Journal of Photochemistry & Photobiology, B: Biology 237 (2022) 112597

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Laser photobiomodulation does not alter clinical and histological characteristics of 4-NQO-induced oral carcinomas and leukoplakia in mice

Gabriela Weirich Neculqueo [a], Marina Estrázulas [b], Karen Cherubini [a], Valesca Sander Koth [a], Fernanda Gonçalves Salum [a,*]

[a] School of Health and Life Sciences, Postgraduate Program in Dentistry, Oral Medicine Division, Pontifical Catholic University of Rio Grande do Sul- PUCRS, Porto Alegre, Rio Grande do Sul, Brazil

[b] Medical School, Postgraduate Program in Medicine, Pontifical Catholic University of Rio Grande do Sul- PUCRS, Porto Alegre, Rio Grande do Sul, Brazil

ARTICLE INFO

Keywords:
4-NQO
Oral carcinogenesis
Mice
Photobiomodulation

ABSTRACT

Objective: This study evaluated the effect of laser photobiomodulation (PBM) on oral leukoplakia and squamous cell carcinomas (OSCC) in a model of oral carcinogenesis.

Materials and Methods: Forty-one C57Bl/6 female mice were distributed in control group, 4-NQO group, Laser group 1.5 J and Laser group 9 J. Oral cancer was induced on the tongue by nitroquinoline oxide (4-NQO), diluted in the water for 16 weeks. In the 18th and 19th weeks, PBM with a diode laser, 0.028 cm^2 spot size, continuous emission mode, 660 nm wavelength was applied on the tongue of animals for seven sessions. Laser group 1.5 J received 30 mW power and 1.5 J energy. In the Laser group 9 J, 100 mW power, and 9 J energy were applied. In the 20th week the animals were euthanized.

Results: All animals exposed to carcinogen developed clinical and histological alterations such as leukoplakia and OSCC on the tongue. There was no significant difference among Laser groups 1.5 and 9 J and 4-NQO group (not irradiated) regarding the area of leukoplakia and carcinomas ($P > 0.05$) or thickness of epithelial tissue and keratin ($P > 0.05$). There were also no association between PBM and histologic classification of the lesions ($P = 0.87$), frequency of OSCC ($P = 0.57$), grade of tumor differentiation ($P = 0.88$) or depth of invasion ($P = 0.45$).

Conclusion: Laser PBM, in both parameters used, does not influence on clinical and histological characteristics of oral leukoplakia and OSCC.

Clinical Relevance: Results suggest that PBM may be a safe treatment for adverse effects of antineoplastic therapies in patients with leukoplakia and OSCC.

1. Introduction

Squamous cell carcinoma is the most frequent neoplasm of the oral cavity, with high rates of incidence, morbidity and mortality in the world population over the years [1]. The main treatments modalities for this neoplasm are still surgical resection, radiotherapy and chemotherapy, alone or in association [2]. These therapies have important adverse effects such as oral mucositis (OM), a debilitating condition, which clinically presents as areas of erythema, erosion and ulceration of the mucosa, in different degrees of severity. It causes painful symptomatology and dysphagia, and can result in weight loss, malnutrition and susceptibility to opportunistic infections [3]. In recent years there has been an increase in the use of laser photobiomodulation (PBM) for the management of OM [4–8]. Based on its positive effects, already demonstrated in the literature, the Multinational Association of Supportive Care in Cancer (MASCC) and International Society of Oral Oncology (ISOO) indicate the clinical use of PBM for the management of OM. However, due to the limited number of clinical studies proving the safety of application on tumor cells, they recommend its use with caution and away from these areas [6].

PBM is a therapeutic modality capable of altering biological activity by photon energy [9]. Light energy interacts with the tissue and promotes effects analgesic, anti-inflammatory and tissue biomodulation that can assist in the process of tissue repair [6,10]. In addition, it is considered a non-invasive therapy, not present cytotoxic effects or drug interaction, has easy application and good acceptance by patients in clinical practice [7,11]. Nevertheless, little is known about its action on dysplastic and neoplastic cells. *In vitro* studies show controversial

* Corresponding author at: Serviço de Estomatologia – Hospital São Lucas, PUCRS, Av. Ipiranga, 6690 Room 231, CEP: 90610-000 Porto Alegre, RS, Brazil.
E-mail address: fernanda.salum@pucrs.br (F.G. Salum).

https://doi.org/10.1016/j.jphotobiol.2022.112597
Received 4 May 2022; Received in revised form 21 October 2022; Accepted 7 November 2022
Available online 11 November 2022

results, while some demonstrate inhibitory effects on tumor cells, others show stimulatory effects on tumor progression [12–14]. However, these models show little similarity to the pathophysiological conditions of an organism, in addition to limited cellular interactions [14,15].

So far, only two *in vivo* studies have analyzed the effect of laser PBM in a model of oral carcinogenesis [16,17]. The results obtained were opposite, one of the studies showed an increase in the degree of tumor differentiation [17], while the other showed a reduction in the incidence of carcinomas *in situ* and invasive, with the PBM [16]. As they were developed in different experimental models, with different chemical carcinogenic and irradiation parameters, it is not possible to carry out an effective comparison between them [12]. Furthermore, studies suggest that the laser may have a biphasic effect, demonstrating different tissue responses with different radiation parameters [18,19].

Therefore, considering the benefits of laser PBM in the management of OM in patients with head and neck cancer and the inconclusive results about the effects of this therapy in oral epithelial dysplasia or in tumor cells, this study aimed to evaluate the effect of laser PBM at the red wavelength (660 nm) in a model of 4-NQO-induced oral carcinogenesis in mice.

2. Materials and Methods

2.1. Animals

The sample consisted of 48 C57Bl/6 female mice (8-week-old), weighing 15 to 20 g, obtained from the Center for Experimental Biological Models (CeMBE/PUCRS, Porto Alegre, RS, Brazil). The study was approved by the Ethics Committee on Animals Use (CEUA) of Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil (protocol number #10277). From 48 mice, one died due to malocclusion, three from stress during laser protocol and three developed cachexia throughout the experiment. Therefore, 41 animals were included in the analysis. They were housed in standard micro-isolators (five per cage), equipped with inlet/outlet air filters, under controlled temperature (23 ± 1 °C) and humidity (50 ± 5%), and a light- dark cycle of 12 h (lights on at 7 a.m., lights off at 7 p.m.). The cages were filled with autoclaved wood chip bedding. The animals received Nuvilab-Cr1 pelleted food (Nuvilab, Colombo, PR, Brasil) and sterile water *ad libitum*. They were submitted to a period of acclimatization of approximately 10 days. Animals were weighed weekly and before euthanasia [16].

