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Original article

The effect of drug holiday on preventing medication-related osteonecrosis of the jaw in osteoporotic rat model

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ABSTRACT

Background: Medication-related osteonecrosis of the jaw (MRONJ) is a severe complication associated with antiresorptive medications managing osteoporosis, such as bisphosphonates (BPs). To date, there is very limited evidence from prospective, controlled studies to support or refute the controversial prevention regimen that if a discontinuation of BPs before dentoalveolar surgery, so called "drug holiday", is effective in reducing the risk of MRONJ development in patients with osteoporosis. We proposed an experimental animal study, aiming to investigate the prevention of MRONJ following tooth extractions in osteoporotic condition, with the implementation of a BP drug holiday.

Methods: Twenty rats were subjected to bilateral ovariectomy. After establishing the osteoporotic condition, all rats were exposed to weekly injections of zoledronate acid (ZA) for 8 weeks. After ZA treatment, 10 rats were subjected to dental extraction and defined as control group, and the rest 10 rats assigned to the DH group had a drug holiday of 8 weeks prior to dental extraction. Eight weeks after the dentoalveolar surgery, bone turnover biomarker in serum, occurrence of MRONJ-like lesion and histomorphometric assessment of osteonecrosis in mandible, and bone microarchitecture indices in femur, were examined.

Results: Eight weeks after dental extraction, the DH group showed a recovered osteoclastic activity, indicated by significantly increased number of osteoclasts in the mandibles and serum level of C-terminal telopeptides of type I collagen, as compared to the control group. No significant differences were observed in the gross-view and histological occurrences of MRONJ-like lesions between the two groups.

There was no significant difference in bone microarchitecture in the femur between the control and DH groups before ZA therapy and 8 weeks after dental extraction.

Conclusion: Our data provided the first experimental evidence in the osteoporotic animal model that the implementation of a BP holiday in prior to dental extractions could partially recover osteoclastic activity, but could not alleviate the development of MRONJ-like lesion or exacerbate the osteoporotic condition in the femur. Longer-term drug holiday, or combination of drug holiday and other prophylaxes to prevent MRONJ in patients with osteoporosis could be worth exploring in future studies, to pave the way for clinical managements.

The translational potential of this article: This *in vivo* prospective study reported that a recovery of osteoclastic activity by a BP drug holiday for 8 weeks in osteoporosis rats did not alleviate the development of MRONJ-like lesion followed by dental extractions. It contributes to the understanding of regimens to prevent MRONJ.

1. Introduction

Bone mass is primarily determined by the balance between the activities of osteoblasts and osteoclasts, which form and resorb bones, respectively. Osteoporosis, resulted from the imbalance of these two activities, is now one of the major age-related skeletal diseases [1]. Antiresorptive medications, such as bisphosphonates (BPs), are widely used to manage osteoporosis [2]. These medications inhibit bone

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remodelling, thereby reducing the risk of osteoporotic fractures and maintaining the patients' quality of life [3]. Although data from randomized trials and clinical experience indicate that antiresorptive medications are generally safe, medication-related osteonecrosis of the jaw (MRONJ) has been observed as a serious adverse effect associated with long-term use of BPs, with an incidence ranging from 0.01% to 0.069% in patients with osteoporosis [4–8].

Many studies suggest that BPs can be considered as the primary risk factor for MRONJ. It is supported by the association between MRONJ and potent antiresorptive medicines, as well as the increased risk and greater dosages of BPs [9,10]. For high-dose treatment, phase 3 studies comparing the use of ZA (4 mg every 4 weeks) in patients with advanced cancers have shown that the incidence of confirmed MRONJ was 1.3% [11–13]. For low-dose treatment (5 mg once a year), an incidence of MRONJ in 5903 patients receiving ZA was 0.007% [14]. In addition, the incidence of MRONJ increases with the length of exposure to antiresorptive medications, according to studies of cancer patients [11,15]. A study evaluated the safety and efficacy of ZA in patients with advanced breast cancer, the incidences of MRONJ in the ZA group at years 1, 2, and 3 were 0.5%, 1.2%, and 1.4%, respectively [16].

Nevertheless, studies have shown that the level of serum BP was extremely low 2 months after the last dose of an oral BP, and the level of serum C-terminal telopeptide (CTX) in patients with MRONJ increased after cessation of BPs for 3 months, indicating the recovery of the bone turnover process [17,18]. For prudent consideration, the American Association of Oral and Maxillofacial Surgeons (AAOMS) recommends that, for patients who receive antiresorptive therapy for longer than 4 years or are concurrently taking steroid and have low fracture risk but a potentially high risk for MRONJ, discontinuation of oral BP (the so-called "drug holiday") for at least 2 months before oral surgery should be considered, if systemic conditions permit, and the BP should not be restarted until osseous healing has occurred [8,19]. Hence, this guideline is also supported by other academic societies including the Korean Society for Bone and Mineral Research and the Korean Association of Oral and Maxillofacial Surgeons [20]. However, as there is no evidence to support that the interruption of drug therapy in patients requiring dental procedures reduces the risk of MRONJ or the progression of the disease, the practice of drug holiday is not recommended by the International Task Force on ONJ [6]. In recent years, more retrospective clinical studies have found no changes in the incidence of MRONJ in patients with osteoporosis who are subjected to BP discontinuation before dental treatment and suggest no need for drug discontinuation before tooth extraction to prevent MRONJ [21–26]. Overall, there is still a considerable controversy regarding whether suspension of BPs for a certain period of time before invasive dental treatment is effective in preventing or reducing the occurrence of MRONJ.

