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REVIEW

Long-term safety of antiresorptive treatment: bone material, matrix and mineralization aspects

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It is well established that long-term antiresorptive use is effective in the reduction of fracture risk in high bone turnover osteoporosis. Nevertheless, during recent years, concerns emerged that longer bone turnover reduction might favor the occurrence of fatigue fractures. However, the underlying mechanisms for both beneficial and suspected adverse effects are not fully understood yet. There is some evidence that their effects on the bone material characteristics have an important role. In principle, the composition and nanostructure of bone material, for example, collagen cross-links and mineral content and crystallinity, is highly dependent on tissue age. Bone turnover determines the age distribution of the bone structural units (BSUs) present in bone, which in turn is decisive for its intrinsic material properties. It is noteworthy that the effects of bone turnover reduction on bone material were observed to be dependent on the duration of the antiresorptive therapy. During the first 2–3 years, significant decreases in the heterogeneity of material properties such as mineralization of the BSUs have been observed. In the long term (5–10 years), the mineralization pattern reverts towards normal heterogeneity and degree of mineralization, with no signs of hypermineralization in the bone matrix. Nevertheless, it has been hypothesized that the occurrence of fatigue fractures (such as atypical femoral fractures) might be linked to a reduced ability of microdamage repair under antiresorptive therapy. The present article examines results from clinical studies after antiresorptive, in particular long-term, therapy with the aforementioned potentially positive or negative effects on bone material.

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Introduction

On the basis of many clinical studies, it is clear that antiresorptives are able to reduce fracture risk in patients with high bone turnover osteoporosis, as in postmenopausal osteoporotic women.[1] However, concerns emerged during recent years that longer bone turnover reduction might favor the occurrence of fatigue fractures[2–4] (fractures that result from repetitive normal loading and not from a singular overloading). Increasing numbers of patients with fatigue fractures after antiresorptive therapy, in particular after long-term bisphosphonate treatment, have been reported. In this context, it has to be mentioned that bisphosphonates have been the longest and most widely used therapy for osteoporosis thus far. However, atypical femoral fractures (AFF) have also been identified in patients after denosumab.[5] Another adverse effect is osteonecrosis of the jaw, which was reported as a rare effect in cancer patients with high-dose bisphosphonate treatment, and it seems to be related to co-therapies such as glucocorticoid therapy and chemotherapy.

In general, the mechanism by which antiresorptives are able to improve the mechanical compentence of osteoporotic bone is not fully understood yet. It is assumed that their action is multifactorial, involving all hierarchical levels of bone (organ, architectural and material level), and material science may contribute to elucidate the efficacy of antiresorptive treatment, in particular that of bisphosphonate treatment.[6,7] This article critically examines the hypotheses on positive and potential negative effects of long-term antiresorptives with the surprisingly sparse available clinical literature. Our focus is on the material property changes under long-term (5 years and longer) antiresorptive treatment of osteoporosis in adult patients. In

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cases in which no information is available from clinical studies, in particular for newer therapeutic agents such as odanacatib or denosumab, we present the results from animal studies.

Importance of Bone Remodeling and Possible Effects of its Reduction on Bone Mechanical Integrity

Bone (re)modeling is an ongoing process in the skeleton, and it occurs at discrete sites in bone. During remodeling, bone is resorbed by osteoclasts, and subsequently the resorption lacuna/space is filled again with new bone matrix by osteoblastic activity. This process facilitates damage repair of older bone matrix. There is some evidence that the site of bone resorption is at least partly determined by apoptotic osteocytes.[8–11] During modeling, the activity of osteoclasts and osteoblasts is spatially uncoupled, and thus, in general, bone formation and resorption take place at different bone sites. This process provides structural adaptation to mechanical demands, and thus bone material is removed from sites at which the local mechanical stimulus is small and added to sites at which it is large (as described by Wolff's law).

