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The effect of low-level helium-neon laser on oral wound healing

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ABSTRACT

Background: The effectiveness of low power lasers on incisional wound healing, because of conflicting results of previous studies, is uncertain. Therefore, the aim of this study was to evaluate the effects of low-level helium-neon (He-Ne) laser irradiation on wound healing in rat's oral mucosa.
Materials and Methods: Sixty-four standardized incisions were carried out on the buccal mucosa of 32 male Wistar divided into four groups of eight animals each. Each rat received two incisions on the opposite sides of the buccal mucosa by a steel scalpel. On the right side (test side), a He-Ne laser (632 nm) was employed on the incision for 40 s. Laser radiation was used just in 1st day, 1st and 2nd day, 1st and 3rd day, and continuous 3 days in groups of A, B, C, and D of rats, respectively. The left side (control side) did not receive any laser. Histological processing and hematoxylin and eosin staining were done on tissue samples after 5 days. Wilcoxon and Kruskal-Wallis tests were used for statistical analysis.
Results: Histological analysis showed that the tissue healing after continuous 3 days on the laser irradiated side was better than the control side, but there was no difference between the two sides in each groups ($P > 0.05$).
Conclusion: This study showed that He-Ne laser had no beneficial effects on incisional oral wound healing particularly in 5 days after laser therapy. Future research in the field of laser effects on oral wound healing in human is recommended.

Key Words: Lasers, Helium, Histological Technique, Low-level laser therapies, oral, wound healings

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INTRODUCTION

The process of tissue healing is very complex and involves vascular and cellular changes, epithelial proliferation, fibroblast proliferation, synthesis and deposition of collagen, production of elastin and proteoglycans, revascularization, and wound contraction.[1-3] The incorporation of laser as a therapeutic tool in the biomedical field has been investigated since 1960 but, in spite of the numerous studies on the effects of laser therapy, it is difficult to justify physical variables such as application technique, dosages, depth, modes, and duration of exposure.[4] It has been observed that photostimulation influences the macrophage production of growth factors, which increases cell proliferation.[5,6] In 1976, Mester *et al*.[7] reported that low-level helium-neon (He-Ne) laser could aid the healing of mechanical injuries. Since then, it was shown that this laser has several effects on living tissue, effects known as laser biostimulation.

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Laser photobiomodulation has been increasingly used with the purpose of improving the quality of wound healing.[8] The therapeutic effects of laser on the different biological types are in a broad range and include trophic-regenerative, anti-inflammatory, and analgesic effects.[1,9] It has also been demonstrated that tissue regeneration becomes more effective when treated with low-level laser.[10-13] There are reports that laser irradiation stimulates the release of fibroblast growth factor and the replication of these cells.[14,15] Irradiation with He-Ne laser would accelerate the healing process, with a better weave of collagen fibers[1,4,16,17] and greater collagen deposition,[18] combined with faster re-epithelialization and neovascularization.[19,20]

In situations of deficient healing, such as ischemia, diabetes, and pressure ulcers, irradiation with a low-level laser could be an alternative for the recovery.[21,22] The objective of the present study was to evaluate the effects of low-level He-Ne laser on the healing process of oral mucosal wounds in rats.

MATERIALS AND METHODS

The animal experimental program was performed under a licensing agreement with the permission of the animal use committee at the School of Dentistry, Rafsnjan University of Medical Sciences, Rafsanjan, Iran.

