Review

Bisphosphonates: The first 40 years

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ABSTRACT

The first full publications on the biological effects of the diphosphonates, later renamed bisphosphonates, appeared in 1969, so it is timely after 40 years to review the history of their development and their impact on clinical medicine.

This special issue of BONE contains a series of review articles covering the basic science and clinical aspects of these drugs, written by some of many scientists who have participated in the advances made in this field.

The discovery and development of the bisphosphonates (BPs) as a major class of drugs for the treatment of bone diseases has been a fascinating story, and is a paradigm of a successful journey from 'bench to bedside'. Bisphosphonates are chemically stable analogues of inorganic pyrophosphate (PPi), and it was studies on the role of PPi as the body's natural 'water softener' in the control of soft tissue and skeletal mineralisation that led to the need to find inhibitors of calcification that would resist hydrolysis by alkaline phosphatase.

The observation that PPi and BPs could not only retard the growth but also the dissolution of hydroxyapatite crystals prompted studies on their ability to inhibit bone resorption. Although PPi was unable to do this, BPs turned out to be remarkably effective inhibitors of bone resorption, both in vitro and in vivo experimental systems, and eventually in humans.

As ever more potent BPs were synthesised and studied, it became apparent that physico-chemical effects were insufficient to explain their biological effects, and that cellular actions must be involved. Despite many attempts, it was not until the 1990s that their biochemical actions were elucidated.

It is now clear that bisphosphonates inhibit bone resorption by being selectively taken up and adsorbed to mineral surfaces in bone, where they interfere with the action of the bone-resorbing osteoclasts. Bisphosphonates are internalised by osteoclasts and interfere with specific biochemical processes. Bisphosphonates can be classified into at least two groups with different molecular modes of action. The simpler non-nitrogen containing bisphosphonates (such as etidronate and clodronate) can be metabolically incorporated into non-hydrolysable analogues of ATP, which interfere with ATP-dependent intracellular pathways. The more potent, nitrogen-containing bisphosphonates (including pamidronate, alendronate, risedronate, ibandronate and zoledronate) are not metabolised in this way but inhibit key enzymes of the mevalonate/cholesterol biosynthetic pathway. The major enzyme target for bisphosphonates is farnesyl pyrophosphate synthase (FPPS), and the crystal structure elucidated for this enzyme reveals how BPs bind to and inhibit at the active site via their critical N atoms. Inhibition of FPPS prevents the biosynthesis of isoprenoid compounds (notably farnesol and geranylgeraniol) that are required for the post-translational prenylation of small GTP-binding proteins (which are also GTPases) such as rab, rho and rac, which are essential for intracellular signalling events within osteoclasts. The accumulation of the upstream metabolite, isopentenyl pyrophosphate (IPP), as a result of inhibition of FPPS may be responsible for immunomodulatory effects on gamma delta ($\gamma\delta$) T cells, and can also lead to production of another ATP metabolite called ApppI, which has intracellular actions. Effects on other cellular targets, such as osteocytes, may also be important.

Over the years many hundreds of BPs have been made, and more than a dozen have been studied in man. As reviewed elsewhere in this issue, bisphosphonates are established as the treatments of choice for various diseases of excessive bone resorption, including Paget's disease of bone, the skeletal complications of malignancy, and osteoporosis. Several of the leading BPs have achieved 'block-buster' status with annual sales in excess of a billion dollars.

As a class, BPs share properties in common. However, as with other classes of drugs, there are obvious chemical, biochemical, and pharmacological differences among the various BPs. Each BP has a unique profile

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in terms of mineral binding and cellular effects that may help to explain potential clinical differences among the BPs.

Even though many of the well-established BPs have come or are coming to the end of their patent life, their use as cheaper generic drugs is likely to continue for many years to come. Furthermore in many areas, e.g. in cancer therapy, the way they are used is not yet optimised. New 'designer' BPs continue to be made, and there are several interesting potential applications in other areas of medicine, with unmet medical needs still to be fulfilled.

The adventure that began in Davos more than 40 years ago is not yet over.

This article is part of a Special Issue entitled Bisphosphonates.

Contents

Introduction

All the bisphosphonates (BPs) currently in use as drugs in clinical medicine possess two P–C bonds, linked through a single carbon to give a geminal bisphosphonate with the core structure made up of P–C–P bonds. They are chemically stable analogues of pyrophosphate compounds, which are found widely in nature. The simplest of the naturally occurring pyrophosphates is inorganic pyrophosphate (PPi), and it was the discovery that this compound circulates in the body as an endogenous 'water softener' that led on to the work with bisphosphonates.

Chemically the bisphosphonates were first synthesised in the 1800s [1], but it is only in the past 40 years that they have been used to treat disorders of calcium metabolism. Even etidronate, which was the first bisphosphonate to be used in humans, was originally synthesised over 100 years ago [2].

The early uses of bisphosphonates were mainly as corrosion inhibitors, also as complexing agents in the textile, fertiliser and oil industries, as well as for many other industrial processes [3]. Their use as 'water softeners' was based on their ability to act as sequestering agents for calcium, and in particular their ability to inhibit calcium carbonate precipitation, as do polyphosphates. This has been applied in the prevention of scaling in domestic and industrial water installations.

A recent search in PubMed under the term 'bisphosphonates' revealed over 19,000 publications, and even this large list this does not cite abstracts, nor all publications and the many books and review articles available that describe the chemistry, pharmacology, and clinical applications of bisphosphonates [4–12].

The discovery of the biological effects of the BPs has its origin in studies of calcification mechanisms and the role of pyrophosphate. It is instructive to trace the steps by which this came about. This review will focus on the historical aspects, and on topics not covered elsewhere in this issue, including aspects of pharmacology, and the inter-relationship between BPs and pyrophosphate metabolism, bearing in mind that disturbances in pyrophosphate metabolism have an important role in several diseases.

How studies on calcification mechanisms and the role of pyrophosphate led to the discovery of the bisphosphonates

The beginning of this story can be traced back to 1962, when Herbert Fleisch spent a postdoctoral year at the University of Rochester with Bill Neuman. W F Neuman (1919–1981)[1] headed the biochemistry section in the Department of Radiation Biology in conjunction with the U.S. Atomic Energy Commission at the university, and with his wife, Margie, had published their landmark book entitled *The Chemical Dynamics of Bone Mineral* in 1958 [13].

In those days studies of bone were dominated by the evolving understanding of the biochemistry of the constituents of bone matrix, and the physical chemistry of bone mineral, in contrast to today's emphasis on genetics and bone cell biology.

The Neuman laboratory had been established to study the effects of radioisotopes in bone in the aftermath of the use of atomic weapons in the second-world war. The prevention of skeletal uptake of hazardous bone-seeking isotopes, such as uranium, radium, and strontium, was a research priority, and the study of calcification mechanisms was part of this endeavour.

Herbert Fleisch had recently graduated in medicine from the University of Lausanne where his father was Professor of Physiology. He had plans to become an orthopaedic surgeon, but his time with the Neumans was to change that forever, much to the benefit of the field of bone research. However Herbert retained close contact and collaboration with the orthopaedic community throughout his career, and Davos was the venue for the AO training courses for many years.

The key observation made by Neuman and Fleisch was that body fluids were super-saturated with respect to calcium phosphate and that the addition of collagen could act as a nucleating agent for the deposition of hydroxyapatite crystals in vitro [14]. They reasoned that

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[1] The William F. Neuman Award is still presented annually by the American Society for Bone and Mineral Research for "outstanding and major scientific research" in bone and mineral research.

Fig. 1. (a & b) The biological effects of bisphosphonates were first studied in Davos, in the Swiss Medical Research Institute (Schweizerisches Forschingsinstut), shown in upper right panel. In Fig. 1b the research labs were on the top floor. Fig. 1c, this group picture from 1967 shows Herbert Fleisch (centre facing), Sylvia Bisaz (top left) and Roman Muhlbauer (bottom left).

