Structure-Activity Relationships for Inhibition of Farnesyl Diphosphate Synthase in Vitro and Inhibition of Bone Resorption in Vivo by Nitrogen-Containing Bisphosphonates

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Received August 22, 2000; accepted October 16, 2000
This paper is available online at http://jpet.aspetjournals.org

ABSTRACT
It has long been known that small changes to the structure of the R² side chain of nitrogen-containing bisphosphonates can dramatically affect their potency for inhibiting bone resorption in vitro and in vivo, although the reason for these differences in antiresorptive potency have not been explained at the level of a pharmacological target. Recently, several nitrogen-containing bisphosphonates were found to inhibit osteoclast-mediated bone resorption in vitro by inhibiting farnesyl diphosphate synthase, thereby preventing protein prenylation in osteoclasts. In this study, we examined the potency of a wider range of nitrogen-containing bisphosphonates, including the highly potent, heterocycle-containing zolendronic acid and minodronate (YM-529). We found a clear correlation between the ability to inhibit farnesyl diphosphate synthase in vitro, to inhibit protein prenylation in cell-free extracts and in purified osteoclasts in vitro, and to inhibit bone resorption in vivo. The activity of recombinant human farnesyl diphosphate synthase was inhibited at concentrations ≥1 nM zolendronic acid or minodronate, the order of potency (zolendronic acid > minodronate > idelalisib > bandronate > inacronate > alendronate > pamidronate) closely matching the order of antiresorptive potency. Furthermore, minor changes to the structure of the R² side chain of heterocycle-containing bisphosphonates, giving rise to less potent inhibitors of bone resorption in vivo, also caused a reduction in potency up to ~300-fold for inhibition of farnesyl diphosphate synthase in vitro. These data indicate that farnesyl diphtophosphate synthase is the major pharmacological target of these drugs in vivo, and that small changes to the structure of the R² side chain alter antiresorptive potency by affecting the ability to inhibit farnesyl diphtophosphate synthase.

Bisphosphonates (BPs) are the most widely used and effective antiresorptive agents currently available for the treatment of Paget’s disease, tumor-associated bone disease, and osteoporosis. All BPs have high affinity for bone mineral as a consequence of their P-C-P backbone structure, which allows chelation of calcium ions (for review, see Ebetino et al., 1998). Following release from bone mineral during acidification by osteoclasts, BPs appear to be internalized specifically by osteoclasts but not other bone cells (Sato et al., 1991). The intracellular accumulation of BP leads to inhibition of osteoclast function, due to changes in the cytoskeleton, loss of the ruffled border (Carano et al., 1990; Sato et al., 1991), and apoptosis (Hughes et al., 1995; Selander et al., 1996; Ito et al., 1999; Reszka et al., 1999).

The ability of BPs to inhibit bone resorption is dependent on the presence of the two phosphonate groups in the P-C-P structure, which appear to be required for interaction with a molecular target in the osteoclast as well as for binding bone mineral (Rogers et al., 1995; van Beek et al., 1998). However, the antiresorptive potency is determined by the chemical and three-dimensional structure of the two side chains, R¹ and R², attached to the central, geminal carbon atom (Geddes et al., 1994; Rogers et al., 2000). Following the discovery that CLO and ETI (BPs with a halogen or methyl group in the R² side chain) could inhibit bone resorption, more potent BPs have been developed by the insertion of a primary, secondary
or tertiary nitrogen function in the R² side chain, for example, PAM, ALN, IBA and INC, which have an alkyl R² side chain (Schenk et al., 1986; Mühlbauer et al., 1991; Rogers et al., 2000), or RIS, ZOL and MIN, which have a heterocyclic R² side chain (Sietsema et al., 1989; Green et al., 1994; Sasaki et al., 1998). The R² side chain, and especially the basic nitrogen group, appear to play an important role in the interaction of BPs with a pharmacological target, since minor modifications to the structure or conformation of the R² side chain (Sietsema et al., 1989; Green et al., 1994; Rogers et al., 1995; Eabetino and Danse-reau, 1995; Rogers et al., 1996). For example, the pairs of heterocycle-containing BPs RIS and NE58051, and NE11808 and NE11809, differ only in the length of the R² side chain (which is increased by -CH₂ in NE58051) or methylation of the heterocyclic ring (as in NE11809) but differ by up to 3000-fold in antiresorptive potency in rodents in vivo (Rogers et al., 1995).