To date, previous studies on drug holiday were mainly based on retrospective clinical studies, and there is very limited scientific evidence from prospective, controlled studies published on the effects of BP drug holiday in preventing MRONJ in patients with osteoporosis. Therefore, we hypothesized that a drug holiday for BP before dental extractions could elevate the osteoclastic activity in an osteoporosis rat model, thus reducing the risk of MRONJ development. We proposed an experimental animal study, aiming to evaluate the prevention of MRONJ following tooth extractions in osteoporotic condition, with the implementation of a BP drug holiday.

2. Materials and methods

2.1. Experimental animals

This study was approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR No. 5191–19) of the University of Hong Kong. Twenty female Sprague–Dawley rats (8-month-old), with an average weight of 437 g (ranging from 375 to 500 g), were included in this study.

2.2. Study design and surgical protocol

The animals were randomly assigned into two groups: control (BP treatment; n = 10), and DH (BP therapy with a drug holiday of 8 weeks; n = 10). All rats were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg), and then subjected to bilateral ovariectomy (OVX). Bone mineral density (BMD) was simultaneously measured by micro-computed tomography (micro-CT). Eight weeks after OVX, BMD was measured again to confirm the establishment of osteoporotic condition. Thereafter, each rat received intraperitoneal injections of zoledronate acid (ZA; 80 μg/kg) once a week for 8 weeks (to mimic patients with osteoporosis who are receiving the antiresorptive therapy for 4 years) [10]. One week after completion of ZA therapy, rats in the control group were subjected to dental extractions to induce the MRONJ; rats in the DH group had a drug holiday of 8 weeks prior to the same surgical intervention. Under general anaesthesia, the three left mandibular molars of each rat were removed using dental forceps and the alveolar sockets were cleaned using a round bur to remove any remaining root fragments. The sockets were compressed to stop the bleeding. No sutures were placed for wound closure. All rats were housed in an environmentally controlled animal-care laboratory after surgery and given analgesics with subcutaneous injection of buprenorphine (0.03 mg/kg) for three consecutive days. No antibiotics were administered. Eight weeks after dental surgery, all rats were euthanized by intraperitoneal injection of pentobarbital (200 mg/kg). The left mandible (L; side receiving dental extractions) and the right mandible (R; contralateral side without surgery) samples and blood samples were harvested. The outline of the study design is shown in Fig. 1.

2.3. In vivo micro-CT analysis of femur

To assess osteoporotic changes, each rat was scanned on the metaphysis region of the left femur at serial time points using a micro-CT (Skyscan1076, Kontich, Belgium) under anaesthesia at: pre-OVX, 8 weeks post-OVX (before ZA therapy), and 8 weeks post-extractions. Two phantom contained rods with the standard densities of 0.25 and 0.75 g/cm^3 were scanned with each sample for calibration. The acquisition settings were at a pixel size of 17.3 μm with an X-ray tube voltage of 88 kVp and an intensity current of 100 μA. Data reconstruction was performed using NRecon software (Skyscan Company), and image processing and analysis were performed using CTAn software (Skyscan Company). In the distal femur region, a series of slices starting at a distance of 2 mm proximal from the higher end of the growth plate with a length of 2 mm were chosen for the evaluation. The trabecular part of the femur was separated using semi-automatically drawn contours. The complete secondary spongiosa of the distal femur was evaluated to avoid sampling errors incurred by random deviations of a single section. Bone mineral density (BMD), bone volume fraction (BV/TV), structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and connectivity density (Conn.D) were calculated using the built-in software.

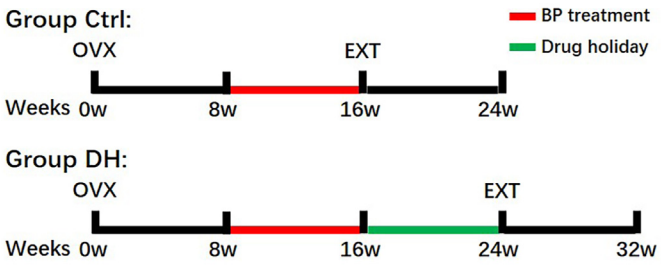

Fig. 1. Schematics of animal grouping with detailed timing of intervention. OVX, ovariectomy; EXT, dental extraction; BP, bisphosphonate.

