Bisphosphonates: Mechanisms of Action

Gideon A. Rodan* and Herbert A. Fleisch‡
**Department of Bone Biology & Osteoporosis, Merck Research Laboratories, West Point, Pennsylvania 19486; and* ‡*Department of Pathophysiology, University of Berne, Berne 3010, Switzerland*

Introduction

Bisphosphonates are pyrophosphate analogues in which the oxygen bridge has been replaced by a carbon with various side chains (P-C-P). These compounds have been known to the chemists since the 19th century, the first synthesis dating back to 1865 (1). They were first used in various industrial procedures, among others as anticorrosive and antiscaling agents (2). After discovering that they can effectively control calcium phosphate formation and dissolution in vitro (3, 4), as well as mineralization and bone resorption in vivo (3, 4), they were developed and used in the treatment of bone diseases, mostly Paget's disease, hypercalcemia of malignancy, and, lately, osteoporosis (for review see reference 5).

General properties

The bisphosphonate group, like pyrophosphate, binds strongly to the bone mineral, hydroxyapatite (6). This explains the specific pharmacological action of these compounds on mineralized tissues, especially bone. Indeed, they deposit where bone mineral is exposed to the surrounding fluids, thus, especially where bone is formed and resorbed. The release of bisphosphonates occurs primarily when the bone where they are deposited is resorbed again, accounting for their long in vivo half-life in humans (7). The P-C-P group is resistant to enzymatic hydrolysis, which explains why bisphosphonates are not metabolized in the body and are excreted unaltered. Bisphosphonates released during bone remodeling might therefore be pharmacologically active. The amount of bisphosphonate released daily in this manner after 10 yr of treatment is estimated at $\sim$ 25% of the amount absorbed from a daily dose and depends upon the location in the skeleton. It will be higher for cancellous, lower for compact bone. The charge and bulk of the bisphosphonate group limits penetration of cell membranes. This may account for the low absorption in the gut (between below 1 and 10%), which is probably paracellular (8), and the limited cellular uptake. Bisphosphonates are rapidly excreted by the kidney, in part by an active tubular secretion process (9).

Address correspondence to Gideon A. Rodan, M.D., Ph.D., Department of Bone Biology & Osteoporosis Research, Merck Research Laboratories, West Point, PA 19486. Phone: 215-652-7477; FAX: 215-652-4328; E-mail: rodan@merck.com

Received for publication 15 April 1996 and accepted in revised form 23 April 1996.

J. Clin. Invest.

0021-9738/96/06/2692/05 $2.00
Volume 97, Number 12, June 1996, 2692–2696

The key pharmacological action of bisphosphonates in clinical use (or development) is inhibition of bone resorption. The mechanism of action of these agents has not been fully elucidated and could differ among bisphosphonates. The aim of this perspective is to review the current knowledge of this subject, to better understand the pharmacology of these compounds and gain further insights into the mechanisms of bone resorption in general.

Bone resorption

The effects of bisphosphonates can be considered at three levels: the tissue, the cellular, and the molecular ones.

Tissue level. At the tissue level, the action of all active bisphosphonates appears to be similar: a reduction in bone turnover. This is evidenced by a decrease in both bone resorption and bone formation, as assessed by biochemical markers. Since during bone loss, there is a negative balance at each turnover unit (BMU), a decrease in turnover leads to a decrease in bone loss. Bone resorption is difficult to measure quantitatively by morphological methods because no specific method, such as double tetracycline labeling used for formation, is available. However, bisphosphonates reduce resorption parameters, such as the extent of the actively resorbing area and the depth of the erosion measured from the bone surface (10–12).

Besides resorption, formation is decreased too, as evidenced by a reduction in the bone formation surface (10–13). Since there is no evidence for reduced osteoblastic activity at individual bone formation sites, as judged by the amount of bone produced per unit time (10–13), it is concluded that the reduction in total bone formation surface is secondary to diminished resorption and reflects reduced remodeling. The link between bone resorption and formation has been called "coupling". Furthermore, the amount of bone formed at each individual basic multicellular unit (BMU), measured by the thickness of the newly formed bone, is not decreased but, if anything, even increased. This interesting observation was reported for several bisphosphonates (10–13). However, since the augmentation is small, it needs to be confirmed. This finding is also consistent with the observation that clodronate increases collagen synthesis by bone and cartilage cells in culture (14) and that alendronate increases mineralized nodule formation in bone marrow cultures (15).