Fig. 2. Group picture from the bisphosphonate meeting held at the Royal College of Physicians in London in 1990, showing key players from that period.

because calcification could be induced by collagen, which was present in many tissues in the body, all tissues should calcify were it not for the presence on inhibitors of calcification in body fluids. They went on to show that body fluids such as plasma and urine did indeed contain inhibitors of calcification. Since it had been known since the 1930s that trace amounts of polyphosphates were capable of acting as water softeners by inhibiting the crystallisation of calcium salts, such as calcium carbonate, they postulated that compounds of this type might be natural regulators of calcification under physiological conditions, and showed that urine contained a phosphatase-labile inhibitor.

After returning to Lausanne, Herbert Fleisch teamed up with Sylvia Bisaz, whose skillful chemistry input enabled them to show unequivocally that the inhibitor in urine was inorganic pyrophosphate (PPi) [15]. In 1964, he was appointed as Director of the Laboratory for Experimental Surgery, in the Schweizerisches Forschungsinstitut, in Davos. This was enabled by Martin Allgower, a surgeon with a particular interest in orthopaedics, then in Chur, and later Professor of Surgery at the University of Basel. The institute in Davos was renowned not only for its pioneering work on orthopaedic implants, but also as a research centre for TB and immunology. Jean Borel, who later went on to discover cyclosporine, was a member of the immunology group at that time (Fig. 1).

I first visited the Davos laboratory in 1964 and joined the Fleisch/Bisaz team full time in 1965 after completing my PhD in the UK. Thus began a lifelong friendship and collaboration. After graduating in Biochemistry from Cambridge, I had joined the MRC unit in Leeds, where I was studying kidney stone formation and other disorders of calcification. It seemed possible that some of these pathologic disorders might be linked to disturbances in PPi metabolism [16]. Prominent among these was the rare and intriguing inherited disorder, hypophosphatasia, in which lack of alkaline phosphatase is associated with mineralisation defects of the skeleton. Thanks to generous help from Professor Charles Dent at University College Hospital in London, I was able to study several children with hypophosphatasia, and showed that PPi levels were elevated in urine [17], thereby indicating that alkaline phosphatase was probably the key extracellular enzyme responsible for hydrolysing pyrophosphate. With the further development of highly specific and sensitive methods for measuring PPi, we later showed elevated concentrations of PPi in plasma [18], further supporting the notion that the activity of alkaline phosphatase regulates circulating amounts of PPi to below the critical levels that would otherwise prevent normal physiological calcification processes. We showed that alkaline phosphatase could indeed act as a pyrophosphatase, with a very low Km at physiological pH, meaning that in the presence of sufficient enzyme and at physiological levels of phosphate, the enzyme could effectively eliminate PPi completely.

Taken together with later studies, the work helped to establish the concept that pyrophosphate (PPi) is the body's own 'water softener' that normally prevents calcification of soft tissues, and regulates bone mineralisation [19]. Inorganic pyrophosphate is a known by-product of many biosynthetic reactions in the body, and it can be calculated that only a tiny fraction of the PPi generated within cells reaches the extracellular compartment, where its turnover is also rapid [20].

Following the early studies that indicated that PPi was a potential endogenous regulator of mineralisation, there have been significant advances in understanding the metabolism of PPi and in identifying clinical disorders in which alterations in PPi may have a pathogenic role.

The concentrations of pyrophosphate in body fluids are probably regulated by hydrolytic enzymes, the levels being set by the balance between formation and destruction. Much of the PPi in the extracellular compartment is likely to be generated at the cell surface by the action of nucleoside triphosphate pyrophosphohydrolases (NTP-PPases), which liberate PPi from NTPs such as ATP. This may also be a mechanism by which concentrations of ATP and other NTPs are kept low in view of their ability to act as ligands for several of the known purinergic receptors on cell surfaces, including cells in bone [21,22].

The major enzyme involved in removing PPi is alkaline phosphatase (TNAP, i.e. Tissue non-specific Alkaline Phosphatase), as has been known for many years. TNAP is also located at cell surfaces and its tissue distribution is restricted particularly to liver, cartilage and bone.

A third regulator of extracellular PPi has been postulated to be a trans-membrane transporter of PPi called ANK, which is thought to extrude PPi from within cells. Its role in PPi metabolism and in regulating extracellular concentrations remains far from clear [23,24].

Genetic mutations of all three of these regulatory proteins are associated with disturbances in PPi metabolism and disordered calcification [25–27]. Skeletal mineralisation is defective when PPi is high e.g. in hypophosphatasia due to many different inactivating mutations in TNAP [28–30]. The recent use of enzyme constructs of alkaline phosphatase to treat hypophosphatasia by enzyme replacement therapy is yielding promising results [31].

Conversely excessive mineralisation and bone formation may occur when NTP-PPase (PC-1) is defective and PPi levels are low as in juvenile vascular calcification [32], and another rare condition called Ossification of the posterior longitudinal ligament (OPLL) [33] of the spine which occurs particularly in Japanese populations.

Mutations of the ANK gene in mice produce a skeletal phenotype of progressive ankylosis and aberrant calcification [34], whilst in human mutations of ANKH are associated with familial chondrocalcinosis, a condition in which calcium pyrophosphate crystals deposit in articular cartilage and other sites [35–37]. Several mutations of the ANKH gene have also been somewhat unexpectedly associated with craniometaphyseal dysplasia (CMD) [38–40]. A third phenotype associated with autosomal recessive mutations in ANKH was recently reported, comprising mental retardation, deafness, ankylosis and mild hypophosphatemia [41]. Recently a further genetic disorder associated with vascular calcification has been identified as due to mutations in the NT5E gene encoding 5′-exonuclease, CD73, which converts AMP to adenosine [42,43].

Contrary to what might be predicted, there is no evidence so far that BPs used clinically interfere with the function of these enzymes and transporters involved in the endogenous metabolism of PPi, either within or outside cells. However from what is now known about how BPs function within cells, it is evident that some simple BPs can substitute for PPi in generating ATP analogues. Furthermore, the N-BPs can displace isoprenoid-PP substrates in enzymes of the mevalonate pathway, notably FPPS.

The therapeutic possibilities opened up by this work on the role of PPi in calcification were obvious, and led to studies to determine whether one might use PPi or polyphosphates to inhibit abnormal calcification. However, 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 [44]. When given by mouth, pyrophosphate and polyphosphates were inactive, due to the hydrolysis of pyrophosphate in the gastrointestinal tract, probably by mucosal brush border phosphatases. During the search for more stable analogues of pyrophosphate that might also have the anti-mineralisation properties of pyrophosphate but that would be resistant to hydrolysis, several different chemical classes of potential analogues were studied, including P−N−P and P−C−C−P compounds. It was only when the bisphosphonates (at that time called diphosphonates) characterised by P−C−P motifs were used that success was achieved. Like pyrophosphate, bisphosphonates had high affinity for bone mineral [45], and were found to prevent the formation and aggregation of calcium phosphate crystals. 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 pathological calcification when given orally to rats in vivo [46].

In these early studies bisphosphonates were shown to not only 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 mineralisation of ectopic bone as well of normal calcified tissues such as bone and cartilage [47]. Bisphosphonates appeared to prevent calcification by physico-chemical mechanisms producing 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 towards the future use of bisphosphonates occurred when we found that bisphosphonates, like we had already shown for PPi [48], also had the novel property of being able to inhibit the dissolution of hydroxyapatite crystals [49]. This led to studies to determine whether they might also inhibit bone resorption, based on the then prevailing notion that the solubility characteristics of bone mineral might affect its rate of removal.

