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Research Paper
TMJ Disorders

Early intra-articular injection of alendronate reduces cartilage changes and subchondral bone loss in rat temporomandibular joints after ovariectomy

K. Chen[1,2], N. Zhang[1], L. Ding[3], W. Zhang[1], J. Hu[1], S. Zhu[1]
[1]State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, China; [2]Department of Oral and Maxillofacial Surgery, Second Hospital of Lanzhou University, Lanzhou, China; [3]Pathology Department, Medical School, University of Michigan, MI, USA

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Abstract. This study investigated the effects of intra-articular injection of alendronate on the mandibular condyle in ovariectomized rats. Sixty rats were divided into five groups: ovariectomy with vehicle treatment alone, early alendronate treatment at ovariectomy, late alendronate treatment at 4 weeks after ovariectomy, sham-operation with vehicle treatment, and normal controls. The changes in cartilage and subchondral bone were evaluated by micro-computed tomography, histology, tartrate-resistant acid phosphatase (TRAP) staining, immunohistochemistry, and real-time quantitative polymerase chain reaction. Compared with late alendronate treatment, early alendronate treatment completely inhibited cartilage thickening (727.6 ± 39.3 vs. 1013.3 ± 51.6; $P = 0.017$) and improved microstructural properties of the subchondral bone, with a higher bone volume ratio (46.4 ± 2.5 vs. 37.5 ± 2.1; $P = 0.038$), trabecular thickness (47.3 ± 1.7 vs. 34.6 ± 1.4; $P = 0.029$), and trabecular number (8.5 ± 0.6 vs. 6.2 ± 0.3; $P = 0.041$) and lower trabecular separation (30.2 ± 1.6 vs. 37.7 ± 2.6; $P = 0.034$). Fewer TRAP-positive cells (4.2 ± 0.2 vs. 6.8 ± 0.4; $P = 0.019$) and a higher OPG/RANKL ratio (0.38 ± 0.01 vs. 0.25 ± 0.03; $P = 0.043$) in the subchondral bone were observed in the animals with early treatment compared to late treatment or ovariectomy/vehicle treatment. In addition, early alendronate treatment blocked the up-regulation of matrix metalloproteinase (MMP)-13 expression in the chondrocytes, whereas late alendronate treatment attenuated the up-regulation of MMP-13 expression. Our results suggest the therapeutic potential of intra-articular alendronate injection in the treatment of osteoporosis-associated temporomandibular disorders.

Key words: cartilage; subchondral bone; temporomandibular joint; osteoporosis; drug delivery.

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Osteoporosis is a systemic disease characterized by reduced bone mass and structural deterioration of bone tissue.[1] Osteoporosis significantly alters bone metabolism in the subchondral bone of the condyle in temporomandibular joints (TMJ).[2–5] The structural integrity of the subchondral bone plays an important role in maintaining the mechanical stability of the joint.[6–8] Clinical data have shown a higher rate of deformation of mandibular condyles and TMJ disorders in elderly women than in men,[4,9,10] suggesting a possible relationship between post-menopausal osteoporosis and TMJ diseases. However, few studies have focused on the treatment of subchondral bone changes in the condyle in the development of osteoporosis.

Alendronate is a nitrogen-containing bisphosphonate and a potent inhibitor of bone loss. A previous investigation by the study team[11] and several other studies[12,13] have shown its chondro-protective effect in experimental osteoarthritis (OA). However, an increasing number of studies have suggested a possible association between the systemic use of alendronate and avascular osteonecrosis of the jaw.[14]

Recent studies have demonstrated the chondro-protective and anti-inflammatory properties of intra-articular injection of low-dose clodronate, another bisphosphonate, in human OA[15] and experimental arthritis.[16] However, the results from knee joints may not be directly applicable to TMJs. In the present study, we investigated whether early or late intra-articular delivery of alendronate can inhibit ovariectomy (OVX)-induced bone loss in the subchondral bone of the mandibular condyle and protect the mechanical integrity of the joint.

Materials and methods

Animal studies

Sixty 7-month-old female Sprague-Dawley rats were randomly divided into five groups: OVX with vehicle treatment ($n = 12$), OVX with an early intra-articular alendronate injection (on the day of OVX) ($n = 12$), OVX with a late alendronate treatment at 4 weeks after OVX ($n = 12$), sham-operated ($n = 12$), and normal controls ($n = 12$). All animal procedures were approved by the institutional animal care and use committee in accordance with the guidelines of the National Institutes of Health.