Bisphosphonates produce a positive calcium balance in animals (16) and increase the amount of bone in animals (17) and in humans (18–22). There are several explanations for this gain, one of which is inherent to bone turnover. Thus, a decrease in bone resorption is not immediately followed by the "coupling-induced" diminution of formation, since formation at remodeling sites initiated before treatment will proceed to

completion. This will produce a relatively rapid (4–6 mo) gain in bone mass, through a reduction in the so-called remodeling space. The second explanation is that after the decrease in turnover, the newly formed bone will have less chance to be remodeled and will therefore have more time to complete mineralization. It has been shown that 70% of mineralization of newly formed bone occurs within the first few weeks, but mineral enrichment continues for a long time, thus "older" bone has a higher mineral content (23). A reduction in turnover would, therefore, increase the average mineral content. Thirdly, if the decrease in resorption depth at individual remodeling sites is not matched by a decrease in formation, the local bone balance will be positive.

To summarize, at the tissue level the data for all active bisphosphonates are consistent with decreased bone turnover resulting from decreased resorption. At the level of individual remodeling units, there may be a positive focal bone balance: formation exceeding resorption.

Cellular level. At the cellular level, there is general agreement that the final target of bisphosphonate action is the osteoclast (Fig. 1). Bisphosphonates could reduce osteoclastic bone resorption through: (*a*) inhibition of osteoclast recruitment to the bone surface; (*b*) inhibition of osteoclast activity on the bone surface; (*c*) shortening of the osteoclast life span; and (*d*) alteration of the bone or bone mineral in ways which reduce, by a pure physicochemical and not a cellular mechanism, the rate of its dissolution. The first three effects could be due to direct action on the osteoclast, or indirectly via action on cells which modulate the osteoclast.

(a) Inhibition of osteoclast recruitment to the bone surface: In vitro, several bisphosphonates inhibit osteoclast differentiation from bone marrow (24). In a coculture system of mouse osteoblast-derived cells and bone marrow cells, alendronate inhibits $1,25(OH)_2D_3$-induced osteoclastogenesis with an IC_{50} of 10–30 μM (25).The bisphosphonate is fully effective in this system when added immediately before the fusion of mononuclear osteoclast precursors to form multinucleated cells. Osteoclast recruitment is also inhibited in cultures of fetal mouse calvaria (26) and mice radii metatarsals (27). Furthermore, bisphosphonates are powerful inhibitors of macrophage proliferation (28). However, several facts suggest that this is not the primary or at least not the only mode of action of bisphosphonates in vivo, since, after bisphosphonate administration, the number of multinucleated osteoclasts on the bone surface often first increases, but the cells look inactive (17). It is only later, after chronic administration, that the osteoclast number decreases. The cause for the initial increase is unknown, it could reflect a stimulation of osteoclast formation to compensate for the decrease in osteoclast activity. Nevertheless, it seems that the potency rank of bisphosphonates, when assessed in vitro, correlates with effects in vivo only when systems which detect osteoclast recruitment, and not only activity, are used.

The effect on osteoclast recruitment may be direct and/or indirect. Recent results speak for the latter. It is now generally accepted that cells of osteoblastic lineage control the recruitment and activity of osteoclasts under physiological and pathological conditions. This control was thought to be due to the production of an as yet unknown activity, generated by osteoblast-lineage cells stimulating bone resorption. Recently, it was shown that when rat osteoblastic cells are briefly (29) or continuously (30) exposed to low concentrations of potent bisphosphonates, their conditioned medium contains a factor(s) that reduces osteoclastic bone resorption in culture.The decrease in resorption was subsequently found to be due to an inhibitor of osteoclast recruitment or survival, with a molecular weight between 1 and 10 kD, released by the osteoblasts (30, 31). Whether the action of the inhibitor also involves cell survival is not yet known, nor is it known which cells of the osteoblastic lineage are involved in this mechanism. The lining cells would be an interesting possibility (32).