2.2. Oral Carcinogenesis Model and Experimental Protocol

Oral carcinogenesis was induced with nitroquinoline oxide (4-NQO – Sigma, St. Louis, USA) dissolved in propyleneglycol (4 mg/ml) and diluted in water with a final concentration of 50 μg/ml. It was administered through water ingestion for 16 weeks. In the 17th week, the administration of 4-NQO was interrupted, and only water *ad libitum* was available to the animals. The protocol of oral carcinogenesis was performed according to the methodology described by Ottaviani et al. [16], which has been used in the induction of oral squamous cell carcinoma, that present a similar appearance to human carcinoma.

Animals ($N = 41$) were randomized into four groups: control group ($N = 9$) received propyleneglycol, for 16 weeks in the drinking water (50 μg/ml); 4-NQO group ($N = 10$) received 4-NQO (50 μg/ml) in the drinking water; Laser group 1.5 J ($N = 12$) received 4-NQO (50 μg/ml) and PBM with 1.5 J energy; Laser group 9 J (N = 10) received 4-NQO (50 μg/ml) and PBM with 9 J energy (Fig. 1).

2.3. Laser Photobiomodulation Therapy

In the 18th and 19th weeks, Laser groups 1.5 J and 9 J received PBM with a diode laser, active medium of indium gallium aluminum phosphide (InGaAlP) (Photon Lase III – DMC Ltda, São Carlos, SP, Brazil), 0.028 cm^2 spot size. In the Laser group 1.5 J the continuous emission mode was used, 660 nm wavelength, 30 mW power, 1.5 J energy per point, 1071.42 mW/cm^2 power density, 53.57 J/cm^2 energy density for 50 s, parameters similar to Monteiro et al. [17] and Petrellis et al. [20], with some modifications. In the Laser group 9 J, continuous emission mode was used, 660 nm wavelength, 100 mW power, 9 J energy per point, 3571.42 mW/cm^2 power density, 321.42 J/ cm^2 energy density for 90 s, parameters similar to Ottaviani et al. [16] and Frigo et al. [21], with modifications (Table 1).

The laser PBM occurred at one point on the dorsum of tongue. For laser application mice were restrained by the scruff method. The spot tip was placed in contact with the mucosa in an angle as perpendicular as possible, minimizing refraction (Fig. 2). We used a power meter to check the output power. The groups performed the treatment for two weeks,

Table 1
Irradiation parameters for the different groups.

Group	Wavelength (nm)	Energy Density (J/cm^2)	Energy (J)	Power (mW)	Power Density (mW/cm^2)	Time (s)
Control	660	53.57	1.5	30	1071.42	50
4-NQO	–	–	–	–	–	–
Laser 1.5 J	660	53.57	1.5	30	1071.42	50
Laser 9 J	660	321.42	9	100	3571.42	90

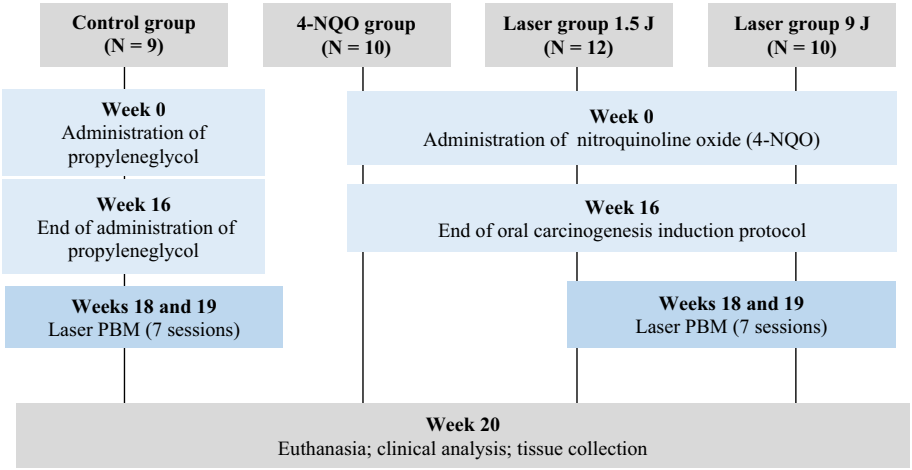

Fig. 1. Protocol performed in the experiment.

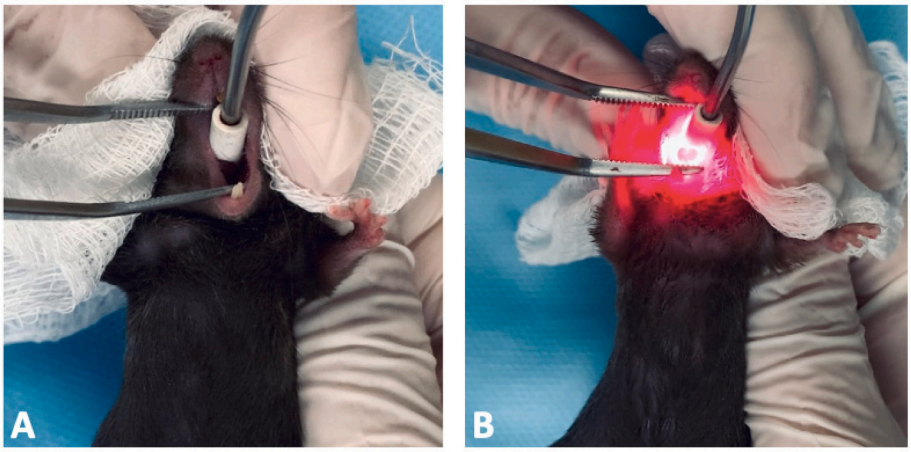

Fig. 2. Laser irradiation. (A) 4-NQO group without laser activation. (B) Irradiation on the dorsum tongue in 1.5 J laser group. A clamp was used to facilitate opening the mouth and gauze to avoid irradiation in the eyes.

on alternate days, completing seven sessions. Sham laser irradiation was performed in 4-NQO group. Animals were restrained by the scruff method, and the spot tip was placed in contact with the mucosa, however laser appliance was not activated. The control group was irradiated with the same parameters of the Laser group 1.5 J.

2.4. Euthanasia, Clinical Analysis and Preparation of Tissue

After the photobiomodulation protocol, at 20th week, the animals were euthanized with an overdose of deep inhalation anesthesia using 4% isoflurane. Immediately after euthanasia, clinical evaluation was performed by a blinded examiner, who evaluated the presence of exophytic lesions, compatible with squamous cell carcinomas, and leukoplastic lesions on the oral mucosa. The tongues were photographed in a standardized way and the images saved in TIFF format for macroscopic analysis. They were collected and immediately fixed in 10% formaldehyde, for 24 h.

