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Original Article

Effect of Laser Therapy on Laryngeal Carcinoma Cell Proliferation (H.Ep-2)

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Abstract

Objective: To evaluate the effect of 660nm and 780 nm laser therapy, with dose of 6.3 J/cm² and 25 mW power, either associated with nutritional stress or not, on laryngeal epidermoid carcinoma cell proliferation (H.Ep. 2). **Material and Methods:** The H.Ep.2 cells were placed in a culture flask and frozen in fetal bovine serum (FBS) at -80°C, with different concentrations of the medium: 5% and 10%. Laser therapy was started 24 h after cell subculturing and performed at time intervals of 6, 12, 24, 48 and 72 hours after the first irradiation, in a period of five days. After this, cell viability was verified using the MTT method. Means and standard deviation were obtained, and the Student's-t and F (ANOVA) statistical tests were used, with Tukey or Tamanhe comparisons. **Results:** The group subjected to 780 nm laser showed significant differences ($p < 0.05$) in growth compared with the controls, in certain periods of the experiment (group 5% = T6 and T12, the group 10%= T0 and T6). Cultures irradiated with 660 nm laser showed no significant differences in their pattern of development in comparison with the controls, at the concentrations of culture media of 5% or 10% ($p > 0.05$). The time influenced the growth of all cultures. **Conclusion:** Low level laser interfered in cell proliferation and this effect could be determined by the wavelength used and the nutritional status of the cells.

Keywords: Dental laser therapy; Low level; Cell proliferation; Head and neck neoplasms.

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Introduction

Low level or non-surgical lasers have wavelengths in the red spectrum (visible) and infrared (invisible) which cause photochemical and photophysical effects respectively on the tissues. Within the group of photochemical effects, biomodulation may be included, which promotes analgesic, anti-inflammatory and healing actions on the irradiated tissues [1].

This type of laser has been used to accelerate the reparative processes in hard and soft tissues [2-7], due to its biomodulation effects on cells and tissues [8], either activating or inhibiting physiological, biochemical and metabolic processes. This capacity to accelerate healing is related to greater cell proliferation, since low level laser acts on the mitochondria stimulating the respiratory system, resulting in greater ATP [9] production, consequently greater DNA activity and production of RNA and proteins [10,11].

In vitro studies have shown that low level laser therapy was capable of increasing the proliferation of different cells, fibroblasts, osteoblasts, Keratinocytes, stem cells and even cells from malignant tumors [2,4,12-16]. Some studies have been conducted with the intention of demonstration the action of laser therapy on stimulating the proliferation of tumor cells [13,17-22], and conflicting results have been observed, seeing that at times laser light stimulated, and at other times inhibited or even made no difference to the proliferation of malignant cells.

With the purpose of using laser therapy with greater safety and efficacy in clinical dentistry, it has become important to study its effect on malignant cells, due to the possibility of unintentional exposure to laser of regions with malignant potential.

Considering the property of laser to promote alterations in cell metabolism, resulting in an increase in mitotic activity, the aim of this study was to investigate whether low level laser at the wavelengths of 660 nm and 780 nm, intensified the proliferative activity of malignant cells (H.Ep-2), submitted to different nutritional conditions.

Material and Methods

Laryngeal epidermoid carcinoma cells (H.Ep-2), obtained from the Department of antibiotics of the Federal University of Pernambuco, were maintained in a culture medium composed of DMEM, 20% of fetal bovine serum (FBS), 1% of L-glutamine and 1% of antibiotic solution (250µg/mL of streptomycin and 80mg/mL of gentamycin sulfate) [15,21], and were stored in plastic culture flasks and incubated at 37°C and 5% of CO2. After 24 hours, the medium was replaced by another with a concentration of 5 or 10% of FBS according to the group. The samples were subcultured every 48 hours.

Twenty-four hours before the first irradiation, 24 plates were prepared (12 plates for each group), with each plate containing 96 wells. In the experimental and control wells 100ml of the suspension containing DMEM (5% or 10% FBS, 1% of L-glutamin, and 1% if solution antibiotic) and 5 x 103cells [19] were placed.

