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Bisphosphonates, Specific Inhibitors of Osteoclast Function and a Class of Drugs for Osteoporosis Therapy

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ABSTRACT

Osteoporosis is a result of the disruption of bone homeostasis that is carried out by bone-forming osteoblasts and bone-degrading osteoclasts. The most common treatment of osteoporosis is N-containing bisphosphonates, a class of non-hydrolyzable pyrophosphate analogs. They have strong affinity to Ca^{2+} of hydroxyapatite with high specificity and can only be liberated from the bone in an acidic environment. These properties bestow them unique pharmacokinetic features including specific and strong retention at bone resorption surface, uptaken specifically by osteoclasts, quick excretion of non-retained free bisphosphonates, long half-life, and recyclability. Such properties underlie the drugs' high efficacy, minor side effects, and intermittent dosing regimens. Further studies show that bisphosphonates inhibit farnesyl pyrophosphate synthase, a critical enzyme required for synthesis of isoprenyl and geranylgeranyl, and inhibit prenylation and geranylgeranylation of small G-proteins such as Rac and Rho. This leads to defective actin ring formation at the sealed zone, a subcellular structure essential for bone resorption, and a decrease in bone resorption. Bisphosphonates are also used to treat Paget's disease of bone, osteolytic bone metastases, and hypercalcemia. Moreover, these properties also make N-BPs a good candidate as a bone-seeking agent. Here we update our understanding of this remarkable class of anti-resorption drugs. J. Cell. Biochem. 112: 1229–1242, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: OSTEOCLAST; OSTEOPOROSIS; BISPHOSPHONATES

Osteoporosis is an aging-related disorder that constitutes a global health threat, as it affects 50% of women and 25% of men over the age of 50. The major features of osteoporosis include decreased bone density and mass, deterioration of microstructure, and increased risk of fractures [Rodan, 1992; Manolagas and Jilka, 1995; Goltzman, 2002]. With ageing, bone regeneration gradually slows down and bone fractures are getting more difficult to heal, leading to morbidity and mortality in aged population. The major organic component of bone is type I collagen and the major inorganic component is hydroxyapatite with a formula of $Ca_5(PO_4)_3(OH)$, with the latter making up of 70% of the bone. Bone formation, including synthesis of bone matrix proteins and matrix mineralization, are carried out by bone forming cells, osteoblasts. Unlike most other organs, the bone contains a class of cells whose function is opposite of osteoblasts, namely osteoclasts. Osteoclasts have bone resorption activity and can cause bone degradation and loss. Due to the two antagonistic cell types, the bone is constantly remodeled, with about 3% of cortical bones and 15% of trabecular bones being replaced by newly formed bones every year, in adulthood [Manolagas and Jilka, 1995]. This remodeling process is believed to help the bone to adapt to mechanical stress and to repair bone microdamages. In normal young adults, bone formation and resorption are coordinated so that the bone density and mass are kept at relatively constant levels. Osteoporosis is a result of disruption of this balance, with bone resorption outweighing formation.

Bone forming osteoblasts are derived from bone marrow mesenchymal stem cells (MSCs), which also have the potential to differentiate into myoblasts, adipocytes, and chondrocytes [Boyle et al., 2003; Harada and Rodan, 2003]. These cell fates are determined by a combination of growth factors, cytokines, and hormones. BMPs and Wnts are two major classes of growth factors

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that control osteoblast differentiation from MSCs [Cao and Chen, 2005; De Biase and Capanna, 2005; Glass and Karsenty, 2007]. They up-regulate the expression of master transcription factors Runx2 and Osterix, which are sufficient and necessary for osteoblast differentiation [Zhang et al., 2000; Lian et al., 2004; Stein et al., 2004; Celil and Campbell, 2005; Wang et al., 2007; Karsenty, 2008]. Bone resorbing osteoclasts are derived from bone marrow hematopoietic stem cells (HSCs) and share the same precursor with macrophages. RANK ligand (receptor activator of nuclear factor κB), M-CSF (macrophage colony-stimulating factor), and some interleukins are essential for osteoclastogenesis, while OPG (osteoprotegerin), a decoy RANKL receptor that binds to RANKL, acts as a negative regulator [Boyle et al., 2003; Martin and Sims, 2005]. The ratio of RANKL to OPG determines the rate of osteoclastogenesis. RANKL is a member of the tumor necrosis family (TNF), and it has been shown that RANKL knockout mice exhibit increased bone mass due to the defect in osteoclastogenesis [Dougall et al., 1999]. On the other hand, OPG knockout mice show increased bone turnover and severe osteoporosis, accompanied by an increase in osteoclastogenesis [Bucay et al., 1998]. Interestingly, RANKL, M-CSF, and OPG can be synthesized by osteoblasts, indicating a coupling mechanism between osteoblasts and osteoclasts.

For the purpose of this review, osteoclasts will be the main focus for discussion. Mature osteoclasts are large multinuclear cells that are rich in vesicles and vacuoles, which facilitate bone resorption [Vaananen et al., 2000; Teitelbaum and Ross, 2003]. The attachment of the osteoclast's plasmalemma to bone surface forms a sealed zone, which is bounded by belts of adhesion structures called podosomes, dynamic structures of integrin-induced actin polymerization. Through digesting the underlying bone, osteoclast action generates a small cavity called Howship's lacunae underneath the cell. Osteoclasts also form a specialized cell membrane, the ''ruffled border,'' which increases its interface area with bones and plays a critical role in removal of the breakdown products of the bone matrix. Osteoclasts release protons into the resorption pits through ruffled border located vacuolar-ATPase, acidifying and dissolving the inorganic components into Ca^{2+}, H_3PO_4, H_2CO_3, and water [Breton and Brown, 2007]. The ions are packaged into small vesicles via endocytosis, transported cross osteoclasts, and released into the extracellular fluid, eventually leading to an increase of these ions in the plasma. In addition, several hydrolytic enzymes, such as members of the cathepsin and matrix metalloprotease (MMP) groups, are released to the resorption lacunae to digest the organic components of the matrix. Of these hydrolytic enzymes, cathepsin K, a papain-like cysteine protease, is the major protease involved in the degradation of type I collagen and other non-collagenous proteins during resorption [Zaidi et al., 2001]. Cathepsin K is mainly expressed in osteoclasts and is most active under acidic conditions, which is present in the resorption pits. Cathepsin K knockout mice exhibit a thicker bone phenotype [Saftig et al., 1998].

The fine balance between bone resorption and formation is maintained via multiple coupling mechanisms between osteoclasts and osteoblasts. For example, osteoblasts produce RANKL, M-CSF, and OPG to regulate osteoclastogenesis [Martin and Sims, 2005]. It is worth noting that bone remodeling is a relatively slow process. It is estimated that bone resorption in an individual remodeling unit in the bone takes about 2 weeks, and it takes much longer for osteoblasts to migrate into the resorption sites and lay down new bones. When bone resorption outperforms formation, osteoporosis occurs. Osteoporosis can be divided into a few types according to its pathogenesis [Manolagas and Jilka, 1995]. Postmenopausal osteoporosis is mainly caused by elevated bone resorption due to estrogen deficiency. In contrast, senile osteoporosis is mainly resulted from a decrease in bone formation due to a decline of the number and activity of osteoblasts. In addition, long-term application of glucocorticoid and lack of mechanical loading can also result in osteoporosis [Harada and Rodan, 2003; Mazziotti et al., 2006; Robling et al., 2006; Canalis et al., 2007]. On the other hand, when bone formation outstrips resorption, bone mass and density will be increased, leading to osteosclerosis or osteopetrosis. Osteosclerosis is caused by enhanced bone formation, while osteopetrosis is a result of compromised bone resorption [Manolagas and Jilka, 1995; Harada and Rodan, 2003].

OSTEOPOROSIS THERAPY WITH BISPHOSPHONATES

Osteoporosis can be prevented or treated in two ways: increasing bone formation (anabolic) or inhibiting bone resorption (catabolic) [Khan, 2003; Li, 2008]. Till date, anti-resorptive drugs are more widely used than anabolic drugs. PTH (1–34 peptide) is the only anabolic drug approved by US FDA [Rosen, 2004; Jilka, 2007]. Anti-resorptive drugs can be classified into several categories based on their chemical nature and working mechanisms. These include estrogen and SERMs (selective estrogen receptor modulators), bisphosphonates, calcitonin, anti-RANKL antibodies (Denosumab, humanized monoclonal ab), etc. [Hamdy, 2008; Gerstenfeld et al., 2009]. Of these, calcitonin is a naturally occurring hormone that regulates blood calcium levels. It slows down the rate of bone thinning and relieves bone pain [Gennari, 2002; Huang et al., 2006]. However, it is not as effective as SERMs or bisphosphonates at preserving the bone and reducing fracture risks.

Estrogen and SERMs, through binding to estrogen receptors, are able to reverse estrogen shortage-induced excessive bone resorption [Miller, 2002; Perez and Weilbaecher, 2006]. Their effects on osteoclastogenesis and bone resorption are much complicated and involve other cell types such as osteoblasts and lymphocytes [Riggs et al., 2002]. Estrogen fade-away leads to up-regulation of IL-6, IL-11, and M-CSF, as well as a decrease in OPG and TGF-β, in osteoblast lineages, and an increase in IL-1 and TNFα in monocytes and T lymphocytes [Clowes et al., 2005; Weitzmann and Pacifici, 2006]. The change in these cytokines and growth factors not only enhances osteoclastogenesis from HSCs, but also inhibits apoptosis of osteoclasts [Nakamura et al., 2007], eventually leading to an increase in the number of osteoclasts and an increase in overall bone resorption. Estrogen and SERMs can reverse these cellular events and rescue the bone loss. However, hormone therapy has been reported to increase the risks of coronary artery disease, and breast and uterine cancer in patients [Labrie, 2007].

