Molecular Mechanisms of Action of Bisphosphonates: Current Status
Anke J. Roelofs, Keith Thompson, Sharon Gordon, and Michael J. Rogers

Abstract

**Purpose:** Bisphosphonates are currently the most important class of antiresorptive agents used in the treatment of metabolic bone diseases, including tumor-associated osteolysis and hypercalcemia. These compounds have high affinity for calcium ions and therefore target bone mineral, where they are internalized by bone-resorbing osteoclasts and inhibit osteoclast function.

**Experimental Design:** This article reviews the pharmacology of bisphosphonates and the relationship between chemical structure and antiresorptive potency. We also describe new insights into their intracellular molecular mechanisms of action, methods for assessing the effects of bisphosphonates on protein prenylation, and their potential as direct antitumor agents.

**Results:** Nitrogen-containing bisphosphonates act intracellularly by inhibiting farnesyl diphosphate synthase, an enzyme of the mevalonate pathway, thereby preventing prenylation of small GTPase signaling proteins required for normal cellular function. Inhibition of farnesyl diphosphate synthase also seems to account for their antitumor effects observed in vitro and for the activation of γδ T cells, a feature of the acute-phase response to bisphosphonate treatment in humans. Bisphosphonates that lack a nitrogen in the chemical structure do not inhibit protein prenylation and have a different mode of action that seems to involve primarily the formation of cytotoxic metabolites in osteoclasts.

**Conclusions:** Bisphosphonates are highly effective inhibitors of bone resorption that selectively affect osteoclasts in vivo but could also have direct effects on other cell types, such as tumor cells. After >30 years of clinical use, their molecular mechanisms of action on osteoclasts are finally becoming clear but their exact antitumor properties remain to be clarified.

Bisphosphonates remain the most widely used and effective antiresorptive agents for the treatment of diseases in which there is an increase in the number or activity of osteoclasts, including tumor-associated osteolysis and hypercalcemia (1). This brief review summarizes our current understanding of the molecular mechanisms of action of bisphosphonates on osteoclasts and their potential to affect other cell types, such as tumor cells, via the same molecular mechanisms.

**General Properties of Bisphosphonates**

Bisphosphonates are synthetic, nonhydrolyzable analogues of PPi (Fig. 1). The P-C-P structure of bisphosphonates imparts the ability to bind divalent metal ions, such as Ca²⁺ (2). For this reason, bisphosphonates are rapidly cleared from the circulation (3, 4) and bind to bone mineral surfaces in vivo at sites of active bone remodeling, particularly areas undergoing osteoclastic resorption (5). The targeting of bisphosphonates to bone, localized release during osteoclastic bone resorption, and efficient uptake into osteoclasts by endocytosis explains why bisphosphonates seem to have a highly selective effect on osteoclasts (2). However, this does not exclude the possibility that small amounts of these drugs are internalized by neighboring cells (such as osteoblasts, bone marrow cells, or tumor cells), particularly with repeated administration over extended periods.

**Metabolites of Simple Bisphosphonates Induce Osteoclast Apoptosis**

Following earlier studies on slime mould amoebae (6), mammalian cells were found to convert some bisphosphonates (only the first-generation bisphosphonates, which closely resemble PPi, such as clodronate and etidronate) intracellularly into methylene-containing (AppCp type) analogues of ATP (Fig. 2; ref. 7). These AppCp-type metabolites accumulate to high concentrations in the cytosol of osteoclasts and other cell types that can effectively internalize bisphosphonates (8). The accumulation of the AppCCl₂p metabolite of clodronate in osteoclasts in vitro inhibits bone resorption by inducing osteoclast apoptosis (Fig. 2; ref. 9), most likely by inhibiting

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ATP-dependent enzymes, such as the adenine nucleotide translocase, a component of the mitochondrial permeability transition pore (10). Induction of osteoclast apoptosis seems to be the primary mechanism by which the simple bisphosphonates inhibit bone resorption because the ability of clodronate and etidronate to inhibit resorption in vitro can be overcome when osteoclast apoptosis is prevented using a caspase inhibitor (11).

