Effect of Low-Level Laser Therapy on Mast Cells in Second-Degree Burns in Rats

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ABSTRACT

Objective: This study sought to investigate whether low-level laser therapy (LLLT) with a helium-neon (He-Ne) laser would affect mast cell number and degranulation in second-degree burns in rats. Background Data: LLLT has been recently applied to stimulate the wound healing process. Materials and Methods: Sixty-five rats were randomly allocated to one of five groups. A deep second-degree burn was inflicted on all rats except those in the control group. In the sham-exposed group burns remained untreated. In the two laser-treated groups, the burns were irradiated every day by LLLT, with energy densities of 1.2 and 2.4 J/cm². In the fifth group the burns were treated topically with 0.2% nitrofurazone cream every day. The unburned skin of the rats in the control group were used for baseline study. The effects on mast cell number and degranulation were assessed by counting the number of intact and degranulated mast cells in sections fixed in formalin and stained with toluidine blue. Results: On the seventh and 16th days post-burn, the type 1 mast cell count in the 2.4-J/cm² laser-treated group was significantly higher than that of the control group. On the 30th day, the total numbers of mast cells in the laser-treated groups were lower than those in the control and sham-exposed groups. Conclusion: LLLT of deep second-degree cutaneous burns in rats significantly increased the number of intact mast cells during the inflammatory and proliferative phases of healing, and decreased the total number of mast cells during the remodeling phase.

INTRODUCTION

The repair process is a normal physiologic response to injury, and generally leads to the restoration of normal structure and function of the tissue. In certain disorders, the repair process leads to alterations in tissue structure that lead to the development of remodeling and fibrosis. 1 Thermal burns are less common than other forms of trauma, but they produce more severe physiological stresses than other forms of traumatic injury. It is estimated that two million people annually suffer from burns in U.S. 2 Keloids and hypertrophic scars are fibrous growths that result from an abnormal connective tissue response to burn injury. 3 Mast cells are related to pathogenesis in many fibrotic disorders. 1 An increased number of mast cells is associated with a variety of pathologic skin conditions in humans. Among these conditions are fibrotic disorders, including hypertrophic scars and keloids. 4, 5 The majority of scar tissue reactions produce the appropriate volume of collagen to fill a dermal defect. Excessive scar formation is a common sequela of abnormal wound healing in both traumatic and surgical injuries. These fibrotic lesions reportedly contain as many as 10 to 100 times more mast cells than normal human skin. 6 However, mast cells have also been shown to participate in physiological wound repair. The physiological role of mast cells in this process could be demonstrated in in vitro and in vivo models of wound healing. 1 Dyson and Lake 7 examined the degree of granulation by identifying three types of mast cells, representing different stages of mast cell degranulation, in skin samples: type 1: intact dark blue cells; type 2: cells that have extruded some granules, but the cell outline is largely intact; and type 3: cells in which degranulation is more extensive and widespread, with complete or partial disintegration of the original cell outline.

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Wound healing is a very complex process that involves interactions of various cell types, such as lymphocytes, monocytes, epithelial cells, and fibroblasts. Three main overlapping phases have been identified in tissue response to injury: inflammation, formation of granulation tissue and matrix, and remodeling. During granulation tissue formation, fibroblasts proliferate and migrate into the wound space. Levi-Schaffer and Kupietzky have demonstrated in vitro that mast cells influence the wound healing process by increasing this fibroblast migration and proliferation. This effect is partially mediated by histamine that acts on H$_2$-receptors on fibroblasts. Besides histamine, mast cell–derived interleukin-4 was also found to stimulate fibroblasts to proliferate and migrate. New investigations are finding that skin mast cells modulate the inflammatory response in healing wounds, play a role in neangiogenesis, and may participate in tissue remodeling in the late phase of wound healing. Recent evidence has shown that skin mast cells and mast cell chymase are important for the formation of granulation tissue and for the synthesis of collagen fibers at the edge of the wound in the burn-healing model in mice.

