

## Low-intensity red laser on the prevention and treatment of induced-oral mucositis in hamsters

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### ABSTRACT

**Objective:** The purpose of this study was to investigate the effects of laser phototherapy as preventive and therapeutic regime on induced-oral mucositis in hamsters.

**Design:** The animals were divided into four groups: preventive cryotherapy, preventive laser, therapeutic laser and therapeutic control group. Mucositis was induced in hamsters by intraperitoneal injection of 5-fluorouracil (5-FU) and superficial scratching. All preventive treatment was performed on the right cheek pouch mucosa. The left pouch mucosa was used for a spontaneous development of mucositis and did not receive any preventive therapy. Laser parameters were:  $\lambda = 660 \text{ nm}$ ,  $P = 30 \text{ mW}$ ,  $D = 1.2 \text{ J/cm}^2$ ,  $\Delta t = 40 \text{ s}$ , spot size  $3 \text{ mm}^2$ ,  $I = 1 \text{ W/cm}^2$ . Cryotherapy was done positioning ice packs in the hamster mucosa 5 min before 5-FU infusion and 10 min afterward. To study the healing of mucositis, the left pouch mucosa of each of the hamsters in the TLG received laser irradiation on the injured area. Irradiation parameters were kept the same as abovementioned. The control hamsters in the TCG did not receive any treatment. The mucositis degree and the animal's body mass were evaluated. An assessment of blood vessels was made based on immunohistochemical staining.

**Results:** The CG animals lost 15.16% of their initial body mass while the LG animals lost 8.97% during the first 5 days. The laser treated animals had a better clinical outcome with a faster healing, and more granulation tissue. The quantity of blood vessels at both LG and CG were higher than in healthy mucosa. Regarding the therapeutic analysis, the severity of the mucositis in the TLG was always lower than TCG. TLG presented higher organization of the granulation tissue, parallel collagen fibrils, and increased angiogenesis.

**Conclusion:** The results suggest that laser phototherapy had a positive effect in reducing mucositis severity, and a more pronounced effect in treating established mucositis.

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### 1. Introduction

Mucositis is a significant clinical problem in patients receiving cancer therapy. The manifestations may range from limited patches of mildly sore erythematous mucosa to frank ulceration and hemorrhage [1,2]. Reports from literature confirm the high incidence of mucositis in patients receiving radiotherapy (RT), or chemo-radiation therapy. In patients receiving standard chemotherapy, 5–15% develop mucositis. When the treatment protocol involves 5-fluorouracil administration (5-FU), with or without leucovorin, this number rises up to 40% [3,4]. Approximately 75–85%

of the patients under conditioning regimen prior to bone marrow transplantation present some degree of mucositis [5]. This treatment-induced complication causes oral discomfort and pain, and can also lead to other complications such as poor nutrition due to pain, delays in drug administration and increased medical costs. It also may be a life-threatening condition due to infection (septicemia) [6].

Few interventions are of proven efficacy in reducing the severity or duration of mucositis, and there are no universally accepted treatment protocols [7]. Many agents and strategies have been used, such as basic oral care, oral rinses, analgesics, antibiotics, cryotherapy, local anesthetics, growth factors and cytokines, biologic mucosal protectants, and anti-inflammatory agents among others. To date, there is not a single treatment capable of preventing or treating mucositis in an efficient way [5–8].

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Some studies have reported beneficial effects of low-intensity laser therapy (LLT) to promote tissue repair and reduce pain and inflammation [9–11]. This therapy has been applied in clinical practice to prevent and treat oral mucositis with some good results been reported [12–15]. Recently, a clinical trial investigated the effects of a red laser on the prevention and reduction of the severity of conditioning-induced-oral mucositis for hematopoietic stem cell transplantation patients [16]. Those results indicated that pretreatment with laser irradiation was effective in reducing the incidence of oral mucositis, although the mechanisms underlying laser effects are still poorly understood [17]. The present study aimed to evaluate clinically and histologically the effects of LLT as a prophylactic tool as well as a treatment agent of 5-fluoracil induced-oral mucositis in hamsters. In the prophylactic study, the effects of LLT were compared with ice chips, a currently acceptable prophylactic agent for oral mucositis [6].

## 2. Methods

Thirty female Golden Syrian hamsters with a mean of 150 g of body mass, and 8 weeks old were used. All animals were acclimated to laboratory conditions for at least 5 days before being dosed with 5-FU. During the acclimatizing period, the general health condition of the hamsters was evaluated, and they were fed with a standard laboratory diet and water *ad libitum*. During the experimental period, all animals received humane care in compliance with the Ethical Principles of Animal Experimentation formulated by the Brazilian College for Animal Experimentation, and in accordance with guidelines approved by the Council of the American Psychological Society for the use of animal in experiments.