A possible explanation for these structure-activity relationships of nitrogen-containing BPs (N-BPs) has only recently been raised, following the discovery that these compounds inhibit the biosynthetic mevalonate pathway, thereby preventing the post-translational prenylation (farnesylation and geranylgeranylation) of small GTP-binding proteins (Luckman et al., 1998a,b; Benford et al., 1999; Reszka et al., 1999; Coxon et al., 2000). Prenylation involves the transfer of an isoprenoid lipid moiety (farnesyl or geranylgeranyl) from farnesyl dipiphosphate (FPP) or geranylgeranyl diphosphate (GGPP) onto a C-terminal cysteine residue of proteins with a characteristic prenylation motif (Zhang and Casey, 1996). Loss of prenylated, especially geranylgeranylated, small GTP-binding proteins such as cdc42, Rac, and Rho in osteoclasts is probably the major route by which N-BPs inhibit bone resorption, since the antiresorptive effect of N-BPs can be overcome in vitro by bypassing the metabolic pathway and replenishing cells with substrate that can be used for protein geranylgeranylation (Fisher et al., 1999; Reszka et al., 1999; van Beek et al., 1999a), and since the effect of BPs in vitro can be mimicked by a specific inhibitor of protein geranylgeranylation (Coxon et al., 2000).

The enzyme of the mevalonate pathway that is inhibited by N-BPs has only just been clarified. van Beek et al. (1999b) and others (Keller and Fliesler, 1999; Bergstrom et al., 2000) recently showed that FPP synthase is inhibited by several N-BPs, although the potent antiresorptive N-BPs ZOL, MIN, and INC were not included. The aim of this study was to determine the potency of a wide range of clinically important N-BPs for inhibition of FPP synthase and, more specifically, to determine whether changes in antiresorptive potency in vivo caused by small changes in the structure of the R² side chain are due to differences in the ability to inhibit FPP synthase.

Materials and Methods

Reagents. Clodronate (CLO), etidronate (ETI), alendronate (ALN), risedronate (RIS), pamidronate (PAM), ibandronate (IBA), inacidronate (INC), also known as YM-175, NE11808, NE11809, and NE58051 were from Procter & Gamble Pharmaceuticals (Cincinnati, OH). Zoledronic acid (ZOL) (the hydrated disodium salt) was from Novartis Pharma AG (Basle, Switzerland), and minodronate (MIN, also known as YM-529) was from Yamanouchi (Tokyo, Japan). The BPs were dissolved in PBS, the pH adjusted to 7.4 with 1 N NaOH, and then filter-sterilized by using a 0.2-μm filter. Mevastatin was purchased from Sigma Chemical Co. (Poole, UK) and converted from the lactone as described by Luckman et al. (1998b). [14C]Mevalonic acid lactone was from Amersham (Aylesbury, UK). Protease inhibitor cocktail and all other reagents were from Sigma Chemical Co., unless stated otherwise.

Incorporation of [14C]Mevalonate into Prenylated Proteins in Osteoclasts. Protein prenylation in purified rabbit osteoclasts in vitro was measured as described recently by Coxon et al. (2000). Briefly, mature osteoclasts were isolated from rabbit long bones and seeded into six-well plates (Costar, Cambridge, MA). Nonosteoclastic cells were removed using pronase/EDTA, and then the osteoclasts were depleted of mevalonate by incubation in α-minimum essential medium containing 10% fetal calf serum and 5 μM mevastatin for 4 h. The medium was replaced with 1.0 ml/well fresh α-minimum essential medium/10% fetal calf serum containing 5 μM mevastatin, 7.5 μCi/ml [14C]mevalonic acid lactone (specific activity 57 mCi/mmol) plus 100 μM RIS, NE58051, NE11808, or NE11809 (duplicate wells per treatment). After 24 h the cells were lysed, and then 50 μg of osteoclast lysate from each treatment was electrophoresed on 12% polyacrylamide-SDS gels under reducing conditions. The gels were fixed and dried, and then labeled proteins were visualized on a Bio-Rad personal FX imager after exposure to a Kodak phosphorimaging screen.