**Nitrogen-Containing Bisphosphonates Act by Inhibiting Farnesyl Diphosphate Synthase**

Nitrogen-containing bisphosphonates (N-BPs), which are several orders of magnitude more potent at inhibiting bone resorption in vivo than the simple bisphosphonates (2, 12), are not metabolized to toxic analogues of ATP (13). Instead, they act by inhibiting farnesyl diphosphate (FPP) synthase, a key enzyme of the mevalonate pathway (Fig. 3). This enzyme is inhibited by nanomolar concentrations of N-BPs (Table 1; refs. 14–16). Zoledronic acid and the structurally similar minodronate are extremely potent inhibitors of FPP synthase (16) and inhibit the enzyme even at picomolar concentrations. Importantly, studies with recombinant human FPP synthase revealed that minor modifications to the structure and conformation of the R² side chain that are known to affect antiresorptive potency (16) also affect the ability to inhibit FPP synthase (2). These studies strongly suggest that FPP synthase is the major pharmacologic target of N-BPs in osteoclasts in vivo.
and help to explain the relationship between bisphosphonate structure and antiresorptive potency.

The exact mechanism by which N-BPs inhibit FPP synthase is only just becoming clear. The recent generation of X-ray crystal structures of the human FPP synthase enzyme, cocrystallized with risedronate or zoledronic acid (17, 18), revealed that N-BPs bind in the geranyl diphosphate (GPP) binding site of the enzyme, with stabilizing interactions occurring between the nitrogen moiety of the N-BP and a conserved threonine and lysine residue in the enzyme. This is consistent with the earlier suggestion by Oldfield et al. (19) that N-BPs mimic the structure of the natural isoprenoid pyrophosphate substrates of the enzyme, GPP and dimethylallyl diphosphate, and compete for binding at the GPP/dimethylallyl diphosphate substrate binding pocket. N-BPs also seem to inhibit bacterial FPP synthase in a similar manner (20). Enzyme kinetic analysis with human FPP synthase indicates that the interaction with N-BPs is highly complex.

Table 1. Potency of N-BPs for inhibiting FPP synthase

<table>
<thead>
<tr>
<th>Bisphosphonate</th>
<th>IC₅₀ (nmol/L), recombinant human enzyme</th>
<th>IC₅₀ (nmol/L), purified recombinant human enzyme</th>
<th>Kᵢ (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamidronate</td>
<td>200</td>
<td>500</td>
<td>ND</td>
</tr>
<tr>
<td>Alendronate</td>
<td>50</td>
<td>340</td>
<td>ND</td>
</tr>
<tr>
<td>Ibandronate</td>
<td>20</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Risedronate</td>
<td>10</td>
<td>3.9</td>
<td>0.34</td>
</tr>
<tr>
<td>Zoledronate</td>
<td>3</td>
<td>ND</td>
<td>0.07</td>
</tr>
<tr>
<td>Minodronate</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*Values of IC₅₀ are from Dunford et al. (16) using partially purified recombinant enzyme.

†Values of IC₅₀ are from Bergstrom et al. (15) using purified enzyme.

‡Values of the overall dissociation constant, Kᵢ, are from Kavanagh et al. (18) using purified enzyme.
and characteristic of “slow tight binding” inhibition (18). Initially, N-BPs seem to compete directly with dimethylallyl diphosphate or GPP for binding to the dimethylallyl diphosphate/GPP binding pocket. This is followed by more complex interactions that promote binding of isopentenyl diphosphate (IPP) in the second isoprenoid binding site of the enzyme, causing conformational changes that stabilize the final ternary complex, helping to explain the extraordinary inhibitory potency of some N-BPs toward this enzyme. These studies are therefore beginning to provide key insights, at the atomic level, into the reasons why minor changes to the structure of the N-BP side chain or the phosphonate groups markedly influence antiresorptive potency (2).

