Int. J. Oral Maxillofac. Surg. 2014; 43: 1030–1034
http://dx.doi.org/10.1016/j.ijom.2014.02.011, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Research Paper
Wound Healing

Effects of low-level laser therapy on the proliferation and apoptosis of gingival fibroblasts treated with zoledronic acid

T. Nogueira Pansani[1], F. Gonçalves Basso[1], A. P. Silveira Turirioni[1], C. Kurachi[2], J. Hebling[1], C. A. de Souza Costa[1]
[1]Araraquara School of Dentistry, UNESP – Universidade Estadual Paulista, Araraquara, SP, Brazil; [2]Physics Institute, USP – Universidade de São Paulo, São Carlos, SP, Brazil

T. Nogueira Pansani, F. Gonçalves Basso, A. P. Silveira Turirioni, C. Kurachi, J. Hebling, C. A. de Souza Costa: Effects of low-level laser therapy on the proliferation and apoptosis of gingival fibroblasts treated with zoledronic acid. Int. J. Oral Maxillofac. Surg. 2014; 43: 1030–1034. © 2014 International Association of Oral and Maxillofacial Surgeons. Published by Elsevier Ltd. All rights reserved.

Abstract. Low-level laser therapy (LLLT) has been indicated as an adjuvant therapy for bisphosphonate-induced osteonecrosis. However, the effects of LLLT on bisphosphonate-treated cells are not yet clear. This study evaluated the effects of LLLT on the proliferation and apoptosis of gingival fibroblasts treated with zoledronic acid (ZA). Cells were exposed to ZA at 5 μM for 48 h. Irradiation was performed using a laser diode prototype (LaserTABLE, InGaAsP; 780 nm ± 3 nm, 25 mW) at 0.5 or 3 J/cm^2, three times every 24 h. Cell proliferation and apoptosis were evaluated by fluorescence microscopy. Data were analyzed by Mann–Whitney test at the 5% level of significance. ZA decreased cell proliferation to 47.62% (interquartile range (IQR) 23.80–57.14%; $P = 0.007$) and increased apoptosis of gingival fibroblasts to 27.7% (IQR 20.9–33.4%; $P = 0.0001$). LLLT increased cell proliferation compared with non-irradiated cells, at 0.5 J/cm^2 (57.14%, IQR 57.14–71.43%; $P = 0.003$) and at 3 J/cm^2 (76.19%, IQR 61.90–76.19%; $P = 0.0001$), but did not increase cell proliferation in ZA-treated cells. Irradiated fibroblasts presented lower apoptosis rates than the ZA-treated cells, but apoptosis was no different in ZA-treated cells compared to those that were ZA-treated and also irradiated.

Keywords: bisphosphonates; cell culture; cell death; cell division; osteonecrosis; laser therapy; zoledronic acid-treated cells.

Accepted for publication 24 February 2014
Available online 19 March 2014

The aetiology of osteonecrosis has been related to the cytotoxic effects of bisphosphonates on bone cells,[1] a decrease in local vascularization and oxygen distribution, as well as the presence of local infection.[2]

Bisphosphonates are analogues of pyrophosphate that are prescribed for diseases associated with intense bone resorption, such as Paget's disease, multiple myeloma, osteoporosis, bone tumours, and metastasis.[3–5] Bisphosphonate-induced osteonecrosis has been characterized as a necrotic bone area in the oral cavity that persists for longer than 8 weeks without a history of head and neck radiotherapy.[3]

Previous studies have shown that bisphosphonates decrease the proliferation rate of endothelial cells, interfering with the tissue repair process.[6] Scheper et al.[7] demonstrated that zoledronic acid (ZA), a high-potency nitrogen-containing bisphosphonate, inhibits cell proliferation and induces apoptosis of fibroblasts.

0901-5027/0801030 + 05 $36.00/0 © 2014 International Association of Oral and Maxillofacial Surgeons. Published by Elsevier Ltd. All rights reserved.

In addition, Ravosa et al.[8] reported a decrease in motility and synthesis of collagen type I by ZA-treated fibroblasts.

Regarding the controversial aspects of the treatment of osteonecrosis, topical and systemic antibiotic therapies, as well as surgical treatment, have been indicated widely.[9] Recently, low-level laser therapy (LLLT) has been used clinically as an adjuvant therapy for this pathological condition, mainly due to its positive effect on pain relief and tissue repair.[9–12]

Several researchers have demonstrated the biostimulatory effects of LLLT on different cell types, such as increased cell proliferation, migration, differentiation, and protein synthesis, as well as gene expression of collagen type I and growth factors.[13–17] Based on the positive effects of LLLT on bisphosphonate-induced osteonecrosis lesions and the toxic effects of these types of drugs on cells, the aim of the present study was to evaluate the proliferation and apoptosis rates of gingival fibroblasts exposed to ZA treatment and subjected to LLLT according to specific parameters.

