

Journal of Biomedical Optics

BiomedicalOptics.SPIEDigitalLibrary.org

Dosimetric study of photobiomodulation therapy in 5-FU- induced oral mucositis in hamsters

Claudia Carrara Cotomacio
Luana Campos
Douglas Nesadal de Souza
Victor Elias Arana-Chavez
Alyne Simões

SPIE.

Claudia Carrara Cotomacio, Luana Campos, Douglas Nesadal de Souza, Victor Elias Arana-Chavez, Alyne Simões, "Dosimetric study of photobiomodulation therapy in 5-FU-induced oral mucositis in hamsters," *J. Biomed. Opt.* **22**(1), 018003 (2017), doi: 10.1117/1.JBO.22.1.018003.

Dosimetric study of photobiomodulation therapy in 5-FU-induced oral mucositis in hamsters

Claudia Carrara Cotomacio, Luana Campos, Douglas Nesadal de Souza, Victor Elias Arana-Chavez, and Alyne Simões*

University of São Paulo, Department of Biomaterials and Oral Biology, School of Dentistry, São Paulo, Brazil

Abstract. Oral mucositis (OM) is a debilitating consequence of cancer treatment that could be treated with photobiomodulation therapy (PBMT); however, there is no consensus about its dosimetric parameters for OM healing. The aim of this study was to compare different PBMT protocols on OM treatment, through clinical and histological analysis. Thirty hamsters were used, in an induced model of OM by 5-fluorouracil (5-FU) and superficial scratching, in seven days of follow-up. The animals were divided into five groups: control (C), which received only anesthesia and chemotherapeutic vehicle; chemotherapy (Ch), which received anesthesia, 5-FU, and scratches; laser 1 (L1), the same as Ch group, PBMT 6 J/cm² and 0.24 J (one point); laser 2 (L2), the same as Ch group, PBMT 25 J/cm² and 1 J (one point); and laser 3 (L3), the same as Ch group, PBMT 4 points of 0.24 J and 6 J/cm² each. The laser used has $\lambda = 660$ nm, 0.04 cm² of spot area, and 40 mW. The best PBMT protocol to maintain lowest OM levels compared to Ch group was L1, followed by L2 and L3. Our results suggest that the application mode of PBMT and the energy delivered per area could interfere with the OM healing. © 2017 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.22.1.018003]

Keywords: oral mucositis; photobiomodulation; photobiomodulation therapy; chemotherapy; hamsters.

Paper 160747R received Oct. 31, 2016; accepted for publication Jan. 9, 2017; published online Jan. 27, 2017.

1 Introduction

Oral mucositis (OM), a clinical condition that usually affects patients undergoing chemotherapy or radiotherapy, is characterized by inflammation of the oral mucosa varying from erythema to confluent ulcers.¹ This condition directly affects the quality of life of these patients due to the pain and difficulties with eating, swallowing, chewing, and speaking, contributing to debilitation of patients with cancer.¹⁻³

Sonis⁴ described the etiological process of OM in five phases: the first phase, called initiation, occurs when either radiation or chemotherapy induces a large production of oxygen reactive species, leading to oxidative stress that can cause cell death or leaves the cell to act as a signaling mediator. In the second and third phases, in response to the primary damage and amplification, in other cells, oxidative stress stimulates death or activation of NF- κ B—a nuclear factor that stimulates the production of inflammatory cytokines such as TNF- α , which in turn stimulate more NF- κ B in other cells, in a positive feedback process, promoting amplification of inflammation. In the fourth phase—ulceration, there is pseudomembrane formation, composed of fibrin, dead cells, and microorganisms, stimulating further proinflammatory activity of macrophages. A few days after oncological treatment ends, the trend is toward healing and reestablishing the epithelium, leading to the last phase: healing itself.

The widely used antimetabolite, 5-fluorouracil (5-FU), affects mitosis in the S phase of DNA replication by cells. This drug specifically affects the synthesis of thymidylic acid, nitrogenous bases of DNA. The nonspecific mechanism of action makes 5-FU attack all cells of the body that frequently

replicate, such as gastrointestinal cells, thus explaining several side effects of the oncological treatment,^{5,6} including OM.

Some studies have been conducted trying to bring more comfort to patients with cancer and help them undergo cancer treatment without oral pain and with more quality of life, decreasing OM severity, for example.⁷ Over the past few years, photobiomodulation therapy (PBMT) has been studied for the management of OM, mainly the red wavelength that seems to be related to tissue repair, delay in the development of OM, or maintenance of the grade of OM at low levels.⁸⁻¹⁰ In addition to low power red laser, the infrared wavelength has also been used, but its use is more related to analgesia, due to its deeper penetration into the tissue and its action mechanism.¹¹

Although many studies have demonstrated the effectiveness of PBMT on chemotherapy or head and neck radiotherapy-induced OM,^{8-10,12,13} several PBMT protocols and application modes have been described in the literature. Thus, due to the wide range of studies that have used different amounts of PBMT energy; different modes of PBMT application, and the characteristics of the tissue before laser irradiation, the aim of this study was to compare the effects of three different parameters of red PBMT on the oral mucosa of hamsters that either received a 5-FU injection and a scratch with the tip of a needle or not.^{14,15}

2 Materials and Methods

This study, conducted at Oral Biology Laboratory, University of São Paulo, São Paulo, Brazil, was approved by the Ethics Committee on Animal Use of University of São Paulo (FO-USP) (Process number 2015.010) and was carried out in

*Address all correspondence to: Alyne Simões, E-mail: lysimoes@usp.br

accordance with the Ethical Principles of Animal Experimentation adopted by the Brazilian Society of Laboratory Animal Science.

