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The local administration of parathyroid hormone encourages the healing of bone defects in the rat calvaria: Micro-computed tomography, histological and histomorphometric evaluation

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ABSTRACT

Objective: To evaluate the effect of a single-dose local administration of PTH on bone healing in rat calvarial bone defects by means of micro-computed tomography, histological and histomorphometric analysis.
Design: Critical-size cranial osteotomy defects were created in 42 male rats. The animals were randomly divided into 3 groups. In the C Group, the bone defect was only filled with a blood clot. In the S Group, it was filled with a collagen sponge and covered with bovine cortical membrane. In the PTH Group, the defect was filled with a collagen sponge soaked with PTH and covered with bovine cortical membrane. The groups were further split in two for euthanasia 15 and 60 days post-surgery. Data was statistically analyzed with *t*-tests for independent samples or the nonparametric Mann-Whitney test when applicable. Intragroup comparisons were analyzed with paired *t*-tests ($p < 0.05$).
Results: Micro-CT analysis results did not demonstrate statistically significant intergroup differences. At 15 days post-surgery, the histomorphometric analysis showed that the PTH Group exhibited a significantly higher percentage of bone formation compared with the S Group. At 60 days post-surgery, a higher percentage of new bone was observed in the PTH group.
Conclusion: The results suggest that the local administration of PTH encouraged the bone healing in critical-size calvarial defects in rats.

1. Introduction

Bone remodeling is the continuous removal of bone followed by the synthesis of bone matrix and mineralization. This process is mediated by calcium homeostasis, which needs three hormones for balance: PTH, Vitamin D (calcitriol) and calcitonin (Aggarwal and Zavras, 2012; Li et al., 2013; Tokunaga et al., 2011).

Parathyroid hormone (PTH) is composed of 84 amino-acid proteins. PTH exhibits both direct and indirect effects, particularly in bone remodeling, kidney and intestines, acts constantly to maintain the optimal endogenous concentration of calcium ions in the bloodstream and is also used in the treatment of osteoporosis (Aggarwal and Zavras, 2012; Alkhiary et al., 2005; Andreassen, Ejersted, & Oxlund, 1999; Li et al., 2013; Mair et al., 2009; Skripitz, Andreassen, & Aspenberg, 2000; Tokunaga et al., 2011; Yun et al., 2010).

Parathyroid hormone exerts both anabolic and catabolic action on the bone (Aggarwal and Zavras, 2012; Andreassen et al., 1999; Kempen et al., 2010; Li et al., 2013; Mair et al., 2009; Ohkawa, Tokunaga, & Endo, 2008; Pensak et al., 2015; Skripitz et al., 2000; Skripitz, Johansson, Ulrich, Werner, & Aspenberg, 2009; Tokunaga et al., 2011; Yun et al., 2010). Preclinical studies have shown that the use of PTH for the treatment of osteoporosis improves bone fracture healing (Alkhiary et al., 2005; Andreassen et al., 1999; Neer et al., 2001). Its intermittent administration increases bone remodeling with greater effect on bone apposition, thereby leading to increased microarchitecture and bone volume rather

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than bone resorption (Gabet, Kohavi, Muller, Chorev, & Bab, 2005; Kneissel, Boyde, & Gasser, 2001). It plays an important role in bone turnover and calcium homeostasis, renal excretion of inorganic phosphate and activation of vitamin D (Aggarwal and Zavras, 2012; Li et al., 2013; Mair et al., 2009; Skripitz et al., 2000; Tokunaga et al., 2011; Yun et al., 2010).

PTH triggers bone resorption due to its effects on osteoblast activity, as PTH receptors are found on osteoblast membranes. PTH binds to PTH1R (parathyroid hormone 1 receptor) on osteoblasts to stimulate the secretion of RANKL (receptor activator of nuclear factor-kB ligand). RANKL binds to receptor activator of RANK on the surface of osteoclasts and promotes cell differentiation and maturation of immature osteoclasts, thus prolonging their survival. PTH also decreases the osteoblasts' secretion of osteoprotegerin. Osteoprotegerin is a protein secreted by osteoblasts that effectively binds to RANKL and prevents its attachment to RANK, thus decreasing the differentiation and resultant maturation of osteoclasts and bone resorption. While PTH and glucocorticoids decrease the production of osteoprotegerin, estrogen increases its expression (Simonet et al., 1997; Yun et al., 2010).

Previous studies have evaluated the effects of systemic PTH administration in rats on bone healing of calvarial critical-size defects (Andreassen and Cacciafesta, 2004; Silva et al., 2015; Stancoven et al., 2013; Tsunori et al., 2015; Tsunori, 2015; Yun et al., 2010), post-extraction socket regeneration (Kuroshima, Al-Salihi, & Yamashita, 2013), bone response around implants in osteoporotic rat maxillae (Heo, Park, Jeon, & Pyo, 2016; Park, Heo, Kim, Min, & Pyo, 2016), periodontal defects repair (Wang, Du, & Ge, 2016) and periodontitis (Barros, Silva, Somerman, & Nociti, 2003). To the best of our knowledge, only one study to date has evaluated the effects of the local administration of PTH in rat gingiva on alveolar bone regeneration (Tokunaga et al., 2011). Thus, the local effects of this drug should be further studied and understood.

