that the acceleration of wound healing and/or early increased tensile strength, associated with the use low power lasers, such as the helium-neon laser, is probably due to a combination of stimulatory effects, specifically on collagen synthesis by fibroblasts, transformation of fibroblasts into myofibroblasts, alteration of lymphoid cells involved in the healing process, and enhanced keratinocyte motility. Release of tissue factors, may additionally account for the beneficial effects seen in the control wounds.

**Conclusions**

The results presented in this communication are the first to demonstrate a beneficial effect of 780 nm diode laser irradiation on wound healing in vivo, as well as on
proliferation of cultured skin cells in vitro. In spite of the small number of patients included in this controlled study, the results imply the potency of this laser of enhancing wound healing in vitro. Further studies are required to determine the modalities, efficacy and the indications for the use of this 780 nm low power diode laser in wound healing as well as the underlying mechanisms involved.

Acknowledgment

This study was supported in part by a grant from the Israeli Ministry of Defense.

References