Bisphosphonates and osteonecrosis of the jaws: Science and rationale

Rajesh Gutta, BDS,[a] and Patrick J. Louis, DDS, MD,[b] Birmingham, AL
UNIVERSITY OF ALABAMA

Bisphosphonates as a group of drugs were introduced for the management of various conditions such as osteoporosis, Paget's disease, multiple myeloma, hypercalcemia of malignancy, breast cancer, prostate cancer, and other tumors. This group of drugs has improved the quality of life in many patients with proven efficacy in limiting pain and skeletal-related events. The controversy of osteonecrosis of the jaws and bisphosphonates is a recent and growing problem. Osteonecrosis of the jaws is recognized as a serious complication of bisphosphonate therapy, more commonly with the intravenous form of the drugs. However, there is limited scientific understanding about the association between osteonecrosis of the jaws and bisphosphonates. In the present article we discuss various mechanisms of action of bisphosphonates, the rationale for occurrence of osteonecrosis in the jaws, and treatment guidelines for the condition. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:186-93)**

Osteonecrosis of the jaws (ONJ) is characterized by the death of bone as a natural consequence of a wide variety of systemic and local factors compromising the blood flow of the bone. Among these factors are hemoglobinopathies, anticardiolipin antibodies, defects in the thrombotic and fibrinolytic systems, fat emboli, alcoholism, systemic lupus erythematosus, and corticosteroids.[1-3] Bisphosphonates (BPs) have recently been the subject of clinical controversies because of the increased incidence of ONJ.

The discovery of BPs as a major class of drugs has extended over a period of 3 decades, although the first synthesis of BP occurred in Germany in 1865.[4] Originally, these drugs were exploited as inhibitors of bone resorption in humans.[5] They have also been used as corrosion inhibitors and fertilizer and in the oil industry. However, their mechanism of action at the cellular and molecular levels remains enigmatic and continues to be investigated. An extensive review of the literature was performed with reference to BPs and ONJ and is summarized in the present paper.

DISCOVERY OF BISPHOSPHONATES AS INHIBITORS OF BONE RESORPTION

In the early 1960s Fleisch proved that inorganic pyrophosphates (PPi) prevent body fluid calcification by binding to hydroxyapatite crystals.[6] They also revealed that the oral forms of PPi were inactivated by the phosphatases secreted by the gastrointestinal brush border.[7] In an attempt to find stable analogs of PPi, diphosphonates (now called bisphosphonates) were among some of the drugs that were investigated during that time.[7] The BPs tolerated the oral route very well without being inactivated, and this became the key element in their success. They also inhibited the dissolution of hydroxyapatite crystals.[8] Further studies even proved that they prevented bone resorption by inhibiting osteoclast activity.[9]

Chemical structure

All bisphosphonates share a common "back bone." Although they are analogous to PPi, structurally BPs differ in that they have a carbon atom bridging the 2 phosphate molecules. This morphology provides the stability for their action and withstands hydrolysis (Fig. 1).

The presence of a hydroxyl group at the R1 chain imparts high clinical affinity for bone, thus selectively localizing to the bone. More potent compounds have been manufactured by altering the R2 side chain. Currently the amino-bisphosphonates are the most powerful drugs that are available (Table I).

Pharmacology

The clinical pharmacology of intravenous (IV) BPs is characterized by low intestinal absorption but highly selective localization and deposition in bone. Oral BPs have a bioavailability of less than 5%.[10] Once in the blood, BPs disappear very rapidly into the bone.[11] After BPs are buried in the skeleton, they are released only when the bone is destroyed in the course of turnover. In humans, the skeletal half-lives of various BPs range from 3 months to as long as 10 years.[12] The inhibition of bone resorption reaches a steady level even when the

[a]Resident, Oral and Maxillofacial Surgery, University of Alabama.
[b]Director, Oral and Maxillofacial Surgery Residency Program, University of Alabama.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2006.12.004

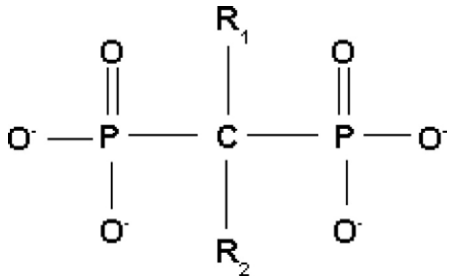


Fig. 1. The basic chemical structure for bisphosphonates.

compounds are given continuously, as has been reported in some animal studies.[13]

Mechanism of action

Different mechanisms have been reported to account for the action of BPs, including induction of apoptosis,[14,15] and disruption of the cell cycle.[16] They also have anti-invasive,[17] antiangiogenic,[18] and antimigration effects. To date, there is still no clear understanding of the molecular targets. Listed below are some of the proposed mechanisms of action of the bisphosphonates.

