

Historical perspectives on the clinical development of bisphosphonates in the treatment of bone diseases

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Abstract

Bisphosphonates (formerly termed diphosphonates) were first synthesized in the late 1800s; however, their clinical use has been relatively recent. The bisphosphonates' affinity for hydroxyapatite crystal surface led Procter & Gamble to test these compounds in dental, then medical applications. With key input from university researchers, this led to the medical use of the first bisphosphonate, etidronate disodium in 1968 to treat a young patient with myositis ossificans progressiva. Further clinical research led to widespread medical application for the bisphosphonate class including use as a diagnostic in radionuclide bone imaging agents, treatment of osteoporosis, Paget's disease of bone, hypercalcemia of malignancy and metastatic bone disease. The historical development of bisphosphonates provides an excellent example of how observations and knowledge obtained at the basic science level were applied and successfully tested in the clinic. The end result of these efforts has provided health care professionals with diagnostic and therapeutic tools to improve the lives of patients.

Keywords: Bisphosphonates, Pyrophosphate, Hydroxyapatite, Etidronate, Osteoporosis

Introduction

Today, bisphosphonates are a widely utilized class of compounds for the treatment of a variety of bone diseases. While medicinal use has been relatively recent, bisphosphonates were first synthesized over a century ago. The purpose of this paper is to provide historical perspectives on the early development of the bisphosphonate class highlighting their contribution to the diagnosis and management of bone diseases.

One of the first geminal bisphosphonates¹ reported to be synthesized was 1-hydroxy-1, 1-ethylidene bisphosphonate disodium salt in 1897 by Von Baeyer and Hoffmann. However, commercial application for these compounds did not take place until 1960 when Blazer and Worms² reported their use

for detergent solutions as complexing agents for calcium and magnesium. In the early 1960s, prior to acquiring a bisphosphonate, Procter & Gamble (P&G) was investigating the mechanism of action of fluoride on enamel and dentin as a therapeutic approach to preventing dental caries. Scientists at P&G discovered that a number of different compounds could radically change the action of acidified fluoride on highly polished enamel surfaces. These compounds changed the action of fluoride from destructive etching of the enamel and deposition of large calcium fluoride (CaF₂) crystals on the surface to an ultra thin closely packed layer (1 to 5 nm) of amorphous CaF₂³. This crystal growth modification resulted in blocked diffusion of calcium and phosphate from the enamel surface and prevented etching. The thin, cohesive layer of CaF₂ completely protected the enamel surface while still providing anticaries⁴ effectiveness. Compounds such as the quaternary ammonium fluoride salts and polyphosphates, including pyrophosphate, were very effective in inhibiting the crystal growth of the surface CaF₂. This phenomenon was also observed with heavy metal salts such as Sn(II) and In(III) fluorides³, where the surface compounds formed were ultra thin layers of heavy metal phosphates protecting the surface from etch damage.

Concurrent with this research, P&G was investigating calcium chelating agents for possible efficacy in removing dental cal-

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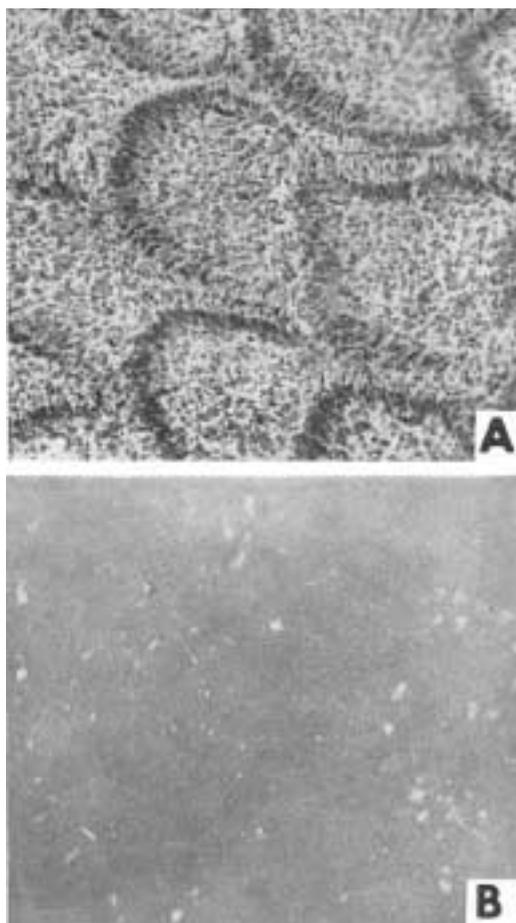


