Protective Effect of Alendronate on Lumbar Facet Degeneration in Ovariectomized Rats

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ACDEF 1,2   Nan Zhang*
ACF 3   Faming Tian*
ABCDEF 1,4   Yu Gou*
BF 5   Tiangang Chen
BD 5   Qingfu Kong
BD 5   Qinglie Lv
BD 5   Hetong Li
AG 1,6   Liu Zhang

1 Department of Orthopedic Surgery, Hebei Medical University, Shijiazhuang, Hebei, P.R. China
2 Department of Orthopedic Surgery, Kailuan General Hospital, Tangshan, Hebei, P.R. China
3 Medical Research Center, North China University of Science and Technology, Tangshan, Hebei, P.R. China
4 Department of Orthopedic Surgery, Tianjin Hospital, Tianjin, P.R. China
5 Department of Orthopedic Surgery, The Affiliated Hospital of North China University of Science and Technology, Tangshan, Hebei, P.R. China
6 Department of Orthopedic Surgery, Meitan General Hospital, Beijing, P.R. China

Corresponding Author:
Source of support:

* Nan Zhang, Faming Tian, and Yu Gou contributed equally to this work
Liu Zhang, e-mail: zhliu130@sohu.com
The study was supported by grants from the National Natural Science Foundation of China (NSFC 31671235), the Major Program of Natural Science Foundation of Hebei Province (H2016209176), the Young Talent Support Foundation of Hebei Province, and the Scientific Innovation Team Foundation of Tangshan City (15130208C)

Background:
Facet joint degeneration (FJD) is a potential source of lower back pain, and estrogen deficiency can accelerate FJD. The present study aimed to investigate the effects of alendronate (ALN) on FJD induced by ovariectomy (OVX) in rats.

Material/Methods:
Thirty female Sprague-Dawley rats underwent either bilateral OVX (n=20) or sham surgery (n=10). The OVX rats subsequently received either subcutaneous ALN (70 μg/kg/week) or vehicle for 12 weeks. Subchondral bone mass and microarchitecture were evaluated by micro-computed tomography. Cartilage degradation was evaluated by toluidine blue staining and histological scoring.

Results:
Compared with the Sham group, the OVX group had significantly decreased bone mineral density, bone volume/trabecular volume, and trabecular thickness, significantly increased trabecular separation in subchondral bone, and significantly higher histological score for cartilage degeneration, particularly loss of cartilage thickness. ALN treatment significantly reversed the changes in subchondral bone, preserved cartilage thickness, and reduced the histological score. Immunohistochemical analyses showed significantly decreased expression of ADAMTS-4, MMP-13, and caspase-3 in the OVX+ALN group compared with the OVX group.

Conclusions:
Treatment with ALN suppressed bone loss, subchondral bone architecture deterioration, and cartilage degeneration in OVX rats, which can be explained by roles of ALN in preservation of subchondral bone mass and microarchitecture, and counteraction of catabolism and chondrocyte apoptosis in cartilage.

MeSH Keywords:
Alendronate • Cartilage • Models, Animal • Ovariectomy

Full-text PDF:
https://www.medscimonit.com/abstract/index/idArt/916978

🖹 2957   ▦ —   📊 6   📚 51

Background

Lower back pain is a major cause of activity limitation and work absence. Osteoarthritis (OA) is a whole-joint disease that affects the articular cartilage, subchondral bone, and synovium. Facet joints (FJs) and their corresponding intervertebral disc comprise a "three-joint complex" that constitutes a "spinal motion segment" [1]. Lumbar spinal FJs (LFJs) were first suggested as a source of lower back and lower-extremity pain in 1911. Since then, so-called "facetogenic back pain" has become a widely accepted, although still controversial, entity in the radiologic and orthopedic literature [2–9].

Facet joint OA accounts for 15–40% of chronic lower back pain cases, which are prevalent worldwide [10,11]. Many risk factors for LFJ arthritis have been identified, including age, sex, and body weight [10]. Among these, sex has been identified as an independent risk factor that contributes to LFJ degeneration, because the degeneration occurs more frequently in women [12,13]. Further studies demonstrated that the prevalence of LFJ arthritis increases dramatically in women at about age 50 years, which coincides with the onset of menopause [14]. In addition, the severity of LFJ arthritis is correlated with estrogen receptor expression in LFJ cartilage [15]. Thus, the cumulative evidence suggests that estrogen plays a pivotal role in regulating metabolic homeostasis in LFJs.

To date, FJ OA has received far less extensive research attention than the dominant phenotype, knee OA. Because there is no targeted pharmacological therapy, clinical management of FJ OA includes analgesic or surgical treatment. In knee OA, the conceptualization has shifted away from a predominant focus on cartilage degeneration toward a view of OA as a whole-joint failure resulting from an imbalance between the breakdown and repair of joint tissues, predominantly cartilage and subchondral bone [16]. Increased bone remodeling is considered a critical event in OA development; therefore, subchondral bone turnover has attracted research attention, with antiresorptive agents (such as bisphosphonates) being potentially disease-modifying therapies. Accordingly, agents with dual roles in modulating both cartilage and bone metabolism have potential as new drugs for OA treatment.

Bisphosphonate has been widely recognized worldwide as a first-line drug treatment for osteoporosis. Although some studies have shown that alendronate (ALN) has a protective effect on degenerative cartilage and subchondral bone in knee OA [17], the effect of ALN on LFJ degeneration has not been evaluated. Cartilage oligomeric matrix protein (COMP) is a non-collagenous extracellular matrix (ECM) protein that is mainly expressed in cartilage [18], and has emerged as a marker for cartilage turnover and joint destruction associated with OA, as well as a marker for the incidence and progression of knee

and hip degeneration [19,20]. The purpose of the present study was to investigate the effects of ALN on subchondral bone by detecting the bone mass and microarchitecture using micro-computed tomography (CT) and on cartilage degradation by histological and immunohistochemical analyses and serum levels of COMP in rats after ovariectomy (OVX).

