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ORIGINAL ARTICLE

Effect of low-intensity laser therapy on mast cell degranulation in human oral mucosa

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Abstract Little is known about the physiological mechanisms related to low-intensity laser therapy (LILT), particularly in acute inflammation and subsequent wound healing. The objective of this study was to verify the effect of LILT on mast cell degranulation. Epulis fissuratum tissues from eight patients were used. One part of the lesion was irradiated with an AsGaAl laser (λ= 670 nm, 8.0 J/cm^2, 5 mW, 4 min). The other part was not irradiated. Then, the specimens were immediately removed, fixed and examined by light microscopy. The number of mast cells was similar in laser-treated samples when compared with non-irradiated specimens. The degranulation indexes of the mast cells observed in the irradiated samples were significantly higher than those of controls ($P<0.05$). LILT with the parameters used increased the number of degranulated mast cells in oral mucosa.

Keywords Laser · Mast cell · Mast cell degranulation

This work was carried out at the University of São Paulo and the Energetic and Nuclear Research Institute (IPEN), São Paulo, Brazil.

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Introduction

Low-intensity laser therapy (LILT), first described by Mester [1], has been reported to be useful in the field of wound healing. It is supposed to be a non-invasive, painless and athermal therapy, which restores functional ability, based on biological estimulative–regenerative, anti-inflammatory and analgesic effects.

Vasodilation is a LILT effect important to improve the wound healing process [2]. Wound healing begins with acute inflammation. In this process, vasodilation is followed by increased vascular permeability. One substance that elicits vascular permeability is histamine, which is an inflammatory mediator released by mast cells present in mucosal and connective tissue [3]. Thus, mast cell degranulation is an important step in wound healing. It is known that LILT can increase mast cell degranulation in animals; however, no information on humans is available. This is probably due to the ethical issues related to the removal of human tissue for histological analysis. Therefore, we developed a research model, where inflamed oral mucosa can be irradiated and immediately removed for histological analysis with no ethical inference. As this model we used inflamed tissues from the oral mucosa, epulis fissuratum, which is an inflammatory oral mucosa lesion the treatment of which is based on surgical removal. Little is known about the physiological mechanisms related to LILT, particularly in acute inflammation and subsequent wound healing. The objective of our study was to verify the effect of LILT on mast cell degranulation in human epulis fissuratum lesions.

Material and methods

Epulis fissuratum tissues from eight patients of the Centro Odontológico Universitário do Norte do Paraná were used, after approval had been obtained from the University of Londrina Ethics Committee.

The patients were selected based on the following features:

a. Aged between 30 years and 60 years
b. Absence of systemic diseases
c. Wearer of ill-fitting complete dentures
d. Presence of epulis fissuratum bigger than 3.0 cm located at the superior or inferior alveolar vestibule
e. No melanic pigmentation of the oral mucosal
f. Not pregnant or breastfeeding

Surgical procedures

The patients were advised not to wear the ill-fitting dentures for 2 weeks before the surgical procedures were to be performed, in order to reduce the inflammation at the lesions. Antisepsis of the oral mucosa with 0.12% chlorhexidine solution was accomplished, and local anesthesia with mepivacaine 2% with levonordefrin 1:20.000 (DFL Indústria e comércio LTDA., Rio de Janeiro, RJ, Brazil) was induced.

Each lesion was divided into two halves, and each part was treated in a random fashion as follows:

Group 1 Control: half of the lesion was removed with a scalpel and immediately fixed in 10% neutral formalin solution.

Group 2 Laser: before removal of the other half, the tissue was irradiated according to the settings listed in Table 1. Immediately after laser irradiation, this part of the lesion was removed by the same process as that described for group 1.

At the end of the surgical procedures the oral mucosa was sutured.

The irradiation was done using all security procedures, such as the use of eye protection for the patients and operators. The Laser Check power meter (Coherent, Inc., Santa Clara, CA, USA) verified the output of our equipment.

Light microscopy

For light microscopy, the specimens were immediately fixed in 10% neutral formalin for 12 h, embedded in paraffin, and cut into 5 μm sections. The sections were stained with hematoxylin and eosin (HE) for diagnostic purposes and with Giemsa stain to identify the mast cells.

Table 1 Laser system and parameters used

Laser	AsGaAl
Device	Kromen (Dentoflex Materiais Odontológicos, São Paulo, Brazil)
Wavelength (nm)	670
Power output (mW)	15
Mode	Continuous
Energy density (J/cm^2)	8.0
Mode of application	Contact
Irradiated area (mm^2)	5
Exposure time (min)	4

Giemsa stain is ordinarily used to identify degranulated mast cell [4].

For each histological section, five consecutive histological fields of ×40 magnification were selected for the analysis. These fields were located immediately below the epithelium, in the connective tissue of the oral mucosa. Each field was then observed at ×400 (Fig. 1a) to locate the mast cells for counting. Then, we checked the degranulated (Fig. 1b) and non-degranulated (Fig. 1c) status of the mast cells, at ×1,000 magnification.

