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# DEVELOPMENT OF ORAL MUCOSITIS MODEL INDUCED BY RADIATION IN HAMSTERS. PREVENTION AND TREATMENT WITH LOW POWER LASER

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## ABSTRACT

*Despite the benefits for the prognosis of patients treated with radiotherapy for oral cancer treatment, it might cause local side effects such as oral mucositis. The oral mucositis is a pathological condition that may appear in affected oral mucosa by ionizing radiation, and the pain related can alter and even stop the antineoplastic treatment, decreasing tumor control rates. Oral mucositis has several treatment modalities, although it remains as a problem since therapies available are not enough to treat efficiently this inflammatory process. Many pharmacological solutions (anti-inflammatory, antibiotics, antiseptic, lubricant agents) are used to alleviate oral mucositis symptoms. Laser treatment has been used as an option, but there is lack of studies to verify the process of laser therapy in oral mucositis caused by ionizing radiation. This work accomplishes follow-up of oral mucositis evolution, comparing laser and benzidamine therapies in an animal model. Forty-two animals were irradiated at head and neck in a single dose of 30 Grays, by means of a $Co^{60}$ source. After irradiation, treatments were applied daily, once a day, for 20 days, in which severity of lesions were clinically classified by two calibrated examiners. Histological evaluation was performed to search for mucosal alterations at treated tissues. Statistical analysis of data showed that laser treatment was more efficient than benzidamine treatment, diminishing severity and duration of oral mucosal lesions caused by ionizing irradiation*

## 1. INTRODUCTION

The treatment for malignant tumor of the oral cavity is based on a multidisciplinary assessment that will determine a treatment plan, either by surgical resection, radiotherapy and or chemotherapy aiming best chances of cure and organ preservation inside the mouth. Prevent or decrease surgical resection is a goal of preoperative radiotherapy combined or not with chemotherapy. Radiation therapy may be unique to tumors in initial staging (T1) or be a therapeutic modality postoperative voiding of cervical lymph nodes, in order to prevent metastasis through regional microcirculation (1,2).

Radiotherapy is widely indicated in cases of malignancies located in the oral cavity, and care should be taken into account before and during treatment. Among the complications that this therapy can cause the patient are xerostomy (dry mouth), skin reactions, mucositis and osteoradionecrosis (3).

Oral mucositis is a complication of toxicity of anticancer therapy of head and neck, whether radiotherapy or chemotherapy. Both therapeutic methods are unspecific, not only because they interfere in the homeostasis of malignant cells, but also normal cells.

Ionizing radiation interferes with the growth and differentiation. Cells carrying high turnover as the oral mucosa and gastrointestinal tract are particularly affected because they proliferate rapidly (4). The division of basal cells of the oral mucosa is inhibited due to cytotoxic therapy, resulting in an atrophic epithelium, susceptible to spontaneous or traumatic ulcers (5). After receiving a cumulative dose of 30 Gy (grays) during radiotherapy induced mucositis can be triggered (3).

The incidence of mucositis in patients undergoing conditioning for bone marrow transplant, infusion therapy for breast and colon tumors and in therapy for head and neck are higher than in other cases. Throughout the oral mucosa can be affected by mucositis, however not keratinized tissues such as labial mucosa, buccal mucosa, floor of the mouth, tongue and soft palate are the most affected by the disease (4).

Painful symptoms resulting from ulcerative oral mucositis interferes in the quality of life and nutrition of the affected individual, as well as significant loss of weight, sometimes making necessary to use a nasogastric tube or gastrostomy for enteral feeding. The disruption of the mucosal barrier increases the risk of infection and is dose-limiting therapy, which can be changed in intensity or even be interrupted in cases of severe mucositis, undermining local control of the tumor malignant and the prognosis of patients (3, 4, 5).

The increased morbidity and cost operating treatment necessitate therapy effective in controlling oral mucositis. Still remains the consensus in the scientific community that there is not a therapy approved for mucositis. As recent studies to develop an understanding of its complex biopathology, mucositis is the target of several potential therapies. However, it is very frequent to intend to reduce symptoms such as pain relief, using narcotic analgesics (6, 7, 8, 9).

Benzidamine is a nonsteroidal anti-inflammatory indicated for attenuation of a number of inflammatory conditions, including radiation-induced oral mucositis or chemotherapy. *In vivo* studies have found reduced levels in plasma of TNF-$\alpha$ and IL-1$\beta$ by the action of benzydamine. These cytokines are found in excess in the mucosa affected by mucositis and *Candida albicans* infections, major microorganism aggravating oral mucositis. Due to be considered an effective analgesic and anti-inflammatory suppressive cytokine, can be used for mitigation of pain and the severity of oral mucositis (10, 11, 12).

