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Zoledronic acid: pharmacologic profile of a potent bisphosphonate

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Abstract

Bisphosphonates are an important class of osteotropic compounds that are effective in treating benign and malignant skeletal diseases characterized by enhanced osteoclast-mediated bone resorption (i.e., osteoporosis, Paget's disease, and tumor-induced osteolysis). The evolution of bisphosphonates has led to compounds with ever-increasing potency. First-generation bisphosphonates, including etidronate and clodronate, contained simple side chains and were relatively weak inhibitors of bone resorption. Second-generation compounds, including pamidronate, alendronate, and ibandronate, have an aliphatic R^2 side chain containing a single nitrogen atom. These nitrogen-containing bisphosphonates (N-BPs) are up to 100-fold more potent than the first-generation compounds. Zoledronic acid, a novel N-BP with an imidazole substituent, has demonstrated more potent inhibition of osteoclast-mediated bone resorption than all other bisphosphonates in both in vitro and in vivo preclinical models. Recent data suggest that N-BPs inhibit farnesyl diphosphate (FPP) synthase, an enzyme in the mevalonate biosynthetic pathway that is critical for protein prenylation and activation of important signaling molecules. Inhibition of FPP synthase also leads to production of triphosphoric acid 1-adenosin-5′yl ester 3-[3-methylbut-3-enyl] ester (ApppI), which induces apoptosis of osteoclasts and tumor cells. Our current knowledge of the pharmacology of N-BPs at the molecular level largely explains their observed effects on bone metabolism and tumor growth in animal models and their clinical activity in the treatment of benign and malignant bone diseases.
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Keywords: Bisphosphonate; Zoledronic acid; Pharmacology

1. Introduction

Bisphosphonates are ideally suited for the treatment of bone disease because they bind avidly to bone mineral at sites of active bone metabolism, where they achieve therapeutic concentrations. Fleisch and colleagues showed that bisphosphonates not only inhibit dissolution of hydroxyapatite crystals, but also affect osteoclast metabolism and function [1–3]. Bone-bound bisphosphonates are released during bone resorption and are internalized by osteoclasts, leading to inhibition of osteoclast activity and induction of osteoclast apoptosis [4–6]. As a result, bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption and have demonstrated clinical utility in the treatment of a wide range of benign and malignant bone diseases, including osteoporosis, Paget's disease of bone, and tumor-induced osteolysis [2]. Although these diseases differ in pathophysiology, they are all associated with increased osteoclast activity and abnormal bone metabolism. There is also extensive preclinical evidence that bisphosphonates have antitumor activity, as evidenced by reduced proliferation and viability of tumor cell lines in vitro and reduced skeletal tumor burden and progression of bone lesions in animal models [7]. This may contribute to the clinical activity of bisphosphonates in patients with malignant bone disease. Currently, a number of bisphosphonates, including zoledronic acid, pamidronate, ibandronate, and clodronate, are widely used to reduce the incidence of skeletal complications in patients with bone metastases.

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This review will focus on the pharmacology of bisphosphonates, particularly the nitrogen-containing bisphosphonates (N-BPs). Preclinical studies have shown that N-BPs have a unique mechanism of action involving the inhibition of farnesyl diphosphate (FPP) synthase, a key enzyme in the mevalonate biosynthetic pathway, and that the inhibition of FPP synthase leads to production of triphosphoric acid 1-adenosin-5′yl ester 3-[3-methylbut-3-enyl] ester (ApppI), which may contribute to the induction of osteoclast apoptosis. These studies have revealed the potential of N-BPs to affect a wide range of cellular processes and help to explain the observed preclinical and clinical activity of these important compounds.

2. The bisphosphonate class of compounds

2.1. Historical overview

Bisphosphonates were first synthesized in 1856 and because of their high affinity for cations, found widespread use as antiscaling agents in various industrial chemical applications. However, beginning in the mid-1960s, they were investigated as pharmaceuticals because of their avidity for bone and their ability to inhibit bone resorption. Initially, bisphosphonates were used to treat Paget's disease, a focal bone disorder characterized by enhanced bone turnover and abnormal structure, and hypercalcemia of malignancy, a potentially life-threatening complication of advanced cancer caused by uncontrolled tumor-induced osteolysis and enhanced calcium reabsorption by the kidney. Now, after more than 30 years of research and development, bisphosphonates have become an essential treatment for a wide range of benign and malignant bone diseases.

Over the past 30 years, several generations of bisphosphonates with increasing potency have been developed (Fig. 1) [8]. First-generation bisphosphonates, such as etidronate and clodronate, are relatively weak inhibitors of bone resorption. Once the clinical utility of bisphosphonates was realized, however, more potent compounds with improved clinical efficacy and tolerability were developed. Second-generation compounds, including pamidronate, alendronate, and ibandronate, are up to 100-fold more potent than the first-generation compounds [9,10], and zoledronic acid, a new-generation compound, has the highest potency in preclinical assays