Dating the anniversary

The first publications appeared as abstracts [50,51] in 1968, and were followed by the two full papers in *Science* in 1969, in which the effects of two representative bisphosphonates, etidronate and clodronate, on crystal formation and dissolution, and on vascular calcification and bone resorption were described [52,53]. These early studies with bisphosphonates were the result of a very fruitful collaboration between the Davos laboratory with Dave Francis (Marion D Francis) of the Procter and Gamble Company in Cincinnati, USA. This introduction had been made in 1966 through James T Irving, who was spending a sabbatical in Davos whilst on leave from the Forsyth Dental Center in Boston. Dave Francis had been studying the physicochemical effects of etidronate on crystal growth with a view to its potential use as a toothpaste additive to combat dental plaque [54,55]. He was also a pioneer in the development of BPs for bone scintigraphy [56], using complexes with stannous ions and the short-lived gamma-emitting isotope, Tc99m.

Although the work on BPs began in 1966, it is probably appropriate to date the birth of the bisphosphonate era from 1969, since in that year the several of the first and important publications appeared.

One published in *Nature* was the first animal model of osteoporosis to be studied, using a model Herbert Fleisch had developed during his thesis studies, namely sciatic nerve section to simulate immobilisation osteoporosis. These studies were later extended to show the efficacy of BPs compared with calcitonin and polyphosphates [57]. Clodronate was subsequently shown to prevent bone loss after spinal cord injury in humans [58].

The other paper published in *Lancet* in 1969 was the first human use of a BP, etidronate, in a child with myositis ossificans progressiva, now called fibrodysplasia ossicans progressiva (FOP).

a

Bisphosphonates. Davos 2004

| Herb Fleisch | Mike Rogers | Graham Russell | Dave Francis | Hal Ebetino |

b

4th Workshop on Molecular Pharmacology & Therapeutics of Bone Disease, St Catherine's College, Oxford, 6-8 July 2009

Fig. 3. (a) Group photo from Bisphosphonate meeting in Davos, 2004. (b) Attendees at Bisphosphonate 40th anniversary meeting held during 4th Molecular Pharmacology of Bone and Therapeutics at St Catherine's College, Oxford in July 2009.
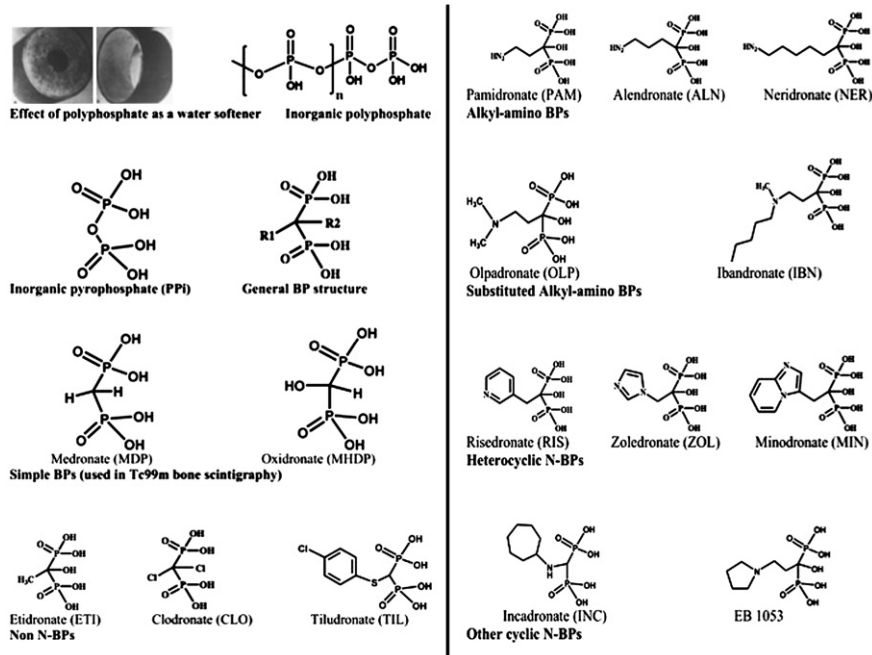
Fig. 4. Structures of polyphosphates, and the bisphosphonates that have been used for clinical applications.

Davos continued to be the venue for meetings on bisphosphonates organised by Herbert Fleisch and his colleagues every two years from the 1980s to 2006 (Figs. 2 and 3).

Bisphosphonates inhibit bone resorption in many different experimental systems, and this enabled the pharmacological development of bisphosphonates

Many studies using a variety of experimental systems showed that bisphosphonates inhibit osteoclast-mediated bone resorption, not only in organ cultures of bone in vitro, but also both in normal animals and in those with experimentally increased resorption. The first experimental model studied was in thyroparathyroidectomized rats treated with parathyroid hormone to stimulate bone resorption in vivo [49,53].

In growing intact rats, the bisphosphonates block the removal of both bone and cartilage, thus retarding the remodelling of the metaphysis, which becomes club-shaped and radiologically denser than normal [59,60]. This effect is the basis of the 'Schenk' model also used to compare the potency of new compounds, named after Robert Schenk who was Professor of Anatomy in Bern, and an outstanding colleague in many of the early studies.

The Schenk model continues to be used as a robust test to assess pharmacological potencies of bisphosphonates [61]. The inhibition of endogenous bone resorption can also be monitored by kinetic studies [62] using radio-calcium (^{45}Ca), and by using biochemical markers of bone resorption.

Bisphosphonates also suppress resorption induced by many other agents such as calcitriol, vitamin D, and retinoids. The effect on retinoid-induced hypercalcaemia has been used to develop a powerful and rapid screening assay for new compounds [63,64], and was the model used to pick ibandronate for clinical development from over 300 compounds tested [65]. Similarly Widler, Green and their colleagues used increases in plasma calcium induced by calcitriol in thyroparathyroidectomized rats to select zoledronate as the lead candidate for clinical development from their medicinal chemistry programme based initially on analogues of pamidronate [66].

The bisphosphonates are also effective in preventing bone destruction in a number of animal models of human disease. Commonly used models of osteoporosis include the prevention of bone loss associated with ovariectomy, so often used because it is a requirement of regulatory agencies. Less commonly used models involve orchidectomy, lactation, low calcium diets, or the administration of agents such as heparin or corticosteroids.

Special features of the pharmacology of bisphosphonates

As drugs bisphosphonates display a few unusual features. Their remarkable selectivity for their target organ of bone is paramount among these and accounts for much of the efficacy and safety of the drug class, as reviewed by Cremers and Papapoulos [67] in this issue. Secondly unlike many drugs, BPs are not metabolised to inactive products, and drug derivatives do not appear in urine. Intracellular conversion of some non-N-BPs to ATP derivatives does occur however, as discussed elsewhere.

Thirdly their oral bioavailability is extremely low, characteristically below 1% for many BPs, and rarely above 5% for others. Nonetheless the property of being active by mouth in early animal studies was key to their future use in man. The mechanism of intestinal absorption of BPs has been ascribed to paracellular transport. BPs are highly charged molecules, and no transporters have been identified. Absorption appears to be enhanced by EDTA, an effect attributed to calcium chelation that opens up gap junctions between intestinal mucosal cells [68].

Finally, the overall safety profile of BPs is good, but the issues of safety are much discussed and debated [69–73] as described by Pazianas and Abrahamsen in this issue [74].

BPs are known to be potentially toxic to the kidneys, which are their major route of elimination from the body, mainly via glomerular filtration, but also possibly by tubular secretion. The mechanisms underlying renal effects are quite well understood, and their impact is well reviewed by Miller in this issue [75].

After the potential clinical value of bisphosphonates had been appreciated, research efforts were devoted to the development of compounds with a more powerful antiresorptive activity, as described above. This was especially true throughout the 1980s, when the efforts

of the medicinal chemists were at their peak. An important aspect of this was to develop compounds that would not inhibit skeletal mineralisation. With compounds such as etidronate there was only a ~10 fold difference between doses that inhibit mineralisation compared with doses that reduce bone resorption. Enhancing this window was readily achieved and among the many hundreds of bisphosphonates that were synthesised, this was only rarely a problem. Indeed 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 mineralisation ceased to be a major clinical concern.

