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Brief Report

The Effect of Laser Therapy on the Proliferation of Oral KB Carcinoma Cells: An *in Vitro* Study

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ABSTRACT

Objective: **The aim of this study was to assess the proliferative effect of carcinoma cells, strain KB, submitted to laser therapy with wavelengths of λ685nm (31 mW; Ø; 0.38 cm², 4 J/cm²) or λ830nm (34.5 mW; Ø; 0.38 cm², 4 J/cm²).** ***Background Data:*** **It is known that the interaction of laser light with living tissues may lead to different results depending upon several factors such as wavelength, dose, potency, and optical properties of the tissue as well as on the condition being treated. The response to the use of laser light may be of stimulation or inhibition. One successful model used to study the effects of laser light on living tissues is the *in vitro* use of different lineages of cells in culture.** ***Methods:*** **Cellular viability was assessed using MTT spectroscopy immediately, and 6, 12, 24, and 72 h after treatment. The irradiations were carried out twice, at 24 h after cell seeding and at 48 h after the first irradiation. The dose of 4 J/cm² was given by a λ685 nm (31 mW, ϕ 0.8 cm²) or λ830 nm (34.5 mW, ϕ 0.8 cm²) diode lasers.** ***Results:*** **The results demonstrated that the time influenced significantly both control ($p = 0.01$) and both cultures irradiated with λ685-nm laser ($p = 0.01$) or λ830-nm laser ($p = 0.09$). The influence of the treatment (laser therapy) was also significant when comparing the results observed in irradiated groups and the control ($p = 0.01$). The influence of the wavelength in the final result, in other words, in the cellular viability of cultures irradiated with the two wavelengths was also significant ($p = 0.01$).** ***Conclusions:*** **It is concluded that laser therapy had a positive biomodulatory effect on the proliferation of KB cells and that this was influenced by the wavelength.**

INTRODUCTION

LASER LIGHT is a peculiar type of electromagnetic radiation, which presents very specific properties and differs from conventional light sources, that is now being widely used in multiple medical applications. It is known that the interaction of laser light with living tissues may lead to different results depending upon several factors, such as wavelength, dose, power, and optical properties of the tissue as well as on the condition being treated. The response to the use of laser light may be stimulation or inhibition. One successful model used to study the effects of laser light on living tissues is the *in vitro* use of different lineages of cells in culture.

Laser therapy demands specifics regarding wavelength, dose, and potency, as the effects produced depend widely on these factors. Dose is considered one of the most important parameters for laser therapy. However, wavelength and power are also of importance for the outcome of the treatment. Laser therapy carried out with the use of therapeutic wavelengths seems not to result in mutagenic effects and may be used repeatedly, without risks.[1] Another advantage of laser therapy is that it may be used as the main treatment or may be a coadjutant of other treatments.

The use of high doses of energy has been considered a potential inhibitor of cellular proliferation within a narrow band of energy and intensity.[2] It is recognized that laser effects are

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limited to a narrow band of the visible spectrum, ranging from λ600 to 700 nm, which appears to be particularly effective for laser therapy.[3] The magnitude of the proliferative effect depends on the wavelength used as well as on the physiologic status of the cell at the irradiation time.[4]

Laser therapy is mostly associated with cell proliferation,[14] however, as laser light is absorbed by chromophores, producing photophysical or photochemical effects that, together or isolated, increase cell stimulation at the mitochondrial membrane level, leading to an increase of both the membrane potential and photonic gradient, and consequently changing optical properties of the mitochondria. This change results in higher activity of NADH, which results in an increase of the production of ATP, RNA, and protein synthesis by the mitochondria.[6]

Previous reports on the effects of the use of laser therapy on cellular proliferation showed that, in HEp-2 cells irradiated with λ635 or λ670 nm using different doses over 7 days, both dose and wavelength influenced cell proliferation, and λ670-nm laser light induced higher proliferative rates.[1]

Several studies have been carried out in order to demonstrate the effect of laser therapy on the stimulation of proliferation cell lines of tumors, and this remains a controversial topic. The effect of laser therapy on cell proliferation has been shown in cell cultures, with conflicting results, as effects have been inhibitory, proliferative, or neutral.

The aim of this study was to assess the effect of two different wavelengths on the proliferation of oral carcinoma cells, strain KB, under nutritional stress using the MTT assay.