Macroscopic analysis of the leukoplakias and neoplastic lesions of the tongue was performed by a blind examiner. Previously, the examiner was calibrated by analyzing 10 images in duplicate at a one-week interval. The measurement of the tumor area as well as of the leukoplakias areas was calculated, by using Image J Software (NIH, Bethesda, MD, USA).

2.5. Histological Analysis

The tongue specimens were subjected to routine histological processing and embedded in paraffin. Sections of 3 μm thick were obtained and stained with hematoxylin and eosin (H&E). Histological analysis was performed by one calibrated and blinded examiner, using a BX50 Olympus binocular microscope (Olympus, Tokyo, Japan). Intra-examiner calibration was performed by reanalysis of 10 slides with an interval of seven days between observations. The histological sections were examined in their entirety and were classified into four groups: no changes; hyperkeratosis and acanthosis; epithelial dysplasia and squamous cell carcinoma [22].

In addition, the squamous cell carcinomas were analyzed to verify the existence of rupture of the basal membrane and were classified according to the degree of differentiation in well-differentiated; moderately differentiated and poorly differentiated [16,17,23]. In invasive tumors, that is, in which there was rupture of the basal membrane, depth of invasion (or tumor thickness) was measured from the tumor surface to the deepest point of invasion by the Image J Software (NIH, Bethesda, MD, USA), under 200 X magnification. Tumor budding, defined as the presence of a single cancer cell or small cluster of <5 cancer cells at the invasive front was also scored [24].

The thickness of the epithelium and keratin was analyzed in all the samples, however, in animals that developed squamous cell carcinoma, evaluation was performed in adjacent areas, where there was no neoplasm. The thickness of the epithelium and keratin were measure by using the Image J Software (NIH, Bethesda, MD, USA), under 200 X magnification. Three fields of the dorsum of the tongue were captured, and three measurements were taken in each field. The mean of the nine measurements was recorded for each specimen. The histological images of the tongue dorsum were obtained with a microscope Axio Imager A1 coupled to an image capture system Axio Vision Rel. 4.4 Software Multimedia (Carl Zeiss, Hallbergmoos, Germany).

2.6. Statistical Analysis

All data were analyzed using SPSS 18.0 software. Kruskal-Wallis non-parametric ANOVA test, followed by the pair-by-pair comparison test, was used to compare tumor and leukoplakia area, depth of invasion, epithelium and keratin thickness, and body weight. Fisher's exact test was used for the histological classification of lesions, presence of tumors clinically and histologically detectable, and the grade of tumor differentiation. P value ≤ 0.05 was considered as an indicative of significance.

3. Results

3.1. Clinical Evaluation

The induction of oral carcinogenesis in the 4-NQO group, Laser groups 1.5 J and 9 J resulted in whitish plaques on the dorsum, edges and ventral tongue of the animals, compatible with leukoplakia. Exophytic whitish nodular lesions with irregular surface, sessile or pedunculated, located on the dorsum, edges and ventral tongue, and floor of the mouth were also found (Fig. 3). These lesions were clinically compatible with squamous cell carcinoma.

In the control group, no alterations were observed in the oral mucosa of the animals. In the 4-NQO group, seven animals (70%) developed clinical evidence of neoplasia, in the Laser group 1.5 J, five (41.6%) mice had a tumor detected during the examination, and in the Laser group 9 J, six (60%) animals developed those lesions. There was no significant difference in the frequency of clinically detectable tumors among the carcinogen-treated groups ($p = 0.45$).

Regarding the tumor and leukoplakia area, there were no significant differences among the groups in which oral carcinogenesis was induced, regardless of the laser PBM ($p > 0.05$). 4-NQO group (18.40 $mm^2 \pm$ 4.34), Laser groups 1.5 J (15.19 $mm^2 \pm$ 5.84) and 9 J (21.26 $mm^2 \pm$

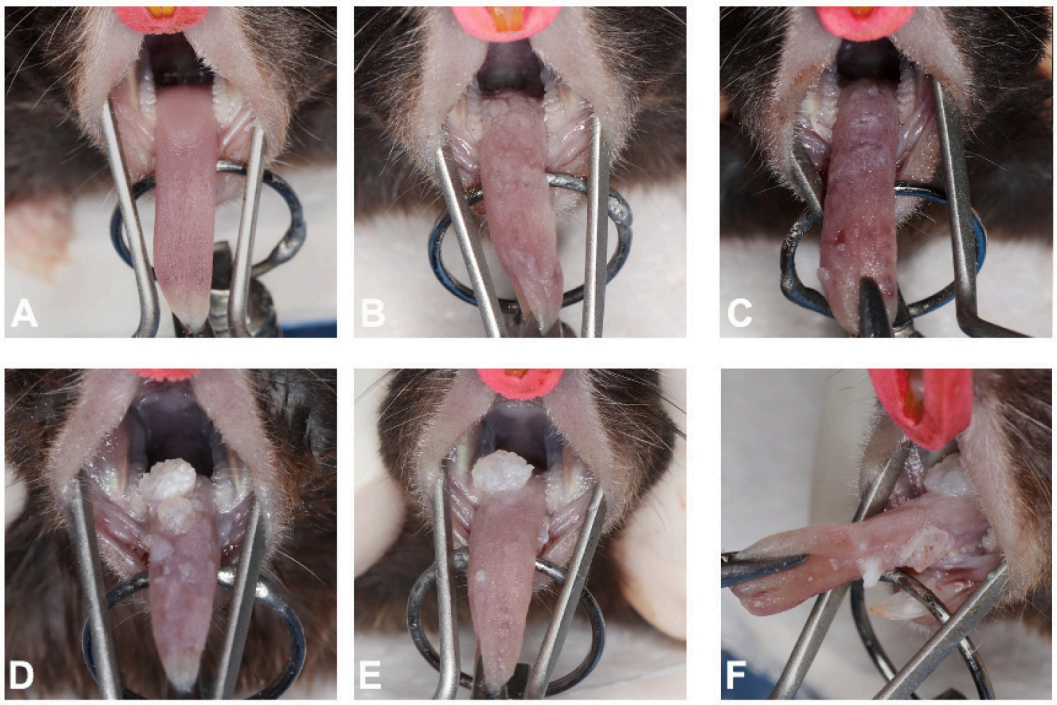

Fig. 3. Representative images of the tongues. Control animal treated with vehicle, without lesions (A). Animals treated with 4-NQO (50 μg/ml) in drinking water showing leukoplakia on the dorsum of the tongue (B,C). Animals treated with 4-NQO presenting multiple neoplastic exophytic lesions with irregular surface on the dorsum (D,E) and ventral tongue (F).