In general a good correlation was observed between potency and structure–activity relationships in vitro and in vivo [76]. Furthermore, the gradation of potency evaluated in the animal models corresponds quite well with that found in humans, although the differences in potency are much smaller in humans.

If not given in excess, bisphosphonates maintain or improve the biomechanical properties of bone both in normal animals and in experimental models of osteoporosis [77]. Many experimental and clinical studies show that BPs conserve bone architecture and strength [78–81]. However there are naturally concerns about whether the use of prolonged high doses of BPs may impair bone turnover to such an extent that bone strength is impaired [82,83]. High doses in animals have been associated with increased microdamage [84,85] and in one early study even fractures [86]. In this issue Allen and Burr provide helpful and comprehensive accounts of current knowns and un-knowns in this area [87,88].

A question often asked is whether BPs inhibit fracture repair. Early stages of fracture repair do not involve steps that one would expect BPs to interfere with. Indeed by acting on osteoclasts it might be predicted that the later stage of removal and remodelling of callus would be more likely to be affected. Over the years, many exper-imental and clinical studies [89] have largely laid this concern to rest. In a sense, this question has now come full cycle with interesting attempts to modify fracture repair in a positive direction [90,91]. In their paper in this issue of Bone, Wilkinson and Little review the data available, and explore the potential applications of BPs in other areas of orthopaedics [92]. There are many potential applications of BPs in orthopaedics which include protection against loosening of prosthe-ses [93], better osseointegration of biomaterials and implants [94–96],

improved healing in distraction osteogenesis [97], and conserving bone architecture after osteonecrosis [98,99] and in Perthes disease [100].

Since bisphosphonates accumulate in bone it is important to know what happens during long-term administration. It is an intriguing but reassuring feature of the bisphosphonates that the inhibition of bone resorption reaches a new steady-state level, rather than becoming progressively lower, even when the compounds are given continu-ously, as first shown by Reitsma et al. over 30 years ago [101]. The level of suppression depends on the administered dose, and this plateau has also been observed repeatedly in human studies [102].

From a clinical point of view this is an extremely important property, since there is no apparent progression of the anti-resorptive effect with time. This suggests that the bisphosphonate buried in the bone is inactive at least as long as it remains buried there. These properties also suggest that, within the therapeutic dosage range, there is little risk of a continuous decrease in bone turnover in the long run, that might lead to an increase in bone fragility. An additional important pharmacological property of BPs is that the total dose administered is a major determinant of their effects. This has been well studied for ibandronate [103] and zoledronate [104]. In both cases the same inhibition of bone resorption has been documented whether the BP is given in small frequent (e.g. daily) doses compared with larger doses given less frequently. This has been the basis for the development of intermittent dosing regimens in man.

BPs can have very long-lasting effects in reducing bone turnover, which seem to be greater and more prolonged with some BPs (e.g. alendronate and zoledronate) compared with others (e.g. etidronate and risedronate). These differences in retention and persistence of effect observed in animal and clinical studies may be related to ob-served differences among BPs in binding to hydroxyapatite. In ex-plaining the long duration of action, it has been proposed that there is continually recycling of BPs off and back onto the bone surfaces. This notion is supported by observations that BPs can be found in plasma and urine many months after dosing.

Another distinct feature of BPs is the well-recognised side effect of the nitrogen-containing bisphosphonates to cause an 'acute phase' response in vivo. This can lead to induction of fever and 'flu'-like symptoms in patients. These effects, now sometimes called 'post-dose' symptoms, are transient and occur predominantly on first exposure to
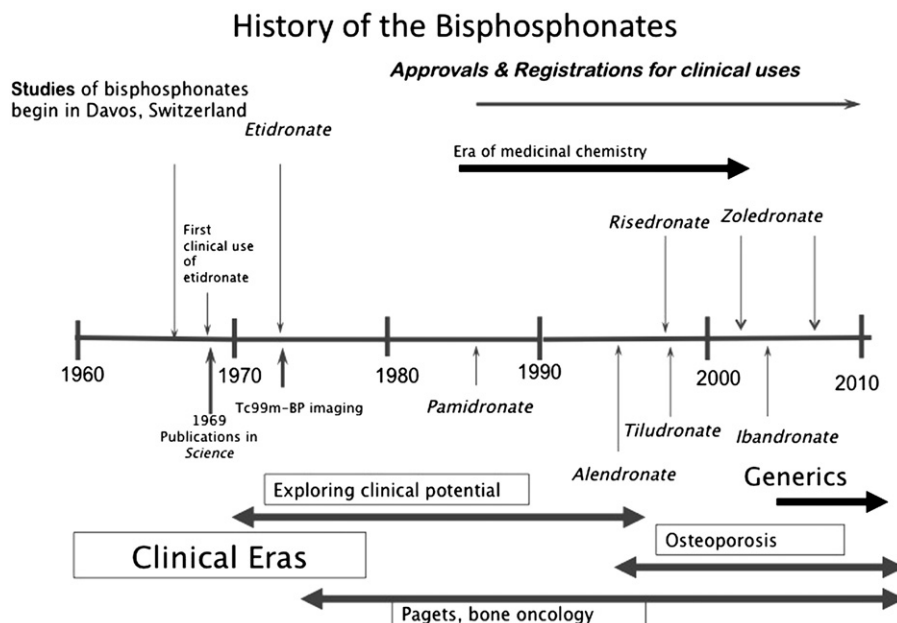
Fig. 5. The history of bisphosphonates.

the drug, especially with iv administration. The mechanism has been attributed to release of pro-inflammatory cytokines, and the mechanism has been further unravelled by showing that it involves release of IPP from monocytes and selective receptor mediated activation of gamma,delta-T-cells leading to their proliferation and activation [105,106] (Fig. 5).

Defining structure activity relationships

The evolution of concepts about the structure activity relationships among BPs has been reviewed in detail elsewhere in this issue [107]. Some of the key historical aspects will be summarised here.

Several of 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 (HAP) and allows for a number of variations in structure based on substitution in the R_1 and R_2 positions on the carbon atom. The ability of the bisphosphonates to bind to HAP crystals, and to prevent both crystal growth and dissolution, was usually enhanced when the R_1 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 R_1 position usually but not always increases the affinity for calcium (and thus bone mineral) due to the ability of bisphosphonates to chelate calcium ions by tridentate rather than bidentate binding [108]. Recent studies on mineral binding have shown that there are hitherto unexpected differences between the BPs indicating that not only the P–C–P structure, but also the R_2 side chains must contribute to mineral binding [109,110].

It was also established many years ago that the ability of bisphosphonates to inhibit bone resorption in vitro and in vivo also requires the P–C–P structure. Monophosphonates, e.g. pentane monophosphonate, or P–C–C–P or P–N–P compounds, are ineffective as inhibitors of bone resorption. Furthermore, the anti-resorptive effect could not 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.

Following the successful clinical use of clodronate and etidronate in the 1970s and 1980s, more potent anti-resorptive bisphosphonates were studied which had different R_2 side chains, but in which R_1 was unaltered. In particular, bisphosphonates containing a basic primary nitrogen atom in an alkyl chain (as in pamidronate and alendronate) were found to be 10–100 fold more potent than etidronate and clodronate. After this in the 1980s, there was a phase in which synthesis of many new 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.

The importance of the nitrogen in the side chain became evident as a feature of the more potent compounds that emerged as clinical candidates, even though the role of the nitrogen remained a mystery for a further decade. Work also continued with new non-nitrogen containing bisphosphonates [111,112].

