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In vitro study on the safety of near infrared laser therapy in its potential application as postmastectomy lymphedema treatment

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ABSTRACT

Clinical studies demonstrated the effectiveness of laser therapy in the management of postmastectomy lymphedema, a discomforting disease that can arise after surgery/radiotherapy and gets progressively worse and chronic. However, safety issues restrict the possibility to treat cancer patients with laser therapy, since the effects of laser radiation on cancer cell behavior are not completely known and the possibility of activating postmastectomy residual cancer cells must be considered.

This paper reports the results of an *in vitro* study aimed to investigate the effect of a class IV, dual-wavelength (808 nm and 905 nm), NIR laser system on the behavior of two human breast adenocarcinoma cell lines (namely, MCF7 and MDA-MB361 cell lines), using human dermal fibroblasts as normal control. Cell viability, proliferation, apoptosis, cell cycle and ability to form colonies were analyzed in order to perform a cell-based safety testing of the laser treatment in view of its potential application in the management of postmastectomy lymphedema. The results showed that, limited to the laser source, treatment conditions and experimental models used, laser radiation did not significantly affect the behavior of human breast adenocarcinoma cells, including their clonogenic efficiency. Although these results do not show any significant laser-induced modification of cancer cell behavior, further studies are needed to assess the possibility of safely applying NIR laser therapy for the management of postmastectomy lymphedema.

1. Introduction

Postmastectomy lymphedema is a discomforting disease [1] whose incidence is approximately 5% after surgery and can increase to 30% following radiotherapy.

Lymphedema is a chronic condition that progressively worsens. It is characterized by increased protein content, excess of intra- and extracellular fluids in the tissues, surplus deposition of fibrous tissue and chronic inflammation, which result in swelling and deformity of the upper limb, accompanied by a brawny edema. The symptoms are: limb heaviness, weakness, pain, restricted shoulder mobility, burning and elevated skin temperature. Lymphedema has a serious adverse impact on quality of life of the patients and can lead to psychological morbidity [2].

Standard treatments for lymphedema include pressotherapy, compression bandaging, manual lymphatic drainage, exercise and skin care. These treatments, generally applied in combination with each other to form a multifaceted intervention known as Complete Decongestive Therapy (CDT), are expensive, time-consuming, require qualified medical professionals, are poorly accepted by patients and have limited effectiveness [3–5].

The majority of alternative methods for the management of lymphedema falls into the category of the Physical Agent Modalities (PAMs), which can be classified as thermical, mechanical or electromagnetic [5]. In a recent review aimed at evaluating the

Abbreviations: NIR, Near-Infrared; CDT, Complete Decongestive Therapy; PAMs, Physical Agent Modalities; MLS, Multiwave Locked System; PI, Propidium Iodide; ROS, Reactive Oxygen Species; LLLT, Low Level Laser Therapy.

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effectiveness of the PAMs, the authors concluded that the current body of published literature lacks sufficient quality to provide the evidence to support the majority of alternative treatment modalities to enhance current treatment protocols. However, some studies on the application of laser therapy to the management of postmastectomy lymphedema met the criteria to be defined "likely to be effective", although the sample size was limited and further research is needed to support a recommendation for practice [5].

Indeed, double-blind, placebo-controlled trials [6–8] have shown that Low Level Laser Therapy (LLLT) can be effective in improving postmastectomy lymphedema symptoms. In these studies, the treatments consisted in irradiating (λ = 904 nm, energy density 1.5 J/cm^2) from 10 to 17 points distributed in the diseased area, with a number of sessions that ranged from 9 to 36. The results showed a significant reduction in the volume of the affected arm [6,7] as well as extracellular fluids and tissue hardness [6], improved shoulder mobility and grip strength [7]. The improvement observed in treated patients, compared to controls, remained significant at 2–3 months after treatment [6,7].

Kozanoglu et al. [8] compared the long-term efficacy of pneumatic compression and LLLT in the management of postmastectomy lymphedema. They found that both treatments were effective in reducing the volume of the limb at the end of the treatment cycle, at 3 and 6 months, but the improvement was greater in the group of patients treated with LLLT. At 12 months, the beneficial effects (decrease of swelling and pain) were still significant only in this group.

A previous study with different treatment modality [9], in which a dual wavelength (632 nm and 904 nm) scanning laser was used and higher energy densities (2–4 J/cm^2) were applied, had already highlighted the effectiveness of laser therapy in significantly reducing both the extracellular fluid and total volume of the affected arm, inducing fibrosis softening and improving heaviness, aching and tightness. Two years follow up showed that volume reduction and tonometry persisted over time [10].