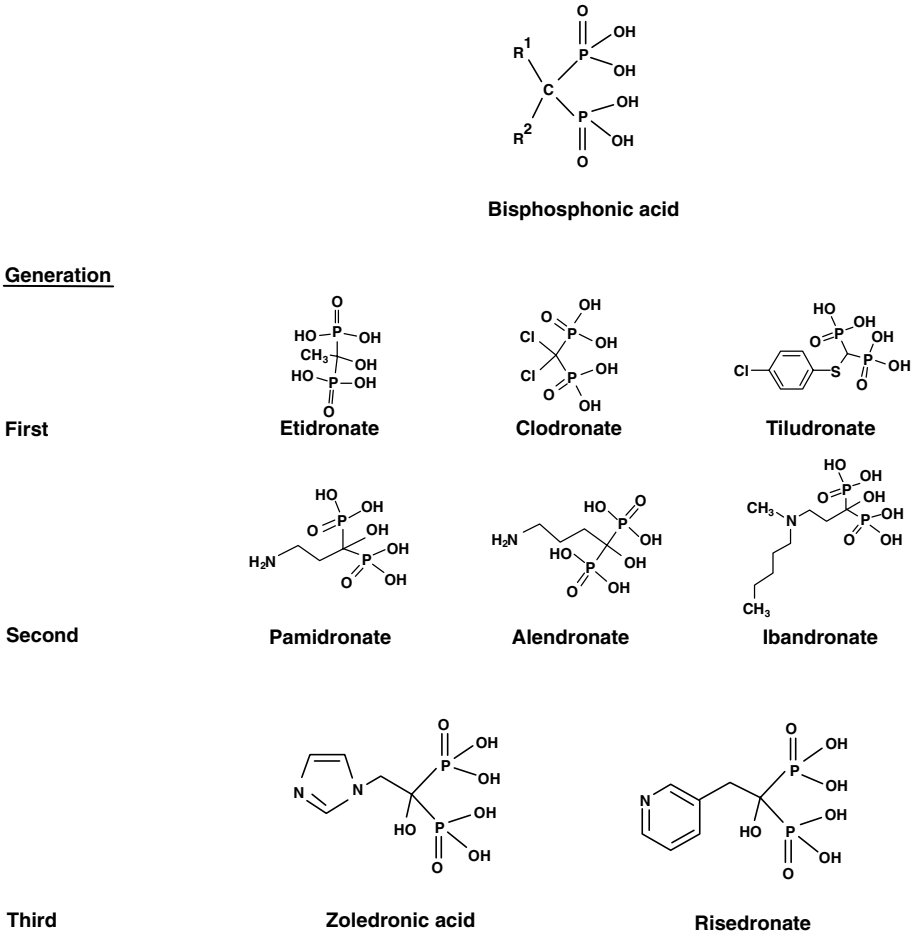

Fig. 1. The structures of selected bisphosphonates. Adapted with permission from Green et al. [8].

[11] and broadest clinical activity in metastatic bone disease of all the available bisphosphonates [12–15].

2.2. Structure–activity relationship

Bisphosphonates are stable, water-soluble, synthetic analogues of naturally occurring inorganic pyrophosphate (P–O–P) in which the central oxygen atom is replaced by a carbon atom (P–C–P), thereby making bisphosphonates resistant to enzymatic degradation [16]. In addition, the presence of the tetravalent central carbon atom facilitates the addition of different side chains, thus permitting synthesis of a wide variety of analogues (Fig. 1). The affinity of bisphosphonates for the bone mineral hydroxyapatite is a property of the highly charged P–C–P motif of the bisphosphonic acid structure [17], and this affinity is enhanced if the R^1 side chain is a hydroxyl group. In general, bisphosphonates can chelate cations such as calcium by bidentate coordination through the oxygen atoms of the phosphonate groups, but if the R^1 side chain is a hydroxyl, the affinity of bisphosphonates for cations is even greater, as this allows tridentate coordination [16].

Given that a hydroxyl group at the R^1 position appears to maximize affinity for bone, attempts to increase antiresorptive potency have focused on the R^2 side chain [18]. First-generation bisphosphonates (e.g., etidronate and clodronate) have simple R^2 side chains and are relatively weak inhibitors of bone resorption. For example, clodronate has two chlorine atoms bound to the central carbon. Second-generation compounds, including pamidronate, alendronate, and ibandronate, have an aliphatic R^2 side chain containing a single nitrogen atom. These N-BPs are 10- to 100-fold more potent than the first-generation compounds [9,10]. Further enhancement of potency was achieved when the nitrogen atom(s) were contained within a heterocyclic ring structure (e.g., risedronate) [19,20]. The addition of a second critically placed nitrogen atom to produce a heterocyclic imidazole substituent resulted in zoledronic acid [11]. This compound was selected from more than 300 candidates based on superior activity in a variety of in vitro and in vivo assays [21]. The presence of the second nitrogen atom may account for the improved potency and therapeutic ratio of zoledronic acid compared with other bisphosphonates.

Pharmacologically as a compound class, bisphosphonates demonstrate low oral bioavailability (~1%), bind rapidly to bone, and have a very long half-life in bone [22]. Approximately 40–60% of the administered dose binds to bone within 24 h and the remaining portion is rapidly excreted unchanged in the urine [22]. Studies with radiolabeled zoledronic acid in rats have shown that it remains readily detectable in bone 1 year after administration, and detailed modeling of the plasma pharmacokinetics of zoledronic acid estimated a terminal elimination half-life >6 months, which is similar to that of pamidronate and alendronate [22]. Bisphosphonates are generally administered orally for the treatment of benign osteoporosis, but because of poor oral bioavailability and the potential to cause intermittent gastrointestinal toxicity, the intravenous (IV) route is an attractive alternative in cancer patients with bone metastases. Given the long half-life of bisphosphonates in bone, a single annual infusion may be sufficient to treat postmenopausal osteoporosis [23], whereas one infusion every 3–4 weeks has been demonstrated to be a highly efficacious treatment regimen for bone metastases [12–15].

3. Mechanism of action

Bisphosphonates accumulate in the mineralized bone matrix at sites of active bone metabolism, are released during bone resorption, and are internalized by osteoclasts [4–6]. The molecular mechanism by which the first-generation, non-N-BPs (eg, etidronate and clodronate) affect osteoclasts appears to involve metabolism of these compounds by osteoclasts and other cells to non-hydrolyzable, cytotoxic adenosine triphosphate (ATP) analogues [24–28], which can induce osteoclast apoptosis [24,29–31]. For example, clodronate is metabolized to $AppCCl_2p$, which at high concentrations inhibits mitochondrial ATP/ADP translocase, thereby causing loss of the mitochondrial membrane potential and direct induction of apoptosis [16,32].

In contrast, nitrogen-containing compounds are not metabolized and must, therefore, affect osteoclasts through a different mechanism [33]. In fact, the potent inhibitory effects of N-BPs on bone resorption appear to be mediated by several different cellular mechanisms [5,34–37]: they directly inhibit osteoclast activity; they inhibit the formation of osteoclasts and their recruitment to the bone surface [34,35]; and they can cause osteoclast apoptosis [30,31]. Current evidence suggests that N-BPs inhibit osteoclast activity by affecting a variety of intracellular processes necessary for osteoclast function, such as organization of the cytoskeleton, membrane transport, and formation of the ruffled border [5]. In vitro studies have shown that N-BPs also directly affect osteoblasts. Pamidronate and zoledronic acid have been shown to promote the differentiation and mineralization of human fetal osteoblasts [38], and low concentrations of these two compounds (10^{-9}–10^{-6} M) can increase production of osteoprotegerin (OPG) from primary human osteoblasts [39]. These effects of N-BPs on osteoblasts can, in turn, effect osteoclastogenesis (i.e., maturation of immature osteoclast progenitors) because the differentiation state of osteoblasts appears to influence their production of OPG and expression of receptor activator of nuclear factor-kappaB (RANK) ligand

(RANKL) [40]. The balance of these molecules is involved in the regulation of osteoclastogenesis. As osteoblasts mature they appear to increase OPG expression and downregulate RANKL, which would inhibit osteoclastogenesis.