Reduction of bone turnover by antiresorptives

With ageing and/or in pathological conditions, bone remodeling becomes unbalanced with an overall negative bone balance; that is, less bone is formed than has been resorbed previously.[12,13] This is amplified in case of systemically increased bone turnover leading to rapid loss in areal or volumetric bone mineral density (BMD; based on the X-ray absorptiometry). In addition, the increase in bone turnover *per se* also decreases bone matrix mineralization, contributing additionally to decreased BMD, as will be discussed later in this article.[14] This underlies the rationale of reducing bone turnover by antiresorptives. In the case of the most commonly prescribed newer-generation (nitrogen containing) bisphosphonates, the antiresorptive action is achieved by interaction of the agent with the mevalonate pathway of the osteoclast, leading to loss of function or apoptosis of the cell.[15] Odanacatib, a newer therapeutic agent, inhibits degradation of collagen by inhibition of cathepsin K, leaving other osteoclastic functions unaffected.[16] Denosumab, a fully human monoclonal antibody, inhibits bone resorption by binding to RANKL, an essential mediator of bone resorption.[16] Although the actions of these agents on bone cells may be different (as for instance, odanacatib was observed to promote periosteal bone formation[17]), all considered agents (bisphosphonates, odanancatib, denosumab) have the ability to reduce the number of remodeling sites, thus leading to a reduction in bone resorption and/or turnover in common. In case of unbalanced bone remodeling, this leads to a deceleration or even eradication (dependent on reduction magnitude) of bone loss. On the other hand, the reduction of bone turnover may prevent structural adaptation and may delay fracture healing (in particular mineralized callus resorption).[18,19] Potentially more worrisome is the elimination of damage repair, which, in concert with some undetermined to date underlying condition(s), could favor fatigue fractures such as AFF especially in the long-term treatment.[2,4]

Effects on cortical versus trabecular bone structure

In view of adverse effects of antiresorptive therapies such as AFF, a discussion is ongoing whether antiresorptive treatment differentially affects bone turnover rates and material properties in cortical and trabecular bone.[3] Long-term studies with bisphosphonate have shown persistent antifracture efficacy and much larger increases in BMD at sites where trabecular bone is abundant (vertebral bone) compared with those where cortical bone is prevalent (femoral neck, total hip…).[1]

Cortical bone has a lower surface-to-bone marrow ratio, and as bone turnover is a surface-based mechanism cortical bone is considered to have lower levels of bone turnover. However, the analysis of bone turnover in cortical bone is challenging, as its structure undergoes distinct changes with ageing (trabecularization)[20] and large differences might exist between different skeletal sites (iliac crest compared to femoral neck for instance), as well as between periosteal, endosteal and intracortical areas.[21] During the first phase of antiresorptive treatment, the residual activity of the osteoblasts results in the filling of resorption space in osteons, thus decreasing pore areas in cortical bone.[22,23] In a recent study on alendronate-treated healthy beagle, the reduction of cortical pore size was associated with impaired mechanical quality during cyclic loading.[24] The authors suggested that the reduced diameter of the cutting cone primarily led to an enlargement of interstitial bone areas of older tissue age, which might be more prone to microdamage.[24] Considering the action of odanacatib, there is experimental evidence for an increase in periosteal bone formation, additionally to a general reduction of bone turnover, resulting in a thickening of the femoral cortical bone in treated monkeys.[17]

Most of the information of antiresorptives' effect is available for cancellous bone, as this compartment has been considered to be most important to osteoporosis and its therapy. Histomorphometric analysis reveals increases in cancellous bone volume by antiresorptives,[7,17] in the case of bisphosphonate treatment owing to the aforementioned filling of remodeling space.[7] Although this is observed in short-term use, an additional increase that plateaus over long-term use was predicted by theoretical models.[25] Structural changes based on high-resolution invasive or *in vivo* imaging were translated into anticipated changes of mechanical properties owing to treatment by means of finite element analyses (FEA).[26] FEA-predicted increases in bone strength have been reported for vertebral bone after therapy with bisphosphonates[27] (for example), and for vertebral and femoral bone after odanacatib[28] or denosumab.[29]

Effects on bone microdamage

Microdamage accumulation might also differ in cortical versus cancellous bone. Cancellous transiliacal bone microcrack frequency was generally low after long-term bisphosphonate treatment.[30] Considering the theoretical and intriguing concept that cortical bone remodeling is primarily initiated by microdamage,[31,32] the reduction in turnover by antiresorptive treatment might affect microdamage accumulation in cortical bone significantly. However, comparative experimental data for both cancellous and cortical compartments are not available yet.

