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Bisphosphonates inhibit bone remodeling in the jaw bones of rats and delay healing following tooth extractions

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SUMMARY

Objective: This study aimed to evaluate the impact of concurrent administration of clinically relevant doses of zoledronic acid (ZA) and dexamethasone (DX) on bone healing after tooth extraction (EXO).
Materials and Methods: Forty-four Sprague–Dawley rats (6–8 month old) were randomized into five groups: ZA + DX = weekly injection of ZA with DX for 7 weeks; WD = ZA with DX for 3 weeks then DX alone for 4 weeks; *C* = control saline for 7 weeks; ZA = ZA alone for 7 weeks and DX = DX alone for 7 weeks. ZA was administered at 0.13 mg/kg/week and DX at 3.8 mg/kg/week and body weights recorded at the time of injection. All rats underwent extraction (EXO) of the mandibular and maxillary first molars at 3 weeks and were euthanized at 7 weeks. The extracted and non-extracted sides of both jaws were harvested for micro-CT analyses.
Results: All rats, particularly those injected with ZA, exhibited weight gain till EXO followed by decline then recovery. ZA + DX group demonstrated highest fractional bone to tissue volume (BV/TV) in the non-extracted side. ZA + DX rats exhibited also highest volume and surface of sequestra. Only sequestra volume was statistically higher in the WD group compared to C group.
Conclusion: Combined treatment with ZA and DX over a prolonged period inhibits bone remodeling and increased sequestra formation to a greater extent than either drug alone. Trauma caused by these sequestra cutting through the mucosa could play a key role in the development of BRONJ by potentially facilitating infection. ZA withdrawal may promote bone-remodeling reactivation following EXO.

Introduction

Bisphosphonates were proven to be effective drugs in the treatment of osteoporosis and cancer [1]. Intravenous bisphosphonates, such as zoledronic acid and pamidronate are given to reduce the skeletal-related events associated with cancer [2–4].

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) was defined in 2006 as an area of exposed bone in the maxillofacial region that does not heal within 8 weeks in a patient under bisphosphonates treatment without history of radiation to the craniofacial region [5].

Bisphosphonates inhibit osteoclast recruitment, differentiation and induce osteoclast apoptosis, which could lead to change in bone microarchitecture and increase the percentage of bone volume to tissue volume [1,6]. Although the pathophysiology of BRONJ remains unknown, previous rat models described in the literature reported that the administration of bisphosphonates could change the jaw bone vascularization [7–10]. In addition, zoledronic acid administration in rats reported to suppress genes associated with lymphoangiogenesis and tissue remodeling, such as VEGF-C and MMP-13 [11]. Another investigation linked increased prevalence of BRONJ with vitamin D deficiency in rats [12]. Based on quantitative histological analysis only, Ali-Erdem et al. reported differences in bone formation, inflammation and necrosis in sites of tooth extractions between rats injected with combinations of zoledronic acid and dexamethasone and a control group [13]. Senel et al. found that the administering bisphosphonates can increase inflammatory responses of the jaw bone without performing tooth extractions or any other dental procedure [14]. However, the effect of bisphosphonates on the microarchitecture and healing of the

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jaw bones is not well described in previous experimental models. In addition, the effect of bisphosphonates withdrawal remains unknown. Bisphosphonates might stay in the bone for an extended period of time and a short holiday might not have a significant impact on the risk of development of BRONJ [1,5].

The bisphosphonate zoledronic acid (ZA) and the glucocorticoid dexamethasone (DX) are commonly used together in the treatment of cancer patients. The combined treatment was reported to induce a BRONJ-like disease in rodents [8,9]. Using a rat model, this study was therefore aimed at the evaluation of the effect of combined administration of ZA and DX on the microarchitecture of jaw bones and the effect of bisphosphonate withdrawal on healing following tooth extractions.

Materials and methods

Drug administration

ZA (0.13 mg/kg/week body weight) and DX (3.8 mg/kg/week body weight) were administered by intra-peritoneal (IP) injections. These doses were converted from human doses of ZA (4 mg/person/3 weeks) and DX (40 mg/person/week) according to the National Institute of Health (NIH) guidelines [15]. Forty-four skeletally mature Sprague–Dawley rats aged 6–8 months were prospectively and randomly divided into the following groups: ZA + DX ($n = 10$) received combined treatment with the drugs throughout the 7-week experiment; WD ($n = 10$) received ZA + DX for 3 weeks prior to EXO, when ZA was withdrawn and DX continued for an additional 4 weeks; C ($n = 8$) was injected with physiological saline solution throughout the experiment to serve as a negative control for ZA + DX therapy; ZA ($n = 8$) and DX ($n = 8$) were treated with the respective drugs alone throughout the experiment as positive controls. Throughout the study, body weights were recorded once a week, at the time of drug administration. All animals underwent extractions (EXO) of the upper and lower left first molars three weeks after starting drug administration and were euthanized 4 weeks post-EXO based on literature indicating bone density in the extraction socket is stable after about 30 days [16].