Thirty-two 1-2-year-old Wistar male white rats were kept in metal cages at room temperature with 12 h of light per day and 37% relative humidity. They received a standard laboratory diet and water ad libitum. Before the experimental procedures, the rats were randomly divided into four groups of eight rats each. After weighing, each animal received an anesthetic injection of 10% ketamine (80 mg/kg) and 1% acepromazine (2.5 mg/kg). After anesthesia, the intraoral surgical field together with the hand piece and fiber of the laser device were sterilized with Betadine solution (Behsa, Arak, Iran). Two parallel incisions by steel scalpel (Bard-Parker number 15) and approximately 10 mm in length were performed in the buccal mucosa of each rat. The buccal mucosa was selected for the oral wounds because of its accessibility. The incisions were not sutured. When we were used laser radiation for each incision, we were covered other incision on the buccal cavity by the steel spatula. The device laser He-Ne Plasmax IV, LHN 9709 (KLD Biossistemas®) was used. The wounds were treated with He-Ne laser at energy density of 1 J/cm^2, the maximal continuous energy level of 5 mW, with the wavelength of 632.8 nm and laser beam area of 0.2 cm^2 that was employed for 40 s. The laser tip beam was kept perpendicular to the irradiated tissue surface. Laser radiation was used just in a 1st day, 1st and 2nd day, 1st and 3rd day, and continuous 3 days in groups of A, B, C, and D of rats, respectively. All of the surgical procedures were performed by the same operator under aseptic conditions. The rats were then returned to their cages, without limitations of activity. To prevent postsurgical infections, antibiotics (penicillin 0.5 mL intramuscular) were administered on the day of the surgical procedure.

The rats were killed by euthanasia (high concentration of CO_2 in air) at intervals of 5 days after the surgical procedure for four groups. Specimens measuring approximately 5 mm × 10 mm were then removed from the control (left) and test (right) sides of each animal. The histological sections were stained with hematoxylin-eosin (H and E) by standard procedures. Briefly, a sample was excised from the mucosal bed of each mouse and was fixed in buffered formalin at 10% for 24 h and later submitted to the routine histological procedure. Through the H and E staining, the general morphological evaluation of the wound was obtained, and the inflammatory pattern was recognized. Ten fields were viewed at ×400 magnification, according to the guidelines described by Vizzotto.[23] For the cell count, the following scale was adopted: No cell = 1; up to 50 cells = 2; 50-100 cells = 3 and more than 100 cells = 4, positive for monomorphonuclear cells and negative for polymorphonuclear cells After the attribution of the indices, their total was calculated so that each group of animals had a final score for classification into three phases of the inflammatory process.[23]

Histological results were displayed as healing grade and processed for Wilcoxon analysis. Kruskal–Wallis tests were used to determine the effect of the groups and the evaluation days on the study variables. A probability of a null hypothesis of <5% ($P < 0.05$) was regarded as statistically significant.

RESULTS

Healing grades in laser-irradiated site in four groups of rats were as follow: A=2, B=2, C=3 and D=4 while these grades in no laser-irradiated site were A=1, B=2, C=3 and D=3. Histological analysis showed that the tissue healing after continuous 3 days on the laser irradiated side was better than the control side, but there was no difference between the two sides in each group. The difference between all groups was not statistically significant ($P > 0.05$).

While the number of inflammatory cells in laser radiation group was lower than the control [Figures 1 and 2] and plumped fibroblast [Figure 3] were very evident in the laser radiated specimens ($P < 0.05$), but there was no significant differences between laser radiated and control group in each divided rats ($P > 0.05$).

DISCUSSION

Open wounds have lost the barrier that protects tissues from bacterial invasion and allow for the escape of vital fluids. Wound healing and tissue repair are complex processes that involve a dynamic series of events including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis, and tissue remodeling.[24]

In recent years, low-intensity laser therapy has gained considerable recognition and importance among treatment modalities for various medical problems including wound repair processes, musculo-skeletal complications, and pain control.[25-28] Clinical studies have shown low energy lasers to be effective as analgesics and to accelerate the healing of injured tissue.[29]

Several studies have been done on low-level laser therapy (LLLT); however, conflicting results and little research on the mouth was an incentive for us to do this research. A Ne-He laser was used in this study, and the results showed those 5 days after the therapy, healing was better on the side receiving laser radiation. This result was similar to the results of some previous studies,[3,25,30,31] although the results were in contrast to many other studies.[4,32] In the previous studies, researchers believed that the differences between fluency energy level in tissues,[3,33] frequency of radiation,[34] systemic effect,[35] and the type of ulcer[36] would influence the results of low level laser exposures and generate conflicting results.