We obtained the degranulation index by dividing the number of degranulated mast cells by the total number of mast cells found in the five fields per specimen.

Statistical analysis

A mean ± standard deviation was obtained per group. The data were compared by the Kruskal–Wallis test complemented by Dunn's test. The level of significance was 5 % ($P \leq 0.05$).

Results

Mast cells were observed in all samples. The total number of mast cells (degranulated and non-degranulated) is presented in Fig. 2. This number was similar in samples of laser-treated oral mucosa and in samples of the control group ($P > 0.05$). Degranulation of mast cells was observed in each sample. The degranulation index of the two groups is graphically expressed in Fig. 3. The degranulation index of the mast cells observed in the irradiated specimens was significantly higher than the index observed in the control group ($P < 0.05$).

Discussion

The epulis fissuratum is a tumor-like hyperplasia of fibrous connective tissue that develops in association with the flange of an ill-fitting complete or partial denture and is

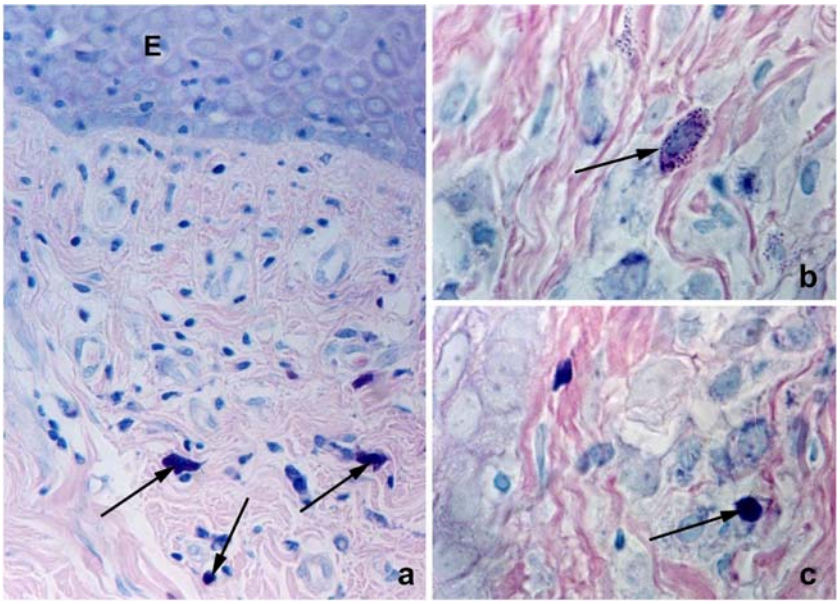

Fig. 1 Photomicrographs of the oral mucosa. **a** Fibrous connective tissue covered by stratified squamous epithelium (*E*). Mast cells are indicated by *arrows*. **b** A degranulated mast cell (*arrow*). **c** A non-degranulated mast cell (*arrow*). **a** Giemsa, ×400; **b**,**c** Giemsa, ×1,000

treated by total surgical removal [5]. Thus, this human tissue is ethically suitable for studies that need histological analysis. For this reason we decided to use this tissue for studying the effect of low-intensity laser therapy (LILT) on mast cell degranulation in human oral mucosa. After laser irradiation of the oral mucosa, it was possible to observe a significant increase in the degranulation index of the mast cells when compared with that of the mast cells in non-irradiated tissue in the same lesion.

Mast cell degranulation may be triggered by a variety of stimuli, which have been linked indirectly with inflammation in the oral cavity. These include IgE, neuropeptides, trauma, and drugs [5, 6]. Histamine increases vascular permeability via structural changes, which include endothelial contraction and intercellular gap formation [7]. In addition, histamine promotes leukocyte adhesion to endothelium via transient mobilization of the adhesion molecule P-selectin (also known as CD62-P or GMP140) to the endothelial surface [8].

Our study showed that the numbers of mast cells were similar in lased and non-lased tissue, probably due to the fact that the time lapse between the laser irradiation and the removal of the lesion was very short (minutes). In fact, El-Sayed and Dyson [9, 10], working with animals, showed that, 2 h after laser irradiation, the total number of mast cells had increased significantly. If mast cells do not proliferate

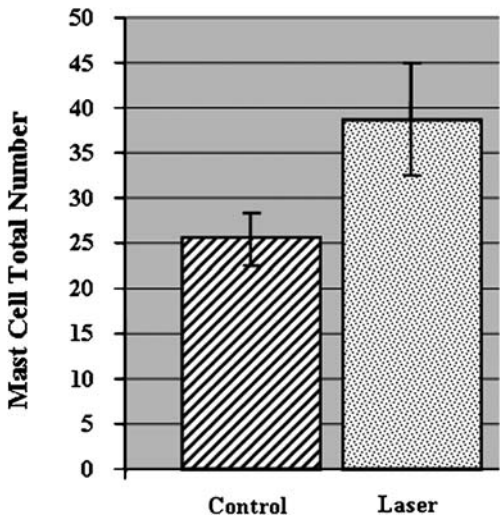

Fig. 2 Graphic representation of the total number of mast cells in the two experimental groups, *Control* (non-irradiated oral mucosa) and *Laser* (irradiated oral mucosa). There are no statistical differences between the groups

