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Effects of green tea and bisphosphonate association on dental socket repair of rats

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ABSTRACT

Objectives: To evaluate the effects of green tea intake and zoledronic acid intravenous therapy on teeth socket repair.
Design: Sixty male *albinus* Wistar rats were divided into 4 groups: C—Control, intravenous (IV) 0.9% saline solution (SS), GT—1% green tea in drinking water and IV SS, BP—IV zoledronic acid (BP), and BP + GT—IV BP and 1% green tea. 0.035 mg/kg of BP was administered every two weeks. After ten weeks, right upper molars were extracted and the green tea started to be offered for GT and BP + GT. After 7, 14, and 28 days the animals were euthanized.
Results: Histopathology analysis revealed lack of socket repair in BP and BP + GT groups, which presented significant increased number of polimorphonuclear leukocytes at day 28, in comparison with C ($p < 0.05$). No significant differences were detected between C and the experimental groups at the same period ($p < 0.05$) when considering mononuclear leukocytes. Immunolabeling revealed that the association of BP and GT caused a slight disturbance in OPG/RANKL system and retarded Runx-2 labeling. Although strong TRAP labeling was observed, most of the positive cells in BP and BP + GT groups were not located on bone surface.
Conclusions: Socket healing of rats treated with BP and regular drinking green tea presented no relevant differences in comparison to those treated with BP alone.

1. Introduction

Nitrogen-containing bisphosphonates (nBPs) are largely known for their high efficiency in treating osteolytic disorders, from osteoporosis to bone metastasis (Russell, 2011). After their administration, nBPs are almost immediately deposited in bone and their metabolism dependends on the dynamics of bone resorption (Fisher, Rodan, & Reszka, 2000; Cremers & Papapoulos, 2011). Low serum concentration and strong bone matrix incorporation of nBPs explain their low toxicity (Lawson et al., 2010). However, some factors influence drug absorption by the organism, such as dose, method of administration, and periodicity (Hadji, 2011), guided by the pathology to be treated.

Most potent nBPs, as zoledronic acid and pamidronate, are intravenously administered (IV), usually indicated for metastatic cancer, multiple myeloma, and severe hypercalcemia (Marx, Sawatari, Fortin, & Broumand, 2005) due to their higher affinity and deposition in bone (Gutta & Louis, 2007). Evidences have been strongly confirmed that these drugs are internalized by the osteoclasts during bone resorption and inhibit farnesyl pyrophosphate synthase, which consequently prevents farnesyl pyrophosphate (FPP) and geranylgeranylphosphate (GGPP) synthesis (Rogers, Crockett, Coxon & Mönkkönen, 2011). The lack of these molecules, mainly GGPPs, prevents the prenylation of small GTPases signaling proteins that regulate osteoclast function, such as cytoskeletal organization, membrane ruffling and apoptosis (Coxon & Rogers, 2003). On the other side, nBPs anti-apoptotic effects on osteocytes and osteoblasts have been observed (Loiselle, Jiang, & Donahue, 2013). However, this efficient capacity of inhibiting bone resorption and maintenance of osteocytes results in a significant change in whole tissue characteristics, since its

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natural turnover is disrupted. In long term, bone matrix becomes more brittle, osteocytes turn old and vascularization decrease, resulting in a poor reactive tissue favoring some adverse conditions as osteonecrosis of the jaws (Marx, 2003). In addition, considering the mechanism of action of anti-RANKL drugs, a recent *in vitro* study on nBP zoledronic acid demonstrated similarity between them. It was revealed that zoledronate inhibited genes related to RANKL- induced osteoclast differentiation, as of the transcription factor of the nuclear factor of the activated T cell, *NFATc1*, and *CAII*, which mediates the hormones responsible for bone resorption and osteoclast formation (Nakagawa et al., 2015).

It is known that alternative therapies represent an important and increasing role in prevention and treatment of a number of pathologies from diverse etiologies (Tomata et al., 2012). Antioxidant effects of poliphenols compounds (catechins) derived from plants as green tea (*Camellia sinensis*) have been proved to be effective in various conditions, including bone tissue disorders (Shen, Yeh, Cao, Chyu, & Wang, 2011). Considering total catechin amount in green tea, epigallocatechin-3-gallate (EGCG) corresponds for up to 59% (Singh, Akhtar, & Haqqi, 2010). Studies have demonstrated the direct influence of catechins in different pathways of osteoclastic differentiation and activation, including via metalloproteases (Oka et al., 2012; Yun et al., 2004) and RANKL (Sato & Takayanagi, 2006), as well as in the improvement of osteoblastic differentiation and activation via Runx-2 (Runt-related transcription factor-2) (Chen, Ho, Chang, Hung, & Wang, 2005). In pathologic condition, such as osteoporosis, EGCG effects seem to inhibit inflammation mediated by eicosanoids, as cyclooxygenase 2 (COX-2), lipoxygenase and nitric oxyde synthase (Tipoe, Leung, Hung, & Fung, 2007). In both situations the beneficial effects of green tea polyphenols in bone tissue is evident.