2.4. Criteria for MRONJ-like lesion diagnosis

The evaluation of MRONJ-like lesions in rats was diagnosed by clinical and histological criteria, respectively. The clinical diagnostic criteria were as follows [27]: (1) Persistent failure of mucosal coverage, with exposed necrotic bone, at week-8, and (2) Sequestration of the alveolar bone by micro-CT examination. The histological diagnostic criteria were as follows [28]: (1) Presence of ulcerative lesions with exposed and necrotic bone and/or osteolysis, (2) Presence of pseudoepitheliomatous-like hyperplasia of the epithelium accompanied by inflammatory cell infiltration, and (3) Presence of sequestrum. The occurrence rates of MRONJ-like lesions were compared between the groups.

2.5. Histomorphometric analysis

The harvested mandibular samples were decalcified in 15% EDTA for 4–6 weeks at room temperature and then dehydrated in a series of ethanol and xylene before being embedded in paraffin. Sections with a thickness of 6 μm were prepared for H&E and tartrate-resistant acid phosphatase (TRAP) staining (#387 A-1 KT, Sigma–Aldrich). In each section focusing on the sites surrounding sockets after dental extraction (Left side receiving surgery) or molars (Right side without surgery), three randomly chosen fields (200 × magnification) were examined, excluding sequestrated and separated bone fragments. Osteoclast were counted on slides assayed for TRAP activity as TRAP-positive multinucleated cells, which were identified as TRAP-positive cells that contained 3 or more nuclei. To quantify the degree of osteonecrosis, the percentage of empty lacunae to total osteocyte lacunae, number of empty lacunae per bone area (N.Empty Lacunae/B.Ar), number of osteocytes per bone area (N.Osteocytes/B.Ar), and number of osteoclasts per bone area (N.Oc/B.Ar), were calculated and compared between the groups.

2.6. Serum level of bone turnover marker

Blood samples were collected from five rats in each group after 6 h' fasting and separated for serum. Serum samples were stored at −80 °C until analysis. The levels of serum C-terminal telopeptides of type I collagen (CTX-I) were determined using an enzyme-linked immunosorbent assay (ELISA) kit (#AC-06F1, RatLaps™ EIA, Immunodiagnostic systems), according to the manufacturer's recommendations. Optical densities were measured at 450 nm with reference at 650 nm using a microplate reader.

2.7. Statistical analysis

All statistical analyses were calculated using SPSS (version 26.0; IBM Corporation, Chicago, IL, USA) and GraphPad Prism (version 8.0; GraphPad Software Inc., San Diego, CA, USA). Data are presented as means ± standard deviation (SD). The paired differences in micro-CT bone analyses between pre-OVX and 8 weeks post-OVX, and histomorphometric analyses between left and right mandibles, were compared using paired-samples T-test. Independent-samples T-test was used to compare micro-CT bone analyses and histomorphometric analysis between the two groups. Chi-square test was used to compare the occurrence rates of MRONJ-like lesions between the two groups. *P* values less than 0.05 were considered statistically significant.

3. Results

Seventeen of 20 rats were included in this study. One rat was euthanized due to an unexpected abdominal tumor development before dental extraction, and two rats died due to anaesthetic accidents during dental surgery.

Eight weeks after dentoalveolar surgery, the DH group showed an elevated osteoclastic activity, as compared to the control group (Fig. 2). The DH group had significantly higher numbers of TRAP-positive osteoclasts in the dental extraction sites and the contralateral sides without surgery than those of the control group ($p = 0.035$, and 0.038, respectively). Serum levels of CTX-I were significantly increased in the DH group than the control group (89.1 ± 22.7 ng/mL, and 67.5 ± 19.5 ng/mL, respectively; $p = 0.013$). Meanwhile, there were no significant differences in the percentage of empty lacunae, number of empty lacunae and number of osteocytes in the non-surgical sides between two groups ($p = 0.342$, 0.311, and 0.479, respectively).

Eight weeks after dental extraction, the MRONJ-like lesion occurrence rates in the control and DH groups were 25.0% and 44.4%, respectively, and the histological MRONJ-like lesion occurrence rates were 75.0% and 88.9%, respectively. No significant differences were observed in the gross-view and histological occurrences of MRONJ-like lesion between the two groups ($p = 0.620$ and 0.576, respectively; Fig. 3A). The representative clinical and histological photos showing necrotic lesions are presented in Fig. 3C and D.