### 2.1. Mucositis induction protocol

Before the beginning of the experiment, two animals were sacrificed in a CO<sub>2</sub> chamber and excisional biopsies of the cheeks pouch mucosa were performed and the specimens were routinely processed for histology to serve as a normal mucosa control. The rest of the animals were anesthetized with Copazine® (Xylazine) 0.075 mL/g and Vetanarcol® (Ketamine) 0.3 mL/g, and the buccal pouches were everted and photographed. Each animal received an intraperitoneal injection of 100 mg/kg of 5-FU on day 1 and 65 mg/kg of 5-FU on day 3. The right and left cheek pouch mucosa were irritated by superficial scratching with the tip of an 18-gauge needle at days 4 and 5. The needle was dragged twice in a linear movement across the everted cheek pouch once a day until erythematous changes were noted. This technique has been repeatedly used to produce ulcerative mucositis, which mimics the human development of mucositis [18,19].

#### 2.1.1. First phase: preventive analysis

Twenty eight hamsters, corresponding to  $n = 28$  right cheek pouch mucosae, were divided into two equally sized groups: (LG) laser group ( $n = 14$ ) and (CG) cryotherapy group ( $n = 14$ ). All preventive treatment was performed only on the right cheek pouch mucosa. The left side was kept untreated and unanalyzed, but with the same mucositis induction protocol. Before any procedure, the animals were anesthetized as previously described.

On the CG group, at days 1 and 3 (chemotherapy infusion days), 14 animals were submitted to applications of ice chips on the right cheek pouch mucosa for 5 min before 5-FU injection and 10 min afterward.

On LG group, 14 animals were irradiated daily from day 1 to day 4, before the scratching after 5-FU injection. The right cheek pouch mucosa was irradiated with a GaAlAs laser (Kondortech, São Carlos,

SP, Brazil) emitting red light ( $\lambda = 660$  nm). The output power was 30 mW at a spot size of 2 mm in diameter corresponding to an irradiance of 1 W/cm<sup>2</sup> per point. The tip of the laser device carefully touched the mucosa for a total of  $\Delta t = 40$  s per day scanning the total area of 1 cm<sup>2</sup> in four different points with 10 S per point. The total energy density (dose) was 1.2 J/cm<sup>2</sup>.

The clinical aspect of the right cheek pouch mucosa was observed by two blind-independent calibrated observers and the degree of mucositis was evaluated through oral mucositis assessment scale (OMAS) modified for hamsters according Wilder-Smith et al. [20] as follows: 0—no ulceration and no erythema; 1—ulceration < 4 mm<sup>2</sup> and slight erythema; 2—ulceration of 4–9 mm<sup>2</sup> and moderate erythema; 3—ulceration > 9 mm<sup>2</sup> and severe erythema; 4—appearance of pseudomembrane. The sores gave by the observers were evaluated by Kappa's test for inter-rater reliability.

The preventive treatment with cryotherapy and laser was performed until day 5, which corresponded to the onset of the mucositis. On day 5, the preventive protocol for CG and LG was ended, but the clinical analysis followed through the 11 days, until the end of the experimental procedure, without any further treatment. The animals were euthanized on day 3 ( $n = 6$ ), day 6 ( $n = 6$ ), day 9 ( $n = 6$ ), and day 11 ( $n = 10$ ), and the right cheek pouch was collected for histological purposes.

The mucositis scores obtained during the 11 days were used to plot a curve and areas under the curves were calculated using the integrate function in Microcal Origin 7.0 software program (Northampton, MA) to provide an overview of the mucositis development after preventive protocol. Statistical analysis was performed using two-tailed *t*-test. The value of  $p < 0.05$  was considered significant. The data are presented as mean  $\pm$  SEM.

The animal's body mass was also evaluated during the first 5 days. A weight loss was expected due to discomfort and pain [21]. The animals were weighed daily. The body mass was recorded and statistical analysis was carried out using one-tailed *t*-test to verify the intra-group performance as well as to compare the weight loss values between groups. The data are presented as mean  $\pm$  SEM.

Histological analysis was performed on specimens from the right cheek pouch mucosa to verify the influence of both treatments on the course of mucositis. After sacrifice, the areas of the mucosa containing the wounds were collected and fixed by immersion in formaldehyde at 4 °C for 24 h. The specimens were dehydrated and thereafter embedded in Paraplast (Oxford, USA) to provide transversal sections of the tissue. Five-micron sections were stained with hematoxylin/eosin (HE). Stained sections were observed and photographed with an optical microscope (Leica DMLP, Germany).

For a quantitative assessment, blood vessels were counted after immunohistochemical staining according to Dako EnVision protocol.

The positive blood vessels localized at the ulcer area were counted in five selected square areas measuring 10,000  $\mu\text{m}^2$  each using the Image J software and the total number of counted blood vessels was divided by the number of squares used. At least three slides from each animal were observed. Healthy mucosa was also evaluated.

Statistical analysis of the number of blood vessels was performed. Values are given as means and bars are standard error bars (SEM). Statistical comparisons between groups were carried out with two-tailed *t*-test, which retains the overall significance level at 5% ( $p < 0.05$ ).

#### 2.1.2. Second phase: therapeutic study

On day 5, the remaining animals ( $n = 22$ , since 6 were euthanized on day 3) were randomly regrouped; thus 22 animals were alienated into two new groups with 11 animals per group and only

the left side was now manipulated to study the therapeutic effects of the laser irradiation over the established mucositis.

