ABSTRACT

The profound effects of the bisphosphonates on calcium metabolism were discovered over 30 years ago, and they are now well established as the major drugs used for the treatment of bone diseases associated with excessive resorption. Their principal uses are for Paget disease of bone, myeloma, bone metastases, and osteoporosis in adults, but there has been increasing and successful application in pediatric bone diseases, notably osteogenesis imperfecta. Bisphosphonates are structural analogues of inorganic pyrophosphate but are resistant to enzymatic and chemical breakdown. Bisphosphonates inhibit bone resorption by selective adsorption to mineral surfaces and subsequent internalization by bone-resorbing osteoclasts where they interfere with various biochemical processes. The simpler, non–nitrogen-containing bisphosphonates (eg, clodronate and etidronate) can be metabolically incorporated into nonhydrolysable analogues of adenosine triphosphate (ATP) that may inhibit ATP-dependent intracellular enzymes. In contrast, the more potent, nitrogen-containing bisphosphonates (eg, pamidronate, alendronate, risedronate, and zoledronate) inhibit a key enzyme, farnesyl pyrophosphate synthase, in the mevalonate pathway, thereby preventing the biosynthesis of isoprenoid compounds that are essential for the posttranslational modification of small guanosine triphosphate (GTP)-binding proteins (which are also GTPases) such as Rab, Rho, and Rac. The inhibition of protein prenylation and the disruption of the function of these key regulatory proteins explains the loss of osteoclast activity. The recently elucidated crystal structure of farnesyl diphosphate reveals how bisphosphonates bind to and inhibit at the active site via their critical nitrogen atoms. Although bisphosphonates are now established as an important class of drugs for the treatment of many bone diseases, there is new knowledge about how they work and the subtle but potentially important differences that exist between individual bisphosphonates. Understanding these may help to explain differences in potency, onset and duration of action, and clinical effectiveness.
The discovery and development of the bisphosphonates as a major class of drugs for the treatment of bone diseases was recently reviewed and represents a fascinating story that has its origins in studies of biological calcification processes. There are many books and review articles available that describe the chemistry, pharmacology, and clinical applications of bisphosphonates.

It had been known since the 1930s that trace amounts of polyphosphates were capable of acting as water softeners by inhibiting the crystallization of calcium salts, such as calcium carbonate, and in the 1960s water softeners by inhibiting the crystallization of calcium salts. It was proposed that inorganic pyrophosphate (PPI) might be the body’s own natural “water softener” that normally prevents calcification of soft tissues and regulates bone mineralization. It subsequently became clear that calcification disorders might be linked to disturbances in PPI metabolism. The first example was an inherited disorder, hypophosphatasia, in which lack of alkaline phosphatase is associated with mineralization defects of the skeleton and elevated PPI levels, indicating that alkaline phosphatase is probably the key extra-cellular enzyme responsible for hydrolyzing pyrophosphate.

Attempts to exploit these concepts by using pyrophosphate and polyphosphates to inhibit ectopic calcification in blood vessels, skin, and kidneys in laboratory animals were successful only when the compounds were injected. Orally administered pyrophosphate and polyphosphates were inactive because of their hydrolysis in the gastrointestinal tract. During the search for more stable analogues of pyrophosphate that might also have the antimineralization properties of pyrophosphate but would be resistant to hydrolysis, several different chemical classes were studied. The bisphosphonates (at that time called diphosphonates), characterized by P-C-P motifs, were among these classes.

Like pyrophosphate, bisphosphonates had high affinity for bone mineral and were found to prevent calcification both in vitro and in vivo but, unlike pyrophosphate, were also able to prevent experimentally induced pathologic calcification when given orally to rats in vivo. This property of being active by mouth was key to their future use in humans.

In these early studies bisphosphonates were shown not only to prevent the experimentally induced calcification of many soft tissues, including skin, kidneys, and blood vessels in vivo but, with some of the compounds (e.g., etidronate), to also inhibit mineralization of ectopic bone as well of normal calcified tissues such as bone and cartilage. Bisphosphonates seem to prevent calcification by physicochemical mechanisms that produce direct impairment of the calcification process by acting as crystal poisons after adsorption to mineral surfaces rather than by effects on the deposition of matrix.