In Vitro Assay of Protein Prenylation. The effect of BPs on protein prenylation in vitro was examined using rabbit reticulocyte lysate (Promega, Madison, WI), which contains all the enzymes necessary for the conversion of mevalonic acid to FPP and GGPP, and the prenyl-protein transferases required for prenylation of exogenous protein substrates (Vorburger et al., 1989). Recombinant H-Ras (Ras-CVLS; Calbiochem, La Jolla, CA) was used as a substrate for prenylation (Fig. 2). Briefly, 10 μl of reticulocyte lysate in replicate tubes were diluted to 30 μl with water. Ras-CVLS (1.5 μg) was added to each tube, together with 0.1 to 100 μM (final concentration) BP or equivalent volume of PBS, and mixed with 0.2 μCi of [14C]mevalonic acid lactone. The lysates were then incubated for 16 h at 37°C. As a negative control, lysates were incubated without Ras substrate. Following incubation, 30 μl of 2× Laemmli sample buffer containing 16 M urea and 5 × 10⁻⁴ M β-mercaptoethanol was added to each tube and boiled for 5 min. The entire contents of each tube were electrophoresed on 12% SDS-PAGE gels under reducing conditions. Radiolabeled Ras-CVLS was visualized after overnight exposure of gels to an imaging screen using a Bio-Rad personal imager and Quantity One software. Densitometric analysis of radiolabeled Ras bands was performed on gels from three independent experiments and values were calculated as a percentage of control (mean ± S.E.M., n = 3).

Expression of Recombinant Human IPP Isomerase and FPP Synthase. The human IPP isomerase clone pFMH12 (Hahn et al., 1996) in the bacterial expression vector pARC906N was used to transform Escherichia coli JM101 (Stratagene Cloning Systems, La Jolla, CA). Bacterial cultures were grown in terrific broth with 50 μg/ml ampicillin at 37°C and vigorous aeration. Bacteria were harvested after the log phase of growth, typically after 7 h, by centrifugation at 2500g for 10 min. The bacterial pellet was resuspended in 5 ml/g wet weight of ice-cold homogenization buffer [50 mM HEPES pH 7.0, 2% (v/v) protease inhibitor cocktail, 0.8 mM dithiothreitol, 10% (v/v) glycerol] and homogenized by sonication on medium power for 3 × 10 s on ice with a cooling period between each 10-s burst. The bacterial lysate was then centrifuged at 13,000g for 20 min at 4°C to remove cell debris. The supernatant was aliquoted and stored at -20°C.

The human farnesyl dipiphosphate synthase clone KIA1293 was a kind gift from the Kazusa DNA Research Institute (Kisarazu, Chiba, Japan). The coding region was excised by PCR using AccuPrime (Biogene Ltd., Kimbolton, Cambs, UK), forward primer: ATGCCCCT-
GTCCCGCTGGTTG, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: TCATCTTCTCGCTGTTGATGGTTT, reverse primer: 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CVLS (Fig. 2, B and C). CLO and ETI slightly inhibited the incorporation of \(^{14}C\)mevalonate into Ras-CVLS at concentrations of 100 and 1000 \(\mu M\) but had no effect at lower concentrations (data not shown). The order of potency for inhibiting farnesylation of Ras-CVLS in vitro was therefore RIS > IBA > ALN > PAM > CLO > ETI, almost identical to the order of antiresorptive potency in vivo (RIS > IBA > ALN > PAM > CLO > ETI) (Geddes et al., 1994). This confirmed that reticulocyte lysates could be used to study the effect of N-BPs on protein prenylation in a cell-free system.