The animals were divided into the groups TCG (no treatment control group) and TLG (therapeutic laser group). Eleven left cheek pouches mucosa from 11 animals on TCG did not receive any treatment and were used as a spontaneous healing control. Eleven animals from TLG received laser irradiation by contact of the laser tip scanning the injured area at days 6–10. The laser parameters were kept the same as mentioned above ( $\lambda = 660$  nm, output power 30 mW, total energy density  $1.2 \text{ J/cm}^2$ , exposure time of 40 s [four points for 10 S], spot size  $3 \text{ mm}^2$ ). During the therapeutic investigation, the left mucosa clinical aspect was evaluated and scored as aforementioned. Areas under the curves were computed.

For both groups, three animals per day were killed on days 6 and 9. The remaining five animals of each group were euthanized on day 11. Mucosa biopsies were carefully collected to include the adjacent healthy mucosa and all the healed tissue in depth. All biopsies were fixed in formalin 10% for 24 h. Thereafter, biopsies were included in paraffin and  $5 \mu\text{m}$  thickness transversal sections were obtained. The specimens were stained with HE using the same procedures described above as well as the immunohistochemical staining for the quantitative assessment of blood vessels.

Statistical analysis for the therapeutic study was achieved in the same way as described in the preventive study.

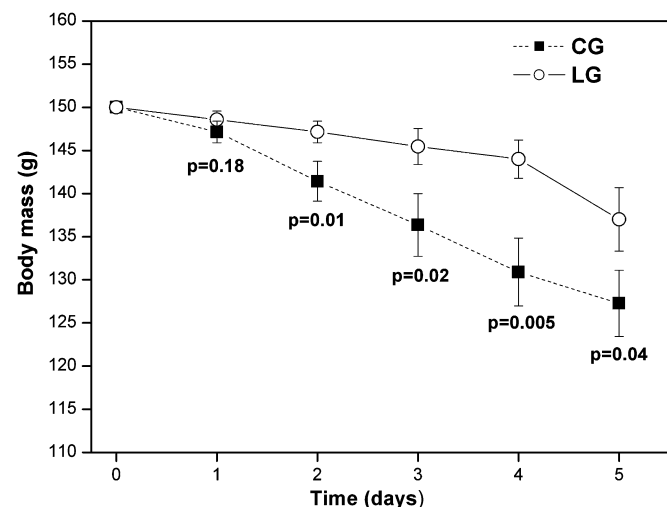
### 3. Results

#### 3.1. Preventive analysis

##### 3.1.1. Body mass

The body mass analysis revealed weight loss for both preventive groups (CG and LG) during the first five days of treatment (Fig. 1). The weight loss of CG was significantly higher than in LG from day 2 to 5.

Regarding the weight loss on CG, a significant difference was found comparing day 1 with the original mean weight on day 0 ( $p = 0.025$ ). Comparing the mean values of day 1 with day 2, a significant difference ( $p = 0.038$ ) was also noted. Comparing the mean weight on day 2 with day 3, day 3 with day 4, and day 4 with day 5, significant differences were not observed. However, comparing



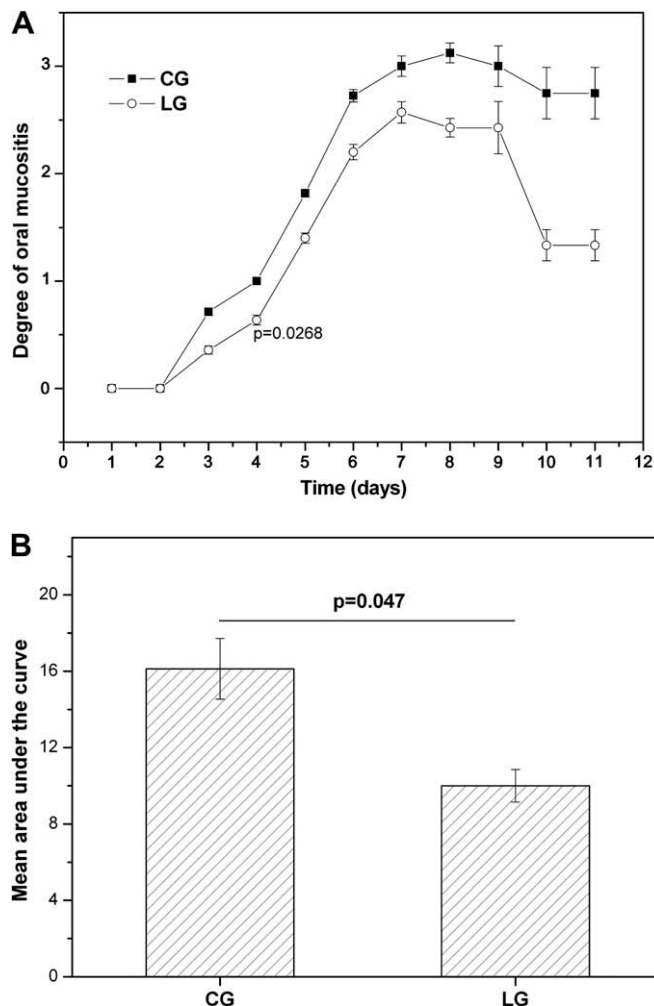
**Fig. 1.** Hamster body mass in cryotherapy (CG) and laser groups (LG) during the first five days of the experiment (preventive period). Note that before irradiation (day 0) and on day 1 the groups presented similar body mass. From day 2 until day 5, the groups were considered statistically different, with a higher mean body mass on LG. Data points are means and SEM. Significance was determined according to one-tailed independent *t*-test.