(b) Inhibition of osteoclast activity on the bone surface: Inhibition of osteoclast activity on the bone surface is strongly supported as part of the mechanism of action, at least for some bisphosphonates, by the fact that osteoclasts show changes in morphology both in vitro (33, 34) and in treated animals in vivo (17). These include changes in the cytoskeleton, especially actin (33), and in the ruffled border (33, 35, 36), the convoluted membrane characteristic of active osteoclasts. This mode of action is also supported by: (*i*) the fact that under certain conditions bisphosphonates can enter cells (37, 38), especially those of the macrophage lineage; and (*ii*) the detection of radioactive alendronate exclusively inside osteoclasts but no other cells on autoradiographs of bone and bone marrow 12 h

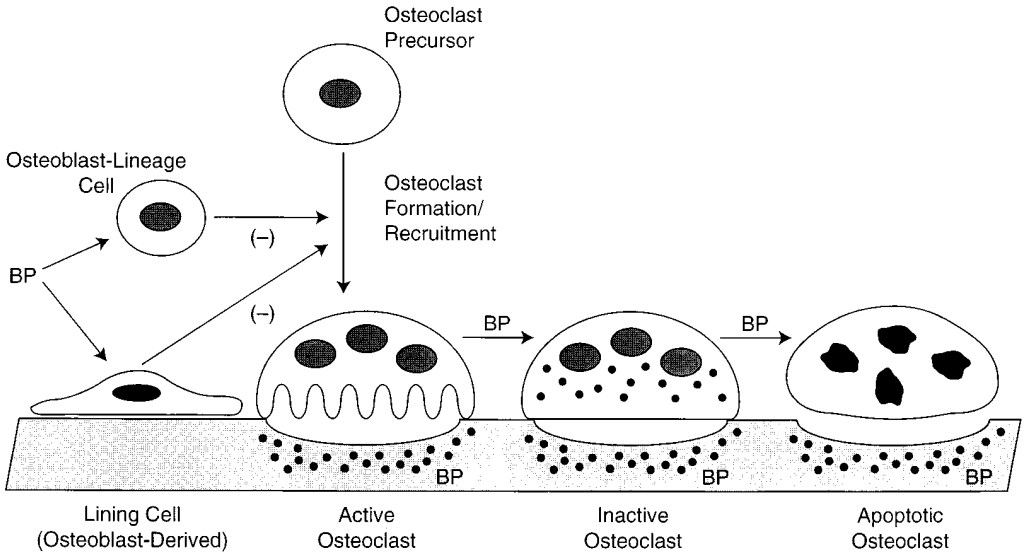

Figure 1. Schematic presentation of proposed mechanisms of action of bisphosphonates.

after administration (36, 39). This selective uptake of bisphosphonates by osteoclasts could be due to the higher concentration present on the bone surface in the resorption space (36, 40) and the phagocytic nature of these cells. At pH 3.5, which is likely to be present in the resorption space, 50% of the alendronate, and probably of other bisphosphonates on the bone surface, is released into the lacunae, thus leading to a local concentration which can reach several hundred μM (36).

(c) Shortening of the osteoclast life span: The life span of osteoclast nuclei has been estimated from histomorphometric studies at ~ 2 wk (32). However, the estimation of the osteoclast life span is difficult and imprecise since it is thought that nuclei are added and released from these cells. It has been proposed that bisphosphonates can reduce the number of osteoclasts through a toxic effect, but the results were obtained at very high concentrations. Changes in morphology, but no osteoclast toxicity, were apparent at the light or EM level in bone sections from animals treated with alendronate (34, 36, 39). Recently, it was reported that risedronate increases osteoclast apoptosis, a phenomenon which is different from toxic cell death, and this was proposed as a general mechanism for bisphosphonate action (41). It is, however, not clear whether apoptosis is directly induced by bisphosphonates and, if so, by what mechanism.

(d) Alteration of bone mineral: The earliest hypothesis for bisphosphonate action on bone proposed physical effects on mineral dissolution. Although bisphosphonates, like pyrophosphate, could affect this process, it is now obvious that the low concentration of bisphosphonates, and especially of the newer more potent ones necessary to produce a pharmacological effect, has no significant impact on mineral dissolution and that their action is therefore cellular. Moreover, structure/activity comparisons (42) showed that inhibition of mineral dissolution by bisphosphonates does not correlate with pharmacological activity, which is due to cellular action.