3.1. The mevalonate pathway

An enzyme of the mevalonate pathway of lipid biosynthesis has recently been identified as the molecular target of N-BPs [41–45]. Several independent studies have now confirmed that FPP synthase is the major enzymatic target of N-BPs [43,46,47]. Dunford and colleagues [46] recently demonstrated that recombinant human FPP synthase is potently inhibited by N-BPs in a concentration-dependent manner. Zoledronic acid and the closely related risedronate were among the most potent bisphosphonates tested in this assay (Fig. 2(a)) [46]. Zoledronic acid demonstrated a 50% inhibitory concentration (IC_{50}) of 3 nM. Interestingly, the potency ranking in this in vitro FPP synthase assay closely matched the rank order of potency of these compounds with respect to inhibition of bone resorption in mouse calvaria cultures (Fig. 2(b)) [11]: zoledronic acid > risedronate > ibandronate > alendronate > pamidronate. This suggests that the ability to inhibit FPP synthase is a major determinant of the antiresorptive potency of N-BPs. Recently, the X-ray crystallographic analysis of risedronate bound to the active site of the bacterial FPP synthase enzyme was reported, showing the molecular interactions between N-BPs and the enzyme [48]. This crystallographic analysis demonstrated how N-BPs mimic a carbocation intermediate to inhibit the enzyme.

Inhibition of FPP synthase affects the synthesis of two isoprenoid lipid intermediates in the mevalonate pathway, farnesyl diphosphate and geranylgeranyl diphosphate, which are required for the post-translational modification (i.e., prenylation) of cellular proteins (Fig. 3) [5,49]. In particular, several small guanosine triphosphate (GTP)-binding proteins such as Ras, Rho, and Rac require prenylation to be localized to the cell membrane, thus allowing them to interact with key cell surface receptors and become activated [50]. These important signaling proteins regulate a variety of cellular processes important for osteoclast function, including cell morphology, integrin signaling, membrane ruffling, transport of endosomes, and apoptosis [41,42,45,50–52]. Zoledronic acid and other N-BPs have been shown to inhibit the mevalonate pathway in purified rabbit osteoclasts in vitro and to prevent the incorporation of [^{14}C]mevalonate into prenylated proteins [43,45].

There is now considerable preclinical evidence that N-BPs exert their effects on osteoclasts by inhibiting protein prenylation, thereby short-circuiting intracellular signaling via small GTPases, such as Ras, which require membrane localization. As a result of their biochemical effects on protein prenylation, N-BPs also induce caspase-dependent apoptosis [29], inhibit matrix metalloproteinase synthesis and secretion [53,54], downregulate $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins [55,56], and stimulate osteoblasts to increase expression of OPG, which antagonizes osteoclastogenesis [39]. Moreover, because the mevalonate pathway is ubiquitous, the effects of bisphosphonates on cell function and survival are not restricted to cells of the monocytic lineage such as osteoclasts and macrophages. Preclinical data indicate that N-BPs also have direct effects on tumor cells in vitro, and N-BPs have been shown to decrease both the viability and proliferation of human breast cancer, multiple myeloma, and prostate cancer cell lines, and to induce apoptosis of tumor cells [57–66].

3.2. Production of ApppI

Recent studies have shown that N-BPs may have yet another mechanism of action: inducing production of the endogenous intracellular ATP analogue ApppI,

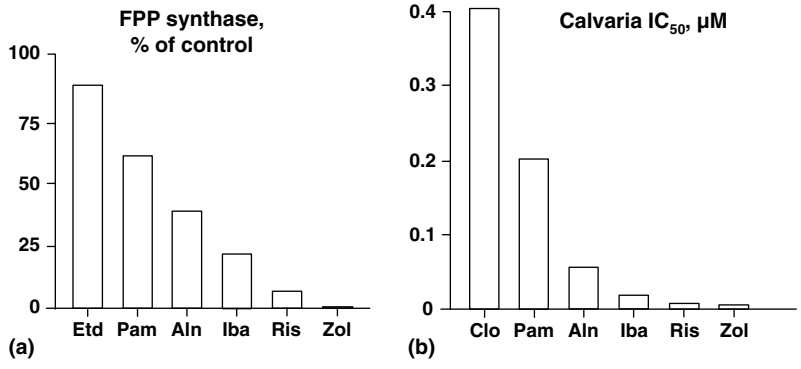

Fig. 2. Relative potency of various bisphosphates based on (a) inhibition of farnesyl diphosphonate (FPP) synthase activity and (b) inhibition of calcium release by osteoclasts in murine calvaria cultures. IC_{50}, 50% inhibitory concentration; Etd, etidronate; Pam, pamidronate; Aln, alendronate; Iba, ibandronate; Ris, risedronate; Zol, zoledronic acid; Clo, clodronate. (a) Adapted with permission from Dunford et al. [46]; (b) data from Green et al. [11].