Examples of BPs that were potent at inhibiting bone resorption included some analogues of pamidronate that contained a tertiary nitrogen atom, such as ibandronate and olpadronate [113]. Even more potent were compounds, such as risedronate [114], minodronate, and zoledronate [115], which contained a nitrogen atom within heterocyclic rings. There is some confusion about the correct nomenclature of the nitrogen containing-BPs; it is not strictly correct to call them amino-BPs, unless they contain amino groups, the best examples being pamidronate and alendronate (Figs. 4 and 5).

It should be remembered that despite the intensive efforts of medicinal chemists throughout the 1980s the identification of promising BPs was largely an empirical exercise. Any new BP had to be tested to determine its biological activity, which could not be predicted from its structure alone. Even quite close structural analogues could show striking differences in biological activity. It is only in the past decade or so, after the molecular mechanisms of action have become much clearer, has it been possible to relate structure to activity on a more scientific basis.

However, by the mid 1990s the clues provided by the analysis of structure–activity relationships did allow the spatial features of the active pharmacophore to be defined in some detail. For maximal potency, it was apparent that the nitrogen atom in the R_2 side chain had to be a critical distance away from the P–C–P group, and in a specific spatial configuration [116]. More recently this has been used quite successfully for predicting the features required in the chemical design of new and more active compounds.

Although the structure of the R_2 side chain was recognised as the major determinant of anti-resorptive potency, it was also known that both phosphonate groups are also required for the drugs to be pharmacologically active. Alterations to one or both phosphonate groups reduce the affinity for bone mineral and this may be one reason why such bisphosphonate analogues are less active. For example, replacement of one of the phosphonate hydroxyl groups with a methyl group (to form a phosphonophosphinate) markedly reduces both bone affinity and anti-resorptive potency. Methylation of both phosphonate groups to form a bisphosphinate leads to loss of bone affinity and loss of anti-resorptive activity in vivo.

Understanding the mechanisms of action of bisphosphonates at a cellular level

The remarkable selectivity of bisphosphonates for bone rather than other tissues is the basis for both their efficacy and safety in clinical medicine. Their preferential uptake by and adsorption to mineral surfaces in bone bring them into close contact with osteoclasts. During bone resorption, bisphosphonates appear to be internalised by endocytosis, along with other products of resorption. The uptake of bisphosphonates by osteoclasts in vivo has been confirmed using radiolabeled [117] and fluorescently labelled alendronate, which was internalised into intracellular vacuoles. Many studies have shown that bisphosphonates can affect osteoclast-mediated bone resorption in a variety of ways that include effects on osteoclast recruitment, differentiation, and resorptive activity, and some may induce apoptosis [118–123]. Following cellular uptake, it was shown that a characteristic morphological feature of bisphosphonate-treated osteoclasts is the lack of a ruffled border, the region of invaginated plasma membrane facing the resorption cavity. Bisphosphonates were also shown to disrupt the cytoskeleton of the osteoclast [124]. These effects can now be explained by the disruption of prenylation-dependent intracellular signalling within osteoclasts.

It is widely accepted that BPs exert their major effects on mature osteoclasts. However since mature, multinucleated osteoclasts are formed by the fusion of mononuclear precursors of haematopoietic origin, bisphosphonates might also inhibit bone resorption by preventing osteoclast formation, in addition to affecting mature osteoclasts. Surprisingly this has been rather neglected as a potential mechanism. Many years ago, it was shown that some bisphosphonates could inhibit the formation of osteoclast-like cells in long-term cultures of human bone marrow in vitro [125], in a dose-dependent manner. In organ culture also, some bisphosphonates can inhibit the generation of mature osteoclasts, possibly by preventing the fusion of osteoclast precursors [126,127].

It is likely that bisphosphonates are selectively internalised 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, whilst the breakdown of the extracellular bone matrix is brought about by the action of proteolytic enzymes. Since bisphosphonates adsorb to bone mineral, especially at sites of bone resorption where the mineral is most exposed [128,129], 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 pharmacological 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. This is much higher than the concentrations of bisphosphonates required to affect osteoclast morphology and cause osteoclast apoptosis in vitro [130].

There are obvious differences between the actions of BPs on osteoclasts compared with denosumab, which is a fully human antibody that neutralises RANK-ligand (RANKL) [131]. Denosumab has recently been approved for the treatment of osteoporosis, and for bone oncology indications. By interfering with the RANKL/RANK system that is central to normal osteoclast development, denosumab prevents osteoclast differentiation, with the result that osteoclasts disappear whilst therapy lasts. In contrast, BPs appear to act mainly by disabling osteoclasts. Not only do osteoclasts not always disappear under treatment with BPs, but "giant" hypernucleated, presumably inactive, osteoclasts can be observed in bone biopsies of osteoporotic patients treated with oral alendronate [132]. Whether these giant cells have any function remains an interesting question. It is also intriguing that inhibitors of cathepsin K, such as odanacatib, under development for osteoporosis [133], also do not ablate osteoclasts, which continue to be present under treatment.

Apart from effects on osteoclasts, BPs may also have actions on osteocytes. Work in this area was pioneered by Teresita Bellido and Lilian Plotkin, and they review the topic in this issue on Bone [134]. In contrast to their potential pro-apoptotic effects in osteoclasts, experimental studies suggest that bisphosphonates are able to prevent osteoblast and osteocyte apoptosis in vitro and in vivo, e.g. when induced by glucocorticoids [135]. This prosurvival effect is apparently independent of gap junctions and results from opening of connexin Cx43 hemichannels. This opening of hemichannels leads to activation of the kinases Src and extracellular signal-regulated kinases (ERKs), followed by phosphorylation of the ERK cytoplasmic target, p90RSK kinase, and its substrates BAD and C/EBPβ, resulting in inhibition of apoptosis [136]. The anti-apoptotic effect of bisphosphonates is separate from the effect of the drugs on osteoclasts, as analogues that lack antiresorptive activity are still able to inhibit osteoblast and osteocyte apoptosis in vitro [137]. The extent to which these effects on osteocytes contribute to the therapeutic effects of BPs in humans is unclear. The possibility that BPs used clinically may get access to osteocytes differentially depending on their mineral binding affinities and inherent structural properties needs to be studied. Interestingly the increased bone formation that occurs under mechanical loading, and which may depend upon mechano-signalling via osteocytes, seems not to be affected by BPs, such as risedronate, even when given to mice at doses greatly in excess of those required to inhibit bone resorption [138].

Understanding the mechanisms of action of bisphosphonates at a biochemical level

Over the years there were many attempts made to explain how bisphosphonates work on cells, especially via inhibitory effects on enzymes. Various studies suggested possible effects on glycolysis [139], or direct or indirect inhibition of the osteoclast proton pumping H$^+$ATPase [140–142], phosphatases [143,144], or lysosomal enzymes [145,146], and even effects on osteoblasts to produce an osteoclast-inhibitory factor [147–150].

Since the early 1990s there has been a systematic effort to elucidate the molecular mechanisms of action of bisphosphonates. Our work in this area was initiated by Michael Rogers [151], starting during his PhD studies on the inhibitory effects of bisphosphonates on the growth of the amoebae of the slime mould *Dictyostelium discoideum* [152–155].

The contemporary view is that there are two major but distinct molecular mechanisms by which bisphosphonates affect osteoclasts, and that bisphosphonates can be classified into at least two major groups based on these different modes of action. These mechanisms are discussed elsewhere in this issue by Rogers et al. [156], who have provided an excellent and comprehensive review of our current knowledge.

The first group comprises the non-nitrogen bisphosphonates, such as clodronate and etidronate, that seem able to most closely mimic pyrophosphate. They behave as PPi analogues by being metabolically incorporated into non-hydrolysable analogues of ATP though the reversal of the actions of aminoacyl-tRNA synthetases. The resulting metabolites contained the P–C–P moiety in place of the β,γ-phosphate groups of ATP, thus resulting in non-hydrolysable (AppCp) nucleotides [157–160]. It is likely that intracellular accumulation of these metabolites within osteoclasts [161,162] inhibits their function and may cause osteoclast cell death, probably by interference with mitochondrial ATP translocases [163]. This group of non-nitrogen-containing bisphosphonates therefore appears to act essentially as prodrugs, being converted to active drug metabolites following intracellular uptake by osteoclasts in vivo.