Other studies, carried out using different NIR laser sources (single and double wavelength, wavelengths ranging from 808 nm to 905 nm) and energy densities similar to those applied in the studies reported above, further confirmed that laser irradiation induced an improvement in lymphedema [11–15].

Therefore, laser therapy has been proposed as an alternative treatment for the management of postmastectomy lymphedema.

Indeed, the effectiveness of laser therapy in the treatment of edema resulting from various causes (trauma, inflammation, surgery, etc.) is known for a long time and has been confirmed by recent studies [7,16–19].

The mechanisms underlying the anti-edema effects of laser therapy are not completely known, but it has been reported that laser radiation may act on microcirculation [20] and lymphatic vessels [10], affects the regulation of inflammation [21], the behavior of fibroblasts [22,23] as well as the production and assembly of extracellular matrix molecules [24,25].

Although there are indications in favor of the application of laser therapy in the treatment of postmastectomy lymphedema and the development of advanced laser systems can further improve the effectiveness of the treatments, safety still remains an open issue. In fact, despite the observation that laser therapy has already been widely used to treat complications developing in cancer patients after surgical tumour resection, chemo- and radiotherapy [26], the long-term safety has been poorly studied. The above clinical studies on laser application in postmastectomy lymphedema did not report the occurrence of adverse effects related to laser exposure during the follow-up period, generally $\leqslant$12 months, but only two studies reported data related to a period $\geqslant$2 years and only one of them reported recurrence and survival at 5 and 10 years [9,15].

On the other hand, the impact of laser radiation on cancer cell behavior is partly unknown and the abundant literature presents controversial results. The main question is whether or not laser irradiation increases proliferative rate and invasiveness of tumor cells, which would constitute an adverse effect of laser radiation that could be potentially harmfull in oncologic patients.

Therefore, it is desirable to increase the limited safety information in order to develop guidelines for laser application in postmastectomy lymphedema.

Although molecular mechanisms induced by laser radiation are not completely understood, they involve mitochondrial photo-acceptors and production of Reactive Oxygen Species (ROS) [27–30]. Furthermore, laser radiation might affect cell cycle progression, protein synthesis, cell energy metabolism, proliferation and apoptosis by modulation of specific kinases and phosphatases [21,28,31].

The effect of laser radiation on tumor cell growth, cell cycle progression and apoptosis has been widely studied and compared to the effect produced on non-neoplastic cells by the use of many different sources (generally low power lasers), wavelengths (mostly red and NIR radiation), energy doses (from tenths to tens of J/cm^2) and treatment modes. These studies produced a large amount of data, sometimes conflicting and difficult to compare, because they were obtained under different conditions, but still very important in the common goal of helping to define systematically guidelines for safely use laser sources when oncologic patients are treated. Some of these results are reported in Table 1 and show that the effects differ not only by changing the treatment conditions but also by applying very similar treatment conditions in different cell populations

The heterogeneity of results suggests:

(1) the need to further increase the current knowledge in this field;
(2) the need of assessing suitable experimental models on the safety of the laser sources and treatment parameters before application in oncologic patients.

In the last decade the application for therapeutic purposes of multi-wavelength sources and high-power lasers has become widespread. In rehabilitation, physical- and sports medicine, these devices are effectively used to decrease inflammation, promote resorption of edema and hematoma as well as stimulate tissue repair. They have been already used to treat secondary disorders in cancer patients [32], but studies on the effects of these emissions on tumor cells are limited.

The aim of the present study was to evaluate the *in vitro* safety (cell-based safety testing) [33] of a high power (Class IV), dual-wavelength, NIR laser system in view of its potential application in the management of postmastectomy lymphedema. After exposure to laser radiation, the behavior of human breast adenocarcinoma cells (MCF-7 and MDA-MB361 cell lines, both characterized by epithelial like morphology) was studied in terms of viability, proliferation, cell cycle progression, apoptosis and cloning efficiency. Human diploid fibroblasts were used as a control of non-cancerous cells.

2. Materials and methods

2.1. Cell cultures

Dermal diploid fibroblasts (a kind gift from Dr. Mocali A., Department of Experimental and Clinical Biomedical Sciences, University of Florence, Italy) were derived from 2-mm punch biopsies taken up from the upper arm of female healthy donors with patient informed permission. Human breast adenocarcinoma cell