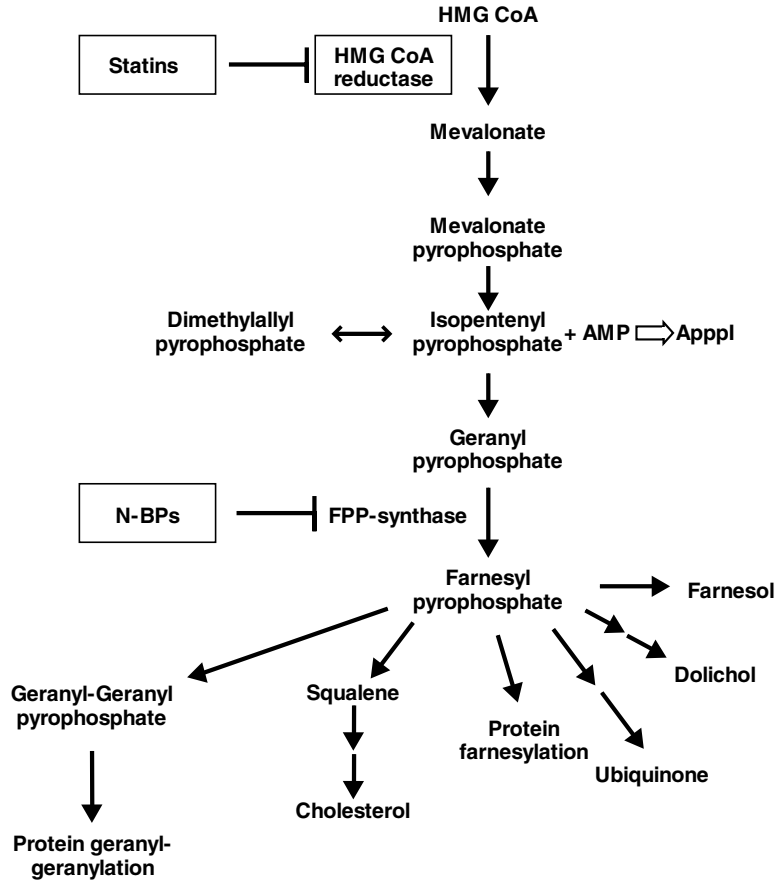

Fig. 3. Schematic diagram of the mevalonate pathway showing that inhibition of FFP synthase by nitrogen-containing bisphosphates blocks synthesis of a isoprenoid lipid intermediate farneysl diphosphate and geranylgeranyl diphosphate, and induces the formation of a cytotoxic IPP-AMP conjugate. HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; PP, diphosphonate; AMP, adenosine monophosphate; ApppI, triphosphoric acid 1-adenosin-5′yl ester 3-[3-methylbut-3-enyl] ester; N-BPs, nitrogen-containing bisphosphonates. Adapted from Rogers et al. [5], and Mönkkönen et al. [49].

which may directly induce apoptosis similar to AppCC1_2p (i.e., a metabolite of clodronate) [67]. Inhibition of FPP synthase results in accumulation of isopentenyl diphosphate, which can be metabolized to IPP-AMP or ApppI (Fig. 3) [49]. The ability of various bisphosphonates to inhibit FPP synthase activity in J774 macrophage cell homogenates correlates well with their ability to induce production of ApppI in J774 cells [46,49]. In this assay, clodronate served as a negative control with little or no effect on FPP synthase activity, and it induced no ApppI production. Of the N-BPs tested, zoledronic acid demonstrated the highest potency in both inhibition of FPP synthase activity and production of ApppI. The implication of this work is that N-BPs can potentially induce apoptosis of osteoclasts and tumor cells by at least two distinct pathways.

3.3. Induction of caspase-dependent osteoclast apoptosis

Zoledronic acid, like other bisphosphonates, can induce apoptosis of osteoclasts in vitro, and the ability to induce apoptosis may contribute to their overall antiresorptive potency in vivo. Benford et al. [29] have shown that zoledronic acid induces apoptosis in purified rabbit osteoclasts, human osteoclastoma-derived osteoclasts, and osteoclast-like cells generated in cultures of human bone marrow in vitro. Osteoclast apoptosis involved characteristic morphologic changes, loss of mitochondrial membrane potential, and the activation of caspase-3-like proteases with the ability to cleave Asp-GluValAsp-containing peptide substrates. Zoledronic acid at a concentration of 100 μM for 48 h increased caspase-3-like activity in rabbit osteoclasts 8-fold compared with untreated osteoclasts and was more potent than other bisphosphonates tested [29]. The induction of osteoclast apoptosis is also likely due to inhibition of protein geranylgeranylation and appears to be dependent on caspase activation because suppression of caspase activity by zVAD-fmk (a broad-range caspase inhibitor) or by SB-281277 (a specific inhibitor of caspase-3 and -7) blocked the characteristic morphologic features of apoptosis after treatment of osteoclasts with zoledronic acid [29].

4. Effects of zoledronic acid on bone metabolism

4.1. Effects on osteoclast-mediated bone resorption in preclinical models

Of the bisphosphonates tested, zoledronic acid has demonstrated the greatest potency in preclinical models of osteoclast-mediated bone resorption. In the mouse calvaria organ culture assay, zoledronic acid inhibited bone resorption induced by 1,25-dihydroxyvitamin D_3 and was more potent than any other bisphosphonate tested in this assay, with an IC_{50} value of 2 nM [11]. Regardless of the stimulus used (1,25-dihydroxyvitamin D_3, parathyroid hormone, parathyroid-related protein, interleukin-1β, or prostaglandin E_2) zoledronic acid was 40- to 100-fold more potent than pamidronate. The ability of zoledronic acid to inhibit bone resorption induced by a variety of mediators implies that it should be effective in a wide range of bone diseases, irrespective of the pathologic mechanism.

In the thyroparathyroidectomized (TPTX) rat model, zoledronic acid demonstrated a dose-dependent inhibition of 1,25-dihydroxyvitamin D_3-induced hypercalcemia, with a half-maximal effective dose (ED_{50}) of 0.07 μg/kg and a 100% effective dose (ED_{100}) of 1.4 μg/kg. In this assay, zoledronic acid was 850-fold more potent than pamidronate and was the most potent compound tested. Comparison of the in vivo TPTX rat data with the in vitro calvaria model data (Table 1) [11] showed a high correlation for all the bisphosphonates tested (correlation coefficient of 0.97). This implies that the hypocalcemic effect of bisphosphonates observed in vivo in the TPTX rat is predominantly due to direct inhibition of bone resorption rather than to any indirect effects on calcium metabolism in the gastrointestinal tract or kidney.

Because some of the earlier bisphosphonates have been reported to impair bone mineralization when administered at high therapeutic doses [67,68], the effect of zoledronic acid and other bisphosphonates on bone mineralization was studied in vitro in calvaria cultures treated with calcium 1,2-glycerophosphate to promote calcium uptake [8]. All of the compounds tested inhibited the incorporation of calcium into calvaria, with IC_{50} values in the range of 10–100 μM in marked contrast to the 2000-fold range in IC_{50} values for the inhibition of bone resorption in the calvaria assay stimulated with 1,25-dihydroxyvitamin D_3. This large dissociation between inhibition of bone resorption and bone mineralization indicates that zoledronic acid has a wide therapeutic ratio that allows it to inhibit bone resorption without affecting bone mineralization.

4.2. Inhibition of bone resorption in growing rats

The in vivo effect of zoledronic acid was compared to that of other bisphosphonates in two short-term experiments with young, growing rats [11]. Treatment for 10 days with zoledronic acid or pamidronate via subcutaneous (SC) injection caused a dose-dependent increase in the radiographic density of the proximal tibial metaphysis; however, zoledronic acid was 230 times more potent than pamidronate (ED_{50} values, 1.7 and 390 μg/kg, respectively) [11]. By contrast, etidronate was ineffective at a dose of 3.2 mg/kg. In a subsequent experiment, bisphosphonates were administered SC for 15 days, after which the distal femur was subjected to chemical analysis. Again, zoledronic acid increased trabecular bone mass as measured by calcium content, hydroxyproline content, and dry weight, and was 87–150 times more potent than pamidronate [8]. In a detailed histomorphometric analysis of bones from rats treated for 10 days with zoledronic acid (0.028–2.8 μg/kg SC) or pamidronate (3.7–370 μg/kg SC), both compounds suppressed cancellous bone turnover and resorption in a dose-dependent manner [69]. Zoledronic acid was 100-fold more potent than pamidronate and, despite its higher antiresorptive potency, resulted in no evidence of impaired bone mineralization.