Bisphosphonates are so far the most commonly used anti-resorptive drugs in osteoporosis therapy [Reszka and Rodan, 2004;

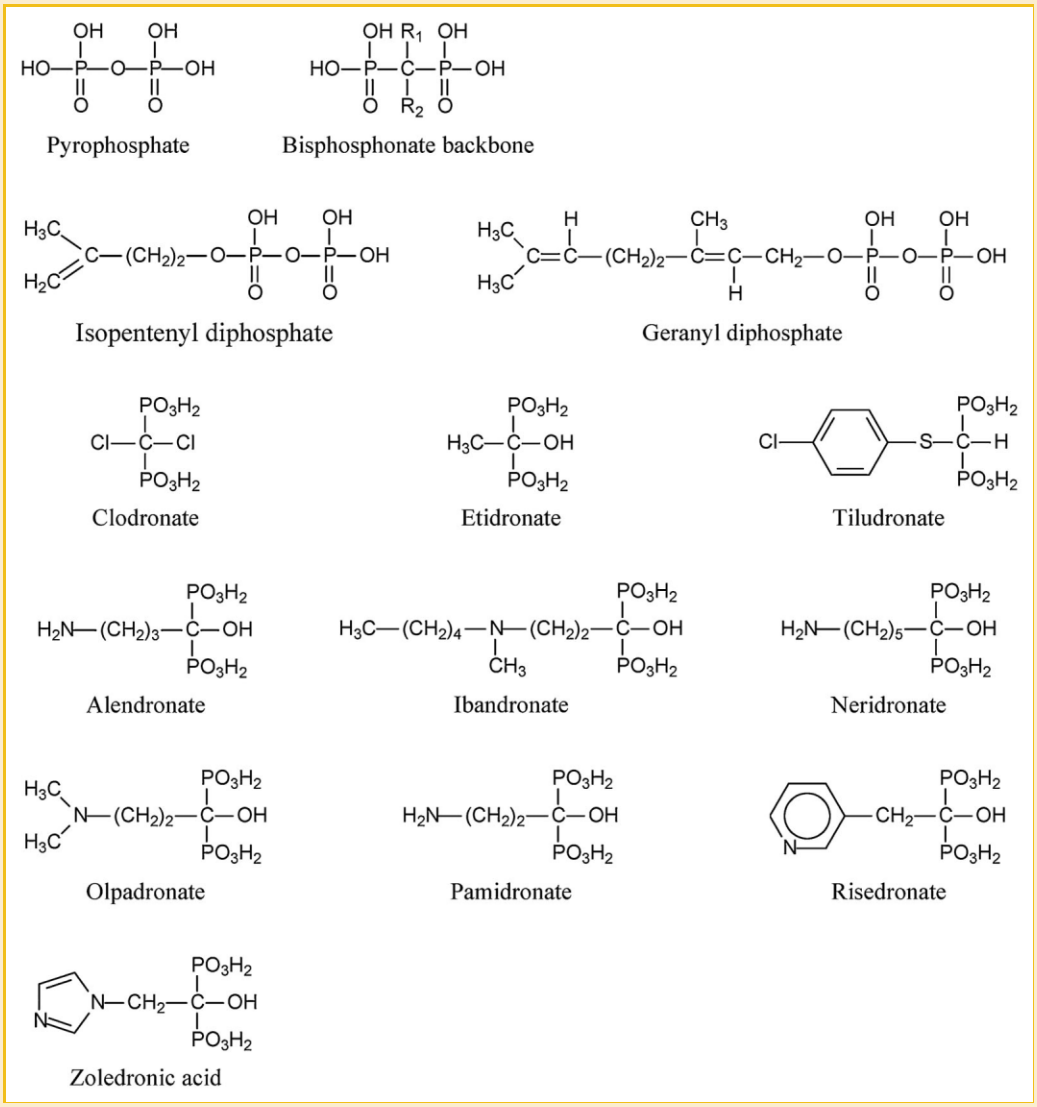

Fig. 1. Structures of pyrophosphate, bisphosphonate backbone, bisphosphonates mimicking intermediates of mevalonate pathway (isopentenyl diphosphate and geranyl diphosphate), non-N-BPs (clondronate, etidronate, and tiludronate), and N-BPs.

Rodan et al., 2004; Russell et al., 2008]. They are synthetic analogs of pyrophosphate (Fig. 1), with a P-C-P backbone and variable R1 and R2 that are covalently linked to the C atom (Fig. 1). Bisphosphonates have two PO_4 groups, which have a strong affinity for Ca^{2+} ions of hydroxyapatite [van Beek et al., 1994]. The binding to hydroxyapatite can be regulated by the pH of the microenvironment, with acidic pH promoting dissociation (Fig. 2). Pyrophosphate was first shown to be able to bind to hydroxyapatite crystals and regulate their calcification more than three decades ago [Fleisch et al., 1966]. The major difference among these bisphosphonates is at the R1 and R2 side chains, which can be classified into N-containing bisphosphonates (N-BPs) and non-N-containing bisphosphonates (non-N-BPs), with the former showing 10–10,000 fold more potency than the latter. Initially it is believed that bisphosphonates inhibit the dissolubility of hydroxyapatite, but it was later demonstrated that the main function of bisphosphonates is to inhibit bone resorption, with non-N-BPs inducing osteoclast apoptosis and N-BPs inhibiting osteoclast activity. Currently bisphosphonates are widely used to treat not only postmenopausal osteoporosis but also other diseases that involve excessive bone resorption, including glucocorticoid-induced osteoporosis, Paget's disease of bone, hypercalcemia of malignancy, and bone metastasis-induced bone loss.

The effects of bisphosphonates on bone resorption in vivo have been extensively studied. In general, they cause a reduction in plasma and urine biochemical markers of bone resorption, including amino- and carboxyl-terminal breakdown products of type I collagen, with maximal suppression peaked within 3 months after uptake [Reszka and Rodan, 2004; Rodan et al., 2004]. On a long-term treatment, bisphosphonates have been shown to prevent bone loss in osteoporotic patients and an increase in bone mineral density at lumbar spine, hip, and femoral neck. More importantly, bisphosphonate administration has been demonstrated to reduce bone fracture risks, especially in vertebrae by 30–70% [Watts et al., 1990;

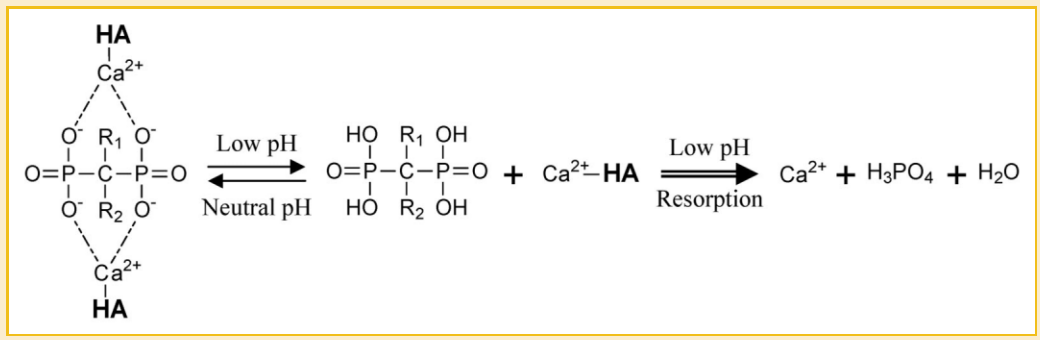

Fig. 2. Chelation of bisphosphonates with hydroxyapatite (Ca^{2+}–HA) under neutral pH, and dissociation under acidic pH in resorption pits.

Liberman et al., 1995; Cummings et al., 1998; Harris et al., 1999; Black et al., 2006; Black et al., 2007]. Some of these drugs have been found to be effective in fighting bone fractures even with monthly or yearly infusion. For example, yearly infusion of zelodronate resulted in a 70% reduction in vertebral fractures, 41% reduction in hip fractures, and 25% reduction in non-vertebral fractures in postmenopausal women [Black et al., 2007].

For their positive outcome in treatment, several bisphosphonates have been approved in anti-bone resorption therapy [Russell et al., 2008]. Etidronate (Didronel) has been used to treat Paget's disease (5–10 mg/kg by mouth, once a day for 6 months), hypercalcemia (7.5 mg/kg, IV infusion, once a day for 3 days). Tiludronate (Skelid) is an S-containing agent used for Paget's disease of bone (40 mg by mouth, once a day). Pamidronate (Aredia) is used for Paget's disease of bone, hypercalcemia of malignancy, and osteolytic bone lesions (e.g., multiple myeloma) (40–80 mg, IV infusion, every 4 months). Alendronate (Fosamax) is used for osteoporosis therapy, in which the tablet (10 mg) is taken on an empty stomach once a day (or 35, 70 mg once a week), and the solution once a week. Risedronate (Actonel) is used for osteoporosis therapy with daily, weekly, or monthly dosing. Ibandronate (Boniva) is used for osteoporosis therapy (2.5 mg once a day, or 150 mg tablet once a month). Zoledronic Acid (Reclast) is also used for osteoporosis therapy (5 mg, IV infusion, once yearly). Clodronate and Olpadronate are not commercially available in the USA. N-BPs are much better than non-N-BPs in terms of potency, with the following ranking order: zoledronate > risedronate > ibandronate > alendronate > pamidronate [Shaw and Bishop, 2005; Russell et al., 2008; Zacharis and Tzanavaras, 2008].

THE PHARMACOKINETICS OF BISPHOSPHONATES

The pharmacokinetics of bisphosphonates, including absorption, tissue distribution, metabolism, and excretion, have been studied in animals and patients with either radio-labeled bisphosphonates (e.g., ^{14}C or ^{3}H) or fluorescein-labeled bisphosphonates (e.g., 6-carboxyfluroscein) [Zacharis and Tzanavaras, 2008]. The pharmacokinetic parameters and molecular pharmacological properties grant N-BPs many of the benefits: (i) high specificity to osteoclasts, minor side effects, and great window between efficacy and toxicity doses; (ii) intermittent dosing regimens; and (iii) a broad spectrum in treating excessive resorption disorders and complications. The pharmacokinetics of N-BPs is best studied with a three-compartment model: blood, bone surface, and deep bone (Fig. 3).

ABSORPTION AND METABOLISM

There are mainly two routes for bisphosphonates administration, oral and intravenous infusion. Oral administration is of extreme low efficacy. Only 0.5–2% of the orally administrated drugs shows bioactivity. This is due to the low efficiency of gastrointestinal uptake and this is further worsened by food uptake (by 60–90%). Therefore, oral bisphosphonates are usually administrated with empty stomach, and excessive amounts of medicine are needed due to poor uptake. In some patients, oral administration can cause esophagus and stomach irritation [Cramer and Silverman, 2006]. Regardless of the administration routes, 40–60% of the plasma bisphosphonates are absorbed into our body and the remainder is excreted through kidney, within 24 h of administration.

Another important point is that bisphosphonates, unlike pyrophosphate that is quickly degraded, are not hydrolyzable. This has been confirmed in laboratory animals and patients. They are not broken down to metabolites and therefore the anti-resorptive effects are directly from the drugs themselves. Non-hydrolyzability is the reason behind drug stability and persistence. However, non-N-BP, as well as a very small amount of N-BP, has been found to be incorporated into non-hydrolyzable ATP analogs, which can induce apoptosis in various cell types [Monkkonen et al., 2006].