### Inhibition of FPP Synthase Prevents the Prenylation of Small GTPases

By inhibiting FPP synthase, N-BPs prevent the synthesis of FPP and its downstream metabolite geranylgeranyl diphosphate (Fig. 3). These isoprenoid lipids are the building blocks for the production of a variety of metabolites, such as dolichol and ubiquinone (21), but are also required for post-translational modification (prenylation) of proteins, including small GTPases (22, 23). The loss of synthesis of FPP and geranylgeranyl diphosphate therefore prevents the prenylation of small GTPases, the majority of which are geranylgeranylated (24–26). Inhibition of protein prenylation by N-BPs can be shown by measuring the incorporation of [14C]mevalonate into farnesylated and geranylgeranylated proteins (13, 27). Risedronate almost completely inhibits protein prenylation in J774 cells at a concentration of 10 μmol/L, which is similar to the concentration that affects osteoclast viability in vitro (28, 29) and has been predicted to be achieved within the osteoclast resorption lacuna in vivo (30). More recently, we and others confirmed that N-BPs (e.g., ≥10 μmol/L zoledronic acid; Fig. 4) inhibit the incorporation of [14C]mevalonate into prenylated small GTPase proteins in purified osteoclasts in vitro (15, 31). Alternatively, the inhibitory effect of N-BPs on the mevalonate pathway can be shown by detecting accumulation of the unprenylated form of the small GTPase Rap1A, which acts as a surrogate marker for inhibition of FPP synthase and which accumulates in cells exposed to N-BPs (Fig. 5A; ref. 32). We have shown the ability of N-BPs to inhibit the prenylation of Rap1A in cultures of all types of primary cells and cell lines studied thus far (Fig. 5), including osteoclasts, osteoblasts, macrophages, epithelial and endothelial cells, and breast, myeloma, and prostate tumor cells. Macrophages and osteoclasts seem to be the most sensitive to low concentrations of N-BPs (1-10 μmol/L) in vitro. In macrophages, treatment with 100 μmol/L N-BP causes the detectable accumulation of unprenylated Rap1A within a few hours (lower concentrations have a delayed effect; the more potent the N-BP, the more rapid the effect; Fig. 5B). In other cell types, such as myeloma cells, the unprenylated form of Rap1A can also be detected within hours of treatment in vitro, but higher concentrations are sometimes required (Fig. 5C). The sensitivity of different cell types to N-BPs most likely depends largely on their ability to internalize sufficient amounts of N-BP to inhibit FPP synthase. Recent studies with a fluorescently labelled bisphosphonate have shown that macrophages and osteoclasts internalize bisphosphonates into membrane-bound vesicles by fluid-phase endocytosis (34). Subsequent acidification of endocytic vesicles is required for bisphosphonates to enter the cytosol, by reducing the negative charge on the phosphonate groups of bisphosphonates and thereby allowing either diffusion or transport of bisphosphonates across the vesicular membrane (34). This mechanism of uptake results in large amounts of N-BP in intracellular vesicles but probably only very small amounts of bisphosphonates in the cytosol or other organelles are available for inhibition of FPP synthase, although the relatively poor uptake of bisphosphonates into the cell cytosol is overcome by their extremely potent inhibition of FPP synthase (16, 18).

### Consequences of Inhibiting Protein Prenylation

Prenylated small GTPases, such as those of the Ras, Rho, and Rab families, are important signaling proteins that regulate a variety of cell processes important for osteoclast function (35). Inhibition of the mevalonate pathway and loss of prenylated proteins, particularly geranylgeranylated small GTPases, seem to be the major mechanism of action of N-BPs because bypassing inhibition of FPP synthase and replenishing cells

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**Fig. 4.** N-BPs inhibit protein prenylation in osteoclasts in vitro. A, purified rabbit osteoclasts were incubated with [14C]mevalonate, which becomes incorporated into [14C]-labeled, prenylated proteins. B, prenylated small GTPase proteins can then be detected by autoradiography following electrophoretic separation. Both alendronate (ALN) and risendronate (RIS) prevent the incorporation of [14C]mevalonate into prenylated proteins, whereas clodronate (CLO) has no effect. Reproduced from Coxon et al. (J Bone Miner Res 2000;15:1467-1476) with permission of the American Society for Bone and Mineral Research.
with an isoprenoid lipid substrate that restores geranylgeranylation can overcome the effects of N-BPs on osteoclast formation, apoptosis, and bone resorption (36–38). In addition, other inhibitors of protein geranylgeranylation, such as statins or GGTI-298 (Fig. 3), mimic the effects of N-BPs (27, 31). However, recent studies by van Beek et al. (39) suggest that pamidronate may have an additional, as yet unidentified, molecular target in osteoclasts because (unlike with other N-BPs) the antiresorptive effect of pamidronate is not dependent on apoptosis at least in vivo (11). Hence, inhibition of protein prenylation remains the most likely explanation for the antiresorptive effects of N-BPs.