Pairs of N-BPs with modifications to the R\(^2\) side chain were then assayed using this approach. Although 10 \(\mu M\) RIS markedly inhibited farnesylation of Ras-CVLS, 10 \(\mu M\) NE58051 (an analog of RIS that did not affect prenylation in osteoclasts) was 3000 times less potent at inhibiting bone resorption in vivo (Luckman et al., 1998a) only partially inhibited farnesylation of Ras-CVLS (Fig. 3). Similarly, 10 \(\mu M\) NE11808 (a potent antiresorptive BP that inhibited prenylation in osteoclasts; Fig. 1B) completely inhibited farnesylation of Ras-CVLS, whereas 10 \(\mu M\) NE11809 (a less antiresorptive analog that had no effect on prenylation in osteoclasts) did not inhibit farnesylation of Ras-CVLS (Fig. 3). Hence, this cell-free assay accurately reflected the structure-activity relationships of BPs for inhibiting protein prenylation in osteoclasts in vitro (Fig. 1B) and for inhibiting bone resorption in vivo.

Nitrogen-Containing Bisphosphonates Do Not Inhibit Recombinant IPP Isomerase. To determine whether N-BPs affected the activity of IPP isomerase, we examined their effect on the activity of recombinant human IPP isomerase. The enzyme was expressed in JM101 cells using the pARC301N bacterial expression system, which was found to constitutively express the protein to a high level (>40% of total bacterial protein). Crude lysates from JM101 cultures expressing recombinant protein were typically found to have specific IPP isomerase activity of 0.13 nmol/min/mg of protein. Native bacterial IPP isomerase activity in JM101 cells under these conditions was negligible. Lysates from J774 cells were also assayed for IPP isomerase activity, which was typically found to be 0.24 pmol/min/mg of protein. Recombinant enzymes and J774 cell lysates were assayed for IPP isomerase activity in the presence of 1 to 100 \(\mu M\) BPs.
All of the BPs tested failed to inhibit IPP isomerase activity even at a concentration of 100 μM (Fig. 4). High (>1000 μM) concentrations of BPs had a slight inhibitory effect, most likely due to chelation of Mg^{2+} ions required for enzyme activity, rather than more specific enzyme inhibition.

**Nitrogen-Containing Bisphosphonates Inhibit FPP Synthase.** To assay FPP synthase in J774 homogenates, typically 10 to 20 mg of protein were used per assay and enzyme activities of 0.6 pmol/min/mg of protein were obtained from the crude homogenate. The use of butan-1-ol as the extracting solvent in which neither IPP nor dimethylallyl diphosphate is soluble ensured that any isotope extracted into the solvent had been incorporated into geranyl diphosphate or isoprenoids of greater chain length, thus minimizing interference from the activity of IPP isomerase. Furthermore, since recombinant IPP isomerase was found to be unaffected by BPs, any inhibition by BPs observed with the enzyme preparations could be ascribed fully to an effect on FPP synthase.

The N-BPs ZOL, RIS, IBA, ALN, and PAM inhibited FPP synthase in J774 cell homogenates with IC_{50} values from 0.02 to 0.85 μM (Fig. 5; Table 1). With recombinant human FPP synthase, the IC_{50} values were consistently about 10-fold less, with values as low as 0.003 μM for ZOL and MIN (Table 1). At a concentration of 0.1 μM there was a clear difference in effectiveness between the N-BPs for inhibiting recombinant human FPP synthase, with MIN, ZOL, and RIS being significantly more effective at inhibiting FPP synthase than ALN or PAM, whereas ALN was significantly more effective than PAM (Fig. 5B). The non-N-BPs CLO and ETI did not have any significant inhibitory effect on FPP synthase activity (Fig. 5, A and B), even at concentrations as high as 100 μM. CLO was still ineffective even at 1000 μM.