Materials and methods

This research was developed in the university laboratory. All experiments were performed using a human gingival fibroblast continuous cell line (HGF). Cells were exposed to ZA and irradiated with a laser diode prototype according to selected parameters. The experimental and control groups are described in Table 1.

Cell culture and zoledronic acid treatment

Cells were seeded in the wells of 24-well plates (3×10^4 cells/cm^2) in culture medium (DMEM–Dulbecco's Modified Eagle's Medium; Gibco, Grand Island, NY, USA), supplemented with 10% foetal bovine serum (FBS; Gibco), and were maintained in an incubator at 37 °C and 5% CO_2 (Isotemp; Fisher Scientific, Pittsburgh, PA, USA).[18] After 48 h, the culture medium was replaced with serum-free DMEM, which was incubated in contact with the cells for 24 h. ZA at 5 μM (8.25 μl/ml) was then added to the serum-free DMEM and kept in contact with the cells for an additional 48 h.[19] The DMEM with ZA was then replaced with fresh DMEM, and the cells were subjected to irradiation by laser diode prototype. Cells not subjected to laser irradiation were used as control groups (G1 and G2). In these groups, the well plates containing the cells were placed in the laser diode prototype, which was not activated.

Table 1. Experimental groups.

Group	Zoledronic acid	Energy dose of LLLT (J/cm^2)
G1 (control group)	No	–
G2	Yes	–
G3	No	0.5
G4	Yes	0.5
G5	No	3.0
G6	Yes	3.0

LLLT, low-level laser therapy.

Low-level laser therapy (LLLT)

Irradiation was performed using a laser diode prototype (LaserTABLE, InGaAsP; 780 nm ± 3 nm, 25 mW).[16,17,19,20] This device is composed of 12 laser InGaAsP (indium–gallium–arsenide–phosphide) diodes that are positioned to irradiate each well in a standardized way and individually. Cells were irradiated three times every 24 h. The parameters selected for LLLT were based on the results of previous studies that have demonstrated increased cell metabolism and proliferation, as well as high migration capacity, after laser irradiation.[17] These parameters are shown in Table 2.

Table 2. LLLT irradiation parameters selected for the experiments.

Parameter	
Potency (W)	0.025
Wave length (nm)	780
Energy doses (J/cm^2)	0.5; 3.0
Irradiation area (cm^2)	2.0
Time (s)	40; 240

LLLT, low-level laser therapy.

Cell proliferation analysis (bromodioxyuridine incorporation)

The evaluation of cell proliferation was performed by the fluorescence method of bromodioxyuridine (BrdU) incorporation; BrdU is an analogue of thymidine. This compound is incorporated into DNA molecules during cell division, demonstrating the cells in DNA synthesis (proliferation). A fluorescence kit, Click-iT EdU Imaging Kit (Invitrogen, Carlsbad, CA, USA), was used for this evaluation, in accordance with the manufacturer's instructions.

Results were analyzed by fluorescence microscopy (Nikon Eclipse TS 100; Nikon Corporation, Tokyo, Japan). Fluorescent nuclei were considered positive. Image analyzer software (Image J 1.45S software; Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) was used to determine the numbers of proliferating cells in four different image fields of each sample.

Analysis of cell apoptosis – TUNEL assay

Apoptotic cells were evaluated by TUNEL assay (terminal deoxynucleotidyl transferase dUTP nick end labelling), which identifies the DNA fragmentation that is characteristic of the apoptotic process.[20] This assay is based on the action of the enzyme terminal deoxynucleotidyl transferase, which adds fluorescent synthetic nucleotides to the DNA fragments, allowing the identification of DNA ruptures that are present in apoptotic cells rather than DNA condensation, observed for cell death by necrosis.

Permeabilization of the plasma cell membrane was performed to permit the influx of fluorescent nucleotides, since this membrane is intact during the apoptotic process.[20] After that, the TUNEL assay was performed with a fluorescence kit, C10245 Click-iT TUNEL (Alexa Fluor 488 Imaging Assay; Invitrogen). A positive group (treated with DNase) was included to improve the comparison. Samples were analyzed by fluorescence microscopy (Nikon Eclipse TS 100; Nikon Corporation). The positive cells were counted and analyzed with image software (Image J 1.45S), and were considered apoptotic cells.

Statistical analysis

The statistical analyses were performed using IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Data were evaluated for normal adherence, and as results did not show normal distribution, the non-parametric Mann–Whitney test was selected for the statistical analysis, using adjusted *P*-values, considering a 5% significance level.

Results

For ZA-treated cells, we observed a significant decrease in cell proliferation compared with the control group ($P < 0.05$).

Download English Version:

https://daneshyari.com/en/article/3132158

Download Persian Version:

https://daneshyari.com/article/3132158

Daneshyari.com