Following the discovery that N-BPs inhibit FPP synthase and prevent protein prenylation, it has been assumed that the antiresorptive effects of N-BPs result from the loss of signaling pathways downstream of prenylated (particularly geranylgeranylated) small GTPases. However, we have shown recently that the unprenylated forms of Rho, Rac, and Cdc42 that accumulate following treatment with N-BPs are in the active, GTP-bound form most likely due to their inability to interact with regulatory proteins, such as Rho GTPase-activating protein (41). Unprenylated small GTPases may therefore affect normal cellular function by inappropriate and sustained activation, rather than inhibition, of downstream signaling pathways, such as p38 (41, 42). A dominant effect of the accumulation of unprenylated proteins, as opposed to the loss of prenylated proteins, would explain why ongoing protein synthesis is required for bisphosphonates to exert their cytotoxic effects (43) because, in all cells, the accumulation of unprenylated proteins is dependent on de novo protein synthesis. Further studies are clearly required to elucidate in more detail the effects of unprenylated proteins on cell function.

**Effects of N-BPs on Tumor Cells**

Numerous studies have described the ability of N-BPs to reduce the survival, proliferation, adhesion, migration, and invasion of tumor cells in vitro (44, 45). Most, if not all, of these antitumor effects of N-BPs in vitro are due to inhibition of FPP synthase because the effects of N-BPs can be largely overcome by replenishing cells with isoprenoid substrates (farnesol or geranylgeraniol) required for protein prenylation (46–49). Furthermore, the structure-activity relationships of N-BPs for affecting tumor cell adhesion and invasion match the structure-activity relationships for inhibiting FPP synthase (50, 51). Similarly, some N-BPs have been shown to affect the viability, migration, and activity of endothelial cells in vitro. Some of these effects could be overcome by replenishing cells with geranylgeranylpyrophosphate and therefore appear to be due to loss of protein prenylation (52, 53). We have recently confirmed that the concentrations of zoledronic acid that affect endothelial cells in vitro (≥10 μmol/L) do indeed inhibit protein prenylation (Fig. 5D). It therefore becomes of increasing importance to determine the in vivo relevance of these in vitro observations.

A variety of intriguing studies in mouse models have shown that treatment with N-BPs can inhibit skeletal metastasis or reduce tumor burden in bone or even at extraskeletal sites in vivo (45, 53–60). In addition, bisphosphonates have been shown to inhibit angiogenesis in experimental models and in animal models of tumorigenesis (45, 55, 61) and to lower circulating levels of proangiogenic vascular endothelial growth factors. However, the pharmacologic significance of this is unclear because restoring prenylation with a substrate for protein geranylgeranylation overcomes the antiresorptive effects of bisphosphonates in vitro (37) but would be unlikely to affect levels of ApppI. Furthermore, unlike the simple bisphosphonates that act by inducing osteoclast apoptosis, the antiresorptive effect of N-BPs is not dependent on apoptosis at least in vivo (11). Hence, inhibition of protein prenylation remains the most likely explanation for the antiresorptive effects of N-BPs.
factor and platelet-derived growth factor in cancer patients (62, 63). However, it has not yet been unequivocally proven that these effects are due to inhibition of protein prenylation following direct internalization of N-BPs by tumor, endothelial, or other cells, such as macrophages, in vivo. Effects of N-BPs on skeletal metastasis, tumor burden in bone, or even angiogenesis could result from inhibition of bone resorption alone (58, 64). Therefore, although N-BPs clearly have the potential for direct antitumor effects, whether this is achievable with clinically relevant doses remains a highly controversial issue and depends on whether tumor cells are able to internalize sufficient amounts of the drugs in vivo.

