

Ga-As (808 nm) Laser Irradiation Enhances ATP Production in Human Neuronal Cells in Culture

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ABSTRACT

Objective: The aim of the present study was to investigate whether Ga-As laser irradiation can enhance adenosine triphosphate (ATP) production in normal human neural progenitor (NHNP) cells in culture. **Methods:** NHNP were grown in tissue culture and were treated by Ga-As laser (808 nm, 50 mW/cm², 0.05 J/cm²), and ATP was determined at 10 min after laser application. **Results:** The quantity of ATP in laser-treated cells was 7513 ± 970 units, which was significantly higher ($p < 0.05$) than the non-treated cells, which comprised 3808 ± 539 ATP units. **Conclusion:** Laser application to NHNP cells significantly increases ATP production in these cells. These findings may explain the beneficial effects of low-level laser therapy (LLLT) in stroked rats. Tissue culture of NHNP cells might offer a good model to study the mechanisms associated with promotion of ATP production in the nervous system by LLLT.

INTRODUCTION

LOW-LEVEL LASER THERAPY (LLLT) has been found to enhance various biological processes *in vivo* and *in vitro*.^{1–3} Modulation of adenosine triphosphate (ATP) production in the cell is suggested as a possible process associated with the beneficial effects of LLLT on cells and tissues. Indeed, in the infarcted/ischemic mammalian heart, LLLT (wavelength 810 nm) was found to significantly preserve mitochondria from damage in the ischemic zone as compared to the non-treated heart.^{4–6} Accordingly, there was also a sixfold increase in ATP production by the cardiomyocytes in the ischemic zone of the laser-treated heart.⁶ In another study, using a relatively high dose (4.8 W/cm² for 15 min) of infrared (830 nm) laser irradiation, ATP was significantly higher in the laser-treated cerebral cortex region relative to the non-treated region.⁷

LLLT has also been shown to biomodulate processes in the nervous system. Anders et al.⁸ recently reviewed the beneficial effects of LLLT (various wavelengths: red [632 nm] and infrared [830 nm]) on functional recovery of injured peripheral nerves. Moreover, it was recently demonstrated that transcranial infrared laser therapy (wavelength 808 nm) applied 6 h after embolic stroke in rabbits and 24 h after ischemic stroke in rats caused a significant improvement of neurological score over sham-treated experimental animals.^{9–11} Light-emitting

diodes (LEDs), at wavelengths of 670, 728, 770, 830, and 880) were shown to regulate cytochrome C oxidase, leading to increased energy metabolism *in vitro* in visual neurons functionally inactivated by toxin (potassium cyanide).¹²

The effect of LLLT on neuronal cells in general and on human progenitor neuronal cells in particular has not yet been investigated. We report here a significant promotion of ATP production by laser irradiation in human neuronal progenitor cells.

METHODS

Cell culture

Normal human neural progenitor (NHNP) cells from Clonetics (Baltimore, MD; catalog no. CC-2599) were thawed and cultured on polyethylenamine (PEI). The cells were plated into 96-well plates (black plastic with clear bottoms; Becton Dickinson, Franklin Lakes, NJ) as spheroids and allowed to differentiate into mature neurons over a period of 2 weeks. An equal number of cells (10⁵) were placed in each well.

Laser treatment

A photon dosing assembly (PDA) was used to provide precisely metered doses of laser light to the NHNP cells in the 96-

well plate. The PDA consisted of a Nikon Diaphot inverted microscope (Nikon, Melville, NY) with a LUDL motorized x,y,z stage (Ludl Electronic Products, Hawthorne, NY). An 808-nm laser (Photothera Inc., Carlsbad, CA; power output of 600 mW) was routed into the rear epi-fluorescent port on the microscope using a custom-designed adapter and a fiber-optic cable. Diffusing lenses were mounted in the path of the beam to create a "speckled" pattern, which was intended to mimic *in vivo* conditions after a laser beam passed through human skin and provide uniform intensity of the laser. The beam diverged to a 25-mm-diameter circle, when it reached the bottom of the 96-well plate. This dimension was chosen so, that a cluster of four adjacent wells could be exposed to the laser at the same time. Stage positioning was controlled by a Silicon Graphics workstation, and laser timing was performed by hand using a digital timer. The measured power density passing through the plate and delivered to the NHNP cells was 50 mW/cm². The duration of irradiation was 1 sec (energy density of 0.05 J/cm²). These optimal parameters of the laser setting were chosen after a series of preliminary experiments (to achieve enhanced ATP production over non-laser-irradiated tissue culture wells) with various power and energy densities of the laser.

ATP determination

CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI) was used to measure the effects of 808-nm laser light on the NHNP cells at 10 min after laser application. This assay generates a "glow-type" luminescent signal produced by a luciferase reaction with cellular ATP. The Cell Titer-Glo reagent was added in an amount equal to the volume of media in the well and resulted in cell lysis followed by a sustained luminescent reaction that was measured using a reporter luminometer (Turner Biosystems, Sunnyvale, CA). Amounts of ATP present in the NHNP cells were quantified in relative luminescent units (RLUs) by the luminometer in each well. The results are expressed as mean \pm standard error of the mean (SEM) of 22 wells for control or laser-treated tissue culture wells. Wells on the same plate were control or laser treated.

