LOW LEVEL LASER IRRADIATION SHOWS NO HISTOPATHOLOGICAL EFFECT ON MYOGENIC SATELLITE CELLS IN TISSUE CULTURE

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It has been found that low energy laser exposure can affect (biostimulate) various processes in cells and tissues. The effects of direct He-Ne laser on the histopathology of a primary culture of satellite cells isolated from rat skeletal muscle was investigated using electron microscopy. The cultured cells were directly exposed to laser light for 3s, and were prepared for electron microscopy 4 days post-irradiation. Non-laser-irradiated (NLI) cells served as a control. The treated cells exhibited well-developed rough endoplasmic reticulum, mitochondria and other cell components, similar to the NLI satellite cells. Previously, we showed that the same dose of laser therapy enhances satellite cell proliferation twofold. Taken together, we suggest that direct He-Ne laser treatment at the power density that can enhance satellite cell proliferation in culture does not cause histopathological changes in the cells, as manifested by their ultrastructure.

Key Words: Laser Therapy, Skeletal Muscle, Satellite cell, Electron Microscopy

Introduction

Low-energy lasers have been found to modulate various biological processes in tissue cultures and animal models^(1, 2). For example, laser therapy has been found to increase mitochondrial respiration and ATP synthesis^(3, 4), accelerate wound healing⁽¹⁾, and promote the process of skeletal muscle regeneration and the neoformation of blood vessels in the injured zone of skeletal muscle⁽⁵⁻⁸⁾. Recently, we showed that Laser treatment induces early cell cycle regulatory protein expression and proliferation in skeletal muscle satellite cells as well as a delay in their dif-

ferentiation⁽⁹⁾, suggesting that laser therapy promotes muscle regeneration at least in part via activation of satellite cells.

Laser therapy, at the proper power density, has been claimed to have no deleterious effects on cells or side effects when used clinically in humans⁽¹⁰⁾. However, there have been no studies evaluating the possible adverse effect of direct laser treatment on cells in tissue culture. Primary cell cultures are considered to be a sensitive system for evaluating possible agents or factors that

may be toxic to cells or may cause adverse effects. In the present study, these effects were analyzed in a primary satellite cell culture derived from rat skeletal muscle⁽⁹⁾ using electron microscopy.

Methods

Cell Preparation

Rat satellite cells were purified from hind-leg muscles of 3-week-old Charles River rats as described previously⁽⁹⁾. Briefly, the muscles were excised, trimmed of fat and connective tissue, and chopped with scissors. The tissue was digested for 1 h at 37°C with 1.25 mg/ml pronase (Boehringer, Mannheim, Germany) and then with 0.25% (w/v) trypsin for another 30 min at 37°C. Tissue fragments were collected by centrifugation at 1500 x g for 5 min and then triturated through a fine pipette. An enriched population of myogenic cells was recovered by differential centrifugation and the pellet was resuspended in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% horse serum (HS). The culture was then passed through a nylon membrane (200 µm) and preplated for 2 h on uncoated cell-culture dishes to enrich the myogenic cell population. Immediately after plating onto petri dishes, cells were irradiated for 3 s (see reference 9). Cells were then counted and plated on 0.1% gelatin-coated plates and maintained in DMEM containing 10% HS.

Laser Irradiation

Cell-containing plates were irradiated through a grid composed of 1.8 mm x 1.8 mm squares to ensure precise irradiation over the entire tissue-culture plate, using a He-Ne laser (632.8 nm, 4.5 mW; 1.8 mm beam diameter; Ealing Electro-Optics, Holliston, MA) at a power density of 180 mW/cm². Primary satellite cells were irradiated

immediately after the preplating period in which cells were not suspended but laid on the bottom of the dish. Cells were then counted and seeded on 35-mm plates. Control, non-irradiated cells were kept in the same conditions as the LASER TREATED cells.

Electron Microscopy

Cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 24 h. Following extensive washing in 0.2 M cacodylate buffer, the cells were stained *en bloc* with uranyl acetate, post-fixed in 1% OsO₄ and embedded in Epon. Cells were separated from the culture dish by several freeze (-70°C)-thaw cycles. Sections were then cut, mounted on grids and viewed in the electron microscope.