4.3. Inhibition of ovariectomy-induced bone loss

Two long-term studies have further shown that zoledronic acid effectively maintains bone mass, strength, and

Table 1
Inhibition of vitamin D_3-induced calcium release from mouse calvaria in vitro and hypercalcemia in the thyroid-parathyroidectomized (TPTX) rat

Compound	Mouse calvaria	TPTX rat
	Mean IC_{50}, μM (no. of experiments)	Mean ± SEM ED_{50}, μg/kg (no. of experiments)
Zoledronic acid	0.002 (5)	0.072 ± 0.018 (7)
Risedronate	0.01 (2)	1.7 ± 0.3 (4)
Ibandronate	0.02 (3)	1.4 ± 0.4 (5)
Alendronate	0.05 (2)	8.2 ± 1.4 (3)
Pamidronate	0.2 (6)	61 ± 7.5 (15)
Clodronate	0.4 (3)	1.200 ± 300 (5)

IC_{50}, concentration of bisphosphonates in the culture medium required to inhibit 1,25-$(OH)_2$ vitamin D_3-induced calcium release by 50%; SEM, standard error of the mean; ED_{50}, dose of bisphosphonate, given on 4 consecutive days subcutaneously, required to inhibit 1,25-$(OH)_2$ vitamin D_3-induced hypercalcemia by 50%. Adapted with permission from Green et al. [11].

architecture in ovariectomized monkeys and rats [70–75]. Long-term treatment of ovariectomized rats with zoledronic acid at doses of 0.3, 1.5, or 7.5 μg/kg/week for 52 weeks prevented the reduction in bone density and maintained mechanical integrity of the femur and lumbar vertebrae in a dose-dependent fashion [74,75]. Zoledronic acid also inhibited the urinary excretion of deoxypyridinium crosslinks and normalized serum osteocalcin, biochemical markers of bone resorption and formation, respectively. Moreover, in a recent long-term study with adult ovariectomized rats, a single IV injection of zoledronic acid dose-dependently prevented bone loss and preserved bone strength for up to 8 months (Fig. 4) [76]. The inhibition of ovariectomy-induced bone loss by zoledronic acid in these animals has been examined in more detail using high-resolution microcomputed tomography [77], a technique that allows the non-destructive quantitation of the three-dimensional bone structure. In ovariectomized control animals treated with saline, there was a significant loss of bone characterized by a diminution of plate-like structures and a transition to more slender, rod-like structures. This bone loss was effectively counteracted in all parts of the vertebral body by weekly low doses of zoledronic acid (1.5 μg/kg) [74].

When ovariectomized adult rhesus monkeys were treated with zoledronic acid (0.5, 2.5, or 12.5 μg/kg) by SC injection weekly for 69 weeks, bone loss (as measured by dual-energy X-ray absorptiometry) was prevented in a dose-dependent fashion [70]. Biochemical markers of bone turnover, including serum osteocalcin, skeletal alkaline phosphatase, and urinary *N*-telopeptide and pyridinoline, were all suppressed by zoledronic acid. Histomorphometric analyses of bone samples from these animals confirmed the suppression of cancellous and cortical bone turnover by zoledronic acid [71,72]. Treatment with zoledronic acid also reduced the porosity of cortical bone, thus preserving bone density and mechanical strength [72,73].

4.4. Inhibition of tumor-induced bone destruction and antitumor effects

Injection of Walker carcinosarcoma 256 cells into the rat is a well-characterized animal model of tumor-induced osteolytic bone destruction in which the osteolysis and associated hypercalcemia are mediated by parathyroid hormone-related protein synthesized by tumor cells [78,79]. Although calcitonin and gallium nitrate were able to rapidly normalize hypercalcemia, both were ineffective in preventing osteolysis [80]. In contrast, treatment of tumor-bearing rats with zoledronic acid, pamidronate, or ibandronate effectively inhibited tumor-induced osteolysis and delayed hypercalcemia [80–82]. Zoledronic acid was 1000-fold more potent than pamidronate in this model.

Zoledronic acid has also been shown to inhibit progression of established bone metastases and development of new bone metastases in two models of breast cancer [83,84]. In nude mice injected with

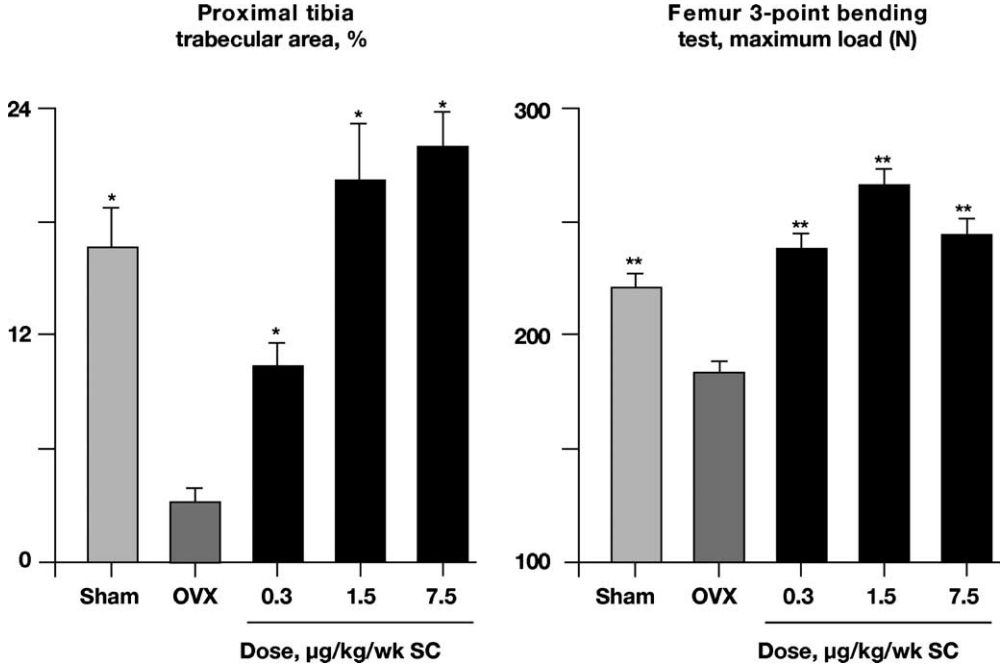

Fig. 4. Long-term treatment with zoledronic acid preserved trabecular bone and bone strength in adult ovariectomized (OVX) rats. SC, subcutaneous. $^{*}P < .05$; $^{**}P < .01$; $n = 16–20$. Adapted with permission from Hornby et al. [75].