Statistical analysis

An unpaired *t*-test was performed on the data.

RESULTS

The average RLUs measured for the control wells was 3808 ± 539 (mean \pm SEM), whereas the laser group showed a twofold increase in ATP content to 7513 ± 970 (Fig. 1). The *t*-test resulted in a *p* value of 0.02.

DISCUSSION

The results of the present study clearly indicate that laser irradiation at the proper power and energy densities can significantly increase ATP production in human neuronal progenitor cells. The power density used (50 mW/cm²) seems to be appropriate to achieve the beneficial effects *in vitro*. It should be noted that this power density is several-fold higher than that

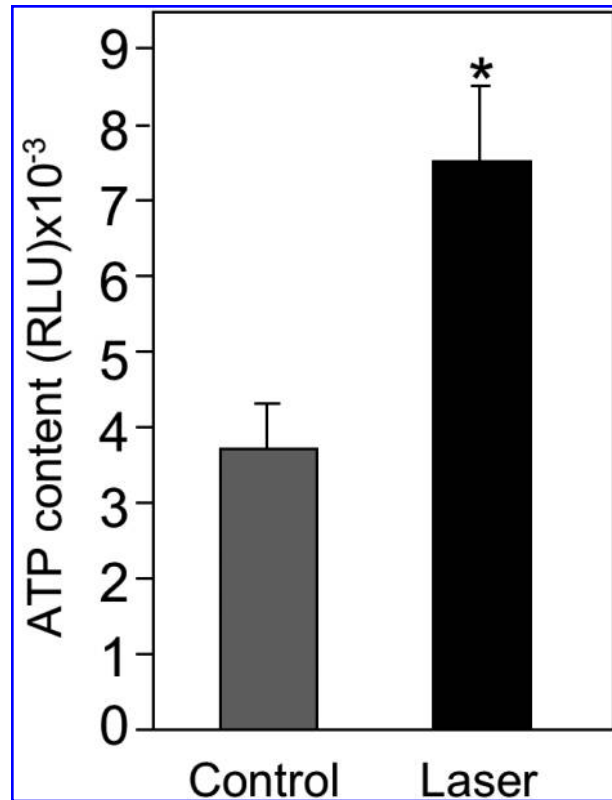


FIG. 1. Adenosine triphosphate (ATP) content in control non-laser-irradiated (dashed column) and laser-irradiated (solid column) human neuronal progenitor cells. Results are expressed as mean \pm standard error of the mean (SEM). **p* < 0.05. RLU, relative luminescent unit.

used (7.5 mW/cm²) for the same type of cells (neurons) *in vivo* in stroked rats.⁹⁻¹¹ Similar findings on differences in dosing of the laser between *in vitro* and *in vivo* were also observed for skeletal muscles.³

The findings of the present study also corroborate results recently published on the positive effects of LLLT on stroked rats and rabbits.⁹⁻¹¹ Increased production of ATP in the treated neurons (and possibly also other cell types in the brain) might contribute to survival of the neurons under ischemic stress conditions in the brain post-stroke. This may in turn partially account for the better functional neurological outcome of the laser-irradiated stroked experimental animals.⁹⁻¹¹ It was also shown in the ischemic heart that LLLT increased production of ATP and better survival of mitochondria in the ischemic zone. The possibility that cells with a better ATP production machinery will also demonstrate upregulation of heat shock proteins and increased antioxidant and antiapoptotic activity (as shown for the ischemic heart and skeletal muscle^{3-6,13}) cannot be ruled out.

The results of the present study may also suggest the *in vitro* system of human neuronal cells as a simple and reliable model to explore the optimal parameters of the laser that may be applied under various physiological conditions of the nervous system. Thus, using the ATP production by the cells as an indicator for beneficial effects, one could investigate the optimal laser irradiation to be applied to neurons in ischemic conditions *in*

vitro or after drug application. This *in vitro* model could save the laborious and costly use of *in vivo* models to explore optimal settings for *in vivo* application of LLLT.

It should be noted that the enhanced production of ATP in the treated neurons was noticeable shortly after treatment at 10 min after laser irradiation of the cells. This rapid response can be beneficial, for example, to neurons in the penumbra (interphase between core and healthy tissue) of the stroke. Light exposure might salvage neurons in the penumbra that are partially injured by the ischemic condition and contribute to cell survival.

CONCLUSION

Low-level laser irradiation enhances ATP production in human neuronal progenitor cells. This phenomenon may partially explain the beneficial effect of laser application obtained in stroked experimental animals. This *in vitro* system for human cells may offer a suitable tool for further elucidation of the mechanism involved in LLLT effects on the nervous system.

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