DISTRIBUTION

Distribution of bisphosphonates has been studied with radio-labeled bisphosphonates in patients and animals. Forty to sixty of the plasma bisphosphonates, or the majority of the bisphosphonates uptaken by patients, are exclusively accumulated in the bone in vivo. Small amounts might accumulate in the soft tissues right after infusion but are quickly redistributed into the bone. Moreover, bisphosphonates bone retention is very stable and long lasting. This is because bisphosphonates have a strong affinity for the bone but not other tissues. The high specificity to the bone explains why almost all the remainder bisphosphonates are quickly cleared via renal excretion. It also appears that all bisphosphonates follow this distribution pattern. For example, after tail vein injection into mice, ^{14}C-labeled clodronate disappeared from blood stream promptly and

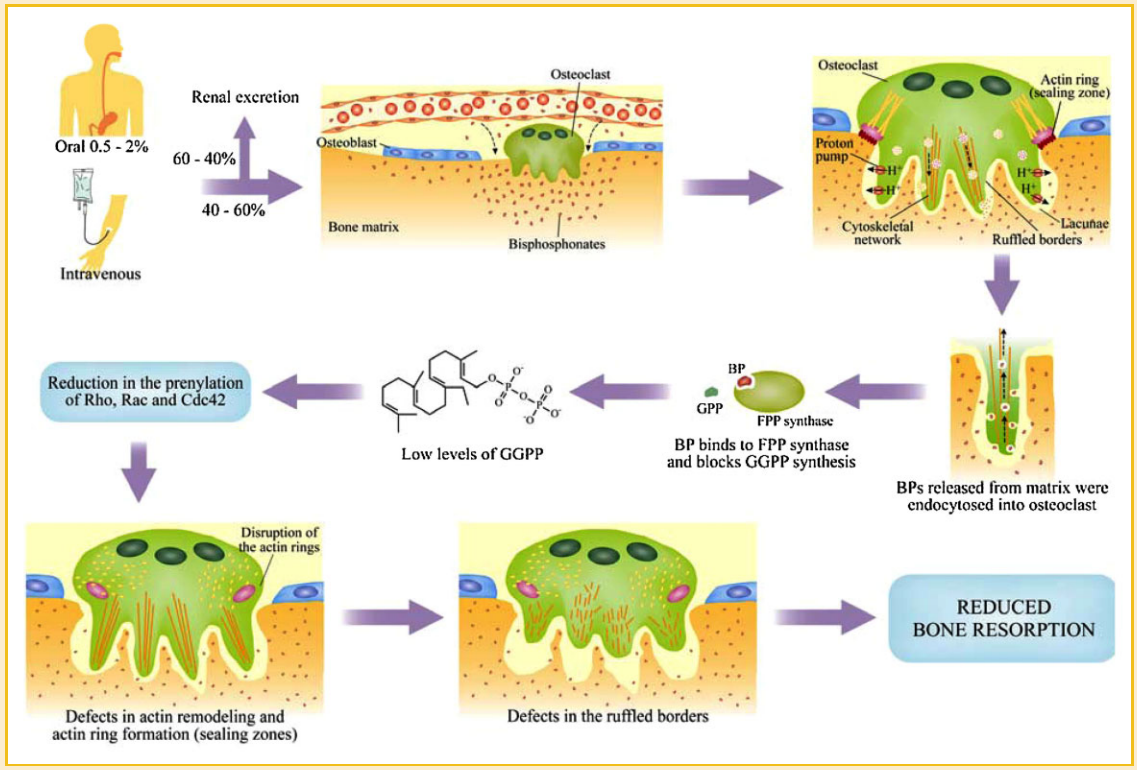

Fig. 3. An illustration showing uptake, distribution, and the anti-resorption function of N-BPs.

the rest were distributed intensively in bones and modestly in the spleen. Bone labeling could be still detectable 90 days after injection [Monkkonen et al., 1987]. A study of pamidronate in cancer patients showed 60–70% bone retention 24 h after injection, and this was not affected by infusion rates [Berenson et al., 1997; Cremers et al., 2002]. A study of alendronate in postmenopausal women also showed that after IV infusion, ^{14}C-labeled alendronate excretion was exclusively by urine, accounting for 47% of the total. The remainder displayed transient and broad distribution in the body, followed by a non-saturable redistribution to the skeleton [Cocquyt et al., 1999]. For ibandronate, about 40–50% of the plasma ibandronate are accumulated in the bone, and the rest into urine [Bauss et al., 2002; Bauss and Russell, 2004]. In patients with cancer and bone metastases, 39 ± 16% of the administered zoledronic acid is recovered in the urine within 24 h, with only trace amounts being found in urine post day 2. The rest of the drug is bound to the bone, which is slowly released back into the circulation, giving rise to the observed prolonged low plasma concentrations [Chen et al., 2002; Weiss et al., 2008]. Bone accumulation of bisphosphonates and renal elimination are not significantly affected by mode of administration, but they are linear to the dose applied.

EXCRETION

Excretion of various bisphosphonates has been examined in patients as well as laboratory animals. Bisphosphonates are non-hydrolyzable. Unless retained in the bone, they are excreted unmetabolized in urine within a couple of days. Initial plasma disposition for most bisphosphonates after infusion is multiple-phased. For example, the half-lives (t1/2) of alendronate is 0.2 h, 39 h, and 4526 h [Lin et al., 1994]. For ibandronate, the initial serum elimination t1/2 is 1.3 h and final elimination t1/2 is 32 h in postmenopausal women [Coleman et al., 1999; Barrett et al., 2004]. The order of urine excretion after 24 h is: clodronate > risedronate > alendronate > zoledronate [Black et al., 2006; Russell et al., 2008]. In general, bone retention of N-BPs is determined by bone turnover rates and renal function, as well as the nature of the N-BPs. More retention is always observed for bisphosphonates with higher affinity for hydroxyapatite.

Moreover, skeleton retained bisphosphonates will be slowly liberated from the bone and excreted in urine over time. After the initial and rapid clearance, the slow elimination of alendronate can take 200 days in rats, 3 years in dogs and 12 years in humans [Harris et al., 1999; Black et al., 2006]. As such, low amounts of N-BPs can still be detectable in the urine long after the infusion. This may explain why in a 10 year study, after 5 years of treatment with alendronate and followed by a 5 year placebo, the levels of bone resorption markers are still below the baseline levels. This suggests that N-BPs still show some activities 5 years after discontinuation [Bone et al., 2004]. Such slow and gradual excretion is also affected by the bone turnover rates of the patients.

BONE RESORPTION SURFACE LOCALIZATION

Bisphosphonates are quickly distributed into the bone after administration, yet the distribution is not even in the bone [Azuma et al., 1995]. More labeling is observed at the bone metastasis sites in cancer patients and high turnover sites of Paget's patients [Azuma

et al., 1995; Coxon et al., 2008]. Under microscope, 10% of the bone surface is densely labeled and 27% is moderately labeled. Dense labeling is observed under osteoclasts or the resorption surface (Fig. 3), whereas the labeling at the osteoblast surface, as well as other surfaces, is much lower [Hoggarth et al., 1991; Ambrosetti et al., 2008]. For example, ^{3}H-labeled alendronate was found to label about 4.8% of the osteoblast surface and 37% of the osteoclast surface in a mouse study. Another study showed that 70% of the osteoclast surface was labeled by ^{3}H-labeled alendronate, while only 2% of osteoblast surface and 13% of other surface were labeled [Sato et al., 1991]. Six days after injection, the labeling was not altered, confirming a long half-life of N-BPs at the resorption surface. The local concentration of N-BPs in the resorption pits can reach up to 0.1–1.0 mM [Azuma et al., 1995]. The reason behind this unique resorption surface localization is believed to be that osteoclast resorption degrades the organic components and therefore exposes hydroxyapatite, which provides the sites for bisphosphonates binding. Thus, the availability of hydroxyapatite surface determines the bone retention rates of bisphosphonates [Sato et al., 1991; Masarachia et al., 1996].

ENTRY OF BISPHOSPHONATES INTO OSTEOCLASTS

The unique bone resorption surface localization pattern suggests that N-BPs are more accessible to osteoclasts. Indeed it has been demonstrated that bisphosphonates are mainly uptaken by osteoclasts [Coxon et al., 2008]. This is inconsistent with the studies using cell culture systems, where bisphosphonates have been reported to get into all sorts of cells besides osteoclasts, e.g., bone marrow stromal cells, osteoblasts, and a variety of cancer cells [Schindeler and Little, 2007]. This discrepancy can be explained by the fact that bisphosphonates are rapidly and strongly sequestered into the bone in vivo, while free bisphosphonates are easily available in cell culture systems. Furthermore, only osteoclasts can protonate bisphosphonates and liberate them from the bone for uptake in vivo.

There is increasing evidence that bisphosphonates enter osteoclasts through the ruffled borders in the resorption pits, where acidic condition liberates bisphosphonates from hydroxyapatite into solution. N-BPs cell entry is mainly mediated by liquid phase endocytosis, as inhibitors of this path block bisphosphonate entry [Thompson et al., 2006; Coxon et al., 2008]. Once inside the cell, the endocytotic vesicles carrying bisphosphonates will be fused with lysosomes and bisphosphonates will be released into the cytosol, where bisphosphonates execute their biological function (Fig. 3). The acidic pH of the lysosomes is also required for the release.

MOLECULAR PHARMACOLOGY OF BISPHOSPHONATES

N-BPs present a great complexity on its mechanism of action in bone remodeling. There are studies suggesting that bisphosphonates might act through various routes. These include blocking osteoclastogenesis, inducing osteoclast apoptosis, and inhibiting osteoclast activity [Rodan and Reszka, 2002; Russell et al., 2008].

OSTEOCLASTOGENESIS

Bisphosphonates can block osteoclastogenesis via altering the expression of RANKL, M-CSF, and OPG by osteoblasts. Cell based studies have shown that bisphosphonates are able to inhibit the expression of RANKL and promote the expression of OPG in osteoblasts. For example, Pan et al. reported that zoledronic acid increased OPG and decreased RANKL expression in osteoblasts [Pan et al., 2004], while Nishida et al. reported that a new bisphosphonate, YM529/ONO-5920, inhibited RANKL expression in osteoblasts [Nishida et al., 2005]. Moreover, Martinin et al. reported that pamidronate treatment led to an increase in serum OPG and RANKL [Martini et al., 2007]. However, the ratio of OPG to RANKL is three-fold higher in patients under treatment, and a long-term (3–6 months) pamidronate treatment led to an increase in OPG and a reduction in RANKL. Since an increase in the OPG/RANKL ratio is known to impede osteoclastogenesis, N-BPs treatment would result in a decrease in the number of mature osteoclasts on the bone surface [Weinstein et al., 2002; Plotkin et al., 2006]. However, there is a lack of in vivo evidence to support this otherwise attractive theory. So far, bisphosphonates have not been reported to reduce the number of mature osteoclasts in patients [Weinstein et al., 2009]. Animal studies have shown that the numbers of osteoclasts are similar in N-BPs treated and untreated normal mice, estrogen-deficient mice, and mice with secondary hyperparathyroidism [Seedor et al., 1991; Weinstein et al., 2002]. This could be due to the fact that osteoblasts accumulate very little amount of bisphosphonates in vivo. Thus, it is believed that this may not be a major route used by bisphosphonates to prevent bone loss.

