THE ROLE OF SUBCHONDRAL BONE REMODELING IN OSTEOARTHRITIS: ALENDRONATE REDUCES CARTILAGE DEGENERATION AND PREVENTS OSTEOPHYTE FORMATION IN THE RAT ANTERIOR CRUCIATE LIGAMENT TRANSECTION MODEL

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Introduction
Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation, subchondral bone sclerosis and osteophyte formation. Recent studies have demonstrated that an increase in subchondral bone turnover is accompanied by specific architectural changes in the trabecular bone in OA joints. Furthermore, epidemiological studies have demonstrated positive correlation between subchondral bone sclerosis and OA progression. These observations suggested a role for subchondral bone changes in the initiation and/or progression of OA, raising the possibility that early intervention that reduces subchondral bone remodeling might retard the progressive loss of articular cartilage. To investigate the potential contribution of bone turnover to OA progression, we evaluated the effect of Alendronate (ALN), a potent bone resorption inhibitor, on cartilage degradation and periarticular bone changes in the rat anterior cruciate ligament transection (ACLT) model of OA.

Methods
All procedures were approved by the Institutional Animal Care and Use Committee of Merck Res. Labs. Ninety-five 20-week old male Sprague-Dawley rats were used for the following study. The rats underwent anterior cruciate ligament transection (ACLT) or sham-operation in the right knee. Rats were divided into 5 groups, ACLT receiving vehicle (ACLT+V, n=12), ACLT with low dose ALN (0.03 mg/kg/wk, ACLT+L, n=12), ACLT with high dose ALN treatment (0.24 mg/kg/wk, ACLT+H, n=12), sham-operated with vehicle (Sham+V, n=12), and sham-operated with high dose ALN (Sham+H, n=12). Rats were sacrificed at 2- and 10-week post-surgery. At both time points, the rats were injected with calcine in the left knee 3 days before necropsy. Another thirty-five rats were used for TGF-β assays and urine collection. These rats were divided into 4 groups, ACLT+V, ACLT+H, Sham+V, and Sham+H and were sacrificed 2-wk post-surgery. After disarticulation, femora were evaluated for incidence of osteophyte formation by gross morphology. Tibiae were cut in half at the center of articular surface along with medial collateral ligament in frontal section with a band saw. Anterior parts were embedded in paraffin for toluidine blue-O and immunohistochemistry and posterior parts were embedded in methylmethacrylate for Masson’s trichrome staining for further bone histomorphometry. Semi-quantitative histopathological grading (modified Mankin criteria) was scored from 3 sections at 100µm apart. To evaluate subchondral bone changes, a macro using Image Pro plus was developed to specifically measure a 600 by 800 µm rectangle area in subchondral region of tibial plateau. Osteophyte surface area was measured in Masson’s trichrome stained sections by manual tracing. We evaluated vascular invasion into calcified cartilage and osteoclast number in subchondral and osteophyte regions. We also determined the levels of serum cartilage oligomeric matrix protein (COMP), urinary C-terminal telopeptide type II collagen (CTX-II) and C-terminal telopeptide type I collagen (CTX-I). Localization of active TGF-β, MMP-13, and MMP-9 in tissue sections were evaluated by immunohistochemical methods. To investigate the potential involvement of TGF-β in the inhibition of osteophyte formation by ALN, active TGF-β levels were quantitated in the supernatants from tibial plateaus and patellae organ cultures with/without ALN treatment using Mink Lung epithelial cell growth inhibition assay.

Results
ALN had significant chondroprotective effects on the ACLT-joints. ALN partially reduced the histological Mankin score (p<0.05, at 0.24 mg/kg/wk) of cartilage damage during OA progression, and suppressed the elevated levels of two specific markers associated with cartilage damage, serum COMP (p<0.05) and urinary CTX-II (p<0.001). Subchondral bone resorption was markedly increased in 2-wk post-surgery and formation was higher in 10-wk post-surgery in the tibial plateau of non-treated ACLT, by comparison to sham-operated joints. ALN at both doses effectively inhibited the ACLT-induced subchondral bone remodeling, as determined by histomorphometry. ALN also markedly reduced the levels of CTX-I in both Sham-operated and ACLT-groups, indicating that ALN inhibited the increased rate of bone turnover in these animals. ALN suppresses vascular penetration into calcified cartilage in subchondral region and also blocked recruitment of TRAP positive cells to calcified cartilage and osteophyte regions. We observed that ALN dose-dependently inhibited osteophyte formation as determined by osteophyte scoring (p<0.05) and osteophyte area (p<0.01). Furthermore, elevated level of active TGF-β was released from ACLT+V patellae relative to sham-operated tibiae and this increase was suppressed by ALN treatment.

Discussion
Regarding cartilage protection, bisphosphonates have been reported to reduce cartilage degradation in collagen- or adjuvant-induced arthritis. Recently, zoledronate was reported to be partially chondroprotective in carrageenan-induced inflammatory arthritis and chymopapain-induced cartilage damage in rabbits. Our results strongly support the role of subchondral bone remodeling in OA pathogenesis in the surgically-induced OA model. We demonstrated that pharmacological inhibition of bone turnover had disease-modifying effects on the osteoarthritic joints, protecting articular cartilage deterioration and preventing osteophyte formation in this model. Although ALN at low dose is sufficient to inhibit subchondral bone remodeling and to partially protect the cartilage, it appears that a higher ALN dose is required to achieve optimal inhibition of cartilage matrix degradation and endochondral osteophyte formation. Recently, in postmenopausal women, ALN treatment (20 mg daily) rapidly suppressed urinary CTX-II level to 50 % of base line (1). Of note the lower 10 mg human osteoporosis dose, which had no significant effect on CTX-II, suffices to suppress CTX-I in these patients (1). The demonstration that treatment with ALN can slow down the progression of OA in ACLT-joints raises the possibility that antiresorptive agents may have therapeutic benefits for OA in humans.

References

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