Perhaps the most important step toward the future use of bisphosphonates occurred when we found that bisphosphonates, as we had already shown for PPI, also had the novel property of being able to inhibit the dissolution of hydroxyapatite crystals. This finding led to studies to determine if they might also inhibit bone resorption, which they did in many different experimental models. In growing intact rats, the bisphosphonates block the removal of both bone and cartilage, thus retarding the modeling of the metaphysis, which becomes club-shaped and radiologically denser than normal. This effect is the basis of the Schenk model and is a phenomenon of interest in pediatrics because it is also observed in children who are treated with high doses of bisphosphonates.

The bisphosphonates are also effective in preventing bone destruction in a number of animal models of human disease, such as immobilization osteoporosis, and the prevention of bone loss associated with ovariectomy. If not given in excess, bisphosphonates do not impair bone growth and can maintain or improve the biomechanical properties of bone in both normal animals and experimental models of osteoporosis.

In general, there is a good correlation between potency and structure-activity relationships in vitro and in vivo. In the presence of bisphosphonates, isolated osteoclasts form fewer and smaller erosion cavities on various mineralized matrices in vitro.

Pharmacology and Cellular Actions

Etidronate was the first bisphosphonate to be used in humans for fibro dysplasia ossificans progressiva and Paget disease. Once the potential clinical value of bisphosphonates had been appreciated, research efforts were devoted to the development of compounds with a more powerful antiresorptive activity but without a corresponding ability to inhibit mineralization. With compounds such as etidronate there was only a 10- to 100-fold difference between doses that inhibit mineralization compared with doses that reduce bone resorption. Enhancing this window was readily achieved, and many hundreds of bisphosphonates have been synthesized; more than a dozen have been used in humans. With the development of bisphosphonates that were more potent inhibitors of bone resorption, these dose differences widened to several orders of magnitude, which meant that inhibition of skeletal mineralization observed with etidronate ceased to be a major clinical concern. The gradation of potency evaluated in the animal models corresponded quite well with that found in humans, although the differences in potency are much smaller in humans.

Bisphosphonates accumulate in bone, so it is impor-
tant to know what happens during long-term administration. From a clinical point of view, it is reassuring that the inhibition of bone resorption reaches a new steady-state level rather than becoming progressively lower, even when the compounds are given continuously. The level of suppression depends on the administered dose and has also been observed in humans. There seems to be no progression of the antiresorptive effect with time, which suggests that the bisphosphonate buried in the bone is inactive for at least as long as it remains buried there. This also means that within the therapeutically effective range, there is little risk of a continuous and progressive decrease in bone turnover in the long run that might lead to an increase in bone fragility. An additional important pharmacologic property of bisphosphonates is that the total dose administered is a major determinant of their effects. This has been well studied for ibandronate and zoledronate. In both cases the same inhibition of bone resorption has been documented regardless of whether the bisphosphonate was given in small frequent (eg, daily) doses compared with larger doses given less frequently. This was the basis for the development of intermittent-dosing regimens in humans.

The pronounced selectivity of bisphosphonates for bone rather than other tissues is the basis for their value in clinical practice. Their preferential uptake by and adsorption to mineral surfaces in bone bring them into close contact with osteoclasts. During bone resorption, bisphosphonates are probably internalized by endocytosis along with other products of resorption. Many studies have shown that bisphosphonates can affect osteoclast-mediated bone resorption in a variety of ways, including effects on osteoclast recruitment, differentiation, and resorptive activity, and may induce apoptosis.

Because mature, multinucleated osteoclasts are formed by the fusion of mononuclear precursors of hematopoietic origin, bisphosphonates could also inhibit bone resorption by preventing osteoclast formation, in addition to affecting mature osteoclasts. In vitro, bisphosphonates can inhibit dose-dependently the formation of osteoclast-like cells in long-term cultures of human bone marrow. In organ culture, also, some bisphosphonates can inhibit the generation of mature osteoclasts, possibly by preventing the fusion of osteoclast precursors.