MATERIALS AND METHODS

KB cells (Cell Bank, Federal University of Rio de Janeiro) were kept frozen at −80°C.[8] After defrosting, the cells were kept in a DMEN medium, with added 20% FBS, at a pH of 7.8, using 10% PBS, 1% L-glutamine, and a 1% antibiotic solution (250 μg/mL streptomycin and 80 mg/mL gentamycin sulfate). The cells were cultured in plastic culture flasks as described previously.[4,12] Cell cultures were re-planted twice a week with DMEN medium, with added 10% FBS, 1% L-glutamine, and a 1% antibiotic solution. Before any experimentation, all cultures were observed under light microscopy, and viability of the culture was confirmed three times using the Trypan blue exclusion test.[4] For cell assay, viability was calculated by the following equation: viability = number of living cells ×100/number of dead cells. At 24 h before irradiation, 50 μL of a cell suspension (2×10^4 cells/mL) was kept in 24 wells of the 96-well plaques containing DMEN medium, with added 5% FBS, 1% L-glutamine, and a 1% antibiotic solution in order to avoid light dispersion to others. The suspension was then irradiated with a dose of 4 J/cm^2 by a λ685-nm (31 mW, ϕ 0.8 cm^2) or a λ830-nm (34.5 mW, ϕ 0.8 cm^2) diode laser at 48-h intervals. The assessment of the cell proliferation was carried out using the MTT method,[7–9] where spectroscopy (λ570 nm) was used to determine the optical density (OD) of both irradiated and control samples.[4]

Statistical analysis

The results were statistically analyzed using the GraphPad Instat&Reg; program. The Kruskal-Wallis test was chosen because of the small sample size.

RESULTS

The results of the MTT assay carried out on non-irradiated and irradiated cells lines can be seen in Table 1 and Figures 1–3.

When the groups were compared simultaneously, a similar decrease of cellular viability was observed in the first 6 h. At 12 h, an increase of cellular population in the cultures irradiated with λ830 nm and in the controls was observed, and in the group irradiated with λ830 nm the increase was detectable up to 48 h. This was not observed in cultures irradiated with λ685 nm or in controls (Fig. 4).

The results demonstrated that time influenced significantly both controls ($p = 0.01$) and the cultures irradiated with λ685-nm ($p = 0.01$) or λ830-nm ($p = 0.09$) lasers. The influence of the treatment (laser therapy) was also significant when comparing the results observed in irradiated groups and the control ($p = 0.01$). The influence of the wavelength on cellular viability of cultures irradiated with the two wavelengths was also significant ($p = 0.01$).

DISCUSSION

Controversies still exist in relation to the effects of the use of laser therapy on biological tissues as conflicting results have been reported in the literature worldwide. On the other hand, it is known that laser therapy has beneficial effects on tissues. Results of previous reports on both humans and animals pointed out several clinical effects such as reduction of both

TABLE 1. MEAN VALUES AND STANDARD ERROR OF THE ABSORBANCE OF THE KB CELLS IN CULTURE

Time/group	*Control*	*685 nm*	*830 nm*
0 h	0.157 ± 0.008	0.138 ± 0.006	0.129 ± 0.003
6 h	0.127 ± 0.006	0.120 ± 0.007	0.122 ± 0.005
12 h	0.138 ± 0.010	0.109 ± 0.008	0.126 ± 0.008
24 h	0.104 ± 0.006	0.101 ± 0.005	0.114 ± 0.004
48 h	0.094 ± 0.005	0.086 ± 0.003	0.102 ± 0.006
72 h	0.095 ± 0.005	0.090 ± 0.002	0.102 ± 0.005

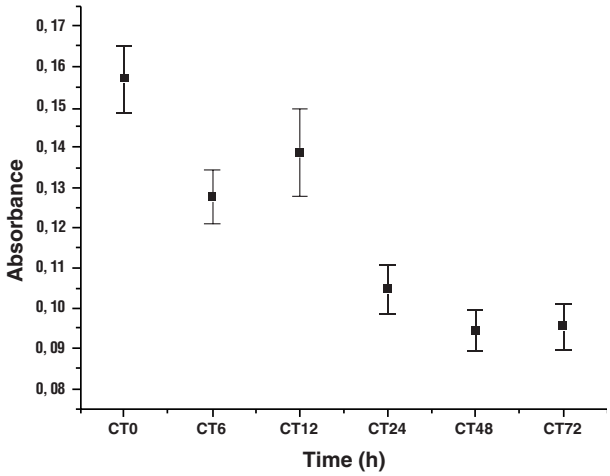

FIG. 1. Result of MTT assay of KB cell not irradiated by laser light. A decrease of the cellular proliferation within the first 6 h of laser irradiation was seen, and it was followed by an increase in the cellular proliferation after 12 h and soon after a progressive decrease of the cellular viability up to 48 h. At the end of the experimental period (72 h), a discreet increase in cell proliferation was seen.