with day 1, the mean weight in all the other moments were different; therefore a significant loss of body mass was detected from day 1 and on. The percentage of body mass lost was 1.91% on day 1, 5.72% on day 2, 9.10% on day 3, 12.73% on day 4 and 15.16% on day 5.

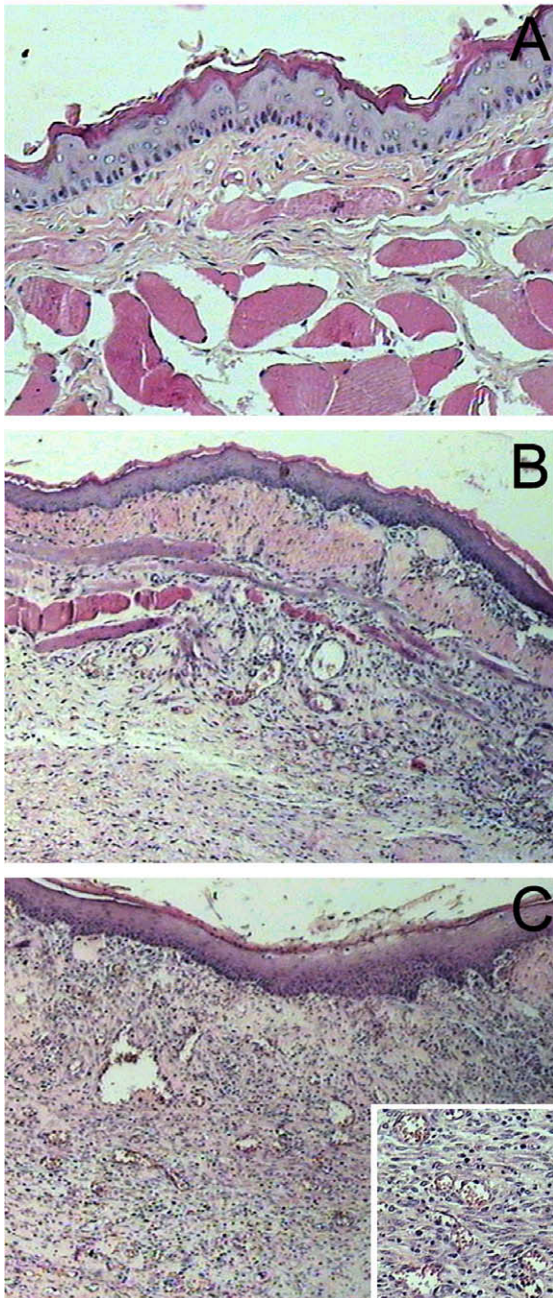
As regards the weight loss on LG, a significant difference was observed between day 0 and day 2 ( $p = 0.025$ ). Comparing the mean values of day 1 with day 2, a non-significant difference ( $p = 0.37$ ) was found. Comparing the mean weight on day 2 with day 3, day 3 with day 4 and day 4 with day 5, no statistically significant differences were perceived. However, comparing with day 1, only following day 4 a significant difference was detected ( $p = 0.047$ ). Therefore a significant loss of body mass was detected from day 4 and day 5. The percentage of body mass lost was 0.96% on day 1, 1.91% on day 2, 3.04% on day 3, 4% on day 4 and 8.97% on day 5.

##### 3.1.2. Mucositis degree

The Kappa's test score (0.69) between observers showed substantial agreement. Fig. 2A displays the mucositis course during experimental period. It is possible to note that the obtained scores



**Fig. 2.** Mucositis development after preventive protocol during experimental period. (A) Differences in the degree of induced-oral mucositis in hamsters between CG and LG. Observe that on the 4th day, the degree of oral mucositis was significantly lower for LG compared to CG. (B) Mean area under the curves obtained for LG and CG. Note that laser phototherapy significantly diminishes the mucositis severity. Data are means and SEM. Significance was determined according to two-tailed independent *t*-test.



**Fig. 3.** Photomicrograph from hamster right mucosa. (A) Healthy mucosa with normal epithelium and no inflammatory infiltrate (original magnification  $\times 40$ ). (B) CG at day 9 showing areas of intense inflammatory infiltrate and granulation tissue (original magnification  $\times 63$ ). (C) LG at day 9 showing intense inflammatory infiltrate (original magnification  $\times 63$ ). The insert shows granulation tissue with expressive angiogenesis (original magnification  $\times 100$ ). HE.

were higher in the CG during all experimental period. The maximum degree obtained from cryotherapy group was 3.1 at day 8. On the group that received preventive laser treatment, the highest degree of mucositis was of 2.6 at day 7. At day 4, a statistically significant difference was observed between groups ( $p = 0.0268$ ). LG presented a mucositis degree significantly lower than CG.

