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USE OF ^{60}CO PANORAMIC SOURCE IN THE INDUCTION OF ORAL MUCOSITIS IN RATS

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ABSTRACT

Oral Mucositis is a well-known side effect of chemo-radiotherapy in cancer patients or transplant recipients that could induce hospitalization or impairs therapy in different levels of severity. This study is devoted to define the first steps in the research of low level laser treatments in oral mucositis, proposing a ^{60}Co radiation to experimentally induce oral mucositis in rats using Panoramic gamma irradiator, simulating usual radiotherapy of head and neck cancer. Fifteen male Wistar rats, above 250g, were irradiated at Centro de Tecnologia das Radiações (IPEN – CNEN/SP) and divided in three experimental groups, with different single doses of radiation (30 Gy, 25 Gy and 20 Gy). The animals were observed for a 20 days period. Animals that received 30 Gy and 25 Gy developed greater severity of mucositis and premature euthanasia was performed in these groups on the 7th and 11th day after the irradiation, respectively. The 20 Gy group developed oral mucositis grading from moderated to severe between the days 7 and 11 after irradiation, with progressive body mass loss and decrease in the intake of food and water. These animals recovered from oral mucositis around the 18th day and clinical remission at the 20th day. The single dose of 20 Gy Gamma radiation proved to be efficient way for inducing oral mucositis in rats, allowing the establishment of an experimental model for oral mucositis in rats for future use on interventions of this serious aspect of radiation therapy, such as laser therapy using different wave lengths and power densities.

1. INTRODUCTION

Oral Mucositis is a well-known inflammatory side effect of chemo-radiotherapy in cancer patients or bone marrow transplant recipients that could induce severe lesions in oral mucosa (lips, cheek and tongue) leading to hospitalization or impairs therapy in different levels of severity (1-5). In fact, around 60% of the cancer patients submitted to chemo-radiotherapy suffer from acute and severe oral mucositis (grades III and IV) [1-8].

The radiation-induced oral mucositis depends on several factors, such as the irradiated region (head and neck), volume of the irradiated tissue and dose (cumulative or single dose) of radiation applied on the tissue [1, 5, 9, 10].

Several studies had been conducted along the years to research the most beneficial treatment to oral mucositis. Low level laser therapy is widely applied in cancer patients that developed such inflammatory reaction and had been proven efficient in its treatment, decreasing the pain [4, 11, 12] and the healing period [9, 13]. More studies using the low level laser therapy are still being conducted, to better understand this technique and its effects on oral mucositis healing process. Due to ethical concerns, intervention or therapeutic studies for clarifying oral mucositis must use experimental animal models. Those models usually use focal X-Ray irradiation for induction of this inflammatory reaction [14, 15] which is diverse for Brazilian radiation protocols, where gamma radiation is still the most conventional radiotherapy used.

The animal model used to study oral mucositis differ accordingly to the research group, given that many species can be used to produce this inflammatory reaction. Studies had been conducted using mice [14], hamsters [15, 16] and rats [17].

The purpose of this study was to determinate the proper dose of gamma radiation to induce oral mucositis in rats, with 3 different doses with the same range of dose rate.

2. MATERIALS AND METHODS

2.1. Animals, source and materials

All experimental *Rattus norvegicus*, Wistar strain male rats were supplied by the colony of Biotechnology Centre IPEN/CNEN. After reception and unless specified, animals were housed in a controlled environment cabinets and maintained at 23°C in individual micro isolator cages, with commercial food and water provided *at libidum*. All the water used in experiments were sterile and without pyrogens. Laboratory reagents were supplied from commercial sources with pro-analysis quality. The panoramic source used in the experiments had a dose rate of 0.56 to 0.60 Gy/min at 20 cm of the source.

2.2 Standardization of irradiation with ^{60}Co for experimental mucositis optimization

2.2.1 – Dosimetry. We standardized the radiation dosis of the panoramic source with lead barrier was performed using an online diode FSH-260 to ensure the accuracy of the dose rates and that no radiation got through the lead protection, thus the only radiation exposed part of the animal was the head and part of the neck. The bodies were protected by two lead blocks (5 cm thick each), leaving only the head and neck regions exposed.

