The effects of low level laser therapy on irradiated cells: a systematic review.

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ABSTRACT

The aim of this systematic review was to elucidate the effects of low level laser on irradiated cells concerning cell structure, viability and DNA damage. A search of health science databases (Cochrane Library and PubMed) was performed. The main key words used were "Low level laser therapies" [Mesh] OR "low intensity laser therapy" OR "low energy laser therapy" OR phototherapy AND "ultra structural changes" OR "ultra structural analysis" OR mitochondria [Mesh] OR "DNA damage" [Mesh]. The inclusion criteria comprised laboratory studies that used LLL with its wavelength ranging from 632 to 1064 nm and a delivery of 1 to16 J/cm², evaluating the cell structure by means of DNA or ultra-structural analysis. The articles selected were carefully read and data of interest were tabulated. Eight studies were included in this review. Three articles have performed cell viability tests showing an increase in viability when low fluences (5 J/cm²) were applied and a decrease with higher fluences (higher than 10 J/cm²). Five articles used transmission electron microscopy to identify any type of ultra structural morphology alteration in irradiated cells and a few differences were found such as the presence of giant mitochondria, and several cytoplasmic collagen-containing phagosomes. Two articles performed a DNA test (Comet Assay) and verified that damage appears when using higher fluences (higher than 10 J/cm²). The effects of LLL irradiation on cell structure and on its DNA vary depending on the laser parameters of irradiation. Adequate dosages can accelerate wound healing, stimulate cell proliferation and decrease the DNA damage. Lower dosages do not seem to stimulate cells and higher dosages seem harmful to cell viability and increase DNA damage.

INTRODUCTION

The use of low intensity laser as a therapeutic modality was originally introduced in Europe by Professor Endre Mester who reported its earliest clinical application in Medicine in 1968. He described that the healing process of chronic ulcer was faster when it was irradiated with argon laser [1]. Since this study was published, the number of studies on the medical application of low-level laser therapy has grown steadily and there has been an increasing clinical use of laser, in vivo and in vitro, for a variety of medical conditions [1].

The laser light emitted is polarized and coherent and may be absorbed by different tissues [1]. Tissue biostimulation is only possible if irradiated cells have molecular photoacceptors that absorb light and enter into a state of excitation triggering an intracellular cascade of signals leading to a measurable biological effect [2]. The transduction of the primary photosignal and its amplification in the cell leads to a photobiological macroeffect, such as an increased cell proliferation or DNA synthesis. Irradiation of cells at certain wavelengths can also activate some inner components and biochemical reactions, and the whole-cell metabolism can be altered [3]. Some studies revealed a new ultra structural conformation of irradiatied cell organelles [4,5] and cell morphology [6-8].

The possible damaging effects of laser irradiation are still highly contested. Light absorption induces the production of reactive oxygen and nitrogen species that are involved in subsequent free radical reactions and lead to a modification of biomolecules and changes in cell function [9].

The present systematic review was focused on this question: what are the effects of low level laser on irradiated cells concerning cell structure, viability and DNA damage?

METHODS

A literature search was performed on Cochrane Library and PubMed. The main key words used were "low level laser therapies" [Mesh] OR "low intensity laser therapy" OR "low energy laser therapy" OR phototherapy AND "ultra structural changes" OR "ultra structural analysis" OR mitochondria [Mesh] OR "DNA damage" [Mesh]. The inclusion criteria comprised laboratory studies that used LLL with wavelengths ranging from 632 to 1064 nm and a delivery of 1 to16 J/cm², evaluating the cell structure by means of DNA or ultra-structural analysis. The articles selected were carefully read and data on the following issues were extracted and