Histologically, the left mandibles receiving dentoalveolar surgery developed typical pathological alterations in the percentage of empty lacunae, number of empty lacunae and number of osteoclasts, as compared to the right mandibles without surgery ($p = 0.004$, 0.005, and 0.035, respectively; Fig. 3B). The control group presented significantly increased percentage of empty lacunae and number of empty lacunae, and decreased number of osteoclasts in the extraction sites ($p = 0.05$, 0.03, and 0.003, respectively); the DH group showed significantly increased percentage of empty lacunae and number of empty lacunae in the extraction sites, but no significant difference in the number of osteoclasts ($p = 0.035$, 0.037, and 0.263, respectively). Comparing the extraction sites between the control and DH groups, there were no significant differences in the percentage of empty lacunae, number of empty lacunae, and number of osteocytes ($p = 0.465$, 0.299, and 0.642, respectively).

Micro-CT analysis showed significantly reduced BMD, BV/TV, SMI and Tb.N in the femurs of rats 8 weeks after OVX (Table 1), confirming the establishment of the osteoporotic model (Fig. 4). There were no significant differences between the control and DH groups in BMD, BV/TV, SMI, Tb.Th, Tb.N, Tb. Sp and Conn. D, at the timepoints before ZA therapy and 8 weeks after dental extraction (Table 2).

4. Discussion

In this study, our data showed that the implementation of a BP holiday for 8 weeks before dental extractions could partially recover osteoclastic activity, but could not alleviate MRONJ-like lesion development or accelerate osteoporotic condition. To the best of our knowledge, this is the first *in vivo* study to investigate the effects of BP drug holiday on MRONJ using an osteoporotic animal model.

There are differences in opinions regarding drug holiday in the prevention of MRONJ, and a full consensus has yet to be reached. On one hand, based on the bone physiology and pharmacokinetics of antiresorptive medications, a BP drug holiday before an invasive dental procedure should be rational because the majority of free BP within the serum is extremely low 2 months after the last dose of an oral BP, and the osteoclast known as the major reservoir of BP only has a life span of 2 weeks [17]. But on the other hand, as BPs have a strong affinity for bone and are slowly released from skeletal tissue for months even years, with an estimated mean terminal half-life of greater than 10 years, so it appears unlikely that a short-term drug holiday will decrease the level of BP binding to the jawbone, thereby preventing MRONJ [29,30]. In our study, we found that the number of osteoclasts and serum level of CTX-I in the DH group were significantly higher than that in the control group, which might indicate a resumed osteoclastic activity and bone turnover after a cessation of BP for 2 months.

There is insufficient clinical evidence supporting that short-term discontinuation of BPs helps prevent the occurrence of MRONJ triggered by invasive dental treatments. Although four retrospective studies

