Archives of Oral Biology 69 (2016) 102–108

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Parathyroid hormone intermittent administration promotes delay on rat incisor eruption

M.A.D. Silva[a,*], D.F.P. Vasconcelos[b,*], M.R. Marques[c], S.P. Barros[d]

[a] Division of Histology and Embryology, Federal University of Campina Grande, Patos, PB, Brazil
[b] Division of Histology and Embryology, School of Biomedicine, Federal University of Piaui, Parnaiba, PI, Brazil
[c] Department of Morphology, Division of Histology, School of Dentistry at Piracicaba, University of Campinas, Piracicaba, SP, Brazil
[d] Center for Oral and Systemic Diseases, Department of Periodontology, UNC School of Dentistry, University of North Carolina at Chapel Hill, USA

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form 2 May 2016
Accepted 25 May 2016

Keywords:
Odontogenesis
Tooth development
Periodontal ligament
Mineralized tissue
Bone biology
Microscopy

ABSTRACT

Objective: This study evaluated the influence of parathyroid hormone (PTH) (1–34) intermittent administration on rat eruption rates of lower incisors under normo, hyper and hypofunctional conditions, Sharpey fibers insertion, and alveolar bone formation.
Materials and methods: Wistar male rats received PTH (1–34) three times a week during the entire experimental period, 31 days. Control animals received the same concentration of the vehicle solution during the same period. Three injections of alizarin were also performed. The experiment evaluated the eruptive rate, the alveolar bone formation and also the morphology, and the area density of Sharpey fibers. After the sacrifice, the mandibles were dissected and samples were prepared for fluorescence and scanning electron microscopy observations.
Results: PTH-treated animals showed significantly reduced eruption rates in all different functional conditions. Analysis evidenced that PTH-treated rats present an increase in bone formation and area density of the Sharpey fibers.
Conclusion: We concluded that the PTH (1–34) intermittent administration reduced the eruptive process rates, through bone formation enhancement and increase in the area density of Sharpey fibers.

1. Introduction

Tooth eruption is considered as complex and tightly controlled process that involves cells of the tooth organ and the neighboring the tissues (Wise, Que, & Huang, 1999). An essential process for tooth eruption is the alveolar bone resorption, where osteoclastogenesis is needed (Yoshino et al., 2003).

The continuously erupting incisors of rats are often used in the studies on eruption. Such experimental model offers conditions in which slight alterations in the eruption rate can be detected, and substances thought to interfere with tooth eruption have been tested (Berkovitz, Migdalski, & Solomon, 1972; de Araujo, Gomes, de Almeida, Klamt, & Novaes, 2014; Masuda et al., 2006; Merzel & Novaes, 2006).

The periodontal tissue of rodent incisors is formed by a periodontal ligament at the surfaces of the tooth where dentine is covered by cementum (mesial, lingual and part of distal), while a tissue comprised by the dental (enamel) organ coated successively by both a densely packed and a loose and largely vascular connective tissue covers the enamel (labial and part of distal) (Marks & Schroeder, 1996). This enamel-related periodontal tissue is similar to the tissue investing the crow of limited growth teeth, such as rodent molars at the time when the crown is completed and eruptive movements begin intraosseously. The eruption process of rat continuously growth incisors can be accelerated or retarded by drugs, functional conditions, radiation or hormones (Gerlach, Toledo, Novaes, Merzel, & Line, 2000; Merzel & Novaes, 2006; Silva & Merzel, 2004).

A study demonstrated that changes in the enamel-related periodontium, in the immobilized incisor of the rat, modify the RANKL/OPG ratio, in the presence of CSF-1, altering the metabolism of cells that participate in the bone remodeling (Neves et al., 2009). Another investigation showed the parathyroid hormone (PTH) (1–34) intermittent administration influenced the RANKL/OPG expression in the bone anabolism of periodontal ligament cells

* Corresponding authors at: Universidade Federal do Piauí—UFPI, Campus Ministro Reis Veloso, Av. São Sebastião, 2819, Reis Veloso, Parnaíba, PI 64204-035, Brazil.
E-mail addresses: silvamad@cstr.ufcg.edu.br (M.A.D. Silva), vasconcelos@ufpi.edu.br (D.F.P. Vasconcelos).

http://dx.doi.org/10.1016/j.archoralbio.2016.05.017
0003-9969/

modifying osteoclastogenesis in co-culture model cells (Lossdörfer, Götz, & Jäger, 2011).

Therefore, the aim of this study was to evaluate the influence of parathyroid hormone (PTH) (1–34) intermittent administration on rat eruption rates of lower incisors under normo, hyper and hypofunctional conditions, area density and insertion of Sharpey fibers, and alveolar bone formation.

2. Materials and methods

2.1. Animal use protocol

Throughout the experiment, the animals were maintained following the "Ethical Principles for Animal Research" established by the Brazilian College for Animal Experimentation (COBEA).

2.2. Experimental design

Sixty Wistar male rats, 4 weeks of age with body weight of 80–90 g, were acquired from CEMIB-UNICAMP. Animals were kept in plastic cages in a 12-h light/dark cycle at 25–30 °C and given tap water and food (Nuvital; Paraná, Brazil) *ad libitum*.