2.1 Animals

All animals were kept in the Animal House of the Laboratory of Oral Biology, School of Dentistry, USP, one per cage, with free access to water and food, in a 12-h day/night cycle at $23 \pm 3^\circ\text{C}$, and were monitored daily. Thirty Syrian hamsters (15 female and 15 male), weighing ~ 150 g each were divided equally, according to gender and number of animals, into five groups: control (six animals), chemotherapy (six animals), laser 1 (six animals), laser 2 (six animals), and laser 3 (six animals), totaling a sample of 12 cheek pouch mucosae analyzed in each group. The control group (C) received only the chemotherapeutic vehicle. All the experimental groups received chemotherapeutic 5-FU, as follows: chemotherapy group (Ch) received OM induction only; laser 1 group (L1) received OM induction and PBMT protocol 1 (the photobiomodulation protocols are shown below); laser 2 group (L2) received OM induction and PBMT protocol 2; and laser 3 group (L3) received OM induction and PBMT protocol 3. The animals were euthanized on day 7 of follow-up. The three PBMT protocols are described below.

2.2 OM Induction

OM induction was performed according to Cruz et al.¹⁵ On days 1 and 3, OM was induced in groups Ch, L1, L2, and L3 by injections of 5-FU (Sigma Chemical Co., St. Louis, Missouri) at doses of 100 and 65 mg/kg body weight, respectively. The animals of C group received only the chemotherapeutic vehicle (ammonia hydroxide, 1 M). On days 4 and 5, the left and right cheek pouch mucosae were scratched with the tip of a needle. The everted cheek pouch [Fig. 1(a)] was scratched within the demarcated area (1 cm^2) by dragging the needle across the mucosa twice, in a linear movement [Fig. 1(c)]. This technique has repeatedly been used to mimic the development of OM in humans.^{14–20}

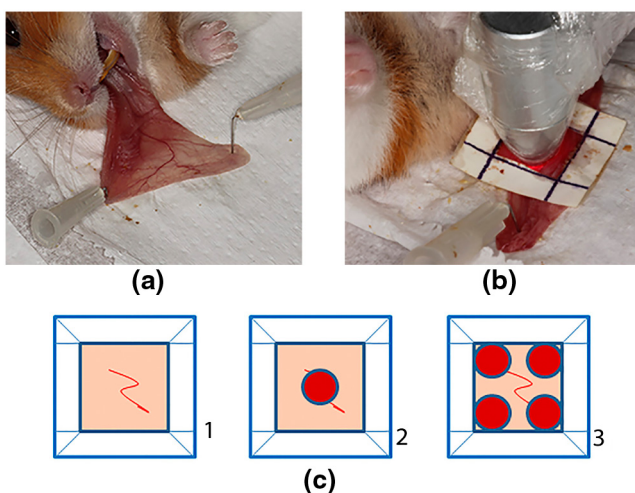


Fig. 1 PBMT application scheme. (a) Everted cheek pouch mucosa, where the scratches and PBMT were performed, (b) PBMT application in contact mode, and (c) PBMT points of application: (1) an example of oral mucosa after scratches; (2) PBMT, using one central point for protocol 1 (L1), PBMT 6 J/cm^2 and 0.24 J per point and for protocol 2 (L2), PBMT 25 J/cm^2 and 1 J per point; and (3) PBMT being applied in 4 points of 6 J/cm^2 and 0.24 J for protocol 3 (L3).

From the day 4, all animals were anesthetized with xylazine (Anazedan[®], Vetbrands, Brazil) 13.8 mg/kg and ketamine (Dopalen[®], Vetbrands, Paulínia, São Paulo, Brazil) 11.6 mg/kg .

2.3 Photobiomodulation Therapy

From days 4 to 7 PBMT was performed in the red wavelength (660 nm), power output of 40 mW , with continuous radiation, and spot area of $\sim 0.04\text{ cm}^2$ (MMOptics[®], São Carlos, Brazil), in contact mode [Fig. 1(b)], with an irradiance of 1 W/cm^2 . The protocols were divided as follows: protocol 1 (L1), a smaller amount of energy in a central point with energy density of 6 J/cm^2 and 0.24 J of energy; protocol 2 (L2), a higher amount of energy in a central point, with energy density of 25 J/cm^2 and 1 J of energy; and the protocol 3 (L3), with higher amount of energy divided into four points of 6 J/cm^2 and 0.24 J each, totaling 24 J/cm^2 and 0.96 J .