Adding weight to the possibility that N-BPs may have direct effects on tumor cells in vivo is the demonstration that an i.v. N-BP infusion results in a sufficiently high peripheral blood concentration [e.g., 1 μmol/L with zoledronic acid (4)] to allow entry into monocytes and/or other highly endocytic cells. This is the cause of the flu-like acute-phase reaction (65) that typically occurs in approximately one third of patients receiving i.v. N-BP treatment for the first time. Inhibition of FPP synthase in these circulating peripheral blood mononuclear cells causes intracellular accumulation of IPP, which is presented to Vγ9Vδ2+ T cells by an as yet unidentified mechanism. This results in the activation and proliferation of these γδ T cells (66–68), triggering cytokine release and thus causing flu-like symptoms (69, 70). Similarly, treatment of tumor cells with N-BPs in vitro also causes the accumulation of IPP, which can activate γδ T cells capable of tumor cell killing (67). These observations raise the possibility that N-BPs could be used as an immune therapy, having indirect antitumor effects in vivo via the activation of γδ T cells (71). However, this mechanism does not seem to explain the antitumor activity of N-BPs in mouse models of tumorigenesis because rodents lack an analogous subset of γδ T cells that can be activated by IPP (72).

Direct uptake of N-BPs by epithelial cells in the gastrointestinal tract followed by inhibition of FPP synthase and loss of prenylated proteins may also explain the ability of some orally administered N-BPs to cause esophagitis and ulceration (2). Taken together, the studies described above suggest that, under certain circumstances, cells other than osteoclasts are indeed capable of internalizing bisphosphonates in vivo. Much therefore remains to be learned about the concentrations of bisphosphonate that occur in vivo in the bone microenvironment and in other tissues, which cell types are capable of internalizing sufficient N-BP to affect protein prenylation, and what effects this might cause, for example, on tumor cells. Importantly, recent studies have shown that bisphosphonates can act in synergy with a variety of currently used anticancer agents at least in vitro (45, 73, 74). This raises the possibility that combination treatment with other cytotoxic drugs could lower the concentration of bisphosphonates needed to directly affect tumor cells and thus increase the potential for N-BPs to have direct antitumor effects in vivo.

Development of New Bisphosphonate Analogues

It has become clear recently that changes to the structure of N-BPs might give rise to compounds capable of inhibiting other enzymes of the mevalonate pathway that use isoprenoid lipids. For example, we recently found that replacement of one of the phosphonate groups of risedronate with a carboxylate group, giving rise to a phosphonocarboxylate analogue (3-PEHPC; Fig. 1), confers the novel ability to specifically inhibit Rab geranylgeranyltransferase (Fig. 3), thereby selectively preventing the prenylation and membrane localization of Rab GTPases without affecting Rho or Ras family GTPases (75, 76). 3-PEHPC is a weak inhibitor of bone resorption, probably by disrupting Rab-dependent vesicular trafficking in osteoclasts (75, 76), induces apoptosis in human myeloma cells (77), and inhibits invasion of breast and prostate cancer cells (51).

Interestingly, the structure-activity relationships of several phosphonocarboxylate analogues for inhibiting Rab geranylgeranyltransferase do not match the structure-activity relationships of the parent bisphosphonates for inhibiting FPP synthase (76), indicating that phosphonocarboxylates represent a new class of antiresorptive and/or antitumor agents with a defined and specific molecular target. Unlike N-BPs, 3-PEHPC does not cause activation of γδ T cells in vitro (the basis of the acute-phase response to N-BPs; ref. 66) and may therefore have a different adverse effect profile. Furthermore, 3-PEHPC treatment of myeloma cells does not induce the S-phase arrest characteristic of N-BPs (77). Although more potent phosphonocarboxylates than 3-PEHPC would be required for further development, the lower bone affinity of such agents compared with the parent N-BPs (78) might be an attractive property in situations where long-term retention in bone is undesirable, for example, in the treatment of pediatric bone disease. In addition, N-BPs with a lower affinity for bone mineral may display higher equilibrium concentrations in the bone marrow microenvironment compared with high-affinity compounds (79), raising the possibility that low bone affinity compounds could act more effectively on tumor cells residing in the bone marrow.