1. The first generation BPs, such as clodronate and etidronate, do not contain an amino group. They are metabolized to form cytotoxic adenosine triphosphate (ATP) analogs that accumulate intracellularly in osteoclasts and induce apoptosis.[14,15]
2. The potent groups of BPs (amino-BPs) are inhibitors of the mevalonate pathway, a biosynthetic pathway for isoprenoid proteins such as farsenyldiphosphate and geranylgeranyldiphosphate. The isoprenoid proteins are required for post-translational phrenylation of the guanosine triphospatases (GTPases), such as Ras, Rho, and Rac. The Ras group of proteins is responsible for cell proliferation and is activated by farnesylation.[19] After internalization, BPs cause disruption of the cytoskeleton by inactivating GTPases such as Ras, Rho, and Rac. The Rho and Rac groups of proteins are responsible for cytoskeleton organization and cell membrane ruffling and are activated through geranylgeranylation.[16,20,21] The cytoskeleton is essential to maintain the "ruffled border," the area with which the osteoclast makes contact with bone and breaks down bone tissue. With the ruffled border compromised, the osteoclast initiates apoptosis, netting a decrease in bone turnover.
3. Bisphosphonates also inhibit various matrix metalloproteinases involved in cancer growth and metastasis.[17,22]
4. Although BPs are also known to have an antiangiogenesis effect, there have been some contradicting reports on this property based on histologic analysis of ONJ samples.[23]
5. There are some reports that BPs may inhibit bone resorption by stimulating osteoblasts to produce an osteoclast-inhibitory factor.[24]
6. Bisphosphonates are also reported to be powerful inhibitors of macrophage proliferation, cells that are of the same lineage as osteoclasts.[25]
7. Other mechanisms include inhibition of the osteoclast's proton ATPase, phosphatases, and other lysozymal enzymes.[26,27]

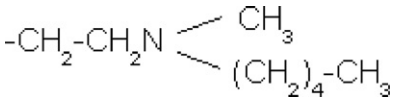
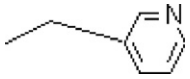
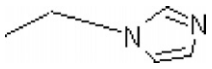
The subcellular space beneath the osteoclast is acidified during the process of bone resorption by the proton-pumps in the ruffled border of the osteoclast membrane.[28] This acidic environment causes dissolution of the hydroxyapatite mineral and releases more BPs, which are then internalized into the osteoclasts through the process of endocytosis.[29] Apart from osteoclasts, some potent BPs, such as pamidronate and zoledronate, are also potent inducers of apoptosis in other cancer cells, such as multiple myeloma, breast cancer, pancreatic cancer, and intestinal epithelial cell lines.[30-32] Many solid tumors cause hypercalcemia of malignancy, and the primary cause of this is often due to release of parathyroid hormone (PTH)–related peptide, which causes extensive bone destruction. Transforming growth factor, a bone-derived factor, also promotes increased production of PTH-related peptide by tumor cells, leading to a vicious cycle of progressive tumor growth and bone destruction. Bisphosphonates are known to interrupt this cycle by inducing osteoclast apoptosis.[33]

Early clinical applications

Etidronate was the first BP used to treat humans for myositis ossificans[34] and to prevent hypertrophic bone formation after total hip replacement surgery.[35] They are also known to prevent dental calculus when used in toothpastes[36-38] and to decrease periodontal bone destruction. However, BPs are predominantly used for osteoporosis, Paget's disease, multiple myeloma, hypercalcemia of malignancy, and metastatic and osteolytic bone disease.[39-42] BPs such as alendronate and tiludronate have been reported to prevent bone loss in healthy postmenopausal women.[43,44] They have also been proven to decrease the accumulation of cholesterol, elastin, and collagen in the arteries.[45]

Bone metastases are common in patients with many types of cancer, particularly with breast and prostate cancer. These 2 combined account for more than 80% of metastatic bone disease. Others include multiple myeloma, lung cancer, and other solid tumors.[46] This condition is associated with considerable skeletal morbidity, including severe bone pain, pathologic fracture,

Table I. Chemical structure, potency and route of administration of various bisphosphonates

Agent	*R1 side chain*	*R2 side chain*	*Relative potency*	*Route*
Etidronate (Didronel)	OH	—CH_3	×1	Oral
Clodronate	Cl	—CL	×10	Oral/IV
Tiludronate (Skelid)	H		×10	Oral
Pamidronate (Aredia)	OH	—CH_2—CH_2—NH_2	×100	IV
Neridronate	OH	—$(CH_2)_6$—NH_2	×100	Oral
Olpadronate	OH	—$(CH_2)_2N(—CH_3)_2$	×1,000	IV
Alendronate (Fosamax)	OH	—$(CH_2)_3$—NH_2	×1,000	Oral
Ibandronate (Boniva)	OH		×5,000	Oral/IV
Risedronate (Actonel)	OH		×5,000	Oral
Zoledronate (Zometa)	OH		×10,000	IV

spinal cord or nerve root compression, and hypercalcemia of malignancy. Among all of these types, breast cancer has the highest incidence of skeletal complications and consequently BPs have become the standard of treatment. Currently, zoledronate has been shown to be significantly effective in patients with metastatic breast, prostate, lung, renal, and other solid cancer types.[47] Zoledronate resulted in a significantly lower incidence of skeletal-related events in most cancer types. A combination therapy with zoledronate and pamidronate has been successfully used in patients with breast cancer, lung cancer, multiple myeloma, lymphoma, and uterine sarcoma.

