Laser Light Prevents Apoptosis In Cho K-1 Cell Line

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J Clin Laser Med Surg. 2003 Aug;21(4):193-6.

OBJECTIVE: The present study investigated the effects of low-level laser therapy (LLLT) on the mitochondria, nucleus, and cytoskeleton of CHO K-1 cells by the use of specific fluorescent probes.

BACKGROUND DATA: The use of LLLT has been recommended by several authors for acceleration of the healing process. The literature on the effects of LLLT in this process is highly contradictory because of difficulties in identifying its effects on cells.

MATERIALS AND METHODS: CHO K-1 cells were cultivated using MEM containing 5% FBS and were irradiated or not with a semiconductor laser (lambda = 830 nm; phi approximately 0.8 mm; 10 mW; 2 J/cm2). The cells were incubated with specific fluorescent probes--0.1 microM for 30 min with 5,5', 6,6'-tetrachloro-1, 1',3,3'-tetraethyl-benzimidazol-carbocyanine iodide (JC-1) for the mitochondria; 5 mM for 5 min of 4',6'-diamidino, 2'-phenylindole (DAPI)for the nucleus, and 0.1 M of 1:100 PHEM of rhodamine-phalloidin during 1 h for the cytoskeleton—and were analyzed by epifluorescence.

RESULTS: Positive biomodulatory effects were observed on irradiated cells compared to their controls as seen on JC-1, DAPI, and rhodamine-phalloidin labeling. Irradiated cells showed an increased level of cellular division, as evidenced by analyzing the intermediary filaments of the cytoskeleton and the chromosomes. Another important observation was that cells maintained under the condition of nutritional deficiency had both membrane and genetic material that was more preserved in comparison to the controls, in which the presence of an apoptotic nucleus could be observed in some cells.

CONCLUSION: The results of the present study demonstrate that LLLT, in addition to providing positive biomodulation, acts in the re-establishment of cellular homeostasis when the cells are maintained under thecondition of nutritional stress; it also prevents apoptosis in CHO K-1 cells.

Effects of infrared and low-power laser irradiation on cell viability, glutathione and glutathione-related enzyme activities in primary rat hepatocytes

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J Formos Med Assoc. 2003 Jul;102(7):486-91.

BACKGROUND AND PURPOSE: Both infrared and low-power laser have been applied to improve circulation, wound repair, and pain control. Infrared and low-power laser therapies have the potential for stimulating enzyme activities which might contribute to increased glutathione (GSH) concentration and provide protection against oxidative damage. This study investigated cell viability, and GSH and its related enzyme activities in rat hepatocytes after irradiation.

METHODS: Hepatocytes were isolated from 8-week- old male Sprague-Dawley rats and the cultures were divided into infrared, laser, and control groups. The cells were treated with infrared and low-power laser at a distance of 35 cm for 20 minutes. The cell morphology, lactate dehydrogenase (LDH) leakage, lipid peroxidation, GSH concentration, GSH peroxidase, GSH reductase (GRd), and GSH S-transferase activities were measured after irradiation.

RESULTS: The morphology and LDH leakage of hepatocytes in the irradiation groups did not differ significantly from those of the control group. After infrared irradiation, a significant decrease in thiobarbituric acid-reactive substances and an increase in GSH concentration were found after 48 hours of incubation compared to the control group ($p < 0.05$). Furthermore, laser irradiation resulted in a significant increase in GRd activity after 48 hours of incubation compared to the control group ($p < 0.05$). A 48-hour incubation period produced greater GRd activity in all groups compared to a 24-hour period ($p < 0.05$).

CONCLUSIONS: Irradiation did not damage rat hepatocytes in this study. Infrared was shown to stimulate GSH production, while laser irradiation increased GRd activity.

Low-level 809 nm GaAlAs laser irradiation increases the proliferation rate of human laryngeal carcinoma cells in vitro

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Lasers Med Sci. 2003;18(2):100-3.