Summary

Bisphosphonates can be grouped into two general classes according to their chemical structure and molecular mechanism of action. The simple bisphosphonates can be metabolically incorporated into nonhydrolyzable analogues of ATP that accumulate intracellularly in osteoclasts, resulting in induction of osteoclast apoptosis. By contrast, the more potent N-BPs inhibit FPP synthase, an enzyme in the mevalonate pathway. Inhibition of this enzyme in osteoclasts prevents the biosynthesis of isoprenoid lipids that are essential for the prenylation of small GTPase signaling proteins. Inhibition of FPP synthase also seems to account for the adverse effects of N-BPs in vivo and for the antitumor effects of N-BPs in vitro. Although N-BPs have been shown to have antitumor activity in various animal models, it remains to be confirmed whether this is directly due to the inhibition of protein prenylation in tumor cells, endothelial cells, or other nonosteoclast cell types in vivo.

Open Discussion

Dr. Boyce: Are there any data that show that accumulation of some unprenylated GTPases could be stimulating tumor cells? There is some evidence that outside the skeleton tumor cell growth may be enhanced, at least in animal models.
Dr. Rogers: What we found in macrophages at least is that activation of p38 and downstream activation of p38 has an antia apoptotic effect. If we treat macrophages with a bisphosphonate and a p38 inhibitor, we get more apoptosis. So, depending on the cell type, if you activate some of them, you might get a prosurvival effect and an antia apoptotic effect, but perhaps activation of others, like Rho, might be proapoptotic, depending again on the cell type. So, yes, under some circumstances it might have antia apoptotic effects on some cell types.

Dr. Boyce: It could vary from one cell type or one tumor type to another.

Dr. Rogers: Absolutely. This effect of p38 activation we found in macrophages prevents apoptosis, but it has the opposite effect in myeloma cells. Certainly, it could be cell type dependent.

Dr. Roodman: Do you think that this activation of p38 MAP kinase explains why bisphosphonates don’t totally inhibit osteoclasts?

Dr. Rogers: That’s a distinct possibility, although it’s difficult to dissect out what all of these GTPases are doing when they accumulate in the unprenylated form.

Dr. Roodman: Have you shown that p38 is activated in osteoclasts?

Dr. Rogers: We’re doing those studies now, but we have looked in a variety of tumor cell types. We see Rho, Rac, and Cdc42 activation in all cell types studied so far. However, what might be different between cell types are the particular signaling pathways then get activated downstream, like p38 or JNK.

Dr. Suva: There was a lot of interest a couple of years ago in osteosarcoma and bisphosphonates. Do you know if anything has come of that? It seems to me like that’s a reasonable cancer target for a bisphosphonate.

Dr. Rogers: I’m not sure that’s been followed or that anyone has reproduced that.

Dr. Coleman: One of the reasons we may not have seen antitumor activity in the clinical situation is because obviously bisphosphonates target so exquisitely to bone. What’s been done about adjusting the affinity to bone, perhaps using the FPP synthase activity alone?

Dr. Rogers: The problem is that the phosphonate groups are essential for binding to FPP synthase because the phosphonate groups bind to those magnesium ions. So as soon as you start modifying the phosphonate groups, most of those compounds no longer inhibit FPP synthase. However, there’s one compound that does inhibit FPP synthase even though it has modified phosphonate groups. These sorts of compounds could be interesting to test in animal models because they could have less propensity to bind to mineral and might detach more readily in a bone microenvironment. Perhaps they could then reach higher local concentrations around tumor cells.

Dr. Weilbaecher: Regarding clodronate, we can’t prove it has a direct antitumor effect, but there certainly is compelling evidence in some of the adjuvant trials with this weak bisphosphonate that there might be effects outside the bone on cancer cells. How does clodronate work if it doesn’t get this pathway on osteoclasts?

Dr. Rogers: Clodronate, like etidronate and tiludronate, does not inhibit FPP synthase or inhibits it very weakly. It has no detectable effects on protein prenylation at sensible concentrations in vitro. Clodronate gets metabolized intracellularly to a nonhydrolyzable ATP analogue. That ATP analogue accumulates in the cytosol of cells and probably inhibits all sorts of ATP-dependent enzymes and seems to trigger apoptosis. If we, for example, use liposomes to introduce the synthetic metabolite into cells, it triggers osteoclast apoptosis. Interestingly, clodronate has lower bone affinity than most of the nitrogen bisphosphonates. On the other hand, it has little effect on tumor cells. It kills endocytic cells like macrophages and osteoclasts, but it doesn’t have the same effects on tumor cell adhesion, invasion, and migration that the nitrogen bisphosphonates do presumably because it doesn’t inhibit prenylation.