6.43) showed similar values regarding those variables (Fig. 4A).

The weight gain of the animals throughout the study was lower in the 4-NQO group (2.18 g ± 2.12), Laser groups 1.5 J (0.11 g ± 1.81) and 9 J (1.90 g ± 1.59), compared to control group (4.27 g ± 2.41). Only Laser group 1.5 J showed a significant difference compared to control group ($p < 0.05$) (Fig. 4B).

3.2. Histological Analysis

There was a statistically significant increase in the thickness of the epithelium and keratin in the dorsum of the tongue in 4-NQO (273.70 μm ± 30.60), Laser 1.5 J (275.40 μm ± 83.70) and Laser 9 J (256.90 μm ± 49.62) groups compared to control (108.00 μm ± 27.85) ($p < 0.001$). Nevertheless, no significant differences were observed among the groups subjected to induction of oral carcinogenesis, receiving or not PBM therapy ($P = 0.89$) (Fig. 4C-G).

In the control group, no alterations were observed in the histopathological analysis (Fig. 5A). In the groups exposed to oral carcinogen, all samples developed some epithelial histological alteration (Fig. 5B-F). In the 4-NQO group, squamous cell carcinoma was detected in eight mice (80%) and epithelial dysplasia in two (20%). In the Laser group 1.5 J, carcinomas were observed in seven animals (58.3%), dysplasia in three (25%) and hyperkeratosis and acanthosis in two (16.7%). In the Laser group 9 J were also diagnosed seven carcinomas (70%), two dysplasias (20%) and one hyperkeratosis and acanthosis (10%) (Fig. 6A). Fisher's Exact test didn't demonstrated association between the histological classification of lesions and laser PBM in animals subjected to induction of oral carcinogenesis ($X^2_{(4)} = 2.084$; $p = 0.87$) (Fig. 6A). In addition, there was no association between the frequency of squamous cell carcinomas and PBM therapy ($X^2_{(2)} = 1.202$; $p = 0.57$) (Fig. 6B).

According to the Fisher's Exact test, it there was also no association between the grade of tumor differentiation and PBM therapy ($X^2_{(4)} = 1824$; $p = 0.88$) (Fig. 6C). Some tumors showed a proliferative growth, without rupture of the basal layer, whereas others showed an invasive character. There was no difference regarding this histological aspect among the groups subjected to induction of oral carcinogenesis, regardless of PBM therapy ($p = 0.80$, Fig. 6D). Depth of invasion (or tumor thickness) was 0.508 mm (± 0.284) in 4-NQO group, 0.340 mm (± 0.146) in Laser 1.5 J group, and 0.535 mm (± 0.213) in Laser 9 J group. There was not significant difference between groups regarding to depth of invasion ($p = 0.455$). Tumor budding were not detected in the samples of squamous cell carcinoma.

4. Discussion

The present study evaluated the effects of laser PBM on leukoplakia and oral squamous cell carcinomas in a 4-NQO-induced carcinogenesis model. Laser with both parameters of energy, 1.5 J and 9 J, did not change the frequency of clinically detectable tumors at the end of the experiment. Despite the slight increase in the area of lesions in the Laser 9 J group, the area of leukoplakia and carcinomas also did not show any changes with PBM in neither parameters. Our study is unprecedented in performing this clinical analysis, as no *in vivo* study evaluating the effects of PBM on leukoplakia and oral carcinomas has analyzed the area of clinically detectable lesions. The results showed that with low energies, like those used in the treatment of oral mucositis, and with high energies, PBM did not change the clinical parameters of oral lesions induced by 4-NQO.

Regarding the histopathological analysis, more cases of squamous cell carcinomas were diagnosed compared to the clinical analysis. In the 1.5 J laser group, two more cases were detected and in the 4-NQO and 9 J laser groups, one more sample. As in previously published studies, in this experiment all animals exposed to the carcinogen showed histological alterations in the tongue, which ranged from hyperkeratosis and acanthosis, dysplasia to squamous cell carcinoma, with distinct grades of differentiation [16,22,25]. PBM therapy did not change the histological presentation of the lesions or the grade of differentiation of squamous cell carcinomas. In addition, the data showed that the laser did not influence in the incidence of tumor invasion among the groups or depth of invasion.

