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The effect of He–Ne and Ga–Al–As lasers on the healing of oral mucosa in diabetic mice

Farahnaz Fahimipour [a,b,*], Behzad Houshmand [c], Parvin Alemi [d], Mohammad Asnaashari [e], Mahmoud Akhavan Tafti [f], Fatemeh Akhoundikharanagh [d], Seyed Emadeddin Najafi Farashah [d], Mohammad Aminisharifabad [g], Aghdas Setoudehnia Korani [d], Mina Mahdian [h], Farshid Bastami [d], Mohammadreza Tahriri [b]

[a] Dental Biomaterials Department, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Developmental Sciences, Marquette University School of Dentistry, Milwaukee, WI 53233, USA
[c] Department of Periodontics, School of Dentistry, Shahid Beheshti University of Medical Science, Tehran, Iran
[d] Dental Research Center, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Department of Endodontic, School of Dentistry, Shahid Beheshti University of Medical Science, Tehran, Iran
[f] Department of Pathology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[g] Department of Computer Science, Wayne State University, Detroit, MI, USA
[h] Department of Oral health and Diagnostic Sciences, School of Dental Medicine, University of Connecticut, Farmington, CT, USA

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ABSTRACT

Delayed wound healing is one of the complications of diabetes mellitus. Low-level laser therapy (LLLT) has been used to accelerate wound healing however the effect of LLLT on the hard palate wound healing in streptozotocin-induced diabetic (STZ-D) mice has not yet been characterized. This study aims to determine the effect of LLLT (He–Ne and Ga–Al–As laser) on the process of wound healing in the hard palate among diabetic and non-diabetic mice. 90 adult male mice were divided into six groups. Type 1 diabetes mellitus was induced in three groups by means of injection of STZ. Of these, one group was irradiated with He–Ne laser (DH group), one with Ga–Al–As laser (DG group) and one did not undergo any LLLT (DC group). The remaining groups were non-diabetic which were allotted to laser therapy with He–Ne laser (NH group) or with Ga–Al–As laser (NG group) or no LLLT (NC group). Five animals from each group were killed on the third, seventh, and fourteenth days after surgery, and biopsies were made for histological analysis. On the 3rd and 7th days after the surgery, the number of polymorphonuclear (PMN) cells in NH, DH, NG, and DG groups was significantly lower than that of the control groups. On the 3rd, 7th and 14th days, the fibroblasts and new blood vessel counts and collagen fibers in diabetic laser treated groups (DG and DH) were significantly higher compared to that of NC, DC, NH and NG groups. On the 7th and 14th days, the fibroblasts and new blood vessel counts and collagen fibers in NH, DH, NG, and DG groups were also significantly higher than that of the control groups, and the fibroblast and new blood vessel counts and collagen density fibers in NH and DH groups were higher than that of the NG and DG groups. LLLT with He–Ne laser compared to Ga–Al–As laser has a positive healing effect on hard palate gingival wounds in STZ-D mice.

1. Introduction

Diabetes is one of the most common diseases in the world [1]. The incidence of this condition is increasing every year with a substantial morbidity and mortality rate. Delayed wound healing is one of the complications in diabetic patients resulting in 15% more amputation than normal population [2].

Wound healing is a complex process that involves a variety of cells, mediators and chemokines and various mechanisms such as fibroblast proliferation, angiogenesis, collagen rearrangement and tissue contraction. Hard palate ulcers can occur due to a variety of reasons including trauma, surgical wound following tooth extraction or tumor resection [3,4].

In the inflammatory phase, inflammatory cells migrate to the wound area followed by the proliferative phase in which the number of fibroblasts and macrophages increases while acute inflammatory reactants decrease. During the final phase of wound healing, fibroblasts mediate the granulation tissue formation via secretion of extra-cellular matrix and collagen the process of tissue reformation occurs fibroblasts help tissue to reformation extra cellular matrix and collagen which lead to create granulation tissue that perfuse with new formed vessels [5].

* Corresponding author at: Dental Biomaterials Department, School of Dentistry, Tehran, University of Medical Sciences, Tehran, Iran.

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Table 1
The specification of the lasers has been used in this research.

Parameters of the lasers	He–Ne	Ga–Al–As
Peak power	5 mW	25 mW
Wavelength	632.8 nm	830 nm
Spot shape	Circular	Circular
Spot size	0.02 cm^2	0.1 cm^2
Frequency	Continuous	Continuous
Exposure time	16 s	16 s
Energy density	4 J/cm^2	4 J/cm^2

Low level laser therapy (LLLT) is a therapeutic method which was initially introduced by Mester [6]. It has been shown to improve wound healing; however, there is controversy in the literature regarding the wound healing efficacy of red and infra-red laser in human tissues [6].

The first laser that was used for this purpose was helium–neon (He–Ne) laser which is based on inert gas. After that, semiconductor diode lasers such as gallium–aluminum–arsenide (Ga–Al–As) were used [7]. A review of literature has shown that although the effects of LLLT on hard palate wound healing have been thoroughly studied in non-diabetic animals [8], the effect of LLLT on the hard palate wound healing in STZ-D mice has not yet been characterized. The results of such studies will assist the clinician in order to render the most efficient and effective treatment for ulcerated areas in the hard palate in diabetic patients. This study aims to determine the effect of LLLT (He–Ne and Ga–Al–As laser) on the process of wound healing in the hard palate among diabetic and non-diabetic mice.