OSTEOCLAST APOPTOSIS

The other possible routes are osteoclast autonomous. Once ingested, bisphosphonates have been reported to induce osteoclast apoptosis and/or inhibit resorption activity. Non-N-BPs, as well as a very small amount of N-BPs, can be incorporated into non-hydrolyzable ATP analogs, which can interfere with ATP-dependent cellular processes and induce apoptosis. This might be the main mechanism by which non-N-BPs inhibit bone resorption and preserve the bone. However, N-BPs treatment does not seem to affect osteoclast numbers in vivo. The consensus view is that the major mechanism by which N-BPs slow down bone resorption is to inhibit osteoclast activity, possibly through disrupting the cytoskeleton structure that osteoclasts rely on to resorb bones.

A very recent study shows that after 3 years of alendronate treatment, the number of osteoclasts was not shown to be reduced in the patients receiving 1 or 5 mg of alendronate. However, it was found that 10 mg of alendronate actually increased the number of osteoclasts by a factor of 2.6 [Weinstein et al., 2009]. This increase is proportional to the cumulative dose of alendronate. However, about 27% of the osteoclasts look larger and have more nuclei (20–40 per cell), and 20–37% of the large cells are apoptotic and are detached from the bone surface. To explain the increase of osteoclasts, the authors proposed that uptake of the breakdown products of bone matrix during bone resorption, especially Ca^{2+}, can cause osteoclast apoptosis. In the presence of N-BPs, osteoclast resorption activity is suppressed and less toxic materials are absorbed. Thus, osteoclasts might actually have a chance to survive longer. Another theory,

described as a self-contained process, has also been proposed. When osteoclasts uptake certain amounts of N-BPs, their resorption activity is inhibited and acidification in resorption pits stops. This will lead to a decline in the uptake of cytotoxic N-BPs, leading to cell survival [Sato et al., 1991]. However, the exact mechanism by which N-BPs directly or indirectly regulate cell survival needs further investigation. Similarly, it is unclear regarding what causes the increase in the number of nuclei per cell in N-BPs treated patients. Is it possible that N-BPs might somehow affect osteoclast biogenesis and maturation in addition to resorption?

BISPHOSPHONATES INHIBIT FPPS AND OSTEOCLAST ACTIVITY

A link of N-BPs to mevalonate pathway and protein lipid modification was first established in a study by Amin and colleagues. They found that ibandronate inhibited squalene synthase of the mevalonate pathway (also known as HMG-CoA reductase or isoprenoid pathway) that is responsible for sterol biosynthesis [Amin et al., 1992] (Fig. 4). However, other N-BPs did not inhibit squalene synthase though they still had an effect on sterol biosynthesis. This prompted researchers to believe that other enzymes of the mevalonate pathway were inhibited by N-BPs. The core of the mevalonate pathway (Fig. 4) involves the synthesis of isoprenyl-diphosphates intermediates, farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP) that are vital for various cellular functions [Goldstein and Brown, 1990] (Fig. 4). The transfer of these isoprenyl groups (C15 farnesyl or C20 geranylgeranyl groups) to the respective cysteine residue within the carboxyl termini of GTP-binding proteins such as Ras, Rho, Rac, and Rab proteins, as well as nuclear lamins [Maltese, 1990; Zhang and Casey, 1996] seems to be a requirement for their localization to the membranes and subsequently their biological function. Indeed, Luckman et al. reported that N-BPs did inhibit the post-translational prenylation of GTP-binding proteins whereas such effects were not seen with clondronate [Luckman et al., 1998]. They further showed that treatment with alendronate resulted in accumulation of unprenylated Ras as well. In addition, the study also revealed that both mevastatin (an inhibitor of HMG-CoA reductase) and N-BPs caused apoptosis in J774 macrophages and murine osteoclasts, which could be rescued by the introduction of FPP or GGPP (Fig. 4). Besides, mevastatin inhibition of osteoclast resorption could also be

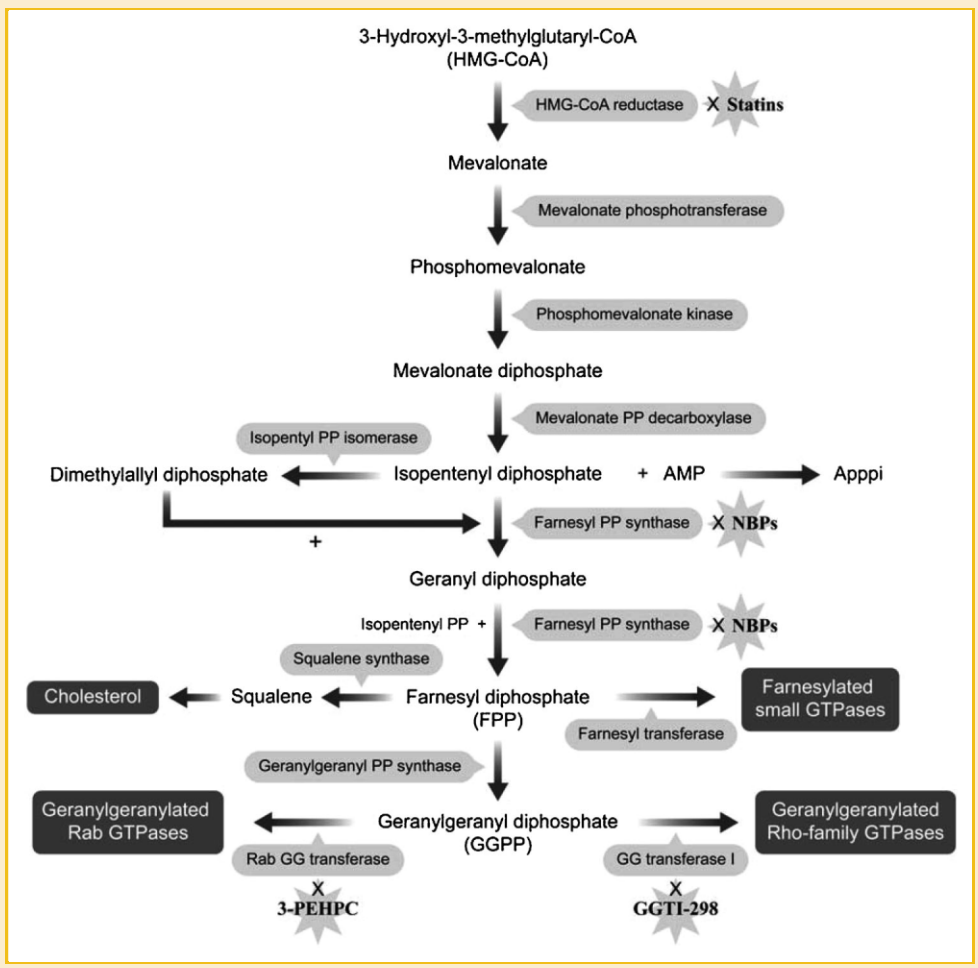

Fig. 4. Schematic diagram of the mevalonate pathway. N-BPs serve to inhibit FPPS activity, which results in the reduced synthesis of geranylgeranyl diphosphates. This in turn, prevents protein prenylation such as geranylgeranylation of Rab and Rho-family GTPases that are crucial for osteoclasts' activity.

overcome by addition of mevalonic acid lactone. These findings suggest that the action of N-BPs lies upstream of FPP but downstream of mevalonate. A later study conducted by van Beek group confirmed that the molecular target of N-BPs is FPP synthase (FPPS) [van Beek et al., 1999b]. N-BPs act through binding to the geranyl diphosphate binding site of FPPS [Kavanagh et al., 2006]. Noteworthy is that although FPPS is inhibited, farnesylation does not form the basis of the anti-resorptive properties of N-BPs. Instead, protein geranylgeranylation is critical for the anti-resorption activity of N-BPs [Coxon et al., 2000]. Through the use of a specific inhibitor of geranylgeranyl transferase I (GGTI-298), Coxon et al. has shown that inhibition of protein geranylgeranylation prevents the formation of osteoclasts, disrupts osteoclast cytoskeleton, and induces apoptosis of osteoclasts, with a subsequent reduction in bone resorption (Fig. 4). In contrast, the specific inhibitor of farnesyl transferase (FTI-277) did not show such effects. This observation is further demonstrated by van Beek et al. in fetal mouse long bone explants and by Fisher et al. through the use of geranylgeraniol (GGOH) to revert the inhibitory effect of N-BPs [Fisher et al., 1999; van beek et al., 1999a; Van Beek et al., 2002].

The post-translational prenylation of the GTP-binding proteins has an influence on the actin cytoskeleton structure of osteoclasts [Sato et al., 1991]. This is especially important as the resorptive property of osteoclasts relies on their ability to form sealing zones and ruffled membranes at the resorptive sites (Fig. 4). The actin-containing sealing zones act as a strong anchorage for the osteoclasts to the bone matrix, help to isolate the resorption lacuna from the extracellular fluid, and permit the maintenance of a specific microenvironment (such as vesicular release of proton and proteases to degrade the mineralized matrix) in the lacuna. Basically the sealing zones are made up of an F-actin core surrounded by a ring of scaffolding proteins such as vinculin, paxillin, or talin, as well as being connected by radial actin fibers [Marchisio et al., 1984; Destaing et al., 2003]. It has been found that Cdc42, Rac and Rho are crucial to initiate the formation of the sealing zones and membrane ruffling [Ridley and Hall, 1992; Zhang et al., 1995]. All these form part of the integrin signaling pathway critical for osteoclast survival and functions. For instance, Cdc42/Rac seems to act on PAK1, leading to the inhibition of cofilin by LIMK-1, which favors actin polymerization [Blanchoin et al., 2000]. Cdc42 can also regulate actin assembly via N-WASP, another interacting partner of Cdc42 [Rohatgi et al., 1999]. This pathway is also disrupted by N-BPs treatment. In the case of Rho, it instead acts on ROCK, which leads to activation of LIMK-1 and deactivation of cofilin [Maekawa et al., 1999]. Therefore, unprenylation of Rho family proteins could result in the activation of cofilin, causing actin depolymerization and the loss of the sealing zones and ruffled membranes. Moreover, Rab plays an important role in vesicular transport that is also supported by the actin cytoskeleton network [Zerial and Stenmark, 1993], without which transport of proteases to the degradation zone will be disrupted. For Ras, its prenylation may have an anti-apoptotic effect on osteoclasts while the lack of prenylation due to N-BPs will induce apoptosis [Oades et al., 2003].