To summarize bisphosphonate effects at the cellular level, there is general agreement that they act directly or indirectly on the osteoclasts. The effect can be on the formation of osteoclasts and/or on their activity. A decrease in osteoclast number can occur either through direct action on osteoclast precursors, or indirectly by stimulating the osteoblasts to produce an inhibitor of osteoclast formation. Osteoclast inactivation is associated with bisphosphonate uptake from the bone surface. Furthermore, the bisphosphonates may act by shortening the life span of the osteoclasts, possibly through apoptosis. At present it is not known which of the mechanisms, the indirect effect on osteoclast recruitment and/or survival or the direct effect on osteoclast activity and/or survival is more important in vivo. The initial theory that resorption is decreased because of a physicochemical decrease in mineral dissolution, due to adsorption of the compound on the crystal surface, is no longer tenable.

Molecular level. The chain of events which leads to osteoclast inactivation or decreased formation, following direct or indirect exposure to a given bisphosphonate, has not yet been elucidated. The possibilities include either bisphosphonate action on a cell surface "receptor" and/or bisphosphonate uptake by the cell, where it interacts with an enzyme or other molecule, affecting cellular metabolism. The receptor hypothesis is favored for the osteoblast-mediated effect, since only a short exposure to low concentrations is needed (29). Candidate receptors would include purinergic receptors, based on (limited) structure homology. One of the signaling pathways via these receptors includes changes in intracellular calcium. To date, there is, however, no evidence for detectable activation of the osteoblast by bisphosphonates via this or any other signal transduction pathway, such as changes in cyclic nucleotides, inositol triphosphate, intracellular calcium, or others, which would support the receptor hypothesis. The other possibility is bisphosphonate modulation of cellular functions through interference with an intracellular biochemical pathway. This would require cellular uptake of the compounds as evidenced for various bisphosphonates (36–39). Since the lipid bilayer of the cell membrane is not readily permeable to bisphosphonates, such uptake should occur via fluid pinocytosis or adsorptive pinocytosis (43). These mechanisms may explain the selective effects of bisphosphonates on macrophages, in addition to osteoclasts, manifested by a decrease in proliferation (28) or as an acute phase response caused in some patients receiving relatively high doses of aminobisphosphonates intravenously (44) and which is thought to act through an increase in IL-6 release (45).

Recently, the slime mold *Dictyostelium discoideum* was shown to take up bisphosphonates by pinocytosis (46). In these cells, clodronate, but not other pharmacologically active bisphosphonates, was incorporated into adenine nucleotides, which could potentially explain why this bisphosphonate sometimes seems to act differently than the other bisphosphonates. There was a good correlation between the effect of various bisphosphonates on the multiplication of the amoebae and their effect on bone resorption in vivo. This correlation suggests that this model may help us understand the uptake by cells and perhaps the intracellular mechanism of action.

It has been known for a long time that bisphosphonates decrease acid production of various cells (37). Since proton extrusion into the resorption space is considered of importance for osteoclast activity, bisphosphonates were investigated as to their effect on this process. Osteoclasts seeded on bone were shown to extrude acid by a sodium-independent mechanism (47), and this process was inhibited by alendronate. The vacuolar type proton ATPase appears to be responsible in part for this extrusion. When this proton pump was assayed in inside-out osteoclast vesicles in vitro, tiludronate was found to be a potent inhibitor (48), while other bisphosphonates more potent in vivo were not inhibitory. However, it should be emphasized again that the various bisphosphonates do not necessarily have a common mechanism of action, especially at the molecular level.

In view of the homology to pyrophosphate, the effect of bisphosphonates on enzyme systems involving pyrophosphate or ATP has been examined. Bisphosphonates did not inhibit alkaline or acid phosphatases (25, 49), which can act as pyrophosphatases or, if they did so, only at millimolar concentrations at which the effect could have been due to metal chelation (49). Nonreceptor tyrosine kinases and serine/threonine phosphatase were not inhibited either (25). Only two groups of enzymes were shown to be inhibited by bisphosphonates: squalene synthase, a lipid metabolism enzyme which has farnesyl pyrophosphate as its substrate (50), and protein tyrosine phosphatases (PTP),[1] which control tyrosine phosphorylation

1. *Abbreviation used in this paper:* PTP, Protein tyrosine phosphate.

involved in signal transduction pathways (25, 51). Squalene synthase inhibition would control cholesterol biosynthesis, but its direct link to the formation of osteoclast ruffled border is not immediately apparent, although a change in membrane rigidity may be induced, but there is no indication that this effect is related to the inhibition of bone resorption. Alendronate did not inhibit farnesyl transferase (unpublished data) which is required for the membrane binding of many proteins.