It is likely that bisphosphonates are selectively internalized by osteoclasts rather than other cell types because of their accumulation in bone and the endocytic activity of osteoclasts. During the process of bone resorption, the subcellular space beneath the osteoclast is acidified by the action of vacuolar-type proton pumps in the ruffled border of the osteoclast membrane. The acidic pH of this microenvironment causes dissolution of the hydroxyapatite bone mineral, whereas the breakdown of the extracellular bone matrix is brought about by the action of proteolytic enzymes, including cathepsin K. Because bisphosphonates adsorb to bone mineral, especially at sites of bone resorption where the mineral is most exposed, osteoclasts are the cell type in bone most likely to be exposed to the highest concentrations of free, non–mineral-bound bisphosphonate as a result of the release of the bisphosphonate from bone mineral in the low-pH environment beneath osteoclasts. It has been estimated that pharmacologic doses of alendronate that inhibit bone resorption in vivo could give rise to local concentrations as high as 1 mM alendronate in the resorption space beneath an osteoclast, which is much higher than the concentrations of bisphosphonates required to affect osteoclast morphology and cause osteoclast apoptosis in vitro.

In contrast to their ability to induce apoptosis in osteoclasts, which contributes to the inhibition of resorptive activity, some experimental studies suggest that bisphosphonates may protect osteocytes and osteoblasts from apoptosis induced by glucocorticoids. Recent evidence suggests that the inhibition of osteocyte apoptosis by bisphosphonates is mediated through the opening of connexin 43 hemichannels and activation of extracellular signal-regulated kinases. The possibility that bisphosphonates used clinically may get access to osteocytes differentially depending on their mineral-binding affinities and inherent structural properties needs to be studied.

**STRUCTURE-ACTIVITY RELATIONSHIPS AND MECHANISM OF ACTION**

The features of the bisphosphonate molecule necessary for biological activity were well defined in the early studies. The P-C-P moiety is responsible for the strong affinity of the bisphosphonates for binding to hydroxyapatite and allows for a number of variations in structure on the basis of substitution in the R1 and R2 positions on the carbon atom (Fig 1). The ability of the bisphosphonates to bind to hydroxyapatite crystals and to prevent both crystal growth and dissolution was enhanced when the R3 side chain (attached to the geminal carbon atom of the P-C-P group) was a hydroxyl group (as in etidronate) rather than a halogen atom such as chlorine (as in clodronate). The presence of a hydroxyl group at the R3 position increases the affinity for calcium (and, thus, bone mineral) because of the ability of bisphosphonates to chelate calcium ions by tridentate rather than bidentate binding.

The ability of bisphosphonates to inhibit bone resorption in vitro and in vivo also requires the P-C-P structure. Monophosphonates (eg, pentane monophosphonate) or P-C-C-P or P-N-P compounds are ineffective as inhibitors of bone resorption. Furthermore, the antiresorptive effect cannot be accounted for simply by adsorption of bisphosphonates to bone mineral and prevention of hydroxyapatite dissolution. It became clear that
bisphosphonates must inhibit bone resorption by cellular effects on osteoclasts rather than simply by physicochemical mechanisms.

After the successful clinical use of clodronate and etidronate in the 1970s and 1980s, more potent antiresorptive bisphosphonates, which had different R² side chains but in which R¹ was unaltered, were studied. In particular, bisphosphonates containing a basic primary amino-nitrogen atom in an alkyl chain (as in pamidronate and alendronate) were found to be 10- to 100-fold more potent than etidronate and clodronate. Then, in the 1980s, there was a phase in which synthesis of novel compounds took place specifically to determine their possible effects on calcium metabolism, with the result that compounds highly effective as inhibitors of bone resorption were identified and studied.

These compounds, especially those that contain a tertiary amino-nitrogen (such as ibandronate and olpadronate), were even more potent at inhibiting bone resorption. Among this generation of compounds that were synthesized to optimize their antiresorptive effects, the most potent antiresorptive bisphosphonates were those containing a nitrogen atom within a heterocyclic ring (as in risedronate and zoledronate), which are up to 10 000-fold more potent than etidronate in some experimental systems (Fig 2).

