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Introduction to Experimental and Clinical Studies Using Low-Level Laser (Light) Therapy (LLLT)

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I am pleased to have the opportunity to serve as guest editor for this special issue of Lasers in Surgery and Medicine that concentrates on low level laser therapy (LLLT) also known as photobiomodulation. I would like to thank Drs. Stuart Nelson and Henry Chan for the invitation to be guest editor and to pay a special tribute to the talented and tireless Ms. Beth Mallen, without whom this would have been an impossible task. LLLT has been known since a few years after the discovery of lasers (incidentally celebrating their 50th anniversary this year). However, it is only in relatively recent times that LLLT has become scientifically and clinically accepted by even a fraction of the medical community. We hope this issue will make a contribution towards increasing the awareness among both physicians and the general scientific public that hard evidence is now available that "sheds light" on the basic mechanisms, pre-clinical applications and clinical benefits of LLLT. It contains a review, basic science and clinical research submissions providing new information concerning cell biology studies after LLLT and highlighting many possible clinical applications of LLLT.

Reviews and mechanistic studies

Hashmi et al have addressed a question that has long intrigued workers in the LLLT field; namely whether pulsed laser or pulsed LED light has any benefits over continuous wave light. They reviewed the literature containing 33 studies of pulsed LLLT and found that out of nine studies that directly compared pulsed with CW light, six found a direct benefit for pulsing. They provided some mechanistic hypotheses for why pulsing could be superior to CW including increased penetration depth and kinetics of ion channels.

Lubart et al used electron paramagnetic resonance (EPR) spin-trapping techniques to follow broadband visible-light (400–800 nm) -induced hydroxyl radicals in various cell types (fibroblasts, sperm cells, cardiomyocytes, and skeletal muscle cells. The concentration of ·OH increased both with illumination time and with cell concentration, and decreased when N2 was bubbled into the cell culture, suggesting that visible light initiates a photochemical reaction via endogenous photosensitizers. Visible light was found to stimulate ROS generation both in membrane and cytoplasm. In addition, fluorescent measurements confirmed the mitochondria to be target for light-cell interaction. Another paper from the same laboratory (Lipovsky et al) compared different wavelengths of light (400–500 nm, 500–800 nm, 415 nm, and 455 nm) with regard to ROS production and antibacterial effects on *S. aureus* and *E. coli*. 415-nm was most effective in killing bacteria at high fluences and stimulating proliferation at low fluences.

Fonseca et al studied the effect of 658-nm diode laser in CW and pulsed modes on *Escherichia coli* bacterial cells including a DNA repair mutant. They found that laser exposure (pulsed

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more than CW) protected the cells against subsequent challenge with hydrogen peroxide and this was not related to DNA damage.

In vitro cellular studies

Houreld and colleagues compared normal and diabetic fibroblasts given in vitro "wounds" and exposed to an 830 nm laser. They found increases in reactive oxygen species and increased production of nitric oxide immediately after irradiation and a reduction in both TNF-alpha and IL-1 at 24 hours together with less apoptosis and more proliferation. The data support the use of LLLT for healing of diabetic wounds.

Kotoe and coworkers asked whether LLLT could be beneficial for the prevention of crestal bone resorption during orthodontic treatment in adult patients. Prostaglandin E2 (PGE2), which has bone-resorptive activity, is produced by human periodontal ligament (PDL) cells in response to mechanical stress. A compressive force of 2.0 g/cm^2 was applied for 24 h to human PDL cells obtained from premolars extracted for orthodontic treatment. LLLT (830 nm laser, 3.82 J/cm^2) was applied 6 h before, 1 h before, and immediately after the application of compressive force. The mRNA expression of cyclooxygenase 2 and phospholipase A2 induced by compression was inhibited by laser.

Chen et al explored the mechanism of histamine release in RBL-2H3 mast cells after laser irradiation (405-nm, 532-nm, 633-nm). They found that 405-nm and 633-nm were effective in causing histamine release consistent with cytochrome oxidase being the photoacceptor. Cytochrome c moved from mitochondria to cytosol reflecting an increased permeability of mitochondrial membrane and cytosolic alkalinization and increased cytosolic calcium occurred.

Hwang and coworkers investigated LLLT increase of bone formation by exposing mouse MC3T3-E1 pre-osteoblasts to a Q-switched, pulsed neodymium-doped yttrium aluminum garnet (Nd:YAG) laser(1064 nm, 1.5, 3 or 5 J/cm^2) energy densities. Alkaline phosphatase increased when combined with bone morphogenetic protein (BMP-2) or not. Cell proliferation declined in the irradiation and combined irradiation/BMP-2 groups. Laser stimulation resulted in significant induction of endogenous BMP-2 protein and gene expression. The increased expression of upstream regulators cbfa1 by laser alone was comparable to exogenous BMP-2 treatment (100 ng/ml). Combined laser/BMP-2 treatment was synergistic in the expression of some genes (IGF-1, cbfa1) and ALP activity. In vitro matrix mineralization was significantly accelerated by laser stimulation compared to that of the control, more so than with the combined laser/BMP-2 treatment.