Figure 1(A and B). Highly polished human dental enamel exposed to two different calcium chelators each at 1×10^{-3} M; ethylenediamine tetraacetic acid (1A) or 1-hydroxyethylidene -1,1-bisphosphonic acid (1B) both adjusted to pH 4.6 with NaOH. Treatment time was 5 minutes, electron microscopy by metal replica, $\times 4300$. Severe damage to the surface of enamel occurred showing rod ends exposed (1A) but the surface of enamel was smooth and undamaged (1B) with the bisphosphonate. (Reprinted with kind permission of Springer Science and Business Media⁵).

culus. While these agents were found to be effective in removing calculus, they chelated enamel calcium⁵ causing surface etch damage (Figure 1A). It was at this point, about 1964, that P&G received 1-hydroxy-1,1-ethylidene bisphosphonate disodium salt (EHDP, etidronate) from Henkel Corporation, Germany as a potential builder for detergency, to chelate calcium and magnesium in the water blocking redeposition of soil in the wash. Because P&G had a major program to examine calculus removing agents, solutions of this material were tested on polished dental enamel. Surprisingly, no damage to the highly polished surface was detected⁵ as being imparted by this extremely effective chelator of calcium (Figure 1B). With this observation we began an extensive research program on the physical chemical and biological properties of the bisphosphonates.

Substance	Calcium in aorta mg/mg dry wt. (n)
Control 1	$5.4 \pm 2.6(26)^*$
Control 2 (Vitamin D ₃)	$68.3 \pm 7.6(51)$
	Subcutaneous administration
$\text{CH}_3\text{C}(\text{OH})(\text{PO}_3\text{HNa})_2$	$2.2 \pm 0.4(9)^*$
$\text{H}_2\text{C}(\text{PO}_3\text{HNa})_2$	$2.5 \pm 1.1(13)^*$
$\text{CH}_3(\text{CH}_2)_4\text{PO}_3\text{HNa}$	$79.1 \pm 25.3(9)$
	Oral administration
$\text{CH}_3\text{C}(\text{OH})(\text{PO}_3\text{HNa})_2$	$2.7 \pm 0.6(6)^*$
$\text{H}_2\text{C}(\text{PO}_3\text{HNa})_2$	$3.8 \pm 1.5(17)^*$
$\text{CH}_3(\text{CH}_2)_4\text{PO}_3\text{HNa}$	$64.0 \pm 16.5(8)$
*significant vs. Control 2; $p < 0.001$	

Table 1. The influence of 1-hydroxy-1,1-ethylidene bisphosphonate, methylenediphosphonate, and n-pentane-1-phosphonate given subcutaneously or orally (10 mg P/kg) on aortic calcification in rats induced by 75,000 I.U. Vitamin D₃/kg. Control 1, no Vitamin D₃; Control 2, Vitamin D₃, only; all other rats were treated with Vitamin D₃ and phosphonates. When given subcutaneously or orally the two bisphosphonates but not the monophosphonate completely prevented the aortic calcification. (Reprinted with kind permission of The American Association for the Advancement of Science¹⁵).

Chemical and biochemical attributes of the bisphosphonates

The central backbone of all the geminal bisphosphonates and pyrophosphate is a "planar W" configuration. Bisphosphonates are capable of both bidentate and tridentate binding to calcium (Figures 2A and B). Of these two types of binding, the tridentate bond is stronger and is especially facilitated through the hydroxy group attached to the central carbon atom. It was observed also that the bisphosphonates were hydrolytically stable⁶ as opposed to the polyphosphates⁷ that are labile in solution and quickly lose their ability to block crystal growth.