Author and year	Experimental model	Laser type and wavelength	Fluence applied (J/cm²)	Viability in irradiated cells (pM ATP*)	Viability in NI cells and survival rate (pM ATP*)	Survival rate in irradiated cells	Survival rate in NI cells	Ultra structure and cell organization in irradiated cells in comparison with control (NI)	DNA damage in irradiated cells	DNA damage in NI cells	Evaluation method
Bayat, 2007	Rabbits	He-Ne (632,8 nm)	13	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Increased number and depth of filopodia and increased density of fibrillary fletwork of extracellular matrix	Not evaluated	Not evaluated	TEM
Delbari, 2007	Rats	He-Ne (632,8 nm)	0.01 1.2	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Larger collagen fibril diameter	Not evaluated	Not evaluated	TEM
De Araújo, 2007	Rats	He-Ne (632,8 nm)	1	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Faster organization of collagen fibrils and inflammatory response	Not evaluated	Not evaluated	TEM
Houreld, 2007	Human fibroblasts	He-Ne (632,8 nm) Diode (830 nm)	5 16	Not evaluated	Not evaluated	96% 90%	95%	Not evaluated	Not evaluated	Not evaluated	Trypan Blue exclusion and apoptosis
Hawkins, 2006	Human fibroblasts	He-Ne (632,8 nm)	0.5 2.5 5 10 16	9.38 10.68 10.46 8.16 8.72	10.31	Not evaluated	Not evaluated	Not evaluated	36.53% 35.82% 32.53% 34.15% 34.55%	33.63%	Comet Assay and CellTiter-Glo
Bortoletto, 2004	Culture cells Hep-2	GaAlAs (635 nm)	10	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Mitochondrial alteration produced increase in ATP synthesis and granular aspect within the first 24 hours.	Not evaluated	Not evaluated	TEM
Kujawa, 2004	Culture cells B14 (hamsters) and human erytrocites	CTL 1106MX (810 nm)	3.75 7.50 11.25 15.00	Not evaluated	Not evaluated	— 98% 96% 95%	100%	Not evaluated	11.5% 13.2% 17.9% 21.8%	11.1%	Method of Mosman and Comet Assay
Manteifel, 1997	Lymphocytes	He-Ne (632,8 nm)	56	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Reduced number of mitochondria, but increased area (giant mitochondria)	Not evaluated	Not evaluated	TEM

*pM ATP indicates the amount of energy a cell can produce, more energy means that the cell is working well, in abnormal condition.
NI= non irradiated cells; TEM= Transmission Electron Microscopy
Cell survival % indicates how many cells were still alive and in normal condition after laser irradiation.

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tabulated: author and publication year; laser type and wavelength, fluence, cell viability, survival rate, ultra structure and DNA damage.

RESULTS

Search for articles retrieved 59 articles, but only eight met the inclusion criteria after title and abstract reading (Manteifel, 1997; Bortoletto, 2004; Kujawa, 2004; Hawkins, 2006; De Araújo, 2007; Bayat, 2007; Delbari, 2007; Houreld, 2007) [10-13,7,8,6,14]

Cell viability

In three of the eight articles included, cell viability tests were performed [12-14]. The results presented an increase in cell viability (%) when low fluences were applied and a decrease with higher fluences.

In the study performed by Hawkins [13], the cell viability assay showed that normal cells exposed to a single dose on 2 consecutive days responded with an increase in cell viability after 0.5 J/cm^2 (P=0.033) and 5 J/cm^2 (P=0.046), while at higher doses (10 and 16 J/cm^2) there was a decrease in cell viability. A dose of 2.5 J/cm^2 did not increase or decrease cell viability when compared with the normal non-irradiated control.

Kujawa [12] affirmed that light irradiation did not produce any substantial change in cell survival. Houreld [14] observed that diabetic-wounded fibroblast cells [human skin fibroblast cell WS1 maintained in a diabetic-induced condition obtained by adding glucose in culture medium (17mMol/l) and characterized by the presence of a central scratch in the monolayer that simulates the wound] irradiated at a wavelength of 632.8 nm with a fluence of 5 J/cm2 showed a significant increase in viability when compared to diabetic-wounded non-irradiated cells (P=0.001) and cells irradiated with 16 J/cm2. Cells irradiated at a wavelength of 830 nm with a fluence of either 5 or 16 J/cm^2

and incubated for one hour showed no significant change in the viability percent. Cell irradiated with 16 J/cm^2 to all three wavelengths showed a decrease in viability. On the other hand, diabetic-wounded cells irradiated with 16 J/cm^2 showed a significant increase in caspase-3 and 7 in comparison with normal and diabetic-wounded non-irradiated cells (P<0.000) and cells irradiated with 5 J/cm^2 (P=0.021). When irradiated at a wavelength of 1064 nm with either 5 or 16 J/cm^2, there was no significant change in viability, although cells irradiated with 16 J/cm^2 showed a decrease when compared with normal and diabetic wounded non-irradiated cells (P=0.066 and P=0.076, respectively) [14]. The results were summarized in Table 1.

