Effect of low-level laser therapy on experimental wounds of hard palate mucosa in mice

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Under general anesthesia and sterile conditions, incision wound was induced in the hard palate mucosa of adult male mice. The wounds of groups 1 and 2 were irradiated daily with He-Ne laser at 3 and 7.5 J/cm² for 120 and 300 s, respectively, while the incision wound of group 3 not exposed served as controls. On day 3 of injury, the laser-treated wounds contained significantly lower neutrophils than the wounds in the control group. By day 7 after injury, the laser-treated wounds contained significantly more fibroblasts and at the same time contained significantly fewer macrophages. In conclusion, an acceleration of the wound healing process of experimental wounds in the hard palate mucosa of mice at low-level laser therapy with a He-Ne laser at energy densities of 3 and 7.5 J/cm² was observed.

Keywords: Hard palate mucosa, Low-level laser therapy, Wound healing

Craniomaxillofacial injuries occur in a significant proportion of trauma patients, either in isolation or in combination with other serious injuries. Transverse maxillary deficiencies are relatively common dentofacial deformity in combination with other maxillary and/or mandibular deformities. Orofacial clefts are defined as congenital defects in which the fusion between two or more of the following structures have failed: the palatal shelves, the maxillary prominences, and the medial nasal prominences. The clefts are surgically closed to restore the integrity of the oral and nasal cavity and to allow for normal feeding and speech development.

Healing of these wounds is associated with the disadvantageous effects in maxillary growth and dento-alveolar development often seen in cleft palate patient. Low-level laser therapy (LLLT) has been used for more than 25 years in clinical practice and is known to modulate various biological processes. LLLT is a non-thermal modality; usually the temperature changes associated with treatment are negligible. A number of different laser light sources, including helium-neon, ruby, and gallium arsenide have been used to deliver LLLT in different treatments and schedules.

In patients, biostimulation with low-level laser radiation has accelerated the healing of gingival incisions and gingivectomy, reduced the severity...
of conditioning-induced oral mucositis in bone marrow transplantation patients. Low-level laser radiation induced formation of new periodontal ligament, cementum, and bone in dogs, and also improved macromolecular clearance via the lymph flow in the hamster gingiva. At the cellular level, studies on cultured human gingival fibroblasts and human oral mucosal fibroblasts have suggested a number of LLLT effects: increased DNA synthesis, induced rapid generation of myofibroblasts from fibroblasts, enhanced proliferation and attachment of human gingival fibroblasts.

The effects of LLLT on the gingiva have been studied quite extensively in vivo and in vitro; however, there is a dearth of data on the effect of LLLT on the mucosa of soft and hard palates. Barasch et al. irradiated He-Ne laser to patients' oral mucositis, including the mucosa of the soft palate. Oral mucositis and pain scores were significantly lower for the treated versus the untreated side. They concluded that He-Ne laser treatment was well tolerated and reduced the severity of oral mucositis in patients. 

The use of LLLT has still not been widely accepted by physicians and a number of controlled studies, and the FDA withdrew its approval for its therapeutic use in the USA in 1983.

The present study sought to address the problem by evaluating whether He-Ne laser radiation at energy densities of 3 and 7.5 J/cm² accelerated the healing of the experimental wounds of the hard palate mucosa in mice.

Materials and Methods

Ninety adult male Albino N mari mice (3 months old, weighing 60±10g) were used. The study protocol was approved by the Medical Ethics Committees of Shahid Sadoghi Medical University, Yazd, Iran. The mice were provided with a standard animal food and water ad libitum. On day zero, all the mice were anesthetized with 50 mg/kg ketamine hydrochloride injected intramuscularly along with 5 mg/kg diazepam. In the hard palate mucosa, 2 mm posterior to the incisive tooth of the upper jaw a 4-mm long and 2-mm deep incision wound was made under sterile conditions. The animals were randomly divided into three groups, of 30 each. The incisions in groups 1 and 2 were treated daily with a He-Ne laser tube with 3 and 7.5 J/cm² energy densities, respectively. LLLT was commenced 24 h after surgery under general anesthesia. The laser used was a He-Ne laser (Meredith Co, Turkey) rated at 25 mW of power.

