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Histological analysis of the periodontal ligament and alveolar bone during dental movement in diabetic rats subjected to low-level laser therapy

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ABSTRACT

Objective: The purpose of this research was to evaluate the histological changes of the periodontal ligament and alveolar bone during dental movement in diabetic rats subjected to low level laser therapy (LLLT).
Methods: The movement of the upper molar was performed in 60 male Wistar rats divided into four groups ($n = 15$): CTR (control), DBT (diabetic), CTR/LT (irradiated control) and DBT/LT (irradiated diabetic). Diabetes was induced with alloxan (150 mg/kg, i.p.). LLLT was applied with GaAlAs laser at 780 nm (35 J/cm^2). After 7, 13 and 19 days, the periodontal ligament and alveolar bone were histologically analyzed.
Results: The mean of osteoblasts ($p < 0.01$) and blood vessels ($p < 0.05$) were significantly decreased in DBT compared with CTR at 7 days, whereas the mean of osteoclasts was lower at 7 ($p < 0.001$) and 13 days ($p < 0.05$). In DBT/LT, only the mean of osteoclasts was lower than in CTR ($p < 0.05$) at 7 days, but no difference was observed at 13 and 19 days ($p > 0.05$). The collagenization of the periodontal ligament was impaired in DBT, whereas DBT/LLT showed density/disposition of the collagen fibers similar to those observed in CTR.
Conclusions: LLLT improved the periodontal ligament and alveolar bone remodeling activity in diabetic rats during dental movement.

1. Introduction

Diabetes mellitus (DM) is one of the most common endocrine disorders. It is characterized by persistently raised blood glucose levels (hyperglycaemia), resulting from deficiencies in insulin secretion, insulin action, or both [1]. Chronic hyperglycaemia is associated with long-term damage, dysfunction, and failure of various organs, such as retinopathy, nephropathy, peripheral neuropathy, and autonomic neuropathy, causing gastrointestinal, genitourinary, and cardiovascular symptoms, as well as sexual dysfunction [2].

It has been reported that moderate to severe damage to the glucose metabolism directly affects the response of bone and connective tissues to injury [3,4]. Orthodontic treatment in adult diabetic patients is thus usually complicated by oral problems such as periodontal degradation and bone loss [5]. Although only a few studies have assessed the histological changes that take place in the periodontal ligament and alveolar bone during dental movement in diabetic experimental animals, they provide evidence that chronic hyperglycaemic status might impair the periodontal ligament response and bone remodeling during orthodontic treatment [6].

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Lasers emit a highly concentrated, non-invasive, non-ionizing radiation that, when in contact with different tissues, promotes thermal, photochemical and nonlinear effects [7]. Several studies have indicated that C (LLLT) modulates different biological activities, such as anti-inflammatory activity [8,9], angiogenesis [10,11] and collagen synthesis [12,13]. In particular, the acceleration of bone regeneration by laser treatment has been a focus of studies [14,15].

It has previously been demonstrated that LLLT can accelerate tooth movement, as well as alveolar bone remodeling in experimental studies [16,17] and clinical trials [18,19]. However, little is known about the effect of laser irradiation on the dynamics of dental movement in diabetic subjects. Therefore, the present study was designed to assess the histological changes taking place in the periodontal ligament and alveolar bone during dental movement in diabetic rats subjected to laser irradiation.

2. Material and methods

2.1. Ethical perspectives

The ethical principles of the COBEA (Brazilian College for Animal Experimentation) for experiments in animals were applied in this study. The institutional review board approved the study (approval n° 341208). The study was carried out at the biotherium and the Laboratory of Morphology and Structural Biology of Tiradentes University (Aracaju/SE, Brazil).

2.2. Biological assay

Sixty adult male rats (*Novergiculs albinus*, Wistar lineage), weighing 250 ± 30 g, were randomly assigned into four experimental groups ($n = 15$) (Table 1). Animals were kept in plastic cages with wood shaving bedding (replaced daily), at a controlled temperature of 22 °C, and a 12 h light/dark cycle, with water and food (diet Labina®, Purina, Sao Paulo, Brazil).