2.2.2 – Dose response experiment

For optimization of experimental mucositis according to the radiation doses, groups of animals were divided submitted to irradiation with different doses of gamma radiation with a panoramic source of ^{60}Co, accordingly as follow:

- First group received 30 Gy single dose
- Second group received 25 Gy single dose
- Third group received 20 Gy single dose.

All the groups were located 20 cm away from the source and equally distant from each other.

15 male Wistar rats, above 250g, were irradiated at Centro de Tecnologia das Radiações (IPEN – CNEN/SP) and divided in three animal groups (5 rats each). The animals were daily anaesthetized by the association of ketamin (75mg/kg) and xylazin (10mg/kg) via intraperitoneal and physical restraint by an appropriated support. The progression of the oral mucositis was documented using 12 Mpixels digital camera. To classify the severity of induced-oral mucositis, this study used the adapted scale from Sonis, S. T. [De®ning mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters, Sonis]. All clinical alterations were noted and carefully described. The animals were observed for the period of 20 days after the irradiation and kept in IPEN animal colony with irradiated commercial food and sterile water ad libitum. Food and water intake was monitored.This study was approved by Ethic Committee in Lab Animals IPEN – CNEN/SP (94/11/CEUA-IPEN/SP).

3. RESULTS

3.1 Dosimetry and Physical Aspects of the Irradiation Procedure.

None of the animals presented or developed any type of inadequate mobile behavior during irradiation procedure that could affect the exposure of irradiation in any of the experimental times.

Dosimeter inside the blocks showed levels of exposure below 10 cGy of animal bodies, allowing that bone marrow and digestory system were protected for radiation.

3.2 Mucositis evolution and systemic effects

The data of mucositis evolution and systemic effects will be presented first as external clinical signs in each experimental group, with subsequent data of systemic effects.

3.2 Irradiation Protocols

3.2.1 30 Gy irradiated animals

All animals in this group presented xerostomia in the first day after irradiation. Inflammatory reactions, such as erythema and/or swelling in the lips and cheeks were observed in the second day. At the third day after irradiation, all the animals presented severe oral mucositis, with ulcers in the tongue, cheeks and abrasions in the lips (Fig. 1). The intake of food and water ceased after day one and body weight loss was intense, as shown at Table 1. Other symptoms related to high dose of gamma radiation were observed, such as blindness (due to ulcerative keratitis), diarrhea, nose bleed and severe secondary bacterial infection. Due to those problems this experimental time was conducted only to the 7^{th} days post-irradiation, when animals were euthanized.

Figure 1. Animal exposed to 30 Gy irradiation at 3 days of revolution clearly showing mucositis and inflammatory process due to tissues damage.

3.2.2 25 Gy irradiated animals

All animals in this group presented swelling of the lips at the third day after irradiation. Xerostomiawas observed at day 4. Tongue, lips and cheeks erythema and swelling were better observed at day 6 followed by acute significant weight loss, as shown in Fig. 2. Severe oral mucositis, with ulceration of 80 to 90% of the tongueepithelial occurred at day 8 concurrently with inflammatory exudates and secondary bacterial infection. Intense weight losses and low intake of food and water ceased after day three (Fig. 4). Formation of pseudo membrane, indicating reepithelization of the tongueand the beginning of the natural healing process was observed at day 10, but the severe ulcers and the inflammation persisted. Two animals presented diarrhea and nose bleed.

Figure 2. Typical evolutivemucositis lesion in 25Gy irradiated animals. Note the intense inflammatory exudate but the relative good aspects of the regenerating mucosa.

3.2.3 Single dose 20 Gy

All animals in this group presented discreet swelling of the lips at the third day after irradiation. Tongue, lips and cheeks erythema and swelling were better observed at day 6, concurrently with xerostomia and progressive weight loss. Severe oral mucositis, with ulceration of 10 to 50% of the tongue epithelial occurred at day 8 with no secondary bacterial infection (Fig. 3). Formation of pseudomembrane, indicating reepithelization of the tongue and the beginning of the natural healing process was observed at day 10, though still incipient. Full reepithelization was observed in two animals at day 18 and the remaining animals at day 20.
The intake of food and water decreased after day three (followed by body weight loss) and restarted after day 13 (Fig. 4).