As hypothesized and experimentally confirmed, bone turnover reduction may increase microdamage accumulation. Studies on human samples are sparse, and information is available from transiliacal bone biopsies, which are less prone to microdamage than other skeletal sites (such as femoral neck and diaphysis).[30] Studies from treated animal models have

shown increased microcrack surface density after higher or lower doses of bisphosphonates at short-term or long-term treatment, as summarized in a review article.[33] It has to be mentioned that most of these animal studies are based on treatment of healthy dogs and not on animal models of high-turnover osteoporosis. In a study on transiliacal bone biopsy samples from long-term alendronate-treated osteoporotic patients, microdamage accumulation was not different from treatment-naive patients without adjustment for potential confounders.[34] After adjustment for age, prevalent fractures, femoral neck BMD and so on, microcrack density was elevated for the bisphosphonate users.

Microdamage accumulation *per se* was considered to impair the mechanical properties of bone.[35] However, it has been hypothesized recently that in particular the progression of microcracks in a homogeneous bone matrix with reduced crack stopping mechanisms might have a role in AFF.[2] In contrast to this hypothesis, X-ray images of AFF fracture sites typically show a broadening of cortical thickness especially at the periosteal site.[4] In an investigation of such bone tissue from the fracture site, no compact dense homogeneous bone tissue, but in contrary a highly porous inhomogeneous bone tissue with relatively lower matrix mineralization, was reported.[36] These observations suggest another AFF mechanism, which may start with microcrack formation/accumulation followed by a period of response of the bone, resulting in the presence of a porous bone tissue, which does not sufficiently meet the mechanical demands leading to AFF.

Effects of Antiresorptive Therapy on Bone Material

Bone material represents a nanocomposite consisting of nano-sized stiff mineral particles embedded in soft collagen fibrils. Owing to ongoing bone resorption and formation at discrete sites (the so-called bone structural units, BSUs), and the maturation of the components collagen and mineral within each BSU, bone material is a highly heterogeneous material. Bone turnover essentially determines the age distribution of these BSUs, and thus the tissue age of the considered bone volume/area. Therefore, bone turnover is essentially influencing the material properties that in turn are determinants of the mechanical performance at the organ level.

Effects on collagen properties

One of the properties that seem to be highly affected by bone turnover alterations is collagen cross-linking, a major post-translational modification of collagen, which has an important role in bone fragility.[37–39] Cross-links might be formed either enzymatically or by glycation, both of which are dependent on tissue age.[40] As a consequence of the reduction of bone turnover by treatment, both enzymatic and nonenzymatic collagen cross-linking might be affected. Spatially resolved information on these collagen properties can be obtained from Fourier transform infrared (FTIR) and Raman microspectroscopy. These vibrational spectroscopic methods are sensitive to bond vibrations, thus providing information on the functional groups present in the mineral and organic matrix components, as well as the short- and medium-range interactions between them ('molecular neighborhood').[41] Measured at large tissue areas (total cross-sectional area of transiliac bone biopsy samples), the aforementioned collagen properties are heavily dependent on turnover rates, whereas measured at sites of comparable tissue age they provide information on possible turnover-independent effects, which antiresorptive therapies may have on the organic matrix. After antiresorptive treatment, collagen cross-link ratios were reported to be increased, and larger accumulation of pentosidine (one of the advanced glycation end products) was observed, in particular after bisphosphonate treatment in healthy canine bone,[33] and in human iliac crest samples.[42] Information on collagen cross-linking under long-term bone turnover reduction is sparse. Analyses of iliac crest biopsy samples of long-term risedronate-treated osteoporotic patients revealed similar cross-link ratios compared with baseline at actively bone-forming trabecular surfaces.[43] In another study, few patients who were treated for more than 5 years with a bisphosphonate and who sustained an AFF revealed the tendency of increased collagen cross-link ratios compared with control levels.[44] Moreover, the heterogeneity of cortical collagen cross-link ratios measured near the site of intertrochanteric and subtrochanteric fractures was reduced in bisphosphonate-treated patients.[45]

Apart from studying the effect of bone turnover reduction by considering larger bone area/volume for analysis of collagen properties, one can also consider bone of a specific tissue age, in particular young bone.[46–48] From the latter, one might obtain information on the state of maturation of the tissue and in turn on eventual changes in the kinetics of tissue maturation in treated versus untreated bone. Collagen cross-link ratios in newly formed tissue from treated patients were observed to be dependent on the type of bisphosphonate: alendronate or risedronate.[46] The outcomes for the risedronate-treated patients revealed that the effect on collagen cross-linking was dependent on the duration of treatment.[46] Comparison of long-term effects on collagen properties in the biopsies from patients of the fracture intervention long-term (10 years) extension study gave evidence for similar collagen properties after 10 years compared with 5 years with alendronate.[47] In another study, zoledronic acid treatment had an influence on tissue maturation compared with placebo.[48] Mineral:matrix ratios, for instance, of few days old bone matrix was higher in the patients on active treatment compared with placebo.