2.3. Alloxan-induced diabetes model

Diabetes status was induced by a single intraperitoneal injection of 150 mg/kg monohydrated alloxan (Sigma, St. Louis, MO, USA) dissolved in sterile 0.9% saline. After 12 h, a 10% glucose solution was offered to the animals to prevent hypoglycaemia. Blood samples were collected from the tail vein of the animals after 72 h in order to assess the plasma glucose levels via the glucose-oxidase enzymatic method, using Accu-Chek Advantage (Boehringer, Germany). Animals with glucose levels above 200 mg/dL were included in the diabetic group. The examinations were repeated every 7 days to confirm the maintenance of glucose levels. Any animals showing reversion of the signs of diabetes (glucose levels below 200 mg/dL) were excluded from this study. The animals in the nondiabetic group (CTR and CTR/LT) received an equivalent volume of citrate buffer. The orthodontic device was applied 6 weeks after diabetes was induced.

Table 1
Distribution of the animals in the experimental groups according to treatment.

Groups	Pre-treatment	Low level laser therapy (energy density)
CTR	Citrat buffer	0 J/cm^2
DBT	Alloxan (150 mg/kg)	35 J/cm^2
CTR/LT	Citrat buffer	0 J/cm^2
DBT/LT	Alloxan (150 mg/kg)	35 J/cm^2

2.4. Experimental tooth movement

At the end of 2 months, anaesthesia was induced with intraperitoneal administration of ketamine–xylazine (100 mg/kg–5 mg/kg), and an appliance exerting force to widen the space between the upper central incisors was fitted to both groups. For mesial movement of the upper left 1st molar, the wire end of a 7.0-mm length of NiTi closed-coil spring (wire size: 0.7 mm, diameter: 1/12 in., Orthometric, Marilia, SP, Brazil) was ligated with the maxillary 1st molar cleat using a 0.010-in. stainless steel ligature wire (Morelli, Sorocaba, SP, Brazil). The other side of the coil spring was also ligated, with the holes in the maxillary incisors drilled laterally just above the gingival papilla with a #1/4 round bar, using the same ligature wire (Fig. 1). The orthodontic force exerted by the appliance was 50 g at the start of the experiment. Tooth movement was performed for 19 days (day 0–19).

2.5. Low-level laser therapy procedures

Animals were subjected to transcutaneous irradiation using a previously calibrated semi-conductor diode laser GaAlAs (Twin Laser, MMOptics, São Paulo, Brazil) with continuous emission at 780 nm wavelength for 60 s (20 s each point). The output power used was 70 mW, with a focal spot of 0.04 cm^2, and a power density of 1.75 W/cm^2. The total energy per session was estimated as 4.2 J (1.4 J/point) and the energy density was 35 J/cm^2 distributed between three different equidistant points in the root portion. The first irradiation was performed immediately after the activation procedures, and then performed every 48 h over the course of 7 days.

2.6. Procedures for histomorphological analysis of the specimens

After 7, 13 and 19 days, animals were euthanized in a CO_2 chamber for post-mortem removal of the maxillae. Tissue specimens were fixed in buffered formaldehyde (10%, pH 7.4) for 48 h, decalcified in 5% nitric acid for 72 h, dehydrated in increasing ethyl alcohol solutions, and diaphanized in xylol for inclusion in paraffin. Subsequently, ten histological sections (5 μm thick) were obtained and stained in hematoxylin-eosin for analysis using a light microscope (Olympus CX31 optic microscope) by three trained observers.

2.7. Histomorphological analysis of the periodontal ligament and alveolar bone

The intensity of the inflammatory response was assessed in histological sections as follows: 0 (lack of inflammatory reaction); 1 (inflammatory cells representing less than 10% of the cell population observed within the wound area); 2 (inflammatory cells representing between 10% and 50% of the cell population observed within the wound area); and 3 (inflammatory cells representing more than 50% of the cell population observed within the wound area). Moreover, the inflammatory profile (IP) was classified as acute (predominance of polymorphonuclear cells) or chronic (predominance of mononuclear cells), and graded as slight/absent, moderate or severe.

2.8. Quantitative analysis of the osteoblast (OsTB)/osteoclast (OsTC) and blood vessel (BvC) count

Counting of osteoblasts, osteoclasts and blood vessels was performed using an image analysis system (Imagelab). All images were sent to a PC using an analogue video camera (PAL system), after being converted to the RGB (red–green–blue) system necessary for digitizing and processing the sections. Five histological