The tyrosine phosphatases balance the activity of tyrosine kinases in signal transduction pathways initiated by growth factors, insulin, and other stimuli. It is noteworthy that in c-src "knock-out" mice the osteoclasts are inactive and lack ruffled border (52) and that dephosphorylation of tyrosine 527 is required for c-src activation (53). All bisphosphonates examined so far (alendronate, etidronate, pamidronate, tiludronate) inhibit PTPs (54). Their relative potency depends on the specific PTP examined and on the substrate used. However, the evidence available so far has not identified a specific PTP as a bisphosphonate target, and the potency of various bisphosphonates in inhibiting the PTPs tested so far (PTPε, PTPσ, PTP1B, and CD45) does not correspond to their pharmacological potency. But the overall in vivo potency could also be influenced by the distribution of bisphosphonates on the bone surface and by their cellular uptake in osteoclasts (33,36) or cells of the mononuclear phagocyte system (28). In view of the involvement of these enzymes in cellular processes associated with cytoskeletal function and vesicular traffic, they are a good choice for possible molecular targets for bisphosphonate action, worthy of further investigation.

In conclusion, although the detailed mechanism of action of bisphosphonates has not been elucidated, it is clear that at the tissue level all active bisphosphonates inhibit bone resorption, bone turnover, and, therefore, bone loss. The effect is due to a decrease in the generation of new bone remodeling units and a decrease in the depth of the erosion cavities. At the cellular level, bisphosphonates inactivate osteoclastic bone resorption directly and/or indirectly. They induce the disappearance of osteoclast ruffled border and inhibit proton extrusion. At the molecular level, tiludronate, but not other bisphosphonates, inhibits vacuolar ATPase; several bisphosphonates inhibit squalene synthase, and all bisphosphonates tested inhibit protein tyrosine phosphatase. The link of these effects to the in vivo action of the respective bisphosphonates remains to be established. Further insights into the molecular basis of bisphosphonate action could come from the identification of other molecules, such as receptors, channels, enzymes, or signal transduction mediators, for which it can be shown that they interact directly with bisphosphonates at pharmacological concentrations and are required for bisphosphonate effects on bone cells.

References

1. Menschutkin, N. 1865. Ueber die Einwirkung des Chlorazetyls auf phosphorige Säure. *Ann. Chem. Pharm.* 133:317–320.

2. Blomen, L.J.M.J. 1995. History of bisphosphonates: Discovery and history of the non-medical uses of bisphosphonates. *In* Bisphosphonate on Bones. O.L.M. Bijvoet, H.A. Fleisch, R.E. Canfield, R.G.G. Russell, editors. Elsevier Science Publishers, B.V., Amsterdam. 11–124.

3. Fleisch, H., R.G.G. Russell, and M.D. Francis. 1969. Diphosphonates inhibit hydroxyapatite dissolution in vitro and bone resorption in tissue culture and in vivo. *Science (Wash. DC).* 165:1262–1264.

4. Francis, M.D., R.G.G. Russell, and H. Fleisch. 1969. Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcification in vivo. Science *(Wash. DC).* 165:1264–1266.

5. Fleisch, H. 1995. Bisphosphonates in bone disease. From the laboratory to the patient. The Parthenon Publishing Group, New York/London. pp. 176.

6. Jung, A., S. Bisaz, and H. Fleisch. 1973. The binding of pyrophosphate and two diphosphonates on hydroxyapatite crystals. *Calcif. Tissue Res.* 11:269–280.

7. Kasting, G.B., and M.D. Francis. 1992. Retention of etidronate in human, dog, and rat. *J. Bone Miner. Res.* 7:513–522.

8. Boulenc, X., E. Marti, H. Joyeux, C. Roques, Y. Berger, and G. Fabre. 1993. Importance of the paracellular pathway for the transport of new bisphosphonate using the human CACO-2 monolayers model. *Biochem. Pharmacol.* 46:1591–1600.

9. Troehler, U., J.P. Bonjour, and H. Fleisch. 1975. Renal secretion of diphosphonates in rats. *Kidney Int.* 8:6–13.

10. Storm, T., T. Steiniche, G. Thamsborg, and F. Melsen. 1993. Changes in bone histomorphometry after long-term treatment with intermittent, cyclic etidronate for postmenopausal osteoporosis. *J. Bone Miner. Res.* 8:199–208.