To show the outcome of preventive therapy on the course of mucositis, the areas under the curves were calculated (Fig. 2B). It is possible to observe that LG shows an area under the curve significantly smaller than CG ( $p = 0.047$ ). Therefore, in this model, LILT shows a more positive effect on diminishing the mucositis severity than cryotherapy.

### 3.1.3. Histological analysis

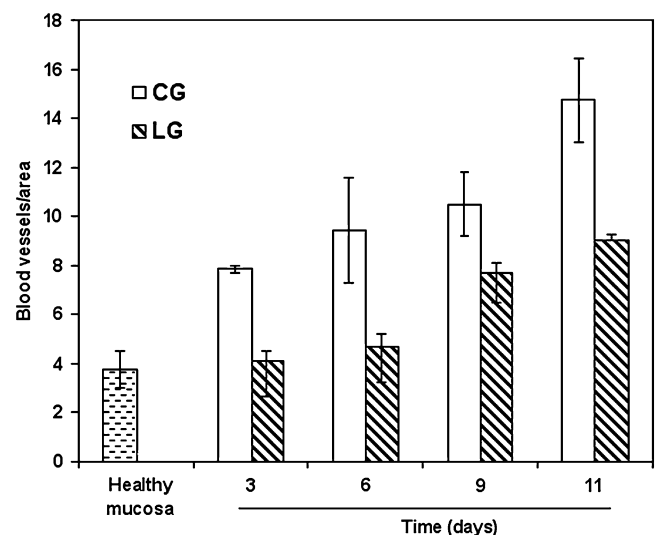
Fig. 3A displays a healthy hamster's mucosa showing normal epithelium and connective tissue with absence of inflammatory infiltrate. On the 3rd day, both groups presented mild epithelial alteration, characterized by congested blood vessels and by a greater amount of keratin. From day 6 until day 9 the differences between the groups became more expressive. At day 6, both groups showed epithelial atrophy, ulceration and mild to moderate unspecific chronic inflammatory infiltrate in the connective tissue. However, laser group presented areas with less inflammatory infiltrate; moreover, the LG presented a more pronounced granulation tissue and the general characteristics indicated a more advanced healing process. At day 9, both groups presented samples with ulcers and chronic infiltrate; all specimens had developed the granulation tissue. Once more, the LG specimens presented more organized collagen fibers characterized by their level of parallelism; it was also noted an expressive angiogenesis (compare Fig. 3B and C). At day 11, there was a complete re-epithelization in the groups, and a scanty presence of lymphocytes and some granulation tissue were also observed. LG showed abundant angiogenesis and mature fibrous connective tissue.

Fig. 4 displays the mean values and SEM of blood vessels during preventive treatment (right mucosa). Healthy mucosa presented an average of 3.75 vessels/area. Regarding comparison with the healthy mucosa, for CG the mean of blood vessels was higher during all the course of mucositis, however, statistically significant differences were observed on days 3, 9 and 11 ( $p < 0.05$ ). LG did not show a significant difference when matched up with the healthy samples on the 3rd and 6th days, but statistically significant differences were observed on days 9 and 11. In both groups, the number of blood vessels increased on day 9 but there was no significant difference between CG and LG at this moment. On day 11, the number of blood vessels continued to increase in the CG group while the LG levels remained the same as day 9. At this moment, the number of blood vessels for LG was significantly lower than CG ( $p = 0.029$ ).

### 3.2. Therapeutic analysis—left cheek pouch mucosa

#### 3.2.1. Mucositis degree

Kappa's statistical measure of inter-rater reliability demonstrated a moderate agreement (0.55) between observers. The re-



