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REVIEW ARTICLE

Bisphosphonates: effects on osteoblast

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Abstract
Purpose Bisphosphonates are synthetic analogues of pyrophosphate usually used in treating bone disorders such as osteoporosis, Paget's disease, fibrous dysplasia, hypercalcemia of malignancy, and inflammation-related bone loss. Though therapeutic effects of bisphosphonates depend primarily on their inhibitory effect on osteoclasts, increasing attention is being given to other effector cells, such as osteoblasts. This review focuses on the presumed effect of bisphosphonates on osteoblasts.
Methods A review of the literature was conducted to evaluate the pharmacodynamic effects of bisphosphonates including inhibition of osteoclasts and apoptosis of osteocytes and osteoblasts as well as their potential stimulatory effects on the proliferation of osteoblasts.
Results Studies have demonstrated that bisphosphonates may stimulate proliferation of osteoblasts and inhibit apoptosis of osteocytes and osteoblasts.
Conclusion Considering that osteoblasts may be involved in bone disorders, such as osteoporosis, osteopetrosis, osteogenesis imperfecta, and Paget's disease, and that bisphosphonates may stimulate proliferation of osteoblasts and inhibit apoptosis of osteocytes and osteoblasts, it is conceivable that a role for bisphosphonates exists in these diseases beyond merely the osteoclast influence.

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Introduction

Bisphosphonates (BPs) are synthetic analogues of pyrophosphate usually used in treating disorders of bone such as osteoporosis, Paget's disease, fibrous dysplasia, hypercalcemia of malignancy, and inflammation-related bone loss [1–5].

BPs can further be separated into nitrogen-containing and non-nitrogen-containing BPs. Nitrogen-containing BPs, such as alendronate, ibandronate, risedronate, and zoledronate, inhibit the mevalonate pathway of cholesterol synthesis via inhibition of the enzyme farnesyl diphosphate synthase and blocking prenylation of small GTPases leading to interruption of osteoclast function [6]. Non-nitrogen-containing BPs, such as etidronate and clodronate, suppress bone resorption by incorporating into intracellular non-hydrolyzable ATP analogues that have no releasable energy content, thus leading to osteoclast death [7, 8]. Moreover, BPs are able to chelate calcium ions and bind to hydroxyapatite crystals on bone surfaces [9].

BPs determine an acidification process at the osteoclast-mineral interface, disrupt the actin attachment sites on the bone surface, and interrupt bone resorption by disturbing the ruffled border function [10–14].

Even if therapeutic effects of BPs depend on their inhibitory effect on osteoclasts, there is increasing evidence that BPs may play a role in osteoblastogenesis [15–21].

Osteoblasts

Osteoblasts are mononuclear specialized cells derived from mesenchymal precursor cells, responsible for formation,

deposition, and mineralization of bone tissue, principally through the deposition of calcium phosphate crystals, such as hydroxyapatite, and extracellular matrix, including proteoglycans and type 1 collagen.

Osteoblasts are involved in osteoclast regulation [22]. The interaction of nuclear factor (NF)-κB ligand (RANKL), a membrane-residing protein on osteoblasts that may also be isolated as a soluble factor as a consequence of matrix metalloproteinases (MMPs) proteolysis, with RANK, a type I transmembrane receptor expressed on osteoclast precursors, is involved in osteoclast precursor differentiation into osteoclasts. The formation of the RANK-RANKL complex results in a cascade characterized by the trimerization of RANK and the activation of TNF receptor-associated factor 6 (TRAF6), which induces NF-κB and mitogen-activated protein kinases (MAPKs), including Jun N-terminal kinase (JNK) and p38, involved in the activation of transcription factors such as c-Fos, c-Src, and microphtalmia transcription factor (MITF) [23–25]. On the other hand, osteoblasts are also involved in osteoprotegerin (OPG) production, a soluble decoy receptor for RANKL, which competitively inhibits the binding of RANKL to RANK on the cell membrane of osteoclasts, thus impeding RANK activation and the subsequent osteoclast activation [26].

The control of osteoclastogenesis by osteoblasts underlines the importance of these cells in the modulation of bone resorption. Moreover, osteoblasts express numerous other molecules involved in the regulation of osteoclastogenesis such as tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), and macrophage-colony stimulating factor (M-CSF).

TNF-α induces the production of RANKL both directly by expressing factors such as RANK, TRAF6, and NF-κB, which are involved in activation of osteoclast precursor cells in the early phase of osteoclast differentiation, and indirectly by stimulating osteoclastogenesis-supporting mesenchymal cells [27–30]. The binding of TNF-α to two receptors, TNF receptor type I (TNFRI) and TNF receptor type II (TNFRII), is responsible for its biological activity. Only the addition of neutralizing anti-TNFRI, but not anti-TNFRII antibodies, suppresses RANKL-induced osteoclastogenesis, suggesting that just TNFRI is involved in RANKL-induced osteoclastogenesis [31, 32].