Clinical studies of irradiation with low intensity lasers (LILT) for the prevention and treatment of oral mucositis, showed this to be a promising therapy (13, 14, 15). Results concluded that not only occurs a decrease pain intensity as well decreasis of the the severity of mucositis, without presenting the side effects (15,16). The biological effects induced by light depend on all the irradiation parameters (wavelength, dose, intensity, irradiation time, operating mode) as well as the optical properties of the target tissue.

Another relevant aspect for the treatment of radiation-induced mucositis is the understanding of its mechanism of action in healthy oral epithelium. Ionizing radiation interferes with cell growth and differentiation, leading to death during the process of mitosis. Irradiation of cells with red light can protect them from damage caused by ionizing radiation, featuring a possible radioprotective property of the He-Ne laser (4,17,18).

The laser treatment for oral mucositis is performed with wavelength in the red and near infrared light sources. Most frequently used lasers are He-Ne (red) or diodes GaAlAs (red or infrared) (17).

The possibility of treating oral mucositis with low intensity lasers have shows positive results, and can be a combined treatment for patients chemotherapy, debilitated by the disease and the therapy. But there are no studies in animals to examine the LILT in lesions produced by radiation. The purpose of this study is to evaluate whether this light source is also effective ionizing radiation-induced mucositis of a $Co^{60}$ source, aiming to expand the indications of lasers in health care.

## 2. MATERIAL AND METHODS

After ethical committee approval (CEUA/IPEN nº 3/2006.), 42 male Golden Syrian hamsters were selected animals , aged 8-12 weeks, with a mass of about 150 mg. The hamsters were separated into four animals per cage. Food and water were available to the animals *ad libitum*. The research followed the ethical principles of animal experimentation of the International Council of Laboratory Animal Science.

### 2.1. Lesion induction by irradiation at the $Co^{60}$ source

For irradiation, the hamsters were sedated and anesthetized with a combination of ketamine (Vetanarcol ®) 100 mg / mL and xylazine (Copazine ®) 20 mg / ml. After sedation, animals were positioned vertically in an adjustable support. Lead blocks of four inches thick were positioned in front of the support for body radiation protection, being exposed only the head of the animal during irradiation. The procedure was performed at CTR (Center for Radiation Technology - IPEN – CNEN/SP), with a panoramic irradiator source $Co^{60}$, with a focal length of 30 cm and a dose of 30 Gy (dose rate of 66.5 Gy / hour) determined in a pilot study.

40 hamsters irradiated as previously described, were separated randomly into two groups, with a total of 20 animals per treatment group. The other 2 remaining irradiated animals were sacrificed immediately after irradiation for verification of the effect of ionizing radiation immediately after irradiation with $Co^{60}$ source.

## 2.2. Laser irradiation treatment

After irradiation the animals were considered to be at day 0 and laser protocols therapy were started. Laser therapy (group I) was performed over 20 days for the 20 animals of laser group, with an interval of 24 hours between each session. Each day only one laser irradiation section was performed in labial mucosa of animals in this group. The laser system used was a GaAlAs diode laser, model Twinlaser (MM Optics, Brazil), beam area of 4 $mm^2$, and wavelength of 780nm, corresponding to the infrared region of the electromagnetic spectrum. The wavelength was selected according to the latest understanding of the pathobiology of oral mucositis (7), as suitable for higher penetration in the tissue compared to the red wavelength (26).

The following laser irradiation parameters were used: mean power of 50 mW, energy density of 6 $J/cm^2$ during 5 sec. Irradiation was punctual, eight points distributed in approximately 2.5 cm extension labial mucosa by about 0.5 cm. During laser irradiation, safety standards were adhered to, such as the use of glasses protection for the operator and assistants. Before each irradiation power was measured with a power/energy meter model Fieldmaster (Coherent, USA).

## 2.3. Benzydamine treatment

From day 0, corresponding to the day of irradiation $Co^{60}$ source, group II also received benzydamine therapy for 20 days for the 20 animals of group, with an interval of 24 hours between each application. Topical application was carried out in the lip, with 0.25 mL of benzydamine 0.15% trade name Benzitrat ® (Biolab / Sanus Pharmaceuticals, Brazil), in cotton swabs, once a day.

## 2.4. Clinical Evaluation and Histology

The severity of oral mucositis in group I was analyzed and compared clinically at the same place in relation to the oral mucosa of Group II. For the histopathological analysis animals were euthanized in a $CO_2$ gas chamber. The biopsied area corresponded to the central region of the lower lip.

Four animals were euthanized from each group on 5 different times, since day zero, corresponding to irradiation until complete regression of the lesions (17, 44). With this parameter irradiation was possible to observe a pattern in the evolution of mucositis and the days were defined for sacrifice: days 8, 10, 15, 18 and 20.

The severity of the lesions on the lip mucosa was classified according to a score given in the literature, where the criterion used is purely clinical (4). Classification was determined by two observers previously calibrated. This grading system was divided into the following: absent (grade 0), mild (grade 1) and severe (grade 3).