Groups 1 and 2 were exposed to 120 and 300s of laser light, respectively. LLLT was applied to one point, covering the whole length of the incision in each session (day). The laser was held 1 cm away from the surface of the target tissue.

The incision wounds of group 3 were treated with the LLLT probe without the laser being switched on, and the group was thus considered the control group. Thereafter, 10 mice of each group were sacrificed 3 days after surgery, 10 mice of each group were sacrificed 7 days after surgery, and the remaining 10 mice were sacrificed on the 15th postoperative day. The mice were sacrificed via the inhalation of chloroform in a closed space.

For histological examinations, a sample was excised from the wound bed and the normal adjacent mucosa of each mouse and was fixed in formaldehyde saline before it was embedded in paraffin blocks. Sagittal sections (5 µm thickness) were cut and stained with the hematoxylin and eosin method. Ten zones from each sample were examined morphometrically using a calibrated ocular on a Nikon light microscope at a magnification of 400X for counting the fibroblasts, macrophages, neutrophils, and endotheliums of the blood vessels. The histological examinations were performed in a blind fashion.

The data were subjected to the one way analysis of variance (ANOVA) and were expressed as mean ± SE. Multiple comparisons were performed by the least significant difference (LSD) test. P values <0.05 were considered statistically significant.

Results

None of the mice showed any sign of swelling or exudation at the surgical site.

Day 3 after surgery—The mean number of fibroblasts in group 1 was higher than those of the other groups, the mean number of blood vessel endotheliums in group 3 was higher than those of the other groups, and the mean number of macrophages in group 2 was higher than those of the other groups. Nevertheless, there were no significant differences in the above-mentioned histological parameters between the studied groups. The ANOVA test showed that the
mean number of neutrophils in group 3 was significantly higher than those of the other groups (P<0.05). The LSD test revealed that there were significant differences between group 3 and groups 1 and 2 (both P<0.05) (Table 1).

Day 7 after surgery—The mean numbers of neutrophils and blood vessel endotheliums were higher in group 3 than those of the other groups. However, there were no significant differences in the above histological parameters between the studied groups. The ANOVA test demonstrated that the mean number of macrophages in group 3 was significantly lower than that of the other groups (P<0.05). The LSD test revealed significant differences between group 3 and groups 1 and 2 (both P<0.05). The ANOVA test also showed that the mean number of macrophages in group 3 was significantly higher than that of the other groups (P<0.05). The LSD test revealed significant differences between group 3 and groups 1 and 2 (both P<0.05) (Table 2).

Day 15 after surgery—The mean numbers of macrophages, neutrophils, and blood vessel endotheliums of group 3 were higher than those of the other groups. The mean number of fibroblasts in group 1 was higher than those of the other groups. Nonetheless, the ANOVA test showed no significant differences in the aforementioned histological parameters between the studied groups (Table 3).

Discussion

The mean number of the neutrophils of the wound in the mice treated with a He-Ne laser was significantly lower than that of the control group. A significant increase in the fibroblasts of the laser-treated groups compared to the control group and also a significant decrease in the number of the macrophages of the laser-treated groups compared to the control group on day 7 after surgery were noted.

Beukelman et al.24 suggested that reducing components of inflammation may positively affect the wound healing process. Young and Dyson25 studied the effect of therapeutic ultrasound on the healing of the dermis of full-thickness excised lesions made in the skin of rats. They quantitatively assessed the healing by means of differential cell counts made in sections of the wound bed 5 and 7 days after injury. The wounds were either control group or exposed to pulsed ultrasound. By day 5 after injury, the ultrasound treated wounds contained more extensive granulation tissue, fewer polymorphonuclear leucocytes and macrophages, and more fibroblasts than the control wounds. Young and Dyson25 suggested that ultrasound therapy could be useful in accelerating the inflammatory and early proliferative stages of repair.