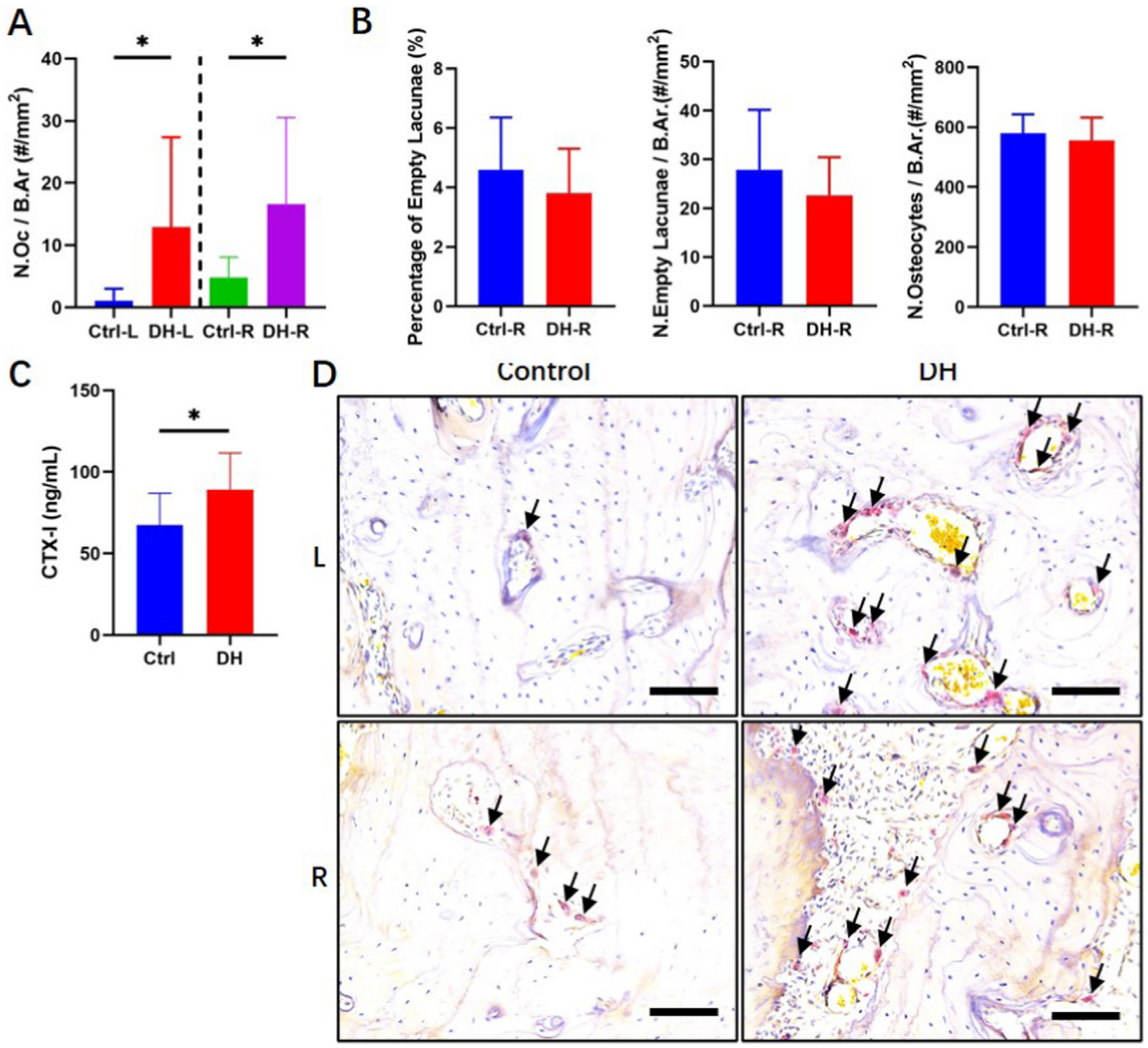

Fig. 2. Eight weeks after dentoalveolar surgery, the DH group showed an elevated osteoclastic activity, indicated by significantly increased numbers of osteoclasts and serum level of CTX-I, as compared to the control group (A) Histomorphometric assessment of TRAP staining on the number of TRAP-positive cells in the dental extraction sites (L) and the contralateral sides without surgery (R) between groups (B) Histomorphometric assessment of H&E staining on the osteonecrotic degree in the contralateral sides without surgery (R) between groups (C) Serum levels of bone turnover marker CTX-I between two groups. Column and bar indicate mean and SD, respectively. Ctrl-L and Ctrl-R groups n = 8, DH-L and DH-R groups n = 9 (A), Ctrl-R group n = 8, DH-R group n = 9 (B), n = 5 in each group (C), by independent-samples T-test. *, $p < 0.05$ (D) Representative TRAP staining images of extraction site (L) and contralateral side without surgery (R) in two groups 8 weeks after dentoalveolar surgery. Arrow points the TRAP-positive stained osteoclast. Scale bar: 100 μm.

recommended BP drug holiday before surgical treatment for MRONJ [31–34], the majority of studies found no influence of a drug holiday in preventing or managing MRONJ [21–26,35,36]. However, this evidence was mainly based on retrospective clinical studies, and no randomized prospective clinical study data have been published on this topic. To date, there are only a few prospective animal studies [37–41] investigating the effects of drug holiday in MRONJ. Studies have shown that the discontinuation of OPG-Fc, a reactive activator of nuclear kappa B ligand (RANKL) inhibitor, prior to tooth extraction altered radiographic and histologic findings of osteonecrosis in mice and rats [37,39,41], which supports the hypothesis that a RANKL inhibitor, such as denosumab, may respond better to a drug holiday due to its quick half-life [5]. Meanwhile, these studies also provided experimental evidence illustrating that ZA discontinuation did not ameliorate ONJ development [37,39]. However, another study demonstrated that ZA holiday significantly decreased the incidence and severity of MRONJ after tooth extraction in rats mimicking patients receiving oncologic antiresorptive therapy [38]. Recently, the first large-animal study using a MRONJ minipig model showed that the implementation of prophylactic measures combined with a drug holiday reduced the occurrence and severity of MRONJ following tooth extraction, indicating that despite the extremely long half-life of BPs in the bone, there might also be a clinical role for a drug holiday [40].

The suppressed bone turnover by the inhibition of osteoclasts, is thought to be one of the most important pathogenic mechanisms of MRONJ [42–44]. The alveolar bone of the jaws is remodelled daily with a high rate of bone turnover and subjected to a wide variety of stresses; while the prolonged use of BPs causes excessive reduction of bone turnover with accumulation of microcracks resulting in an increased risk of bone necrosis in osseous repair [7,45–47]. This theory partially explains the exclusive occurrence of MRONJ in jawbone. However, it fails to explain why MRONJ does not appear to occur more frequently in patients treated with denosumab, whose biochemical and histological indices of bone turnover are lower than those seen in patients treated with ZA [48]; besides, reduced osteoclastic bone resorption and bone remodelling are not always typically detected in affected tissues from patients with MRONJ, and ONJ does not appear to occur in other