Material and Methods

Animal model and treatment

Thirty 3-month-old female Sprague-Dawley rats (body weight, 265±14 g; Vital River Experimental Animal Technical Co., China) were used in the study. The rats were randomly assigned to undergo either a sham operation (Sham group; n=10) or bilateral OVX (n=20). The OVX rats were subcutaneously injected with either vehicle (OVX group; n=10) or 70 µg/kg/week ALN (Sigma-Aldrich, USA) [21] (OVX+ALN group; n=10), beginning immediately after OVX. Body weights were recorded weekly, and ALN doses were adjusted accordingly. During the whole experiment, all animals were kept at 21±1°C on a 12-hour/12-hour light/dark cycle. All rats were allowed free access to water and a maintenance diet (HFK Biotechnology Co., China). All cages contained wood shavings, bedding, and a cardboard tube for environmental enrichment. All experimental procedures in the present study were approved by the Institutional Animal Care and Use Committee of our institution (No. LX201809).

Surgical procedure

For OVX surgery, the rats were premedicated and given general anesthesia with isoflurane (2% in oxygen). The ovaries were accessed through a ventral abdominopelvic skin incision and exteriorized through the muscle wall on each side. Finally, the fascia and skin were closed. All animals were given a prophylactic antibiotic (Penicillin-G; 40,000 U) starting soon after surgery for 3 days.

Follow-up

All rats were euthanized at 12 weeks postoperatively. The L4–L5 segments of the spine were removed, and the muscles were dissected away from the bone. Blood samples were obtained after the animals were euthanized, and serum was separated by centrifugation and stored at -80°C. Specimens were fixed in formalin for micro-CT scanning, histologic examination, and histomorphometric and immunohistochemical analyses of the L4–L5 FJs.

Biomarker assays

An enzyme-linked immunosorbent assay (ELISA) kit (Cusabio Biotech Co., China) was used to assess the serum concentration

of COMP in accordance with the manufacturer's instructions. The data were collected using an iMARK Reader (Bio-Rad Laboratories, Inc., USA).

Micro-CT analysis

The L4–L5 FJs underwent micro-CT analysis of the subchondral bone mass and microarchitecture using SkyScan 1176 (Bruker, Belgium). The subchondral cancellous bone located under the subchondral bone plate was defined as the region of interest. Bone mineral density (BMD), trabecular separation (Tb.Sp), trabecular number (Tb.N), bone volume/trabecular volume (BV/TV), and trabecular thickness (Tb.Th) were calculated.

Histological assessments

The L4–L5 spine segments (including the FJs) were fixed in 10% formalin solution and then decalcified in 10% EDTA-2Na for 10 weeks, embedded in paraffin, and cut into 6-μm sections for toluidine blue staining and subsequent histological observation. The histological appearance of the right L4–L5 FJs was evaluated in accordance with the modified Mankin grading system (grade 0: intact surface; grade 1: surface fissures; grade 2: surface fissures to mid-zone; grade 3: surface fissures to deep zone; and grade 4: complete destruction) [22]. The cartilage thickness of the right L4–L5 FJs was also calculated. Histological scoring and cartilage thickness calculation were blindly performed by 2 independent researchers.

Immunohistochemical analysis

Immunohistochemical analyses were performed to detect the expression levels of aggrecan (1: 500; GeneTex, Inc., USA), collagen II (Col-II) (1: 100; II-II6B3 deposited in DSHB by T.F. Linsenmayer), caspase-3 (1: 200; Boster Co., China), matrix metalloproteinase (MMP)-13 (1: 200; GeneTex, Inc., USA), and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 (1: 200; Abcam, Inc., USA) in cartilage. Paraffin sections were deparaffinized with xylene and rehydrated with ethanol. After antigen retrieval with 0.05% trypsin and inactivation of endogenous peroxidases with 0.3% H_2O_2, the sections were incubated at 4°C overnight with the target protein antibody. The remaining experimental procedures were conducted in accordance with the protocols provided with the PV-6000 DAB detection kit and ZLI-9018 DAB kit (both from ZSGBBIO Corp., China), before being counterstained with hematoxylin. Images were captured at 100× magnification using a BX53 microscope (Olympus, Japan) and semiquantitatively analyzed with Image-Pro Plus version 6.0 software (Media Cybernetics, USA). The average optical density intensity of each protein, expressed as the integrated optical density/mm^2, was defined as the value of the integrated optical density divided by the cartilage area.
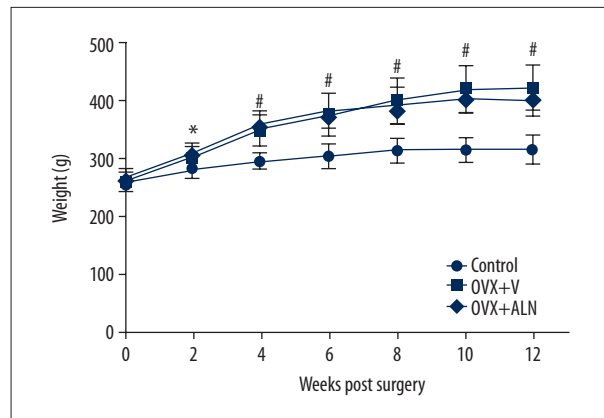

Figure 1. Comparison of body weights among the 3 groups. * P<0.05 *vs.* control group; # P<0.001 *vs.* control group.

