

Whither goest the lifeless osteoclast?

Evaluation of: Weinstein RS, Roberson PK, Manolagas SC: Giant osteoclast formation and long-term oral bisphosphonate therapy. *N. Engl. J. Med.* 360, 53–62 (2009). Giant osteoclasts are pathognomonic in Paget's Disease, secondary hyperparathyroidism, giant cell tumors and fibrous dysplasia. Weinstein *et al.* provided a detailed histological analysis of abnormal hypernucleated osteoclasts in half of a set of biopsies from patients who received clinical doses of alendronate, a bisphosphonate inhibitor of bone resorption. In addition, 30% of the osteoclasts were apoptotic, a feature not found in the other clinical conditions. Their report reveals new information about skeletal effects of alendronate in addition to known effects to inhibit osteoclastic bone resorption. Their report raises new questions about the lifecycle of the human osteoclast, effects of bisphosphonates on cell fusion and on efferocytosis (removal of apoptotic cells), significance, if any, of the abnormal osteoclasts, and distinguishing features of the set of alendronate-treated subjects whose biopsies showed these abnormalities.

KEYWORDS: alendronate ■ apoptosis ■ bisphosphonate ■ efferocytosis ■ giant cells ■ osteoclasts

Weinstein *et al.* compared osteoclasts in bone biopsies from control and alendronate-treated women [1]. Osteoclasts are the large, multinucleated cells responsible for bone resorption. They are formed by fusion of mononuclear precursors that are derived from hematopoietic stem cells of the monocytic lineage and are specialized to attach to bone surfaces and to degrade the bone matrix. Active osteoclasts adhere to bone matrix with a membrane specialization of integrins that are organized into a ring-like sealing zone. This zone surrounds the ruffled border and defines the resorption lacuna where bone is degraded. Acidification of the lacuna is achieved by the action of a membrane-bound vacuolar-type ATPase proton pump and results in solubilization of the mineral phase. Thereupon, proteolytic enzymes of two major classes, lysosomal cysteine proteases (especially cathepsin K) and matrix metalloproteinases (MMP-9), degrade collagen and other bone matrix proteins. Degradation products are endocytosed into vesicles and large vacuoles and are carried to the secretory surface of the cell. During development and growth, osteoclasts resorb calcified cartilage and contour the shapes of enlarging bones; thereafter, they act in coordination with bone-forming osteoblasts to remodel and renew bone tissue. It is likely that osteoclasts undergo cycles of resorption and rest, but *in vivo*, details of kinetics are lacking. The osteoclast lifespan is limited by apoptosis – the controlled process

that removes cells and is evident by pycnotic nuclei, loss of ruffled border and detachment from the bone surface. Apoptosis is distinct from cell necrosis, which occurs in groups of swollen cells, often with intracellular precipitation of calcium. Apoptosis occurs rapidly, with phagocytes ingesting and eliminating the apoptotic bodies; apoptotic osteoclasts may be difficult to detect in normal bone biopsies, even with specialized stains that detect early stages of DNA fragmentation.

Bisphosphonates are potent inhibitors of osteoclastic bone resorption and were first introduced to retard bone turnover in patients with Paget's disease. Seven bisphosphonates have been approved by the US FDA and new, more potent compounds continue to appear. Intravenous nitrogen-containing formulations are routinely used for treating bone metastases and hypercalcemia of malignancy in patients with cancer. The most common indications for bisphosphonates are the treatment and prevention of osteoporosis. Off-label uses are expanding, particularly in pediatric conditions associated with low bone mass, such as osteogenesis imperfecta.

Bisphosphonates are chemically stable analogs of pyrophosphate, and all have two phosphate groups that bind to the calcium phosphate mineral of bone's extracellular matrix [2]. The fact that they accumulate in bone is the reason for their potent effects on the skeleton. They selectively target the osteoclast because they

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preferentially bind to bone mineral exposed at resorbing surfaces, as the osteoclast acidifies the bone surface, which dissolves the bisphosphonate, and because the osteoclast can uptake the released bisphosphonates along with the matrix products of resorption. Like pyrophosphate, bisphosphonates can inhibit mineralization *in vitro* and *in vivo*, but the newer ones, especially the nitrogen-containing ones, such as alendronate and zoledronate, can inhibit bone resorption at doses far lower than those that inhibit mineralization. Several mechanisms account for the effects of bisphosphonates on osteoclasts. Etidronate and clodronate are metabolized into ATP analogs that induce osteoclast apoptosis. It is now understood that the nitrogen-containing bisphosphonates bind to the enzyme farnesyl diphosphate (FPP) synthase, thereby inhibiting prenylation of essential GTPase proteins and inducing osteoclast apoptosis. These effects of bisphosphonates would occur in all cell types, but their accumulation on bone surfaces targets their actions to osteoclasts.