In biological systems the bisphosphonates chemisorb to the calcium of hydroxyapatite in bones, teeth and soft tissue calcifications. The bisphosphonates form an ultra thin layer on bone surfaces as shown in the schematic (Figure 3). This surface adsorption on bone is shown in the microautoradiographic thin sections⁸ of a spicule of bone (Figure 4) from a rabbit administered parenterally with tritiated 1-hydroxyethylidene bisphosphonate. Note that even where the bone surface in the spicule is covered with osteoid, the deposit of the bisphosphonate is below the osteoid and on the surface of the apatite spicule. This is to be expected because the concentration gradient of bisphosphonate is high in the extracellular fluid and approaching zero at the inorganic surface of the bone due to the stronger adsorption of the bisphosphonate on the inorgan-

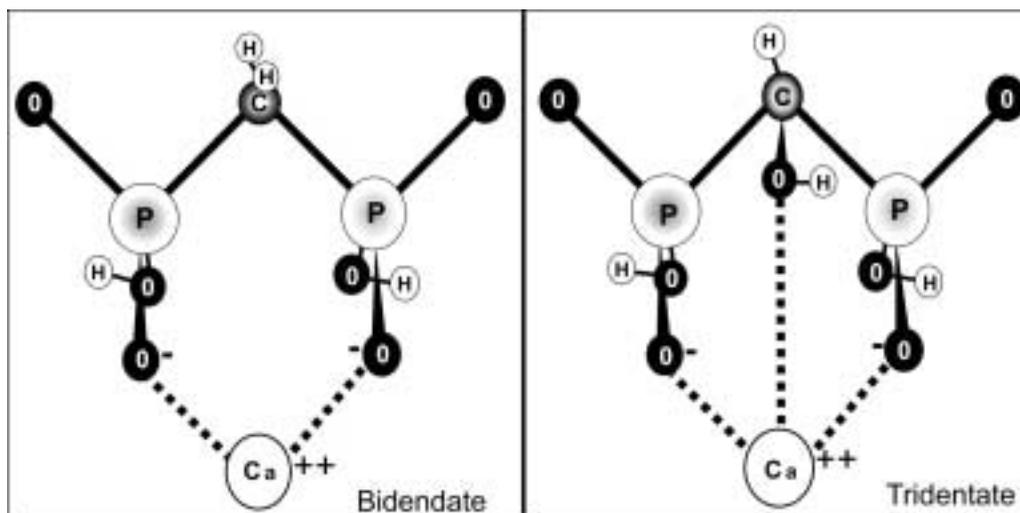


Figure 2(A and B). The fundamental planar "W" (O-P-C-P-O) as evident in both 2(A and B) and providing bidentate binding to calcium with methylidene bisphosphonate (2A) and the stronger tridentate binding through the hydroxy group of hydroxymethylidene bisphosphonate (2B).

ic hydroxyapatite than on the organic osteoid. The quantitative adsorption ratio⁹ between hydroxyapatite and osteoid is 40:1.

The dissolution rate of pure hydroxyapatite crystals is significantly slowed after treatment with bisphosphonate *in vitro*, in tissue culture, and *in vivo*^{7,10}. Another characteristic of the bisphosphonates is their ability to block crystal growth of forming hydroxyapatite^{6,11} from solutions of calcium and orthophosphate (Figures 5A,B,C). Note in Figure 5B that the consumption of base (NaOH) is blocked by the inability to grow crystals of hydroxyapatite in the presence of the adsorbing bisphosphonate (EHDP) on the surface of the forming nuclei. Even after long periods of time the calcium phosphate precipitate in the presence of the bisphosphonate does not give a crystal pattern of hydroxyapatite⁶ (it is amorphous). The monophosphonate n-pentane-1-phosphonate (PMP) does not block crystal growth (Figure 5C). The application of this property of the bisphosphonates in dental health is their ability to block the formation of calculus. When the bisphosphonate is administered either in the calculus forming diet or topically applied to the teeth of the animals, supragingival calculus (hydroxyapatite) is inhibited¹² from forming on the teeth of the animals. The most obvious principle involved in this *in vivo* blocking of the deposit of crystalline calculus, is that the particle size of the deposit is so small, that surface free energy is consequently very high and redissolution occurs readily according to the principle of the Kelvin effect^{13,14}.