The aim of this study was to evaluate the effect of a single-dose local administration of PTH on bone healing in rat calvarial bone defects by means of micro-computed tomography as well as histological and histomorphometric analysis.

2. Material & methods

2.1. Experimental design

Ethical approval was obtained from the Ethics Research Committee of Positivo University. The experiment was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Forty-two male rats (Rattus norvegicus, Albinus, Wistar) aged 3–5 months and weighing an average of 387 g were used. The animals had ad libitum access to water and a standard laboratory diet.

Throughout the trial period, the animals were triple-housed in cages in purpose-designed rooms. The environmental conditions of light, temperature and humidity were controlled. The animals were randomly divided into three groups: C Group (control), S Group (collagen sponge) and PTH Group (parathyroid hormone). The groups were further split in two (n = 7) for euthanasia 15 and 60 days post-surgery.

2.2. Surgical procedure and PTH administration

For the experimental surgical procedures, the animals were sedated with oxygen and isoflurane 3 l/min (Cristália, Itapira, SP, Brazil) and then anesthetized by intramuscular injection in the back of the thigh with Xylazine 10 mg/kg (Vetbrands, Paulinia, SP, Brazil) and ketamine 80 mg/kg (Vetbrands). Anesthesia was maintained by isoflurane vaporization as needed. The surgical procedure was performed by one single trained and calibrated operator.

After the induction of anesthesia, the dorsal part of the cranium was shaved and aseptically prepared for surgery. A U-shaped incision was performed with a scalpel blade No. 15c (Advantive, Xishan City, China) for surgical access to the calvaria area and a full-thickness flap was raised in the posterior direction.

A critical-size (5 mm diameter), through-through, cranial osteotomy defect was created using a trephine bur (Neodent, Curitiba, Brazil) engaged in an implant hand piece (20:1, Kavo, Joinville, Brazil) under profuse irrigation with sterile saline. A defect was created on the skull of each animal for a total of 42 bone defects. The defect was centered along the median sagittal suture 1 mm anterior to the occipital bone. The resulting calvarial block was then carefully removed to avoid tearing the dura.

In the C Group, the bone defect was only filled with a blood clot. In the S Group, it was filled with a 5 mm in diameter absorbable hemostatic collagen sponge (Hemospon, Technew, Rio de Janeiro, RJ, Brazil) that was cut with the trephine bur so that its size was compatible with the defect, and then covered with bovine cortical membrane (GenDerm, Baumer, São Paulo, SP, Brazil) cut to fully cover the sponge and defect (size 8 × 8 mm). In the PTH Group, the defect was filled in a manner similar to the S Group. However, the sponge was soaked with 20 μg PTH (Forteo, Indianapolis, Indiana, United States). Finally, tissues were repositioned and sutured using 4-0 silk (Ethicon Inc. Somerville, NJ, USA).

The animals received a single 0.1 ml intramuscular dose of antibiotic (Pentabiotic 24,000 IU/kg body wt) and 0.1 ml subcutaneous morphine immediately post-surgery. For 3 days, they were given paracetamol dissolved in water (20 drops/400 ml).

The animals were euthanized at either 15 or 60 days post-surgery using a CO_2 chamber for 5–10 min. The original area of surgical defects in the calvaria were then harvested and fixed in 10% buffered formalin solution for 24 h, after soft tissue has been removed.

2.3. Micro-computed tomography analysis

Micro-computed tomography (micro-CT) scans containing the surrounding defects of all samples were obtained 60 days post-surgery using a micro-CT apparatus (Skyscan, 1174 v.2, Kontich, Belgium).

The micro-CT was set as follows: 0.5 mm aluminum filter; 1° rotation steps and an isotropic voxel size of 19.7 μm; voltage, 50 kV; and electrical current, 800 μA.

Three-dimensional images were produced using NRecon 1.66 computer software (Skyscan). The computer software DataViewer (Skyscan) was then utilized for the evaluation of three-dimensional reconstructions (linear measurements) of the coronal, transaxial and sagittal axes as well as the linear definition of the original bone defect in the anterior-posterior direction, lateral from the right parietal to the left parietal and linear extension of the original bone defect.

For volumetric analysis (tissue volume (TV), bone volume (BV) or percentage of bone volume (BV/TV)), an axis of interest (transaxial) was chosen using the CTAn 1.10 software (Skyscan) to measure the newly formed bone volume. To analyze the bone defects, only the 5 mm elliptical shape was chosen to select the area of interest.

2.4. Tissue preparation

The blocks used for histological and histomorphometric analysis were submitted to histotechnical preparation. The