The decrease in the number of inflammatory cells and the increase in the number of fibroblasts in the laser–treated wounds in the present study on days 3 and 7 after injury chime in with the Beukelman et al.24 and Young and Dyson25 studies. Bisht et al.5 studied the biostimulatory effect of He-Ne laser irradiation on various parameters of the healing process in open skin wounds of rats. They produced two full thickness skin wounds on either side of the

Table 2—Fibroblasts, macrophages, neutrophils, and blood vessel endotheliums of the wounds of the studied groups on the 7th postoperative day.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fibroblasts</th>
<th>Macrophages</th>
<th>Neutrophils</th>
<th>Blood vessel endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.1(First laser group)</td>
<td>834.8±30.1</td>
<td>31.5±3.9</td>
<td>77.4±6.2</td>
<td>102.4±5.6</td>
</tr>
<tr>
<td>Gr. 2(Second laser group)</td>
<td>827.6±25.1</td>
<td>73.8±4.4</td>
<td>79.0±6.5</td>
<td>101.1±5.5</td>
</tr>
<tr>
<td>Gr.3(Control group)</td>
<td>739.7±29.1</td>
<td>88.1±5.9</td>
<td>96.4±6.6</td>
<td>112.2±5.3</td>
</tr>
</tbody>
</table>

*P < 0.05

Table 3—Fibroblasts, macrophages, neutrophils, and blood vessel endotheliums of the wounds of the studied groups on the 15th postoperative day.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fibroblasts</th>
<th>Macrophages</th>
<th>Neutrophils</th>
<th>Blood vessel endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.1(First laser group)</td>
<td>897.5±18.6</td>
<td>55.9±3.9</td>
<td>47.7±9.2</td>
<td>91.4±5.8</td>
</tr>
<tr>
<td>Gr. 2(Second laser group)</td>
<td>852.3±43.1</td>
<td>57.6±4.5</td>
<td>79.2±3.2</td>
<td>96.1±19.4</td>
</tr>
<tr>
<td>Gr.3(Control group)</td>
<td>822.1±27</td>
<td>65.5±5.4</td>
<td>52.0±5.1</td>
<td>98.2±4.7</td>
</tr>
</tbody>
</table>

*P < 0.05
midline. The wounds on the left side were irradiated daily with He-Ne laser at 4J/cm², whereas the wounds on the right side were not exposed and thus served as controls. Early epithelialisation with increased fibroblastic reaction and neovascularization was seen in the laser–treated wounds. Wound histology also showed a significant increase in leukocyte infiltration until day 9. Bisht et al. 8 reported that their results had established the biostimulatory effects of low-intensity laser radiation on the healing of the skin wound. Increased leukocytic reaction, reported by Bisht et al., is in disagreement with decreased neutrophil and macrophage counts, observed in the present study. The literature, however, shows that photobioactivation accelerates inflammation and promotes fibroblast proliferation, and even accelerates the healing process. 9

Recent investigations using advanced experimental techniques have tried to define the precise biostimulatory effects of LLLT. Recently, an upregulation of genes leading to an inverse in the proliferation of laser-treated fibroblasts has been demonstrated. 10

Irradiation of human fibroblasts at 628 nm was reported to have caused an increase in 7 categories of genes already known to play roles in the enhancement of cell proliferation and suppression of apoptosis. 11 Another related study succeeded in demonstrating in real time that LLLT could cause intracytosolic increase in pH and calcium as well as changes in reactive oxygen species. 12

These findings have laid a new groundwork for attempting to understand the comprehensive effects of LLLT on the biochemical milieu of the injured tissue. 13 To conclude, acceleration in the healing of experimental wounds in the hard palate mucosa of mice at LLLT with He-Ne laser at energy densities of 3 and 7.5 J/cm² was observed.

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