The analysis of structure-activity relationships allowed the spatial features of the active pharmacophore to be defined in considerable detail even before the molecular mechanism of action was fully elucidated. For maximal potency, the nitrogen atom in the R² side chain must be a critical distance away from the P-C-P group and in a specific spatial configuration. This principle was used successfully for predicting the features required in the chemical design of new and more active compounds.

Although the structure of the R² side chain is the major determinant of antiresorptive potency, both phosphonate groups are also required for the drugs to be pharmacologically active.
In summary, studies of the relationships between bisphosphonate structure and antiresorptive potency suggested that the ability of bisphosphonates to inhibit bone resorption depend on 2 separate properties of the bisphosphonate molecule. The 2 phosphate groups, together with a hydroxyl group at the R1 position, impart high affinity for bone mineral and act as a “bone hook,” which allows rapid and efficient targeting of bisphosphonates to bone mineral surfaces. Once localized within bone, the structure and three-dimensional conformation of the R2 side chain (as well as the phosphonate groups in the molecule) determine the biological activity of the molecule and influence the ability of the drugs to interact with specific molecular targets. Our understanding of what these molecular targets might be has become much clearer as a result of recent work.

Over the years there have been many efforts to explain how bisphosphonates work on cells, especially via inhibitory effects on enzymes (eg, by direct or indirect inhibition of the osteoclast proton-pumping H+ ATPase, phosphatases, or lysosomal enzymes). Because osteoclasts are highly endocytic, bisphosphonate present in the resorption space is likely to be internalized by endocytosis and thereby affect osteoclasts directly. The uptake of bisphosphonates by osteoclasts in vivo has been confirmed by using radiolabeled and fluorescently labeled alendronate, which was internalized into intracellular vacuoles. After cellular uptake, a characteristic morphologic feature of bisphosphonate-treated osteoclasts is the lack of a ruffled border, the region of invaginated plasma membrane facing the resorption cavity. Bisphosphonates also disrupt the cytoskeleton of the osteoclast. Early explanations for these effects invoked the inhibition of protein kinases or phosphatases that regulate cytoskeletal structure, such as protein tyrosine phosphatases. However, a more likely mechanism by which the cytoskeleton may be affected involves loss of function of small GTPases such as Rho and Rac.

Since the early 1990s there has been a systematic effort by our group and others to elucidate the molecular mechanisms of action of bisphosphonates and we have proposed that bisphosphonates can be classified into at least 2 major groups with different modes of action (Fig 3). The first group comprises the non–nitrogen-containing bisphosphonates that perhaps most closely resemble pyrophosphate, such as clodronate and etidronate, and these can be metabolically incorporated into nonhydrolyzable analogues of adenosine triphosphate (ATP) by reversing the reactions of aminoacyl–transfer RNA synthetases. The resulting metabolites contain the P-C-P moiety in place of the B,γ-phosphate groups of ATP, thus resulting in nonhydrolyzable (AppCp) nucleotides. It is likely that intracellular accumulation of these metabolites within osteoclasts inhibits their function and may cause osteoclast cell death. The AppCp-type metabolites of bisphosphonates are cytotoxic when internalized and cause similar changes in morphology to those observed in clodronate-treated cells, possibly by interference with mitochondrial ATP translocases. Overall, this group of bisphosphonates, therefore, seem to act as prodrugs, being converted to active metabolites after intracellular uptake by osteoclasts in vivo.

In contrast, the second group contains the more potent, nitrogen-containing bisphosphonates such as alendronate, risedronate, and zoledronate. Members of this group interfere with other metabolic reactions, notably in the mevalonate biosynthetic pathway, and affect cellular activity and cell survival by interfering with protein prenylation and, therefore, the signaling functions of key regulatory proteins. These mechanisms have been reviewed in detail elsewhere. The mevalonate pathway is a biosynthetic route responsible for the production of cholesterol, other sterols, and isoprenoid lipids such as isopentenyl diphosphate (also known as isopentenyl pyrophosphate), as well as farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP). FPP and GGPP are required for the posttranslational modification (prenylation) of small GTPases such as Ras, Rab, Rho, and Rac, which are prenylated at a cysteine residue in characteristic C-terminal motifs. Small GTPases are important signaling proteins that regulate a variety of cell processes important for osteoclast function, including cell morphology, cytoskeletal arrangement, membrane ruffling, trafficking of vesicles, and apoptosis. Prenylation is

