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Inhibited osteoclastic bone resorption through alendronate treatment in rats reduces severe osteoarthritis progression

M. Siebelt [a,*], J.H. Waarsing [a], H.C. Groen [b], C. Müller [c], S.J. Koelewijn [b], E. de Blois [b], J.A.N. Verhaar [a], M. de Jong [b,e], H. Weinans [d,f]

[a] Department of Orthopaedics, Erasmus University Medical Center, Rotterdam, The Netherlands
[b] Department of Nuclear Medicine, Erasmus University Medical Center, The Netherlands
[c] Center for Radiopharmaceutical Sciences ETH-PSI-USZ, Paul Scherrer Institute, Villigen-PSI, Switzerland
[d] Department of Biomechanical Engineering, Delft University of Technology, Delft, The Netherlands
[e] Department of Radiology, Erasmus University Medical Center, The Netherlands
[f] Dept. Orthopaedics & Dept. Rheumatology, UMC Utrecht, The Netherlands

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ABSTRACT

Osteoarthritis (OA) is a non-rheumatoid joint disease characterized by progressive degeneration of extra-cellular cartilage matrix (ECM), enhanced subchondral bone remodeling, osteophyte formation and synovial thickening. Alendronate (ALN) is a potent inhibitor of osteoclastic bone resorption and results in reduced bone remodeling. This study investigated the effects of pre-emptive use of ALN on OA related osteoclastic subchondral bone resorption in an *in vivo* rat model for severe OA. Using multi-modality imaging we measured effects of ALN treatment within cartilage and synovium. Severe osteoarthritis was induced in left rat knees using papain injections in combination with a moderate running protocol. Twenty rats were treated with subcutaneous ALN injections and compared to twenty untreated controls. Animals were longitudinally monitored for 12 weeks with *in vivo* μCT to measure subchondral bone changes and SPECT/CT to determine synovial macrophage activation using a folate-based radiotracer. Articular cartilage was analyzed at 6 and 12 weeks with *ex vivo* contrast enhanced μCT and histology to measure sulfated-glycosaminoglycan (sGAG) content and cartilage thickness.
ALN treatment successfully inhibited subchondral bone remodeling. As a result we found less subchondral plate porosity and reduced osteophytosis. ALN treatment did not reduce subchondral sclerosis. However, after the OA induction phase, ALN treatment protected cartilage ECM from degradation and reduced synovial macrophage activation. Surprisingly, ALN treatment also improved sGAG content of tibia cartilage in healthy joints. Our data was consistent with the hypothesis that osteoclastic bone resorption might play an important role in OA and may be a driving force for progression of the disease. However, our study suggest that this effect might not solely be effects on osteoclastic activity, since ALN treatment also influenced macrophage functioning. Additionally, ALN treatment and physical activity exercised a positive effect in healthy control joints, which increased cartilage sGAG content. More research on this topic might lead to novel insights as to improve cartilage quality.

Introduction

Osteoarthritis (OA) is characterized by articular cartilage degradation and has long been seen as primarily a cartilage disorder. However, nowadays OA is considered as a 'whole joint disease' and it is thought that pathological changes in one joint tissue might compromise structure and function of other joint tissues. Changes within the subchondral bone have been known for a long time to play a role within OA development [1].

Within a healthy joint, the thin dome-like shaped subchondral plate is supported by vertical oriented trabeculae and plays an important role to evenly distribute forces from weight-bearing. Healthy subchondral bone protects cartilage from high peak stresses and possible matrix damage. Animal studies showed that during early OA there is a marked reduction in subchondral bone thickness [2,3] and there are increased numbers of subchondral pores [4,5]. On TRAP-stained histology sections, bone resorption and pore formation as a consequence of increased osteoclast activity [6], result in loss of integrity and plasticity at the osteochondral junction. This compromises its biomechanical function and could promote cartilage damage. Due to all the evidence that subchondral bone remodeling is involved in disease progression, bisphosphonates were suggested to be useful as an interesting intervention strategy to treat OA.

* Corresponding author at: Department of Orthopaedics, Erasmus University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. Fax: +31 10 7044690.
E-mail address: m.siebelt@erasmusmc.nl (M. Siebelt).

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Alendronate (ALN), risedronate and zoledronate are all nitrogen-containing bisphosphonates and potent inhibitors of osteoclastic resorption used clinically for the treatment of osteoporosis [7]. Both alendronate and zoledronate have demonstrated positive results when used as an OA modifying agent in preclinical animal studies [8–12]. It is suggested that osteoclast-mediated resorption of mineralized cartilage at the subchondral bone-cartilage interface is an early initiating event in OA pathobiology and that only early bisphosphonates use after OA induction will result in the observed positive effect on cartilage health [12]. If in fact osteoclast activation during OA is time-dependent and reduces with ongoing OA stages, this might explain the disappointing results from large clinical trials on the role of bisphosphonates as treatment for OA. These trials included a very heterogeneous patient population, in which a large portion of patients had already severely progressed OA. Therefore, it is less likely that these patients benefit from osteoclast inhibition through bisphosphonates [13–18].

Late or progressive OA shows a different type of subchondral bone remodeling. Several animal studies showed that an initial thinning of the subchondral bone plate [19,20] is followed by a recovery phase leading to subsequent thickening of the subchondral plate due to enhanced osteoblast activity [20–22]. During this un-physiological high bone turnover in OA joints, there is an altered phenotypic expression of osteoblasts, which results in the production of sclerotic bone together with cyst formation and osteophyte development [4,9,23]. It has been hypothesized that as a result of the functional coupling between osteoclasts and osteoblasts, increased osteoclastic bone resorption induces a rise in osteoblast activity leading to increased subchondral bone thickness and sclerosis [24]. If true, bisphosphonate intervention to inhibit osteoclastic bone resorption might intervene with eventual formation of subchondral sclerosis by osteoblasts.