Statistical analysis

All data were analyzed using SPSS 20.0 software (SPSS, Inc., USA) and presented as mean ± standard error of the mean. Differences between groups were compared by one-way analysis of variance followed by Fisher's least-significant difference test. Differences in modified Mankin scores between groups were analyzed by the Kruskal-Wallis H test, followed by the Dunn-Bonferroni post hoc test. Pearson correlation analysis was used to evaluate the correlation of serum COMP concentration with cartilage thickness. Two-tailed *P*-values of less than 0.05 were considered statistically significant.

Results

Body weight

The body weights showed no significant difference at baseline between any 2 groups, and increased over time in all groups. The body weights in the OVX group and OVX+ALN group were significantly higher than those in the Sham group at 2 weeks after surgery (*P*<0.05) and became more obviously different with increasing age (*P*<0.001), with no marked differences between the OVX group and OVX+ALN group (Figure 1).

Histological examination of articular cartilage

Toluidine blue staining showed no notable lesions in the cartilage surface in the Sham group: the chondrocyte structure was normal and the ECM was uniformly stained. The OVX group had obvious cartilage degeneration, characterized by surface irregularities and small fissures. After ALN administration, the degree of cartilage injury in OVX rats was alleviated. The OVX group had significantly higher modified Mankin scores than the Sham group and OVX+ALN group (all *P*<0.05).
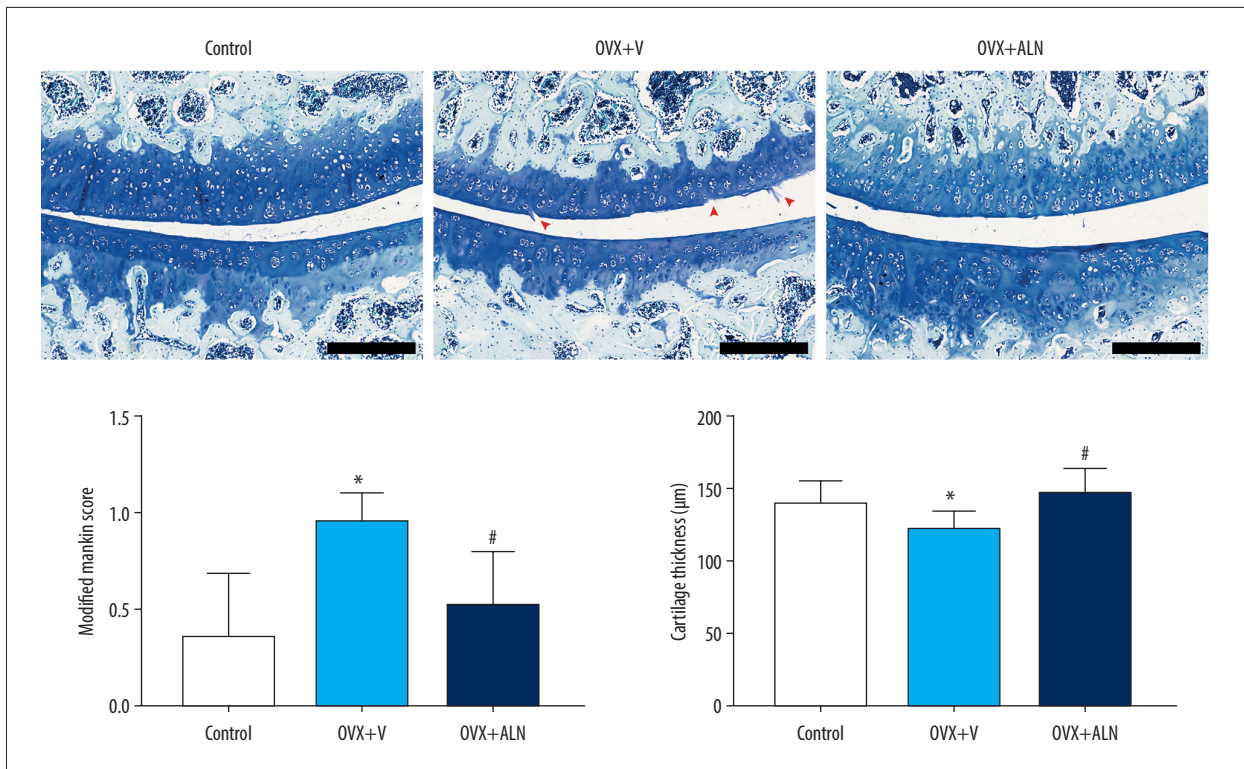
Figure 2. Histological charts of the right facet joints of L4–5 segments of the lumbar spine in different groups. The cartilage surface, the chondrocyte structure and the extracellular matrix (ECM) were observed by toluidine blue staining. The OVX+V group uses red arrows to indicate small fissures. Bar=100 mm. The lower left figure shows different groups modified Mankin score, * P<0.05 *vs.* control group; # P<0.05 *vs.* OVX+ALN group. The lower right is cartilage thickness. * P<0.05 *vs.* control group; # P<0.05 *vs.* OVX+ALN group.

There were no significant differences between the Sham group and OVX+ALN group (*P*>0.05) (Figure 2).

The cartilage was significantly thinner in the OVX group than in the Sham group (*P*<0.05). The cartilage was significantly thicker in the OVX+ALN group than in the OVX group (*P*<0.001), but did not differ significantly between the OVX+ALN group and Sham group (*P*>0.05) (Figure 2).

Immunohistochemical assessments

Compared with the Sham group, the OVX group had significantly lower expression of aggrecan (*P*<0.01), but significantly higher expression of ADAMTS-4, MMP-13, and caspase-3 (all *P*<0.001). Compared with the OVX group, the OVX+ALN group had significantly lower expression of ADAMTS-4, MMP-13, and caspase-3 (all *P*<0.001), but significantly higher expression of aggrecan (*P*<0.05) (Figure 3).