11. Boyce, R.W., C.L. Paddock, J.R. Gleason, W.K. Sietsma, and E.F. Eriksen. 1995. The effects of risedronate on canine cancellous bone remodeling: Three-dimensional kinetic reconstruction of the remodeling site. *J. Bone Miner. Res.* 10:211–221.

12. Ott, S.M. 1993. Clinical effects of bisphosphonates in involutional osteoporosis. *J. Bone Miner. Res.* 8:S597–S606.

13. Balena, R., B.C. Toolan, M. Shea, A. Markatos, E.R. Myers, S.C. Lee, E.E. Opas, J.G. Seedor, H. Klein, D. Frankenfield, et al. 1993. The effects of 2-year treatment with the aminobisphosphonate alendronate on bone metabolism, bone histomorphometry, and bone strength in ovariectomized nonhuman primates. *J. Clin. Invest.* 92:2577–2586.

14. Guenther, H.L., H.E. Guenther, and H. Fleisch. 1981. The influence of 1-hydroxyethane-1,1-diphosphonate and dichloromethanediphosphonate on lysine hydroxylation and cross-link formation in rat bone, cartilage and skin collagen. *Biochem. J.* 196:303–310.

15. Giuliani, N., G. Girasole, M. Pedrazzoni, G. Passeri, C. Gatti, and M. Passeri. 1995. Alendronate stimulates b-FGF production and mineralized nodule formation in human osteoblastic cells and osteoblastogenesis in human bone marrow cultures. *J. Bone Miner. Res.* 10(Suppl. 1):S171. (Abstr.)

16. Gasser, A.B., D.B. Morgan, H.A. Fleisch, and L.J. Richelle. 1972. The influence of two diphosphonates on calcium metabolism in the rat. *Clin. Sci.* 43: 31–45.

17. Schenk, R., W.A. Merz, R. Mühlbauer, R.G.G. Russell, and H. Fleisch. 1973. Effect of ethane-1-hydroxy-1,1-diphosphonate (EHDP) and dichloromethylene diphosphonate (Cl_2MDP) on the calcification and resorption of cartilage and bone in the tibial epiphysis and metaphysis of rats. *Calcif. Tissue Res.* 11:196–214.

18. Watts, N.B., S.T. Harris, H.K. Genant, R.D. Wasnich, P.D. Miller, R.D. Jackson, A.A. Licata, P. Ross, G.C. Woodson, M.J. Yanover, et al. 1990. Intermittent cyclical etidronate treatment of post menopausal osteoporosis. *N. Engl. J. Med.* 323:73–79.

19. Harris, S.T., N.B. Watts, R.D. Jackson, H.K. Genant, R.D. Wasnich, P. Ross, P.D. Miller, A.A. Licata, and C.H. Chesnut III. 1993. Four-year study of intermittent cyclic etidronate treatment of postmenopausal osteoporosis: Three years of blinded therapy followed by one year of open therapy. *Am. J. Med.* 95: 557–567.

20. Giannini, S., A. D'Angelo, L. Malvasi, R. Castrignano, T. Pati, R. Tronca, L. Liberto, M. Nobile, and G. Crepaldi. 1993. Effects of one-year cyclical treatment with clodronate on postmenopausal bone loss. *Bone (Tarrytown).* 14:137–141.

21. Reid, I.R., D.J. Wattie, M.C. Evans, G.D. Gamble, J.P. Stapleton, and J. Cornish. 1994. Continuous therapy with pamidronate, a potent bisphosphonate, in postmenopausal osteoporosis. *J. Clin. Endocrinol. & Metab.* 79:1595–1599.

22. Liberman, U.A., S.R. Weiss, J. Bröll, H.W. Minne, H. Quan, N.H. Bell, J. Rodriguez-Portales, R.W. Downs, Jr., J. Dequekker, M. Favus, et al. 1995. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. *N. Engl. J. Med.* 333:1437–1443.

23. Amprino, R., and A. Engstrom. 1952. Studies on x-ray absorption and diffraction of bone tissue. *Acta Anatomica.* 15:1–21.