From these studies, it is now evident that the anti-resorptive activity of N-BPs is affected through FPPS, resulting in the loss of geranylgeranylation of Rab, Rac, Ras, and Rho proteins. This eventually leads to a defect in actin dynamics crucial for osteoclast function (Fig. 3). However, exceptions are some N-BPs such as pamidronate, whose effect could only be partially reversed with GGOH, thus suggesting that alternative pathway exists that has yet to be realized [van Beek et al., 2003]. Interestingly, Dunford et al. reported that loss of prenylation in the presence of N-BPs seemed to cause sustained activation of Cdc42, Rac, and Rho-GTPases, instead of inhibition. Unprenylated forms of Cdc42, Rac, and Rho were found to be GTP-bound, leading to the accumulation of active GTPases and disruption of actin remodeling [Dunford et al., 2006]. Furthermore, p38 MAPK can be activated by Rac in the presence of N-BPs. One function of the activated p38 MAPK could be to partially suppress N-BPs induced osteoclast apoptosis [Dunford et al., 2006]. However, how unprenylated G-proteins are activated and how they disrupt actin cytoskeleton warrant further investigation. Nonetheless, the fact that the effects of N-BPs on bone resorption can be reversed by addition of geranylgeraniol, and that N-BPs' anti-resorption effects can be mimicked by other reagents that inhibit protein prenylation, such as statins, support the concept that N-BPs inhibit bone resorption mainly through disrupting small G-protein mediated actin cytoskeleton assembly/dynamics [Luckman et al., 1998; Fisher et al., 1999; van beek et al., 1999a; Coxon et al., 2000]. The rank order of FPPS enzyme inhibition by bisphosphonates is as followed: zoledronate > risedronate > ibandronate > alendronate > pamidronate > etidronate = clodronate [Russell et al., 2008].

EFFECT ON BONE FORMATION?

While much effort has been focused on osteoclasts and bone resorption in the study of N-BPs, there are some studies suggesting that N-BPs might act on osteoblasts as well. While extremely high doses of N-BPs have been shown to have a negative effect on mineralization in vivo, most likely due to their strong mineral binding activity [Nancollas et al., 2006], almost none of the N-BPs have been found to impair bone mineralization in vivo at the doses effective for anti-resorption treatment. One reason could be that osteoblasts can only accumulate a very small amount of N-BPs in vivo. Indeed, it has been reported that prenylation of small GTP binding proteins are not inhibited in osteoblasts. The quick distribution of N-BPs to the bone resorption surface, rather than the osteoblast surface, limits osteoblast uptake of N-BPs. Moreover, osteoblasts uptake of N-BPs might need the nearby osteoclasts to release N-BPs from the bone into extracellular fluid. This is supported by in vitro studies with bone slices. Under this setting, bisphosphonates are strongly absorbed to the bone chips. While osteoclasts and macrophages could uptake bisphosphonates, osteoblasts failed to do so. In contrast, co-culture with osteoclasts promoted N-BPs uptake by osteoblasts. This low concentration of N-BPs in osteoblasts might even contribute to the drugs' bone preservation function, as it has been shown that low amounts of N-BPs could prevent osteoblast/osteocyte apoptosis [Plotkin et al., 1999]. However, it is believed that the direct effects of N-BPs on bone formation should be minor, and the altered bone formation observed in patients receiving N-BPs treatment should be secondary to the slowed bone turnover caused by N-BPs [Russell et al., 2008].

RECYCLING OF BISPHOSPHONATES AND INTERMITTENT DOSING REGIMENS

It is known that N-BPs bind rapidly to the bone after administration with the remainder excreted, and these N-BPs are mainly located at the bone resorption surface. As we know that bone resorption in an individual bone unit takes about 2 weeks, this implies that N-BPs will be staying there for a long time. Once liberated from the bone matrix, N-BPs can have three fates theoretically. Some will enter osteoclasts and are transported across the cell, eventually entering blood stream. A portion of the recycled N-BPs (~50%), just like the newly infused N-BPs, will be systematically redistributed to the bone surfaces, while the remainder are eliminated via renal excretion. N-BPs that are not up-taken by osteoclasts, as well as the non-liberated N-BPs, will stay and be embedded deeply in the bone after new bone is formed at this location. The embedded N-BPs are away from the bone surface and will not be active, unless new bone remodeling occurs at these sites. Therefore, once bound to the bone, bisphosphonates will reside there for a considerable period of time and can be slowly recycled. As such, daily, weekly, or even monthly dosing should give a similar effectiveness as long as the cumulative doses are similar. A study on ibandronate confirmed that the total cumulative dose determines the response, independent of the administration frequency [Barrett et al., 2004]. Moreover, an once yearly IV infusion of zelodronate has shown its efficacy in preserving bone mass and reducing bone fractures [Amanat et al., 2007; Black et al., 2007]. The intermittent dosing regimens with higher doses can reduce the risk of upper gastrointestinal irritation that is associated with daily oral administration, and thus increases patient adherence to N-BPs therapy [Bone et al., 2000].

THE STRUCTURES OF BISPHOSPHONATES DETERMINE THEIR PHARMACOKINETICS

All bisphosphonates have comparable pharmacokinetic parameters with some differences. The contribution of the structural elements, the PCP-core structure and R1 and R2 side chains, to the pharmacokinetic profiles can be learnt by comparing different bisphosphonates. Some studies have been carried out to compare the potency, bone retention, bone affinity, and excretion of various bisphosphonates, although most have not been studied in a head-to-head manner [Papapoulos, 2006]. In general, all bisphosphonates share pharmacokinetic features including specific retention at the bone, rapid elimination of non-retained bisphosphonates unmetabolized, and long lasting efficacy. These features must be determined by the P-C-P core structure, rather than the R1 or R2 side chains. Moreover, it is the chemical properties of PCP that form the basis for its specificity to osteoclasts. Under neutral pH, bisphosphonates are bound to Ca^{2+} of hydroxyapatite in the bone matrix, especially at the resorption surface, where osteoclast activity exposes hydroxyapatite (Fig. 2). Only under acidic pH, the phosphate groups of bisphosphonates can be protonated, decreasing their affinity for calcium ions and leading to their release into solution. Low pH is a property unique to the resorption pits formed by osteoclasts. Thus, only osteoclasts can uptake and transport N-BPs, and evidently osteoclasts are the main cell type that is affected by N-BPs. An essential role for low pH is demonstrated by oc/oc mice, which have a defective vacuolar-ATPase gene [Scimeca et al., 2000]. Although these mice lack bone resorption, they do have properly formed resorption pits [Murakami et al., 1995]. It was found that tiludronate could not affect the actin ring of these osteoclasts, as the drug cannot be taken in by osteoclasts [Takami et al., 2003; Akiyama et al., 2004]. Therefore, the core structure and its affinity to Ca^{2+} account for its high affinity to hydroxyapatite, osteoclast uptake, and recycling.

On the other hand, there exist differences among bisphosphonates in terms of bone retention and elimination, suggesting that the R1 and R2 side chains also contribute to their affinity to the bone [van Beek et al., 1994]. Using fetal mouse long bones, it was found that bisphosphonates bind to the bone with the highest affinity when R1 is a hydroxyl group. This might be the reason why almost all N-BPs have a hydroxyl group at the R1 position. In addition, R2 also seems to have an influence on N-BPs' affinity to bones. Further studies show the ranking order of bisphosphonates with regard to their affinity to hydroxyapatite as: zoledronate > pamidronate > alendronate > ibandronate > risedronate > etidronate [Henneman et al., 2008]. Zoledronate has the longest retention time in vivo, due to a nitrogen within a heterocyclic ring at R2. It is now proposed that the three dimension structure and the orientation of N atom determine the affinity of various bisphosphonates to bones [Russell et al., 2008].

While it is predictable that bisphosphonates with higher affinity to bones would show stronger bone retention and greater potency, some N-BPs show different bioactivities even though they have similar affinity to hydroxyapatite. This suggests that affinity to the bones may not be a major factor in determining the bioactivity of N-BPs. Instead, a correlation between potency and anti-FPPS activity has been observed, suggesting that the inhibitory effect on FPPS, which is decided by the R2 side chains, determines the bioactivity of bisphosphonates.

SIDE EFFECTS OF BISPHOSPHONATES

A 5–10 year follow-up study on postmenopausal women with bisphosphonates treatment supports that N-BPs are relatively safe drugs, with negligible adverse effects [Bone et al., 2004]. Three factors might have contributed to the low toxicity: (i) high specific affinity for bones but no other organs, (ii) not absorbed by any other cells except osteoclasts in vivo, and (iii) quick excretion of the free bisphosphonates. However, it has also been reported that bisphosphonates do have some side effects. For oral administration, stomach upset and inflammation of the esophagus are common, so patients need fasting overnight before dosing and remain upright for 30 min after dosing [Cramer et al., 2007]. Due to these reasons, persistence and adherence to oral N-BPs treatment is poor. On the other hand, IV infusion causes acute inflammatory response including fever-like syndrome, in 10–30% of the first time N-BPs users. The incidence rate goes down dramatically from the second infusion onwards. Such response is believed to be mediated by monocytes, which also uptake N-BPs during early drug adminis-

tration, leading to an accumulation of the substrate of FPPS, isopentenyl pyrophosphate (IPP). IPP can act as a ligand for T cell receptor and trigger acute proinflammation response [Zgani et al., 2004]. Intermittent dosing regimens (IV infusion) can provide partial help to these problems, promoting patients' adherence to therapy. In addition, N-BPs have been reported to cause ocular inflammation, renal toxicity, and osteonecrosis of jaw (ONJ) [Hess et al., 2008; Ruggiero and Mehrotra, 2009]. The incidence rate of ONJ is very low, with 1 in 10,000–1,00,000 patients. It is unclear at the moment how N-BPs cause ONJ, as N-BPs, e.g., ibandronate, accumulation in the jaw is no different from other bones [Bauss et al., 2008].