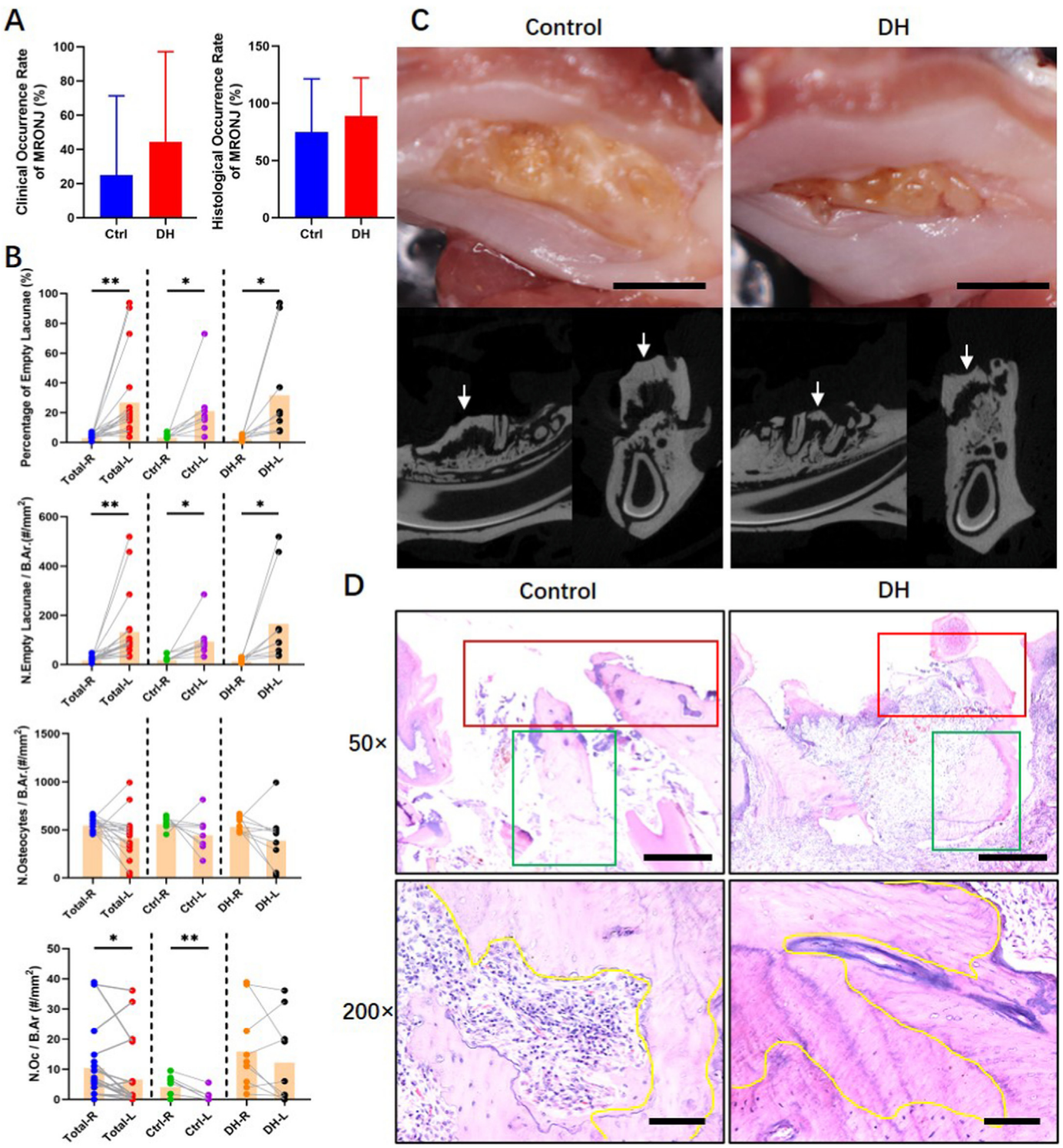

Fig. 3. Eight weeks after dentoalveolar surgery, the DH group did not show an alleviation in the development of MRONJ-like lesion, as compared to control group (A) Clinical and histological occurrence rates of MRONJ-like lesion in two groups at 8 weeks after dental extraction. Control group n = 8, DH group n = 9, by Chi-square test. Column and bar indicate mean and SD, respectively (B) Histomorphometric assessments of H&E staining on the osteonecrotic degree and TRAP staining on the number of TRAP-positive cells between groups. Column indicates mean. n = 17 for total, n = 8 for control group, and n = 9 for DH group. Comparisons between right and left mandibles were using paired-samples T-test. Comparisons between two groups were using independent-samples T-test. *, $p < 0.05$. **, $p < 0.01$ (C) Representative clinical photographs and micro-CT images from two groups, 8 weeks postoperatively. Large open wounds with exposed necrotic bone involving all extraction sites were noted in rats developing MRONJ-like lesion. Micro-CT images showed sequestered alveolar bone fragments (white arrows) at the extraction region and widened periodontal ligament at each remaining root fragment in rats developing MRONJ-like lesion. Scale bar in clinical photos: 1 mm (D) Representative histological photos from two groups, 8 weeks postoperatively. H&E staining images showed ulcerative gingival lesions with exposed and necrotic bone (red), presence of sequestrum (green), and osteonecrotic area (yellow) in the extraction region. Scale bar in 50 × and 200 × images: 500 μm and 100 μm, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
In vivo micro-CT analysis of femurs between pre-OVX and 8-week post-OVX.