In that way, the L1 group received 6 J/cm^2 and 0.24 J per point, 6 s of application in a central point of the OM area; L2 received 25 J/cm^2 , 1 J per point, 25 s, in a central point of the OM area; and L3 received a total of 24 J/cm^2 and 0.96 J divided into four points of 6 J/cm^2 and 0.24 J , 6 s per point, around the induced OM area [Fig. 1(c)]. The procedures complied with the health and safety regulations, including the use of protective glasses, gloves, and PVC film to cover the laser pen tip.

2.4 OM Evaluation

OM clinical severity was evaluated by two calibrated examiners and degree of severity was determined by using the scales proposed by Sonis et al.²⁰—modified OM Assessment Scale (OMAS)—adapted to hamsters, according to Wilder-Smith et al.²¹ Both right and left oral mucosa tissues were classified, totaling 12 mucosal samples per group. For classification, the ulcerated area was estimated, considering the demarcated area of 1 cm^2 , as 0 (nothing), $<4\text{ mm}^2$; $4\text{ to }9\text{ mm}^2$ or $>9\text{ mm}^2$; and the severity of erythema was classified as 0 (nothing); not severe (mild erythema, close to bright red color); and severe (severe erythema, close to dark red and purple color). After crossing these two items of information, it was possible to determine the grade of OM in each mucosa.

2.5 Food and Water Intake

Food and water was measured on the first and last day of follow-up to calculate the intake in this period. The body mass of each animal was also assessed daily.

2.6 Morphological Analysis

The left and right cheek pouch mucosae of all animals were removed immediately before euthanasia and fixed in 4% formaldehyde (freshly prepared from paraformaldehyde) and 0.1% glutaraldehyde (Polysciences, Pennsylvania) buffered in 0.1 M phosphate at pH 7.2. The tissues were prepared for histological analysis by light microscopy in increasing concentrations of alcohol for subsequent paraffin embedment. Four-micrometer sections were cut using a low profile knife (Crystal Plus[™]) on a microtome (MICROM HM 360, Germany) and stained with hematoxylin and eosin. Images were captured and examined using an Olympus BX 60 light microscope at $20\times$ magnification.

2.7 Statistical Analysis

The Kruskal-Wallis and Friedman statistical tests were used to analyze the clinical outcomes, with a power value of 80%. Values with significance less than 5% ($p < 0.05$) were considered for the study. For all tests, statistical software Action Stat Pro (version 3.1, 2016) was used.

3 Results

3.1 OM Evaluation

With regard to results of OM evolution and grades distribution, on day 4, the first day of scratches, the Ch and L2 groups presented most grades equal to 2 (just one classification grade 1 and one grade 3), L1 group presented grades between 1 and 2 (mean and median closer to grade 2), and L3 group presented grades between 2 and 3 (mean and median closer to grade 2). On day 5, the L1 and L2 groups increased the distribution between 1 and 3 (mean and median closer to grade 2). Likewise, groups Ch and L3 increased OM values, but mean and median were closer to grade 3. On day 6, L1 and L2 groups maintained the distribution of OM grades, but most of the classifications in L1 group were placed between grades 1 and 2, while for L2 group were between grades 2 and 3 (mean and median closer to grade 2). Ch group increased the distribution between grades 3 and 4, being mean and median closer to 4. L3 group showed most of the grades classifications between grades 3 and 4. On day 7, the last day of follow-up, the Ch group increased the grade classifications, mean and median near to grade 5 while all the PBMT protocols presented better OM results than no treatment (Ch group). The protocol 1 (L1 group), with the lowest amount of energy in one central point, showed the best results, followed by L2 and L3 protocols. The L1 group decreased the OM classification, between 0 and 2 (mean and median equal to 1); L2 group maintained the distribution between 1 and 2 (just one grade value equal to 3 and mean and median equal to 2); and L3 group, that after an OM

grade increase at the first days, maintained the grades between 3 and 4 (just one OM value equal to 1). The control group, that did not receive any OM induction, showed grade 0 during all the experimental time, as expected [Fig. 2(a)].

On the following days, the Ch group showed a tendency to increase OM grade while all the PBMT groups showed better results in mucositis grades than Ch group, which only received mucositis induction [Fig. 2(a)]. On day 5, the mean of the OM grade in groups L1 and L2 was ~20% lower than that of group Ch ($p = 0.064$). On days 6 and 7, group L1 showed a lower grade of OM than that of group Ch at 52% on day 6 ($p < 0.001$) and 78% on day 7 ($p < 0.001$). L2 group showed scores ~40% ($p < 0.001$) and 60% ($p < 0.001$), respectively, lower than group Ch on days 6 and 7. The L3 group showed scores 33% lower than Ch group ($p < 0.001$). The L1 group showed better OM grades than L2 group ($p = 0.001$) and L3 group ($p < 0.001$) on day 7 [Fig. 2(a)].