BP AND ONJ

Every year, an estimated 30 million BP prescriptions are written in the U.S. alone. It is estimated that pamidronate and zoledronate have been used in over 2.5 million patients worldwide.[48] The first case report in oral and maxillofacial surgery on BP was the failure of osseointegrated dental implants.[49] The exact incidence of ONJ is unknown. However, some reports have estimated it to be about 1 in 10,000.[50] A single-center study has reported 11% incidence of ONJ in patients with multiple myeloma on BP therapy. In that study, it was also concluded that zoledronic acid produced a 9.5-fold greater risk for developing ONJ than pamidronate alone and 4.5-fold greater risk than subsequent use of pamidronate + zoledronic acid. Use of thalidomide and a number of bisphosphonate infusions also increased the risk for ONJ 2.4-fold and 4.9-fold, respectively.[51] The incidence of developing ONJ increases with time of exposure, and this risk increases dramatically in patients using zoledronic acid.[52] It is estimated that with each decade of life, there is a 9% increased risk of developing ONJ in patients on BP therapy for multiple myeloma.[53] In higher doses, BPs are also known to affect the mineralization of enamel, dentin, and cementum of the tooth.[54-56] In large doses, etidronate or clodronate cause an increase in fracture rate if given over a year.[57] Some oral forms of BPs affect osteoclastic function with less severity, because of their high intestinal absorption and poor bioavailability. However, few oral BPs (alendronate, ibandronate, and risedronate) are more potent than the IV form of BP, such as pamidronate.

In 2003, the American Society of Clinical Oncology recommended that once initiated, IV BPs be continued until there is evidence of substantial decline in a patient's general performance status.[58] At this time, we do not have enough data to determine the long-term risks of oral or IV BPs or which subset of the patient population is at risk of developing ONJ. There are little data on the activity of IV BPs beyond 2 years of administration. In one report, there was an extremely low incidence of skeletal-related events in a patient population with bone metastasis that was followed for up to 10 years.[59] In a very interesting clinical study, Coleman et al.[47] investigated the correlation between bone metabolism and clinical outcome during BP therapy by measuring urinary N-telopeptide. They estimated the correlation between this bone resorption marker and the

relative risks for negative clinical outcomes. Interestingly, N-telopeptide levels correlated with the risk of skeletal complications and disease progression. This serves as an excellent prognostic tool. This could lead to the selection of cancer patients who might benefit from therapy with BPs by evaluating urine or serum markers that predict who is at highest risk for skeletal complications and disease progression.[46]

Importance of proper nomenclature

Terms such as avascular necrosis of the jaws, bisphossy jaw, bisphosphonate osteomyelitis, bisphosphonate osteonecrosis, bisphosphonate osteochemonecrosis, and osteochemonecrosis have all been used in the literature. An analogy drawn to phossy jaw was also reported[23]: Osteonecrosis of the jaws caused by BPs was compared with the phossy jaw, more commonly seen in workers in the match industry in the 19th and early 20th centuries. Phossy jaw was caused by chronic exposure to white phosphorus, and the average period from first exposure to diagnosis was 5 years.[60] Reports of phossy jaw were first reported in the literature as early as 1845.[61] However, similar to phossy jaw necrosis, the bone morphology in ONJ is more like a pumice stone or of a moth-eaten appearance.

Some investigators have commented that ONJ is similar to osteoradionecrosis (ORN). However, there is a clear histologic difference between the 2 conditions. As reported by Hellstein and Marek,[23] in ONJ there is relative paucity of Howhip's lacunae and sparse reversal lines. In contrast, there is a higher incidence of Howship's lacunae and reversal lines in cases of ORN. Although there have been some reports in the literature about osteonecrosis caused by steroids, this form is different from ONJ in the sense that steroid-induced osteonecrosis does not cause bone exposure.[62]

Scientific rationale for ONJ

The oral cavity possesses some unique demands with constant bone remodeling. This requires adaptability and up-regulation of the osteoblast and osteoclast function. Osteoclasts have a life span of about 150 days, after which they resorb the mineral matrix of bone and release bone morphogenetic protein (BMP) and insulin-like growth factors, which in turn induce local stem cells to differentiate into osteoblasts and form new bone. This is a very critical cycle and remains in a tight temporal zone. If the osteoclast function is too severely impaired, dead and dying osteoclasts are not replaced and the capillary network in the bone is not maintained, resulting in bone necrosis. Besides resorption at the sites of bone turnover, formation is also decreased, as noted by a reduction in the bone formation surface.[63,64]