	Pre-OVX	8 weeks post-OVX	Paired difference (n = 19)	*P* value
BMD (mg HA/cm^3)	0.29 ± 0.05	0.26 ± 0.05	0.03 ± 0.03	<0.001***
BV/TV (%)	22.85 ± 7.35	20.26 ± 7.12	2.59 ± 4.96	0.035*
SMI	1.77 ± 0.58	2.05 ± 0.61	−0.28 ± 0.33	0.002**
Tb.Th (mm)	0.12 ± 0.02	0.12 ± 0.01	0.00 ± 0.01	0.563
Tb.N (#/mm)	1.91 ± 0.56	1.72 ± 0.55	0.19 ± 0.34	0.024*
Tb.Sp (mm)	0.37 ± 0.17	0.37 ± 0.14	0.00 ± 0.09	0.977
Conn.D (mm^{-3})	53.41 ± 32.99	47.26 ± 32.00	6.15 ± 23.58	0.271

*, $p < 0.05$; **, $p < 0.01$; ***$p < 0.001$.

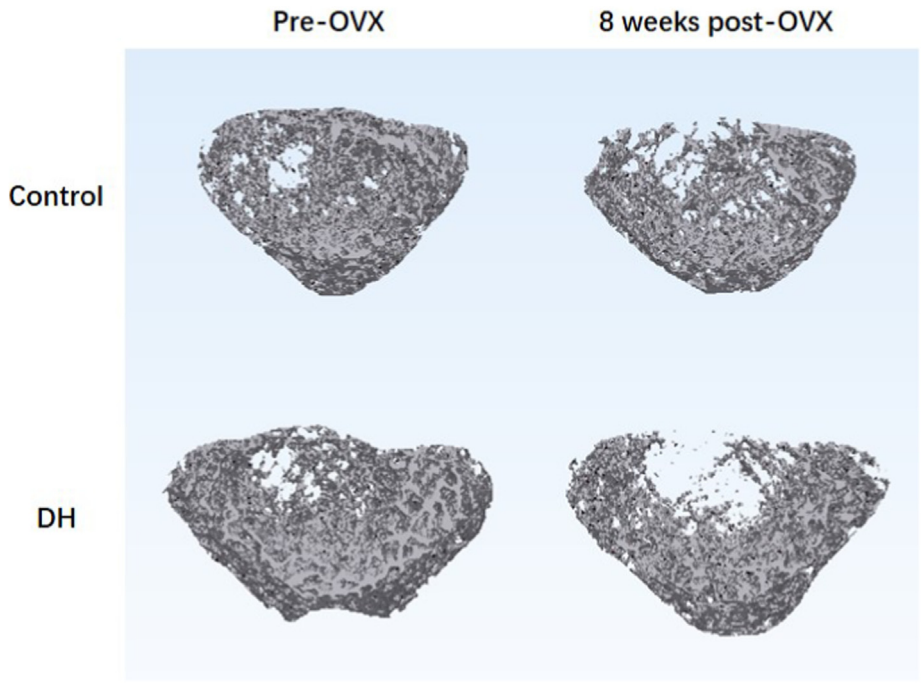

Fig. 4. Three-dimensional morphology of the trabecular part in the distal femur before OVX and 8 weeks after OVX. OVX, ovariectomy; DH, drug holiday.

Table 2
In vivo micro-CT analysis of femurs between the control and DH groups.

	Before ZA therapy			8 weeks post-extraction		
	Ctrl (n = 8)	DH (n = 10)	*P*-value	Ctrl (n = 8)	DH (n = 9)	*P*-value
BMD (mg HA/cm^3)	0.26 ± 0.06	0.26 ± 0.05	0.914	0.26 ± 0.07	0.27 ± 0.06	0.815
BV/TV (%)	20.10 ± 8.54	20.50 ± 6.67	0.912	20.25 ± 8.47	22.11 ± 7.12	0.635
SMI	1.77 ± 0.55	2.27 ± 0.62	0.096	1.85 ± 0.41	1.52 ± 0.38	0.116
Tb.Th (mm)	0.13 ± 0.01	0.11 ± 0.01	0.053	0.13 ± 0.01	0.13 ± 0.01	0.405
Tb.N (#/mm)	1.56 ± 0.57	1.86 ± 0.56	0.282	1.53 ± 0.55	1.66 ± 0.48	0.609
Tb.Sp (mm)	0.42 ± 0.10	0.34 ± 0.11	0.174	0.46 ± 0.18	0.56 ± 0.29	0.406
Conn.D (mm^{-3})	37.31 ± 16.64	56.30 ± 26.04	0.093	32.66 ± 15.64	29.33 ± 17.07	0.680

