Effect of Alendronate on Development of Post-Traumatic Osteoarthritis Induced by Non-Invasive Joint Injury in Mice

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Disclosures:

Introduction: During the development of osteoarthritis (OA), subchondral bone undergoes considerable changes in structure and strength prior to the onset of cartilage damage. With this in mind, bone anti-resorptive agents such as bisphosphonates have been investigated for possible mitigating effects on the progression of OA. The results thus far have been promising, showing a strong bone turnover-inhibiting capability. Additionally, bisphosphonates have been shown to have chondroprotective effects, either by downregulating Matrix Metalloproteinase 13 (MMP13) in subchondral bone, MMP9 in cartilage in order to inhibit Transforming Growth Factor Beta (TGFβ), or by preventing angiogenesis [1]. Unlike other bisphosphonates, however, alendronate (ALN) has also shown the ability to hinder osteophyte formation in a dose-dependent manner [1]. Previous studies investigating the use of ALN to inhibit OA development are limited because they used invasive or non-physiologic methods to induce OA development, and/or they did not investigate early time points during which bone is rapidly turned over prior to the onset of cartilage damage. The current study utilized a previously described non-invasive model of joint injury in mice [2] to investigate post-traumatic osteoarthritis (PTOA) in its early stages, and if ALN would reduce subchondral bone turnover and OA development. We hypothesized that ALN treatment would inhibit early trabecular bone loss from the epiphysis, and would decrease long-term osteophyte formation and cartilage degeneration after joint injury.

Methods: Non-invasive, unilateral ACL injury via tibial compression overload was performed on 54 mice (female C57BL/6, 10 weeks old at the time of injury) as previously described [2]. Another 36 mice were subjected to sham injury. Mice were treated subcutaneously with low-dose ALN (0.08 mg/kg/week), high-dose ALN (2.0 mg/kg/week), or vehicle, starting immediately after injury and lasting until sacrifice at 7, 14 or 56 days. Blood was collected immediately prior to sacrifice, and serum was analyzed for cross-linked C-terminal telopeptide of type I collagen (CTX-I), a biomarker of bone resorption, and procollagen type 1 N-terminal propeptide (P1NP), a biomarker of bone formation. Whole joints were scanned using micro-computed tomography (μCT), and structural analysis was performed on the trabecular bone of the femoral epiphysis. Trabecular bone volume per total volume (BV/TV), trabecular thickness (Tb.Th), bone mineral density (BMD; mg Hydroxyapatite/cm3 TV), and other trabecular bone parameters were directly measured using the manufacturer’s analysis tools. Osteophyte volume was also measured for 56 day samples, and included all mineralized tissue in and around the joint space, excluding naturally ossified structures (patella, fabella, and menisci). Following μCT analysis, whole-joint histology was performed on knees to analyze degeneration of articular cartilage. Knees were decalcified for 4 days in 10% buffered formic acid and processed for standard paraffin embedding. Sagittal 6 micron sections were cut across the medial aspect of the joint, separated by 250 microns (4 sections for each bone). All sections were stained with Safranin-O and Fast Green. Blinded slides were graded independently by three readers using the osteoarthritis research society international (OARSI) scale. For all outcomes, data were analyzed at each time point using a 2-way ANOVA stratified by injury status and treatment.

Results: μCT analysis revealed significant loss of trabecular bone from the femoral epiphysis due to injury by 7 and 14 days post-injury, consistent with our previous study [2]. This loss of epiphysial trabecular bone was not inhibited by low-dose ALN treatment, however high-dose ALN treatment was able to fully prevent the initial loss of trabecular bone associated with knee injury (Fig. 1a-b). By 56 days post-injury, vehicle treated mice still exhibited a deficit in trabecular bone volume in the injured knee compared to the uninjured knee. However, both low-dose and high-dose ALN treatment were able to maintain trabecular bone volume at this time point. A systemic increase in trabecular bone volume due to ALN treatment was also observed at the 56 day time point. Contrary to previously reported results, ALN treatment was not able to reduce osteophyte volume at 56 days post-injury (Fig. 1c). We observed no significant differences in osteophyte volume between treatment groups, with a trend toward greater osteophyte volume in ALN treated mice. Whole-joint histology scores of tibial and femoral articular cartilage revealed considerable degeneration in all injured knees, with no differences in OA development due to ALN treatment (Fig. 1d). Consistent with our previous study [3], we observed nearly complete degeneration of articular cartilage from both the tibia and femur, with the greatest degeneration occurring at the posterior tibia. Analysis of serum biomarkers revealed a nearly 5-fold increase in CTX-I (bone resorption) induced by joint injury by 14 days post-injury (Fig. 1e). ALN treatment was able to significantly reduce this bone resorption at early time points, although CTX-I levels remained higher than in uninjured mice. By 56 days post-injury, serum CTX-I levels were not significantly higher in injured mice compared to uninjured mice, although a
trend remained toward greater bone resorption in these mice (Fig. 1f). We observed no changes in P1NP levels (bone formation) due to injury or ALN treatment at any time point.

**Discussion:** Consistent with our initial hypothesis, alendronate treatment was able to prevent early trabecular bone loss following non-invasive joint injury, particularly at high dose. However, contrary to our hypothesis, neither low- nor high-dose alendronate treatments were able to prevent osteophyte formation, nor were they able to affect long-term articular cartilage loss or joint degeneration. These results are in contrast to previous studies, which showed that ALN treatment was chondroprotective and inhibited osteophyte formation. Our results also suggest that subchondral bone changes initiated by joint injury are due to increased bone resorption, with little change in bone formation at any of the time points quantified.

**Significance:** Altogether, these data contribute to understanding the role of bone in osteoarthritis development, and call into question the efficacy of using anti-resorptive agents for slowing or preventing osteoarthritis. It is possible that it may be more effective to target the underlying pathways activated by injury that lead to bone resorption, rather than targeting the process of bone resorption alone.

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**References:**
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