MDA-MB-231 breast cancer cells, which form characteristic osteolytic lesions, treatment with zoledronic acid (0.2, 1.0, or 5.0 μg/day SC) for 10 consecutive days beginning on day 23 after tumor cell injection significantly reduced radiographic bone lesion area by >80% compared with controls [83]. Ibandronate (1.0 μg/day) and alendronate (10 μg/day) resulted in non-significant reductions in bone lesion area of 65% and 55%, respectively. These findings were confirmed using highly sensitive in vivo imaging of MDA-MB-231 cells genetically engineered to express a photon-emitting protein. Administration of zoledronic acid from days 18 to 29 after tumor cell injection markedly inhibited the progression of established osteolytic lesions [84]. In another murine model, treatment with IV zoledronic acid (5 μg/day) for 7 days after injection of 4T1 murine mammary tumor cells (i.e., prevention setting) markedly decreased the formation of new bone metastases assessed at day 28 [83]. The observed decrease in radiographic lesion area was accompanied by a concomitant increase in the number of apoptotic osteoclasts and tumor cells in bone lesions. Similar studies in a murine model of multiple myeloma have demonstrated that zoledronic acid reduces osteolysis, tumor burden and angiogenesis, with a concomitant increase in survival [85]. These and a number of other animal models of malignant bone disease have consistently demonstrated the local antitumor activity of N-BPs on bone metastases [7].

5. Conclusions

Bisphosphonates are highly effective inhibitors of osteoclast-mediated bone resorption. Over the years it has been demonstrated that changes in the chemical structure of these compounds can profoundly affect their potency and mechanism of action. For example, the addition of a nitrogen atom significantly modifies the mechanism of action, resulting in greater potency and therapeutic ratio. An enzyme of the mevalonate pathway has been identified as the molecular target of N-BPs. Inhibition of FPP synthase activity inhibits protein prenylation and can also lead to production of the ATP analogue ApppI. As a result, N-BPs inhibit osteoclast activity and induce osteoclast apoptosis. Moreover, these effects extend to tumor cells growing in bone, thereby explaining the observed antitumor activity of bisphosphonates. Preclinical studies provide a strong rationale for the clinical application of bisphosphonates for the treatment of both benign and malignant bone diseases. In particular, zoledronic acid is a highly potent, new-generation bisphosphonate that has broad clinical activity in patients with bone metastasis. Zoledronic acid has been shown to reduce the incidence and to delay the onset of skeletal complications in patients with breast cancer, multiple myeloma, prostate cancer, lung cancer, renal cancer, and other solid tumors [12–15]. Zoledronic acid has also been shown to inhibit bone loss associated with estrogen and androgen suppression for the treatment of breast or prostate cancer [86,87]. Low doses of zoledronic acid have also been shown to inhibit bone loss in benign bone diseases such as post-menopausal osteoporosis [23] and Paget's disease [88]. The evolution of bisphosphonates has led to tremendous improvements in their clinical activity, and ongoing studies continue to investigate their clinical application in a number of new indications.

References

[1] S.A. Stronski, L. Bettschen-Camin, A. Wetterwald, R. Felix, U. Trechsel, H. Fleisch, Calcif. Tissue Int. 42 (1988) 248.
[2] H. Fleisch, Breast Cancer Res. 4 (2002) 30.
[3] R.G. Russell, P.I. Croucher, M.J. Rogers, Osteoporos. Int. 9 (Suppl. 2) (1999) S66.
[4] A.M. Flanagan, T.J. Chambers, Calcif. Tissue Int. 49 (1991) 407.
[5] M.J. Rogers, S. Gordon, H.L. Benford, F.P. Coxon, S.P. Luckman, J. Monkkonen, J.C. Frith, Cancer 88 (Suppl.) (2000) 2961.
[6] M. Sato, W. Grasser, N. Endo, R. Akins, H. Simmons, D.D. Thompson, E. Golub, G.A. Rodan, J. Clin. Invest. 88 (1991) 2095.
[7] P. Clezardin, Semin. Oncol. 29 (Suppl. 21) (2002) 33.
[8] J.R. Green, M.J. Rogers, Drug Dev. Res. 55 (2002) 210.
[9] H. Shinoda, G. Adamek, R. Felix, H. Fleisch, R. Schenk, P. Hagan, Calcif. Tissue Int. 35 (1983) 87.
[10] R. Schenk, P. Eggli, H. Fleisch, S. Rosini, Calcif. Tissue Int. 38 (1986) 342.
[11] J.R. Green, K. Müller, K.A. Jaeggi, J. Bone Miner. Res. 9 (1994) 745.
[12] L.S. Rosen, D. Gordon, M. Kaminski, A. Howell, A. Belch, J. Mackey, J. Apffelstaedt, M.A. Hussein, R.E. Coleman, D.J. Reitsma, B.-L. Chen, J.J. Seaman, Cancer 98 (2003) 1735.
[13] F. Saad, D.M. Gleason, R. Murray, S. Tchekmedyian, P. Venner, L. Lacombe, J.L. Chin, J.J. Vinholes, J.A. Goas, M. Zheng, For the zoledronic acid prostate cancer study group, J. Natl. Cancer Inst. 96 (2004) 879.
[14] L.S. Rosen, D. Gordon, N.S. Tchekmedyian, R. Yanagihara, V. Hirsh, M. Krzakowski, M. Pawlicki, P. de Souza, M. Zheng, G. Urbanowitz, D. Reitsma, J. Seaman, Cancer 100 (2004) 2613.
[15] A. Lipton, M. Zheng, J. Seaman, Cancer 98 (2003) 962.
[16] M.J. Rogers, D.J. Watts, R.G.G. Russell, Cancer 80 (Suppl.) (1997) 1652.
[17] A. Jung, S. Bisaz, H. Fleisch, Calcif. Tissue Res. 11 (1973) 269.
[18] A.D. Geddes, S.M. D'Souza, F.H. Ebetino, K.J. Ibbotson, J. Bone Miner. Res. 8 (1994) 265.
[19] W.K. Sietsema, F.H. Ebetino, A.M. Salvagno, J.A. Bevan, Drugs Exp. Clin. Res. 15 (1989) 389.
[20] E.R. van Beek, C.W.G.M. Lowik, F.H. Ebetino, S.E. Papapoulos, Bone 23 (1998) 437.
[21] L. Wilder, K.A. Jaeggi, M. Glatt, K. Muller, R. Bachmann, M. Bisping, A.R. Born, R. Cortesi, G. Guiglia, H. Jeker, R. Klein, U. Ramseler, J. Schmid, G. Schreiber, Y. Seltenmeyer, J.R. Green, J. Med. Chem. 45 (2002) 3721.