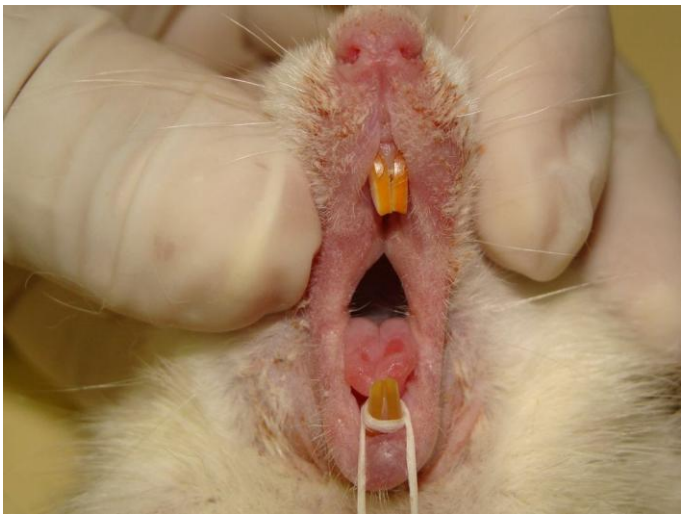

Figure 3 – Mucosistis in good evolution in 20Gy irradiated rats. Note the small lesions in tongue and the absence of inflammatory exudate.

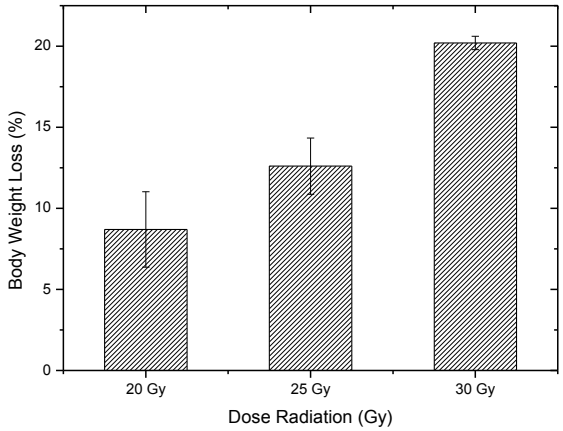

Figure 4 – Body weight and food intake in irradiated rats for mucositis

4. DISCUSSION

The induction of oral mucositis in animal model is highly important to the study of this inflammatory reaction that affects cancer patients undergoing chemotherapy, radiotherapy or the association of both therapies. The great number of studies on this subject allow the research groups to choose different species to work with. The most common species are hamsters, mice and rats. The present study chose to work with rats due to its easy handling, maintenance and mouth size, bigger and easier to access when compared to mice.

The effects of gamma radiation exposure were observed in the three groups of this study. The severity of the disease was related to the level of pain of the rats. Body weight loss was used to deduce the intake of food and water and the percentage of weight loss used to compare the groups. The first group, irradiated with 30 Gy presented severe oral mucositis at early stages of the experiment, presenting higher percentage of weight loss, when compared to the group irradiated with 20 Gy (showed at Fig. 4).

Daily anaesthesia protocols along with xerostomia can decrease the natural grooming habits of the rats, favoring the body weight loss and the accumulation of inflammatory exudates.

The severity level of mucositis, the body weight loss and pathogenic period were carefully considered in this study. The groups irradiated with 30 Gy and 25 Gy presented the most severe cases of oral mucositis resulting in premature euthanasia while the group irradiated with 20 Gy was able to ensure the natural healing process after single dose radiation.

5. CONCLUSIONS

Based on the results of this study 20 Gy single dose gamma irradiation from a ^{60}Co panoramic source can successfully be used for inducing a reproductive experimental model of oral mucositis in rats.

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