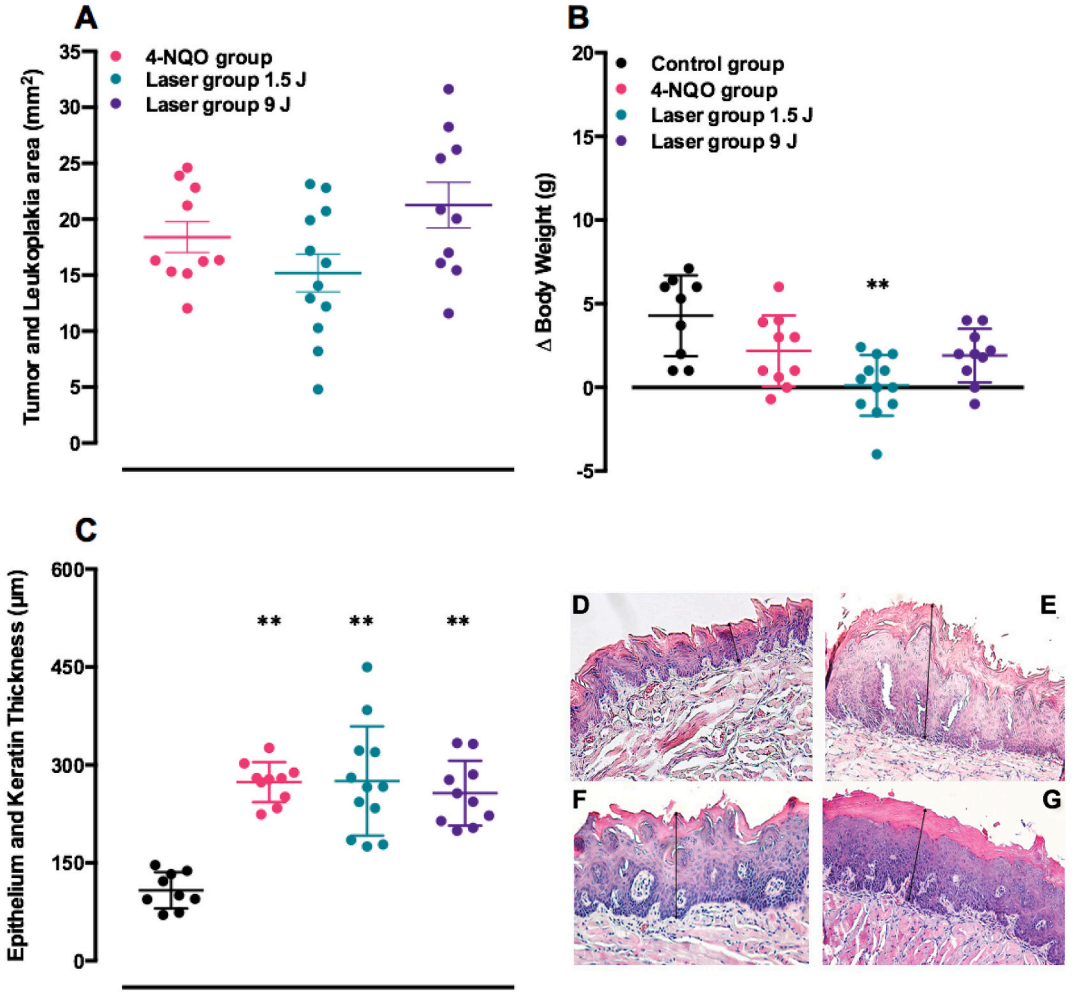

Fig. 4. Effects of 4-NQO-induced oral carcinogenesis and photobiomodulation (PBM). (A) The tumor and leukoplakia area did not differ in animals carcinogen-treated, receiving or not PBM. (B) Weight gain of C57Bl/6 mice throughout the study (Δ body weight). (C) Epithelium and keratin thickness in 4-NQO, Laser 1.5 J and Laser 9 J groups compared to the control group. (D-G) Representative images showing epithelium and keratin thickness (bidirectional arrow) in control (D), 4-NQO group (E), Laser group 1.5 J (F) and Laser group 9 J (G) (H&E staining). Original magnification 200 X, scale bar (—) 100 µm. ** $p < 0.01$ compared to control group.

In contrast, Monteiro et al. [17] observed an increase in the histological grade of squamous cell carcinoma with laser PBM. However, in his study, oral carcinomas were induced in the cheek pouch of golden Syrian hamsters in an experimental model with 9,10-dimethyl 1,2-benzanthracene (DMBA). The laser was applied for 133 s, with energy of 4 J, for four weeks, on alternate days. This carcinogenic model, induced in hamster cheek pouch, does not represent the environmental influences occurred in the oral cavity. Besides, epithelium in this anatomical region is thinner, compared to the epithelium of the oral cavity in humans, which may justify the contrasting results [23,25]. In the present study, we opted for the experimental model of induction of oral carcinogenesis with 4-NQO in mice, as it presents good results already described in the literature [16,22,25,26], added its similarity to human carcinomas [22,25]. Futhermore, 4-NQO has advantages over other chemical carcinogenesis such as DMBA, for being water-soluble, allowing the administration in the drinking water, causing less stress during the experiment [22,26].

Ottaviani et al. [16] also found that PBM with laser is a safety procedure in dysplastic and neoplastic lesions. The authors performed an *in vivo* study in a 4-NQO-induced model of oral carcinogenesis. However, different from our data, the authors observed a reduction in the appearance of dysplastic lesions, close to the areas of carcinomas, in addition to a lower incidence of *in situ* and invasive carcinomas. They used irradiation substantially different parameters from those of the present study such as infrared wavelength (970 nm), 2.5 W power, 75 J energy, for 30 s, applied for four consecutive days. This extremely high power may reduce the effect of photobiomodulation and cause thermal alterations, which may explain the reduction of the incidence of the lesions observed in the study by Ottaviani et al. [16]. Furthermore, these parameters could not be used in the management of OM, as they are not recommended by MASCC and ISOO. Nonetheless laser has biphasic effects, being able to demonstrate contrasting responses with different doses in the same tissue [18,19]. For this reason, in the present study, we opted to use two laser protocols, one with lower energy, similar to that used in clinical practice, and the other with higher energy, which may have inhibitory effects, without thermal effects.

In vitro studies as demonstrated by Gomes Henriques et al. [14] tested two protocols of red laser on tumor cells (SCC25) with a 0.99 J energy. A stimulatory effect on tumor proliferation and invasion occurred. Our study, using an *in vivo* model of oral carcinogenesis, with similar irradiation patterns, did not show significant differences in the tumor incidence, invasion and differentiation. The difference among the results can be attributed to the *in vitro* model, which show little similarity to the pathophysiological conditions [14,15].

This study demonstrated multifocal leukoplastic and neoplastic lesions after the induction with 4-NQO in the drinking water (50 µg/ml),