Results

Electron microscopy of the satellite cells 4 days post laser irradiation or non-irradiated cells is demonstrated in Figs.1 and 2. It can be seen (Fig 1a) that the NLI cells have a spindle shape appearance and some of them were fusing with neighboring cells, typical for satellite cells at day 4 in culture⁽⁹⁾. The mitochondria were numerous and usually around the nucleus. Ribosomes and polyribosomes organized in rosette-like structures are scattered throughout the cytoplasm. The rough endoplasmic reticulum and mitochondria are the predominant organelles in the cytoplasm of the satellite cells (Figs. 1A, 2A). In general, the electron microscopy characteristics of the laser-irradiated cells did not differ from the non-irradiated cells (Figs.1a vs. 1b, and 2a vs. 2b). Furthermore, as seen by high power magnification, the ultrastructural features of the intracellular structures (mitochondria, rough endoplasmic reticulum, etc.) were similar to those of the NLI satellite cells (Figs. 2 a & 2b).

Fig 1A: Electronmicrograph showing a group of control non-irradiated cells. Resin section stained with uranyl acetate and lead citrate. Magnification: X 3,300. Scale bar = 10 μ m.

Fig 1B: A group of three laser irradiated cells. Note that the ultrastructure of the cell components is indistinguishable from that of the controls. Resin section stained with uranyl acetate and lead citrate. Magnification: X 3,300. Scale bar = 10 μ m.

Fig 2A: Higher magnification electronmicrograph showing typical cytoplasmic components of a non-irradiated cell. Ribosomes and polyribosomes are scattered throughout the cytoplasm, and rough endoplasmic reticulum (RER) and mitochondria (Mit) are the predominant organelles. Resin section stained with uranyl acetate and lead citrate. Magnification: X 25,000. Scale bar = 1.0 μ m.

Fig 2B: Higher magnification electronmicrograph showing typical cytoplasmic components of an irradiated cell. The cell contents appear essentially the same as those in the control non-irradiated cell above. Mit = mitochondria, RER = rough endoplasmic reticulum. Resin section stained with uranyl acetate and lead citrate. Magnification: X 25,000. Scale bar = 1.0 μ m.

Discussion

This is the first study to report the absence of histopathological changes up to 4 days post-laser irradiation with a power density (180 mW/cm²) previously shown to be optimal for the promotion of proliferation of these cells in culture⁽⁹⁾. The fact that the same level of irradiation enhanced satellite cell proliferation further supports the notion that low intensity laser treatment does not damage the cell machinery. The results of the present study may also have implications on laser irradiation *in vivo*. The power density of low intensity laser *in vivo* decreases markedly with tissue depth due to dispersion of the laser beam and absorption of the energy by the cell layers⁽¹¹⁾. Thus, it could be postulated that the cells *in vivo* receive the laser irradiation at an energy, that is similar to (for the first layer of cells that is directly exposed to the laser) or lower than (for deeper layers) that received by cells in culture. Taken together, it may be proposed that low intensity laser treatment will have no deleterious effects (in terms of histopathological changes) on cells *in vivo*. Furthermore, we previously employed the same He-Ne laser irradiation with the same power density in *in vivo* studies, and achieved enhancement of skeletal muscle regeneration⁽⁵⁻⁸⁾, bone repair⁽¹²⁾, and beneficial effects on the ischemic heart⁽¹³⁾.

The present study contributes to knowledge about the safety of using low intensity lasers in medicine for external use, as well as for its application to internal organs by either direct exposure (i.e. during surgery) or delivery of the energy via optic fiber. Indeed, we have recently demonstrated that low intensity laser treatment delivered to infarcted myocardium can cause a profound reduction in the infarcted size⁽¹⁴⁾ and

also has beneficial effects on the ischemic heart in mice, rats and dogs⁽¹³⁾.

In conclusion, the results of the present study indicate that direct irradiation of cells *in vitro* with low-energy laser at an energy density that enhances their proliferation, does not have destructive effects on the cells as manifested by histopathological analysis by electron microscopy. Thus, this power density of laser irradiation can be considered safe for primary cultures and most probably for cells *in vivo*.

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