The aim of the study was to investigate the effect of low-level 809 nm laser irradiation on the proliferation rate of human larynx carcinoma cells in vitro. Epithelial tumor cells were obtained from a laryngeal carcinoma and cultured under standard conditions. For laser treatment the cells were spread on 96-well tissue culture plates. Sixty-six cell cultures were irradiated with an 809 nm GaAlAs laser. Another 66 served as controls. Power output was 10 mW(cw) and the time of exposure 75-300 s per well, corresponding to an energy fluence of 1.96-7.84 J/cm2. Subsequent to laser treatment, the cultures were incubated for 72 h. The proliferation rate was determined by means of fluorescence activity of a redox indicator (Alamar Blue Assay) added to the cultures immediately after the respective treatment. The indicator is reduced by metabolic activity related to cellular growth. Proliferation was determined up to 72 h after laser application. The irradiated cells revealed a considerably higher proliferation activity. The differences were highly significant up to 72 h after irradiation (Mann-Whitney U test, $p < 0.001$). A cellular responsiveness of human laryngeal carcinoma cells to low-level laser irradiation is obvious. The cell line is therefore suitable for basic research investigations concerning the biological mechanisms of LLLT on cells.

Increased Fibroblast Proliferation Induced By Light Emitting Diode And Low Power Laser Irradiation

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Lasers Med Sci. 2003;18(2):95-9.

BACKGROUND AND OBJECTIVE: As Light Emitting Diode (LED) devices are commercially introduced as an alternative for Low Level Laser (LLL) Therapy, the ability of LED in influencing wound healing processes at cellular level was examined.

STUDY DESIGN/MATERIALS AND METHODS: Cultured fibroblasts were treated in a controlled, randomized manner, during three consecutive days, either with an infrared LLL or with a LED light source emitting several wavelengths (950 nm, 660 nm and 570 nm) and respective power outputs. Treatment duration varied in relation to varying surface energy densities (radiant exposures).

RESULTS: Statistical analysis revealed a higher rate of proliferation ($p < 0.001$) in all irradiated cultures in comparison with the controls. Green light yielded a significantly higher number of cells, than red ($p < 0.001$) and infrared LED light ($p < 0.001$) and than the cultures irradiated with the LLL ($p < 0.001$); the red probe provided a higher increase ($p < 0.001$) than the infrared LED probe and than the LLL source.

CONCLUSION: LED and LLL irradiation resulted in an increased fibroblast proliferation in vitro. This study therefore postulates possible stimulatory effects on wound healing in vivo at the applied dosimetric parameters.

Effect Of Low-Level Laser Irradiation On Osteoglycin Gene Expression In Osteoblasts
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Lasers Med Sci. 2003;18(2):78-82.

Many studies have attempted to elucidate the mechanism of the biostimulatory effects of low-level laser irradiation (LLLI), but the molecular basis of these effects remains obscure. We investigated the stimulatory effect of LLLI on bone formation during the early proliferation stage of cultured osteoblastic cells. A mouse calvaria-derived osteoblastic cell line, MC3T3-E1, was utilised to perform a cDNA microarray hybridisation to identify genes that induced expression by LLLI at the early stage. Among those genes that showed at least a twofold increased expression, the osteoglycin/mimecan gene was upregulated 2.3-fold at 2 h after LLLI. Osteoglycin is a small leucine-rich proteoglycan (SLRP) of the extracellular matrix which was previously called the osteoinductive factor. SLRP are abundantly contained in the bone matrix, cartilage cells and connective tissues, and are thought to regulate cell proliferation, differentiation and adhesion in close association with collagen and many other growth factors. We investigated the time-related expression of this gene by LLLI using a reverse transcription polymerase chain reaction (RT-PCR) method, and more precisely with a real-time PCR method, and found increases of 1.5-2-fold at 2-4 h after LLLI compared with the non-irradiated controls. These results suggest that the increased expression of the osteoglycin gene by LLLI in the early proliferation stage of cultured osteoblastic cells may play an important role in the stimulation of bone formation in concert with matrix proteins and growth factors.

Low-Level Laser Irradiation Attenuates Production Of Reactive Oxygen Species By Human Neutrophils
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J Clin Laser Med Surg. 2003 Jun;21(3):165-70.