In contrast, the second group of bisphosphonates contains all of the more potent, nitrogen-containing compounds (N-BPs), which are not metabolised to AppCp-type metabolites as described above. In contrast, members of this group of N-BPs interfere with specific metabolic reactions, notably in the mevalonate biosynthetic pathway that leads to the synthesis of cholesterol and other sterols. The enzymes in this pathway metabolise pyrophosphate-containing isoprenoid lipids, which are progressively condensed into longer chains. Bisphosphonates are able to inhibit several enzymes in this pathway to varying extents [164,165], but the major target for the anti-resorptive N-BPs is farnesyl pyrophosphate synthase (FPPS).

The isoprenoid lipids produced in the mevalonate pathway include isopentenyldiphosphate (also known as isopentenylpyrophosphate IPP), as well as farnesyldiphosphate (FPP) and geranylgeranyldiphosphate (GGPP). FPP and GGPP are required for the post-translational modification (prenylation) of small GTPases such as Ras, Rab, Rho and Rac, which are prenylated at a cysteine residue in characteristic C-terminal motifs [166,167]. Small GTPases are important signalling proteins which regulate a variety of cell processes important for osteoclast function, including cell morphology, cytoskeletal arrangement, membrane ruffling, trafficking of vesicles and apoptosis [168–171]. Prenylation is required for the correct function of these proteins, since the lipid prenyl group serves to anchor the proteins in cell membranes and may also participate in protein: protein interactions (Fig. 6).

The inhibitory effects of bisphosphonates on the mevalonate pathway are now widely known, and this represents a very important step forward in understanding how these drugs work. Many observations point to the importance of the mevalonate pathway for osteoclast function, and validate the notion that N-BPs act by inhibition of this pathway [172–177].

There is an interesting relationship between statins and bisphosphonates, in that both inhibit enzymes in the mevalonate pathway. It was predicted that if inhibition of the mevalonate pathway could account for the anti-resorptive effects of bisphosphonates, then the statin drugs should also inhibit bone resorption. Statins are inhibitors
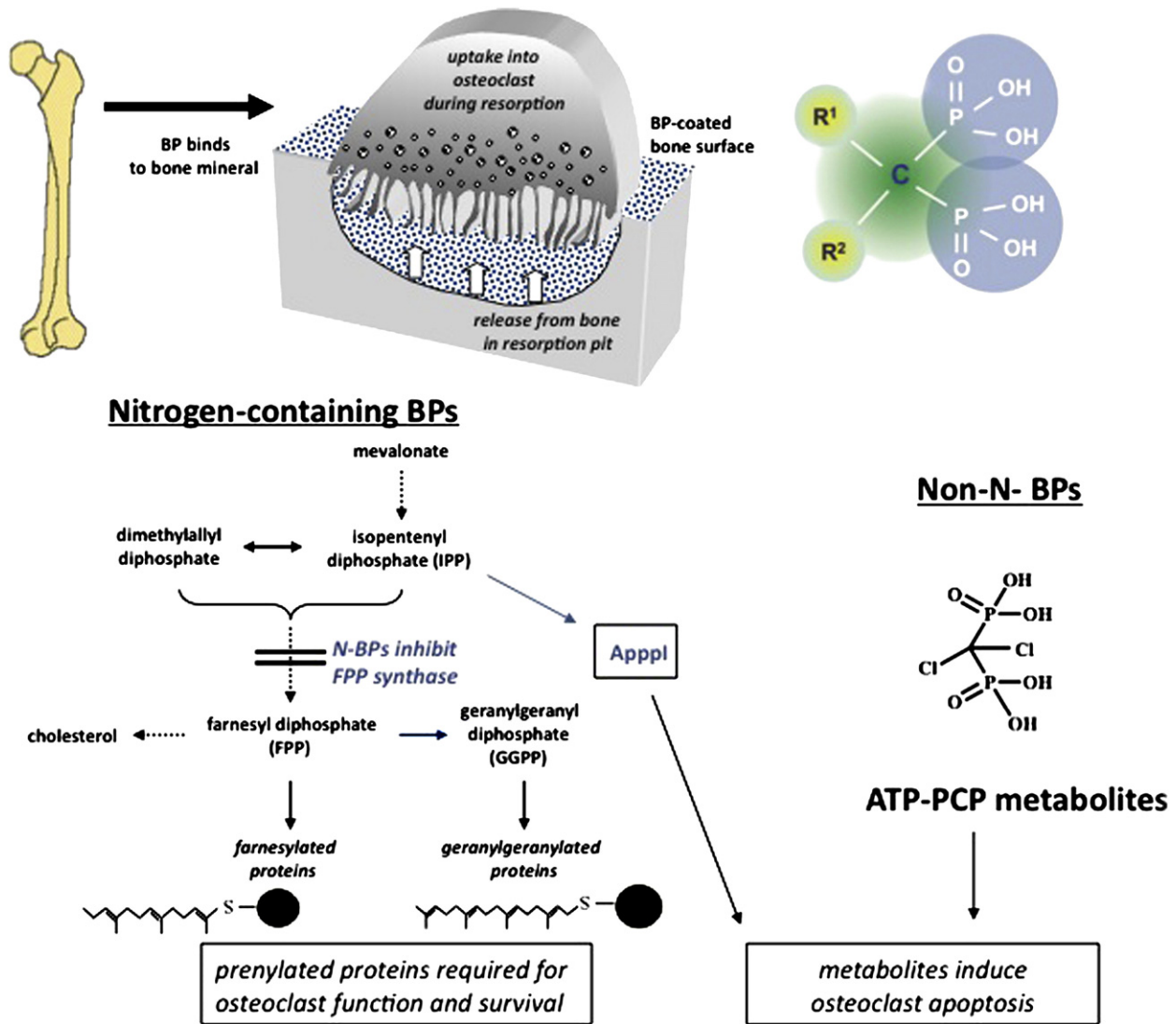
Fig. 6. The cellular and biochemical mechanisms of action of bisphosphonates.

of HMG-CoA reductase, one of the first steps in the mevalonate pathway. In fact they proved to be even more potent than bisphosphonates at inhibiting osteoclast formation and bone resorption in vitro [178], an effect that could also be overcome by the addition of geranylgeraniol (which is used for protein geranylgeranylation) but not farnesol (which is utilised for protein farnesylation), suggesting that 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 Rab in processes that are fundamental to osteoclast formation and function, e.g. cytoskeletal rearrangement, membrane ruffling and vesicular trafficking [179], and further work has confirmed this, particularly the importance of Rab proteins (Fig. 7).

The comparison between bisphosphonates and statins is informative for another reason. Even though they both inhibit enzymes in the same biochemical pathway their pharmacological effects are quite distinct. The statins are widely used as cholesterol-lowering drugs, through their ability to lower cholesterol biosynthesis by inhibiting HMG-CoA reductase, but N-BPs have no marked effects on circulating cholesterol levels. Conversely despite several studies there is no substantial evidence that statins have effects on bone when used clinically. The most likely explanation is that statins are selectively and efficiently taken up by liver rather than bone, which is the con-verse of the case for bisphosphonates. This is therefore an excellent example of how drug specificity is achieved by highly selective tissue targeting.

Clinical applications of bisphosphonates

The most impressive clinical application of bisphosphonates has undoubtedly been as inhibitors of bone resorption, often for diseases where no effective treatment existed previously, but it took many years for them to become well established.