Weinstein *et al.* had access to bone biopsies from a 3-year trial of oral alendronate for preventing bone loss in healthy postmenopausal women of 40–59 years of age [1]. The original study described an increase in bone mineral density and a decrease in the biochemical markers of bone turnover in the groups of subjects treated with alendronate at 5 or 10 mg/day for 3 years, or in those given 20 mg/day for 2 years followed by placebo for 1 year [3]. Some of the subjects volunteered for transiliac bone biopsy at the end of the 3-year period; the specimens were used in the original report to demonstrate that alendronate reduced bone turnover, as expected. For this study, which was published more than 10 years after the original one, 51 specimens were processed for the specialized examination of osteoclast morphology, staining for tartrate-resistant acid phosphatase and staining for DNA fragmentation. These are not routine assays and require fastidious technique to avoid distortions and misinterpretation. Professor Weinstein (University of Arkansas for Medical Sciences, AR, USA) disclosed the many virtuoso measures he took to ensure the highest quality of histology needed for these analyses of nondecalcified bone biopsies.

It is clear from the biochemical markers of bone turnover that bone resorption was decreased in subjects receiving alendronate [3]. Because bisphosphonates inhibit bone resorption and promote apoptosis of osteoclasts *in vitro* [4], it could be expected that biopsies from subjects

taking bisphosphonates may show few osteoclasts. Normally, the number of osteoclasts is used as an index of bone resorption [5]. Nonetheless, Weinstein *et al.* reported that there were significantly more osteoclasts in samples from subjects treated with 10 mg/day alendronate than placebo [1]. It must be concluded that those osteoclasts did not resorb bone well. Morphological abnormalities suggesting this was found in the treated groups: the osteoclasts were detached from the bone, often with intervening cells; resorption lacunae were shallow or absent. In addition, they had more than eight nuclear profiles, often as many as 40, and they had pycnotic nuclei. The number of detached osteoclasts was statistically elevated in the group receiving 10 mg/day alendronate for 3 years. The number in the group receiving 20 mg/day for 2 years followed by 1 year of placebo was almost normal; this suggests some measure of recovery in the drug-free period. Apoptotic nuclei were present in all treatment groups. The authors speculated that alendronate may prolong apoptosis. We offered another possible mechanism, that phagocytes that should be recruited to dispose of the apoptotic osteoclasts are themselves inhibited by alendronate in the bone microenvironment [6]. *In vitro* evidence demonstrates that bisphosphonates inhibit macrophage development [7] and induce apoptosis in macrophage-like cells [8].

The report by Weinstein *et al.* is the first detailed publication on the numbers and features of osteoclasts in humans receiving alendronate, although it cites similar observations reported by others in abstract form. Some animal studies have noted, in passing, that administration of bisphosphonates increased osteoclast numbers. For example, in a study demonstrating that nitrogen-containing bisphosphonates reduced immunoreactivity for a product downstream of FPP synthase, it was also noted that osteoclast numbers were significantly elevated in bones from the groups of rats treated with the nitrogen-containing bisphosphonates but not with the bisphosphonates lacking nitrogen [9].

Although osteoclast apoptosis may be a real effect of alendronate, biopsies from ten of the 17 subjects receiving the highest doses of alendronate exhibited no abnormal osteoclasts. The mean prevalence of apoptotic osteoclasts was 30% in the group receiving 10 mg/day. It would be very useful for future studies to delve into whether the occurrence of these abnormal cells is related to, for example, subjects' clinical features (e.g., lowest body weight) or subjects' responses to alendronate (e.g., greatest suppression of bone turnover). Alendronate treatment of

2 or 3 years duration is not considered long-term for osteoporosis. Growing concerns regarding skeletal complications of oral bisphosphonates, such as presentation with unusual subtrochanteric or shaft fracture through thick cortices [10] and osteonecrosis of the jaw [101], highlight the need for more information about these agents.