A comparison was also made between the potency of RIS and NE58051, and between NE11808 and NE11809, for inhibiting recombinant human FPP synthase. RIS was found to be almost 300-fold more potent at inhibiting recombinant FPP synthase than NE58051 (Fig. 6), whereas NE11808 was 73-fold more potent than NE11809 (Fig. 6; Table 1). Similar results were obtained with J774 cell homogenates (Table 1).
TABLE 1
Antiresorptive potency of bisphosphonates in vivo and their ability to inhibit FPP synthase in vitro
The values for lowest effective dose (LED) are for inhibition of bone resorption in rats in vivo (Geddes et al., 1994; Ebetino and Dansereau, 1995; Luckman et al., 1998a). The values for IC₅₀ (mean ± S.E.M. from three experiments) were calculated from dose-response plots of inhibition of FPP synthase in J774 cell homogenates or inhibition of recombinant human FPP synthase. CLO and ETI were not included since inhibition was negligible at concentrations ≤100 μM. Linear regression analysis demonstrated a significant correlation between antiresorptive potency of N-BPs and potency for inhibition of FPP synthase (r = 0.95, p < 0.0001; Spearman’s rho correlation). The relative ability of N-BPs to inhibit FPP synthase was determined by preincubating recombinant human FPP synthase with 0.001 to 10 μM BPs for 10 min before addition of [14C]IPP substrate. Data are the mean ± S.E.M. of three independent experiments, expressed as a percentage of FPP synthase activity in the absence of BP. *p < 0.05 versus NE58051/NE11809; **p < 0.01 versus NE58051/NE11809 (paired t test).

<table>
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<th>Compound</th>
<th>LED (mg phosphorus/kg)</th>
<th>IC₅₀, J774 Cells (μM)</th>
<th>IC₅₀, rhFPP Synthase (μM)</th>
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<tr>
<td>NE58051</td>
<td>1.0</td>
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<tr>
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<tr>
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<td>MIN</td>
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N.D., not determined.

was carried out using the nonparametric Spearman’s rho correlation test. The value of r was 0.94, with p = 0.0001, indicating a highly significant correlation between the ability to inhibit FPP synthase and the ability to inhibit bone resorption in vivo.

Discussion
We and others have recently shown that N-BPs inhibit bone resorption by preventing protein prenylation in osteoclasts (Fisher et al., 1999; van Beek et al., 1999a; Coxon et al., 2000), owing to inhibition of FPP synthase, an enzyme in the mevalonate pathway (van Beek et al., 1999b; Bergstrom et al., 2000). In this study, we sought to determine the potency of a wider range of N-BPs for inhibiting FPP synthase, and determine whether changes in antiresorptive potency in vivo caused by altering the structure of the R₂ side chain of N-BPs are due to differences in potency for inhibiting FPP synthase.

Using a cell-free protein prenylation assay, we found that the order of potency of BPs for inhibiting prenylation of a recombinant Ras substrate (Ras-CVLS) matched the order of antiresorptive potency, with the N-BPs RIS and IBA being more potent than ALN or PAM. Furthermore, CLO and ETI had little effect on prenylation of Ras-CVLS, consistent with the inability of CLO and ETI (which lack a nitrogen in the chemical structure) to inhibit protein prenylation in intact cells (Luckman et al., 1998b; Benford et al., 1999; Coxon et al., 2000) and the finding by van Beek et al., 1999c; Bergstrom et al., 2000) that CLO and ETI have little effect on the activity of FPP synthase in vitro.

Although many previous studies have demonstrated the importance of the structure and conformation of the R₂ side chain of N-BPs in determining antiresorptive potency, an explanation for these structure-activity relationships has not been fully established. In this study we found that for two pairs of heterocycle-containing N-BPs with minor differences in the R₂ side chain (RIS versus NE58051, and NE11808 versus NE11809), the ability to inhibit prenylation of Ras-CVLS in the cell-free prenylation assay matched the ability to inhibit protein prenylation in purified osteoclasts in vitro and also matched the relative potency for inhibition of bone resorption in vivo. This difference appears to be due to the ability to inhibit FPP synthase, since both RIS and NE11808 were potent inhibitors of recombinant human FPP synthase and FPP synthase in macrophage lysates, whereas NE58051 and NE11809 were up to 293-fold less potent at inhibiting the enzyme. We recently reported a similar correlation between the ability of these compounds to inhibit protein prenylation in macrophage cells and their antiresorptive potency (Luckman et al., 1998a), although differences in cellular uptake between the pairs of N-BPs could have accounted for the differences in ability to inhibit protein prenylation. Since we found in the present study that these pairs of compounds also differ in their ability to inhibit recombinant FPP synthase and protein prenylation in cell-free lysates, this demonstrates for the first time that the structure-activity relationships of N-BPs for inhibition of bone resorption in vivo are related to differences in the ability to inhibit FPP synthase rather than to differences in cellular uptake or bioavailability.