Biomarker analysis

The OVX group had significantly higher serum COMP concentrations than the Sham group (*P*<0.01). However, there were

no significant differences in serum COMP concentrations between the OVX+ALN group and OVX group or Sham group (all *P*>0.05) (Figure 4).

Micro-CT analysis of subchondral bone

Compared with the Sham group, the OVX group had significantly lower BMD (*P*<0.001), BV/TV (*P*<0.001), and Tb.Th (*P*<0.05), but significantly higher Tb.Sp (*P*<0.001). Compared with the OVX group, the OVX+ALN group had significantly higher BMD, BV/TV, and Tb.Th, but significantly lower Tb.Sp (all *P*<0.001) (Figure 5).

Correlation analysis

The serum COMP concentration was significantly negatively correlated with cartilage thickness (r=-0.380, *P*=0.038) (Figure 6).

Discussion

In the present study, we examined the role of estrogen deficiency-induced subchondral bone deterioration in FJ cartilage
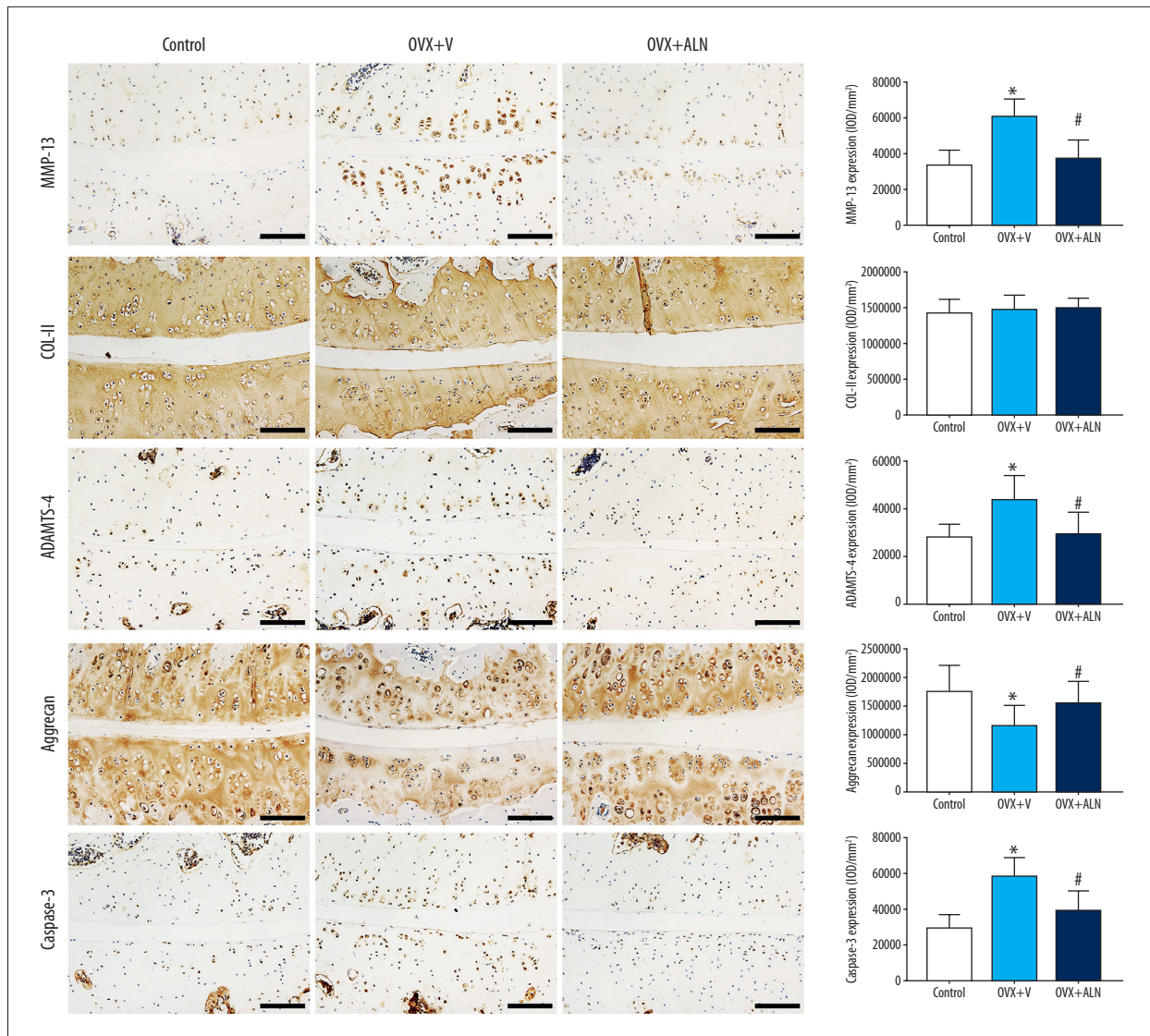
Figure 3. Immunohistochemistry assay for the expression levels and IOD values of COL-II, ADAMTS-4, MMP-13, caspase-3 and Aggrecan in different groups. * $P<0.001$ *vs.* control group; # $P<0.001$ *vs.* OVX+ALN group. Bars=100 um.
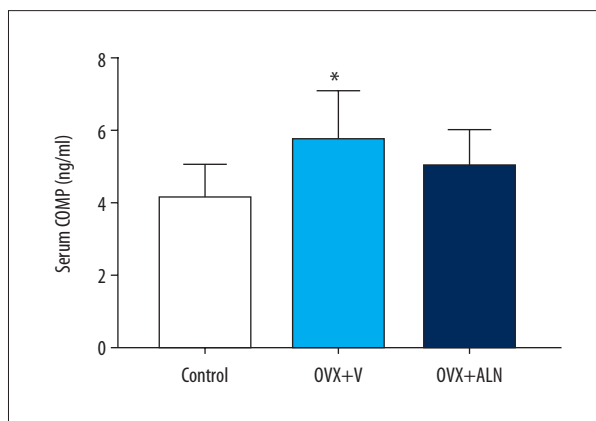

Figure 4. The serum COMP in different group. * $P<0.05$ *vs.* control group.

degeneration, and further evaluated the effect of ALN in this model, focusing mainly on the morphology and molecular changes in both the subchondral bone and cartilage. We first demonstrated that estrogen deficiency induced by OVX led to osteopenia in subchondral bone and cartilage degeneration. Furthermore, ALN treatment retarded these degenerative changes by preventing deterioration of the subchondral bone microarchitecture and inhibiting catabolic metabolism in cartilage.

