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Effect of radiotherapy on the eruption rate and morphology of the odontogenic region of rat incisors

Amanda Maria Medeiros de Araujo [a,*], *Carolina Cintra Gomes* [b], *Solange Maria de Almeida* [b], *Carla Beatriz Klamt* [b], *Pedro Duarte Novaes* [c]

[a] *Department of Clinic and Social Dentistry, School of Dentistry, Federal University of Paraíba, João Pessoa, Paraíba, Brazil*

[b] *Department of Oral Diagnosis, Division of Oral Radiology, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil*

[c] *Department of Morphology, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil*

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ABSTRACT

Objective: The goal in this study was to evaluate the results of doses of 5 and 15 Gy of radiation in odontogenic region of the rats inferior mandibular-incisors by a histological analysis and the rate of eruptions.

Design: Animals were divided into three groups: control, radiotherapy 5 Gy and radiotherapy 15 Gy. In which tooth-eruption-rate was measured every two days.

Results: Animals in Group 5 Gy presented values similar to those of the control group. Animals in Group 15 Gy presented reduction in tooth-eruption-rate as of the sixth day of the experiment, vast disorganization of odontoblasts and ameloblasts, apparent reduction in cell population in the follicle region and alterations in cervical loop formation of the dental organ.

Conclusions: It was concluded that there was a difference between the researched doses, and histological alteration at 15 Gy lead to statistical reduction in tooth-eruption-rate.

1. Introduction

Cancer is a set of diseases that have in common the disorderly growth of cells that invade tissues and organs and may spread by metastasis to other regions. Paediatric tumours account for approximately 2.5% of the total number of tumours expected among Brazilians.[1] In the United States, cancer in childhood occurs in fewer than 2% of cases.[2]

Various modalities of therapies have been applied in children with cancer, which are associated with toxic effects resulting in complications.[3] Radiotherapy is widely used as an efficient form of treatment, either alone or associated with chemotherapy and/or surgery.[4] There is concern due to the

* *Corresponding author at*: Rua Joakim Schuller, 40/Edf Oasis Plaza – Apt 304, 58.037-760 Bairro Jardim Oceania, João Pessoa, Paraíba, Brazil. Fax: +55 8332167409.

E-mail addresses: araujo.amm@gmail.com, amandammaraujo@yahoo.com.br (A.M.M. de Araujo).

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high percentage of acute or delayed oral complications that may occur during and/or after radiotherapy treatment.[5]

In cancer of the head and neck, the applicability and efficacy of radiotherapy is determined by parameters such as the histological type of the neoplasia (slow × rapid response), location and volume of the area to be irradiated, dose, rhythm of application, type of radiation and the patient's general conditions.[6]

The most frequent oral complications in patients submitted to radiotherapy in the region of the head and neck and/or chemotherapy are haemorrhage, infection, exacerbation of pre-existent infection, mucositis, xerostomia, radiation caries and interruption in tooth development. They lead to alterations in odontogenesis[3] such as enamel hypoplasia, cessation of tooth development, non formation of teeth,[7,8] microdontia and alterations in rhizogenesis, such as interruption, thinning and widening of the pulp chamber.[9,10]

The effect of radiation on permanent teeth and its degree of severity depend on the stage of tooth formation, as well as the dose and extent of the irradiated area. Innumerable dental alterations may occur in children submitted to antineoplastic treatment, capable of affecting shape, number or root formation, such as hypoplasia, microdontia, hypodontia, taurodontia, conical roots, among other.[8]

Odontogenesis is the stage of tooth development that involves morphogenesis, histogenesis and cell differentiation. The consecutive stages of tooth development are the bud, cap and bell stages.[11,12] Tooth development is a complex process and it can undergo various alterations during its formation.[12]

Rat incisors have an odontogenic region that presents cells with elevated metabolism and constant differentiation. This region has a compartment of stem cells and one of proliferative cells with innumerable molecules involved in kinetic processes such as cell proliferation, migration, differentiation and cell death.[13,14] In the epithelium of the cervical loop intense cell proliferation occurs, from which cells migrate and differentiate into ameloblastic and odontoblastic cells.[9] The growth, calcification and eruption of rat incisors occur continuously throughout life, showing all the stages of formation in a single tooth, and is therefore a model for evaluating the effect of X radiation on progenitor cells.[9]