The relative ability of N-BPs to inhibit FPP synthase appears to be dependent on the orientation of the nitrogen atom in the heterocyclic group relative to the phosphonate groups. The potent antiresorptive N-BP NE58025 adopts a rigid...
three-dimensional conformation with the nitrogen atom located at a fixed position (Ebetino et al., 1993). A comparison between the three-dimensional structure of NE58025 and possible conformations of RIS and NE11808 (Fig. 7) shows that a close overlap is possible between the position of the nitrogen in NE58025, RIS, and NE11808. In contrast, a close overlap of the nitrogen is not energetically favored with NE58051 or NE11809, which are less potent inhibitors of FPP synthase than RIS or NE11808. This suggests that the nitrogen atom in N-BPs interacts with amino acid residues in a binding site in FPP synthase. Martin et al. (1999) recently suggested that N-BPs could inhibit IPP isomerase or FPP synthase because the nitrogen atom of N-BPs may mimic a carbocation in the transition state of IPP, GPP, or FPP in the active site of IPP isomerase or FPP synthase. Our data confirm that the orientation of the nitrogen atom is indeed essential for inhibition of FPP synthase. However, none of the BPs examined inhibited recombinant human IPP isomerase in vitro at concentrations up to 1000 μM [i.e., 100-fold higher than concentrations that inhibit prenylation in intact osteoclasts or macrophages (Luckman et al., 1998b; Bergstrom et al., 2000; Coxon et al., 2000)], confirming that IPP isomerase is not a target of N-BPs (van Beek et al., 1999b; Bergstrom et al., 2000). Further crystallographic studies are therefore necessary to identify the exact manner in which N-BPs bind to and inhibit FPP synthase rather than IPP isomerase.

Our studies also demonstrate that FPP synthase is a molecular target of the antiresorptive N-BPs ZOL, MIN, RIS, IBA, INC, ALN, and PAM dose dependently inhibited FPP synthase activity in lysates of J774 macrophages [a murine cell line in which the inhibitory effect of N-BPs on protein prenylation was first demonstrated (Luckman et al., 1998b)], or recombinant human FPP synthase, with a statistically significant correlation between the order of potency for inhibition of recombinant human FPP synthase in vitro and the order of antiresorptive potency in vivo. ZOL and MIN inhibited recombinant human FPP synthase at concentrations ≥1 nM. This is consistent with our finding that a concentration of 10 μM ZOL or RIS completely inhibits prenylation in intact J774 cells and osteoclasts (Luckman et al., 1998b; Coxon et al., 2000). Similar values of IC50 for inhibition of FPP synthase and FPP synthase/IPP isomerase by RIS, IBA, ALN, and PAM were recently reported by Bergstrom et al. (2000) and van Beek et al. (1999c).

Interestingly, INC and IBA have also been shown to inhibit squalene synthase, another enzyme in the mevalonate pathway required for cholesterol biosynthesis (Amin et al., 1992, 1996). However, these N-BPs appear to inhibit bone resorption due to inhibition of FPP synthase (resulting in the loss of the downstream metabolite GGPP required for geranylgeranylation of small GTPases essential for osteoclast function (Coxon et al., 2000) rather than squalene synthase, since the addition of cholesterol (hence bypassing the requirement for squalene synthase) could not rescue osteoclasts in vitro from the effect of N-BPs (Fisher et al., 1999; van Beek et al., 1999a).

Our observations demonstrate that the antiresorptive property of N-BPs in vivo results from their ability to prevent...
protein prenylation in osteoclasts following inhibition of FPP synthase. Furthermore, the changes in antiresorptive potency that arise due to changes in the R² side chain of N-BPs can also be explained largely by differences in the ability to inhibit FPP synthase rather than differences in cellular uptake or pharmacokinetics.

Acknowledgment

We thank Dr. Bobby Barnett, Procter & Gamble Pharmaceuticals, for providing the structures shown in Fig. 7.

References


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