The groups of this experiment were organized in the following manner: One group was composed of cells irradiated with InGaAIP laser (MM Optics Ltda., São Carlos, SP, Brazil) at a wavelength of 660 nm; in the other group, the cells were irradiated with the same laser appliance, but at a wavelength of 780 nm. In both irradiations, the dose used was 6.3 J/cm² and power of 25 mW. Each experimental group was composed of 12 plates, of which 6 plates contained cells nourished with 5% of FBS (nutritional deficit) and the remaining 6 plates, cells nourished with 10% FBS. All irradiations were performed at time intervals of 6 (T6), 12 (T12), 24 (T24), 48 (T48) and 72 (T72) hours after the first application of laser (T0), with a duration of 10s in each well, using the punctual irradiation mode. In each plate, 10 wells were irradiated. The Control Group did not receive any type of irradiation; it was situated in the same plates as those belonging to the experimental groups, and was formed of 8 wells in each plate, maintained under the same nutritional and storage conditions as the irradiated wells.

Cell growth was evaluated by the MTT colorimetric method {3-[4,5-dimethylthiazol-2-yl)3,5-diphenyl]tetrazolium}, twenty-four hours after the last irradiation, with determination of optical density [22]. The values were provided by the microplate reader - Thermo Plate TP-Reader.

In the analysis of the data, the following statistical measurements were obtained: mean and standard deviation (descriptive statistical techniques) and the Student's-t statistical tests with equal or unequal variances and F test (ANOVA) were used, with Tukey or Tamanhe [23] paired comparisons (inferential statistical techniques), with a level of significance of 5% ($p<0.05$).

Results

The group submitted to 780 nm laser showed significant differences ($p<0.05$) in growth, in comparison with its controls at certain time intervals in the experiment. In the group maintained at 5%, the cells irradiated with 780 nm laser presented an increase in growth at the time intervals of T6 and T12; a different situation could be observed at T48, in which the irradiated cells presented a lower degree of development in comparison with their control. In the group maintained at 10%, the cells belonging to the control group grew significantly in comparison with those irradiated with 780 nm laser in the time intervals T0 and T6 only (Table 1).

The cultures irradiated with 660 nm laser presented no significant difference in their pattern of development in comparison with their controls, at the culture medium concentrations of 5% or 10% ($p>0.05$) (Table 2).

When comparing the groups irradiated with 660 and 780nm, significant differences in cell growth were observed in the groups maintained with 5% FBS ($p<0.05$) in the time intervals T0, T6 and T12. The same situation was observed in the group maintained with 10%, but only for the time interval T48. The cells irradiated with 780 nm laser presented a greater degree of growth in these time intervals (Table 3).

The non irradiated H.Ep. 2 cells presented maximum development at the end of the experiment (T72); irrespective of being maintained with 5% or 10% of FBS (Figure 1).

Table 1. Optical Density of cells in each experimental time interval, according to group: control at 5% SFB (n=48 wells), control at 10 % SFB (n= 48 wells), irradiated (780 nm) at 5% SFB (n= 60 wells) or irradiated (780 nm) at 10% SFB (n= 60 wells.).

Time/ Subgroup:	Concentration of the medium (FBS) 5% Mean ± SD (2)	10% Mean ± SD (2)
• **T0**		
Control	0.255±0.010	0.331±0.013
Experimental (780 nm)	0.299±0.016	0.267±0.015
p-Value	p(1) = 0.001	**p(1) < 0.001***
• **T6**		
Control	0.255±0.010	0.331±0.013
Experimental (780 nm)	0.299±0.016	0.316±0.015
p-Value	**p(1) < 0.001***	**p(1) = 0.040***
• **T12**		
Control	0.279±0.022	0.308±0.019
Experimental (780 nm)	0.300±0.013	0.315±0.014
p-Value	**p(1) = 0.021***	p(1) = 0.404*
• **T24**		
Control	0.329±0.023	0.349±0.012
Experimental (780 nm)	0.334±0.020	0.340±0.011
p-Value	p(1) = 0.631	p(1) = 0.129
• **T48**		
Control	0.374±0.046	0.374±0.037
Experimental (780 nm)	0.335±0.018	0.403±0.020
p-Value	**p(1) = 0.024***	p(1) = 0.052
• **T72**		
Control	0.358±0.015	0.358±0.025
Experimental (780 nm)	0.355±0.016	0.364±0.025
p-Value	p(1) = 0.761	p(1) = 0.613

(*) Significant difference at 5.0%. (1): Student's-t test with equal variances.