Cell ultra structure and organization

Five articles used transmission electron microscopy to identify any type of ultra structural morphology alteration in irradiated cells [5-8,10,11]. The articles were divided into two subgroups: the first with two studies, which focused their analysis on mitochondria [5,11], and the second with the other three, which concentrated on cell morphology and characteristics [6-8].

Mitochondrial alteration after irradiation produced an increase in ATP synthesis and granular aspect during the first hours, but after 24 hours the aspect was similar to that of the control group [11]. Another aspect was the presence of giant mitochondria in the irradiated group; the number of mitochondria was reduced when compared with non-irradiated cells, but the total area of all mitochondria was similar for experimental and control groups [5].

Delbari [6] studied the difference between fibril diameter of transected medial collateral ligament (MCL) in rats irradiated and non irradiated with He-Ne laser and observed that fibril diameter in the irradiated group was larger than in control group but density was similar. Bayat [7] used transmission electron microscopy

on rabbit articular cartilage to evaluate chondrocytes and demonstrated that the nucleus of control and experimental groups presented the same characteristics: euchromatin nucleus in all cells. He observed a significant difference in the quantity and depth of filopodia, which means that irradiated chondrocytes worked more than normal chondrocytes. Another observation was that the experimental group had a moderate density of fibrillary fletwork of extracellular matrix while the control group presented a low density. De Araújo [8] observed that laser radiation reduced the local inflammation and appears to influence the organization of collagen fibrils in the repairing areas. The results were summarized in Table 1.

DNA damage

Two articles performed the Comet Assay test to verify DNA damage [12,13]. Damage appeared when high fluences were applied: Kujawa [12] observed DNA damage using 15 J/cm^2 and Hawkins [13] using 10 J/cm^2 and 16 J/cm^2. On the other hand, dosages that are too low do not seem to stimulate wounded fibroblast cells (cells characterized by the presence of a central scratch in the monolayer that simulates the wound). Hawkins [13] found DNA damage on wounded fibroblast cells irradiated by 0.5 J/cm^2 .
The dose used was possibly too low to stimulate cell function restoration and to initiate the healing process. When different doses were used in this study (2.5, 5 and 10 J/cm^2), there was no significant increase in DNA damage. On the contrary, there was a decrease in DNA damage when a dose of 5 J/cm^2 was used, indicating a repair process [12]. These results indicates that, as the dose increases beyond the optimum dose, the amount of DNA damage also increases [12,13]. The results were summarized in Table 1.

DISCUSSION

As we approach the fiftieth year of laser therapy use, there are still many open

questions such as the exact mechanism of its action; the correct dosage for a certain medical condition; the effect in cellular viability; DNA damage; and influence on surrounding tissues. This review was focused on verifying the effects of LLL irradiation on cell structure and organization and possible DNA damage.

Five articles used transmission electron microscopy to identify ultra structural morphological alterations in irradiated cells [5-8,10,11]. No harmful effects were observed in these studies. Only desired effects, such as the presence of giant mitochondria [5], increased fibril diameter [6], increased number and depth of filopodia and increased density of fibrillary network of extracellular matrix [7], faster organization of collagen fibrils and inflammatory response [8] were observed.

The selected articles reported the observation of significant DNA damage when high fluences were applied (higher than 10 J/cm2) and an inability to heal wounded cells when applying low fluence [12,13]. An adequate dosage maintained cell viability, improved cell proliferation and slightly decreased DNA damage [13]. The secret of LLL therapy seems to be the choice of the adequate type of laser and its parameters of irradiation. Using the correct laser device, wavelength, fluence rate and radiant exposure, no degenerative or lethal alterations occur within the cells or the tissue surrounding the irradiated areas [5-8,11,12-14].

CONCLUSION
This review concluded that the effects of LLL irradiation on cell structure and on its DNA vary depending on the laser parameters of irradiation. Adequate dosages can accelerate wound healing, stimulate cell proliferation and decrease DNA damage. Lower dosages do not seem to stimulate cells and higher dosages seem to be harmful to cell viability and increase DNA damage.

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