However, the first clinical uses of bisphosphonates were as inhibitors of calcification. Etidronate was the only BP to be used in this way, first in fibrodysplasia ossicans progressiva (FOP, formerly known as myositis ossificans) [180,181]. Etidronate showed some promise in patients who had undergone total hip replacement surgery to prevent subsequent heterotopic ossification and to improve mobility [182]. It was also used to prevent ectopic calcification and ossification, after spinal cord injury and in topical applications in toothpastes to prevent dental calculus. There is a recent and renewed interest in devising effective treatments for calcification in renal failure and vascular disease [183].

One of the other early clinical uses of bisphosphonates was as agents for bone imaging, "bone scanning," for which they still remain

Mevalonate pathway.
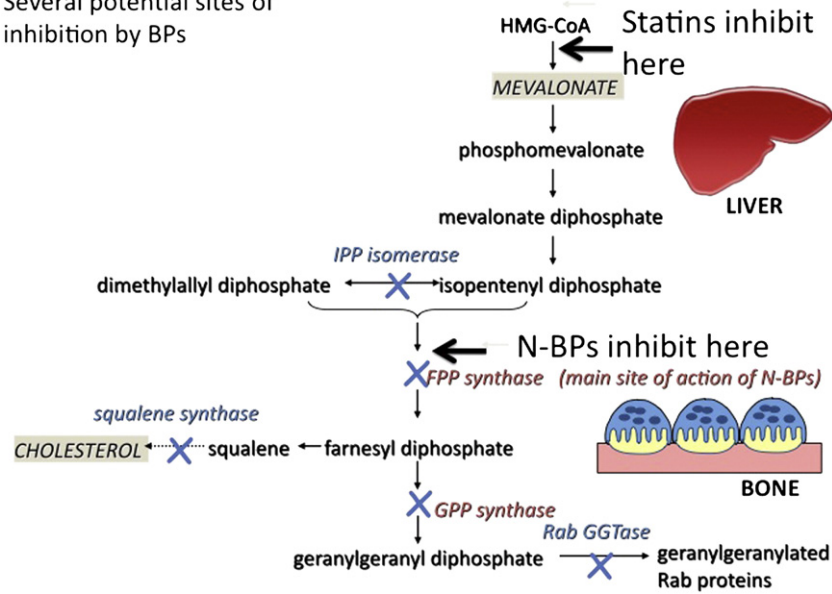### Several potential sites of inhibition by BPs

Fig. 7. The different effects of statins and BPs in the mevalonate pathway, indicating how tissue selectivity of uptake determines their pharmacological specificity.

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 gamma-emitting technetium isotope [184,185] (Fig. 8).

Bisphosphonates have become the treatment of choice for a variety of bone diseases in which excessive osteoclast activity is an important pathological feature, including Paget's disease of bone, metastatic and osteolytic bone disease, and hypercalcaemia of malignancy, as well as osteoporosis.

Lessons from Use of Technetium-99m BP Bone Scans
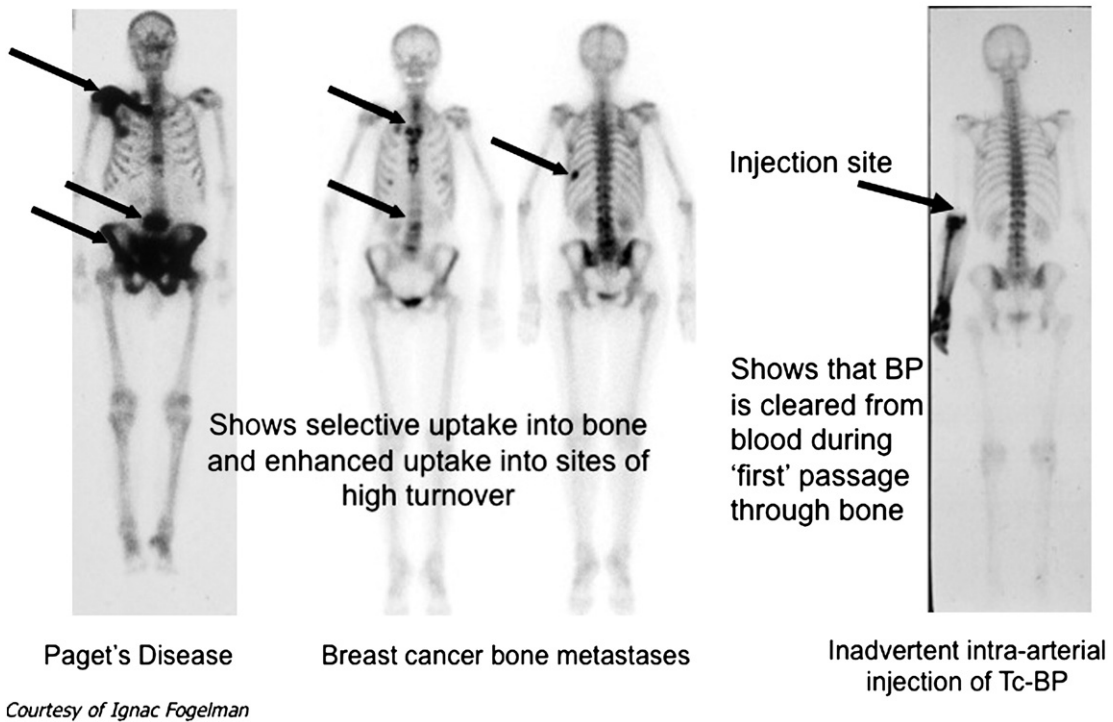
Fig. 8. The use of Technetium-99m bisphosphonate bone scans. Uptake into sites of high bone turnover occurs in Paget's disease (left) and bone metastases from breast cancer (centre). Image on right shows extensive uptake into forearm bones after inadvertent injection of scanning agent into the brachial artery. This illustrates the efficient clearance of BP from the circulation by 'first pass' clearance through bone (images by courtesy of Ignac Fogelman).

Fig. 9. Effect of Binding Affinity of Bisphosphonates on their Uptake and Detachment from bone surfaces and their re-cycling.

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 practise different compounds have come to be favoured for the treatment of different diseases. Currently there are at least eleven bisphosphonates (etidronate, clodronate, tiludronate, pamidronate, alendronate, ibandronate, risedronate, and zoledronate, and also to a limited extent olpadronate, neridronate and minodronate) that have been registered for various clinical applications in various countries, but not all in USA or Europe. To a major extent, the diseases in which they are used reflects the history of their clinical development, the role of 'champions', and the degree of commercial interest in and sponsorship of the relevant clinical trials.

Paget's disease was the first clinical disorder in which a dose-dependent inhibition of bone resorption could be demonstrated using bisphosphonates in man [186,187], and was well established by the 1980s [188–191]. The medical treatment of Paget's disease is now reliant almost exclusively on the use of the bisphosphonate class of drugs. There have been gradual improvements in the ability of these drugs to keep the disease under control, starting with etidronate in the 1970s, and progressing through the use of other BPs given by mouth, such as clodronate [192,193], tiludronate, alendronate, and risedronate. These days most patients are treated with BPs given by infusion, either as pamidronate or more recently as zoledronic acid. The effects of zoledronate are truly remarkable, not only in reducing the excessive destructive activity taking place within the bone, but in producing often very long lasting effects [194]. Many patients will not need further treatment after just one infusion of zoledronic acid [195,196]. Even though it is known that BPs can reside in bone for long periods, the real reasons for these long-lasting effects are not well understood (Fig. 9). Reid and Hosking have provided a thoughtful review of the use of bisphosphonates in Paget's disease as part of this issue of Bone [197].

The use of bisphosphonates in cancers can also be traced back to the early 1980s. Several groups showed the impressive efficacy, particularly of clodronate [198,199] and pamidronate [200], in the treatment of hypercalcaemia of malignancy, associated with myeloma and bone metastases. But it took many more years before the large-scale trials were done that enabled the registration of these drugs for the prevention of skeletal related events associated with a variety of cancers, as reviewed by Coleman and McCloskey in this issue of Bone [201].