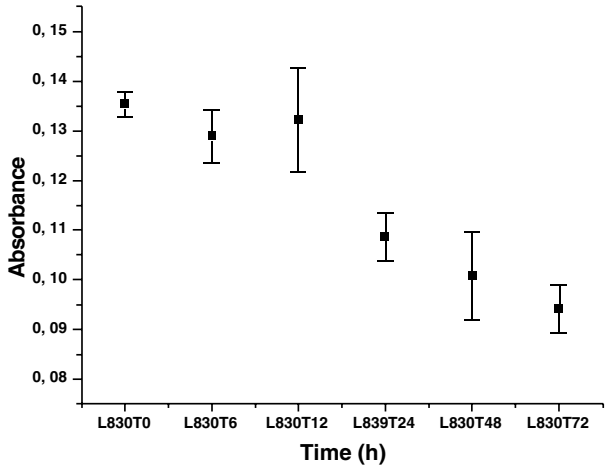

FIG. 3. Result of MTT assay of KB cell irradiated with λ830-nm laser light. A decrease of the cellular viability in the first 6 h after the irradiation was followed by an increase in the viability up to 12 h. A decrease in the count was seen up to the end of the experimental period.

inflammation and pain, and also on the proliferation of cells.[13] Studies have shown that the effect on cells is a result of improved local circulation, which results in better nutritional status of the tissue as well as in increased cell activity probably due to a higher production of ATP induced by laser light.[5]

In the present study, it the influence of time was assessed in the proliferative process of KB cells submitted to λ685 or λ830 laser light (dose of 4 J/cm^2) using the MTT assay. The results demonstrated that the cells, independent of the effect of the laser irradiation, obeyed their regular life cycle for this lineage as described previously.[10] This cell line presents a logarithmic phase growth in approximately 30 h in culture medium containing 10% human serum.[11] The results of the present study show that nutritional status (5% BFS) influenced the proliferation as described previously by our group in HeP-2 cell lines.[12] A decrease in cell number in the first 6 h was seen, which was followed by a quick increase after 12 h and subsequent decreases. The increase on the proliferation rate may be a result of the effect of the laser therapy on the cells. The proliferative effect observed in HeP-2 cells submitted to laser therapy at λ635 and λ670 nm and different doses was verified by Pinheiro et al.[1] The authors concluded that λ670 nm laser light showed increased proliferative response of the cultured cells, but not the λ635 nm. When compared on the present experiment the two wavelengths of λ685 nm and λ830 nm showed different results, which demonstrated that the higher wavelength (λ830 nm) presented a larger proliferative effect. It is

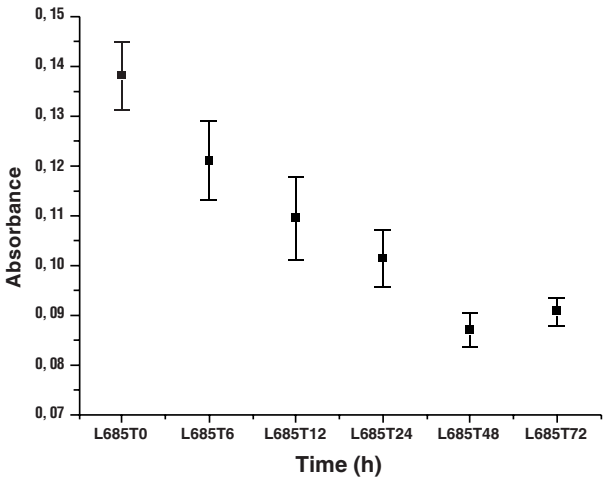

FIG. 2. Result of MTT assay of KB cell irradiated with λ685-nm laser light. A constant decline of the cellular viability was observed up to 48 h. At the end of the experimental period of 72 h, a discreet increase of the cellular viability was observed.

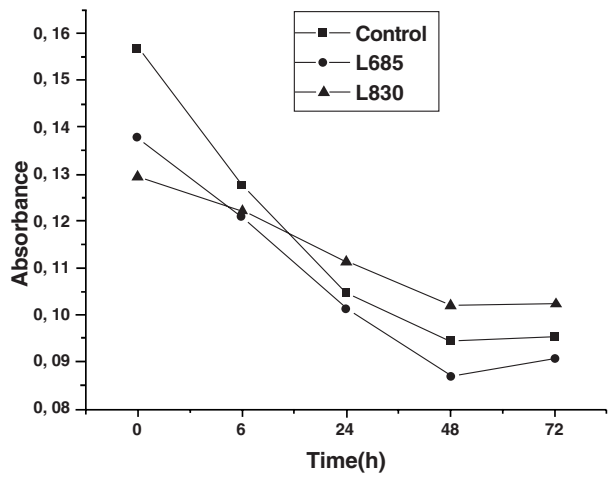

FIG. 4. Result of MTT assay of KB cell irradiated with λ685-nm or λ830-nm laser light and their controls.

important to observe that the two wavelengths possess different effects on the cells: one results in a photochemical effect, that is, directly on the mitochondria, and the other presents a photophysical effect, that is, on the cellular membrane.

It is concluded that laser therapy had a positive biomodulatory effect on the proliferation of KB cells and that this was influenced by wavelength.

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