Low-level laser therapy (LLLT) has recently been used to stimulate the wound healing process. In laboratory animals, biostimulation of the wound healing process as reported by several investigators results in the stimulation of fibroblast proliferation, significant increases in re-epithelialization, increased collagen synthesis and granulation tissue formation, acceleration of wound closure, improved tensile strength of scars, and faster healing of burns.

There are few studies regarding the effects of light on skin mast cells. In their first study, El Sayed and Dyson showed that mast cells can be activated and their total number increased by exposure to light of certain wavelengths. In their second study, El Sayed and Dyson investigated the effects of different frequencies of laser light (wavelength = 820 nm) on the total number and percentage of degranulation of mast cells of injured skin. They observed that the total number of mast cells was increased significantly by all the pulse rates compared to those in the sham-irradiated group, but there was no significant difference between pulse rates. However, although the number of degranulated mast cells was higher in all laser-treated wounds, only the 20 Hz and 292 Hz frequencies were significantly effective. El Sayed and Dyson concluded that increases in mast cell number are not pulsing-frequency dependent, whereas degranulation is.

Until now, there have been no histological studies of the effect of helium-neon laser energy on mast cell morphology in burned skin. The aim of the present study was to evaluate the effect of LLLT with a helium-neon laser on the total number of mast cells and their degranulation in second-degree burns in rats using histological methods.

### Table 1. Specifications of the Laser Used in the Study

| Laser source: 10 mW helium-neon laser |  |
| Wavelength: 632.8 nm |  |
| Frequency: Continuous |  |
| Spot area: 3.14 mm$^2$ |  |
| Exposure: 120 s for the 1.2 J/cm$^2$ laser-treated group daily |  |
| 240 s for the 1.2 J/cm$^2$ laser-treated group daily |  |

### Materials and Methods

Sixty-five adult male Wistar rats weighing 250 ± 30 g each were kept in separate cages and fed ad libitum. Rats were randomly divided into five groups: (1) sham-exposed, (2) 1.2 J/cm$^2$ laser-treated, (3) 2.4 J/cm$^2$ laser-treated, (4) nitrofurazone-treated, and (5) control groups. On day zero, rats in the first four groups were anesthetized with 50 mg/kg ketamine hydrochloride and 5 mg/kg diazepam injected IM. Hair on the dorsum of each rat was shaved and cleaned with povidone iodine. Each rat was held in a special box with a 3 × 3-cm opening. The back of each rat was exposed through the opening for 3 s to the external lip of a cylinder 22 mm in diameter connected to a source of boiling water. Post-burn histological examination showed that the epidermis and most of the dermis were burned. The surface area of burned skin was 3.8 cm$^2$. All procedures were approved by Institutional Medical Ethics Committee.

Rats in the sham-exposed group (n = 15) were left untreated. The rats in the two laser-treated groups (each n = 15) were exposed daily to 1.2 J/cm$^2$ and 2.4 J/cm$^2$ LLLT, respectively, from the first day post-burn until the experimental time period was completed. The burned tissues received laser energy every day during the process of burn healing to ascertain if the therapy increased repair activity. The specifications of the laser used (made by the Iranian Atomic Energy Agency, Tehran, Iran) and exposure times are shown in Table 1. To apply the laser treatment, the burned area was divided into several equal squares (1 cm × 1 cm). The same squares were treated during each treatment session. The tip of the laser was lightly in contact with the surface of the burned skin at the center of each square, and directed perpendicularly to the target tissue for the designated time period (120 s in the 1.2 J/cm$^2$ laser-treated group and 240 s in the 2.4 J/cm$^2$ laser-treated group). The output of laser source was monitored with a power meter. The total energy applied (fluence) of the LLLT for the laser-treated groups are shown in Table 2. The rats in the fourth group (n = 15) were treated daily with topical 0.2% nitrofurazone cream (Tehran Daru Co., Tehran, Iran) from the first day post-burn until the experimental time period was completed.

