



INDUCTION OF BONE PRODUCTION BY SUPERPULSED LASER IRRADIATION IN A HUMAN OSTEOBLAST CELL LINE

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INTRODUCTION - The effect of superpulsed low-level laser therapy (SLLLT) on bone regeneration has been the focus of more recent research in dentistry. Studies investigated the ability of low-level laser in stimulating both bone production and bone-implant interactions. Regarding bone production, "in vivo" experiments demonstrated that pulsed laser irradiation with high peak powers increase osteoblast activity and decrease osteoclast numbers¹. These results have been confirmed by "in vitro" studies evidencing that low-level lasers can stimulate proliferation of nodule-forming osteoblasts and differentiation of osteoblast precursors². Regarding the bone-implant interactions, both "in vivo" and "in vitro" studies suggested that low-level laser might improve tissue healing and attachment of implants, increasing implant success³.

The present research aimed to investigate the effect of superpulsed laser irradiation on proliferation and bone formation in human osteoblast-like cells MG-63

MATERIALS AND METHODS

Superpulsed low-level laser irradiation: human osteoblast-like cells MG-63 were seeded (10,000/cm²) in MEM medium containing 2 mM L-glutamine, 1% (v/v) antibiotic/antimycotic solution, 1mM sodium pyruvate, and 10% (v/v) FBS (fetal bovine serum). Twenty four h after seeding, cells were exposed to superpulsed low-level laser in a laminar flow hood. **LUMIX 2 DENTAL** (USA Laser Biotech Inc., Richmond, Virginia) was used. Experimental parameters used were: modulation 100%, frequency 30 kHz, energy 60 J, exposure time 5 minutes. Cells were exposed to laser irradiation every 24 h for the times reported in the Figures.

Cell proliferation: cell growth was evaluated in the monolayer and in the culture medium using a Burker chamber and expressed as number of cells/cm².

Markers of osteoblast activity: cells detached from the monolayer were examined for parameters marker of osteoblast activity. Alkaline phosphatase (ALP) and osteocalcin mRNA content was analyzed by Real Time PCR technique.

The fold change was defined as the relative expression compared to that at time 0 (time of seeding cells), calculated as $2^{-\Delta\Delta Ct}$, where $\Delta Ct = Ct_{\text{sample}} - Ct_{\text{GAPDH}}$ and $\Delta\Delta Ct = \Delta Ct_{\text{sample}} - \Delta Ct_{\text{time 0}}$.

EXPERIMENTAL PROTOCOL



LUMIX 2
COLD LASER



Cells MG-63

Modulation: 100%
Frequency: 30 kHz
Energy: 60 J
Time: 5 min

Cells treated every 24 h

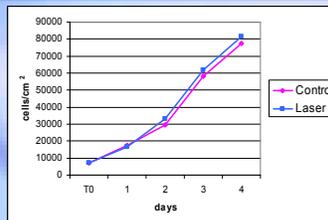
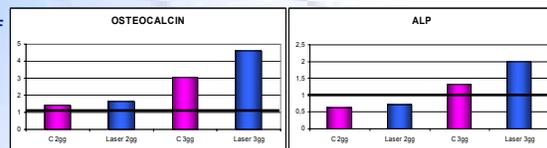


Figure 1: number of MG-63 cells treated or not with superpulsed low-level laser irradiation

Figure 2: mRNA content of osteocalcin and ALP in MG-63 cells treated or not with superpulsed low-level laser irradiation



RESULTS

Figure 1 reports the effect of superpulsed low-level laser irradiation on proliferation of MG-63 cells. An increase of cell number was evident in laser-treated cells starting from the 2 day treatment and remained evident in the following days. No induction of cell death was evidenced. The ability of low-level laser irradiation in stimulating bone production has been evaluated by determining the expression of some proteins involved in calcium nodule formation: osteocalcin and ALP. Figure 2 refers the mRNA content of osteocalcin and ALP determined at 2 and 3 day treatment with low-level laser irradiation. In both untreated- and treated-cells osteocalcin increased in comparison with cells at time 0, but the increase was much higher in laser-treated cells than in untreated ones. As regards to ALP, the values were higher in laser-treated cells than in untreated ones even after 2 days post-treatment. The ALP values in laser-treated cells were initially lower than that of untreated cells at time 0.

Preliminary results obtained in this research evidenced that repeated superpulsed low-level laser irradiation stimulates cell proliferation in human osteoblasts-like cells, and more importantly, increases the expression of proteins essential in bone formation

- 1) Ninomiya T., Hosoya A., Nakamura H., Sano K., Nishisaka T., Ozawa H. Bone. 2007;40:140-148.
- 2) Ozawa Y., Shimizu N., Kariya G., Abiko Y. Bone. 1998;22:347-354.
- 3) Khadra M., Ronald H.J., Lyngstadaas S.P., Ellingsen J.E., Haanaes H.R. Clin. Oral Implants Res. 2004;15:325-332.