The experimental groups were assigned into six groups (n = 10): (1) (Cnor group) normofunctional teeth, control, the animals received 1% acetic acid solution; (2) (Chypo group) hypofunctional teeth, the animals received 1% acetic acid solution; (3) (Chype group) hyperfunctional teeth, the animals received 1% acetic acid solution; (4) (Pnor group) normofunctional teeth, the animals received 40 μg/kg PTH (1–34) (Sigma-Aldrich Co., St. Louis, USA), to promote bone anabolism and periodontal ligament fiber reattachment in previous studies (Barros, Silva, Somerman, & Nociti, 2003; Marques et al., 2005; Silva et al., 2015; Vasconcelos et al., 2014) in this dose; (5) (Phypo group) teeth hypofunctional, the animals received 40 μg/kg PTH (1–34); (6) (Phype group) teeth hyperfunctional, the animals received 40 μg/kg PTH (1–34). The treatment period was from day 3 to 28 of the experiment for all animals, with three times a week by subcutaneous injection, and sacrificed after 31 days of the beginning (Fig. 1).

2.3. Eruption conditions

The hypofunctional and hyperfunctional conditions of the teeth were performed maintained under normofunctional status and in every 2 days the left lower incisors were sectioned at the level of the gingival papilla three times a week so that these teeth could be kept out of occlusion, a condition named hypofunctional (unimpeded). It is considered that the right lower incisors, still under occlusion, became hyperfunctional (impeded) through this procedure. The hypofunctional (unimpeded) and hyperfunctional (impeded) conditions in this study were performed in the same animal, using the left incisor for hypofunctional and right incisor for hyperfunctional, both in lower teeth (Silva & Merzel, 2004; Steigman, Michaeli, Yitzhaki, & Weinreb, 1989).

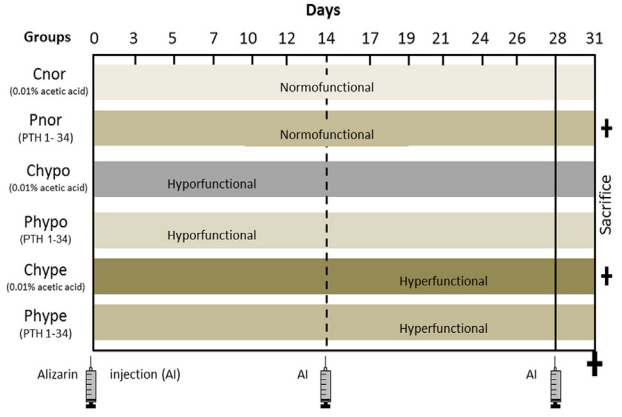

Fig. 1. Animal experiments demonstrate the group division, beginning treatments, administration of marker alizarin, and the time of sacrifice.

2.4. Eruption rate

A calibrated grid in a microscope eyepiece was used to measure eruption at ×10 magnification. A trained examiner unaware of the group to which each animal belonged measured the distances. The eruption rates of the lower incisors were measured three times a week, all at the same period of time, by recording the distance from the gingival margin to marks made on the impeded and normal teeth, the trimmed edge on unimpeded incisors. The reference marks and the trimmed oclusal edge of right incisors were made after each measurement. For all procedures, the animals were anaesthetized with ketamine (1.0 mL/kg). The eruption rates were recorded 12 times.

2.5. Fluorescence microscope analysis

The animals received intraperitoneal injections of the fluorescent bone marker alizarin at 40 mg/Kg (Sigma-Aldrich Co., St. Louis, USA), in three different experimental times: day 0, day 14 and day 28 (Fig. 1), counting from the beginning of the experiment. After mandibles were dissected and immersed in 4% buffered formalin overnight, the anterior region was dehydrated in alcohol solutions and infiltrated with resin, both processes under low pressure. The samples were included in acrylic resin and cut into 0.8 mm sections. These sections were polished to yield obtain sections of 30 μm. These samples were analyzed under fluorescence microscopy (Leica, DMLP), and the bone formation labeled by alizarin was evidenced. Areas created between the first and last alizarin deposition were measured.

2.6. Scanning electronic microscope analysis

The mandibles were dissected and immersed in 4% buffered formalin overnight. Samples were sectioned and the incisors were carefully removed to expose the lingual internal wall of the socket related to the periodontal ligament. The specimens were rendered anorganic by immersion for 2 h in 5.25% sodium hypochlorite. After dehydration with graded acetone, they were mounted on stubs, and coated with gold (about 10 nm thick, Sputter Coater MED 010, Balzers, Liechtenstein). The specimens were observed and photographed in a JSM 5600 LV (Jeol, Japan).

The area density of the Sharpey fibers (D), a quantitative measure, was determined through point counting (Weibel, Kistler, & Scherle, 1966). A grid containing 108 equidistant points (total grid area = 24607.8 μm^2) was used in an image analysis system (KS400-2.0, Kontron Electronic, Germany). Analysis was performed on lingual face for three random images, at 300× magnification, in two different regions of the alveolar socket: diastema (A) and adjacent to the first lower molar (B). The area density corresponds to the fraction of the area occupied by Sharpey fibers:

$$D = p_A/3 \times 24607.8\ \mu m^2/108$$

p_A = the number of points hitting Sharpey fibers in three images of the same animal.