IL-1 is a potential regulator of osteoclastogenis only in the presence of adequate levels of RANKL and induces the activation of a p38 MAP-kinase in osteoclast precursor and marrow stromal cells that is involved in TNF-α-mediated osteoclastogenesis [33].

M-CSF is involved in recruiting osteoclasts, as demonstrated in mice with mutation in the coding region of the M-CSF; the mice were characterized by osteoclast-deficient osteopetrosis [34]. M-CSF stimulates RANK expression on the cell surface of pre-osteoclasts rendering them reactive to RANKL [35]. Moreover, M-CSF induces osteoclast differentiation via binding to c-fms, a tyrosine kinase receptor that in turn induces ERK1/2 and PI3-K/AKT activation [36].

Role of osteoblasts in bone disorders

Several studies have showed that osteoblasts may be involved in bone disorders, such as osteoporosis, osteopetrosis, osteogenesis imperfecta, and Paget's disease.

Even if osteoporosis is primarily characterized by an imbalance of bone turn-over favoring osteoclastic bone resorption, osteoblasts play a role in this disease. Osteoporosis is mainly observed in postmenopause because of the hormonal modification related to menopause. The decreased levels of estrogen in postmenopausal women are responsible for an increased osteoclastogenesis. In fact, estrogen has been demonstrated to increase OPG levels [37]. Moreover, estrogen has an inhibitory effect on osteoblast production of numerous paracrine factors, including IL-1, IL-6, and TNF-α, which are involved in osteoclastogenesis, and inhibits the transcription factor Egr-1, which is responsible for M-CSF production [38, 39]. In postmenopausal women, the decreased levels of estrogen are correlated with increased levels of IL-1, IL-6, TNF-α, and M-CSF, responsible for an augmented bone resorption.

Osteopetrosis is a descriptive term that refers to a group of rare, heritable disorders of the skeleton where the rate of bone formation is higher than the rate of bone resorption. Osteopetrosis is caused by failure of osteoclast development or function, and mutations in at least 10 genes have been identified as causative in humans, accounting for 70% of all cases. It has been hypothesized that osteopetrosis is characterized by an imbalance between bone formation and bone resorption due to an altered communication between osteoblasts and osteoclasts. It has been demonstrated that in cultured osteoblast-like cells from patients affected by osteopetrosis, the production of osteocalcin, which is a marker for mature osteoblasts, and M-CSF, which is involved in osteoclastogenesis, was inhibited, while normal levels of alkaline phosphatase were detected [40]. These results suggest that osteopetrosis is characterized by a deficient differentiation of pre-osteoblasts into mature osteoblasts, and a reduced maturation and differentiation of osteoclasts.

Osteogenesis imperfecta is a group of genetic bone disorders characterized by fractures with minimal or absent trauma, dentinogenesis imperfecta, and hearing loss. About 90% of individuals with osteogenesis imperfecta types I, II, III, and IV have an identifiable mutation in either gene *COL1A1* or *COL1A2*. In recent years, a role for osteoblasts has been described in three new types of osteogenesis imperfecta. Differently from types I, II, and III, the new types, V, VI, and VII, do not have mutations within type

1 collagen and are characterized by reduced levels of alkaline phosphatase and normal levels of osteocalcin, suggesting the presence of an altered osteoblast differentiation, rather than bone formation [41–44].

A role for osteoblasts has been hypothesized also in Paget's disease. Although its etiology is still unknown, alterations in osteoclast function have been described [45]. Nevertheless, considering that Paget's disease is characterized by a high alkaline phosphatase activity, which is an indicator of high osteoblast activity, the presence of a contextual imperfection in osteoblast function and in the osteoclast-osteoblast interaction has been hypothesized [45, 46]. Some data seem to support the hypothesis that a defect in the RANKL-OPG system is responsible for abnormal osteoclast activity that results in a further osteoblast activation in order to balance the augmented bone resorption. In fact, in patients affected by Paget's disease, high RANKL mRNA transcripts, lower OPG levels, and a major responsiveness of osteoclast precursors to RANKL stimulation have been found in osteoblast-like cells compared to cells from normal patients [47–49].

BPs and osteoblasts

BPs are used to treat numerous diseases, such as osteoporosis, Paget's disease, osteogenesis imperfecta, bone metastases, hypercalcemia of malignancy, fibrous dysplasia, and inflammation-related bone loss.

Already in the 1990s, in vitro studies showed that osteoblasts treated with BPs inhibit osteoclastogenesis [50, 51]. Although their primary action may be an inhibitory effect on osteoclasts, increasing attention is being given to other effector cells that may be influenced by BPs [52]. In recent years, it has been hypothesized that a further target of BPs may be osteoblasts, which subsequently influence osteoclasts. In fact, BPs control osteoblast metabolism, albeit with varying or conflicting effects, depending on the type of BPs used and the different experimental models.