OBJECTIVE: The aim of this study was to examine the effects of low-level laser therapy (LLLT) on production of reactive oxygen (ROS) species by human neutrophils.

BACKGROUND DATA: LLLT is an effective therapeutic modality for inflammatory conditions.

MATERIALS AND METHODS: The laser device used was the infrared diode laser (GaAlAs), 830-nm continuous wave (150 mW/cm(2)). After irradiation, ROS production by neutrophils was measured using luminol-dependent chemiluminescence (LmCL) and expression of CD11b and CD16 on neutrophil surface was measured by flow cytometry.

RESULTS: The LmCL response of neutrophils was reduced by laser irradiation at 60 min prior to the stimulation with opsonized zymosan and calcium ionophore. The attenuating effect of LLLT

was larger in neutrophils of smokers than non-smokers, while the amount of produced ROS was larger in neutrophils of smokers. Expression of CD11b and CD16 on neutrophil surface was not affected by LLLT.

CONCLUSION: Attenuation of ROS production by neutrophils may play a role in the effects of LLLT in the treatment of inflammatory tissues. There is a possible usage of LLLT to improve wound healing in smokers.

Effect of low-level GaAlAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an in vitro study.
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J Clin Periodontol. 2003 Apr;30(4):353-8.

AIM: The aim of this in vitro study was to evaluate a potential stimulatory effect of low-level laser irradiation on the proliferation of human periodontal ligament fibroblasts (PDLF).

MATERIALS AND METHODS: PDLF obtained from third molar periodontal ligaments were cultured under standard conditions and spread on 96-well tissue culture plates. Subconfluent monolayers were irradiated with an 809-nm diode laser operated at a power output of 10 mW in the continuous wave (cw) mode at energy fluences of 1.96- 7.84 Jcm-2. The variable irradiation parameters were the time of exposure (75-300 s per well) and the number of irradiations (1-3). After laser treatment, the cultures were incubated for 24 h. The proliferation rate of the lased and control cultures was determined by means of fluorescence activity of a reduction-oxidation (REDOX) indicator (Alamar Blue Assay) added to the cell culture. Proliferation, expressed in relative fluorescence units (RFU), was determined 24, 48 and 72 h after irradiation.

RESULTS: The irradiated cells revealed a considerably higher proliferation activity than the controls. The differences were significant up to 72 h after irradiation (Mann-Whitney U-test, $p<0.05$).

CONCLUSION: A cellular effect of the soft laser application is clearly discernible. Clinical studies are needed to evaluate whether the application of low-level laser therapy might be beneficial in regenerative periodontal therapy.

Effect of He-Ne Laser (632.8 nm) and Polygen on CHO cells
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J Clin Laser Med Surg. 2000 Jun;18(3):145-50.

OBJECTIVE: We determined the effect of He-Ne laser biostimulation in combination with Polygen (PG) on Chinese hamster ovary (CHO) cells.

BACKGROUND DATA:
Several studies have shown that He-Ne laser (632.8 nm), growth factors, and growth hormone can enhance cellular proliferation and that the use of low-level laser stimulation combined with growth factor stimulation has scientific support. PG, an animal protein extract containing a blend of growth factors and growth hormone, was used together with a He-Ne laser to determine their efficacy in the enhancement of cellular proliferation.

METHODS: The dose-response curves for the colony-forming ability of CHO cells in 5% FCS-MEM with 6-125 microg/ml PG and He-Ne laser with an optimum power density of 1.25 mW/cm2 and cumulative doses of 60-600 mJ/cm2 was given for 3 consecutive days. The combined effects of He-Ne laser 180 mJ/cm2 with 6 and 12 microg/ml PG were determined. Quadruplicate cultures were performed. The student's t-test was used to ascertain differences of treated groups from controls.

RESULTS: The mean number of colonies (MNC) was increased using 180 mJ/cm2 laser by 13.2% ($p < 0.01$); 6 and 12 microg/ml PG by 19.2% ($p < 0.0025$) and 13.2% ($p = 0.01$); laser + PG 6 microg/ml by 23.2% ($p < 0.001$) and laser + PG 12 microg/ml by 20.5% ($p < 0.001$). An additional significant increase of 8.8% ($p < 0.05$) and an insignificant 6.4% ($p = 0.086$) by laser + PG 6 microg/ml and laser + PG 12 microg/ml were observed, respectively, when compared to the solitary effect of laser.