OTHER USES OF BISPHOSPHONATES

BISPHOSPHONATES AND HCM

Hypercalcemia of malignancy is a condition of an abnormally high concentration of calcium in the blood of cancer patients. It is a common complication that affects approximately 10–20% of cancer patients of various stages and 20–40% of patients with advanced cancer. One cause is that cancer may release certain hormones to systemically increase the calcium level in the blood. For example, multiple myeloma, which grows in the bone marrow, can lead to hypercalcemia. Another cause is that cancer has spread to the bones, leading to an enhanced local bone resorption at the metastasis sites. This is also referred to as bone metastasis-induced bone loss. Breast and prostate cancers frequently spread to the bone. Tumor cells enter bones through blood or lymphatic vessels, where they alter bone metabolism to facilitate cancer metastasis. Breast cancers express high levels of parathyroid hormone related protein (PTHrP), which can promote bone breakdown. Local or global excessive bone resorption results in bone pain and fractures, in addition to hypercalcemia. Consistent with this scenario, inhibition of osteoclast activity not only decreases bone lesions but also reduces tumor burden. Bisphosphonates have been used to treat hypercalcemia of malignancy and bone metastasis-induced bone loss. Recent studies also suggest that bisphosphonates might have anti-tumor functions, and they are shown to directly affect tumor growth and metastasis. In vitro studies show that bisphosphonates inhibit growth, attachment and invasion of various cancer cell lines, through the synthesis of non-hydrolyzable ATP analogs [Giraudo et al., 2004; Miwa et al., 2005; Monkkonen et al., 2006]. Moreover, bisphosphonates, in combination with certain anti-tumor drugs, can have a synergistic effect in killing tumor cells. For example, zoledronic acid, in combination with Gleevec, appears to improve the efficacy of killing leukemia cell [Leyvraz et al., 1992; Kuroda et al., 2003]. Thus, bisphosphonates hold a lot of potential for combinational treatment of cancer.

BISPHOSPHONATES AND PAGET'S DISEASE THERAPY

Paget's disease of bone is a chronic disorder of focal bone remodeling, affecting 1–2% of people over age 55 in Western countries [Whyte, 2006; Ralston, 2008]. They show excessive bone resorption, especially at the sites of axial skeleton, and is followed by imperfect bone formation, leading to bone weakening, bone pain, arthritis, fractures, and enlarged and deformed bones. Osteoclasts in these patients show an increase in number and size, with more nuclei per cell. Human genetics studies have linked Paget's disease to mutations in Sequestosome 1 (SQSTM1), a scaffold protein that controls NFκB signaling, RANK, and RANKL [Ralston, 2008]. Hearing loss and headache may also occur when the skull bones are affected. Paget's disease of bone can be treated with bisphosphonates, which suppress bone resorption, reduce bone pain, and restore normal bone histology. The rank of potency for various bisphosphonates in treating Paget's disease is similar to that for osteoporosis therapy. OPG−/− mice is an animal model of Paget's disease that show progressive hearing loss [Kanzaki et al., 2006]. Due to increased resorption, the bones in the middle ear, the malleus, incus, and stapes, which conduct sound from the tympanic membrane to the inner ear, are defective. Risedronate treatment not only inhibited bone loss in these bones but also improved hearing in these mice [Kanzaki et al., 2009]. Thus, N-BPs can be used to treat osteoporosis-induced conductive hearing loss.

OTHER DISORDERS

N-BPs have been used to treat glucocorticoid induced osteoporosis. Zoledronic acid has been tried in treatment of rheumatoid arthritis and Osteogenesis Imperfecta (OI) as well, with positive results being obtained. Zoledronic acid has also been found to enhance endochondrial fracture repair and increase callus volume and mechanical strength [Rauch and Glorieux, 2005; Breuil and Euller-Ziegler, 2006; Corrado et al., 2007].

BISPHOSPHONATES AS BONE-SEEKING AGENTS

Due to their high specificity to bones, bisphosphonates are now being developed as bone-seeking agents to specifically deliver drugs into bones. Bisphosphonates have been reported to faithfully target the compounds or proteins that are fused to them to the bone. Fujisaki et al. reported that 62% of CF-BP (6-carboxyfluorescein-BP) was taken up by bones, with the rest excreted in the urine. Similar to bisphosphonates, regeneration of CF was observed as CF-BP could also be deeply buried in the bone matrix [Fujisaki et al., 1995], which could be later released near osteoclast or resorption surface. Tsushima et al. reported that SM-16896, a hybrid between estrogen and bisphosphonate, showed strong labeling in the bone but little in the uterus 24 h after injection into rats. It was also observed to reduce bone loss [Tsushima et al., 2000]. Targeting estrogen to osteoblast could minimize the adverse effects associated with estrogen replacement therapy. Moreover, specifically targeting anabolic agent such as PTH to the bone is an attractive idea. This can promote osteoblast function and bone formation at the resorption site, while minimizing the adverse effects on other tissues. Another study also shows that gencitabine-bisphosphonate are mainly (67%) retained in the bone 8 h after administration. This can be used to specifically target bone metastasis [Sawicki et al., 2008].

CONCLUSIONS

Bisphosphonates have transformed the clinical care of osteoporosis as well as complications caused by excessive bone resorption. N-BPs

are now the first choice for treatment of Paget's disease of bone and osteoporosis. Although N-BPs have been implicated in regulating osteoblast function, osteoclastogenesis, osteoclast apoptosis, and osteoclast activity, they inhibit bone resorption mainly by disrupting cytoskeleton structure and the resorption activity of osteoclasts. The unique chemical nature of these compounds determines their pharmacokinetic parameters and therefore their efficacy, specificity, and perdurability. The greatness of resorption suppression is mainly determined by the R2 group and the length of persistence of the drug is determined by the R1 group as well as the entire structure. In search for new anti-resorptive drugs, bisphosphonates with higher affinity to both hydroxyapatite and FPPS will hold the most potential.

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REFERENCES

Akiyama H, Lyons JP, Mori-Akiyama Y, Yang X, Zhang R, Zhang Z, Deng JM, Taketo MM, Nakamura T, Behringer RR, McCrea PD, de Crombrugghe B. 2004. Interactions between Sox9 and beta-catenin control chondrocyte differentiation. Gene Dev 18:1072–1087.

Amanat N, McDonald M, Godfrey C, Bilston L, Little D. 2007. Optimal timing of a single dose of zoledronic acid to increase strength in rat fracture repair. J Bone Miner Res 22:867–876.

Ambrosetti D, Holmes G, Mansukhani A, Basilico C. 2008. Fibroblast growth factor signaling uses multiple mechanisms to inhibit Wnt-induced transcription in osteoblasts. Mol Cell Biol 28:4759–4771.

Amin D, Cornell SA, Gustafson SK, Needle SJ, Ullrich JW, Bilder GE, Perrone MH. 1992. Bisphosphonates used for the treatment of bone disorders inhibit squalene synthase and cholesterol biosynthesis. J Lipid Res 33:1657–1663.

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

Barrett J, Worth E, Bauss F, Epstein S. 2004. Ibandronate: a clinical pharmacological and pharmacokinetic update. J Clin Pharmacol 44:951–965.

Bauss F, Lalla S, Endele R, Hothorn LA. 2002. Effects of treatment with ibandronate on bone mass, architecture, biomechanical properties, and bone concentration of ibandronate in ovariectomized aged rats. J Rheumatol 29:2200–2208.

Bauss F, Pfister T, Papapoulos S. 2008. Ibandronate uptake in the jaw is similar to long bones and vertebrae in the rat. J Bone Miner Metab 26:406–408.

Bauss F, Russell RG. 2004. Ibandronate in osteoporosis: preclinical data and rationale for intermittent dosing. Osteoporos Int 15:423–433.

Berenson JR, Rosen L, Vescio R, Lau HS, Woo M, Sioufi A, Kowalski MO, Knight RD, Seaman JJ. 1997. Pharmacokinetics of pamidronate disodium in patients with cancer with normal or impaired renal function. J Clin Pharmacol 37:285–290.

Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, Cosman F, Lakatos P, Leung PC, Man Z, Mautalen C, Mesenbrink P, Hu H, Caminis J, Tong K, Rosario-Jansen T, Krasnow J, Hue TF, Sellmeyer D, Eriksen EF, Cummings SR. 2007. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med 356:1809–1822.

Black DM, Schwartz AV, Ensrud KE, Cauley JA, Levis S, Quandt SA, Satterfield S, Wallace RB, Bauer DC, Palermo L, Wehren LE, Lombardi A, Santora AC, Cummings SR. 2006. Effects of continuing or stopping alendronate after 5 years of treatment: the Fracture Intervention Trial Long-term Extension (FLEX): a randomized trial. JAMA 296:2927–2938.

Blanchoin L, Robinson RC, Choe S, Pollard TD. 2000. Phosphorylation of Acanthamoeba actophorin (ADF/cofilin) blocks interaction with actin without a change in atomic structure. J Mol Biol 295:203–211.

Bone HG, Adami S, Rizzoli R, Favus M, Ross PD, Santora A, Prahalada S, Daifotis A, Orloff J, Yates J. 2000. Weekly administration of alendronate: rationale and plan for clinical assessment. Clin Ther 22:15–28.

Bone HG, Hosking D, Devogelaer JP, Tucci JR, Emkey RD, Tonino RP, Rodriguez-Portales JA, Downs RW, Gupta J, Santora AC, Liberman UA. 2004. Ten years' experience with alendronate for osteoporosis in postmenopausal women. N Engl J Med 350:1189–1199.

Boyle WJ, Simonet WS, Lacey DL. 2003. Osteoclast differentiation and activation. Nature 423:337–342.

Breton S, Brown D. 2007. New insights into the regulation of V-ATPase-dependent proton secretion. Am J Physiol Renal Physiol 292:F1–10.

Breuil V, Euller-Ziegler L. 2006. Bisphosphonate therapy in rheumatoid arthritis. Joint Bone Spine 73:349–354.

Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. 1998. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268.

Canalis E, Mazziotti G, Giustina A, Bilezikian JP. 2007. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int 18: 1319–1328.

Cao X, Chen D. 2005. The BMP signaling and in vivo bone formation. Gene 357:1–8.

Celil AB, Campbell PG. 2005. BMP-2 and insulin-like growth factor-I mediate Osterix (Osx) expression in human mesenchymal stem cells via the MAPK and protein kinase D signaling pathways. J Biol Chem 280:31353–31359.

Chen T, Berenson J, Vescio R, Swift R, Gilchick A, Goodin S, LoRusso P, Ma P, Ravera C, Deckert F, Schran H, Seaman J, Skerjanec A. 2002. Pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with bone metastases. J Clin Pharmacol 42:1228–1236.

Clowes JA, Riggs BL, Khosla S. 2005. The role of the immune system in the pathophysiology of osteoporosis. Immunol Rev 208:207–227.