The rats in each group were divided into three equal subgroups. Subgroups A, B, and C were sacrificed by neck dislocation following anesthesia with chloroform on the seventh, 16th, and 30th days post-burn, respectively. The unburned skin of the control rats were used for baseline study. A sample for histological examination was excised from the burned skin of each rat. Samples were fixed in formalin, embedded in paraffin blocks, and sagittal sections were cut from all regions of the samples and stained with 1% toluidine blue dissolved in dis-

### Table 2. Total Energy Applied to the Laser-Treated Groups (J/cm$^2$)

<table>
<thead>
<tr>
<th>Group</th>
<th>7 days</th>
<th>16 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2 J/cm$^2$</td>
<td>8.4</td>
<td>19.2</td>
<td>36</td>
</tr>
<tr>
<td>2.4 J/cm$^2$</td>
<td>16.8</td>
<td>38.4</td>
<td>72</td>
</tr>
</tbody>
</table>
Laser Therapy for Mast Cells of Burns

tilled water for 2 min. Histological examination was performed in a blind fashion.

Slides from different regions of each sample were randomly chosen and 100 zones in each sample were examined morphometrically by light microscopy (1000× magnification with oil-immersion). Intact mast cells showed deep blue staining. Based on the degree of granulation, it was possible to identify the three types of mast cells, representing different stages of mast cell degranulation, in the skin samples.

The data were subjected to one-way analysis of variance (ANOVA), and expressed as mean ± SEM. Multiple comparisons were performed using the least significant difference (LSD) test, and p < 0.05 was considered statistically significant.

RESULTS

Statistical analysis of the histological examination is shown in Figs. 1, 2, and 3.

7 Days post-burn

Type 1 mast cells. The numbers of type 1 mast cells in the nitrofurazone-treated, 1.2 J/cm² laser-treated, and 2.4 J/cm² laser-treated groups were significantly higher than that of the control group (LSD test: p = 0.003, p = 0.021, and p = 0.031, respectively).

Types 2 and 3 and the total number of mast cells. The numbers of type 2 and 3 mast cells, as well as the total number of mast cells, were higher in the 1.2 J/cm² laser-treated group than in the other groups. However, there were no significant differences in the numbers of type 2 and 3 mast cells, or in the total number of mast cells, between the studied groups.

16 Days post-burn

Type 1 mast cells. The numbers of type 1 mast cells in the 2.4 J/cm² laser-treated and sham-exposed groups were significantly higher than that of the control group (LSD test: p = 0.004 and p = 0.01, respectively).

Type 2 and total number of mast cells. The number of type 2 and the total number of mast cells in the 2.4 J/cm² laser-treated group were higher than those of the other groups. However, there were no statistically significant differences between the study groups.

Type 3 mast cells. The number of type 3 mast cells was higher in the nitrofurazone-treated group than in any of the other study groups. However, there were no statistically significant differences between the study groups.

30 Days post-burn

Type 1 and 2 mast cells. The numbers of type 1 and 2 mast cells in the sham-exposed group were higher than in any of the other study groups. There were no statistically significant differences in the numbers of type 1 mast cells between the study groups. The number of type 2 mast cells in the sham-exposed group was significantly higher than in the control group (LSD test: p = 0.029).

Type 3 mast cells. The number of type 3 mast cells in the sham-exposed group was significantly higher than in the 1.2 J/cm² laser-treated, the 2.4 J/cm² laser-treated, the nitrofurazone-treated, and the control groups (LSD test: p = 0.04, p = 0.004, p = 0.032, and p = 0.019, respectively).

Total number of mast cells. The total number of mast cells in the sham-exposed group was significantly higher than in the control group (LSD test: p = 0.005). The total numbers of mast
posed group at 16 and 30 days post-burn. The results of less in the laser-treated groups compared to the sham-exposed and the depth of new epidermal growth were significantly lower in the laser-treated groups on the seventh and the 16th day post-burn. The number of macrophages, fibroblasts, and mast cells in both laser-treated groups and in the nitrofurazone-treated group were lower than those of the sham-exposed and control groups. However, there were no statistically significant differences between the study groups.