Dr. Suva: From the crystal structure of FPP synthase, is there any evidence for other binding pockets on the surface?

Dr. Rogers: In the molecular modeling studies, it looked as if one molecule of bisphosphonate is bound to each of those substrate-binding pockets in the enzyme. However, in the crystal structures, it looks as if the bisphosphonates only binds to the GPP pocket and not to the IPP pocket.

Dr. Suva: Theoretically, you could target a molecule that might target the other pocket.

Dr. Rogers: Yes. We’ve been trying to do some enzyme kinetic studies, but this is a really complicated enzyme to study.

Dr. Suva: Has anybody been able to take advantage of the conformational change and develop allosteric inhibitors?

Dr. Rogers: Maybe that’s for the future.

Dr. Body: What are the effects of these drugs on osteoblasts and on the dialog between osteoblasts and osteoclasts?

Dr. Rogers: We found effects on apoptosis in tumor cells, but knowing now that these compounds inhibit a ubiquitous metabolic enzyme, it’s not surprising that you get apoptosis and growth inhibition of any cell type in vitro with high enough concentrations, including osteoblasts. At the moment, we are limited by the sensitivity of the method for detecting changes in protein prenylation because we can only see an effect on prenylation above about 1 μmol/L or 5 μmol/L of zoledronate. However, almost certainly below those concentrations, there are still very subtle effects on prenylation that we can’t detect. It’s a limitation of the Western blot approach. So are these effects relevant in vivo? Do osteoblasts take up enough bisphosphonates in vivo to have an effect on prenylation? We don’t have those answers yet.

Dr. Bruland: Did you achieve these molar concentrations by the standard approved doses? Is it realistic to expect antitumor activity?

Dr. Rogers: Studies with zoledronate show that about 1 to 3 μmol/L is the maximum circulating concentration with a standard dose of zoledronate in patients. Concentrations above that, for example, 10, 50, or 100 μmol/L, are almost certainly not relevant, at least not in the peripheral circulation. We still don’t know what’s happening in the bone microenvironment. Beneath the resorbing osteoclasts, there may well be hundreds of micromolar perhaps even millimolar concentrations of bisphosphonates in the resorption lacuna, but we don’t know what sort of concentrations are reached around a resorbing osteoclast. Certainly, 1 μmol/L of zoledronate or risedronate is sufficient to affect tumor cell adhesion and migration and invasion in vitro.

Dr. Vessella: Why don’t we know what the concentrations of the bisphosphonates are in the bone? If we take a bone...
biopsy specimen after giving a course of bisphosphonates, isn’t there a way of determining how much bisphosphonate is in the bone?

Dr. Rogers: The problem is, what does that mean? You can estimate how much is bound to bone, but when it’s bound to bone it’s pharmacologically inactive. It’s only when it’s released and it can be taken up by cells that it becomes active. The bisphosphonates are constantly desorbing from the bone surface and retaッチting and then you have a resorbing osteoclast that’s releasing lots of bisphosphonate. Does some of that diffuse into the bone marrow? Does it then reattach? Does it enter the circulation? Is it recycled?

Dr. Weilbacher: Once you’ve given your dose of bisphosphonate and have presumably inhibited osteoclasts, would you then have less release of this bisphosphonate, because you don’t have the osteoclasts to resorb the bone anymore?

Dr. Rogers: Yes, in other words they probably inhibited their own release eventually. However, it gets more complicated, because endocytosis, which is the way that these bisphosphonates get into cells, is dependent on small GTPases, and when you block prenylation of those, you get less endocytosis. Therefore, bisphosphonates also inhibit their own uptake in cells, such as macrophages.

Dr. Lipton: You commented that 10-minute exposure is enough for maximum inhibition. Have you done that with multiple cell lines? We did some crude experiments years ago with the breast cancer cell line where it looked like you needed a 24-hour period of prolongation to get maximum cell inhibition.

Dr. Rogers: We haven’t done an extensive comparison. Certainly, with endoécytic cells like macrophages, and probably osteoclasts, you only need a short exposure because they’re so endoécytic and internalize bisphosphonate very quickly. The less endoécytic the cell type (such as tumor cells), the longer you need to expose them to get sufficient bisphosphonate into the cell to have an effect.

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