24. Hughes, D.E., B.R. MacDonald, R.G.G. Russell, and M. Gowen. 1989. Inhibition of osteoclast-like cell formation by bisphosphonates in long-term cultures of human bone marrow. *J. Clin. Invest.* 83:1930–1935.

25. Schmidt, A., S.J. Rutledge, N. Endo, E.E. Opas, H. Tanaka, G. Wesolowski, C.T. Leu, Z. Huang, C. Ramachandaran, S.B. Rodan, and G.A. Rodan. 1996. Protein-tyrosine phosphatase activity regulates osteoclast formation and function: inhibition by alendronate. *Proc. Natl. Acad. Sci. USA.* 93:3068–3073.

26. Evans, C.E., and I.P. Braidman. 1994. Effects of two novel bisphosphonates on bone cells in vitro. *Bone Miner.* 26:95–107.

27. Boonekamp, P.M., L.J.A. van der Wee-Pals, M.M.L van Wijk-van Lennep, C.W. Thesing, and O.L.M. Bijvoet. 1986. Two modes of action of bisphosphonates on osteoclastic resorption of mineralized matrix. *Bone Miner.* 1:27–39.

28. Cecchini, M.G., R. Felix, H. Fleisch, and P.H. Cooper. 1987. Effects of bisphosphonates on proliferation and viability of mouse bone marrow-derived macrophages. *J. Bone Miner. Res.* 2:135–142.

29. Sahni, M., H.L. Guenther, H. Fleisch, P. Collin, and T.J. Martin. 1993.

Bisphosphonates act on rat bone resorption through the mediation of osteoblasts. *J. Clin. Invest.* 91:2004–2011.

30. Nishikawa, M., T. Akatsu, Y. Katayama, Y. Yasutomo, S. Kado, N. Kugai, M. Yamamoto, and N. Nagata. 1996. Bisphosphonates act on osteoblastic cells and inhibit osteoclast formation in mouse marrow cultures. *Bone (Tarrytown).* 18:9–14.

31. Vitté, C., H. Fleisch, and H.L. Guenther. 1996. Bisphosphonates induce osteoblasts to secrete an inhibitor of osteoclast-mediated resorption. *Endocrinology*. 137:2324–2333.

32. Parfitt, A.M., G.R. Mundy, G.D. Roodman, D.E. Hughes and B.F. Boyce. 1996. A new model for the regulation of bone resorption, with particular reference to the effects of bisphosphonates. *J. Bone Miner. Res.* 11:150–159.

33. Murakami, H., N. Takahashi, T. Sasaki, N. Udagawa, S. Tanaka, I. Nakamura, D. Zhang, A. Barbier, and T. Suda. 1995. A possible mechanism of the specific action of bisphosphonates on osteoclasts: tiludronate preferentially affects polarized osteoclasts having ruffled borders. *Bone (Tarrytown).* 17:137–144.

34. Sato, M., and W. Grasser. 1990. Effects of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. *J. Bone Miner. Res.* 5: 31–40.

35. Plasmans, C.M.T., P.H.K. Jap, W. Kuypers, and T.J.J. Slooff. 1980. Influence of a diphosphonate on the cellular aspect of young bone tissue. *Calcif. Tissue Int.* 32:247–256.

36. Sato, M., W. Grasser, N. Endo, R. Akins, H. Simmons, D.D. Thompson, E. Golub, and G.A. Rodan. 1991. Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure. *J. Clin. Invest.* 88: 2095–2105.

37. Fast, D.K., R. Felix, C. Dowse, W.F. Neuman, and H. Fleisch. 1978. The effects of diphosphonates on the growth and glycolysis of connective-tissue cells in culture. *Biochem. J.* 172:97–107.

38. Felix, R., H.L. Guenther, and H. Fleisch. 1984. The subcellular distribution of ^{14}C dichloromethylenebisphosphonate and ^{14}C 1-hydroxyethylidene-1,1-bisphosphonate in cultured calvaria cells. *Calcif. Tissue Int.* 36:108–113.

39. Masarachia, P., M. Weinreb, R. Balena, and G.A. Rodan. 1995. Comparison of the distribution of ^{3}H-alendronate and ^{3}H-etidronate in rat and mouse bones. *J. Bone Miner. Res.* 10(Suppl. 1):S250. (Abstr.)