CONCLUSIONS: Results suggest that the He:Ne laser or PG can stimulate CHO cell proliferation and that further stimulation can be achieved by using the He:Ne laser and PG simultaneously. This combination could be useful as a new treatment modality.

The Effects Of Low Level Laser Irradiation On Osteoblastic Cells

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Clin Orthod Res. 2001 Feb;4(1):3-14.

Low level laser therapy has been used in treating many conditions with reports of multiple clinical effects including promotion of healing of both hard and soft tissue lesions. Low level laser therapy as a treatment modality remains controversial, however. The effects of wavelength, beam type, energy output, energy level, energy intensity, and exposure regime of low level laser therapy remain unexplained. Moreover, no specific therapeutic window for dosimetry and mechanism of action has been determined at the level of individual cell types. The aim of this study was to investigate the effects of low level laser irradiation on the human osteosarcoma cell line, SAOS-2. The cells were irradiated as a single or daily dose for up to 10 days with a GaAlAs continuous wave diode laser (830 nm, net output of 90 mW, energy levels of 0.3, 0.5, 1, 2, and 4 Joules). Cell viability was not affected by laser irradiation, with the viability being greater than 90% for all experimental groups. Cellular proliferation or activation was not found to be significantly affected by any of the energy levels and varying exposure regimes investigated. Low level laser irradiation did result in a heat shock response at an energy level of 2 J. No significant early or late effects of laser irradiation on protein expression and alkaline phosphatase activity were found. Investigation of intracellular calcium concentration revealed a tendency of a transient positive change after irradiation. Low level laser irradiation was unable to stimulate the osteosarcoma cells utilised for this research at a gross cell population level. The heat shock response and increased intracellular calcium indicate that the cells do respond to low level laser irradiation. Further research is required, utilising different cell and animal models, to more specifically determine the effects of low level laser irradiation at a cellular level. These effects should be more thoroughly investigated before low level laser therapy can be considered as a potential accelerator stimulus for orthodontic tooth movement.

The Effect Of The Blood Serum From Patients Subjected To Intravenous Laser Therapy On The Parameters Of Synaptic Transmission

[Article in Russian]

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Biull Eksp Biol Med. 1993 Aug;116(8):149-51.

The effect of serum of patients with myocardial ischemia after low-level laser therapy on parameters of synaptic conductance of rat hippocampal neurons was investigated. The serum from patients with an initially low level of neuronal activity obtained after determination of laser irradiation increased the amplitude and that from patients with high activity. Thus the process of normalization of these parameters was observed. Our results may help to optimize the course of medical treatment, and subsequently give an insight to understanding of the mechanism of therapeutic effect of laser irradiation.

Effects Of Visible And Near-Infrared Lasers On Cell Cultures

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J Photochem Photobiol B. 1992 Feb 28;12(3):305-10.

The effect of 360, 632 and 780 nm light on NIH fibroblast cells was examined. Mitosis counts of irradiated cells at various energy doses were taken. Scanning electron micrographs of these cells were studied. It is suggested that low-level laser therapy in the visible and in the near-infrared region is due to cell respiration stimulation by either the endogenous porphyrins in the cell, or by the cytochromes.

Low-Intensity Near-Infrared Laser Radiation-Induced Changes Of Acetylcholinesterase Activity Of Human Erythrocytes

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J Clin Laser Med Surg. 2003 Dec;21(6):351-5.

OBJECTIVE: The aim of the present study was to investigate the transformations of red blood cells produced by low-intensity infrared laser radiation (810 nm).

BACKGROUND DATA: Low-intensity (the output power of a laser device in the milliwatt range) laser radiation as a local phototherapeutic modality is characterized by its ability to induce non-thermic, nondestructive photobiological processes in cells and tissues. However, the exact theory concerning the therapeutic effects of laser biostimulation has not been developed.