![FIGURE 3](attachment:image.png)

Two distinct molecular mechanisms of action of bisphosphonates (BPs) that both inhibit osteoclasts (see text for details). GTP indicates guanosine triphosphate.
required for the correct function of these proteins because the lipid prenyl group serves to anchor the proteins in cell membranes and may also participate in protein-protein interactions.70

Many observations point to the importance of the mevalonate pathway for osteoclast function and strengthen the proposal that the nitrogen-containing bisphosphonates inhibit osteoclastic bone resorption predominantly by inhibition of this pathway. These bisphosphonates inhibit the synthesis of mevalonate metabolites including FPP and GGPP, and thereby impair the prenylation of proteins,71 and cause alteration of function of small GTPases. There is a strong structure-activity relationship such that changes to the structure of the nitrogen-containing R² side chain or to the phosphonate groups, which alter antiresorptive potency, also influence the ability to inhibit protein prenylation to a corresponding degree.72 An important verification of the critical importance of this pathway has come from showing that the addition of intermediates of the mevalonate pathway (such as FPP and GGPP) could overcome bisphosphonate-induced apoptosis and other events in many cell systems. Another prediction was that if inhibition of the mevalonate pathway could account for the antiresorptive effects of bisphosphonates, then the statin drugs should also inhibit bone resorption. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, one of the first steps in the mevalonate pathway. They proved to be even more potent than bisphosphonates at inhibiting osteoclast formation and bone resorption in vitro,73,74 an effect that could also be overcome by the addition of geranylglyceraniol (which can be used for protein geranylgeranylation) but not farnesol (which is used for protein farnesylation). Hence, it seems that although nitrogen-containing bisphosphonates can prevent both farnesylation and geranylgeranylation of proteins (probably by inhibiting enzymes required for synthesis of FPP and GGPP), loss of geranylgeranylated proteins in osteoclasts is of greater consequence than loss of farnesylated proteins. This is consistent with the known role of geranylgeranylated proteins such as Rho, Rac, and Rabin processes that are fundamental to osteoclast formation and function (eg, cytoskeletal rearrangement, membrane ruffling, and vesicular trafficking75), and further work has confirmed this, particularly the importance of Rab proteins.

The comparison between bisphosphonates and statins is interesting. The statins are widely used as cholesterol-lowering drugs (they are able to lower cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase). Despite several studies, there is no substantial evidence that statins have effects on bone when used clinically, perhaps because they are selectively taken up by the liver rather than bone, which is the converse of the case for bisphosphonates. Therefore, this is an excellent example of how drug specificity is achieved by highly selective tissue targeting.

The exact enzymes of the mevalonate pathway that are inhibited by individual bisphosphonates have been partially elucidated. Several enzymes of the pathway use isoprenoid diphosphates as a substrate (isopentenyl pyrophosphate isomerase, FPP synthase, GGPP synthase, squalene synthase) and, thus, are likely to have similar substrate-binding sites. Thus, if nitrogen-containing bisphosphonates act as substrate analogues of an isoprenoid diphosphate, it is possible that these bisphosphonates will inhibit more than 1 of the enzymes of the mevalonate pathway. Early studies revealed that incadronate and ibandronate, but not other bisphosphonates, are inhibitors of squalene synthase, an enzyme in the mevalonate pathway that is required for cholesterol biosynthesis.76,77 Inhibition of squalene synthase, however, would not lead to inhibition of protein prenylation.