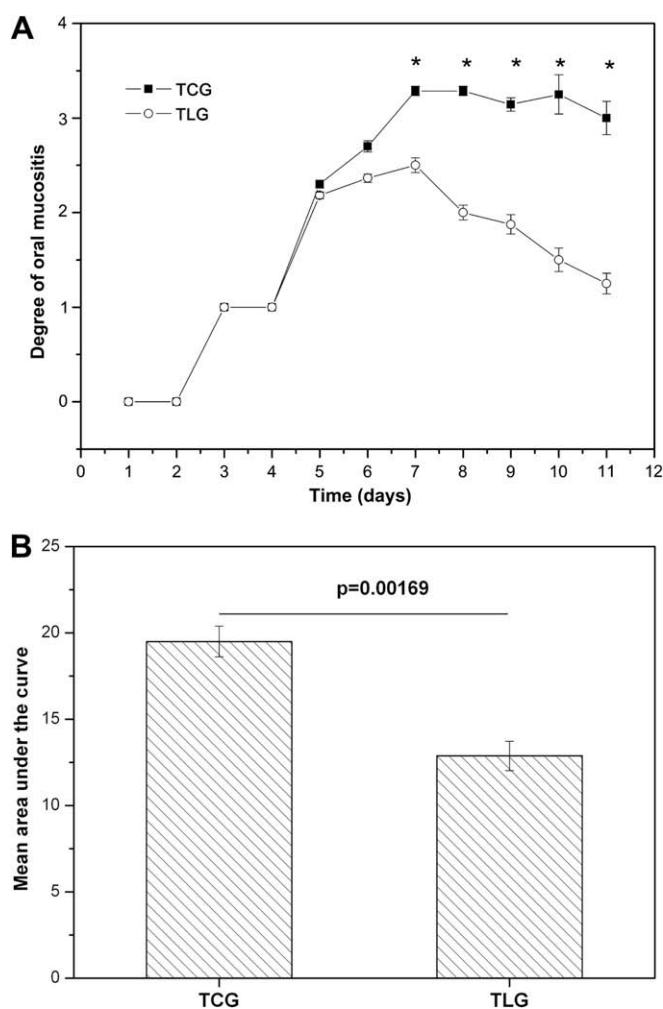
**Fig. 4.** Number of blood vessels in hamster right mucosa during the course of the preventive treatment for LG and CG. On the 11th day, LG presented a significantly lower number of blood vessels compared to CG. Data points are means and SEM. Significance was determined according to two-tailed independent *t*-test.

sults showed that following day 7, the degree of mucositis in the laser group was significantly lower than the degree of mucositis in control group. Laser group had a maximum degree of mucositis of 2.5 at day 7, while control group showed a peak of 3.3 at day 7 and remained around this value until day 10. A noteworthy remark was that until day 5, when the samples were redistributed, the degree of oral mucositis was equal for both groups on the left cheek pouch, suggesting that the preventive intervention at the right cheek pouch mucosa did not interfere with the left side (Fig. 5A). Moreover, from day 7, the scores obtained from TLG were significantly lower than those obtained from TCG ( $p < 0.05$ ). Grade 4 mucositis was not observed in the TLG through all experimental period.

The areas under the curves for the therapeutic protocol are plotted in Fig. 5B. A statistically significant difference was detected between control and irradiated left cheek pouches ( $p = 0.00169$ ). These data suggest that LILT under the studied parameters has a pronounced effect to improve mucosa healing since a minor area size is observed.

### 3.2.2. Histological analysis

At day 6, both groups presented ulcers, acute inflammatory infiltrate, areas of necrosis and granulation tissue (Fig. 6A and B).



**Fig. 5.** Mucositis development during therapeutic procedure. (A) Differences in the degree of induced-oral mucositis in hamsters between TCG and TLG. Observe that after the 7th day, the degree of oral mucositis was always significantly lower in TLG compared to TCG ( $p < 0.05$ ). (B) Mean area under the curves obtained for TCG and TLG. Note that LILT significantly improves mucosa healing. Data are means and SEM. Significance was determined according to two-tailed independent *t*-test.

At day 9 there was a moderate chronic inflammatory infiltrate, some organization on the granulation tissue with expressive angiogenesis and fibrogenesis in the laser group. At day 11, the control group showed a more cellular connective tissue, with mild to moderate chronic inflammatory infiltrate. At the same period, the laser group presented abundant blood vessels, scanty lymphocytes and parallelism of the collagen fibrils and fibroblasts (compare Fig. 6C and D).

Fig. 7 shows the mean values and SEM of blood vessels during therapeutic analysis (left mucosa). Note that the number of vessels for experimental groups is significantly higher than healthy mucosa on days 6, 9 and 11; however, despite TLG present a lower average of blood vessels than TCG on days 9 and 11, no significant differences were observed between these groups throughout the treatment.

## 4. Discussion

The effects of prophylactic and therapeutic laser irradiation on mucositis establishment and development were evaluated in this study. The results showed that under the conditions proposed in this work, LILT has a positive effect in reducing mucositis severity, and a more pronounced effect in treating established mucositis.