MATERIALS AND METHODS: The suspensions of human erythrocytes in PBS (10% hematocrit) were irradiated with near-infrared (810 nm) therapy laser at different light doses (0-20 J) and light power (fluence rate; 200 or 400 mW) at 37 degrees C. As the parameters characterizing the cell structural and functional changes membrane acetylcholinesterase (AchEase) activity, the membrane potential, the level of intracellular glutathione, the level of products of membrane lipid peroxidation, and the cell osmotic stability were measured.

RESULTS: It was found that near-infrared low-intensity laser radiation produced complex biphasic dose-dependent changes of the parameters of AchEase reaction in the dose-dependent manner: at smaller doses of radiation (6 J) the maximal reaction rate and Michaelis-Menten constant value decreased, and at higher radiation doses these parameters increased. No

significant changes of erythrocyte stability, cellular redox state (reduced glutathione or lipid peroxidation product levels), or cell membrane electrochemical potential were observed.

CONCLUSION: Low-intensity near-infrared laser radiation (810 mn) produced AchEase activity changes, reflecting the effect of light on the enzyme due to energy absorption. Protein molecule conformational transitions and enzyme activity modifications in cells have been suggested as laser radiation-induced events.

Effects Of Infrared And Low-Power Laser Irradiation On Cell Viability, Glutathione And Glutathione-Related Enzyme Activities In Primary Rat Hepatocytes

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J Formos Med Assoc. 2003 Jul;102(7):486-91.

BACKGROUND AND PURPOSE: Both infrared and low-power laser have been applied to improve circulation, wound repair, and pain control. Infrared and low-power laser therapies have the potential for stimulating enzyme activities which might contribute to increased glutathione (GSH) concentration and provide protection against oxidative damage. This study investigated cell viability, and GSH and its related enzyme activities in rat hepatocytes after irradiation.

METHODS: Hepatocytes were isolated from 8-week-old male Sprague-Dawley rats and the cultures were divided into infrared, laser, and control groups. The cells were treated with infrared and low-power laser at a distance of 35 cm for 20 minutes. The cell morphology, lactate dehydrogenase (LDH) leakage, lipid peroxidation, GSH concentration, GSH peroxidase, GSH reductase (GRd), and GSH S-transferase activities were measured after irradiation.

RESULTS: The morphology and LDH leakage of hepatocytes in the irradiation groups did not differ significantly from those of the control group. After infrared irradiation, a significant decrease in thiobarbituric acid-reactive substances and an increase in GSH concentration were found after 48 hours of incubation compared to the control group ($p < 0.05$). Furthermore, laser irradiation resulted in a significant increase in GRd activity after 48 hours of incubation compared to the control group ($p < 0.05$). A 48-hour incubation period produced greater GRd activity in all groups compared to a 24-hour period ($p < 0.05$).

CONCLUSIONS: Irradiation did not damage rat hepatocytes in this study. Infrared was shown to stimulate GSH production, while laser irradiation increased GRd activity.

Biostimulation Of Human Chondrocytes With Ga-Al-As Diode Laser: 'In Vitro' Research.

Artificial Cells, Blood Substitutes, and Immobilization Biotechnology. 2000; 28(2):193-201.
Morrone G, Guzzardella G A, Tigani D et al.

The aim of the study was to verify the effects of lllt performed with GaAlAs (780 nm, 2500 mW) on human cartilage cells in vitro. The cartilage sample used for the biostimulation treatment was taken from the fight knee of a 19-year-old patient. After the chondrocytes were isolated and suspended for cultivation, the cultures were incubated for 10 days. The culture were divided into four groups. Groups I, II, III were subject to biostimulation with the following laser parameters: 300J, 1W, 100Hz,10 min. exposure, pulsating emission; 300J, 1W, 300Hz, 10 min. exposure, pulsating emission; and 300J, 1W, 500Hz, 10 min. exposure, pulsating emission, respectively. Group IV did not receive any treatment. The laser biostimulation was conducted for five consecutive days. The data showed good results in terms of cell viability and levels of Ca and Alkaline Phosphate in the groups treated with laser compared to the untreated group. The results

obtained confirm our previous positive in vitro results that the GaAlAs Laser provides biostimulation without cell damage.