The samples of labial mucosa for future histological analysis, was randomly selected from the central lower lip. The fragments were processed routinely. The histological analysis considered the following:

• the morphology of tissues (histology descriptive analysis)
• cellularity of the tissue adjacent to the basal layer of the epithelium
• amount of collagen fibers
• amount of white space, corresponding to edema fluid, vascular spaces or technical artifacts
• mast cells count secreting pro-inflammatory cytokine TNF-?

## 3. RESULTS

Three aspects of the lamina propria were taken into account: a cellularity, presence of collagen fibers and empty space. The cellularity can be represented mainly by inflammatory cells, endothelial cells or fibroblasts. The empty space may correspond to the interior of blood vessels, edema, technical artifact or extracellular matrix consisting of proteoglycans, glycosaminoglycans, glycoproteins and water. According be verified in descriptive histological analysis, both the presence of endothelial cells, as space on the blood vessels were homogeneous - in quantity and size - in labial mucosa for all subgroups. Therefore, we considered the fibroblasts and inflammatory cells as possibly responsible for the change in cellularity. For voids, was disregarded in the analysis space inside the blood vessels.

With the results, we calculated the mean and standard deviation in all subgroups individually. The animals were individualized in order to obtain a final average that takes into account not only the statistical distribution of the data, as well as the individual characteristics of each animal.

The subgroups that received laser treatment for low power oral mucositis showed a lower frequency of injury to lip, as well as severity / degree of mucositis was lower when compared with the subgroups treated with topical benzydamine (Figure 1).

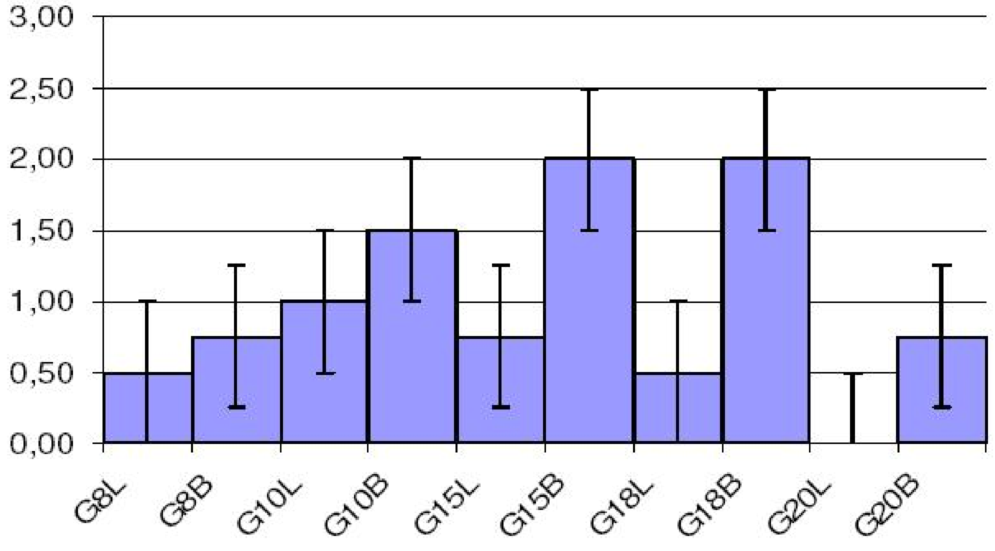


**Figure 1: Degree of mucositis grading from 0 to 3 (0: absent, 1: mild, 2: moderate, 3: severe) according to treatment group / day and sacrifice day for histological analysis. Bars represent the standard deviation.**

Statistical analysis of the group G15 shows a dramatic improvement in treatment with laser compared to treatment with benzydamine and there was statistical difference significant among them. Thus, we conclude that within a range of significance of 5%, the data related to G15L and G15B are different.

Statistical data analysis of clinical coincide with the histological specification, which revealed that the tissue corresponding to the G10L groups and G15L had the higher number of collagen fibers modeled, probably due to stimulation of fibroblast activity by laser. The arrangement of collagen fibers observed from day 10 suggests more advanced repair the lamina propria, and faster contraction wound. Benzydamine group G10B collagen still had scarce fibers arranged in spaced, indicating a probable cause for the delay repair observed at day 15 compared to the laser group. It is possible that the difference in regeneration of tissue between the two groups due to fibroblasts turn into myofibroblasts in the tissue irradiated with the low power laser, facilitating the regeneration of the adjacent epithelium.

The analysis of the groups G18 and G20 also showed improvement in laser groups relation to benzydamine groups, this difference was statistically significant for the significance of 5%. Despite the difference in the clinical outcome, histological analysis for these groups was similar.

## 3. CONCLUSIONS

The effectiveness in the reduction of the clinical OM severity was higher for low-power laser treated group than the topical benzydamine one. It was observed a larger epithelial area and advancement in collagenization and cellularity indexes in the laser group. Further studies must be conducted in order to better elucidate the mechanisms of low-power laser and benzydamine in OM control.

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