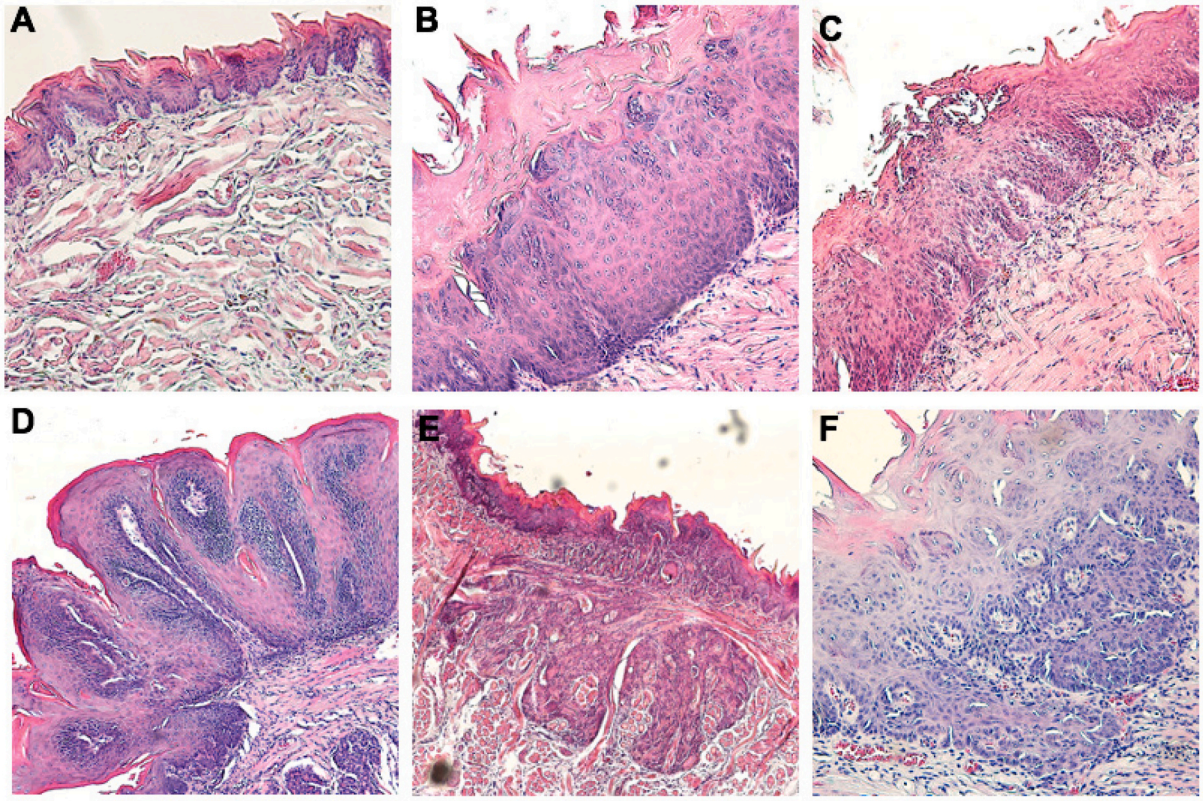

Fig. 5. Representative images of histological aspect of tongues after treatment with 4-NQO and vehicle. (A) Control group without alterations, sample treated with vehicle. (B) Hyperkeratosis and acanthosis in group laser 1.5 J. (C) Epithelial dysplasia in group laser 9 J. (D) Squamous cell carcinoma with exophytic growth in laser group 9 J. (E and F) Invasive squamous cell carcinoma in laser group 1.5 J. (H&E staining). Original magnification 100 X (A, D and E) and 200 X (B, C and F).

in the 20th week of the experiment. These lesions developed throughout the entire dorsum of the tongue of animals. Furthermore, some of the animals also developed lesions in the ventral tongue, in the edges of tongue and in the mouth floor. Such lesions are compatible with what is described in the literature using the same experimental model [16,22]. Therefore, we decided to assess the thickness of epithelium and keratin from the dorsum of the tongue of the animals, in fields where there was no squamous cell carcinoma. The data showed an increase in these variables in the groups that used the carcinogen, but no differences among them. This indicates that laser PBM, in the presented parameters, does not have the ability to change the thickness of epithelium and keratin. *In vivo* studies that evaluated the effects of PBM on oral carcinogenesis model did not analyze these parameters until then. On the other hand, a study in a skin carcinogenesis model, which evaluated the application of LED for 30 min, 642 nm wavelength, 21.6 J/cm^2 energy density, demonstrated an increase in the thickness of the epithelium, compared to the control group [27].

According to the literature, the induction of oral carcinogenesis with 4-NQO causes a drastic reduction in the weight of the animals [22]. At the end of the experiment, the animals in the 4-NQO, laser 1.5 J and laser 9 J groups showed lower weight gain than the control group. Nevertheless, only the laser 1.5 J group differed statistically from the control. In the laser 1.5 J group, some animals developed cachexia at the end of the experiment. In the 4-NQO and laser 9 J groups, the condition also developed, but the animals were euthanized before the end of the experiment and were not included in the data analysis. Some of these animals developed exophytic tumors of large proportions, causing difficulty in feeding, associated with cachexia. It is worth noting that the present study presented limitations like the small sample and a short time of follow-up after PBM therapy, which may be a factor to consider, as tumor development and progression are time dependent [22,23,25,28]. To overcome this limitation, it would be possible to euthanize the animals at different times, that is, after the PBM protocol (as performed) and a few months later. Thus, it would be possible to evaluate the effects of PBM on tumor development and on clinical and histological changes over the time. Another issue to be discussed is that the carcinomas observed in this study had a leukoplakia and exophytic clinical appearance. It would also be interesting to observe the effect of PBM on lesions with an erythroplakia and endophytic clinical appearance.

The laser PBM, in the parameters of the present study, did not show significant influence in relation to the area of oral leukoplakia and carcinomas, or in relation to the histological classification of lesions, tumor incidence, grade of tumor differentiation, thickness of the epithelium and keratin. This implies that laser PBM in these parameters is not able to change the clinical and histopathological features of oral squamous cell carcinomas and leukoplakia in the proposed model. There is still a need for further studies, with a larger size sample, a longer follow-up period after PBM therapy, and in other oral carcinogenesis models.

Author Contributions

Gabriela Weirich Neculqueo: conceptualization, methodology, formal analysis and investigation, writing – figures preparation, resources

Marina Estrázulas: methodology, formal analysis

Valesca Sander Koth: formal analysis and investigation, writing – figures preparation

Karen Cherubini: writing - review and editing, supervision

Fernanda Gonçalves Salum: conceptualization, methodology, writing – figures preparation, writing - review and editing, supervision