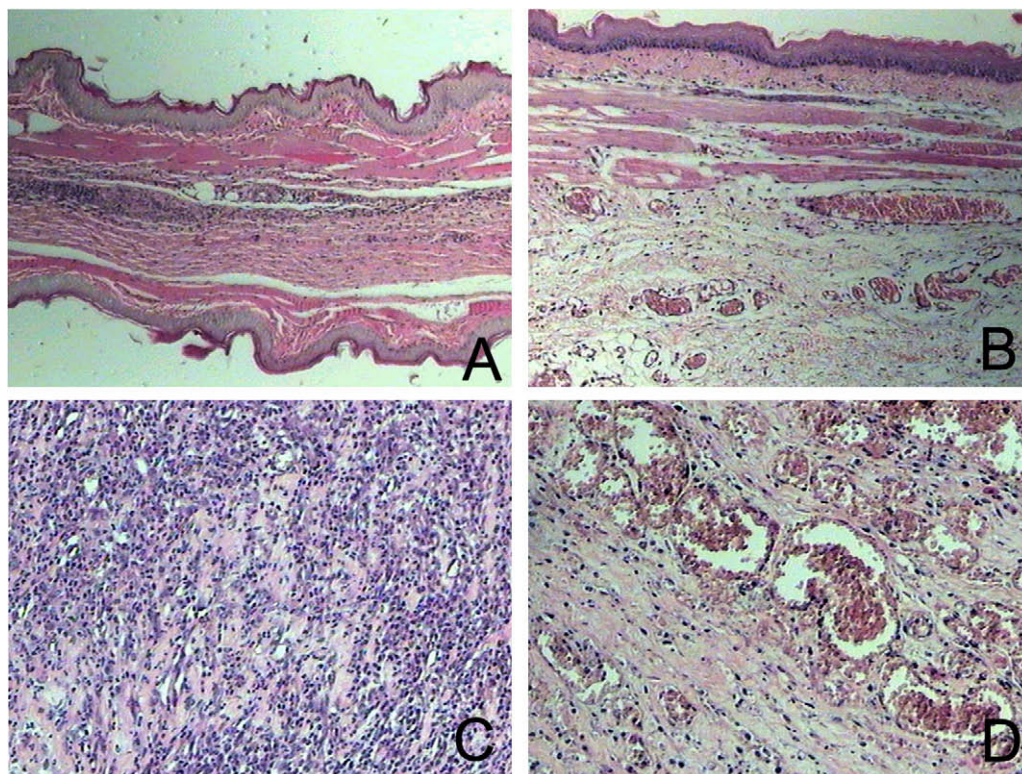
Chemotherapy and radiotherapy-induced-oral mucositis represents a major side effect, frequently encountered in cancer patients. This side effect causes significant morbidity and may postpone the treatment plan. Due to its high incidence, a search for methods that could prevent this side effect is a real necessity; moreover the treatment compliance is also an important issue, owing to the general health conditions of the affected patients. Up to now, a single effective intervention for the prophylaxis or even management of oral mucositis has not been identified.

In this study, on the prophylactic assay, LILT was compared to ice chips application. The idea behind cold application is to promote a temporary vasoconstriction on the oral mucosa reducing the exposure of the oral epithelium cells to serum peak levels of cytostatic agents with a relatively short half-life in plasma as 5-FU [2,4,8]. Systematic reviews have pointed out the use of ice chips or popsicles as a possible method to prevent oral mucositis [6].

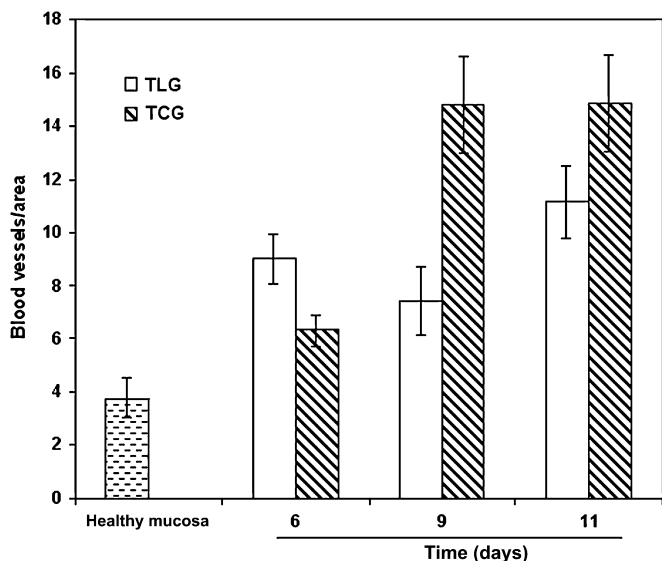
The results suggest that LILT is more effective than the cold application as prophylactic agent. The noteworthy data is the animals' body weight. On the cryotherapy group, the animals lost on the first 5 days approximately 15% of their initial body weight; conversely, in LG group the animals lost approximately 9%. The significance of this weight maintenance may include less pain and consequently less discomfort, which leads to better alimentary conditions [21].

The effects of the red and near infrared laser radiation on oral mucositis has been studied by several authors [11,12,16]. Many mechanisms have been proposed to explain the clinical findings; so far it seems that several different pathways may be involved [22]. Clinical trials performed with low-intensity laser to prevent or to treat oral mucositis had demonstrated via patient's assessment of pain scores, morphine requirement and ability of swallow, the analgesic effect and mucositis incidence reduction through LILT [12,14,16]. Significant points in favor of LILT as a prophylactic agent for oral mucositis are its compliance, as reported by Wong [12], its low cost and the so far lack of reported side effects [23].