Interactions between Procter and Gamble and the University of Berne

In the early stages of our physical-chemical research on bisphosphonates, M. D. Francis attended The International

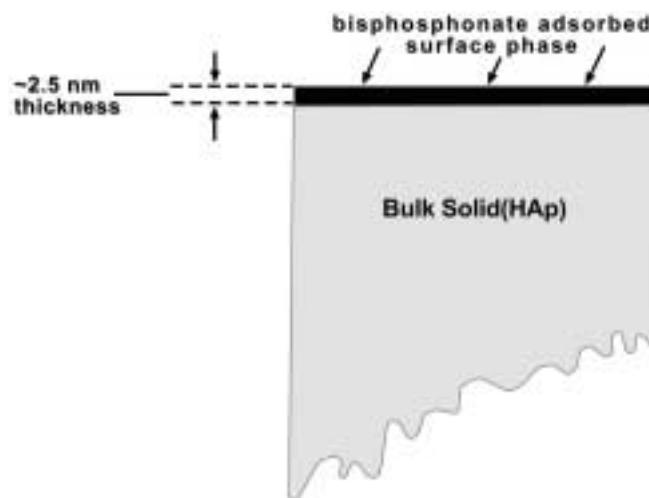


Figure 3. A schematic presentation of the ultra thin layer (~2.5 nm) of a bisphosphonate (black) adsorbed on the surface of the bulk solid hydroxyapatite (HAp).

Conference on Dental Research in Miami and met with the late Professor James Irving who was on sabbatical at the the Davos Research Institute in Switzerland with Professor Herbert Fleisch, who had recently been working with the late Professor William Neuman at the University of Rochester. Information was exchanged regarding the complete blocking effect of polyphosphates on crystal growth of hydroxyapaite. These data included electron microscopy and diffraction studies generated by P&G and the blocking effect of these compounds on soft tissue calcification of small animals from

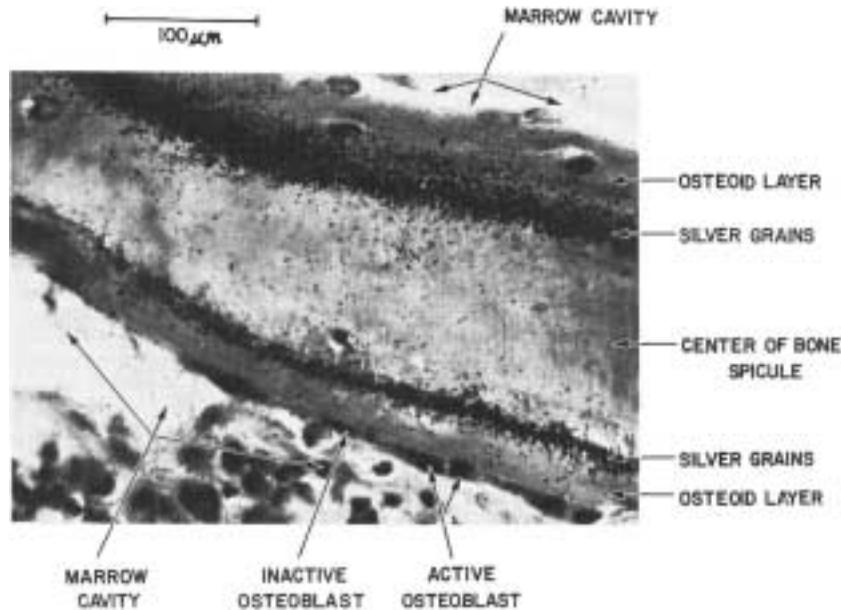


Figure 4. A microautoradiograph of a spicule of bone surrounded by marrow 24 hours after administration of a single dose of ^3H -1-hydroxy-1-1-ethylidene bisphosphonate. The silver grains (black) are on the surface of the hydroxyapatite of the bone below the lamellar osteoid layer of the active bone spicule. (Reprinted with kind permission of Elsevier⁸).

studies at the Davos laboratories. Both parties at that time recognized the hydrolytic instability of the polyphosphates.

Subsequently, Professor Fleisch was invited to P&G's Miami Valley Laboratories in Cincinnati to discuss mutual interests and research capabilities. Professor Fleisch was provided with a number of P&G's synthesized bisphosphonates and co-operation between the two laboratories on a number of biological and physical-chemical experiments were explored between the two research institutes. Together with Professor Graham Russell, who had joined the Davos laboratory in 1964 as a research fellow, Professor Fleisch conducted studies showing the *in vivo* inhibitory effect of two geminal bisphosphonates on ectopic calcification of aortic tissue in hypervitaminosis D rats (Table 1) which was published¹⁵ as a landmark paper in *Science* in 1969. The two laboratories continued to explore the biological and toxicological properties of a number of structurally different bisphosphonates.