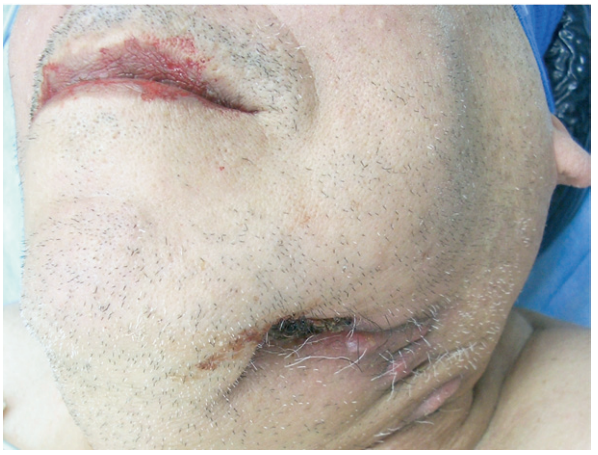


Fig. 2. Chronic orocutaneous fistula with necrosed bone.

This reduction in bone formation surface is secondary to diminished resorption.

Risk factors for developing ONJ

The risk factors for developing ONJ include trauma, female gender, advanced age, edentulous regions, radiotherapy, chemotherapy, steroid therapy, blood dyscrasias/metastatic disease, anemia, coagulopathy, surgical dental procedures, alcohol or tobacco use, prior infection, and bisphosphonate therapy.[65]

Clinical signs and symptoms

Patients may remain asymptomatic for many weeks or months, and the condition may only be recognized by the presence of exposed bone in the oral cavity. These lesions are most frequently symptomatic when sites become secondarily infected or there is trauma to the soft tissues via the sharp edges of the exposed bone. Typical signs and symptoms include pain, bone exposure, soft-tissue swelling, infection, loosening of teeth, and drainage (Figs. 2-5).

Treatment

Based on the articles published to date, the following recommendations are made by the American Association of Oral and Maxillofacial Surgeons for management of patients on BP therapy and patients with proven ONJ.[66]

Management of patients receiving BP therapy without clinical symptoms

a. Individuals on IV BP therapy:
   i. Avoid procedures that involve direct osseous injury.
   ii. Nonrestorable teeth may be treated with endodontic therapy.

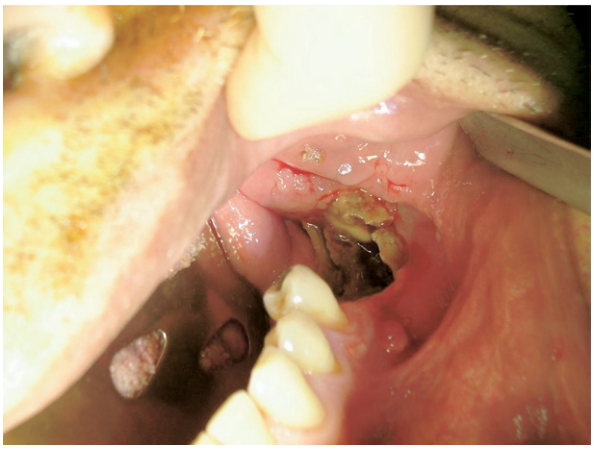


Fig. 3. Intraoral view revealing osteonecrosis.

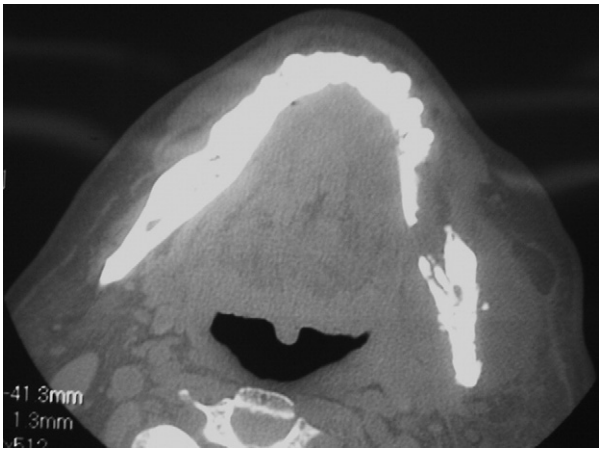


Fig. 4. Axial CT imaging showing continuity defect in the left mandible.

iii. Avoid placement of dental implants in patients on potent IV form of BPs.

b. Individuals on oral BP therapy:
   i. Elective dentoalveolar surgery is not contraindicated.
   ii. Patients should be adequately informed about the risk of compromised bone healing.
   iii. Alteration in surgical planning is not necessary in patients on oral BPs for less than 3 years and no other risk factors.
   iv. For a patient on oral BP and corticosteroids for less than 3 years, consider discontinuing the oral BP therapy for 3 months. Treatment may be resumed after bone healing is completed. This decision should be made after consulting the treating physician and only if systemic conditions permit. The same strategy applies for patients on oral BPs and steroids for more than 3 years.