Cocquyt V, Kline WF, Gertz BJ, Van Belle SJ, Holland SD, DeSmet M, Quan H, Vyas KP, Zhang KE, De Greve J, Porras AG. 1999. Pharmacokinetics of intravenous alendronate. J Clin Pharmacol 39:385–393.

Coleman RE, Purohit OP, Black C, Vinholes JJ, Schlosser K, Huss H, Quinn KJ, Kanis J. 1999. Double-blind, randomised, placebo-controlled, dose-finding study of oral ibandronate in patients with metastatic bone disease. Ann Oncol 10:311–316.

Corrado A, Santoro N, Cantatore FP. 2007. Extra-skeletal effects of bisphosphonates. Joint Bone Spine 74:32–38.

Coxon FP, Helfrich MH, Van't Hof R, Sebti S, Ralston SH, Hamilton A, Rogers MJ. 2000. Protein geranylgeranylation is required for osteoclast formation, function, and survival: inhibition by bisphosphonates and GGTI-298. J Bone Miner Res 15:1467–1476.

Coxon FP, Thompson K, Roelofs AJ, Ebetino FH, Rogers MJ. 2008. Visualizing mineral binding and uptake of bisphosphonate by osteoclasts and non-resorbing cells. Bone 42:848–860.

Cramer JA, Gold DT, Silverman SL, Lewiecki EM. 2007. A systematic review of persistence and compliance with bisphosphonates for osteoporosis. Osteoporos Int 18:1023–1031.

Cramer JA, Silverman S. 2006. Persistence with bisphosphonate treatment for osteoporosis: finding the root of the problem. Am J Med 119:S12–S17.

Cremers S, Sparidans R, den HJ, Hamdy N, Vermeij P, Papapoulos S. 2002. A pharmacokinetic and pharmacodynamic model for intravenous bisphosphonate (pamidronate) in osteoporosis. Eur J Clin Pharmacol 57: 883–890.

Cummings SR, Black DM, Thompson DE, Applegate WB, Barrett-Connor E, Musliner TA, Palermo L, Prineas R, Rubin SM, Scott JC, Vogt T, Wallace R, Yates AJ, LaCroix AZ. 1998. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial. JAMA 280:2077–2082.

De Biase P, Capanna R. 2005. Clinical applications of BMPs. Injury 36 (Suppl 3):S43–S46.

Destaing O, Saltel F, Geminard JC, Jurdic P, Bard F. 2003. Podosomes display actin turnover and dynamic self-organization in osteoclasts expressing actin-green fluorescent protein. Mol Biol Cell 14:407–416.

Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. 1999. RANK is essential for osteoclast and lymph node development. Gene Dev 13:2412–2424.

Dunford JE, Rogers MJ, Ebetino FH, Phipps RJ, Coxon FP. 2006. Inhibition of protein prenylation by bisphosphonates causes sustained activation of Rac, Cdc42, and Rho GTPases. J Bone Miner Res 21:684–694.

Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE, Masarachia PJ, Wesolowski G, Russell RG, Rodan GA, Reszka AA. 1999. Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci USA 96:133–138.

Fleisch H, Russell RG, Straumann F. 1966. Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. Nature 212: 901–903.

Fujisaki J, Tokunaga Y, Takahashi T, Hirose T, Shimojo F, Kagayama A, Hata T. 1995. Osteotropic drug delivery system (ODDS) based on bisphosphonic prodrug. I: synthesis and in vivo characterization of osteotropic carboxyfluorescein. J Drug Target 3:273–282.

Gennari C. 2002. Analgesic effect of calcitonin in osteoporosis. Bone 30:67S–70S.

Gerstenfeld LC, Sacks DJ, Pelis M, Mason ZD, Graves DT, Barrero M, Ominsky MS, Kostenuik PJ, Morgan EF, Einhorn TA. 2009. Comparison of effects of the bisphosphonate alendronate versus the RANKL inhibitor denosumab on murine fracture healing. J Bone Miner Res 24:196–208.

Giraudo E, Inoue M, Hanahan D. 2004. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest 114:623–633.

Glass DA 2nd, Karsenty G. 2007. In vivo analysis of Wnt signaling in bone. Endocrinology 148:2630–2634.

Goldstein JL, Brown MS. 1990. Regulation of the mevalonate pathway. Nature 343:425–430.

Goltzman D. 2002. Discoveries, drugs and skeletal disorders. Nat Rev Drug Discov 1:784–796.

Hamdy NA. 2008. Denosumab: RANKL inhibition in the management of bone loss. Drug Today (Barc) 44:7–21.

Harada S, Rodan GA. 2003. Control of osteoblast function and regulation of bone mass. Nature 423:349–355.

Harris ST, Watts NB, Genant HK, McKeever CD, Hangartner T, Keller M, Chesnut CH 3rd, Brown J, Eriksen EF, Hoseyni MS, Axelrod DW, Miller PD. 1999. Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. Vertebral Efficacy With Risedronate Therapy (VERT) Study Group. JAMA 282:1344–1352.

Henneman ZJ, Nancollas GH, Ebetino FH, Russell RG, Phipps RJ. 2008. Bisphosphonate binding affinity as assessed by inhibition of carbonated apatite dissolution in vitro. J Biomed Mater Res A 85:993–1000.

Hess LM, Jeter JM, Benham-Hutchins M, Alberts DS. 2008. Factors associated with osteonecrosis of the jaw among bisphosphonate users. Am J Med 121: 475–483 e3.

Hoggarth CR, Bennett R, Daley-Yates PT. 1991. The pharmacokinetics and distribution of pamidronate for a range of doses in the mouse. Calcif Tissue Int 49:416–420.

Huang CL, Sun L, Moonga BS, Zaidi M. 2006. Molecular physiology and pharmacology of calcitonin. Cell Mol Biol (Noisy-le-grand) 52:33–43.

Jilka RL. 2007. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. Bone 40:1434–1446.

Kanzaki S, Ito M, Takada Y, Ogawa K, Matsuo K. 2006. Resorption of auditory ossicles and hearing loss in mice lacking osteoprotegerin. Bone 39:414–419.

Kanzaki S, Takada Y, Ogawa K, Matsuo K. 2009. Bisphosphonate therapy ameliorates hearing loss in mice lacking osteoprotegerin. J Bone Miner Res 24:43–49.

Karsenty G. 2008. Transcriptional control of skeletogenesis. Annu Rev Genomics Hum Genet 9:183–196.

Kavanagh KL, Guo K, Dunford JE, Wu X, Knapp S, Ebetino FH, Rogers MJ, Russell RG, Oppermann U. 2006. The molecular mechanism of nitrogen-containing bisphosphonates as antiosteoporosis drugs. Proc Natl Acad Sci USA 103:7829–7834.

Khan A. 2003. Advances in osteoporosis therapy 2003 update of practical guidelines. Can Fam Physician 49:441–447.

Kuroda J, Kimura S, Segawa H, Kobayashi Y, Yoshikawa T, Urasaki Y, Ueda T, Enjo F, Tokuda H, Ottmann OG, Maekawa T. 2003. The third-generation bisphosphonate zoledronate synergistically augments the anti-Ph+ leukemia activity of imatinib mesylate. Blood 102:2229–2235.

Labrie F. 2007. Drug insight: breast cancer prevention and tissue-targeted hormone replacement therapy. Nat Clin Pract Endocrinol Metab 3:584–593.

Leyvraz S, Hess U, Flesch G, Bauer J, Hauffe S, Ford JM, Burckhardt P. 1992. Pharmacokinetics of pamidronate in patients with bone metastases. J Natl Cancer Inst 84:788–792.

Li B. 2008. Bone morphogenetic protein-Smad pathway as drug targets for osteoporosis and cancer therapy. Endocr Metab Immune Disord Drug Target 8:208–219.

Lian JB, Javed A, Zaidi SK, Lengner C, Montecino M, van Wijnen AJ, Stein JL, Stein GS. 2004. Regulatory controls for osteoblast growth and differentiation: role of Runx/Cbfa/AML factors. Crit Rev Eukaryot Gene Expr 14:1–41.

Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW Jr, Dequeker J, Favus M. 1995. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group. N Engl J Med 333:1437–1443.

Lin JH, Chen IW, deLuna FA. 1994. Nonlinear kinetics of alendronate. Plasma protein binding and bone uptake. Drug Metab Dispos 22:400–405.

Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. 1998. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. J Bone Miner Res 13:581–589.

Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K, Narumiya S. 1999. Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science 285: 895–898.

Maltese WA. 1990. Posttranslational modification of proteins by isoprenoids in mammalian cells. Faseb J 4:3319–3328.

Manolagas SC, Jilka RL. 1995. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 332:305–311.

Marchisio PC, Cirillo D, Naldini L, Primavera MV, Teti A, Zambonin-Zallone A. 1984. Cell-substratum interaction of cultured avian osteoclasts is mediated by specific adhesion structures. J Cell Biol 99:1696–1705.

Martin TJ, Sims NA. 2005. Osteoclast-derived activity in the coupling of bone formation to resorption. Trend Mol Med 11:76–81.

Martini G, Gennari L, Merlotti D, Salvadori S, Franci MB, Campagna S, Avanzati A, De Paola V, Valleggi F, Nuti R. 2007. Serum OPG and RANKL levels before and after intravenous bisphosphonate treatment in Paget's disease of bone. Bone 40:457–463.

Masarachia P, Weinreb M, Balena R, Rodan GA. 1996. Comparison of the distribution of 3H-alendronate and 3H-etidronate in rat and mouse bones. Bone 19:281–290.

Mazziotti G, Angeli A, Bilezikian JP, Canalis E, Giustina A. 2006. Glucocorticoid-induced osteoporosis: an update. Trend Endocrinol Metab 17:144–149.

Miller CP. 2002. SERMs: evolutionary chemistry, revolutionary biology. Curr Pharm Des 8:2089–2111.

Miwa S, Mizokami A, Keller ET, Taichman R, Zhang J, Namiki M. 2005. The bisphosphonate YM529 inhibits osteolytic and osteoblastic changes and CXCR-4-induced invasion in prostate cancer. Cancer Res 65:8818–8825.

Monkkonen H, Auriola S, Lehenkari P, Kellinsalmi M, Hassinen IE, Vepsalainen J, Monkkonen J. 2006. A new endogenous ATP analog (ApppI) inhibits the mitochondrial adenine nucleotide translocase (ANT) and is responsible for the apoptosis induced by nitrogen-containing bisphosphonates. Br J Pharmacol 147:437–445.

Monkkonen J, Ylitalo P, Elo HA, Airaksinen MM. 1987. Distribution of [14C]clodronate (dichloromethylene bisphosphonate) disodium in mice. Toxicol Appl Pharmacol 89:287–292.