Bilateral OVX animals are widely used as models of postmenopausal osteoporosis [23], in which bone turnover is abnormally increased. Emerging evidence from clinical and animal studies indicates that abnormal subchondral bone remodeling and subsequent deterioration of the bone microarchitecture and quality greatly contributes to degeneration of the
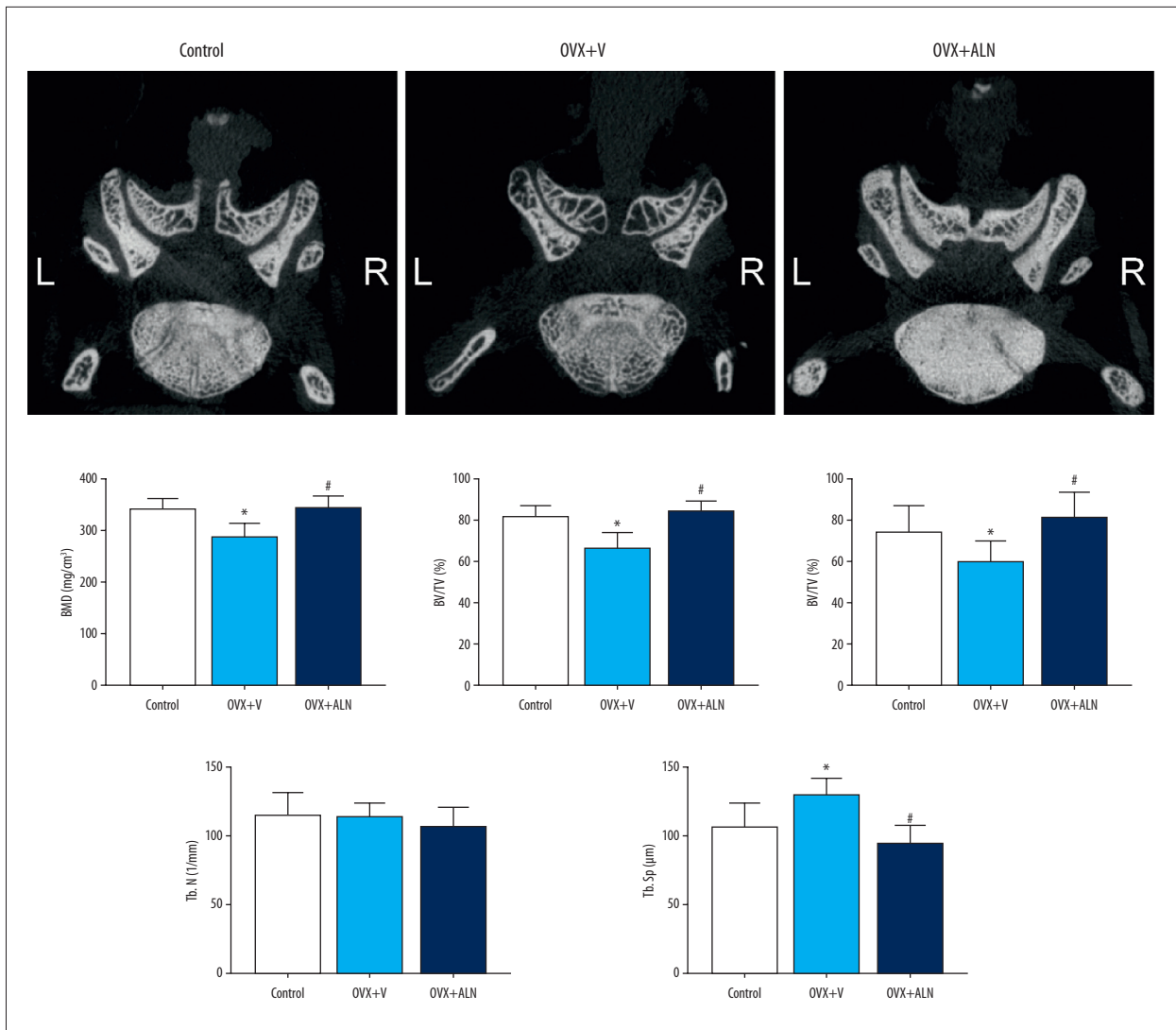
Figure 5. Micro-CT analysis of subchondral bone in different group. * P<0.001 *vs.* control group. # P<0.001 *vs.* OVX+ALN group.