Management of patients with proven ONJ based on staging of the condition

a. Stage 1: Exposed/necrotic bone in patients who are asymptomatic and have no evidence of infection.
b. Stage 2: Exposed/necrotic bone in patients with pain and clinical evidence of infection.
c. Stage 3: Exposed/necrotic bone in patients with pain, infection, and 1 or more of the following: pathologic fracture, extraoral fistula, or osteolysis extending to the inferior border.

Treatment of patients with established ONJ:

i. Patients with stage 1 ONJ: Conservative management with oral rinse such as 0.12% chlorhexidine.
ii. Patients with stage 2 ONJ: Manage with antibiotics and antimicrobial oral rinses.
iii. Patients with stage 3 ONJ: Surgical debridement/resection in combination with antibiotic therapy. Extraction of symptomatic teeth can be performed without any additional risks of worsening the condition.

The International Myeloma Foundation recommends suppression of the drug for 2-4 months to facilitate recovery in patients with ONJ. They also advise discontinuing BP therapy if a patient must undergo any oral surgery. However, at this time it is unclear if this would have any beneficial effects. It has been reported that ONJ appears long after discontinuation of therapy, in some cases as long as 12 months.[53]

Prevention

Before initiating BP therapy, all medical and dental practitioners are encouraged to follow these guidelines:

1. All patients should undergo a routine dental exam to rule out any dental source of infection.
2. All medical practitioners also should perform a baseline oral exam.
3. Invasive dental or/and oral surgical procedures should be completed before initiating therapy.
4. Practice preventive dentistry, involving procedures such as oral prophylaxis, dental restorations, and endodontic therapy, and check dentures for irritational foci.
5. Schedule routine follow-up every 3 months to check for any signs of developing ONJ.
6. The risks associated with oral surgical procedures such as dental implants, extractions, and extensive periodontal surgeries must be discussed with the patient and weighted against the benefits.

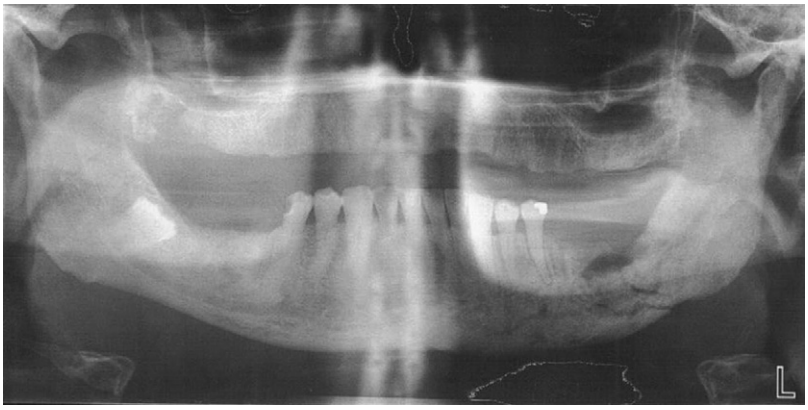


Fig. 5. Panoramic x-ray showing osteonecrosis and multiple fistulous tracts.

7. Because the incidence of ONJ may vary between the oral and IV forms of BPs, procedures must be considered with caution especially for patients on the IV form of BPs.

CAN WE DESIGN SAFER COMPOUNDS?

It is possible to design BPs with activity against selective GTPases excluding the Rho and Ras groups of proteins. This will prevent the disruption of isoprenylation of the mevalonate pathway, thus sparing the osteoclastic activity. Based on the evidence that BPs act as inhibitors of farsenyldiphosphate and geranylgeranyldiphosphate, a later-generation aminobisphosphonate, NE10790, was developed. This has the specific ability to inhibit the activity of other proteins than the Ras and Rho groups. The NE10790 BP was able to inhibit bone resorption without affecting osteoclast number or the activity of osteoclasts.[67] Also, agents such as stealth liposomes could be used as drug delivery systems for BPs. Doxirubicin is one of the first anticancer drugs encapsulated in pegylated liposoomes and delivered to specific sites of action.[68] This property could be used for BPs as well. Pegylated liposomes could be used as vehicles for BPs for specific accumulation in tumor sites rather than being accumulated in bone tissue. Currently, there are new compounds being added to this group of drugs. New-generation antibodies, such as denosumab, have been developed to treat patients with bone metastasis, multiple myeloma, and osteoporosis. Denosumab is a human IgG2 molecule with long circulatory residence time that facilitates a rapid and sustained decrease in bone resorption. This drug does not have a direct inhibitory effect on osteoblasts, unlike the current generation bisphosphonates.[69,70] Also, other issues, such as choice of regimen (continuous vs. intermittent dosing), oral route, combination therapy, and extension of use to other conditions, are all being considered.

