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Short-term perioperative teriparatide therapy for the prevention of medication-related osteonecrosis of the jaw: A randomized, controlled preclinical study in rats

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ABSTRACT

Objective: Dentoalveolar procedures in patients receiving bisphosphonates and other antiresorptive agents are associated with an increased risk of medication-related osteonecrosis of the jaw (MRONJ). The aim of present study was to evaluate the effects of perioperative teriparatide (TPD) therapy in prevention of MRONJ.

Subjects and methods: Two protocols of TPD therapy were studied. For protocol A, 25 TPD-treated (AT) and 25 control (AC) rats received 5 weekly injection of 0.06 mg/kg zoledronate. At the end of week 5, extraction of bilateral mandibular first molars was performed for all rats, and 4-week TPD (20 μg/kg/day) and saline therapy was started for AT and AC rats, respectively. For protocol B, 25 TPD-treated (BT) and 25 control (BC) rats received 5 weekly injection of 0.06 mg/kg zoledronate. One week later, 4-week TPD and saline therapy was started for BT and BC rats, respectively. Both groups underwent tooth extraction at the end of week 7 of the experiment. All rats were sacrificed 8 weeks after tooth extraction and assessed clinically for bone exposure/fistula, and histologically for density of osteocytes in newly formed bone and empty osteocyte lacunae in alveolar bone.

Results: Incidence of bone exposure/fistula and mean numbers of osteocytes and empty lacunae per 25 mm^2 (at 400× magnification) were 20%, 15.36, and 2.63 in AT group; 78%, 5.78, and 6.81 in AC group; 14%, 16.94, and 2.08 in BT group; and 78%, 7.54, and 5.95 in BC group; respectively. The differences between AT and AC and between BT and BC were statistically significant ($P < 0.001$). However, no statistically significant difference between AT and BT and between AC and BC was found.

Conclusion: Four weeks of TPD therapy, beginning at the same day or 2 weeks before tooth extraction, had a potential role in prevention of ONJ.

1. Introduction

Osteoporosis has become one of the most prevalent and costly global health problem and bisphosphonates (BPs) are the most widely prescribed agents for treatment of postmenopausal and steroid-induced osteoporosis. BPs are also used to manage cancer-related conditions such as bone metastasis from solid tumors and lytic lesions in multiple myeloma (Ruggiero et al., 2014; Schiodt et al., 2014; Brown et al., 2014). A serious complication associated with this class of medications is BP-related osteonecrosis of the jaw (BRONJ). Because of growing number of osteonecrosis cases involving the jaws associated with other antiresorptive (denosumab) and antiangiogenic therapies, the use of the term medication-related osteonecrosis of the jaw (MRONJ) was recommended by the

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American Association of Oral and Maxillofacial Surgeons position paper updated in 2014 (Ruggiero et al., 2014).

MRONJ can be a devastating disease causing severe morbidity and significant financial cost, and may adversely affect the patient's quality of life. The management of ONJ can be difficult and the therapeutic strategies that have been recommended by different associations are partly contradictory (Voss et al., 2012; Otto et al., 2015). Therefore, great emphasis is placed on prevention of this serious complication (Ripamonti et al., 2009).

It is well documented that preventive measures such as hygiene control, detailed dental examination and treatment before starting BP therapy, use of alternative dosing schedules that reduce BP exposure; avoidance of oral surgical procedures that expose the jaw bone, perioperative drug holiday, and antibiotic prophylaxis effectively reduced the incidence of ONJ (Saia et al., 2010; Ruggiero et al., 2014; Schiodt et al., 2014; Bodem et al., 2015; Zandi et al., 2015). However, these preventive measures are sometimes not very successful.

Teriparatide (TPD) is a biosynthetic polypeptide hormone composed of the first 34 amino acids of human parathyroid hormone. TPD is an anabolic agent that its intermittent administration initially stimulates bone formation by osteoblasts and subsequently bone resorption by osteoclasts; thus promotes bone remodeling. These osteoanabolic effects of TPD counteract the antiosteoclast property of BPs (Kwon et al., 2012; Kim et al., 2014). The successful use of intermittent low-dose parathyroid hormone in resolution of ONJ was firstly reported by Harper and Fung (2007). Thereafter, several studies demonstrated the beneficial effects of TPD therapy in management of ONJ, but the number of the publications is limited and their results are inconsistent (Cheung and Seeman, 2010; Kyrgidis and Antoniades, 2010; Lee et al., 2010). Most of these investigations were case studies reporting the effects of TPD in treatment of ONJ (Cheung and Seeman, 2010; Lee et al., 2010; Kwon et al., 2012; Neuprez et al., 2014: Kakehashi et al., 2015); few investigations have evaluated the beneficial role of this medication in prevention of osteonecrosis (Dayisoylu et al., 2013; Keskinruzgar et al., 2016). Therefore, the evidence in support of the use of TPD in prevention of ONJ is currently limited, and more investigations using well-designed randomized, controlled trials are required.