Ghalayani *et al*.[35] assessment the effect of diode laser therapy on incisional wound healing and expression of iNOS and eNOS on rat oral tissue. Results showed that the tissue healing after 7 days on the laser-irradiated side was better than the control side, but there was no significant difference between the two sides on the days 2, 14, and 21 after surgery. The difference in the amounts of iNOS between the groups was significant; it was more in the laser-irradiated

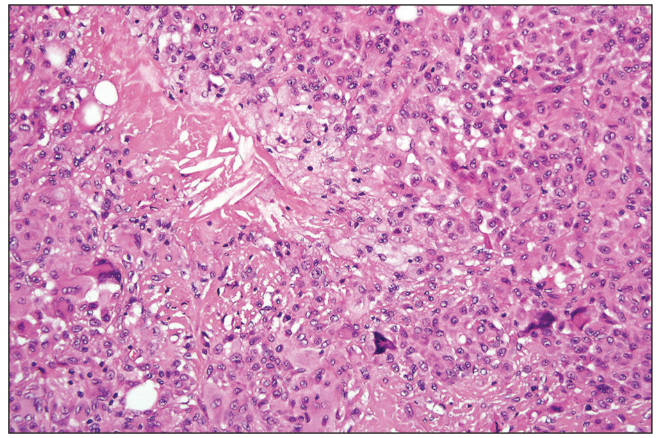

Figure 1: Laser therapy after 5 days in the nonradiated group showing smaller fibroblast and more inflammatory cells.

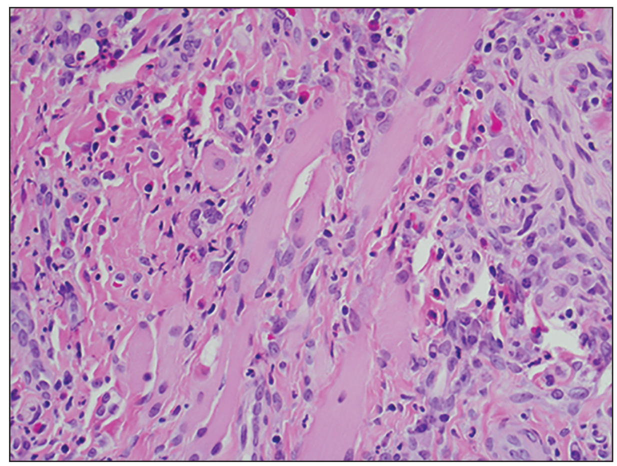

Figure 2: Laser therapy after 5 days in radiated group showing a decreasing number of inflammatory cells and enlarged fibroblasts.

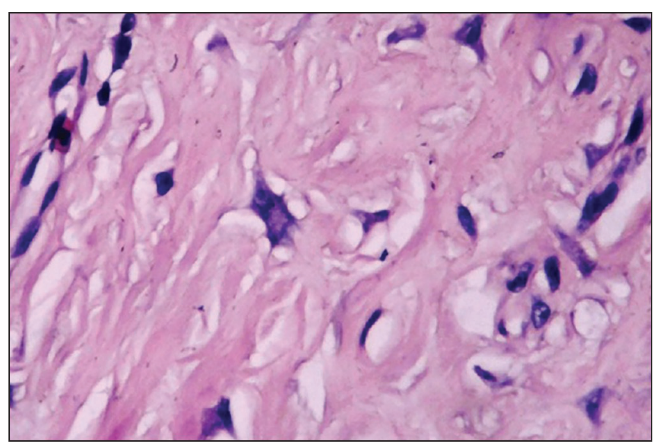

Figure 3: Higher magnification of plumped fibroblasts.

side than the control side.[35]