However, it is now clear that farnesyl pyrophosphate synthase (FPPS) is a major site of action of the nitrogen-containing bisphosphonates (N-bisphosphonates).78 FPPS catalyzes the successive condensation of isopentenyl pyrophosphate with dimethylallyl pyrophosphate and geranyl pyrophosphate. There is a strong relationship among individual bisphosphonates between inhibition of bone resorption and inhibition of FPPS, with the most potent bisphosphonates having IC₅₀ values (concentration that inhibits response by 50%) in the nanomolar range.79 Modeling studies have provided a molecular rationale for bisphosphonate binding to FPPS.80 Our recent studies using protein crystallography, enzyme kinetics, and isothermal titration calorimetry led to the first published high-resolution radiograph structures of the human enzyme in complexes with risedronate and zoledronate.81 These agents bind to the dimethylallyl/geranyl pyrophosphate ligand pocket and induce a conformational change. The interactions of the N-bisphosphonate cyclic nitrogen with Thr201 and Lys200 suggest that these inhibitors achieve potency by positioning their nitrogen in a proposed carbocation82 binding site. This explains how the nitrogen moiety is so important to the potency of these bisphosphonates. Kinetic analyses reveal that inhibition is competitive with geranyl pyrophosphate and is of a slow, tight-binding character, which indicates that isomerization of an initial enzyme-inhibitor complex occurs after binding of the N-bisphosphonate.

Taken together, these observations clearly indicate that bisphosphonates can be grouped into 2 classes: those that can be metabolized into nonhydrolyzable analogues of ATP (the least potent bisphosphonates) and those that are not metabolized but can inhibit protein prenylation (the potent, nitrogen-containing bisphosphonates). The identification of 2 such classes may help to explain some of the other pharmacologic differences between the 2 classes.
CLINICAL APPLICATIONS OF BISPHOSPHONATES

After it was shown that bisphosphonates inhibited experimentally induced calcification and bone resorption, their potential application to clinical disorders was obvious, but it took many years for them to become well established.

The earliest clinical applications of bisphosphonates included use of etidronate as an inhibitor of calcification in fibro dysplasia ossificans progressiva (formerly known as myositis ossificans) and in patients who had undergone total hip replacement surgery to prevent subsequent heterotopic ossification and improve mobility.83

One of the other early clinical uses of bisphosphonates was as agents for bone imaging, “bone scanning,” for which they still remain outstandingly useful for detecting bone metastases and other bone lesions. The application of pyrophosphate and simple bisphosphonates as bone-scanning agents depends on their strong affinity for bone mineral, particularly at sites of increased bone turnover, and their ability to be linked to a γ-emitting technetium isotope.84,85

The most impressive clinical application of bisphosphonates has been as inhibitors of bone resorption, especially for diseases in which no effective treatment existed previously. Thus, bisphosphonates became the treatment of choice for a variety of bone diseases in which excessive osteoclast activity is an important pathologic feature, including Paget disease of bone, metastatic and osteolytic bone disease, and hypercalcemia of malignancy, as well as osteoporosis.

The clinical pharmacology of bisphosphonates is characterized by low intestinal absorption (~1%–4%) but highly selective localization and retention in bone. Significant adverse effects of bisphosphonates are minimal.86–88 Although there are more similarities than differences between individual compounds and each bisphosphonate is potentially capable of treating any of the disorders of bone resorption in which they are used, in practice different compounds have come to be favored for the treatment of different diseases. There are currently at least 10 bisphosphonates (etidronate, clodronate, tiludronate, pamidronate, alendronate, risedronate, zolendronate, and ibandronate and, to a limited extent, olpadronate and neridronate) that have been registered for various clinical applications in various countries. To a major extent, the diseases in which they are used reflects the history of their clinical development and the degree of commercial interest in and sponsorship of the relevant clinical trials.

Paget disease was the first clinical disorder in which a dose-dependent inhibition of bone resorption could be demonstrated by using bisphosphonates in humans.89,90 Bisphosphonates have become the most important drugs used in the treatment of Paget disease.91 For many years pamidronate given by intravenous infusion was used extensively,92 but the newer and more potent bisphosphonates can produce even more profound suppression of disease activity than was possible with the bisphosphonates available in previous years.93,94

The latest advance is with zolendronate,95 which, when given as a single 5-mg infusion, produced a greater and longer-lasting suppression of excess bone turnover than even oral risedronate given at 30 mg/day over 2 months, hitherto one of the most effective treatments.