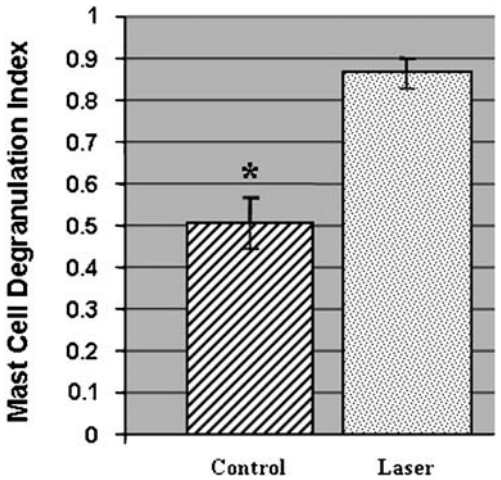

Fig. 3 Graphic representation of the degranulation indexes of the mast cells in the two experimental groups. The degranulation index of the non-irradiated group (*Control*) is significantly smaller than the index of the lased group (*asterisk*)

locally, it can be suggested that laser irradiation promotes a process of directed migration of mast cells. In our study, it was not possible to wait until 2 h after irradiation because it would not be ethical for us to keep the patients for such a time before the surgical removal of their oral lesions.

In relation to mast cell degranulation, the indexes observed in the irradiated specimens were significantly higher than those in the control group. This increase should have an impact on the inflammatory process, because preformed mediators released after degranulation of mast cells can serve to promote inflammation via a multiplicity of actions. In fact, according to Karu [11], the increase in mast cell degranulation can be related to an increase in ATP production, stimulated by LILT.

Other authors have also observed increases in the degranulation indexes of mast cells; however, our study is the first one performed with human tissue [10, 12, 13]. El-Sayed and Dyson [10] showed an increase of this index in the irradiated injured skin of rats that was mostly related to the repetition rate of the irradiation.

Low-intensity laser therapy, under the conditions used in this study, promotes human mast cell degranulation in significant amounts and, therefore, may increase the inflammation process. This suggests that laser may be a therapeutic agent, targeting the mobilization and secretion of mast cells. However, more studies using different LILT parameters must be done to elucidate further the effects of this therapy on mast cell degranulation and on other important cells of the inflammatory process.

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References

1. Mester E (1966) The use of the laser beam in therapy. Orv Hetil 107:1012–1016
2. Schindl A, Schindl M, Schon H, Knobler R, Havelec L, Schindl L (1998) Low-intensity laser irradiation improves skin circulation in patients with diabetic microangiopathy. Diabetes Care 21:580–584
3. Walsh LJ, Kaminer MS, Lazarus GS, Lavker RM, Murphy GF (1991) Role of laminin in localization of human dermal mast cells. Lab Invest 65:433–440
4. Montagna W, Carlisle KS, Brenner RM (1987) Histological techniques for the study of granular cells during wound healing. Am J Dermatopathol 9:26–29
5. Neville BW (2002) Oral and maxillofacial pathology. Saunders, Philadelphia
6. Walsh LJ, Savage NW, Ishii T, Seymour GJ (1990) Immunopathogenesis of oral lichen planus. J Oral Pathol Med 19:389–396
7. Majno G, Palade GE, Schoefl GI (1961) Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: a topographic study. J Biophys Biochem Cytol 11:607–626
8. Johnson GI, Cook RG, McEver RP (1989) Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. Cell 56:1033–1044
9. El-Sayed SO, Dyson M (1990) Comparison of the effect of multi-wavelength light produced by a cluster of semiconductor diodes and of each individual diode on mast cell number and degranulation in intact and injured skin. Lasers Surg Med 10:559–568
10. El-Sayed SO, Dyson M (1996) Effect of laser pulse repetition rate and pulse duration on mast cell number and degranulation. Lasers Surg Med 19:433–437
11. Karu TI (1992) Local pulsed heating of absorbing chromophores as a possible primary mechanism of low power laser effects. Abstract book of 3rd World Congress of the International Society for Low-Power Laser Applications in Medicine, Bologna, Italy, September 9–12
12. Tan OT, Whitaker D, Garden JM, Murphy G (1988) Pulsed dye laser (577 nm) treatment of portwine stains: ultrastructural evidence of neovascularization and mast cell degranulation in healed lesions. J Invest Dermatol 90:395–398
13. Ercocen AR, Kono T, Kikuchi Y, Kitazawa Y, Nozaki M (2003) Efficacy of the flashlamp-pumped pulsed-dye laser in nonsurgical delay of skin flaps. Dermatol Surg 29:692–699