CONCLUSION

It is important that all health professionals, especially dentists, oncologists, and oral surgeons, be aware of the possibility that patients being considered for dental extractions or other oral surgery may be undergoing BP therapy. Most medical practitioners are unaware of this serious and potentially permanent complication. Also, patients should be informed of the risk of ONJ, so that they have the opportunity to assess their need for dental treatment before starting therapy. All patients using BPs should be under vigilant surveillance.

REFERENCES

1. Bouquot JE, McMahon RE. Neuropathic pain in maxillofacial osteonecrosis. J Oral Maxillofac Surg 2000;58:1003-20.
2. Assouline-Dayan Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. Semin Arthritis Rheum 2002;32:94-124.
3. Gruppo R, Glueck CJ, McMahon RE, Bouquot J, Rabinovich BA, Becker A, et al. The pathophysiology of alveolar osteonecrosis of the jaw: anticardiolipin antibodies, thrombophilia, and hypofibrinolysis. J Lab Clin Med 1996;127:481-8.
4. Menschutkin N. Ueber die Einwirkung des Chloracetyls auf phosphorige Säure. Ann Chem Pharm 1865;133:317-20.
5. Francis MD, Russell RGG, Fleisch H. Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcification in vivo. Science 1969;165:1264-6.
6. Fleisch H, Russell RGG, Straumann F. Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. Nature 1966;212:901-3.
7. Fleisch H, Russell RGG, Bisaz S, Muhlbauer RC, Williams DA. The inhibitory effect of phosphonates on the formation of calcium phosphate crystals in vitro and on aortic and kidney calcification in vivo. Eur J Clin Invest 1970;1:12-8.
8. Fleisch H, Russell RGG, Francis MD. Diphosphonates inhibit hydroxyapatite dissolution in vitro and bone resorption in tissue culture and in vivo. Science 1969;165:1262-4.
9. Schenk R, Eggli P, Fleisch H, Rosini S. Quantitative morphometric evaluation of the inhibitory activity of new aminobisphosphonates on bone resorption in the rat. Calcif Tissue Int 1986;38:342-9.