When the means of each group were analyzed during the time [Fig. 2(b)], Ch group presented a difference in the grades of OM on days 4 and 5 in comparison with day 7 (an increase of 143% and 65% of OM) and days 4 to 6 (an increase of 91%) ($p < 0.001$). L1 group tended to show a decrease from 1.8 to 1 in the OM scores from days 4 to 7; the significant difference was between grades assessed on days 5 and 6, when OM started to decrease (a decrease of 30%— $p = 0.038$). Group L2 maintained the OM scores near to 2 ($p > 0.05$). Group L3 score increased from 2 to 3, and the significant difference was between grades assessed on days 4 to 6, when this group reached its highest score (on day 6 the OM grade was 48% greater than OM grade assessed on day 4— $p = 0.01$) [Fig. 2(b)].

The mean of OM grades in male and female hamsters was analyzed separately too. Male and female hamsters showed the same evolution in the experimental time ($p > 0.05$) in each group: Ch [Fig. 3(a)], L1 [Fig. 3(b)], L2 [Fig. 3(c)], and L3 [Fig. 3(d)], with similar trend lines.

Clinically, on day 5, last day of scratches, the aspect of the oral mucosa was similar among groups Ch, L1, L2, and L3. On

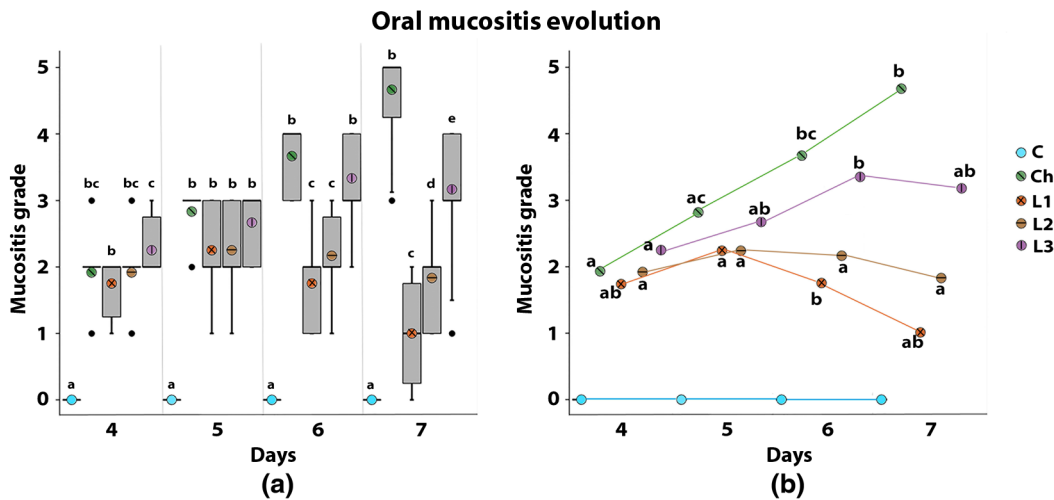


Fig. 2 (a) Mucositis grades distributions in each group from days 4 to 7, according to W-Smith scale for mucositis grade and considering both genders together (male and females). Different letters indicate statistical relevance among all groups in each day ($p < 0.05$ -Kruskal-Wallis test); (b) OM evolution by means of each group from days 4 to 7. Different letters indicate statistical relevance among days (Friedman test). C, control; Ch, chemotherapy; L1, PBMT 6 J/cm² and 0.24 J—one point; L2, PBMT 25 J/cm² and 1 J—one point; L3, PBMT four points of 6 J/cm² and 0.24 J; circle, mean; bold line, median; dark dot, maximum and minimum single value.