Numerous worldwide population under nBPs therapy and the imminent risk of osteonecrosis of the jaws have raised the necessity of the development of a number of clinical and experimental studies in order to characterize and elucidate it histopathology. In the field of experimental researches, different animals are used and rats are the majority (Barba-Recreo et al., 2014; Sharma, Hamlet, Pectu, & Ivanovski, 2013). Despite the systemic commitment that the patients under nBPs therapy usually present, when it comes to animal models this is not a mandatory condition for inducing osteonecrosis of the jaws, as proved by previous reports (Biasotto et al., 2010; Hokugo et al., 2010; Marino et al., 2012; Senel et al., 2010). Therefore, considering the high consumption of both substances by world population and the risk of osteonecrosis, their simultaneous ingestion was investigated in this study in order to observe their effects on socket bone healing after teeth extractions, and serve as an indicative on how simple daylife associations can interfere in healing mechanisms.

2. Materials & methods

2.1. Study design

The present study has been approved by the Ethical Committee for Animal Care (protocol 037/2012) of Bauru School of Dentistry, São Paulo University, Bauru, Brazil. All experimental protocols involving animals followed the National Institutes of Health guide for the care and use of Laboratory animals (NIH), as well as Brazilian Society of Laboratory Animal Science (COBEA). Sixty male Wistar rats, mean weight 300 g, average age of 5 months, were kept in controlled environment of 21–22 °C temperature and 12 h light-dark ycles, with food and water *ad libitum*, except for the animals treated with green tea.

2.2. Total phenol compounds analysis from 1% green tea infusion

Green tea infusion was daily prepared and its total phenol compounds was analyzed under spectrophotometry according to Folin-Ciocalteu method (Mah et al., 2014), using galic acid as pattern. Three different temperatures were observed, 40, 60 and 100 °C, under 5 and 10 min-infusion. The combination that resulted in the highest phenolic compounds concentration was chosen for green tea preparation in the present study.

2.3. Treatment protocols

The animals were divided into four groups (n = 15 each), according to the treatment, as follows: Group C: negative control, intravenous (IV) 0.9% saline solution (SS), Group GT: 1% green tea (Mate Leão, The Coca Cola Company, Curitiba, Brazil) in drinking water and IV 0.9% SS, Group BP: IV zoledronic acid (BP) (Zometa, Novartis Pharma AG, Basel, Switzerland), Group BP +GT: 1% green tea, and IV BP.

The animals of groups BP and BP + GT were treated with 0.035 mg/kg of zoledronic acid IV (Hokugo et al., 2010), every two weeks. Those of groups GT and BP + GT received 20 ml of 1% (w/v) green tea in drinking water (de Moraes et al., 2004), daily prepared in which 1 g of tea leaves were infused in 100 ml at 100°C filtered water for 5 min. After five doses of BP, all animals had their right upper molars extracted, from when they started receiving green tea.

2.4. Surgical procedure

All animals underwent surgical procedure for dental extraction following strict asseptic protocol. Sedation was induced with intramuscular (IM) administration of 1% ketamine (Frankotar, Virbac Ltda, São Paulo, Brazil) along with 2% chloridrate of xylazine (Virbaxyl 2%, Virbac Ltda, São Paulo, Brazil) in the recommended dose according to each animal weight. Once sedated, antisepsy with topic 1% polyvinylpyrrolidone was performed before dental extractions. A delicate sindesmotomy was made in the gingival sulcus of the upper right molars with 3S hollemback spatula, followed by teeth luxation with an apical elevator (EW1, Hu-Friedy, Chicago, USA) adapted in medial and distal bone crests, until total teeth luxation was achieved for further extraction with a Backaus clamp that perfectly adapts in molars furcation, in order to avoid root fractures. After that, sockets were cleaned with sterile 0.9% saline solution and spontaneously filled with blood clot.

2.5. Histologic procedures

After 7, 14, and 28 days from tooth extraction, five animals of each period and group were euthanized with anesthetic overdose for specimens' retrieval, which were immediately immersed in 10% formaline (Merck, Darmstadt, Germany) for 48 h. Macroscopical aspect of the extraction sites was registered (Fig. 1). After fixation, specimens were washed in tap water for 24 h, immersed in buffered 4% EDTA for demineralization. Semi-serial histological slices were obtained considering long-axis of the sockets and stained with hematoxylin-eosin and Goldner trichrome. For this, the first slices that presented the entire sockets were selected. Then, every each 30 μm slices were obtained to be analyzed. A descriptive histopathological analysis was performed based on the analysis of all slices obtained from each animal of each group, taking into consideration: inflammation, bone formation, maturation and remodeling, bone viability, biofilm adhesion, and overlying mucosa.