**DISCUSSION**

Many of the components of mast cell granules may be involved in the body’s response to injury. The induction of mast cell degranulation in injured tissue and the consequent release of their components may significantly affect the rate of repair. However, in our study there were significantly more type 1 mast cells in the sham-exposed group was significantly higher than that of the 1.2 J/cm² laser-treated, 2.4 J/cm² laser-treated, nitrofurazone-treated, or control groups (LSD test: $p = 0.004$, $p = 0.002$, and $p = 0.019$, respectively). The total number of mast cells in the sham-exposed group was significantly higher than that of the control group (LSD test: $p = 0.005$).

In previous research, we have studied the effects of the same LLLT regimen on the healing of second-degree burns, and we found that both the number of macrophages and the depth of new epidermal growth were significantly less in the laser-treated groups compared to the sham-exposed group at 16 and 30 days post-burn. The results of the present study are in agreement with those of that study. It appears that the optimal energy density and wavelength of the laser energy used in this study were not suitable for effective LLLT on the few macrophages, fibroblasts, and mast cells, or on the capillaries and blood vessels remaining in injured tissue.

Mast cells are known to accumulate in healing wounds, and in the present study the total number of mast cells in the sham-exposed group 30 days post-burn was significantly higher than in the control group. However, we also found at 30 days post-burn that LLLT at the two energy densities we used did not increase the total number of mast cells compared to the control group. The ability of LLLT to decrease the total number of mast cells may be related to the reduction of either proliferation or migration of these cells, and may prove to be a valuable therapeutic technique. Increased numbers of mast cells are associated with a variety of pathological skin conditions in humans, including fibrotic disorders such as hypertrophic scars and keloids. The majority of scar reactions produce an appropriate volume of collagen to fill the dermal defect, but excessive scar formation is a common sequela of abnormal wound healing in both traumatic and surgical injuries. These fibrotic lesions reportedly exhibit as much as 10 to 100 times more mast cells than normal human skin. However, the mechanism for the proliferation or recruitment of mast cells in fibrotic lesions has not yet been elucidated. Sasaki et al. studied incisional wounds in rats that were divided into two groups that were killed 7 or 14 days post-wounding. The wounds were treated with charged cross-linked diethylaminoethyl dextran (CLDD) beads. At the designated times post-wounding, biopsy specimens were tested for wound-breaking strength, or processed for histological testing and counting of mast cells. They found significant increases in the number of mast cells and wound-breaking strength after implantation of the CLDD beads. Sasaki et al. concluded that these findings are clinically germane to the assessment of proposed therapeutic applications of CLDD beads for a variety of impaired wound healing states. Furthermore, if an increased mast cell population is intimately linked to hypertrophic scar and keloid formation, the results of their study suggest that CLDD bead therapy for cutaneous wounds may lead to improved wound healing in humans.

The results of this study are in agreement with findings of study by Al-Watban and Gonzaga. They made one burn on back of each rat. Rats were divided into one control and four treatment groups. Treatments were given at actual doses of 0.201, 1.007, and 4.028 J/cm² using 670 nm diode laser. They found treatment with 0.201, 1.007, and 4.028 J/cm² produced healing deceleration of 26.52%, 16.69%, and 9.87%, respectively. While most of treatments exacerbated the normal healing rate, 2.014 J/cm² gave 5.43% acceleration. However, no significant difference on healing rates was noted between control and treatments groups. Al-Watban and Gonzaga concluded that burn healing stimulation with low-power diode laser appeared to be dose dependent, where a minimal stimulating effect was discerned from 2.014 J/cm². Although it did not apparently hasten the healing process on normal rats, it demonstrated its role on the cosmetic aspect of healing when less scar tissue formation was observed on laser-treated group.

**CONCLUSION**

Low-level laser therapy of deep second-degree cutaneous burns in rats significantly increased the number of intact mast cells during the inflammatory and proliferative phases of healing, and decreased the total number of mast cells during the re-
modeling phase. These findings may have significance in the future treatment of wound healing in humans, as well as in reducing the formation of excessive fibrotic tissue in scars and keloids.

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