Comparison of oral mucositis in different genders

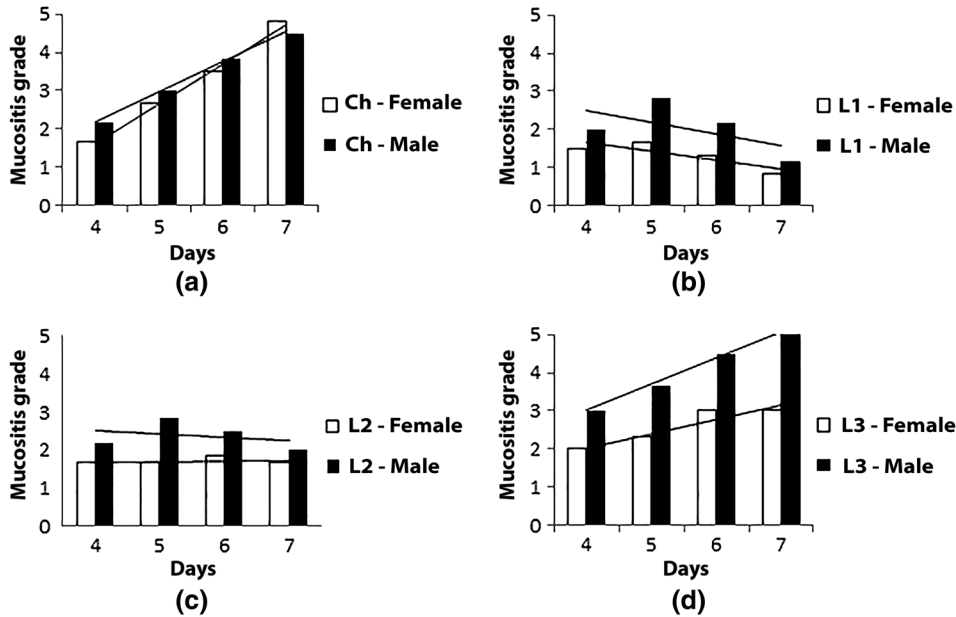


Fig. 3 Comparison of OM evolution between male and female genders in each group: (a) chemotherapy group (Ch); (b) PBM protocol 1 group (L1); (c) PBM protocol 2 group (L2); (d) PBM protocol 3 group (L3), from days 4 to 7, with tendency lines of OM evolution ($p > 0.05$, Friedman test).

day 7, Ch group, that received only mucositis induction, presented ulcers, necrosis, and severe erythema while in L1 and L2 groups it was possible to observe an improvement of OM. L3 group showed lesions, but in a better stage than Ch group (Fig. 4).

3.2 Food, Water Intake, and Body Mass

No statistical difference was found in food and water intake. However, group L1 showed food and water intake closer to group C, which did not receive OM induction. Group C had the highest water intake, 28% more than group Ch; 7% more than group L1; 60% more than group L2; and 90% more than group L3. For food intake, group L1 was the one with the highest intake: 8% more than group C; 43% more than group Ch; 31% more than group L2; and 43% more than group L3. Nevertheless, group C was the one that showed the lowest weight loss: 69% less than group Ch and L1 ($p = 0.005$); 74% less than group L2 group ($p = 0.05$); and 67% less than group L3 ($p = 0.05$) (Fig. 5).

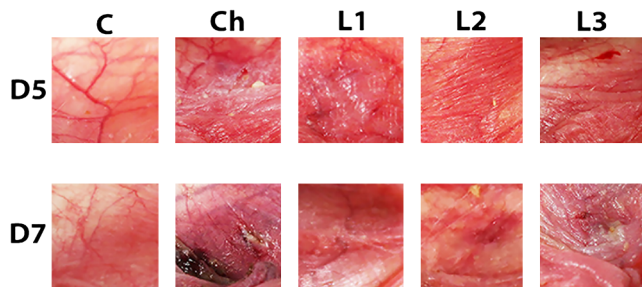


Fig. 4 Clinical comparison of the hamster cheeks pouch mucosa in each group, between days 5 (D5) and 7 (D7).

3.3 Histological Analysis

Some histological aspects were observed in the experimental groups. On day 7, group C presented nondamaged tissue: intact epithelium and connective tissue of the lamina propria, with significant muscle fibers and blood vessels. Group Ch showed evident ulcer and a disrupted epithelium; the subjacent connective tissue exhibited moderate to intense inflammatory infiltrate, with presence of extravasation of blood cells and necrosis below the ulcer. Group L1 showed an aspect similar to that of group C, with continuous and more organized epithelium than group Ch, and absence of inflammatory cells in the connective tissue. Group L2 presented the same pattern as those of groups C and L1, with the aspect of a continuous thin epithelium and few blood vessels in the connective tissue. The pattern of group L3 seemed to be an intermediate stage between those of group Ch and groups L1 and L2, due to the appearance of epithelial regeneration in the ulcer region, in which the basal layer seemed to be forming and not all of the epithelium was

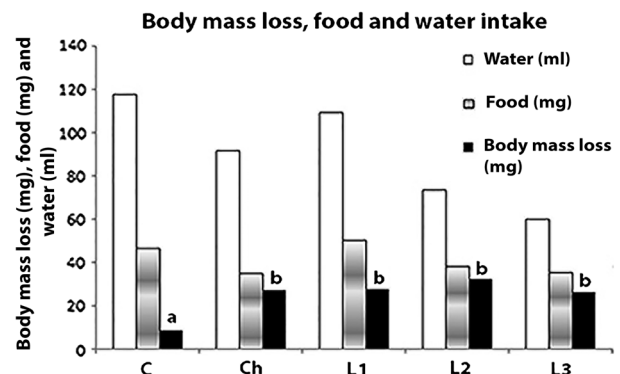


Fig. 5 Comparison of food, water intake, and body mass on day 7 ($p > 0.05$). Kruskal-Wallis test.