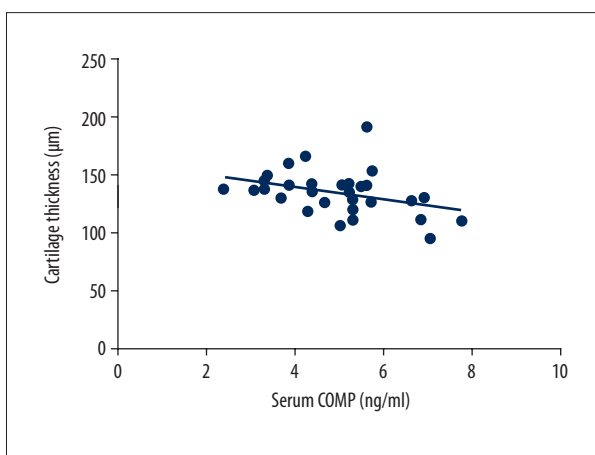**Figure 6.** The serum COMP concentration was significantly negatively correlated with cartilage thickness (r=−0.380, P=0.038).

anatomically adjacent cartilage [24,25]. In the present study, at 12 weeks postoperatively, OVX rats had significantly lower BMD, BV/TV, and Tb.Th, and significantly greater Tb.Sp in subchondral bone compared with those who underwent sham surgery. These findings demonstrate that activated bone remodeling and deterioration of the subchondral bone architecture, which are considered to change the biomechanical properties of subchondral bone and thereby alter its ability to absorb stress from cartilage and even transmit aberrant stress to articular cartilage, finally lead to cartilage deterioration, which in turn transmits more loading to the subchondral bone, resulting in whole-joint degeneration as OA [26,27]. Interestingly, prior induction of osteoporosis by OVX increased the severity of cartilage damage in an experimental OA rabbit model, and cartilage damage was positively correlated with bone loss [28]. Further evidence suggests that estrogen deficiency not only leads to enhanced bone remodeling, and thus bone loss and

deterioration of biomechanical properties, but also activates catabolism in cartilage [29,30].

In line with the abovementioned studies, the histological data in the present study demonstrated that OVX-induced catabolic metabolism in cartilage in rat FJs led to histological changes suggestive of OA, such as much thinner cartilage compared with the Sham group. Immunohistochemical analyses provided more evidence for the effect of OVX on cartilage metabolism. MMP-13 and ADAMTS-4 are important matrix-degrading enzymes that play major roles in cartilage turnover and degradation of Col-II and aggrecan [31–34]. Caspase-3 is an apoptotic executive protease [35], and is increased in chondrocyte apoptosis [36]. Our results demonstrated that OVX rats had significantly increased expression of MMP-13, ADAMTS-4, and caspase-3, and significantly decreased expression of aggrecan compared with the Sham group. However, it is interesting that the degenerative cartilage changes in OVX rats were mainly manifested as small fissures confined to the surface, and did not extend to the deeper cartilage, indicating relatively modest cartilage lesions compared with those secondary to mechanical instability or chemical stimulation [37–39]. Our findings are consistent with previous studies, in which the main feature of FJ cartilage degeneration was loss of thickness, but not damage to superficial cartilage [40,41], thereby supporting the hypothesis that estrogen can prevent cartilage loss. Based on the present findings and those from previous studies, we conclude that estrogen deficiency stimulated subchondral bone remodeling with resultant deterioration of the subchondral bone microarchitecture and thus impaired the biomechanical properties, and also led to high cartilage turnover with increased chondrocyte apoptosis and catabolic metabolism, which finally caused the cartilage degeneration in this model.

The important role of the subchondral bone in OA development enlarges the window for screening of new agents for its treatment, particularly for subtypes with obviously abnormal bone remodeling activity [42]. ALN is widely used for osteoporosis treatment as a bone resorption-inhibiting agent, while accumulating evidence from a series of animal studies supports the potential protective effects of ALN in OA [43–45]. Our previous study evaluated the effects of ALN on subchondral bone quality and cartilage degeneration in an anterior cruciate ligament transection-induced rabbit model of knee OA, and proved that ALN plays an important role in cartilage protection in OA joints by inhibiting subchondral bone resorption and thereby improving subchondral bone quality [46]. In the OVX rat model, ALN treatment inhibited both subchondral bone loss and cartilage degeneration in the knee joints [47]. However, to date, no studies have shown the effect of ALN on FJ degeneration in OVX rats. As expected, the micro-CT results in the present study demonstrated that ALN treatment completely prevented the bone loss and microarchitecture deterioration of

the subchondral bone. Furthermore, the BMD in the OVX+ALN group was comparable to that in the Sham group, and significantly higher than that in the OVX group. These results are consistent with previous studies [46,47]. In addition, the parameters for the subchondral bone structure further proved the preservation effect of ALN in this model. The decreased BV/TV and Tb.Th and increased Tb.Sp in OVX rats were completely retarded by ALN treatment, which reversed these changes to normal levels because no differences were observed between the OVX+ALN group and Sham group.

A previous study showed that deterioration in subchondral bone (low BV/TV and Tb.Th, high Tb.Sp, and rod-like structure) occurs before the onset of cartilage degeneration [48]. Thus, we speculate that ALN protects the subchondral bone against resorption and fragility, and thereby preserves its structure and biomechanical properties, finally contributing to the beneficial effects of ALN on cartilage in this OVX rat model, because the histological cartilage analyses showed that ALN treatment prevented the morphological changes in FJ cartilage in OVX rats. In particular, ALN significantly inhibited the loss of cartilage thickness. Another mechanism by which ALN protects cartilage is inhibition of the cartilage degradation accelerated by prostaglandins, leukotrienes, and certain growth factors produced by osteoblasts during subchondral bone remodeling [49].

The immunohistochemical analyses further explored the mechanism for ALN protection of FJ cartilage in OVX rats, and revealed that intervention with ALN significantly decreased the expression levels of MMP-13, ADAMTS-4, and caspase-3 in the FJ cartilage of OVX rats, and significantly increased the expression of aggrecan. These findings indicate that ALN positively affects the maintenance of the balance between cartilage ECM metabolism and inhibition of chondrocyte apoptosis in FJ OA. Stimulation of chondrocyte proliferation is another positive function of ALN [50]. Although the exact details of the mechanism are unclear, this function may be associated with the direct or indirect effects of ALN on the OPG/RANKL pathway [51]. Therefore, the direct effects of ALN in maintaining the structural integrity of cartilage and inhibiting the loss of cartilage thickness may be attributed to its protection of cartilage chondrocytes and ECM metabolism.

