Understanding Photobiomodulation (cont’d)
Thursday, April 15, 2010, 11:15 a.m. – 11:35 a.m., Breakout Session
Student Scholarship Award Presentation

310-DM4 – Low-Level Er:YAG Laser Irradiation Enhances Osteoblast Proliferation Through Activation of MAPK/ERK

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Based on various advantageous effects, the Er:YAG laser has been recently considered as one of the most promising laser systems for periodontal and peri-implant therapy.

It has been reported in an animal study that an increased amount of new bone formation was significantly enhanced following Er:YAG laser irradiation (Mizutani K, Aoki A, Takasaki AA, Kinoshita A, Hayashi C, Oda S, Ishikawa I. Periodontal tissue healing following flap surgery using an Er:YAG laser in dogs. Lasers Surg Med 2006;38(4):314-324). One of the potential explanations for significant new bone formation might be related to the effect of low-level Er:YAG laser irradiation (low-level laser therapy, photobiomodulation).

In osteogenesis, several in vitro studies using different laser devices have previously demonstrated the positive effects of low-level irradiation in promoting new bone formation by inducing proliferation and differentiation of osteoblasts. Since no studies have reported the effect of low-level Er:YAG irradiation on osteoblasts, the aim of this study is to investigate the potential photobiomodulatory effect of the Er:YAG laser on osteoblasts.

Materials and Methods: An Er:YAG laser apparatus (VersaWave®, HOYA ConBio®, Fremont, Calif., USA) has a wavelength of 2.94 μm, an output energy range of 30 to 350 mJ/pulse, a maximum pulse repetition rate of 50 Hz, and a pulse duration of 200 μs. Laser irradiation was performed perpendicularly to the bottom of a culture dish at a distance of 15 cm. The laser energy was emitted from the handpiece without mounting a cover sleeve and contact tip in order to completely irradiate the MC3T3-E1 mouse osteoblast cells in a 35-mm tissue culture dish.

Experiment 1: Effect of low-level Er:YAG laser on cell proliferation

First, the laser was fixed at 30 Hz and 30 sec, and energy levels of 23 to 68 mJ/pulse (fluence: 2.1 to 6.4 J/cm²) was applied. Second, the laser was fixed at 30 Hz and 23 mJ/pulse, and irradiation time was 30-120 sec (fluence: 2.1 to 8.6 J/cm²). Third, the laser was fixed at 23 mJ/pulse and 30 sec and the pulse rate was 10 to 50 Hz (fluence: 0.7 to 3.6 J/cm²). All irradiations were performed in the absence of the culture medium. Irradiation in the presence of culture medium was also performed by applying 0.5 ml of medium, slightly covering the cell surface. The energy level was set to 23 mJ/pulse, pulse rate to 30 Hz, and irradiation time was 1 to 4 minutes (fluence: 4.3 to 17.2 J/cm²). At days 1 and 3 following Er:YAG laser irradiation,
cell viability was determined by cell counting. The degree of cell death at day 1 was determined by measuring the lactate dehydrogenase (LDH) levels.

Experiment 2: Effect of low-level Er:YAG laser on mitogen-activated protein kinase (MAPK) pathways

The involvement of MAPK pathways in laser-enhanced cell proliferation was investigated by examining the effect of specific MAPK inhibitors (added prior to irradiation) and phosphorylation of MAPKs by Western blotting. Er:YAG laser irradiation was performed at 23 mJ/pulse and 30 Hz for 60 sec (fluence: 4.3 J/cm²) in the absence of medium.

The one-way analysis of variance (ANOVA) test was used for all group comparisons, and post hoc Tukey’s test was used to compare differences between each group. A p value of < 0.05 was considered significant.

Results: The low-level Er:YAG laser enhanced the proliferation of osteoblasts in an energy-, time-, and pulse-dependent manner. At various combinations of irradiation parameters, significantly increased cell proliferation was observed at fluences of approximately 1.0 to 15.1 J/cm², with no increase in LDH activity.

Regarding the effect of low-level Er:YAG laser on MAPK pathways, inhibition of laser-enhanced proliferation was observed after cell treatment with MAPK/ERK (extracellular signal-regulated kinase) inhibitor U0126. Further, Western blotting analysis revealed induction of MAPK/ERK phosphorylation 5 min following irradiation compared to nonirradiated control cells.

Conclusions: At various combinations of irradiation parameters, low-level Er:YAG laser irradiation promotes osteoblast proliferation mainly by the activation of the MAPK/ERK pathway. These findings suggest faster bone tissue healing following Er:YAG laser therapy, as well as a number of advantageous clinical therapeutic effects.

This presentation discusses investigational devices that have not yet received U.S. FDA approval or clearance for the specified clinical indications, or describes off-label uses.

Biography: Dr. Verica Aleksic has graduated as the best student of her generation from Faculty of Dentistry, University of Banjaluka, Bosnia and Herzegovina, in 2004. She joined Tokyo Medical and Dental University’s (TMDU’s) Periodontology Department for a PhD course as a winner of the Monbukagakusho Scholarship in 2005. Additionally, she is a member of Advanced International Super Students (AISS) of the Global Center of Excellence (GCOE) Program, “International Research Center for Molecular Science in Tooth and Bone Diseases,” TMDU. Dr. Aleksic is married and has one child.

Disclosure: Dr. Aleksic has no commercial or financial interest relative to this presentation.

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