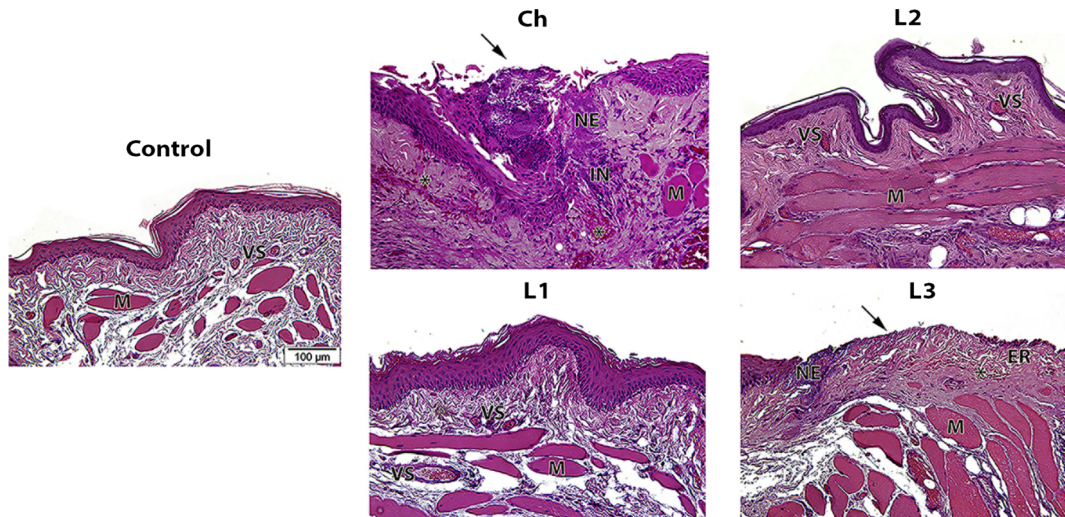


Fig. 6 Photomicrographs of the cheek pouch mucosa of animals from the different experimental groups. Representative images from control, Ch (chemotherapy), L1 (PBMT 6 J/cm² and 0.24 J—one point), L2 (PBMT 25 J/cm² and 1 J—one point), and L3 (PBMT 24 J/cm² and 0.96 J—four points of 6 J/cm², 0.24 J each) groups on day 7. Arrows, ulcers; asterisks, extravasation of blood cells; IN, inflammatory infiltrate; VS, blood vessels; M, muscle fibers; NE, necrosis; ER, epithelial regeneration. Hematoxylin and eosin staining. Original magnification: 20 \times .

organized; some extravasation of blood cells and small region of necrosis were observed in the lamina propria (Fig. 6).

4 Discussion

In the past few years, PBMT has been shown to be a good alternative for OM management. Animal models have been successfully used to determine which is the best PBMT protocol (wavelength, power, frequency, time, and mode of application) to keep OM at lower levels or speed up the healing process. In the present study, all of the three protocols were capable of maintaining lower grades of OM than that of group Ch, but only group L1, which had received only one central point of PBMT application, with 0.24 J of energy, showed significant difference on day 7, followed by L2 and L3 protocols, in comparison to Ch group.

With use of the OM induction model in hamsters, proposed by Sonis et al.,¹⁴ some studies have tested the effects of PBMT on OM. França et al.¹⁶ observed that the group that received PBMT (660 nm, 30 mW, 1.2 J/cm² in four points of \sim 0.4 J of energy on each one) on OM lesions presented faster healing than the group that had not received treatment. Another study¹⁷ that used 96 hamsters divided into control (no treatment) and experimental groups (PBMT for OM prevention; PBMT to treat OM, and a combination of both), showed positive results, reducing OM levels when the therapeutic PBMT protocol was applied. In this study, PBMT was used with a power output of 40 mW, 6.6 J/cm² of energy density, 0.24 J of energy, and 6 s of irradiation time, per point, with six points being applied in contact mode.

In the present study, group L1 that received 6 J/cm²/0.24 J, 6 s of application, in a central point of the OM area, showed the best results in OM healing, followed by groups L2 (25 J/cm²/1 J, 25 s, in a central point) and L3 (6 J/cm²/0.96 J divided into four points, 6 s each, around the induced OM area). The authors were able to infer that the central and localized application mode of L1 and L2 groups appeared to be more effective than the application around the OM lesion, as was performed in group L3, and the less

amount of energy of L1 group seems to be better than a higher amount of energy of L2 group in a central point, or divided in four points, as performed in L3 group. However, it is possible to suppose that if group L3 had had a fifth application point, or more localized points in the center of the lesion, this group would perhaps have had better results, such as the results obtained by Lopez et al.,¹⁷ who applied six points distributed over the entire OM surface area, with 0.24 J of energy per point, or like the results found by Campos et al.¹⁹ that applied five points of 0.24 J each, covering all the surface area of OM induction.

Based on the literature over the past few years, Simões et al.²² suggested that there are some aspects and parameters of PBMT that ensured the results obtained. One of these was application in contact mode perpendicular to the tissue, because it guaranteed that the amount of energy calculated would reach the tissue. Furthermore, taking into account the results obtained in the present study, the position of the laser beam in relation to the center of the lesion and the amount of energy delivered to the tissue could also be important aspects to consider during PBMT irradiation.