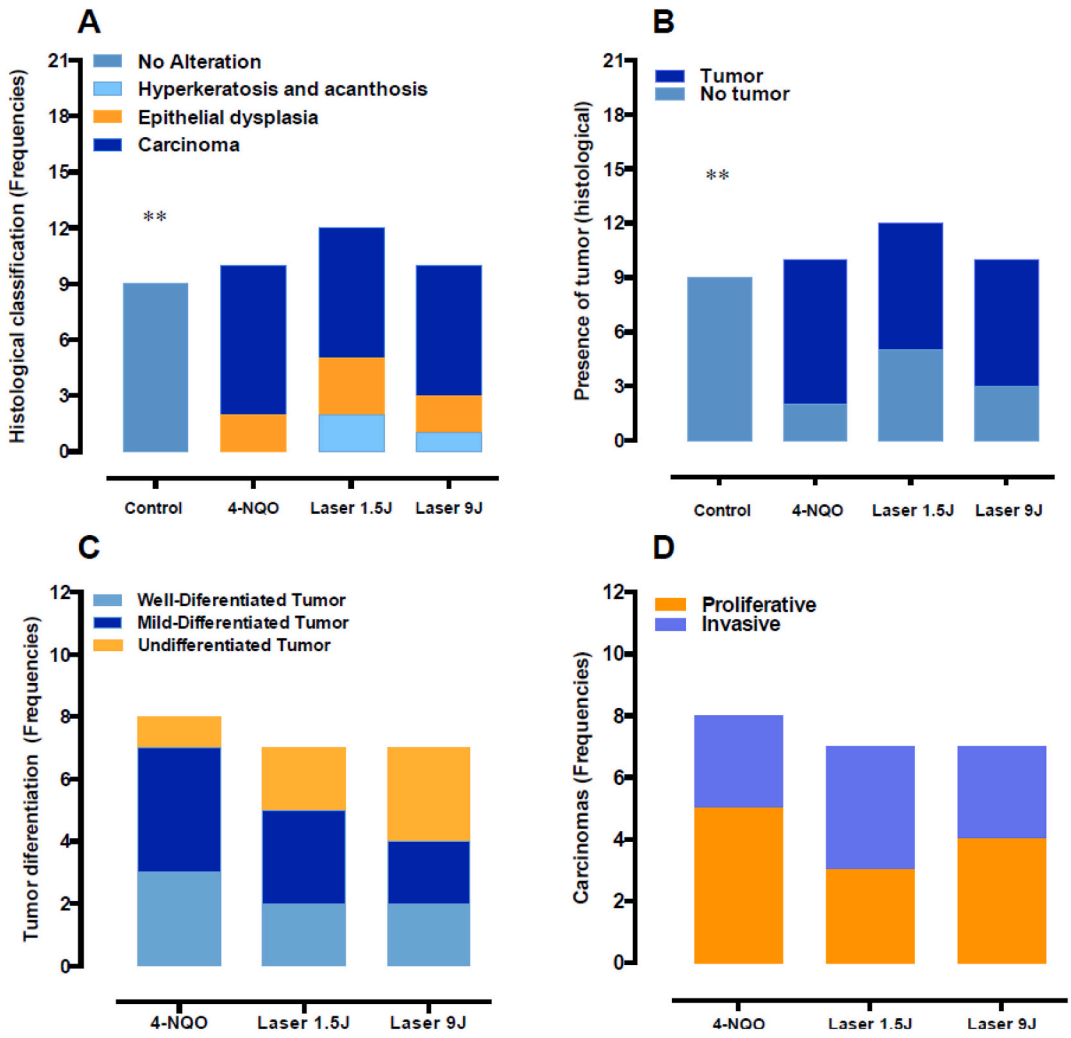

Fig. 6. Effects of 4-NQO-induced oral carcinogenesis and PBM therapy in the histological classification of lesions (A), tumor incidence (B) and grade of tumor differentiation (C) in each group. Assessment of the frequency of invasive and proliferative carcinomas in the groups (D). ** $p < 0.01$.

Funding

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)- finance code 001.

Declaration of Competing Interest

The authors declare there is no conflict of interests.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotobiol.2022.112597.

References

[1] Global Cancer Observatory (n.d.), https://gco.iarc.fr/tomorrow/en (accessed March 12, 2022).
[2] A. Chamoli, A.S. Gosavi, U.P. Shirwadkar, K.V. Wangdale, S.K. Behera, N. K. Kurrey, K. Kalia, A. Mandoli, Overview of oral cavity squamous cell carcinoma: risk factors, mechanisms, and diagnostics, Oral Oncol. 121 (2021), 105451, https://doi.org/10.1016/j.oraloncology.2021.105451.
[3] C. Pulito, A. Cristaudo, C. La Porta, S. Zapperi, G. Blandino, A. Morrone, S. Strano, Oral mucositis: the hidden side of cancer therapy, J. Exp. Clin. Cancer Res. 39 (2020) 1–15, https://doi.org/10.1186/s13046-020-01715-7.
[4] J.A.E.M. Zecha, J.E. Raber-Durlacher, R.G. Nair, J.B. Epstein, S. Elad, M. R. Hamblin, A. Barasch, C.A. Migliorati, D.M.J. Milstein, M.T. Genot, L. Lansaat, R. van der Brink, J. Arnabat-Dominguez, L. van der Molen, I. Jacobi, J. van Diessen, J. de Lange, L.E. Smeele, M.M. Schubert, R.J. Bensadoun, Low-level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 2: proposed applications and treatment protocols, Support Care Cancer 24 (2016) 2793–2805, https://doi.org/10.1007/s00520-016-3153-y.
[5] J.A.E.M. Zecha, J.E. Raber-Durlacher, R.G. Nair, J.B. Epstein, S.T. Sonis, S. Elad, M. R. Hamblin, A. Barasch, C.A. Migliorati, D.M.J. Milstein, M.T. Genot, L. Lansaat, R. van der Brink, J. Arnabat-Dominguez, L. van der Molen, I. Jacobi, J. van Diessen, J. de Lange, L.E. Smeele, M.M. Schubert, R.J. Bensadoun, Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 1: mechanisms of action, dosimetric, and safety considerations, Support Care Cancer 24 (2016) 2781–2792, https://doi.org/10.1007/s00520-016-3152-z.
[6] Y. Zadik, P.R. Arany, E.R. Fregnani, P. Bossi, H.S. Antunes, R.J. Bensadoun, L. A. Gueiros, A. Majorana, R.G. Nair, V. Ranna, W.J.E. Tissing, A. Vaddi, R. Lubart, C.A. Migliorati, R.V. Lalla, K.K.F. Cheng, S. Elad, Systematic review of photobiomodulation for the management of oral mucositis in cancer patients and clinical practice guidelines, Support Care Cancer 27 (2019) 3969–3983, https://doi.org/10.1007/s00520-019-04890-2.
[7] S.S. Rezk-Allah, H.M.A. Elshafi, R.J. Farid, M.A.E. Hassan, S.A. Alsirafy, Effect of low-level laser therapy in treatment of chemotherapy induced oral mucositis, J. Lasers Med. Sci. 10 (2019) 125–130, https://doi.org/10.15171/jlms.2019.20.
[8] R. Hanna, S. Dalvi, S. Benedicenti, A. Amaroli, T. Sălăgean, I.D. Pop, D. Todea, I. R. Bordea, Photobiomodulation therapy in oral mucositis and potentially malignant oral lesions: a therapy towards the future, Cancers (Basel) 12 (2020) 1–14, https://doi.org/10.3390/cancers12071949.

[9] G.C. Louzeiro, K. Cherubini, M.A.Z. de Figueiredo, F.G. Salum, Effect of photobiomodulation on salivary flow and composition, xerostomia and quality of life of patients during head and neck radiotherapy in short term follow-up: a randomized controlled clinical trial, J. Photochem. Photobiol. B Biol. 209 (2020), https://doi.org/10.1016/j.jphotobiol.2020.111933.

[10] K.A.M. Kalhori, F. Vahdatinia, M.R. Jamalpour, P. Vescovi, C. Fornaini, E. Merigo, R. Fekrazad, Photobiomodulation in oral medicine, photobiomodulation, photomedicine, Laser Surg. 37 (2019) 837–861, https://doi.org/10.1089/photob.2019.4706.