Notably, we started the ALN treatment immediately after OVX, and thus it remains unclear whether ALN treatment would still be effective if it was commenced after cartilage degeneration had occurred. One previous study that tested the effects of ALN on monosodium iodoacetate-induced knee OA in rats using different intervention time-points found that ALN treatment started at study initiation was beneficial for both bone and cartilage, while ALN treatment started at 2 or 6 weeks after study initiation showed protective effects on bone but not cartilage [17]. Therefore, the timing of ALN treatment initiation

is crucial for treatment of OA. It is clinically more beneficial to protect both the subchondral bone and cartilage in OA.

Our previous study demonstrated that COMP is also a bio-marker for FJ degeneration in OVX rats [41]. The present study confirmed that the serum COMP concentration was negatively correlated with cartilage thickness. However, an unexpected result was that the COMP level in the OVX+ALN group did not differ significantly from that in the OVX group or Sham group, and this may be because ALN partially prevented the COMP increase in OVX rats. Further studies with more follow-up time-points are needed to provide more information with which to clarify this phenomenon.

Conclusions

In summary, OVX resulted in overactive subchondral bone re-modeling and cartilage catabolism, finally causing the development of OA. ALN treatment at the dosage and time-points used in the present study suppressed the degenerative changes in both the subchondral bone and cartilage and inhibited chon-drocyte apoptosis. All of these factors contributed to the pre-ventive effects of ALN on OA in this OVX rat model.

Acknowledgments

References:

1. Gellhorn AC, Katz JN, Suri P: Osteoarthritis of the spine: the facet joints. Nat Rev Rheumatol, 2013; 9: 216–24

2. Lewinnek GE, Warfield CA: Facet joint degeneration as a cause of low back pain. Clin Orthop, 1986; 213: 216–22

3. Helbig T, Lee CK: The lumbar facet syndrome. Spine, 1988; 13: 61–64

4. Carrera GF, Haughton VM, Syvertsen A et al: Computed tomography of the facet joints. Radiology, 1980; 134: 145–48

5. Raskin SP: Degenerative changes of the lumbar spine: Assessment by computed tomography. Orthopedics, 1981; 4: 186–95

6. Nachemson AL: Newest knowledge of low-back pain: A critical look. Clin Orthop, 1992; 179: 8–20

7. Schwarzer AC, Aprill C, Derby R et al: Clinical features of patients with pain stemming from the lumbar zygapophyseal joints. Is the lumbar facet syndrome a clinical entity? Spine, 1994; 10: 1132–37

8. Manchkanti L, Pampati V, Fellows B et al: Prevalence of facet joint pain in chronic low back pain. Pain Physician, 1999; 2: 59–64

9. Manchikanti L, Pampati RR, Fellows B et al: The diagnostic validity and ther-apeutic value of medial branch blocks with or without adjuvants. Curr Rev Pain, 2000; 4: 337–44

10. Gellhorn AC, Katz JN, Suri P et al: Osteoarthritis of the spine: The facet joints. Nat Rev Rheumatol, 2013; 9: 216–24

11. Dreyer SJ, Dreyfuss PH: Low back pain and the zygapophysial (facet) joints. Arch Phys Med Rehabil, 1996; 77: 290–300

12. Suri P, Miyakoshi A, Hunter DJ et al: Does lumbar spinal degeneration be-gin with the anterior structures? A study of the observed epidemiology in a community-based population. BMC Musculoskelet Disord, 2011; 12: 202

13. Goode AP, Marshall SW, Renner JB et al: Lumbar spine radiographic fea-tures and demographic, clinical, and radiographic knee, hip, and hand os-teoarthritis. Arthritis Care Res (Hoboken), 2012; 64: 1536–44

14. Sniekers YH, Weinans H, Bierma-Zeinstra SM et al: Animal models for os-teoarthritis: the effect of ovariectomy and estrogen treatment a system-atic approach. Osteoarthr Cartil, 2008; 16: 533–41

15. Ha KY, Chang CH, Kim KW et al: Expression of estrogen receptor of the fac-et joints in degenerative spondylolisthesis. Spine, 2005; 30: 562–66

16. Hunter DJ, Felson DT: Osteoarthritis. BMJ, 2006; 332: 639–42

17. Mohan G, Perilli E, Parkinson IH et al: Pre-emptive, early, and delayed alen-dronate treatment in a rat model of knee osteoarthritis: Effect on subchon-dral trabecular bone microarchitecture and cartilage degradation of the tib-ia, bone/cartilage turnover, and joint discomfort. Osteoarthritis Cartilage, 2013; 21: 1595–604

18. Hecht JT, Deere M, Putnam E et al: Characterization of cartilage oligomeric matrix protein (COMP) in human normal and pseudoachondroplasia mus-culoskeletal tissues. Matrix Biol, 1998; 17: 269–78

19. Sharif M, Kirwan JR, Elson CJ et al: Suggestion of nonlinear or phasic pro-gression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. Arthritis Rheum, 2004; 50: 2479–88

20. Mobasheri A, Bay-Jensen AC, van Spil WE et al: Osteoarthritis year in review 2016: Biomarkers (biochemical markers). Osteoarthritis Cartilage, 2017; 25: 199–208

21. Chen H, Zhu H, Zhang K et al: Estrogen deficiency accelerates lumbar fac-et joints arthritis. Sci Rep, 2017; 7: 1379

22. Wu T, Ni S, Cao Y et al: Three-dimensional visualization and pathologic char-acteristics of cartilage and subchondral bone changes in the lumbar facet joint of an ovariectomized mouse model. Spine J, 2018; 18: 63–73

23. Komori T: Animal models for osteoporosis. Eur J Pharmacol, 2015; 759: 287–94

24. Aso K, Shahtaheri SM, Hill R et al: Associations of symptomatic knee OA with histopathologic features in subchondral bone. Arthritis Rheumatol, 2019; 71(6): 916–24