Regarding the food and water intake, although there were no statistical differences among the groups, group C and the group that received less energy per point (L1) showed similar results, indicating better healing of OM in group L1, which enabled the hamsters to eat and drink more than the animals in the other groups, as reported by Campos et al.¹⁹ As expected, group C showed the lowest loss of body mass, differently from the groups that received OM induction (Ch, L1, L2, and L3). Meanwhile, the body mass loss could have been caused by OM induction and consequent difficulty with feeding and systemic effects of 5-FU, in addition to the daily manipulation, application of anesthesia, and stress of the animals.^{15,16}

With regard to the histological findings, other animal studies in the literature have demonstrated similar aspects to those observed in the present study. França et al.¹⁶ showed intense inflammatory infiltrate and scanty granulation tissue in the mucosa without treatment; whereas, in the mucosa that received

PBMT the cited authors observed organization of the collagen fibrils, less inflammatory infiltrate, and expressive angiogenesis. These results were endorsed by Lopes et al.,¹⁸ who showed that PBMT-induced organized collagen, and it also appeared to be related to the modulation of inflammation by reducing the neutrophil infiltrate. Furthermore, Cruz et al.,¹⁵ in a similar study performed with hamsters that received the OM induction protocol and PBMT with a higher dose of laser irradiation (120 J/cm², 40 mW, and 4.4 J of energy), showed similar histological results in the laser group, on day 10, compared with those observed for groups L1 and L2 in the present study, on day 7. Thus, the lower level of energy applied in the present study demonstrated a faster wound healing process when compared with those observed by Cruz et al., which is in agreement with the idea that there are several dosimetric parameters that could interfere with the effect of PBMT on the irradiated tissue.

Huang et al.^{22,23} reviewed the biphasic dose response in photobiomodulation, following the principles of Arndt Schulz model, and showed that insufficient doses and energies have no effect on the treated surface area, in the same way that too high energies seem to have inhibitory effects.^{15,22–26} There are no defined limits of energies to stimulate healing or to produce inhibitory response. Although there is a lack of studies in dosimetric field, some studies, mainly in animal models, started to give us directions to an ideal amount of energy to each desired clinical application. Corazza et al.²⁵ analyzed the effects of photobiomodulation with low level laser and light-emitting diode (LED) therapies on skin wounds in rats and showed that the dose of 5 J/cm² was better than 20 J/cm² for wound healing, similar to the results obtained in the present study for groups L1 (6 J/cm²) and L2 (25 J/cm²). These results showed that PBMT promoted tissue healing, but the difference in speed and effectiveness of the results could be correlated to the amount of energy delivered to the tissue surface and high energies could induce inhibitory effects.^{22–26}

Although protocols with high energies are not the best protocols to speed up healing, they could be related to analgesic effects due to the inhibitory process. Some studies in humans have used high energies to treat OM (2 to 3 J per point)^{12,27,28} and, despite finding benefit in OM healing, they reported greater pain relief than studies using lower energies (closer to 0.24 J, as used in this study),^{8,29,30} which focused the results on the effects of PBM in OM healing.

All the protocols, in the present study, were better than chemotherapy control group to OM healing; however, the group that received less energy showed a faster healing than the other two protocols with high energies. Nevertheless, if it was possible measure the pain associated to OM, maybe the groups with higher energies would presented more pain relief, as reported by the studies in humans and by Yan et al., who showed that PBMT in 650 nm, 35 mW, and 1 J per point produced an analgesic effect in rat's sciatic nerves.³¹

In conclusion, within the limitations of this study, our results suggest that PBMT, mainly with regard to the amount of energy delivered per area and application mode, could interfere in OM healing, being the protocol with less energy in a central point better than the other protocols used for tissue repair. Thus, a further dosimetric study is necessary in the field of PBMT to elucidate the mechanisms of action and biological effects of PBMT following different treatment protocols, to support the results found in this present study.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Acknowledgments

This work has been supported in part by CAPES, University of Sao Paulo and Fundação de Amparo à Pesquisa do Estado de Sao Paulo (FAPESP, Grant No. 2011/14013-1).