A key observation was that the bisphosphonates blocked the dissolution of hydroxyapatite crystals which led to the prediction that they might retard bone resorption. This was subsequently demonstrated in organ cultures and various experimental models *in vivo* leading to a second seminal paper in *Science*⁷.

In 1967, Dr. Andrew Bassett, who was familiar with Professor Fleisch's work, contacted him and explained that he had a 16-month-old female patient with myositis ossificans progressiva (MOP), a condition involving calcification of soft tissue. Muscles in her chest were calcifying and her condition became critical due to respiratory insufficiency. Professor Fleisch indicated that the polyphosphates that he had worked

with would not be effective in humans because of the rapidity of hydrolysis of the polyphosphates by alkaline phosphatases. He suggested that Dr. Bassett contact Dr. Francis to discuss the new compounds being explored at P&G. Dr. Bassett made this contact and was immediately invited to P&G laboratories and made aware of all the information the company had on the geminal bisphosphonates. Dr. Francis wrote a short memo on the nature of the child's pathology and the potential inhibitory action on the heterotopic calcification of the MOP. He requested P&G upper management approve the use of etidronate to block the advancement of the calcification. Within 36 hours, approval was granted and Dr. Bassett was advised. At that time, P&G was not a pharmaceutical company so Dr. Bassett obtained an Investigative New Drug Application from the Food and Drug Administration. Dr. Francis formulated the drug in an appropriate formulation to facilitate the child's ability to take the drug easily and to insure its retained activity. The girl was treated orally at 10 mg/kg daily with etidronate. Dr. Bassett called Dr. Francis after her third daily treatment and indicated that her inflammatory ectopic lesions were decreasing. She was given etidronate intermittently for years as needed to treat her periodic exacerbations; her condition continued to be controlled.

The clinical development of bisphosphonates

The treatment of the child with MOP was the first human use of a geminal bisphosphonate, etidronate¹⁶ in medicine. At a subsequent meeting in the Davos laboratory, Drs.

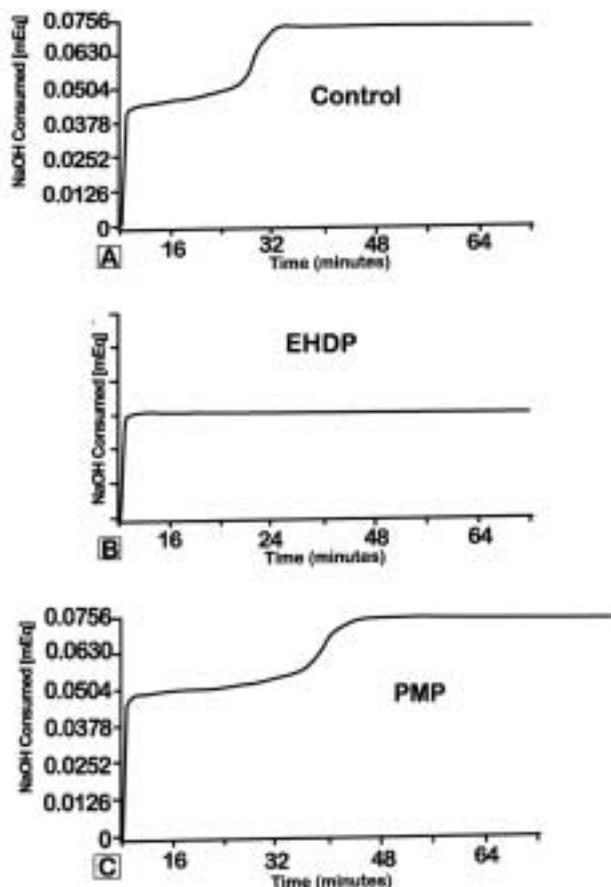


Figure 5. Rate of formation of hydroxyapatite (HAp) using pH Stat titrations of NaOH consumed (ordinate) versus time (abscissa) at constant pH 7.4 and calcium chloride and sodium phosphate initially at 4×10^{-3} M and no inhibitor, producing the biphasic graph (control). Initial calcium and phosphate as in the control but 2×10^{-4} M inhibitor present and inhibitors were 1-hydroxy-1,1-ethylidene bisphosphonate (EHDP) and n-pentane-1-phosphonate (PMP). Crystal growth was inhibited by the EHDP as evidenced by the monophasic curve and decreased base consumption. The monophosphonate (PMP) did not inhibit crystal growth and produced a similar biphasic curve to the control. (Reprinted with kind permission of CRC Press Inc.¹¹).

