ULTRASTRUCTURAL CHANGES OF THE MOUSE PERIODONTIUM AFTER HeNe LASER RADIATION: A TRANSMISSION ELECTRON MICROSCOPIC STUDY

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Helium-neon (HeNe) laser radiation is usually utilized in dentistry for diagnostic and therapeutic purposes, principally in Europe. No studies on the ultrastructural effects of HeNe laser irradiation of the periodontal tissues have been reported. This study shows the ultrastructural effects of HeNe laser radiation in mouse periodontium in two different conditions of irradiation. Twenty-four forty days old albino mice were irradiated with a HeNe laser source. They were divided into two groups according to dosage and time (two and six minutes of irradiation. 10.50566 J/cm² and 31.51698 J/cm² respectively). Each group was subdivided depending on the time of animal sacrifice (immediately after irradiation or 10 days later, respectively). A further 12 mice served as controls using the same handling procedures and times as the experimental groups, but without radiation. In transmission electron microscopy (T.E.M.) ultrathin sections showed epithelial alterations only in the group irradiated for six minutes, and was more manifested in the sacrificed animals ten days after the irradiation. In the low dose irradiated animals, the bone showed two osteocyte populations according to their response to radiation. The connective tissue also showed two types of fibroblast population. This study showed abnormal cellular effects after HeNe laser irradiation in healthy rat periodontium, being more predominant at higher doses, although also present at doses usually utilized in clinical practice.

Key words: electron microscopy; gingival tissues; HeNe laser irradiation

Introduction

In the past ten years, diagnostic oral applications of helium-neon (HeNe) laser have been studied.^(13,15-18,21,22) These studies are based on direct irradiation over the teeth and periodontal tissues. However, very few studies have analyzed the interaction of laser radiation with periodontal tissues, and these few have used previously diseased tissues.^(8,19) Therefore, the effects of laser radiation on healthy periodontal tissues are not known and healthy tissues are often involved in therapeutic laser applications.

The purpose of this study is to describe tissue modifications after HeNe laser radiation of healthy mouse periodontium *in vivo*.

Materials and Methods

Thirty six forty day old albino mice were selected for this study and were divided into three groups: (a) control group with no irradiation; (b) experimental group 1, irradiated with a HeNe laser of 4.64 mW power measured at the distal end of the fibreoptic with an exposure time of 2 minutes giving a dose (energy density) of 10.50566 J/cm²; and (c) experimental group 2 with the same laser power but 6 minutes' irradiation giving a dose of 31.51698 J/cm². The mice in the three groups were sacrificed at two different periods. Half were killed immediately after laser irradiation, and the other half, 10 days after irradiation. The laser parameters are summarized in Table 1.

After anaesthesia, (Nembutal® solution, 4 mg/cc in physiologic saline solution, dose 4 mg/100 g), the animals were perfused with a dextran solution containing 6% NaCl (Macrodex®) for 7-10 minutes till the

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Table 1: Laser used and physical parameters.

Parameter	Value
Type	HeNe
Wavelength	632.8 nm
Output power (C/W)	
- at laser	7 mW
- at distal end of fibre	4.64 mW
Stabilization time	30 $\pm$ 3 min
Diameter of fibre optic	2.6 mm
Beam transverse section	0.053 cm ²
Irradiance	0.0874 W/cm ²

perfusate appeared clear. Perfusion was continued with 1.7% cacodylate buffered glutaraldehyde with a pH of 7.2 for 15 minutes. The mandibles were removed and block sections of the molar area, corresponding to the irradiated zone, were introduced in a glutaraldehyde solution for histologic processing. The biopsies were decalcified in a 10% nitric acid solution in distilled water during 2-3 hours. They were then postfixed in osmium tetroxide for 2 h, dehydrated in graded acetone concentrations and embedded in Vestopal. Ultrathin sections (approximately 400 Å) were obtained and were observed under a Philips EM 300 Transmission Electron Microscope.

The criteria for defining a modification in the observed tissues was its appearance in at least 2 from each 3 animals, always using for each animal the same areas of epithelium, connective tissue and bone.

Results

Epithelium

In the epithelium, tissue alterations were only observed in the animals irradiated with the higher dose. These alterations consisted in the appearance of basal and spinous epithelial cells with a lack, or very minute presence, of cytoplasmic synthesizing organella (rough endoplasmic reticula [RER] and mitochondria) (Figure 1). These images appeared in both the animals sacrificed immediately after irradiation and in those sacrificed at the later stage. A certain degree of desmolysis (intercellular union system destruction) was seen in the animals of the experimental group 2, both in the immediately sacrificed animals and in those sacrificed at the later stage (Figure 2).