10. Conte P, Guarneri V. Safety of intravenous and oral bisphosphonates and compliance with dosing regimens. Oncologist 2004; 9(Suppl 4):28-37.
11. Bisaz S, Jung A, Fleisch H. Uptake by bone of pyrophosphate, diphosphonates and their technetium derivatives. Clin Sci Mol Med 1978;54:265-72.
12. Kasting GB, Francis MD. Retention of etidronate in human, dog, and rat. J Bone Miner Res 1992;7:513-22.
13. Reitsma PH, Bijvoet OLM, Verlinden-Ooms H, van der Wee-Pals LJ. Kinetic studies of bone and mineral metabolism during treatment with (3-amino-1-hydroxy-propylidene)-1,1-bisphosphonate (APD) in rats. Calcif Tissue Int 1980;32:145-57.
14. Lehenkari PP, Kellinsalmi M, Napankangas JP, Ylitalo KV, Monkkonen J, Rogers MJ, et al. Further insight into mechanism of action of clodronate: inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. Mol Pharmacol 2002;61:1255-62.
15. Reszka AA, Rodan GA. Mechanism of action of bisphosphonates. Curr Osteoporos Rep 2003;1:45-52.
16. Murakami H, Takahashi N, Sasaki T, Udagawa N, Tanaka S, Nakamura I, et al. A possible mechanism of the specific action of bisphosphonates on osteoclasts: tiludronate preferentially affects polarized osteoclasts having ruffled borders. Bone 1995;17: 137-44.
17. Body JJ. Rationale for the use of bisphosphonates in osteoblastic and osteolytic bone lesions. Breast 2003;12(Suppl 2):S37-44.
18. Fournier P, Boissier S, Filleur S, Guglielmi J, Cabon F, Colombel M, et al. Bisphosphonates inhibit angiogenesis in vitro and testosterone-stimulated vascular regrowth in the ventral prostate in castrated rats. Cancer Res 2002;62:6538.
19. Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 1996;65: 241-9.
20. Hall A. Rho GTPases and the actin cytoskeleton. Science 1998;279:509-14.
21. Sato M, Grasser W, Endo N, Akins R, Simmons H, Thompson DD, et al. Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure. J Clin Invest 1991;88:2095-105.
22. John A, Tuszynski G. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. Pathol Oncol Res 2001;7:14-23.
23. Hellstein JW, Marek CL. Bisphosphonate osteochemonecrosis (bis-phossy jaw): is this phossy jaw of the 21st century? J Oral Maxillofac Surg 2005;63:682-9.
24. Niskikawa M, Akatsu T, Katayama Y, Yasutomo Y, Kado S, Kugal N, et al. Bisphosphonates act on osteoblastic cells and inhibit osteoclast formation in mouse marrow cultures. Bone 1996;18:9-14.
25. Cecchini MG, Felix R, Fleisch H, Cooper PH. Effect of bisphosphonates on proliferation and viability of mouse bone marrow–derived macrophages. J Bone Miner Res 1987;2:135-42.
26. David P, Nguyen H, Barbier A, Baron R. The bisphosphonate tiludronate is a potent inhibitor of the osteoclast vacuolar H^+ ATPase. J Bone Miner Res 1996;11:1498-507.
27. Felix R, Russell RGG, Fleisch H. The effect of several diphosphonates on acid phosphohydrolases and other lysosomal enzymes. Biochim Biophys Acta 1976;429:429-38.
28. Suda T, Nakamura I, Jimi E, Takahashi N. Regulation of osteoclast function. J Bone Miner Res 1997;12:869-79.
29. Russell RG, Rogers MJ. Bisphosphonates: from the laboratory to the clinic and back again. Bone 1999;25:97-106.
30. Tassone P, Tagliaferri P, Viscomi C, Palmieri C, Caraglia M, D'Alessandro A, et al. Zoledronic acid induces antiproliferative and apoptotic effects in human pancreatic cancer cells in vitro. Br J Cancer 2003;88:1971-8.
31. Senaratne SG, Mansi JL, Colston KW. The bisphosphonate zoledronic acid impairs Ras membrane localisation and induces cytochrome c release in breast cancer cells. Br J Cancer 2002;86:1479-1486.
32. Twiss IM, Pas O, Ramp-Koopmanschap W, Den Hartigh J, Vermeij P. The effects of nitrogen-containing bisphosphonates on human epithelial (Caco-2) cells, an in vitro model for intestinal epithelium. J Bone Miner Res 1999;14:784-91.
33. Mundy GR, Yoneda T, Hiraga T. Preclinical studies with zoledronic acid and other bisphosphonates: impact on the bone microenvironment. Semin Oncol 2001;28:35-44.
34. Bassett CAL, Donath A, Macagno F, Preisig R, Fleisch H, Francis MD. Diphosphonates in the treatment of myositis ossificans. Lancet 1969;2:845.
35. Bijvoet O, Fleisch H, Canfield RE, Russell RGG. Bisphosphonates on bone. Amsterdam: Elsevier; 1995.
36. Briner WW, Francis MD, Widder JS. The control of dental calculus in experimental animals. Int Dent J 1971;21:61-73.
37. Mühlemann HR, Bowles D, Schatt A, Bernimoulin JP. Effect of diphosphonate on human supragingival calculus. Helv Odont Acta 1970;14:31-3.
38. Sturzenberger OP, Swancar JR, Reiter G. Reduction of dental calculus in humans through the use of a dentifrice containing a crystal-growth inhibitor. J Periodontol 1971;42:416-9.
39. Delmas PD, Meunier PJ. The management of Paget's disease of bone. N Engl J Med 1997;336:558-6.
40. McCloskey EV, MacLennan ICM, Drayson M, Chapman C, Dunn J, Kanis JA. MRC Working Party on Leukaemia in Adults. A randomized trial of the effect of clodronate on skeletal morbidity in multiple myeloma. Br J Haematol 1998;100:317-25.
41. Mundy GR, Yoneda T. Bisphosphonates as anticancer drugs. N Engl J Med 1998;339:398-400.
42. Ralston SH, Gallacher SJ, Patel U, Dryburgh FJ, Fraser WD, Cowan RA, et al. Comparison of 3 bisphosphonates in cancer-associated hypercalcaemia. Lancet 1989;2:1180-2.
43. Keen RW, Spector TD. Alendronate: a new bisphosphonate for the treatment of osteoporosis. Br J Clin Pract. 1996 Jun;50(4):211-7.
44. Reginster JY, Lecart MP, Deroisy R, Sarlet N, Denis D, Ethgen D, et al. Prevention of postmenopausal bone loss by tiludronate. Lancet 1989;2:1469-71.
45. Kramsch DM, Chan CT. The effect of agents interfering with soft tissue calcification and cell proliferation on calcific fibrous-fatty plaques in rabbits. Circ Res 1978;42:562-71.
46. Caraglia M, Santini D, Marra M, Vincenzi B, Tonini G, Budillon A. A Emerging anticancer molecular mechanisms of aminobisphosphonates. Endocr Relat Cancer 2006;13:7-26.
47. Coleman RE, Major P, Lipton A, Brown JE, Lee KA, Smith M, et al. Predictive value of bone resorption and formation markers in cancer patients with bone metastases receiving the bisphosphonate zoledronic acid. J Clin Oncol 2005;23:4925-35.
48. Tarassoff P, Csermak K. Avascular necrosis of the jaws: risk factors in metastatic cancer patients. J Oral Maxillofac Surg 2003;61:1238-9.
49. Starck WJ, Epker BN. Failure of osseointegrated dental implants after diphosphonate therapy for osteoporosis: a case report. Int J Oral Maxillofac Implants 1995;10:74-8.
50. Novartis Pharmaceuticals Co. Updated safety: possible relationship of Aredia (pamidronate disodium) and/or Zometa (zoledronic acid) with osteonecrosis of the jaw [letter to health care professionals]. Ottawa: Health Canada; Nov 2004. Available at: www.hc-sc.gc.ca/hpfb-dgpsa/tpd-dpt/aredia_zometa_hpc_e.html. (accessed May 26, 2005).