The histopathological results obtained from the preventive assay demonstrated that LILT acted, until certain point, as a protective agent since a less severe damage to the oral epithelium was observed followed by a more advanced healing. According to Wong [12], LILT does not alter the oral mucosa perfusion, thus the observed effects may not be linked with less 5-FU exposure, as it seems to be the case with cryotherapy, therefore LILT effects are



**Fig. 6.** Photomicrograph from hamster left mucosa. (A) TCG at day 6 with areas of moderate inflammatory infiltrate with scanty granulation tissue ( $\times 40$ ). (B) TLG at day 6 showing few inflammatory cells and intense angiogenesis ( $\times 40$ ). (C) A detail of the granulation tissue from control group at day 11 ( $\times 100$ ). (D) Mucosa repair in TLG at day 11, with less inflammatory infiltrate and more blood vessels ( $\times 100$ ). HE.



**Fig. 7.** Number of blood vessels in hamster left mucosa during the course of the therapeutic analysis for TLG and TCG. No statistically significant differences were observed between the groups at the investigated moments. Data points are means and SEM. Significance was determined according to two-tailed independent *t*-test.

probably connected with cellular metabolism and host defenses [24]. The blood vessels quantification enforces the histological and clinical findings since in laser treated groups the smaller vascular reactions observed may indicate a less severe injury.

Regarding the therapeutic analysis, the histopathological observation showed that specimens from laser group presented higher organization of the granulation tissue, parallel collagen bundles,

besides an increased angiogenesis. These findings are in agreement with other reports, which observed differences in the healing process between non-irradiated and irradiated wounds [10,17].

Pathophysiologic theories have been classifying oral mucositis as three phase pathology with an initial vascular-inflammatory phase, an epithelial phase and ulcerative phase and a healing phase. During the inflammatory phase, the injury induces the release of several tissue irritants as free radicals, pro-inflammatory cytokines including interleukin- $1\beta$ , prostaglandins and tumor necrosis factor- $\alpha$  by epithelial, endothelial, and connective tissue cells [23]. The free radicals importance in the mucositis course seems to be more and more evident. In a recent study, Severin et al. demonstrated that a reduced antioxidant capability of blood plasma after radiotherapy had a significant relation with mucositis severity. According to the authors, an improvement on the antioxidant status may counteract the damage caused by reactive oxygen species (ROS) formed as a result of the chemo/radiotherapy [25].

The effect of the red radiation over antioxidant enzymes has been investigated. It was demonstrated that under low pH, the cellular superoxide dismutase (Cu-Zn-SOD) is rendered inactive, but under red laser irradiation this enzyme undergoes complete reactivation [26]. Superoxide dismutase reduces the concentration of superoxide radicals, and as it is well known, ROS as superoxide radicals represent a threat to biological systems due to their oxidative reactivity that may damage redox-sensitive components on cells as proteins, lipids and nucleic acids [27]. In fact, studies have demonstrated the action of the red laser on ROS and suggested ROS as responsible or at least partially responsible for the effects observed in LILT studies [24].

As aforementioned, oral mucositis takes place probably via several and concomitant pathways. An important mechanism currently pointed out is the apoptotic route [28]. The tissue damage observed during mucositis course appears to be consequence of

cellular apoptosis rather than necrosis. Apoptotic changes have been noted in fibroblasts and endothelial cells [28]. Therefore, apoptosis and its regulation may be crucial factors in the development of oral mucositis. In the same context, in a recent clinical study, Xanthinaki and collaborators demonstrated that patients receiving head and neck radiotherapy showed an increased expression of pro-apoptotic protein and a decreased expression on anti-apoptotic proteins [29].

The results of this study as well as the results of Desmet et al., that found that a light-emitting diode (LED) with a 670 nm central emission may be an effective preventive countermeasure to the development of oral mucositis in cancer patients, provided a further clue to investigate the role of red light on oral mucositis and probably apoptosis prevention [30]. Besides, Liang et al. showed the effects of a LED (670 nm central emission) over cyanide-induced apoptosis over visual cortical neurons [31]. The results of their study showed that LED significantly decreases cyanide-induced apoptosis via several different pathways as the reduction of ROS generation, down-regulation of apoptotic proteins and up-regulation of anti-apoptotic proteins, as well as through the activation of cytochrome oxidase and energy metabolism. Their study also showed that the prevention of apoptosis is not complete since some degree of apoptotic cells was found on irradiated groups. Therefore, in a parallel consideration with this study, LILT would prevent a more severe damage due to apoptosis-induction by mucositis, but it would not avoid the onset of it.

Several possible pathways may lead to the clinical findings reported on trials conducted with LILT to prevent or treat mucositis; most likely several mechanisms may take place at the same time. Even though control clinical studies are scarce in the literature, the evidence suggests that LILT may be useful to control and/or treat mucositis [5]. In this context, besides control clinical studies, a clear explanation for this positive effect must be identified. Thus, basic research has to be performed in this field to clarify the exact scope of the LILT in this clinical condition. The experimental design proposed in this study seems to be suitable for this end and the obtained data demonstrated that the results achieved in clinical trials can be simulated with this model. To the best of our knowledge, this is a pioneer study that may serve as one possible model to answer primary questions about the action of LILT on mucositis induced by 5-FU. Therefore, this study design may serve as a reproducible research model that could be used for further testing of light therapy and other therapeutic modalities for the prevention and healing of oral mucositis.

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