Few studies have been conducted to evaluate the effects of radiation on the in the stage of development. As there is a scarcity in the literature of studies on the irradiated odontogenic region, it is important to evaluate the effect of radiotherapy on the odontogenic region of rat incisors. The aim of this study was to evaluate the morphologic alterations caused by the action of radiation on the odontogenic region of rat incisors, with doses of 5 and 15 Gy, and find out whether these alterations may interfere in the eruption rate of these teeth.

2. Materials and methods

Thirty adult male rats (Rattus norvegicus, Albinus Wistar), with a mean weight of 220 grams, obtained from the central vivarium of UNICAMP were used in the study. While the research was conducted, the rats were maintained in polycarbonate cages, in which they remained in an environment with controlled temperature and humidity, and an alternating 12-h light–dark cycle. The animals' diet consisted of standard balanced rations and water *ad libitum*, daily. This study was approved by the Ethics Committee on Animal Experimentation of the State University of Campinas, Protocol Number 1843-1.

The animals were divided into three groups: Group 1; Group 2 in which the animals were submitted to a 5 Gy dose of radiotherapy in the region of the head and neck; and Group 3 in which the animals were submitted to a 15 Gy dose of radiotherapy in the region of the head and neck.

The experiment lasted 16 days, in which the tooth eruption rates were measured every two days. On the second day of the experiment, the animals were submitted to radiotherapy in the region of the head and neck. The eruption rate was measured in all the animals. To do this, a linear mark was made on the vestibular face of the mandibular incisors with cylindrical diamond bur adapted to a high speed handpiece under cooling. With the aid of a millimetre eyepiece (Ernest Leitz Wetzlar Germany 12.5×) adapted to a stereoscopic loupe, and the use of a millimetre ruler for calibration (Carl Zeiss 5+ 100/100 mm), the distance from the gingival margin up to the mark was measured. To obtain the measurements, the animals were anesthetized with Halothane (Cristália, Brazil). When the mark approximated the incisal edge of the tooth that was being worn by the friction, a new mark was made. For better confirmation of the data, the eruption rate was verified both on the mandibular right incisor and on the left and the final daily eruption value was given by the mean of these values. The values were transformed into millimetres and statistically analyzed by ANOVA.

Before all the irradiation procedures, all the animals were anesthetized with intramuscular injection of 80 mg/kg of Ketamine Hydrochloride (Dopalen® Agribrands do Brasil Ltda.; Paulínia, São Paulo, Brazil) and 8 mg/kg of Xylazine Hydrochloride (Rompum®, Bayer S.A., São Paulo, SP, Brazil). The animals irradiated after beginning the tooth eruption rate measurement received a single dose of 5 Gy and 15 Gy of X radiation, depending on their group, from a linear accelerator, Varian brand, model Clinic 6/100 at a focal distance of 100 cm. The collimation field was 15 × 30 cm, so that only the head and neck region was irradiated.

All the animals were sacrificed by cervical dislocation at the end of the experiment. The rat hemimandibles were removed and fixed by immersion in 10% buffered formalin. After collection, each hemimandible was divided into two parts, anterior and posterior, using a high concentration, dual faced diamond cutting disc, measuring 4″ × 0.12″ × 1/2 (102 mm × 0.3 mm × 12.7 mm) (EXTEC Corp., Diamond Wafering Blade) and kept in 10% buffered formalin for 24 h under agitation to promote better fixation.

The posterior half of each hemimandible was decalcified with 4.13% EDTA in phosphate buffer pH 7.4, for 30 days. After decalcification, the odontogenic region was separated with a scalpel from the mesial face of the third molar, dehydrated in increasing grades of ethanol solutions, diaphanized and embedded in Paraplast Plus®: tissue embedding medium (McCormick™ Scientific, Leica Biosystems St. Louis LLC, USA).

Transverse cuts in relation to the long axis of the incisor were made from the beginning of the odontogenic region in