51. Zervas K, Verrou E, Teleioudis Z, Vahtsevanos K, Banti A, Mihou D, et al. Incidence, risk factors and management of osteonecrosis of the jaw in patients with multiple myeloma: a single-centre experience in 303 patients. Br J Haematol 2006;134:620-3.
52. Bamias A, Kastritis E, Bamia C, Moulopoulos LA, Melakopoulos I, Bozas G, et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. J Clin Oncol 2005;23:8580-7.
53. Badros A, Weikel D, Salama A, Goloubeva O, Schneider A, Rapoport A, et al. Osteonecrosis of the jaw in multiple myeloma patients: clinical features and risk factors. J Clin Oncol 2006; 24:945-52.
54. Larsson A. The short-term effects of high doses of ethylene-1-hydroxy-1,1-diphosphonates upon early dentin formation. Calcif Tissue Res 1974;16:109-27.
55. Weile V, Josephsen K, Fejerskov O. Effects of single doses of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) on the mineralizing front of rat incisor enamel: a microradiographic and scanning electron microscopic study. Arch Oral Biol 1990;35:857-67.
56. Alatli I, Hammarström L. Root surface defects in rat molar induced by 1-hydroxyethylidene-1,1-bisphosphonate. Acta Odontol Scand 1996;54:59-65.
57. Flora L, Hassing GS, Cloyd GG, Bevan JA, Parfitt AM, Villanueva AR. Comparative skeletal effects of two diphosphonates in dogs. Metab Bone Dis Rel Res 1980;2:389-407.
58. Hillner BE, Ingle JN, Chlebowski RT, Gralow J, Yee GC, Janjan NA, et al. American Society of Clinical Oncology 2003 update on the role of bisphosphonates and bone health issues in women with breast cancer. J Clin Oncol 2003;21:4042-57.
59. Guarneri V, Donati S, Nicolini M, Giovannelli S, D'Amico R, Conte PF. Renal safety and efficacy of i.v. bisphosphonates in patients with skeletal metastases treated for up to 10 Years. Oncologist 2005;10:842-8.
60. Legge T. Industrial maladies. London: Oxford University Press; 1934.
61. Phosphorus necrosis under control [Editor's Note]. Br Dent J 1944;76:343.
62. Zigic TM, Marcous C, Hungerford DS, Dansereau JV, Stevens MB. Corticosteroid therapy associated with ischemic necrosis of bone in systemic lupus erythematosis. Am J Med 1985;79:596.
63. Storm T, Steiniche T, Thamsborg G, Melsen F. Changes in bone histomorphometry after long-term treatment with intermittent, cyclic etidronate for postmenopausal osteoporosis. J Bone Miner Res 1993;8:199-208.
64. Balena R, Toolan BC, Shea M, Markatos A, Myers ER, Lee SC, et al. The effects of 2-year treatment with the aminobisphosphonate alendronate on bone metabolism, bone histomorphometry, and bone strength in ovariectomized nonhuman primates. J Clin Invest 1993;92:2577-86.
65. Damato K, Gralow J, Hoff A, Huryn J, Marx RE, Ruggiero S, et al. Available at: http://www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4095B2_02_12-Novartis-Zometa-App-11.pdf. (Accessed May 4, 2005).
66. AAOMS Position Paper on Bisphosphonate-Related Osteonecrosis of the Jaws. Available at: http://aaoms.org/docs/position_papers/osteonecrosis.pdf. (Accessed October 5, 2006).
67. Coxon FP, Helfrich MH, Larijani B, Muzylak M, Dunford JE, Marshall D, et al. Identification of a novel phosphonocarboxylate inhibitor of Rab geranylgeranyl transferase that specifically prevents Rab prenylation in osteoclasts and macrophages. J Biol Chem 2001;276:48213-22.
68. Caraglia M, Addeo R, Costanzo R, Montella L, Faiola V, Marra M, et al. Phase II study of temozolomide plus pegylated liposomal doxorubicin in the treatment of brain metastases from solid tumours. Cancer Chemother Pharmacol 2005;12:1-6.
69. McClung M, Lewiecki EM, Cohen SB, Bolognese MA, Woodson GC, Moffett AH, et al. Denosumab in postmenopausal women with low bone mineral density. N Engl J Med 2006;354:821-31.
70. Body JJ, Facon T, Coleman RE, Lipton A, Geurs F, Fan M, et al. A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. Clin Cancer Res 2006;12:1221-8.

Reprint requests:

Rajesh Gutta, BDS
#419 School of Dentistry Building
1919, 7th Avenue South
Birmingham, AL 35294
rajeshg@uab.edu