[11] M.D. Martins, F.M. Silveira, L.P. Webber, V.P. Wagner, M.A.T. Martins, C. H. Squarize, R.M. Castilho, The impact of photobiomodulation therapy on the biology and behavior of head and neck squamous cell carcinomas cell lines, J. Photochem. Photobiol. B Biol. 209 (2020), https://doi.org/10.1016/j.jphotobiol.2020.111924.

[12] F.M. Silveira, M.D.P. Paglioni, M.M. Marques, A.R. Santos-Silva, C.A. Migliorati, P. Arany, M.D. Martins, Examining tumor modulating effects of photobiomodulation therapy on head and neck squamous cell carcinomas, Photochem. Photobiol. Sci. 18 (2019) 1621–1637, https://doi.org/10.1039/c9pp00120d.

[13] J.L. da Silva, A.F.S. Silva-de-Oliveira, R.A.C. Andraus, L.P. Maia, Effects of low level laser therapy in cancer cells—a systematic review of the literature, Lasers Med. Sci. 35 (2020) 523–529, https://doi.org/10.1007/s10103-019-02824-2.

[14] Á.C. Gomes Henriques, F. Ginani, R.M. Oliveira, T.S.L. Keesen, C.A. Galvão Barboza, H.A. Oliveira Rocha, J.F.L. De Castro, R. Della Coletta, R. De Almeida Freitas, Low-level laser therapy promotes proliferation and invasion of oral squamous cell carcinoma cells, Lasers Med. Sci. 29 (2014) 1385–1395, https://doi.org/10.1007/s10103-014-1535-2.

[15] J.P. Gillet, S. Varma, M.M. Gottesman, The clinical relevance of cancer cell lines, J. Natl. Cancer Inst. 105 (2013) 452–458, https://doi.org/10.1093/jnci/djt007.

[16] G. Ottaviani, V. Martinelli, K. Rupel, N. Caronni, A. Naseem, L. Zandonà, G. Perinetti, M. Gobbo, R. Di Lenarda, R. Bussani, F. Benvenuti, M. Giacca, M. Biasotto, S. Zacchigna, Laser therapy inhibits tumor growth in mice by promoting immune surveillance and vessel normalization, EBioMedicine. 11 (2016) 165–172, https://doi.org/10.1016/j.ebiom.2016.07.028.

[17] J.S. Monteiro, A.L.B. Pinheiro, S.C.P.S. De Oliveira, G.T.S. Aciole, J.A.C. Sousa, M. C.T. Cangussú, J.N. Dos Santos, Influence of laser phototherapy (λ660nm) on the outcome of oral chemical carcinogenesis on the hamster cheek pouch model: histological study, Photomed. Laser Surg. 29 (2011) 741–745, https://doi.org/10.1089/pho.2010.2896.

[18] M.R. Hamblin, Y.Y. Huang, S.K. Sharma, J. Carroll, Biphasic dose response in low level light therapy - an update, Dose-Response. 9 (2011) 602–618, https://doi.org/10.2203/dose-response.11-009.Hamblin.

[19] N.N.F. Lopes, H. Plapler, M.C. Chavantes, R.V. Lalla, E.M. Yoshimura, M.T.S. Alves, Cyclooxygenase-2 and vascular endothelial growth factor expression in 5-fluoro-uracil-induced oral mucositis in hamsters: evaluation of two low-intensity laser protocols, Support Care Cancer 17 (2009) 1409–1415, https://doi.org/10.1007/s00520-009-0603-9.

[20] M.C. Petrellis, L. Frigo, R.L. Marcos, R.C. Pallotta, M.H.C. de Carvalho, M. N. Muscará, D.A. Maria, R.Á.B. Lopes-Martins, Laser photobiomodulation of pro-inflammatory mediators on Walker tumor 256 induced rats, J. Photochem. Photobiol. B Biol. 177 (2017) 69–75, https://doi.org/10.1016/j.jphotobiol.2017.09.011.

[21] L. Frigo, J.M. Cordeiro, G.M. Favero, D.A. Maria, E.C.P. Leal-Junior, J. Joensen, J. M. Bjordal, D.C. Roxo, R.L. Marcos, R.A.B. Lopes-Martins, High doses of laser phototherapy can increase proliferation in melanoma stromal connective tissue, Lasers Med. Sci. 33 (2018) 1215–1223, https://doi.org/10.1007/s10103-018-2461-5.

[22] X.H. Tang, B. Knudsen, D. Bemis, S. Tickoo, L.J. Gudas, Oral cavity and esophageal carcinogenesis modeled in carcinogen-treated mice, Clin. Cancer Res. 10 (2004) 301–313, https://doi.org/10.1158/1078-0432.CCR-0999-3.

[23] B.L. Hawkins, B.W. Heniford, D.M. Ackermann, M. Leonberger, S.A. Martinez, F. J. Hendler, 4NQO carcinogenesis: a mouse model of oral cavity squamous cell carcinoma, Head Neck 16 (1994) 424–432, https://doi.org/10.1002/hed.2880160506.

[24] R.D. Almangush, I.O. Coletta, C. Bello, H. Bitu, L.K. Keski-Säntti, J.H. Mäkinen, M. Kauppila, J. Pukkila, J. Hagström, S. Laranne, Y. Tommola, V.M. Soini, P. Kosma, L.P. Koivunen, P. Kowalski, R. Nieminen, I. Grénman, T. Leivo, Salo, a simple novel prognostic model for early stage oral tongue cancer, Int. J. Oral Maxillofac. Surg. 44 (2015) 143–150, https://doi.org/10.1016/j.ijom.2014.10.004.

[25] J.M. Nauta, J.L.N. Roodenburg, P.G.J. Nikkels, M.J.H. Witjes, A. Vermey, Comparison of epithelial dysplasia-the 4NQO rat palate model and human oral mucosa, Int. J. Oral Maxillofac. Surg. 24 (1995) 53–58, https://doi.org/10.1016/S0901-5027(05)80857-4.

[26] M.C.A. Rivera, 4NQO carcinogenesis: a model of oral squamous cell carcinoma, Int. J. Morphol. 30 (2012) 309–314, https://doi.org/10.4067/s0717-95022012000100055.

[27] H. Goo, S. Mo, H.J. Park, M.Y. Lee, J.-C. Ahn, Treatment with LEDs at a wavelength of 642 nm enhances skin tumor proliferation in a mouse model, Biomed. Opt. Express. 12 (2021) 5583, https://doi.org/10.1364/boe.427205.

[28] N.E. Steidler, P.C. Reade, Experimental induction of oral squamous cell carcinomas in mice with 4-nitroquinolone-1-oxide, Oral Surg. Oral Med. Oral Pathol. 57 (1984) 524–531, https://doi.org/10.1016/0030-4220(84)90312-8.