Effects on bone mineral

Information on changes in bone mineral characteristics such as crystal structure, maturity, crystallinity, elemental composition, mineral particle size, shape and arrangement is given by techniques such as wide- and small-angle X-ray scattering, X-ray fluorescence analysis and Raman and FTIR spectroscopy. So far, no changes in mineral particle size parameters were reported for bisphosphonate-treated bone.[49–52] In particular, no change in particle size after long-term alendronate[49] could be detected. This is, for instance, in contrast to the recently revisited bone anabolic therapy with fluoride, which was shown to significantly alter the nanocomposite material resulting in the occurrence of enlarged and less oriented mineral particles,[53] likely responsible for the failure to reduce the bone fragility in these patients despite significantly increased bone mass.

Considering the effects of antiresorptives on maturity/crystallinity of the bone mineral, lowered values after long-term alendronate compared with untreated osteoporosis were reported,[54] although the spectroscopic method used is in

conflict with other published ones.[55,56] In this work, maturity/crystallinity was characterized for comparable mineral content among the treatment groups by separating the measured BSUs qualitatively into low, medium, and high mineral content.[54] In contrast, in newly formed bone matrix defined by the presence of fluorescence labels, maturity/crystallinity was similar between long-term (5 years and longer) and short-term bisphosphonate use, although it was dependent on the type of bisphosphonate (alendronate versus risedronate).[46] This bone quality metric was also similar between patients who were on alendronate for either 5 or 10 years.[47]

Effects on bone matrix mineralization

The aforementioned collagen properties and the amount of mineral particles embedded in the collagenous matrix are important determinants of overall material elasticity, toughness and strength. The weight or volume fraction of mineral in the bone matrix is measured spatially resolved by methods such as microradiography, synchrotron μCT and quantitative backscattered electron microscopy typically obtained from the entire volume or cross-sectional area of bone biopsy samples. Another measure of the degree of mineralization is the mineral:matrix ratio based on Raman or FTIR microspectroscopy extracted typically from distinct microanatomical areas of cancellous or cortical bone.

During the formation of mineralized bone matrix, the osteoblasts first form the unmineralized osteoid, which starts to mineralize after a certain lag time (about 15 days).[57] The primary phase of mineralization to about 70% of full mineralization is a rapid process, whereas the secondary phase to full mineralization is slower, and it lasts for several months to years.[58–61] These processes of mineral accumulation within each site of newly formed bone together with the activation frequency of remodeling sites are causing a specific mineralization pattern of the bone material. In cases of increased bone turnover, such as in postmenopausal osteoporosis, the overall mineralization density is lower owing to the higher number of active remodeling sites, thus leading to a larger amount of bone areas undergoing primary mineralization[22] or being in an early stage of secondary mineralization. The reduction of bone turnover by treatment significantly affects this mineralization pattern during the first few years of treatment. Although for most of these treatment studies the abnormally low degree of mineralization was shifted toward normal,[22,42] the heterogeneity of mineralization (and other material properties) was found to be lower than normal.[22,42,45,62] Material heterogeneity in general is thought to be an important prerequisite for the prevention of crack propagation and the biomechanical function of bone.[63–67] In the long-term antiresorptive studies, it was shown that the mineralization densities were either higher than those for untreated osteoporosis[54,62] or similar to normal.[49,62,68] Essentially, in two previous studies, we found heterogeneity of mineralization similar to normal after long-term treatment with risedronate[62] or alendronate.[49] In addition, the latter study showed that bone matrix mineralization was in the normal range and similar between long-term treatment of 5 years with alendronate plus 5 subsequent years with placebo compared with 10 years with the active drug,[49] suggesting that the material properties were normalized already after 5 years on therapy and were not further altered by longer drug administration. This is consistent with the recommended initial treatment period of postmenopausal osteoporosis[69] and in line with the report of the FDA's Drug Safety and Risk Management Committee on long-term use released in 2011, which reveals 'that most women with osteoporosis bisphosphonate treatment can safely stop taking bisphosphonates after 5 years, as they have similar levels of increased bone density and reduced fracture risk as those who continue taking them.'