Another study examined the same in vitro system of mouse MC3T3-E1 osteoblasts. Fujimoto irradiated cells at the subconfluent stage using a low-intensity Ga-Al-As laser (830-nm, 0.96 to 3.82 J/cm^2). One day after laser irradiation expression of BMPs, transcription factors (Runx2, Osterix, Dlx5, Msx2) and phosphorylation of Smad1 were increased by 1.91 J/cm^2 laser. Noggin, a BMP receptor blocker, inhibited the laser-induced Runx2 expression and phosphorylation of Smad1. Moreover, laser irradiation significantly increased the calcium content of cell cultures, and noggin inhibited this increase.

A third study also looked at osteoblasts and laser therapy together with other cell types of importance in dentistry. Chellini and colleagues used Nd:YAG laser (pulse energy 20 mJ at 50–70 Hz) on Saos-2 osteoblasts, H-end endothelial cells and NIH/3T3 fibroblasts pre-treated or not with photosensitizing dye methylene blue (MB). Nd:YAG laser irradiation did not affect cell viability in all the tested cell types, even when combined with pre-treatment with MB, and efficiently stimulated cell growth in the nonsensitized osteoblasts. Moreover, a significant induction in the expression of osteopontin, ALP and Runx2 in osteoblasts, type I collagen in

fibroblasts and vinculin in endothelial cells was observed in the irradiated cells. There was also an increase in the intracellular Ca2+ in osteoblasts through the activation of TRPC1 ion channels. Moreover, the pharmacologic or genetic inhibition of these channels strongly attenuated laser-induced osteopontin expression, suggesting a role for the laser-mediated Ca2 + influx in regulating osteoblast differentiation.

MacDaniel and colleagues explored the use of two-wavelengths (590 nm and 870 nm) LED photobiomodulation on human skin fibroblasts. With a fixed combined irradiance of 4 mW/cm^2 and fluence of 0.1 J/cm^2 they varied the ratios of each wavelength. They used an 80-gene array to show that the greatest increase in collagen I and decrease in collagenase (MMP-1) was observed with 75%/25% ratio of 590nm to 870-nm.

Preclinical animal studies

Xavier et al investigated the effects of LLLT (880±10nm LED, 7.5J/cm^2) on inflammatory process in a rat model of Achilles tendinitis induced by collagenase.

LLLT was initiated 12h after the tendinitis induction, with a 48-hour interval between the irradiations and continued for 7 or 14 days or given for 7th to 14th day. LLLT decreased the inflammatory cells influx and mRNA expression of IL-1β, IL-6, TNF-alpha in both phases, and COX-2 in the initial phase.

Lopes and colleagues studied the effect of LLLT on chemotherapy-induced oral mucositis in a hamster cheek pouch model. Hamsters received systemic injections of 5-fluorouracil followed by scratching of the cheek pouch mucosa using a needle. The cheek pouches were illuminated with an InGaAIP diode laser (660nm). They delivered a power output of 35mW or 100mW to five points each of which received 2.7 J on days 3, 4, 5 and 6 of the experiment. The reduced peak clinical severity of mucositis in the 35mW laser group was accompanied by a decrease in the number of neutrophils and an increase in the proportion of mature collagen as compared to the other two groups.

Servetto and coworkers tested LLLT on rats with experimental myopathy induced by injecting adrenaline in the left posterior limb muscle at the same point on 5 consecutive days. They used a helium-neon (He-Ne, 632.8-nm) or a pulsed gallium arsenide (Ga.As, 904-nm) laser delivering 9.5 J/cm^2 daily for 7 consecutive days with either laser. Both lasers reduced inflammatory biomarkers associated with oxidative stress, fibrinogen, L-citrulline and superoxide dismutase (SOD). Nitric oxide that was lower in myopathy was increased by LLLT. Histology showed a lower inflammatory infiltrate after LLLT.

Wood and others asked whether LLLT, ultrasound (US) or the combination could be used to improve tendon injuries. Rats received a traumatic injury to the Achilles (calcaneal) tendon and were treated for 5 consecutive days with US (3 MHz, pulsed mode, 20% duty cycle, 2 ms on and 8ms off, pulse repetition frequency 100 Hz, spatial average temporal peak intensity of 1.0 W/cm^2) and/or LLLT (GaAlAs 830 nm, 40 mW, power density of 1.4 W/cm2, total energy dose of 0.12 J). The amount of type I collagen found all treated groups was significantly higher than that in the control group. US was also effective in increasing collagen organization in the early stages of the healing process.

Clinical studies

Hofling et al carried out a clinical trial of LLLT for chronic autoimmune thyroiditis (CAT). Fifteen patients who had hypothyroidism caused by CAT and were undergoing levothyroxine (LT4) treatment received 10 applications of LLLT (830 nm, CW output power 50 mW) twice a week, using either the punctual technique (8 patients) or the sweep technique (7 patients),

with fluences in the range of 38 J/cm^2 to 108 J/cm^2. Ultrasound was performed prior to and 30 days after LLLT. All patients' reduced LT4 dosage needs, including 7 who did not require any LT4 through the 9-month follow-up. Antibodies against thyroid peroxidase also decreased and a post-LLLT increase in the ultrasound echogenicity were observed.

Barolet and Boucher reported on three patients who received near-IR prophylactic LLLT to alter the wound healing process in order to avoid or attenuate the formation of hypertrophic scars or keloids. Following scar revision by surgery or CO^2 laser ablation on bilateral areas, one scar was treated daily by the patient at home with non-thermal, non-ablative NIR LED (805 nm at 30mW/cm^2) for 30 days. Significant improvements on the NIR-treated versus the control scar were seen in all efficacy measures. No significant treatment-related adverse effects were reported.