conditions associated with low bone turnover, such as hypoparathyroidism [49–53]. Taken together, it suggests that the inhibition of osteoclastic bone resorption and remodelling might provide an underlying condition for developing MRONJ, but there are still some factors exclusively in the jaw which could trigger the onset of MRONJ. In terms of pathological findings of MRONJ in rodents, although significantly decreased numbers of osteoclasts in the tooth extraction socket are the most commonly reported pathological feature, several studies have reported increased numbers of osteoclasts or varied numbers of osteoclasts at different timepoints [54,55]. In our study, we compared the number of osteoclasts in the same sides between the groups with or without drug holiday (Fig. 2A), which demonstrated significant increases in the number of osteoclasts induced by the drug holiday, both in surgical side and non-surgical side. Then we compared the number of osteoclasts between non-surgical side and surgical side within the same group, showing the pathological alterations of osteoclasts in MRONJ induced by the dental extraction. The significant decrease of osteoclasts was presented in the control group, which was thought to be the important evidence supporting the pathogenesis of developing MRONJ, but not in the DH group. Besides, although not statistically significant, the occurrence of MRONJ-like lesions in the DH group was slightly higher than the control group, regardless of whether the number of osteoclasts in the DH group was significantly higher than that in the control group. Therefore, it is likely that the development of MRONJ was facilitated by a combination

of different mechanisms, and the partially recovered osteoclastic activity caused by the drug holiday was insufficient to achieve a significant beneficial impact on preventing MRONJ.

There is another concern on BP drug holiday from the perspective of orthopedics. Even the discontinuation of BP before invasive dental treatment is effective, does the benefit of preventing or reducing the occurrence of MRONJ outweigh the risk of aggravating osteoporosis? In patients with osteoporosis who take BP drug holiday, exacerbation of osteoporosis, including decreased bone mineral density and increased incidence of fractures, has been observed [56–59]. Given the low incidence of MRONJ in patient with osteoporosis who are treated with BPs, the benefit-to-risk ratio for MRONJ prevention is negative for most women with osteoporosis, and drug holidays may only be considered for patients at a lower risk of fracture after 5 years of alendronate therapy or 3 years of ZA therapy [2]. Less is understood about the benefits and harms of initiating long-term osteoporosis drug treatments, and consensus is lacking regarding which patients should have BP holidays, when, and for how long, as well as criteria for restarting treatment [4,60,61]. In this study, we used ovariectomized rats as the osteoporosis animal model. One reason for this model is that ovariectomized rats are useful for type I primary osteoporosis, which is closer to the clinical scenario in our project [62]. Another reason is that a study demonstrated that ovariectomized rats showed a higher MRONJ incidence than sham rats, indicating that the MRONJ rat model with underlying osteoporosis showed different pathological characteristics than the conventional MRONJ model [28]. Although significantly increased serum level of CTX-I was shown in the DH group at the endpoint, there were no significant differences in microarchitectural indices of femur between the rats receiving or not receiving a drug holiday, indicating that a BP drug holiday of 8 weeks before dental extraction might not significant enough to accelerate osteoporotic condition in rats.

In this study, we provided the evidence of the effects of BP holiday on MRONJ in an osteoporosis model, with a strength of the randomized, prospective, controlled experimental design. However, there are certain limitations of our study that should be addressed. One of the limitations of this study was the small sample size of animals. The negative findings of the comparison of MRONJ-like lesions between groups should be carefully interpreted. Further studies with a larger sample size and well-controlled compounding factors are needed. Although most MRONJ studies used rodent animals, the dosage of ZA and the anatomy and metabolism of rodents are different from those of humans. Therefore, prospective clinical studies are needed to validate our results.

In conclusion, our experimental study reported that a BP drug holiday before dental extractions in osteoporosis rats could partially recover osteoclastic activity, but could not alleviate the development of MRONJ-like lesion or exacerbate the osteoporotic condition in the femur. The reverted osteoclastic activity due to the BP drug holiday might be good context for ameliorating the risk of developing MRONJ; therefore, longer-term drug holiday, or combination of drug holiday and other prophylaxes to prevent MRONJ in patients with osteoporosis could be worth exploring in future studies, to provide more *in vivo* evidence for paving the way for clinical managements.

Authorship

Category 1

Conception and design of study: Wang-yong Zhu, Ling Qin, Yu-xiong Su, Acquisition of data: Wang-yong Zhu, Wei-fa Yang, Leilei Wang, Xinmiao Lan, Zhuo-ying Tao, Jiaxin Guo; Analysis and/or interpretation of data: Wang-yong Zhu, Leilei Wang

Category 2

Drafting the manuscript: Wang-yong Zhu; Revising the manuscript critically for important intellectual content: Jiankun Xu, Ling Qin, Yu-xiong Su.

Category 3

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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