Bilateral OVX were performed under general anaesthesia as described previously.[11] For local administration of alendronate (Novartis Pharma Stein AG, Switzerland), a small incision was made in the skin between the eye and ear and the location of the articular capsules of the TMJ. A needle was then inserted from a posterosuperior direction into the inferior joint cavity, so that the disc was avoided.[17] Alendronate was injected on the day of OVX for the early alendronate treatment group or at week 4 after OVX for the late alendronate treatment group. Subsequent injections were administered weekly for 4 weeks at a dosage of 0.1 mg alendronate in 0.02 ml saline solution (5 mg/ml). We chose week 4 after ovariectomy to define early vs. late intervention, because previous studies have shown significant osteoporosis-like changes in the condyle by this time-point.[4] The dose of alendronate was chosen based on the local effective concentration established in a previous in vivo preliminary study, in which local delivery of 1 mg/ml alendronate gel significantly improved bone fill compared with placebo gel in patients with perodontitis.[18] In the present study, we set the concentration at 5 mg/ml, five times higher than the local effective concentration, because of the potential drug dilution within the articular cavity. The rats in the sham-operated and the OVX/vehicle groups were injected with an equivalent volume of saline. Six animals per group were sacrificed at week 10 and week 16 after OVX, respectively (Fig. 1).

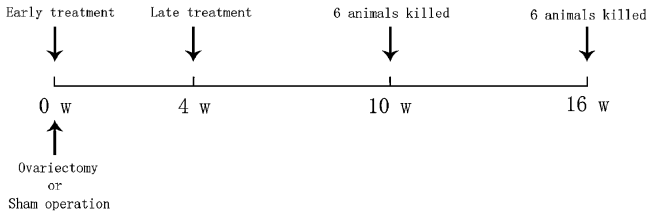

Fig. 1. Experimental design. Early alendronate treatment was started on the day of ovariectomy, while late treatment was started at 4 weeks after the ovariectomy. Six animals in each group were killed at 10 and 16 weeks after ovariectomy, respectively.

Micro-computed tomography (micro-CT)

The condyles were scanned using a micro-CT system (μ-CT 80 Scanner; Scanco Medical, Brüttisellen, Switzerland). The system was set to 55 kV, 145 mA, and 500 ms integration time. The binary images obtained with a resolution of 1024 × 1024 pixels and isotropic voxel size of 18 μm were reconstructed to three dimensions for qualitative and quantitative evaluations (threshold 250–700). Two regions were defined as the sampling sites: (1) a 'subchondral region', comprising the region connected to cartilage, and (2) a 'central region', comprising the region beneath the former (Fig. 2A).[3] The following parameters were calculated to describe the bone structure: bone volume ratio (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp).

Histology and tartrate-resistant acid phosphatase (TRAP) staining

The condyles were paraffin-embedded and every fifth slide was stained with haematoxylin and eosin (H&E) or TRAP (Sigma–Aldrich, St. Louis, MO, USA). The thickness of the cartilaginous layer of the condyle was measured (Fig. 3A and B).[19] TRAP-positive cells were counted in the subchondral bone region or the central bone region of three serial sections per sample.[7]

Immunohistochemistry

Primary antibody for matrix metalloproteinase (MMP)-13 was purchased from Biovision (Milpitas, CA, USA). Immunohistochemical staining was performed according to the manufacturer's instructions. Briefly, tissue sections were washed twice with phosphate-buffered saline (PBS) containing 0.3% Tween 20 for 1 h and then incubated with anti-MMP-13 polyclonal antibodies, followed by horseradish peroxidase-labelled secondary antibody (Dako, Carpinteria, CA, USA); the colour (brown) was developed using 0.5 mg/ml 3,3′-diaminobenzidine tetrahydrochloride. The same procedures were carried out without the primary antibody for negative controls.

Real-time quantitative polymerase chain reaction (QRT-PCR)

Total RNA was isolated from the subchondral bone using Trizol reagents (Invitrogen Life Technologies, Carlsbad, CA, USA). Reverse transcription was performed using the PrimeScript RT Reagent Kit (Takara Bio, Shiga, Japan) and the first-strand cDNA was synthesized using oligo(dT)15 primers (Takara Bio, Shiga, Japan). Primer