40. Azuma, Y., H. Sato, Y. Oue, K. Okabe, T. Ohta, M. Tsuchimoto, and M. Kiyoki. 1995. Alendronate distributed on bone surfaces inhibits osteoclastic bone resorption in vitro and in experimental hypercalcemic models. *Bone (Tarrytown).* 16:235–245.

41. Hughes, D.E., K.R. Wright, H.L. Uy, A. Sasaki, T. Yoneda, G.D. Roodman, G.R. Mundy, and B.F. Boyce. 1995. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J. Bone Miner. Res.* 10:1478–1487.

42. Shinoda, H., G. Adamek, R. Felix, H. Fleisch, R. Schenk, and P. Hagan. 1983. Structure-activity relationships of various bisphosphonates. *Calcif. Tissue Int.* 35:87–99.

43. Duncan, R., and M.K. Pratten. 1985. Pinocytosis: mechanism and regulation. *In* Mononuclear Phagocytes: Physiology and Pathology. Research Monographs in Cell and Tissue Physiology 11. J.T. Dingle and J.L. Gordon, editors. Elsevier Science Publishers, B.V., Amsterdam/New York. 27–51.

44. Adami, S., A.K. Bhalla, R. Dorizzi, F. Montesanti, S. Rosini, G. Salvagno, and V. Lo Cascio. 1987. The acute-phase response after bisphosphonate administration. *Calcif. Tissue Int.* 41:326–331.

45. Schweitzer, D.H., M. Oostendorp-van de Ruit, G. van der Pluijm, C.W.G.M. Löwik, and S.E. Papapoulos. 1995. Interleukin-6 and the acute phase response during treatment of patients with Paget's disease with the nitrogen-containing bisphosphonate dimethylaminohydroxypropylidene bisphosphonate. *J. Bone Miner. Res.* 10:956–962.

46. Rogers, M.J., D.J. Watts, R.G.G. Russell, X. Ji, X. Xiong, G.M. Blackburn, A.V. Bayless, and F.H. Ebetino. 1994. Inhibitory effects of bisphosphonates on growth of amoebae of the cellular slime mold dictostelium discoideum. *J. Bone Miner. Res.* 9:1029–1039.

47. Zimolo, Z., G. Wesolowski, and G.A. Rodan. 1995. Acid extrusion is induced by osteoclast attachment to bone: inhibition by alendronate and calcitonin. *J. Clin. Invest.* 96:2277–2283.

48. David, P., H. Nguyen, H. Barbier, and R. Baron. 1995. Tiludronate is a potent and specific inhibitor of the osteoclast vacuolar H^{+}ATPase. *Bone (Tarrytown).* 16: 166S. (Abstr.)

49. Felix, R., R.G.G. Russell, and H. Fleisch. 1976. The effect of several diphosphonates on acid phosphohydrolases and other lysosomal enzymes. *Biochim. Biophys. Acta.* 429:429–438.

50. Ciosek, C.P., Jr., D.R. Magnin, T.W. Harrity, J.V.H. Logan, J.K. Dickson, Jr., E.M. Gordon, K.A. Hamilton, K.G. Jolibois, L.K. Kunselman, R.M. Lawrence, et al. 1993. Lipophilic 1,1-bisphosphonates are potent squalene synthase inhibitors and orally active cholesterol lowering agents in vivo. *J. Biol. Chem.* 268:24832–24837.

51. Endo, N., S.J. Rutledge, E.E. Opas, R. Vogel, G.A. Rodan, and A. Schmidt. 1996. Human protein tyrosine phosphatase-σ: alternative splicing and inhibition by bisphosphonates. J. Bone Min. Res. 11(4):535–543.

52. Boyce, B.F., T. Yoneda, C. Lowe, P. Soriano, and G.R. Mundy 1992. Requirement of pp60$^{c\text{-}src}$ expression for osteoclasts to form ruffled borders and resorb bone in mice. *J. Clin. Invest.* 90:1622–1627.

53. Cooper, J.A., and B. Howell. 1993. The when and how of src regulation. *Cell.* 73:1051–1054.

54. Schmidt, A., S.J. Rutledge, E.E. Opas, G. Wesolowski, C.T. Leu, S.B. Rodan, and G.A. Rodan. 1995. Protein tyrosine phosphatase activity regulates osteoclast formation and function: inhibition by alendronate. *Mol. Biol. Cell.* 6(Suppl.):136*a*. (Abstr.)