In their accompanying review of the scientific basis of using BPs in cancers, Clezardin et al. [202] discuss the relative contribution of direct anti-tumour effects versus effects mediated through inhibition of bone resorption. An exciting possibility is that synergistic anti-tumour effects may be achievable in the presence of other chemotherapeutic agents.

The use of BPs in osteoporosis is the most recent area of development and became established in the 1990s, first with etidronate [203–205] in many countries, and then with alendronate [206–208] on a world-wide basis. Three more BPs have been introduced since then, risedronate [209,210], ibandronate [211], and most recently zoledronate [212]. Minodronate is used in Japan [213].

As a drug class the bisphosphonates have emerged as the leading treatments for postmenopausal and other forms of osteoporosis [214]. Eastell et al. provide an up-to-date review of their use in this issue [215].

In general the % reduction of vertebral fractures is greater than for non-vertebral fractures, but the reduction in hip fractures of up to 40% achieved with alendronate, risedronate and zoledronate is reassuring. It is difficult to compare the relative efficacy of the different BPs because prospective head-to-head comparative trials have unfortunately not been done.

A major issue is the poor adherence to therapy with the oral drugs, even though various modes of administration are available in daily, weekly, or monthly formats. The recent introduction of an enteric-coated version of risedronate [216] with a small amount of EDTA that can be given with meals represents another means of trying to improve patient's willingness to take therapy. The availability of yearly intravenous treatment with zoledronate [217,218] as an effective treatment has the attraction of delivering a defined dose without the variability associated with oral administration as well as avoiding potential gastrointestinal intolerance.

Apart from oral and parenteral routes of administration, other routes have been explored, e.g. transdermal delivery, but not so far adopted into clinical practise.

Bisphosphonates are mainly used in women with postmenopausal osteoporosis, but also in men, in patients on glucocorticoids, and in children with the inherited 'brittle bone' disorder, osteogenesis imperfecta [219,220].

Current challenges and new directions with bisphosphonates

There are many ongoing issues with clinical aspects of the treatment of bone diseases. In osteoporosis, issues under consideration

Where next with Bisphosphonates?

- Now possible to synthesise "designer" BPs with defined properties in terms of
  - **mineral affinity**
  - **Inhibition of FPPS (farnesyl pyrophosphate synthase)**
  - **actions on other mevalonate pathway enzymes**
- BPs with lower mineral affinity and super-potency on FPPS may enable optimisation of clinical potential
  - less reduction of bone turnover,
  - optimal skeletal distribution and anti-fracture effects
- Many unmet medical needs and new opportunities
  - Combinations with other drugs
  - Rheumatoid erosions, inflammatory bone loss
  - Osteoarthritis,
  - Implant fixation, orthopaedic and dental
  - Fracture healing
  - Skeletal drug delivery
  - Immunological effects
  - Anti-parasite effects
- Enhance current uses eg
  - Anti-cancer effects
    - Oral
    - Combination with chemotherapeutic agents

Fig. 10. Future opportunities for bisphosphonates.

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 teraparatide, and their extended use in related indications e.g. glucocorticosteroid-associated osteoporosis, male osteoporosis, childhood osteopenic disorders, arthritis, and other disorders. There is still much that can be done to improve the way in which existing drugs are used, e.g. in terms of compliance with therapy. Issues of safety need ongoing vigilance. The introduction of new therapies at a time when BPs are becoming generic and more affordable poses other challenges.

The clinical use of bisphosphonates in bone diseases is well established, and their use in oncology, rheumatoid arthritis, osteoarthritis and orthopaedics offers opportunities for further success. A particularly exciting series of recent experimental and clinical observations indicates that bisphosphonates may prevent various cancers and also reduce mortality and increase life span [221–236].

From the research angle, there remain many other interesting directions for future work. Despite the advances, there are still interesting mechanistic issues to be solved. Whilst we have confidence that the N-BPs inhibit FPPS, how the bisphosphonates reach this intracellular target is still unclear. In addition, there are other potential intracellular targets that may contribute to lesser degrees. Furthermore, characterization of new cell surface targets will be necessary to explain how bisphosphonates block apoptosis of osteocytes and osteoblasts at low concentrations.

There are numerous examples of BPs having effects on cells and tissues outside the skeleton. The effects on osteoclast precursors, tumour cells, macrophages and gamma delta T cells are examples, and in some cases may be explained by sufficient BPs entering cells to inhibit the mevalonate pathway.

A particularly interesting aspect of these non-skeletal effects is the observations made on protozoan parasites, the growth of several of which can be inhibited by BPs acting on FPPS [237–239]. The therapeutic potential is enticing given the importance of these diseases. The range of eligible protozoa includes *Entamoeba* [240], *Plasmodia* [241], Trypanosomes [242], *Toxoplasma* [243], *Cryptosporidia* [244], and *Leishmania* spp. [245].

It has long been thought that BPs might be used as carriers to deliver other pharmacological agents to the bone [246]. This has been achieved with some radiopharmaceuticals, but the future challenge will be to devise better means of releasing active drugs from bisphosphonate conjugates, a topic beyond the scope of this manuscript (Fig. 10).

Reflections on the past, present and future

It is now 40 years since the discovery of the profound effects of the bisphosphonates on calcium metabolism. It has taken a long time for them to become well established as clinically successful antiresorptive agents, which has enabled new approaches to the therapy of bone diseases.

Studies of the structure–activity relationships over many years have led to a much better understanding of the unique properties of bisphosphonates and how they work. These studies have culminated in the identification of their molecular mechanisms of action, with the FPPS enzyme the likely primary target of N-BPs in the bone cell. The bisphosphonates have exceptional selectivity for their target organ. With the advanced understanding we now have of differences among the bisphosphonates in bone affinity and cell effects, we can more clearly explain their clinical features, and assess the future utility of this series of drugs. Advances in synthesis, crystallography, and visualisation of drug distribution have created new opportunities in this field. There are potential new therapeutic targets that will benefit from optimised drug design.

In looking back one has to wonder whether the bisphosphonates would have won through to become successful drugs if they were re-discovered today. They started as simple chemicals, intended for non-biological uses. They had no defined receptor, and there was no clear identification of eventual therapeutic target, and no predictable commercial value. With a bioavailability in the range of 1% they would be unlikely to pass today's hurdle set for putative oral drugs.

Furthermore, the setbacks encountered by the early bisphosphonates could have easily de-railed development. Etidronate struggled to become an osteoporosis drug based on the narrow window between inhibition of bone mineralisation and bone resorption, and fracture efficacy based only on small trials. Clodronate was abandoned for some time after the leukaemia scare, which was later shown to be groundless.

Pamidronate as the first of the alkyl amino-BPs produced the acute phase responses, which were a problem in that they represented an unexplained potential toxicity. Even alendronate, despite the largest trials then done in osteoporosis, had set backs in terms of oesophageal

adverse events. The reality was that the drug class survived these setbacks due to the enthusiasm and tenacity of a small number of clinical investigators who pioneered the early studies, and acted as champions for the drug class.

It was not until the 1980s, more than a decade after their first clinical use, that several pharmaceutical companies made a serious commitment in terms of medicinal chemistry and clinical development.

Even then, the difficulties of bringing these drugs to the market were not always straightforward, as illustrated by those that fell by the wayside, such as oral pamidronate and tiludronate. There are important lessons to be learnt from the need to do good dose–response studies during Phase 2 development and to make appropriate choices of doses.

So where does the field go from here? Despite the considerable potential for developing 'better' bisphosphonates based on current knowledge of their structure–activity properties, it is uncertain, given the high cost of development, whether further agents will be developed unless they offer distinct advantages over currently available bisphosphonates.

However there are still unmet medical needs, and several clinical leads and opportunities ripe for further study. Serendipity played an important part in the early stages of this odyssey, and the next part of the journey can take advantage of all that has been learnt over this period.

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