25. Hayami T, Pickarski M, Zhuo Y et al: Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament tran-section and meniscectomized models of osteoarthritis. Bone, 2006; 38: 234–43

26. Herrero-Beaumont G, Roman-Blas JA, Largo R et al: Bone mineral density and joint cartilage: Four clinical settings of a complex relationship in os-teoarthritis. Ann Rheum Dis, 2011; 70: 1523–25

27. Li G, Yin J, Gao J et al: Subchondral bone in osteoarthritis: Insight into risk factors and microstructural changes. Arthritis Res Ther, 2013; 15: 223

28. Calvo E, Castañeda S, Largo R et al: Osteoporosis increases the severity of cartilage damage in an experimental model of osteoarthritis in rabbits. Osteoarthritis Cartilage, 2007; 15: 69–77

29. Li S, Niu G, Wu Y et al: Vitamin D prevents articular cartilage erosion by regulating collagen II turnover through TGF-β1 in ovariectomized rats. Osteoarthritis Cartilage, 2016; 24: 345–53

30. Zhou J, Zhong P, Liao Y et al: Electroacupuncture ameliorates subchondral bone deterioration and inhibits cartilage degeneration in ovariectomised rats. Acupunct Med, 2018; 36: 37–43

31. Ismail HM, Miotla-Zarebska J, Troeberg L et al: Brief report: JNK-2 controls aggrecan degradation in murine articular cartilage and the development of experimental osteoarthritis. Arthritis Rheumatol, 2016; 68: 1165–71

32. Kiani C, Chen L, Wu YJ et al: Structure and function of aggrecan. Cell Res, 2002; 12: 19–32

33. Li H, Wang D, Yuan Y et al: New insights on the MMP-13 regulatory net-work in the pathogenesis of early osteoarthritis. Arthritis Res Ther, 2017; 19: 248

34. Mobasheri A, Bay-Jensen AC, van Spil WE et al: Osteoarthritis year in review 2016: Biomarkers (biochemical markers). Osteoarthritis Cartilage, 2017; 25: 199–208

35. Xue H, Tu Y, Ma T et al: Lactoferrin inhibits IL-1beta-induced chondrocyte apoptosis through AKT1-induced CREB1 activation. Cell Physiol Biochem, 2015; 36: 2456–65

36. Bao G, Xu L, Xu X et al: SGTB promotes the caspase-dependent apoptosis in chondrocytes of osteoarthritis. Inflammation, 2016; 39: 601–10

37. Cope PJ, Ourradi K, Li Y et al: Models of osteoarthritis: The good, the bad and the promising. Osteoarthritis Cartilage, 2019; 27: 230–39

38. Bagi CM, Berryman ER, Teo S et al: Oral administration of undenatured native chicken type II collagen (UC-II) diminished deterioration of articular cartilage in a rat model of osteoarthritis (OA). Osteoarthritis Cartilage, 2017; 25: 2080–90

39. Philpott HT, O'Brien M, McDougall JJ: Attenuation of early phase inflammation by cannabidiol prevents pain and nerve damage in rat osteoarthritis. Pain, 2017; 158: 2442–51

40. Chen H, Zhu H, Zhang K et al: Estrogen deficiency accelerates lumbar facet joints arthritis. Sci Rep, 2017; 7: 1379

41. Gou Y, Tian F, Kong Q et al: Salmon calcitonin attenuates degenerative changes in cartilage and subchondral bone in lumbar facet joint in an experimental rat model. Med Sci Monit, 2018; 24: 2849–57

42. Roman-Blas JA, Castaneda S, Largo R et al: An OA phenotype may obtain major benefit from bone-acting agents. Semin Arthritis Rheum, 2014; 43: 421–28

43. Acar N, Balkarli H, Soyuncu Y et al: The determination of apoptosis rates on articular cartilages of ovariectomized rats with and without alendronate treatment. Histol Histopathol, 2016; 31: 635–45

44. Khorasani MS, Diko S, Hsia AW et al: Effect of alendronate on post-traumatic osteoarthritis induced by anterior cruciate ligament rupture in mice. Arthritis Res Ther, 2015; 17: 30

45. Shirai T, Kobayashi M, Nishitani K et al: Chondroprotective effect of alendronate in a rabbit model of osteoarthritis. J Orthop Res, 2011; 29: 1572–77

46. Zhang L, Hu H, Tian F et al: Enhancement of subchondral bone quality by alendronate administration for the reduction of cartilage degeneration in the early phase of experimental osteoarthritis. Clin Exp Med, 2011; 11: 235–43

47. Zhu S, Chen K, Lan Y et al: Alendronate protects against articular cartilage erosion by inhibiting subchondral bone loss in ovariectomized rats. Bone, 2013; 53: 340–49

48. Muraoka T, Hagino H, Okano T et al: Role of subchondral bone in osteoarthritis development: A comparative study of two strains of guinea pigs with and without spontaneously occurring osteoarthritis. Arthritis Rheum, 2007; 56: 3366–74

49. Lajeunesse D, Reboul P: Subchondral bone in osteoarthritis: A biologic link with articular cartilage leading to abnormal remodeling. Curr Opin Rheumatol, 2003; 15: 628–33

50. Heino TJ, Chagin AS, Takigawa M et al: Effects of alendronate and pamidronate on cultured rat metatarsal bones: Failure to prevent dexamethasone-induced growth retardation. Bone, 2008; 42: 702–9

51. Chen K, Zhang N, Ding L et al: Early intra-articular injection of alendronate reduces cartilage changes and subchondral bone loss in rat temporomandibular joints after ovariectomy. Int J Oral Maxillofac Surg, 2014: 43: 996–1004