2. Materials and Methods

2.1. Animals

This was a randomized controlled trial approved by the ethics committee of Iran center for dental research, Shahid Beheshti University of Medical Science, Tehran, Iran. The study consisted of ninety adult male albino mice with an average weight of 60 ± 1 g. The mice were housed in air-conditioned room with 22 c temperature and diurnal cycle (12 h light/12 h dark).

The mice were randomly divided to six groups; NC (non-diabetic, control), NH (non-diabetic, He–Ne laser), NG (non-diabetic, Ga–Al–As laser), DC (diabetic, control), DH (diabetic, He–Ne laser) and DG (diabetic, Ga–Al–As laser).

3. Induction of Type I Diabetes Mellitus (DM)

Type I DM was induced in groups DC, DH and DG via administration of 55 mg/kg of body weight pancreatin β-cell STZ (Zanosar Pharmacia and Upjohn Co., Kalamazoo, MI, USA) through intraperitoneal injection. Non-diabetic mice received control injections of distilled water. Presence of diabetes was confirmed by measuring blood glucose level after 7 days post-injection, and the mice with glucose levels above 250 mg/dl in a distal tail small injury sample were included in the study [9].

4. Wound Creation

The wound model was identical to that of D'Arcangelo's study [10]. Mice were anesthetized with intramuscular injection of ketamine hydrochloride (40 mg/kg) and diazepam (4 mg/kg) and then hard palate was disinfected with iodine solution. Subsequently, an incision (4 mm long and 2 mm deep) was made in the median raphe, 2 mm posterior to the lingual surface of incisor teeth using a scalpel number 15. Postoperative antibiotic was administered to the mice for 4 days.

5. Laser Therapy Application

In this study two types of lasers were used; helium neon laser (He–Ne) with wavelength 632.8 nm, peak power 5 mW, and spot size 0.02 cm^2 and gallium–aluminum–arsenide laser (Ga–Al–As) with wavelength 830 nm, peak power 25 mW, and spot size 0.10 cm^2(low-level laser apparatus). It is worth mentioning that the specification of the lasers has been used in this research are summarized in Table 1.

Lasers were applied to two areas of the wound at a 1 cm distance and perpendicular to it. Application time and dose of laser irradiation were 16 s and 4 J/cm^2 respectively [9]. NH and DH groups were irradiated with He–Ne laser and NG and DG groups with Ga–Al–As laser. The NC and DC groups did not receive any laser therapy. The four experimental groups received the first dose of irradiation immediately after the surgeries and were subsequently irradiated once a day every day following the surgery, identically.

6. Histologic Procedures

Five mice from each group were sacrificed on the 3rd, 7th, and 14th postoperative days using chloroform in a closed space and prepared for histologic analysis. The samples were fixed in 10% formalin (Merck, Darmstadt, Germany) for 24 h, dehydrated in gradate ethyl alcohol

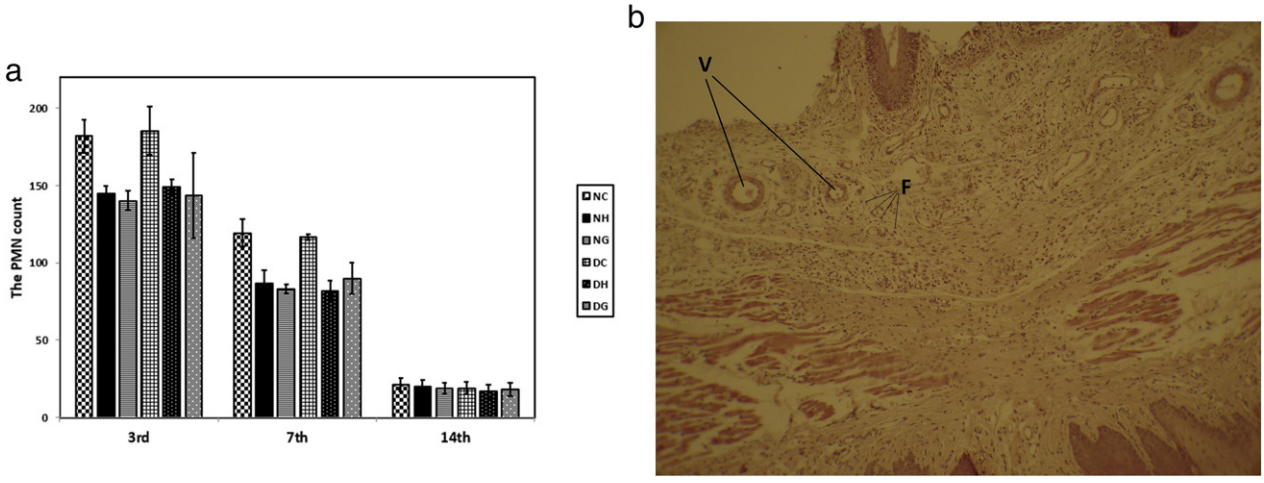

Fig. 1. (a) Mean ± SEM number of PMN cells on the 3rd, 7th, and 14th days post-operation and (b) a photomicrograph of oral mucosa in the control group on day 14 after surgery. On the 14th day, inflammatory cells could hardly be observed (F: fibroblast and V: blood vessel).