In terms of commercial success, the use of bisphosphonates in oncology has been preeminent. Many cancers in humans are associated with hypercalcemia (raised blood calcium) and/or increased bone destruction. Bisphosphonates are remarkably effective in the treatment of bone problems associated with malignancy96 and are now the drugs of choice.97–99 Clinical trials that investigate the benefit of bisphosphonate therapy use a composite end point defined as a skeletal-related or bone event, which typically includes pathologic fracture, spinal cord compression, radiation or surgery to bone, and hypercalcemia of malignancy. Bisphosphonates significantly reduce the incidence of these events in myeloma100 and in patients with breast cancer metastases101,102 and in metastatic prostate cancer,103 lung cancer, renal cell carcinoma, and other solid tumors. The goals of treatment for bone metastases are also to prevent disease-related skeletal complications, palliate pain, and maintain quality of life. Zoledronate,104 pamidronate, clodronate, and ibandronate105,106 have demonstrated efficacy compared with placebo.

There is the important possibility that the survival of patients may be prolonged107–109 in some groups of patients. Recently, osteonecrosis of the jaw110 was identified as a potential complication of high-dose bisphosphonate therapy in malignant diseases.

The other area of outstanding commercial success with bisphosphonates has been in the therapy of osteoporosis, which is a major public health problem.111,112 Up until the 1990s, there were few treatments for osteoporosis. As a drug class the bisphosphonates have emerged in the past few years as the leading effective treatments for postmenopausal and other forms of osteoporosis. Etidronate was the first of these,113–115 followed by alendronate116–118 and then risedronate.119,120 All have been approved as therapies in many countries and can increase bone mass and reduce fracture rates at the spine by 30% to 50% and at other sites in postmenopausal women.121 The reduction in fractures may be related not only to the increase in bone mass arising from the inhibition of bone resorption and reduced activation frequency of bone-remodeling units but also to enhanced osteon mineralization.122 These bisphosphonates also prevent bone loss associated with glucocorticosteroid administration.123,124

Among the newer bisphosphonates, ibandronate125 was introduced recently as a once-monthly tablet. In addition to formulations to be taken by mouth weekly or
monthly, new routes of administration are being studied, especially periodic (e.g., 3 monthly) injections with ibandronate and once-yearly treatment with zoledronate.\textsuperscript{126} This has the attraction of delivering a defined dose without the variability associated with oral administration as well as avoiding potential gastrointestinal intolerance. If these approaches are accompanied by greater compliance and convenience, they are likely to become popular methods of treatment.

Other clinical issues under consideration with bisphosphonates include the choice of therapeutic regimen (e.g., the use of intermittent dosing rather than continuous, intravenous versus oral therapy), the optimal duration of therapy, the combination with other drugs such as teriparatide, and their extended use in related indications (e.g., glucocorticoid-associated osteoporosis, male osteoporosis, childhood osteopenic disorders, arthritis, and other disorders). Therefore, there is much that needs to be done to improve the way in which existing drugs can be used and to introduce new ones.

In pediatrics, pamidronate has proved remarkably effective in increasing bone in children with the inherited “brittle-bone” disorder, osteogenesis imperfecta.\textsuperscript{127,128}

**SOME CURRENT ISSUES WITH BISPHOSPHONATES: BONE ARCHITECTURE, STRUCTURE, AND STRENGTH, AND ON BONE HEALING AND FRACTURE REPAIR**

Many experimental and clinical studies show that bisphosphonates conserve bone architecture and strength.\textsuperscript{129–133} However, there have been concerns about whether the use of prolonged high doses of bisphosphonates may impair bone turnover to such an extent that bone strength is impaired. High doses in animals are associated with increased microdamage\textsuperscript{134,135} and even fractures.\textsuperscript{136} It has been suggested that bisphosphonates might prevent naturally occurring microscopic cracks in bone from healing. There have been isolated reports of adynamic bone associated with bisphosphate usage,\textsuperscript{137} but long-term use of the bisphosphonates in the therapy of osteoporosis seems to be safe.\textsuperscript{138} Case reports of induction of osteopetrosis-like lesions in children who were treated with excessive doses of pamidronate have been published.\textsuperscript{139}