Table 2. Optical density of cells according to concentration of the medium, time interval of the experiment, and group: control at 5% SFB (n=48 wells), control at 10 % SFB (n= 48 wells), irradiated (660 nm) at 5% SFB (n= 60 wells) or irradiated (660 nm) at 10% SFB (n= 60 wells.).

Concentration of the medium (5% or 10 % of FBS)	Experimental Time Interval	Group Control Mean ± S.D	Experimental (660 nm laser) Mean ± S.D	p-Value
• 5%	0 h	0.201 ± 0.012	0.197 ± 0.015	p(1) = 0.514
	6 h	0.208 ± 0.010	0.203 ± 0.011	p(1) = 0.322
	12 h	0.208 ± 0.010	0.207 ± 0.015	p(1) = 0.778
	24 h	0.207 ± 0.012	0.207 ± 0.016	p(1) = 0.919
	48 h	0.202 ± 0.012	0.200 ± 0.011	p(1) = 0.728
	72 h	0.214 ± 0.013	0.214 ± 0.014	p(1) = 0.965
• 10%	0 h	0.206 ± 0.010	0.199 ± 0.012 (a)	p(1) = 0.155
	6 h	0.198 ± 0.011	0.196 ± 0.013 (a)	p(1) = 0.803
	12 h	0.198 ± 0.012	0.197 ± 0.012 (a)	p(1) = 0.838
	24 h	0.202 ± 0.012	0.202 ± 0.015 (ab)	p(1) = 0.991
	48 h	0.204 ± 0.011	0.200 ± 0.015 (ab)	p(1) = 0.537
	72 h	0.221 ± 0.010	0.217 ± 0.013 (b)	p(1) = 0.481

Table 3. Optical density of cells in each time interval of the experiment, according to the experimental group (irradiated with 660 nm or 780 nm laser) and concentration of the medium.

Time/Experimental Group	Concentration of the medium (5% or 10% of FBS)	
	5% Mean ± SD (2)	**10%** Mean ± SD (2)
• **T0**		
660nm	0.284±0.011	0.273±0.023
780nm	0.299±0.016	0.267±0.015
p-Value	**p(1) = 0.021***	p(1) = 0.505
• **T6**		
660nm	0.284±0.011	0.326±0.018
780nm	0.299±0.016	0.316±0.015
p-Value	**p(1) = 0.021***	p(1) = 0.182
• **T12**		
660nm	0.268±0.021	0.309±0.012
780nm	0.300±0.013	0.315±0.014
p-Value	**p(1) = 0.001***	p(1) = 0.333
• **T24**		
660nm	0.332±0.016	0.339±0.016
780nm	0.334±0.020	0.340±0.011
p-Value	p(1) = 0.866	p(1) = 0.860
• **T48**		
660nm	0.336±0.018	0.378±0.017
780nm	0.335±0.018	0.403±0.020
p-Value	p(1) = 0.872	**p(1) = 0.007***
• **T72**		
660nm	0.341±0.017	0.366±0.027
780nm	0.355±0.016	0.364±0.025
p-Value	p(1) = 0.075	p(1) = 0.864

(*) Significant difference at 5.0%. (1): Student's-t test with equal variances.