[22] J.H. Lin, Bone 18 (1996) 75.
[23] I.R. Reid, J.P. Brown, P. Burckhardt, Z. Horowitz, P. Richardson, U. Trechsel, A. Widmer, J.P. Devogelaer, J.M. Kaufman, P. Jaeger, J.J. Body, P.J. Meunier, N. Engl. J. Med. 346 (2002) 653.
[24] J.C. Frith, J. Monkkonen, S. Auriola, H. Monkkonen, M.J. Rogers, Arthritis Rheum. 44 (2001) 2201.
[25] J.C. Frith, J. Monkkonen, G.M. Blackburn, R.G. Russell, M.J. Rogers, J. Bone Miner. Res. 12 (1997) 1358.
[26] S. Pelorgeas, J.B. Martin, M. Satre, Biochem. Pharmacol. 44 (1992) 2157.
[27] M.J. Rogers, X. Ji, R.G. Russell, G.M. Blackburn, M.P. Williamson, A.V. Bayless, F.H. Ebetino, D.J. Watts, Biochem. J. 303 (1994) 303.
[28] M.J. Rogers, R.G. Russell, G.M. Blackburn, M.P. Williamson, D.J. Watts, Biochem. Biophys. Res. Commun. 189 (1992) 414.
[29] H.L. Benford, N.W. McGowan, M.H. Helfrich, M.E. Nuttall, M.J. Rogers, Bone 28 (2001) 465.
[30] D.E. Hughes, K.R. Wright, H.L. Uy, A. Sasaki, T. Yoneda, G.D. Roodman, G.R. Mundy, B.F. Boyce, J. Bone Miner. Res. 10 (1995) 1478.
[31] K.S. Selander, J. Monkkonen, E.K. Karhukorpi, P. Harkonen, R. Hannuniemi, H.K. Vaananen, Mol. Pharmacol. 50 (1996) 1127.
[32] P.P. Lehenkari, M. Kellinsalmi, J.P. Napankangas, K.V. Ylitalo, J. Monkkonen, M.J. Rogers, A. Azhayev, H.K. Vaananen, I.E. Hassinen, Mol. Pharmacol. 61 (2002) 1255.
[33] H.L. Benford, J.C. Frith, S. Auriola, J. Mönkkönen, M.J. Rogers, Mol. Pharmacol. 56 (1999) 131.
[34] C.E. Evans, I.P. Braidman, Bone Miner. 26 (1994) 95.
[35] C.W. Lowik, G. van der Pluijm, L.J. van der Wee-Pals, H.B. van Treslong-de Groot, O.L. Bijvoet, J. Bone Miner. Res. 3 (1988) 185.
[36] C.W.G.M. Lowik, P.M. Boonekamp, G. van de Pluym, L. van der Wee-Pals, H. Bloys van Treslong-de Groot, O.L. Bijvoet, Adv. Exp. Med. Biol. 208 (1986) 275.
[37] P.M. Boonekamp, L.J. van der Wee-Pals, M.M. van Wijk-van Lennep, C.W. Thesing, O.L. Bijvoet, Bone Miner. 1 (1986) 27.
[38] G.G. Reinholz, B. Getz, L. Pederson, E.S. Sanders, M. Subramaniam, J.N. Ingle, T.C. Spelsberg, Cancer Res. 60 (2000) 6001.
[39] V. Viereck, G. Emons, V. Lauck, K.H. Frosch, S. Blaschke, C. Gründker, L.C. Hofbauer, Biochem. Biophys. Res. Commun. 291 (2002) 680.
[40] G.J. Atkins, P. Kostakis, B. Pan, A. Farrugia, S. Gronthos, A. Evdokiou, K. Harrison, D.M. Findlay, A.C.W. Zannettio, J. Bone Miner. Res. 18 (2003) 1088.
[41] S.P. Luckman, D.E. Hughes, F.P. Coxon, R. Graham, R.G. Russell, M.J. Rogers, J. Bone Miner. Res. 13 (1998) 581.
[42] S.P. Luckman, F.P. Coxon, F.H. Ebetino, R.G. Russell, M.J. Rogers, J. Bone Miner. Res. 13 (1998) 1668.
[43] J.D. Bergstrom, R.G. Bostedor, P.J. Masarachia, A.A. Reszka, G. Rodan, Arch. Biochem. Biophys. 373 (2000) 231.
[44] D. Amin, S.A. Cornell, S.K. Gustafson, S.J. Needle, J.W. Ullrich, G.E. Bilder, M.H. Perrone, J. Lipid Res. 33 (1992) 1657.
[45] F.P. Coxon, M.H. Helfrich, R. Van't Hof, S. Sebti, S.H. Ralston, A. Hamilton, M.J. Rogers, J. Bone Miner. Res. 15 (2000) 1467.
[46] J.E. Dunford, K. Thompson, F.P. Coxon, S.P. Luckman, F.M. Hahn, C.D. Poulter, F.H. Ebetino, M.J. Rogers, J. Pharmacol. Exp. Ther. 296 (2001) 235.
[47] E. van Beek, E. Pieterman, L. Cohen, C. Lowik, S. Papapoulos, Biochem. Biophys. Res. Commun. 264 (1999) 108.
[48] D.J. Hosfield, Y. Zhang, D.R. Dougan, A. Broun, L.W. Tari, R.V. Swanson, J. Finn, J. Biol. Chem. 279 (2004) 8526.
[49] H. Mönkkönen, P.P. Lehenkari, M. Kellinsalmi, I.E. Hassinen, S. Auriola, J. Vepsäläinen, J. Mönkkönen, Bone 34 (2004) S66.
[50] F.L. Zhang, P.J. Casey, Annu. Rev. Biochem. 65 (1996) 241.
[51] R.G. Russell, M.J. Rogers, J.C. Frith, S.P. Luckman, F.P. Coxon, H.L. Benford, P.I. Croucher, C. Shipman, H.A. Fleisch, J. Bone Miner. Res. 14 (Suppl. 2) (1999) 53.
[52] A. Oliff, Biochim. Biophys. Acta 1423 (1999) C19.
[53] O. Teronen, P. Heikkila, Y.T. Konttinen, M. Laitinen, T. Salo, R. Hanemaaijer, A. Teronen, P. Maisi, T. Sorsa, Ann. N. Y. Acad. Sci. 878 (1999) 453.