The greatest clinical significance of this priority paper evaluation is the alert to pathologists concerning the occurrence of these cells in the setting of oral bisphosphonate use for osteoporosis. These cells should not be confused with other types of hypernucleated cells. Giant osteoclasts are features of bone in Paget's disease and in hyperparathyroidism, but those conditions are associated with elevated osteoid and osteoblast levels. The hypernucleated cells of giant cell tumors and fibrous dysplasia are located in fibrous stroma, distant from the bone. Only in the instance of bisphosphonate therapy are there high numbers of apoptotic osteoclasts. A case study recently published by Jain and Weinstein exemplifies the issues in histological differential diagnoses of giant cells in bone [11].

Several mysteries need to be solved. Apoptosis is a mechanism for the controlled removal of injured or worn-out cells that usually occurs quickly. Cell death, removal, and replenishment are essential for homeostasis in healthy organisms. Finding large numbers of apoptotic osteoclasts in the alendronate groups may signify increased formation and/or decreased removal. The increased numbers of nuclei may indicate continued fusion of mononuclear precursors with osteoclasts in the presence of alendronate. It also indicates that the cell membrane of the apoptotic osteoclast has

not lost all functionality. Efferocytosis is a term used to describe the process by which phagocytes dispose of apoptotic cells. It is notable that Rho GTPases in phagocytes play crucial roles in activating efferocytosis and pinocytosis [12]. Another important regulator of cell survival is osteoprotegerin (OPG). OPG is well known as a decoy receptor, produced by marrow stromal cells and osteoblasts, that blocks the binding of RANK ligand (RANKL) to RANK in osteoclast progenitors [13]. In addition, OPG can stimulate cell survival by binding to TNF-related apoptosis-inducing ligand (TRAIL), thereby allowing cells to escape cell death [14]. We recently reported that alendronate upregulated OPG in human marrow stromal cells *in vitro*, and that OPG was constitutively elevated (by 1.53-fold) in marrow from women treated with alendronate, compared with age-matched untreated women [15].

Efferocytosis has been demonstrated to be impaired in a number of disorders, such as rheumatoid arthritis [16], advanced atherosclerosis [17] and chronic lung diseases [12]. In those settings, it is believed that impaired efferocytosis perpetuates inflammation. Although Weinstein described no evidence of inflammation or fibrosis in the bone biopsies (possibly attributable to the anti-inflammatory actions of bisphosphonates), the persistence of apoptotic cells may ultimately have untoward effects when, and if, the bisphosphonates are eliminated from the bone matrix. It has been calculated that following discontinuation of 10 years of treatment with 10 mg/day alendronate, skeletal release of stored alendronate approximates the equivalent of an oral daily dose of 2.5 mg with a very gradual increase in bone

Executive summary

Findings from the study

- Biopsies available from a previous controlled trial showed elevated numbers of osteoclasts in samples from subjects receiving 10 mg/day alendronate for 3 years.
- The biopsies showed that, in a dose-related manner, alendronate therapy was associated with abnormal-appearing osteoclasts (detached from bone surfaces, greater number of nuclei or prevalence of nuclear apoptosis).
- Only half of the specimens revealed abnormalities and only 30% of the giant osteoclasts demonstrated evidence of apoptosis. The findings need to be correlated with clinical parameters and measures of bone turnover.

Impact of the study

- The use of bisphosphonates should be added to the differential diagnosis of finding giant cells in bone biopsies.
- Questions are raised regarding the lifecycle of the human osteoclast, the process of cell fusion in the bisphosphonate-containing milieu, and the possible impact of abnormal cells.
- Bisphosphonate treatment is added to the list of disorders of apoptosis/efferocytosis.

Conclusion

- This histopathological study revealed skeletal effects of alendronate in addition to the known effects inhibiting osteoclastic bone resorption.

Future perspective

- More information is needed about the relative effects of the various bisphosphonates on the osteoclast and macrophage lifecycle and whether those effects are related to antiresorptive potency.
- The potential pathophysiological impact of these abnormal osteoclasts should be investigated.

turnover [18]. Unlike with discontinuation of conjugated estrogen, bone mineral density was maintained in the year following discontinuation after 2 years of 10 mg/day alendronate [19].

Weinstein's observations raise many new questions about the life and death of human osteoclasts that can be addressed experimentally. Without doubt, more information is needed regarding all of the effects of bisphosphonates on bone and bone marrow cells.

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The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Website

- FDA site containing memorandum for the matter of osteonecrosis of the jaw www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4095B2_03_04-FDA-TAB3 Accessed 8 July 2009