Recently, we established a novel rat OA model using a combination of papain injections with a running protocol which induces severe knee joint articular cartilage degradation together with activation of synovial macrophages and prominent involvement of subchondral bone [25]. In this particular study we found that papain injection alone induced moderate OA features, like sGAG and slight cartilage matrix loss, enhanced loss of the subchondral cortical plate. As a result of OA induction through papain injections and running, there was a complete different response and rats develop a pronounced sclerotic bone phenotype within the lateral compartment of the proximal tibia plateau combined with severe loss of cartilage matrix. In the current study, we investigated whether pre-emptive inhibition of osteoclast function through bisphosphonate treatment could prevent the development bone sclerosis, and possibly could prevent or reduce the development of OA. We used longitudinal *in vivo* microCT scans to measure effects of ALN treatment on subchondral sclerosis development and *ex-vivo* microCT on cartilage samples to see if cartilage was protected against degradation. Besides marked changes of articular cartilage and subchondral bone in this model for OA, we know there is also abundant activation of synovial macrophages [25]. Therefore, we also measured whether ALN treatment had an effect on synovial macrophage activation using a folate-based radiotracer for *in vivo* SPECT/CT imaging [26].

Methods

Effect of systemic alendronate treatment on severe osteoarthritis progression

Forty 16-week-old male Wistar rats (Charles River Netherlands BV, Maastricht, the Netherlands) were housed in the animal facility of the Erasmus University Medical Centre, with a 12-h light–dark regimen, at 21 °C during the experimental period, and received standard food pellets and water *ad libitum*. Severe osteoarthritis was induced in all animals using intra-articular papain injections in their left knee joints combined with exposure to a moderate exercise protocol as described before [25]. In short, all animals received three intra-articular injections in their left knee joints with 30 μl papain/L-cystein solution [27]. Their right knee joint served as an internal healthy control. All rats were forced to run on a motorized rodent treadmill (LE-8700; Panlab Harvard Apparatus, Barcelona, Spain) 500 m/day during 5 days/week, for six weeks covering a distance of 15 km in total [25].

Animals were divided over two groups: twenty rats served as untreated controls and twenty rats were treated during the experiment with three times weekly subcutaneous ALN injections (2.4 μg/kg) (alendronate, Sigma-Aldrich, St. Louis, MO, USA) to inhibit osteoclast bone resorption, a dose previously reported to be comparable to the clinical dose of 10 mg/day prescribed for the treatment of postmenopausal osteoporosis [28] (Fig. 1). Sterile water was used as the vehicle for dissolving ALN. Untreated animals did not receive placebo injections.

During the study all animals were longitudinally monitored with microCT to measure subchondral bone changes. At six and twelve weeks, ten rats in both groups were selected for a full analysis sequence. This sequence consisted of a SPECT/CT using a folate-based radiotracer to quantify macrophage activation *in vivo* [28], and *ex vivo* EPIC-μCT and histology to measure cartilage quality [29]. For all procedures, the exact same procedures were followed as described earlier [25]. The animal ethic committee of the Erasmus University Medical Center, Rotterdam, the Netherlands, approved all conducted procedures. A detailed planning scheme of all groups and conducted tests is given in Fig. 1.

Subchondral bone measurements on μCT scans

Both knees of all animals were μCT scanned under isoflurane anesthesia, using a Skyscan 1176 *in vivo* μCT scanner (Skyscan, Kontich, Belgium). 10 min of scan time was required per knee at an isotropic voxel size of 18 μm, at a voltage of 65 kV, a current of 385 mA, field of view of 35 mm, using a 1.0 mm aluminum filter, over 198° with a 0.5° rotation step, and a 270 msec exposure time. All datasets were segmented with a local threshold algorithm [30]. Cortical and trabecular bone were automatically separated using in-house software [31]. Using Skyscan software, both subchondral plate thickness (Sb. Pl. Th. in μm) and subchondral plate porosity (Sb. Pl. Por. in mm^3) of the medial and lateral compartment of the tibial plateau were measured [24]. In the tibial epiphysis, the trabecular thickness (Tb. Th. in μm) and trabecular bone volume fraction (BV/TV), representing the ratio of trabecular bone volume (BV, in mm^3) to endocortical tissue volume (TV, in mm^3). We additionally quantified the amount of ectopic bone formation as a measure for osteophyte growth (mm^3) on longitudinal μCT scans.

Determination of activated macrophages by SPECT/CT using ^{111}In-EC0800

Activated macrophages express the folate receptor-β allowing monitoring macrophages *in vivo* using folate-based radiotracers [32–34]. Phosphate saline-buffered (PBS, pH 6.5) DOTA-Bz-folate (EC0800, kindly provided by Endocyte Inc., West Lafayette, USA) [35] was labeled with $^{111}InCl_3$ (Covidien, Petten, The Netherlands) as described previously [25]. Quality control was performed with ITLC-SG and revealed a radiochemical yield of >95% at a specific activity of 50 MBq/μg. ^{111}In-EC0800 (55 MBq) was administered *via* the tail vein 20 h prior to scanning. SPECT/CT scans were performed with a 4-head multiplex multi-pinhole small-animal SPECT/CT camera (NanoSPECT/CT™, Bioscan Inc., Washington DC, USA). All knee joints were scanned with both helical μCT (acquisition time 5 min) and SPECT (acquisition time 30 min). All scans were analyzed using InVivoScope post-processing software (Bioscan Inc.). To reduce inter-individual variation, the absolute difference in measured radioactivity (kBq/mm^3) of the OA knee joint compared to the contralateral control joint was calculated. This absolute difference was used when comparing mean values of untreated animals with ALN treated animals.