Vinck *et al*.[37] carried out a cell culture study to observe the influence of diode light emission and LLLT in the process of wound healing, in which cultures of fibroblasts obtained from 8-day-old chicken embryos were treated for 3 consecutive days. Infrared laser gallium-aluminum arsenate was applied with three wavelengths emitted separately. All three applications covered an area of 18 cm^2 and used a frequency of 0–1, 500 Hz, at an application distance of 0.6 cm. LLLT was used with the following parameters: One 5 s emission with a peak potency of 40 mW resulting in an exposure of 1 J/cm^2. Infrared, the light spectrum with red light, has a radiation exposure of 0.53 J/cm^2. Green light emits 0.1 Jcm^2, corresponding to an exposure time of 1, 2, and 3 min, respectively, with peak potencies equivalent to 160, 80, and 10 mW, respectively. Statistical analysis showed a high proliferation of fibroblasts *in vitro*. Therefore, this study presupposed the possibility of stimulatory effects on *in vivo* wound healing when treated with LLLT. Demir *et al*.[38] investigated the effects of electrical stimulation (ES) and LLLT in wound healing in an experimental study using Swiss-albino female rats which were divided into four groups of 30 animals each. A 6 cm linear incision was made in the dorsal region of each rat. Results showed ES and laser treatments have positive effects on inflammation, proliferation, and maturation of wounds and can be successfully used for decubitus ulcers and chronic wounds.

An experimental study conducted by Khadra[39] observed the effects of gallium-aluminum arsenate lasers, a diode laser, on bone healing process in 20 rats with 2.7 mm diameter bone defects in the parietal region treated for 4 weeks. On the 28th day, the animals were euthanized for histological assessment of bone defects. There was a significant increase in calcium, phosphorus, and protein. Similarly, histological analyses showed a marked growth of angiogenesis and connective tissue. They concluded, therefore, that LLLT can favor bone formation in rats with bone defects.

In the present study, the test of choice for assessing the healing grade was histological observation. The healing was compared in different times after the laser therapy. In Group D (3 continuous days of laser therapy), there was histologically better healing on the irradiated side compared to the control side. Improvement in the healing in Group D compared to the other groups on the laser irradiated side could be due to the fact that in the other groups, the rats did not receive enough laser radiation and according to the high rate of repair in rats, there was complete healing in Groups C and D, and there was no difference between the groups. This may explain that use of laser in contentious days was noticed and increase the proliferative rate of fibroblast. However, other studies should be done in human samples that the amount of radiation, radiation time, area to be determined. This study was consistent with the researches of Parirokh, *et al*.,[3] and Rezende, *et al*.[40]

Results showed that fibroblast proliferation was significantly more evident in the laser radiated than the control group in 5 days interval which was in agreement with previous studies in which low power laser beneficial effects were demonstrated.[33,41] To ensure the effects of low-level laser therapy on the healing process, there is still a need for consensus on the standards for the physical variables: Application times and techniques, energy densities, output powers, and wavelengths. The comparison of the results of several authors has been hindered by their use of different methodologies. The use of He-Ne laser (632.8 nm), applied with different densities can lead to different cellular responses, and this may preclude comparisons. Hawkins and Abrahamse[42] applied doses of 0.5, 2.5, 5, 10, and 16 J/cm^2 to human skin fibroblasts on 2 consecutive days and found that 5 J/cm^2 stimulated mitochondrial activity, cell proliferation, and fibroblast migration. However, higher doses decreased cell viability and proliferation and damaged the cell membrane and DNA.[42]

CONCLUSION

This study showed that He-Ne laser has had beneficial effects on incisional wound healing. However this result is not significant statistically. More research with the high sample on acute and chronic ulcers is recommended.

Ethical approval

The presented study was conducted in accordance with Helsinki Declaration (1974) and recommendation of the college of Veterinary, Kerman University, Kerman, Iran, an entity as associated with the International Council for Laboratory Animal Sciences. This project was approved by the Animal Research Ethics Committee of Rafsanjan University of Medical Sciences under the protocol number 418.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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