A question often asked is whether bisphosphonates inhibit fracture repair. By reducing bone turnover one might expect bisphosphonates to interfere with fracture healing. However, a recent long-term study in a beagle dog model that simulated fracture repair has demonstrated that ibandronate treatment did not adversely affect normal bone healing.\textsuperscript{140} Studies of repair processes after creating drill-hole defects in dogs also showed no impairment with ibandronate.\textsuperscript{141}

Several other recent studies raised the intriguing possibility that bisphosphonates may enhance fracture repair and related processes.\textsuperscript{142} In studies of the osseointegration of metal implants in ovariectomized rats, treatment with ibandronate resulted in improved osseointegration rather than impairment of the healing process.\textsuperscript{143} Potential applications of bisphosphonates in orthopedics include protection against loosening of prostheses,\textsuperscript{144} better integration of biomaterials and implants, improved healing in distraction osteogenesis,\textsuperscript{145} and conserving bone architecture after osteonecrosis\textsuperscript{146,147} and in Perthes disease.\textsuperscript{148}

There are potentially important differences between clinically useful bisphosphonates regarding their potency and duration of action. Efficacy is closely related to affinity for bone mineral and ability to inhibit FPP synthase. Recent studies showing that there are marked differences among bisphosphonates in binding to hydroxyapatite\textsuperscript{149} may explain the variations in retention and persistence of effect that have been observed in animal and clinical studies. In the case of zoledronic acid, in particular, the remarkable magnitude of effect and prolonged duration of action can be explained in part by these new observations. In explaining the long duration of action, it has been proposed that there is continual

**FIGURE 4**

Bisphosphonate (BP) uptake and detachment from bone surfaces: effect of binding affinity on recirculation of bisphosphonate on and off bone surfaces. The differences in mineral-binding affinity may affect distribution into different bone compartments and persistence of drug action at bone surfaces. (Adapted from Nancollas GH, Tang R, Phipps RJ, et al. Bone. 2006;38:617–627.)

<table>
<thead>
<tr>
<th>High-affinity BP (eg, alendronate, zoledronate)</th>
<th>More avid uptake</th>
<th>Lower desorption</th>
<th>Higher reattachment</th>
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<td>BP</td>
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Higher-affinity BPs may diffuse less well in bone and remain nearer accessible surfaces.

BPs can be detected in body fluids many months after injection.
recycling of bisphosphonate off and back onto the bone surface. This notion is supported by observations that bisphosphonates can be found in plasma and urine many months after dosing (Fig 4).

There are numerous examples of bisphosphonates having effects on cells and tissues outside the skeleton. The effects on osteoclast precursors, tumor cells, macrophages, and γδ-T cells are examples and in all cases are probably explained by sufficient bisphosphonates entering cells to inhibit the mevalonate pathway. A well-recognized adverse effect of the nitrogen-containing bisphosphonates is that they cause an acute-phase response in vivo,150,151 which can lead to induction of fever and “flu-like” symptoms in patients. These effects are transient and occur predominantly on first exposure to the drug, especially with intravenous administration. The mechanism has been attributed to release of proinflammatory cytokines, and the mechanism has been further unraveled by showing that it involves selective receptor-mediated activation of γδ-T cells, leading to their proliferation and activation.152 The bisphosphonate effect involves the mevalonate pathway in vitro and can be overcome by using statins.153

Another interesting aspect of these nonskeletal effects are the observations made on protozoan parasites, the growth of which can be inhibited by bisphosphonates having effects on cells and tissues outside the skeleton.

In the future, other clinical indications ripe for future study include the prevention of bone loss and erosions in rheumatoid arthritis, possible applications in other joint diseases, the reduction of bone loss associated with periodontal disease, and loosening of joint prostheses.

The recent elucidation of the likely mode of action of bisphosphonates within cells opens up the possibility of exploiting the subtle and potentially important differences between the classes of bisphosphonates and individual compounds.

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