Tooth extraction (EXO)

As described previously [8,16,17], animals were anesthetized with a single IP injection of rodent anesthetic cocktail (1 mL/kg) composed of 5.0 mL ketamine (100 mg/mL), 2.5 mL xylazine (20 mg/mL), 1.0 mL acepromazine (25 mg/mL) in 1.5 mL saline. The first molars were removed from the left side of the maxilla and mandible using a dental explorer No. 5, adapted elevators and cotton pliers while the tongue was retracted to one side of the mouth using a mosquito hemostat and sutures. Teeth selected for extractions were first loosened by running the tip of the explorer around the cervical portion and then extracted by luxation with a cotton plier. The extraction sockets were cleaned of residual roots using a medium straight headpiece (5100-015-250) and round fluted SST bur with diameter of 1.0 mm (1608-006-155, Stryker Canada). Following extraction, the bone ridge was shaped and smoothed using surgical rongeurs. Only one upper and one lower molar tooth was extracted in each animal to minimally interfere with the normal eating habits and result in minor post-operative complications [9]. To reduce pain and discomfort, rats were injected with carprofen (10 mg/kg) before the intervention and at 1 and 2 days post-EXO. Wet food was also offered to the animals to ensure regular nutritional intake. Rats were observed twice/week throughout the post-operative period till euthanasia.

Radiologic imaging

X-rays were captured (Kubtec, Milford, CT) at the time of EXO and 3 weeks post-EXO. Although serial X-rays cannot provide precise quantitative information, they were favored over *in vivo* micro-CT images to reduce the amount of irradiation to the soft and hard tissues surrounding the area of EXO, which could significantly interfere with the healing process. Following euthanasia using CO_2 inhalation, both, the EXO and NON-EXO sides of the mandible and maxilla were excised, cleaned, fixed overnight at 4 °C in 4% paraformaldehyde, rinsed thoroughly in three changes of sterile phosphate buffered saline (PBS) and stored at 4 °C while waiting for micro-computed tomography (micro-CT) scan and analysis (Skyscan 1172, Kontich, Belgium). Specimens were scanned at 8.9-μm resolution with exposure time 550 ms, electric voltage 59 kV and current 167 μA adjusted to allow maximum differentiation between mineralized and non-mineralized tissues and using a 0.5 mm thickness aluminum filter. The instrument was equipped with a 1.3 MP camera to capture high-resolution 2D images that were assembled into 3D reconstructions using NRecon software supplied with the instrument. In the NON-EXO side, a region of interest (ROI) covering the area extending from the mesial aspect of the second molar to the mesial aspect of the first molar, including both the cortical and trabecular bone and excluding the first molar itself, was selected for quantitative analyses (Fig. 1). In the mandible, the ROI extended towards the lower border of the mandible to cover the bone till the beginning of the incisor's enamel, which provided a clear anatomical reference (Fig. 1b). Since visual and threshold separations between trabecular and cortical bones in the mandible and maxilla were not reliably reproducible, the outlined ROI included both the cortical and trabecular bones (Fig. 1). In the EXO side, the ROI was extended 3.0 mm mesially from the mesial surface of the second molar to cover the EXO area including the separated bone fragments (sequestra) excluding the alveolar ridge. The percentage of bone volume to tissues volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), trabecular pattern factor (Tb.Pf) and bone structure model index (SMI) were quantified using the CTAn Skyscan software. The number, volume and surface area of the isolated bone fragments in the EXO sites were also measured.

Statistical analysis

The number of rats used for this study was based on our pilot investigations and on previous studies [8,9]. A total of 10 rats in the ZA + DX group and 8 rats in the C group were estimated to detect a 6% difference in the BV/TV in the NON-EXO side at the site of the first mandibular molar. This sample size was calculated based on a *t*-test, a standard deviation of 4.2%, 80% power and type 1 error of 0.05, with an increase of 15% to accommodate for the asymptotic relative efficiency of the Mann–Whitney-U test. All groups were compared individually to group C to determine the effect of drug administration on the jaw bone micro-architecture. Wilcoxon signed rank test was used for a within-group comparison of animal weights. Statistically significant difference was set at a p value less than 0.05.

Results

Animal weight

All rats exhibited weight gain for the first 2–3 weeks with weight loss after EXO. Gradual recovery and weight gain was noted in the post-EXO period in all study groups. Statistically significant increase in the animals' weight was noted at day 15 in the ZA and