[54] P. Heikkila, O. Teronen, M. Moilanen, Y.T. Konttinen, R. Hanemaaijer, M. Laitinen, P. Maisi, G. van der Pluijm, J.D. Bartlett, T. Salo, T. Sorsa, Anticancer Drugs 13 (2002) 245.
[55] K. Bonjean, A. Bellahcene, R. Locigno, J. Green, V. Castronovo, Proc. Am. Assoc. Cancer Res. 42 (2001) 106.
[56] M. Bezzi, M. Hasmim, G. Bieler, O. Dormond, C. Ruegg, J. Biol. Chem. 278 (2003) 43603.
[57] A. Aparicio, A. Gardner, Y. Tu, A. Savage, J. Berenson, A. Lichtenstein, Leukemia 12 (1998) 220.
[58] O. Fromigue, B. Siwek, J.J. Body, Calcif. Tissue Int. 64 (Suppl. 1) (1999) S115.
[59] O. Fromigue, L. Lagneaux, J.J. Body, J. Bone Miner. Res. 15 (2000) 2211.
[60] S.G. Senaratne, G. Pirianov, J.L. Mansi, T.R. Arnett, K.W. Colston, Br. J. Cancer 82 (2000) 1459.
[61] C.M. Shipman, M.J. Rogers, J.F. Apperley, R.G. Russell, P.I. Croucher, Br. J. Haematol. 98 (1997) 665.
[62] C.M. Shipman, P.I. Croucher, R.G. Russell, M.H. Helfrich, M.J. Rogers, Cancer Res. 58 (1998) 5294.
[63] P. Tassone, S. Forciniti, E. Galea, G. Morrone, M.C. Turco, V. Martinelli, P. Tagliaferri, S. Venuta, Leukemia 14 (2000) 841.
[64] E. Corey, L.G. Brown, J.E. Quinn, M. Poot, M.P. Roudier, C.S. Higano, R.L. Vessella, Clin. Cancer Res. 9 (2003) 295, Erratum in: Clin. Cancer Res. 9 (2003) 1575.
[65] L.G. Brown, R.L. Vessella, E. Corey, J. Bone Miner. Res. 15 (Suppl. 1) (2000) S446.
[66] M.V. Lee, E.M. Fong, F.R. Singer, R.S. Guenette, Cancer Res. 61 (2001) 2602.
[67] B.B. Adamson, S.J. Gallacher, J. Byars, S.H. Ralston, I.T. Boyle, B.F. Boyce, Lancet 342 (1993) 1459.
[68] C.J. Gibbs, J.E. Aaron, M. Peacock, Br. Med. J. (Clin. Res. Ed.) 292 (1986) 1227.
[69] A. Pataki, K. Müller, J.R. Green, Y.F. Ma, Q.N. Li, W.S. Jee, Anat. Rec. 249 (1997) 458, Erratum in: Anat. Rec. 250 (1998) 250.
[70] N. Binkley, D. Kimmel, J. Bruner, A. Haffa, B. Davidowitz, C. Meng, V. Schaffer, J. Green, J. Bone Miner. Res. 13 (1998) 1775.
[71] S. Bare, D. Kimmel, N. Binkley, V. Schaffer, J. Green, J. Bone Miner. Res. 12 (Suppl. 1) (1997) S473.
[72] C. O'Rourke, S. Bare, D. Kimmel, N. Binkley, V. Schaffer, J. Green, J. Bone Miner. Res. 12 (Suppl. 1) (1997) S473.
[73] M.D. Grynpas, M. Kasra, N. Binkley, Bone 22 (Suppl.) (1998) S47.
[74] M. Glatt, A. Pataki, G.P. Evans, S.B. Homby, J.R. Green, Osteoporos. Int. 15 (2004) 707.
[75] S.B. Hornby, G.P. Evans, S.L. Hornby, A. Pataki, M. Glatt, J.R. Green, Calcif. Tissue Int. 72 (2003) 519.
[76] J.A. Gasser, J.R. Green, Bone 30 (Suppl.) (2002) S41.
[77] M. Glatt, Eur. Cell Mater. 1 (2001) 18.
[78] B. Krempien, F. Wingen, T. Eichmann, M. Müller, D. Schmahl, Oncology 45 (1988) 41.
[79] T. Schilling, E. Blind, R. Baier, H.P. Sinn, E. Moallem, J. Silver, R. Ziegler, F. Raue, J. Bone Miner. Res. 10 (1995) 7.
[80] B. Krempien, G. Wipfler, C.H. Manegold, J. Cancer Res. Clin. Oncol. 118 (Suppl.) (1992) R81.
[81] M. Juraschek, M.J. Seibel, H.W. Woitge, B. Krempien, F. Bauss, Bone 26 (2000) 475.
[82] G. Wipfler, R. Büchele, B. Krempien, In: Proceedings of the International Conference on Calcium Regulating Hormones, Siena, 1992.

[83] J. Green, H. Gschaidmeier, T. Yoneda, G. Mundy, Ann. Oncol. 11 (Suppl. 4) (2000) 14.
[84] O. Peyruchaud, B. Winding, I. Pecheur, C.M. Serre, P. Delmas, P. Clezardin, J. Bone Miner. Res. 16 (2001) 2027.
[85] P.I. Croucher, R. De Hendrik, M.J. Perry, A. Hijzen, C.M. Shipman, L. Lippitt, J. Green, E. Van Marck, B. Van Camp, K. Vanderkerken, J. Bone Miner. Res. 18 (2003) 482.
[86] M. Gnant, H. Hausmaninger, H. Samonigg, B. Mlineritsch, S. Taucher, G. Luschin-Ebengreuth, R. Jakesz, Breast Cancer Res. Treat. 76 (Suppl. 1) (2002) S31.
[87] M.R. Smith, J. Eastham, D.M. Gleason, D. Shasha, S. Tchekmedyian, N. Zinner, J. Urol. 169 (2003) 2008.
[88] M. Arden-Cordone, E.S. Siris, K.W. Lyles, A. Knieriem, R.A. Newton, V. Schaffer, K. Zelenakas, Calcif. Tissue Int. 60 (1997) 415.