The aim of the present study was to evaluate the effectiveness of short-term perioperative TPD therapy in prevention of ONJ in rats.

2. Material and methods

This research protocol has been reviewed and approved by the Hamadan University of Medical Sciences Ethics Committee.

A total of 100 male Wistar rats were obtained from the Animal House of the University. The animals were 10 weeks old and weighed 350 ± 20 g. From 10 days before the start of the study, the rats were kept in a temperature- and humidity-controlled environment, with food and water supplies *ad libitum*.

In the present investigation, two TPD therapy protocols for prevention of ONJ were evaluated as follows:

Protocol A: Four weeks of TPD therapy starting on the day of tooth extraction and Protocol B: Four weeks of TPD therapy starting 2 weeks before tooth extraction.

A total of 100 rats were randomly distributed into two groups of A and B (each 50 rats), and each group was further divided into two subgroups of TPD and control (AT, AC, BT, and BC; 25 rats in each). The random assignment of the rats into groups and subgroups was done by the use of a lottery (http://www.graphpad.com/quickcalcs/randomize1.cfm).

In group A, all 50 rats received intraperitoneal injection of 0.06 mg/kg zoledronate (Zometa, Novartis Pharma, Basel, Switzerland), once a week for 4 weeks (a total of 5 BP injections), after which bisphosphonate was discontinued. The protocol for ONJ induction was as described in an earlier study by Zandi et al. (2016). At the end of week 5 of the experiment, extraction of bilateral mandibular first molar teeth was performed for both AT and AC subgroups, and daily subcutaneous injection of 20 μg/kg TPD (Cinnopar, Cinnagen, Iran) and the same volume of normal saline was started for the AT and AC subgroups, respectively, and continued for 28 days. The dose and interval of TPD administration was in accord with previous animal studies (Reynolds et al., 2011; Dayisoylu et al., 2013; Qiu et al., 2013). Eight weeks after surgical intervention (at the end of week 13 of the experiment), all of the 50 rats in study group A were sacrificed using an intraperitoneal injection of 200 mg/kg sodium pentobarbital.

In study group B, all of the 50 rats received intraperitoneal injection of 0.06 mg/kg zoledronate, once a week for 4 weeks (a total of 5 injections), after which bisphosphonate was discontinued. At the end of week 5 of the experiment, the rats in BT and BC subgroups received subcutaneous injection of 20 μg/kg/day TPD and the same volume of saline for 28 days, respectively. All of the rats in study group B underwent extraction of bilateral mandibular first molar teeth at the end of week 7 of the experiment and were sacrificed 8 weeks after surgical intervention (at the end of week 15 of the experiment) using intraperitoneal injection of 200 mg/kg sodium pentobarbital. The outline of the study design is illustrated in Fig. 1.

In the present study, tooth extraction was conducted under intraperitoneal general anesthesia using 75 mg/kg of Ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 7.5 mg/kg of midazolam (Midazolex, Exir, Iran). After placing the rat in a supine position, the gingiva around the right and left mandibular first molars was detached and the teeth were removed using a sharp dental explorer.

Following euthanasia, all of the 100 rats were clinically examined by two trained examiners, independently, for the presence of bone exposure at the extraction sites, abscess formation, and intraoral or extraoral fistulae that could be probed to bone. The mandibles of 100 sacrificed rats were harvested and sectioned at the midline. The 200 hemimandibles were fixed in 10% formalin solution and decalcified with EDTA. After dehydration, all samples were processed for paraffin embedding and 6 serial sections, 4 μm thick, were cut in a sagittal plane along the center of the extraction socket and stained with haematoxylin and eosin (H&E). For each sample, two H&E sections including the maximum length of the socket and a lack of artefact tissues were selected. All sections were assessed by an experienced pathologist for new bone formation in the extraction socket and dead bone (area of bone having lacunae without osteocyte) in the surrounding alveolar bone.

To evaluate the new bone formation, three different areas of the extraction socket were randomly selected in each histological section under brightfield microscopy at 400× magnification, and the average number of osteocytes per 25 mm^2 was calculated using a 10 mm × 10 mm eyepiece grid reticule. For evaluation of dead bone, three different areas of alveolar bone mesial and distal to the extraction socket were randomly selected in each histological section and the average number of empty osteocyte lacunae per 25 mm^2 was calculated using the same method. Ten percent of the sections were randomly selected and evaluated by the same examiner, and an excellent Intra-class Correlation Coefficient (ICC = 0.87) was observed. In the present study, the dental procedures and the clinical and histological examinations were performed in a blinded fashion.

Statistical tests were performed using SPSS 16.0 (SPSS Inc., Chicago, IL) statistics software. Data were expressed as the mean ± standard deviation. According to skewed distribution of the