The bone material findings also indicate that after long-term treatment (longer than 5 years) a new steady state of bone turnover rate at a lower level is achieved, which might explain why the mineralization pattern is different from that after short-term treatment.[70] Moreover, no signs of hypermineralization could be detected after long-term bone turnover reduction in these cohorts[49,62] or in other patients.[71] It is noteworthy that the postmenopausal osteoporotic patients from the HORIZON study (zoledronic acid treatment of 3 years) revealed higher average mineral content in their bone matrix compared with normal.[22] These patients were reported to have decreased fracture risk; however, it remains unknown whether further increases in mineral content have to be expected for long-term zoledronic acid therapy (in particular as a consequence of the aforementioned accelerated mineral accumulation in newly formed bone matrix in this treatment). Further, it is unclear whether such an increase in degree of mineralization is beneficial for fracture risk reduction in the long term.

For the new therapies with odanacatib and denosumab, no information on their effects on bone material is available on long-term therapy effects, and the short-term treatment effects were obtained from animal studies. Short-term treatment with odanacatib showed similarly increased degree of bone mineralization in odanacatib or alendronate versus control monkeys.[72]

It is difficult to speculate on general differences between antiresorptive treatment effects in material properties (in particular tissue mineralization) in cancellous versus cortical bone compartments, as few comparative data are available. Considering the mineralization pattern, cortical bone was found to be affected by bisphosphonate in a similar manner as cancellous bone,[73] whereas others report an increase in the mineral-to-matrix ratio of cortical bone only.[74] These works analyzed bone from short-term bisphosphonate treatment. No information on eventual differences between mineralization changes in cortical versus cancellous bone is available for long-term treatment.

Effects on mechanical properties

Antiresorptive therapies are well known to affect bone material properties at lower size scales. Considering intrinsic mechanical properties that can be directly obtained from the material based on microindentation or nanoindentation, the results from long-term bisphosphonate-treated human bone are not definite. Increased hardness was reported after short-term bisphosphonate,[75] whereas reduced hardness was found after long-term treatment compared with untreated osteoporosis.[54,76] In contrast to the latter finding, others observed greater plastic deformation resistance, as well as harder and stiffer trabecular bone from patients with long-term bone turnover reduction.[77] *In vivo* mechanical testing at the tibia was able to discriminate bisphosphonate-treated patients with AFF, as well as untreated patients with typical osteoporotic fractures from untreated postmenopausal controls in their *in vivo*

indentation properties, whereas indentation properties from long-term bisphosphonate users without AFF were not different from those of controls.[78]

Conclusion

Bone material studies contributed to the understanding of the mechanisms of antiresorptive therapy on the skeleton. Relatively sufficient information is available from short-term antiresorptive treatment studies, in contrast to long-term treatment, where investigations of bone samples are sparse. Bone matrix mineralization was shifted towards normal, or were normalized with alendronate or risedronate in patients who had decreased bone matrix mineralization at baseline. In particular, mineralization heterogeneity was not decreased in these treated patients. Moreover, material maturation including mineral and collagen properties was not found to be negatively affected during long-term antiresorptive treatment. The effects on local mechanical properties reported are conflicting, showing either decreased or increased hardness and elasticity after long-term treatment. Only little information on microdamage accumulation after long-term treatment in patients is available yet. Animal studies point toward increased microdamage accumulation owing to reduced bone turnover levels. However, insufficient evidence exists whether these observations occur also at fracture-relevant sites in the human skeleton and whether these are related to the occurrence of adverse events such as AFF. The mechanisms of antiresorptive therapy on bone material are still under investigation; in particular, long-term treatment awaits further studies.

Conflict of Interest

Since 2010 the work on osteoporosis and treatment at the Ludwig Boltzmann Institute of Osteology has received funding from MSD, Amgen, Eli Lilly and Roche. There are no other conflicts of interest.

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