Connective tissue

In the connective tissue, two well-differentiated fibroblastic populations were seen depending on the irradiation dosage. The first cell population showed a dilated RER and a high number of mitochondria, suggesting cells in an active phase of collagen synthesis

(Figures 3, 4 and 5). These cells were seen in the animals of experimental group 1 that were sacrificed immediately after irradiation and in experimental group 2 in both stages. The second fibroblastic cell population showed signs of cell degeneration with cytoplasmic lysis and perinuclear dilatation (Figure 6). These fibroblasts were seen mostly in the experimental group 2 at both periods of sacrifice, but very seldom in the experimental group 1 in the later period, which showed a mature connective tissue with fibroblasts in a quiescent phase.

Bone

The Bone also showed two very well differentiated osteocytic populations. The first population consisted of osteocytes compatible with those in the normal state (Figures 7 and 8). These cells were seen in the control group, in experimental group 1 at both time periods and in experimental group 2 in the later period. The second population showed osteocyte alterations suggestive of a degenerative process. The cells appeared retracted showing a star-like image (Figure 9). The nuclei showed a progressive chromatin condensation, and in some cases complete nuclear destruction (Figure 10). In the cytoplasm, lysosomal bodies and myelin figures could be noted, also suggestive of cell degeneration (Figure 10). These cell alterations were seen mostly in experimental group 2 at both time periods but very seldom in experimental group 1.

Discussion

The effects of high power laser radiation on cells have been previously studied with transmission electron microscopy.^(3,4,10) Although the main mechanism of action of these laser sources may be partially thermal, different authors agree on assigning also a non-thermal effect,^(2,6,9) although its precise cause is unknown. In this study we have described in the epithelium the finding of cells with cytoplasmic changes consisting of a lack of synthesizing organelles and lack of intercellular union complexes. These findings have been described previously, although not in relation to laser radiation. Kubasova, Myers & Schenk,^(11,14,19) have also described alterations in the cellular structures of membranes that could explain these changes. However, from this study we cannot conclude any cause/effect relationship. The epithelial alterations appeared mostly in the second stage of experimental group 2, which suggests a direct relationship between dosage and irradiation time with the pathologic changes found.

The connective tissue presented a dual response, showing hyperactive and degenerative fibroblasts. This dichotomous response has also been reported by Ab-ergel and Bosatra^(2,5) in *in vitro* biochemical studies

Fig 1: Electron photomicrograph showing cells of the stratum granulosum in the gingival epithelium, showing a decrease in the synthesizing organella (RER and mitochondria) x 13,500 (reference bar = 1.0 μm).

Fig 2: Breakdown of intercellular systems in gingival epithelium present in animals treated with high dose laser radiation. x 13,500 (reference bar = 1.0 μm).

Figs 3, 4 & 5:

Fibroblasts belonging to the first group of response to laser irradiation, showing dilated RER with an increase in mitochondria. x 20,500, x 37,500, x 13,500 respectively. (reference bars = 1.0 μ m, 0.5 μ m and 1.0 μ m respectively).

Fig 6: Fibroblasts belonging to the second group of response to radiation showing cellular destruction and perinuclear dilatation. x 30,500 (reference bar = 0.5 μ m).

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Fig 7: Osteoblast compatible with normal state after high dose HeNe laser irradiation. x 30,500. (Reference bar = 0.5 μ m).

Fig 8: Detail of elongated processes of osteoblast after laser irradiation. x 75,000. (Reference bar = 0.25 μ m).

Figs 9 & 10:

Osteoblasts irradiated with high dose HeNe laser showing signs of necrobiosis and cellular degeneration. x 13,500 and x 20,500, respectively. (Reference bars = 1.0 μ m and 1.0 μ m respectively).

analyzing the collagen synthesis of fibroblast cells irradiated with the GaAs laser. These authors related the dual response in the different stage of cell differentiation from the mesenchymal cells.

In bone, we also demonstrated a dual kind of response, with osteocytes showing clear signs of degeneration and cell lysis, together with normal osteocytes compatible with those found in the control group. This different response of the bone cells to radiation suggests a response dependent on intrinsic cellular factors, independent from the experimental conditions. In experimental group 2 at the second stage the number of normal osteocytes was very low, with clear predominance of damaged cells, which suggests that these alterations are also dependent on dosage and time, in a similar manner to the findings in the epithelium, but the bone seems to be more sensitive to low doses of radiation.

Conclusion

In conclusion this study shows certain effects of HeNe laser radiation on healthy periodontal tissues, more predominant at higher doses, but also appearing at the dose recommended for clinical use. Therefore, the use of low level laser therapy sources should be further studied mostly in relation to long term response and to different doses.

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