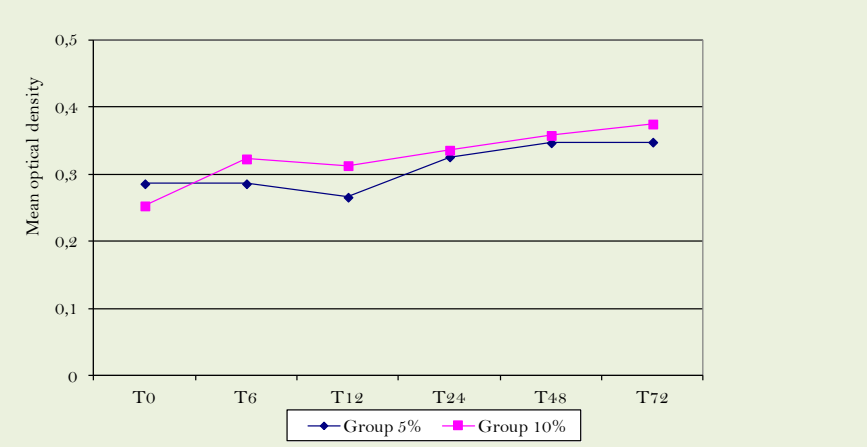

Figure 1. Mean optical density of cells belonging to the control group according to the time intervals of the experiment and concentration of the medium in which they were maintained (5% or 10% of FBS).

Discussion

The capacity of low level laser to increase the proliferation of healthy cells is known [24,25]. However, there is still controversy about its biomodulatory effect when interacting with malignant cells. Due to mutations in their genetic material, tumor cells multiply in a disorderly manner [26].

In view of this, studies that seek to define the effects of low level laser on malignant cells lead to greater safety for their use, and are of great importance.

In this study, malignant cells from one and the same lineage were submitted to a culture medium. Irrespective of being under nutritional stress, the cells proliferated in a standard manner, the same situation as was observed in a previous study [17]. Therefore, in the control group (non irradiated), the nutritional status did not appear to have any influence. A different situation was, however, observed in the group irradiated with 780 nm laser, seeing that only the cells maintained with 5% nutrition experienced a significant increase in growth.

When we evaluated the effect of laser at 660 nm on the proliferation of malignant cells, we observed that this did not provide a higher level of growth of the irradiated cells, in comparison with the cells belonging to the control group, irrespective of the concentration of the medium in which they were maintained. This result is similar to that of another study, which evaluated the effects of laser therapy at 660 nm on oral carcinoma cells [22]. As regards treatment with laser at 780 nm, differences in the growth pattern of cells in the experimental group were observed in comparison with the cells in the control group.

The 780 nm laser is situated in the infrared radiation band, and is not visible to the naked eye. Various studies [20,29,30] have discoursed about the effects on cell proliferation caused by the application of infrared lasers; A laser with a wavelength of 808 nm was capable of accelerating the healing of wounds of irradiated rats [29] treatment with laser at the same wavelength, however, in glioblastoma cells caused inhibition of their proliferation [30]. Working in the same way with cultured malignant cells (KB cells), other authors [20] proved the positive biomodulating effect of 830 nm laser, by the larger degree of growth in the groups irradiated with it.

We know that different factors, such as the laser wavelength, dose received during the entire experiment, the type of tissue studied, conditions under which the cells are maintained, among others, may influence the results found [31],which would justify the diverse findings in the literature. In the present research the increase in cell proliferation was caused only by the 780 nm laser, which is mainly absorbed by the cell membrane and causes photophysical and photoelectric effects, differently from 660 nm laser, which is absorbed by chromophores of the mitochondrial membrane, causing a photochemical effect [10].

The significant growth provided by irradiation with 780 nm laser was observed only in the group maintained with 5% of FBS. It is known that laser is capable of increasing the production of ATP, particularly when the cells are under stress, which may justify this fact. In the present research, the growth of cells irradiated with 780 nm laser was not observed in a uniform manner, since it was significant only at some time intervals of the experiment.

Conclusion

Laser therapy with a wavelength of 780 nm, dose of 6.3J/cm² and power of 25mW had a positive biomodulating effect on cultured laryngeal epidermoid carcinoma cell proliferation (H.Ep.2), maintained under a condition of nutritional deficit, at certain time intervals of the experiment. The same effect was not observed in any of the time intervals for any of the concentrations of the medium in the cultures irradiated with 660 nm laser, with the same dose and power previously mentioned. The proliferative rate of this cell lineage suffered the influence of time, both in the experimental and control groups, irrespective of the nutritional degree of the cell culture.

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