Superpulsed Laser Irradiation Increases Osteoblast Activity Via Modulation of Bone Morphogenetic Factors

Silvia Saracino, BSc,[1] Marco Mozzati, DDS,[2] Germana Martinasso, PhD,[1] Renato Pol, DMD,[2] Rosa A. Canuto, MD,[1] and Giuliana Muzio, PhD[1]*

[1]*Department of Experimental Medicine and Oncology, University of Turin, Corso Raffaello 30, 10125 Turin, Italy*
[2]*Department of Biomedical Sciences and Human Oncology, Section of Prosthetic Dentistry, School of Dentistry, University of Turin, Lingotto, Via Nizza 230, 10126 Turin, Italy*

Background and Objective: Laser therapy is a new approach applicable in different medical fields when bone loss occurs, including orthopedics and dentistry. It has also been used to induce soft-tissue healing, for pain relief, bone, and nerve regeneration. With regard to bone synthesis, laser exposure has been shown to increase osteoblast activity and decrease osteoclast number, by inducing alkaline phosphatase (ALP), osteopontin, and bone sialoprotein expression. Studies have investigated the effects of continuous or pulsed laser irradiation, but no data are yet available on the properties of superpulsed laser irradiation. This study thus aimed to investigate the effect of superpulsed laser irradiation on osteogenic activity of human osteoblast-like cells, paying particular attention to investigating the molecular mechanisms underlying the effects of this type of laser radiation.

Study Design/Materials and Methods: Human osteoblast-like MG-63 cells were exposed to 3, 7, or 10 superpulsed laser irradiation (pulse width 200 nanoseconds, minimum peak power 45 W, frequency 30 kHz, total energy 60 J, exposure time 5 minutes). The following parameters were evaluated: cell growth and viability (light microscopy, lactate dehydrogenase release), calcium deposits (Alizarin Red S staining), expression of bone morphogenetic factors (real-time PCR).

Results: Superpulsed laser irradiation decreases cell growth, induces expression of TGF-β2, BMP-4, and BMP-7, type I collagen, ALP, and osteocalcin, and increases the size and the number of calcium deposits. The stimulatory effect is maximum on day 10, that is, after seven applications.

Conclusions: Reported results show that superpulsed laser irradiation, like the continuous and pulsed counterparts, possesses osteogenic properties, inducing the expression of molecules known to be important mediators of bone formation and, as a consequence, increasing calcium deposits in human MG-63 cells. Moreover, the data suggest a new potential role for PPARγ as a regulator of osteoblast proliferation. Lasers Surg. Med. 41:298–304, 2009.

Key words: BMPs; calcium deposits; MG-63 cells; superpulsed laser; TGF-β2

INTRODUCTION

Laser therapy is a new approach in different medical fields, including orthopedics and dentistry, when bone loss occurs, that is, in cases of bone fracture and tooth extraction [1]. Recent studies have reported the benefits of low level laser therapy (LLLT), which has been used to induce soft-tissue healing, for pain relief, bone, and nerve regeneration [2], although the molecular mechanisms triggered are not yet fully clear.

With regard to bone synthesis, in vivo experiments on rat femur have shown that pulsed laser irradiation with high peak power stimulates bone formation by increasing osteoblast activity and decreasing osteoclast numbers [3]. Beneficial properties have been ascribed to LLLT's anti-inflammatory effect, postulating that the treatment modulates transcription factors and regulates the expression of pro-inflammatory cytokines [4]. A recent study on the subplantar tissue of rat's paw evidenced that LLLT decreased mRNA content of TNF-α, IL-1β, and IL-6 [5]; it was suggested that an early target of radiation was TNF-α which, in turn, activates other cytokines. These authors also reported a decreased expression of kinin receptors in the same experimental model [6].

It has also been reported that LLLT induces the formation of small amounts of reactive oxygen species (ROS), which can trigger cell stimulation via increased mitochondrial respiration and ATP formation [7].

The stimulatory effect of LLLT has also been confirmed in vitro in different cell lines. In osteoblast-like cells isolated from fetal rat calvariae, LLLT stimulated proliferation and differentiation, inducing alkaline phosphatase (ALP), osteopontin (OP), and bone sialoprotein expression [8]. Similar results have been obtained in cultured human SaOS-2 cells, where early induction of ALP, type I collagen,

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*Correspondence to: Giuliana Muzio, PhD, Department of Experimental Medicine and Oncology, University of Turin, Corso Raffaello 30, 10125 Turin, Italy. E-mail: giuliana.muzio@unito.it
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