Laboratory data suggest that BPs have a mitogenic effect on osteoblasts [19–21]. Studies have demonstrated that BPs inhibit the expression of RANKL and increase the expression of OPG in human osteoblastic cells, suggesting that the antiresorptive effect of BPs is mediated by osteoblast influence on RANKL signaling [53, 54]. Moreover, other studies have showed that BPs may increase or decrease osteoblastogenesis in relation to their concentration: a pro-osteoblastogenic effect has been seen at lower concentrations of BPs ranging from 10^{-9} to 10^{-6} M, whereas the inhibitory effect has been found at concentrations higher than 10^{-5} M [21, 55–62].

Moreover, BPs may be involved in the treatment of skeletal conditions where macrophage-derived cytokines are important, including arthritis and implant loosening, by reducing the inhibitory effects of macrophages on osteoblasts, as demonstrated in vitro by addition of BPs to co-cultures of osteoblasts and macrophages [63].

By using in vitro models, Im et al. [21] have demonstrated the anabolic effect of alendronate and risedronate on osteoblasts. Following the proliferation of a primary human trabecular bone cell culture and MG-63 osteoblast-like cell line after treatment with BPs, the maturation of osteoblasts was assayed using alkaline phosphatase bioassay and reverse transcription-polymerase chain reaction for markers of osteoblast differentiation. Treatment with BPs appreciably increased the cell number and alkaline phosphatase activity over controls.

Pamidronate and zolendronate are responsible for increasing protein synthesis, secretion of type I collagen, and activity of alkaline phosphatase in osteoblasts even if they inhibit proliferation of other cells, including macrophages, linfocites, myeloma, and lung cancer cells. Their mechanism of action directly involves the cellular metabolism. On the other hand, other BPs, such as etidronate, are characterized by a different mechanism of action based on chelation of divalent ions in the culture medium, which probably is responsible for a different function. In fact, etidronate inhibits in vitro osteoblast proliferation at concentrations greater than pamidronate and zoledronic acid [64].

BPs are involved in inducing osteoblast precursor proliferation and stimulate the development of mineralized nodules in murine and human bone marrow cultures in vitro [65]. In particular, clodronate promotes osteoblast differentiation in cultures of osteoblast-like line cells, such as ST2 and MC3T3-E1 cells, and in rat organ cultures, while etidronate stimulates osteoblast differentiation only in MC3T3-E1 cells [66]. Moreover, etidronate stimulates osteoblastogenesis and wound closure in rat calvaria [67]. In contrast, alendronate and pamidronate show no effect on ST2 and MC3T3-E1 cells or in rat organ cultures of osteoblasts, while alendronate and risedronate significantly increase osteoblast and osteoblast progenitor proliferation in primary human trabecular bone cell culture and in MG-63 osteoblast-like cell line, suggesting that BPs may have different effects on osteoblast formation [66].

By using primary human osteoblast cultures obtained from cancellous bone of osteoarthritic and osteoporotic patients and a corresponding healthy control group, Corrado et al. [68] found that neridronate can modify the metabolic activity of human osteoblasts by enhancing or decreasing their biosynthetic activity, both in normal and in pathological conditions, depending on compound concentration and on different cell types. Recently, these data have been confirmed by using primary human osteoblast cultures obtained from cancellous bone of healthy subjects undergoing bone marrow biopsy, treated with increasing concentrations of zoledronate,

with and without 1,25$(OH)_2$ vitamin D_3. The results of this study have demonstrated that BPs have different cellular biochemical effects depending on dosage and sustain the hypothesis that their positive effect on bone mineral density could be partially due to an anabolic action on osteoblasts [69].

Administration of BPs has been related to apoptosis inhibition on cells of the osteoblastic lineage [70–77]. The existence of different mechanisms of action for the prosurvival effect of BPs has been hypothesized. Recently, Bellido and Plotkin [78] demonstrated that the expression of connexin (Cx) 43 on osteoblast surface is responsible for this anti-apoptotic effect. In fact, the opening of Cx43 hemichannels leads to the sequential phosphorylation of kinases such as Src kinase, extracellular signal-regulated kinases (ERKs), ERK cytoplasmic target p90RSK kinase, BAD, and C/EBPβ, resulting in inhibition of apoptosis.

Concluding remarks

Though therapeutic effects of BPs depend primarily on their inhibitory effect on osteoclasts, increasing attention is being given to other effector cells, such as osteoblasts. BPs may stimulate proliferation of osteoblasts and inhibit apoptosis of osteocytes and osteoblasts. Although in vitro studies have demonstrated a role for BPs in osteoblast stimulation, effects of BPs on osteoblasts in vivo remain unclear because of numerous indirect effects on the remodeling cycle mediated through reduction of bone resorption. Indeed, in vitro studies with BPs require a careful interpretation with regard to the presence of many confounding factors, such as different BP concentrations and different models used, which may explain the presence of contrasting results. The significance of these data needs to be assessed considering that in vivo osteoblasts are exposed to different BPs concentrations in the bone microenvironment.

Considering that osteoblasts may be involved in bone disorders such as osteoporosis, osteopetrosis, osteogenesis imperfecta, and Paget's disease, it is conceivable that there is a role for BPs in these diseases that goes beyond the mere osteoclast influence.

Conflict of interest The authors declare that they have no conflict of interest.

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