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

Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, Harada Y, Azuma Y, Krust A, Yamamoto Y, Nishina H, Takeda S, Takayanagi H, Metzger D, Kanno J, Takaoka K, Martin TJ, Chambon P, Kato S. 2007. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. Cell 130:811–823.

Nancollas GH, Tang R, Phipps RJ, Henneman Z, Gulde S, Wu W, Mangood A, Russell RG, Ebetino FH. 2006. Novel insights into actions of bisphosphonates on bone: differences in interactions with hydroxyapatite. Bone 38:617–627.

Nishida S, Tsubaki M, Hoshino M, Namimatsu A, Uji H, Yoshioka S, Tanimori Y, Yanae M, Iwaki M, Irimajiri K. 2005. Nitrogen-containing bisphosphonate, YM529/ONO-5920 (a novel minodronic acid), inhibits RANKL expression in a cultured bone marrow stromal cell line ST2. Biochem Biophys Res Commun 328:91–97.

Oades GM, Senaratne SG, Clarke IA, Kirby RS, Colston KW. 2003. Nitrogen containing bisphosphonates induce apoptosis and inhibit the mevalonate pathway, impairing Ras membrane localization in prostate cancer cells. J Urol 170:246–252.

Pan B, Farrugia AN, To LB, Findlay DM, Green J, Lynch K, Zannettino AC. 2004. The nitrogen-containing bisphosphonate, zoledronic acid, influences RANKL expression in human osteoblast-like cells by activating TNF-alpha converting enzyme (TACE). J Bone Miner Res 19:147–154.

Papapoulos SE. 2006. Bisphosphonate actions: physical chemistry revisited. Bone 38:613–616.

Perez EA, Weilbaecher K. 2006. Aromatase inhibitors and bone loss. Oncology (Williston Park) 20:1029–1039 discussion 1039-40, 1042,1048.

Plotkin LI, Manolagas SC, Bellido T. 2006. Dissociation of the pro-apoptotic effects of bisphosphonates on osteoclasts from their anti-apoptotic effects on osteoblasts/osteocytes with novel analogs. Bone 39:443–452.

Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T. 1999. Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. J Clin Invest 104:1363–1374.

Ralston SH. 2008. Pathogenesis of Paget's disease of bone. Bone 43:819–825.

Rauch F, Glorieux FH. 2005. Bisphosphonate treatment in osteogenesis imperfecta: which drug, for whom, for how long? Ann Med 37:295–302.

Reszka AA, Rodan GA. 2004. Nitrogen-containing bisphosphonate mechanism of action. Mini Rev Med Chem 4:711–719.

Ridley AJ, Hall A. 1992. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell 70:389–399.

Riggs BL, Khosla S, Melton LJ 3rd. 2002. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279–302.

Robling AG, Castillo AB, Turner CH. 2006. Biomechanical and molecular regulation of bone remodeling. Annu Rev Biomed Eng 8:455–498.

Rodan G, Reszka A, Golub E, Rizzoli R. 2004. Bone safety of long-term bisphosphonate treatment. Curr Med Res Opin 20:1291–1300.

Rodan GA. 1992. Introduction to bone biology. Bone 13(Suppl 1):S3–6.

Rodan GA, Reszka AA. 2002. Bisphosphonate mechanism of action. Curr Mol Med 2:571–577.

Rohatgi R, Ma L, Miki H, Lopez M, Kirchhausen T, Takenawa T, Kirschner MW. 1999. The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. Cell 97:221–231.

Rosen CJ. 2004. What's new with PTH in osteoporosis: where are we and where are we headed? Trend Endocrinol Metab 15:229–233.

Ruggiero SL, Mehrotra B. 2009. Bisphosphonate-related osteonecrosis of the jaw: diagnosis, prevention, and management. Annu Rev Med 60:85–96.

Russell RG, Watts NB, Ebetino FH, Rogers MJ. 2008. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. Osteoporos Int 19:733–759.

Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W, Moritz JD, Schu P, von Figura K. 1998. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci USA 95:13453–13458.

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

Sawicki M, Lecercle D, Grillon G, Le Gall B, Serandour AL, Poncy JL, Bailly T, Burgada R, Lecouvey M, Challeix V, Leydier A, Pellet-Rostaing S, Ansoborlo E, Taran F. 2008. Bisphosphonate sequestering agents. Synthesis and preliminary evaluation for in vitro and in vivo uranium(VI) chelation. Eur J Med Chem 43:2768–2777.

Schindeler A, Little DG. 2007. Bisphosphonate action: revelations and deceptions from in vitro studies. J Pharm Sci 96:1872–1878.

Scimeca JC, Franchi A, Trojani C, Parrinello H, Grosgeorge J, Robert C, Jaillon O, Poirier C, Gaudray P, Carle GF. 2000. The gene encoding the mouse homologue of the human osteoclast-specific 116-kDa V-ATPase subunit bears a deletion in osteosclerotic (oc/oc) mutants. Bone 26:207–213.

Seedor JG, Quartuccio HA, Thompson DD. 1991. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. J Bone Miner Res 6:339–346.

Shaw NJ, Bishop NJ. 2005. Bisphosphonate treatment of bone disease. Arch Dis Child 90:494–499.

Stein GS, Lian JB, van Wijnen AJ, Stein JL, Montecino M, Javed A, Zaidi SK, Young DW, Choi JY, Pockwinse SM. 2004. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene 23:4315–4329.

Takami M, Suda K, Sahara T, Itoh K, Nagai K, Sasaki T, Udagawa N, Takahashi N. 2003. Involvement of vacuolar H+ -ATPase in incorporation of risedronate into osteoclasts. Bone 32:341–349.

Teitelbaum SL, Ross FP. 2003. Genetic regulation of osteoclast development and function. Nat Rev Genet 4:638–649.

Thompson K, Rogers MJ, Coxon FP, Crockett JC. 2006. Cytosolic entry of bisphosphonate drugs requires acidification of vesicles after fluid-phase endocytosis. Mol Pharmacol 69:1624–1632.

Tsushima N, Yabuki M, Harada H, Katsumata T, Kanamaru H, Nakatsuka I, Yamamoto M, Nakatsuka M. 2000. Tissue distribution and pharmacological potential of SM-16896, a novel oestrogen-bisphosphonate hybrid compound. J Pharm Pharmacol 52:27–37.

Vaananen HK, Zhao H, Mulari M, Halleen JM. 2000. The cell biology of osteoclast function. J Cell Sci 113(Pt 3):377–381.

van Beek E, Hoekstra M, van de Ruit M, Lowik C, Papapoulos S. 1994. Structural requirements for bisphosphonate actions in vitro. J Bone Miner Res 9:1875–1882.

van beek E, Lowik C, van der Pluijm G, Papapoulos S. 1999a. The role of geranylgeranylation in bone resorption and its suppression by bisphosphonates in fetal bone explants in vitro: A clue to the mechanism of action of nitrogen-containing bisphosphonates. J Bone Miner Res 14:722–729.

van Beek E, Pieterman E, Cohen L, Lowik C, Papapoulos S. 1999b. Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. Biochem Biophys Res Commun 264:108–111.

van Beek ER, Cohen LH, Leroy IM, Ebetino FH, Lowik CW, Papapoulos SE. 2003. Differentiating the mechanisms of antiresorptive action of nitrogen containing bisphosphonates. Bone 33:805–811.

Van Beek ER, Lowik CW, Papapoulos SE. 2002. Bisphosphonates suppress bone resorption by a direct effect on early osteoclast precursors without affecting the osteoclastogenic capacity of osteogenic cells: the role of protein geranylgeranylation in the action of nitrogen-containing bisphosphonates on osteoclast precursors. Bone 30:64–70.

Wang X, Goh CH, Li B. 2007. p38 mitogen-activated protein kinase regulates osteoblast differentiation through osterix. Endocrinology 148:1629–1637.

Watts NB, Harris ST, Genant HK, Wasnich RD, Miller PD, Jackson RD, Licata AA, Ross P, Woodson GC 3rd, Yanover MJ, Mysiw WJ, Kohse L, Rao MB, Steiger P, Richmond B, Chesnut CH 3rd. 1990. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. N Engl J Med 323:73–79.

Weinstein RS, Chen JR, Powers CC, Stewart SA, Landes RD, Bellido T, Jilka RL, Parfitt AM, Manolagas SC. 2002. Promotion of osteoclast survival and antagonism of bisphosphonate-induced osteoclast apoptosis by glucocorticoids. J Clin Invest 109:1041–1048.

Weinstein RS, Roberson PK, Manolagas SC. 2009. Giant osteoclast formation and long-term oral bisphosphonate therapy. N Engl J Med 360:53–62.

Weiss HM, Pfaar U, Schweitzer A, Wiegand H, Skerjanec A, Schran H. 2008. Biodistribution and plasma protein binding of zoledronic acid. Drug Metab Dispos 36:2043–2049.

Weitzmann MN, Pacifici R. 2006. Estrogen regulation of immune cell bone interactions. Ann NY Acad Sci 1068:256–274.

Whyte MP. 2006. Clinical practice. Paget's disease of bone. N Engl J Med 355:593–600.

Zacharis CK, Tzanavaras PD. 2008. Determination of bisphosphonate active pharmaceutical ingredients in pharmaceuticals and biological material: a review of analytical methods. J Pharm Biomed Anal 48:483–496.

Zaidi M, Troen B, Moonga BS, Abe E. 2001. Cathepsin K, osteoclastic resorption, and osteoporosis therapy. J Bone Miner Res 16:1747–1749.

Zerial M, Stenmark H. 1993. Rab GTPases in vesicular transport. Curr Opin Cell Biol 5:613–620.

Zgani I, Menut C, Seman M, Gallois V, Laffont V, Liautard J, Liautard JP, Criton M, Montero JL. 2004. Synthesis of prenyl pyrophosphonates as new potent phosphoantigens inducing selective activation of human Vgamma9Vdelta2 T lymphocytes. J Med Chem 47:4600–4612.

Zhang D, Udagawa N, Nakamura I, Murakami H, Saito S, Yamasaki K, Shibasaki Y, Morii N, Narumiya S, Takahashi N, Suda T. 1995. The small GTP-binding protein, rho p21, is involved in bone resorption by regulating cytoskeletal organization in osteoclasts. J Cell Sci 108(Pt 6):2285–2292.

Zhang FL, Casey P. 1996. Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 65:241–269.

Zhang YW, Yasui N, Ito K, Huang G, Fujii M, Hanai J, Nogami H, Ochi T, Miyazono K, Ito Y. 2000. A RUNX2/PEBP2alpha A/CBFA1 mutation displaying impaired transactivation and Smad interaction in cleidocranial dysplasia. Proc Natl Acad Sci USA 97:10549–10554.