Francis, Russell and Fleisch discussed the possible extensions of the applications of the bisphosphonates. Dr. Russell suggested that etidronate might be effective in Paget's disease of bone, in which abnormally accelerated bone turnover (both resorption and formation) results in weak and misshapened bone. Together with Dr. Roger Smith at the Nuffield Orthopaedic Center in Oxford, they conducted a small human clinical study in patients with Paget's disease of bone using biochemical indices of bone turnover including serum alkaline phosphatase and urinary hydroxyproline as the defining parameters for reduction of the pagetic syndrome. This study was the first evidence of the effectiveness of the bisphosphonates for treatment of Paget's disease¹⁷ of



Figure 6. Example of a Tc-99m MDP bone scintigram. There is an intense localization of the radiopharmaceutical in areas of elevated bone turnover.

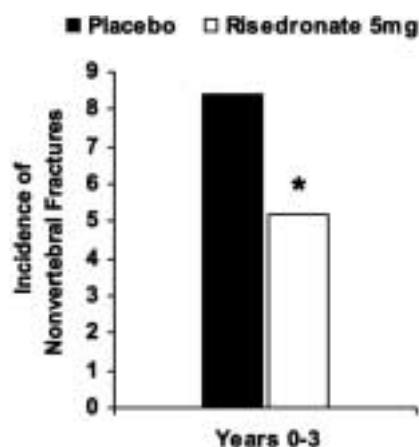


Figure 7. Effect of risedronate treatment on the incidence of non-vertebral fractures over 3 years in women with postmenopausal osteoporosis. Treatment resulted in a significant 39% reduction of risk in non-vertebral fractures. (Significant compared with placebo, $p=0.02$). (Reprinted with kind permission of The Journal of the American Medical Association²³).

bone and this class of compounds is now considered standard treatment. This early work and subsequent larger studies conducted in the 1970-80s led to regulatory agency approvals for etidronate (Didronel®) for treatment of Paget's disease of bone¹⁸, prevention and treatment of heterotopic ossification due to total hip replacement or spinal cord injury¹⁹ as well as the intravenous form for the treatment of hypercalcemia of malignancy²⁰.

In addition, Dr. Andrew Tofe and Dr. Francis discovered and developed the use of etidronate disodium and other bisphosphonates such as methylenehydroxy bisphosphonate, to bind reduced 140 keV technetium-99m, parenterally administered, for bone scanning. The bi and tridentate binding capacity of the bisphosphonates²¹ resulted in the ability to detect abnormal metabolic activity of bones, such as, in Paget's disease of bone, bone metastases and in detecting soft tissue calcifications (Figure 6).

In the mid-1980s, a clinical program was undertaken at P&G to determine the efficacy and safety of a cyclical regimen of etidronate in the prevention and treatment of postmenopausal osteoporosis. These early studies demonstrated that etidronate significantly increased bone mineral density and reduced the rate of vertebral fractures in patients who were at high risk for fracture²². This led the company to develop risedronate sodium (Actonel®), a highly potent pyridinyl bisphosphonate. Clinical studies in postmenopausal osteoporotic women have established its long-term efficacy and safety profile in the prevention of vertebral and non-vertebral osteoporotic fractures²³ (Figure 7). The efficacy and safety of risedronate sodium has also been established in osteoporotic men²⁴ as well as in patients receiving glucocorticoids²⁵.

Subsequent to the discovery and early development of bisphosphonates by P&G, there have been numerous investigative studies with other differently-structured geminal bisphosphonates. The more commonly available compounds include: alendronate, clodronate, ibandronate, pamidronate, and zoledronate. The investigation of these compounds has led to other clinical uses, most notably in the treatment of multiple myeloma and bone metastases from solid tumors²⁶ in conjunction with standard anti-neoplastic therapy.

Summary

The bisphosphonates have provided significant medical breakthroughs in the treatment of bone diseases. The mechanism of action, coupled with its unique bone-specific targeting, have led to the widespread clinical use of the bisphosphonates. The historical development of bisphosphonates provides an excellent example of how observations and knowledge obtained at the basic science level have been applied and successfully tested in the clinic. The end result of these efforts has been to provide health care professionals with diagnostic and therapeutic tools to improve the lives of patients.

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