References

1. M. A. Mazzeo et al., "Oral signs of intravenous chemotherapy with 5-fluorouracil and leucovorin calcium in colon cancer treatment," *Med. Oral Patol. Oral Cir. Bucal.* **14**(3), E108–E113 (2009).
2. L. Chen and A. Malhotra, "Combination approach: the future of the war against cancer," *Cell Biochem. Biophys.* **72**(3), 637–641 (2015).
3. A. M. Shih et al., "A research review of the current treatments for radiation-induced oral mucositis in patients with head and neck cancer," *Oncol. Nurs. Forum.* **29**(7), 1063–1080 (2002).
4. T. S. Sonis, "Oral mucositis," *Anti-Cancer Drugs* **22**(7), 607–612 (2011).
5. L. S. Goodman et al., *The Pharmacological Basis of Therapeutics*, McGraw Hill, New York, New York (1995).
6. V. L. Almeida et al., "Câncer e agentes antineoplásicos ciclo-celular específicos e ciclo-celular não específicos que interagem com o DNA: uma introdução," *Quím. Nova* **28**(1), 118–129 (2005).
7. T. S. Sonis et al., "Could the biological robustness of low level laser therapy (photobiomodulation) impact its use in the management of mucositis in head and neck cancer patients," *Oral Oncol.* **54**, 7–14 (2016).
8. A. Simões et al., "Laser phototherapy as topical prophylaxis against head and neck cancer radiotherapy-induced oral mucositis: comparison between low and high/low power lasers," *Lasers Surg. Med.* **41**(4), 264–270 (2009).
9. H. S. Antunes et al., "Low-power laser in the prevention of induced oral mucositis in bone marrow transplantation patients: a randomized trial," *Blood* **109**(5), 2250–2255 (2007).
10. P. A. G. Carvalho et al., "Evaluation of low-level laser therapy in the prevention and treatment of radiation-induced mucositis: a double-blind randomized study in head and neck cancer patients," *Oral Oncol.* **47**(12), 1176–1181 (2011).
11. T. I. Karu, "Multiple roles of cytochrome c oxidase in mammalian cells under action of red and IR-A radiation," *IUBMB Life* **62**(8), 607–610 (2010).
12. A. P. Gautam et al., "Low level helium neon laser therapy for chemoradiotherapy induced oral mucositis in oral cancer patients: a randomized controlled trial," *Oral Oncol.* **48**(9), 893–897 (2012).
13. A. G. Lima et al., "Oral mucositis prevention by low-level laser therapy in head-and-neck cancer patients undergoing concurrent chemoradiotherapy: a phase III randomized study," *Int. J. Radiat. Oncol. Biol. Phys.* **82**(1), 270–275 (2012).
14. S. T. Sonis et al., "An animal model for mucositis induced by cancer chemotherapy," *Oral Surg. Oral Med. Oral Pathol.* **69**(4), 437–443 (1990).
15. E. P. Cruz et al., "Clinical, biochemical and histological study of the effect of antimicrobial photodynamic therapy on oral mucositis induced by 5-fluorouracil in hamsters," *Photodiagnosis Photodyn. Ther.* **12**(2), 298–309 (2015).
16. C. M. França et al., "Low-intensity red laser on the prevention and treatment of induced-oral mucositis in hamsters," *J. Photochem. Photobiol. B* **94**(1), 25–31 (2009).
17. T. C. Lopez et al., "Effect of laser phototherapy in the prevention and treatment of chemo induced mucositis in hamsters," *Braz. Oral Res.* **27**(4), 342–348 (2013).
18. N. N. F. Lopes et al., "Effects of low-level-laser therapy on collagen expression and neutrophil infiltrate in 5-fluorouracil-induced oral mucositis in hamsters," *Lasers Surg. Med.* **42**(6), 546–552 (2010).
19. L. Campos et al., "Comparative study among three different phototherapy protocols to treat chemotherapy-induced oral mucositis in hamsters," *J. Biophotonics* 1–10 (2016).

20. S. T. Sonis et al., "Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group," *Cancer* **85**(10), 2103–2113 (1999).
21. P. Wilder-Smith et al., "In vivo imaging of oral mucositis in an animal model using optical coherence tomography and optical Doppler tomography," *Clin. Cancer Res.* **13**(8), 2449–2454 (2007).
22. Y. Y. Huang et al., "Biphasic dose response in low level light therapy," *Dose Response* **7**(4), 358–383 (2009).
23. Y. Y. Huang et al., "Biphasic dose response in low level light therapy—an update," *Dose Response* **9** (4), 602–618 (2011).
24. J. Tuner and L. Hode, *The New Laser Therapy Handbook*, Prima Books, Grängesberg, Dalarna County, Sweden (2010).
25. A. V. Corazza et al., "Photobiomodulation on the angiogenesis of skin wounds in rats using different light sources," *Photomed. Laser Surg.* **25**(2), 102–106 (2007).
26. P. M. Freitas and A. Simoes, *Lasers in Dentistry: Guide for Clinical Practice*, Wiley Blackwell, New York, New York (2015).
27. M. M. Abramoff et al., "Low-level laser therapy in the prevention and treatment of chemotherapy-induced oral mucositis in young patients," *Photomed. Laser Surg.* **26**(4), 393–400 (2008).
28. T. Zanin et al., "Use of 660-nm diode laser in the prevention and treatment of human oral mucositis induced by radiotherapy and chemotherapy," *Photomed. Laser Surg.* **28**(2), 233–237 (2010)
29. F. P. Eduardo et al., "Severity of oral mucositis in patients undergoing hematopoietic cell transplantation and an oral laser phototherapy protocol: a survey of 30 patients," *Photomed. Laser Surg.* **27**(1), 137–144 (2009).
30. G. B. Silva et al., "The prevention of induced oral mucositis with low-level laser therapy in bone marrow transplantation patients: a randomized clinical trial," *Photomed. Laser Surg.* **29**(1), 27–31 (2011).
31. W. Yan et al., "Inhibitory effects of visible 650-nm and infrared 808-nm laser irradiation on somatosensory and compound muscle action potentials in rat sciatic nerve: implications for laser-induced analgesia," *J. Peripher. Nerv. Syst.* **16**(2), 130–135 (2011).

Biographies for the authors are not available.