CHAPTER 3 Bisphosphonates

Historical Review

The bisphosphonates constitute a group of pharmacological agents first synthesised in the 1880s but developed over the past 30 years for diagnosis and treatment of disorders of bone and anomalies of calcium metabolism. The fundamental research carried out by H. Fleisch in the 1960s laid the ground work for the rapid development of the bisphosphonates in medicine.

The starting point was provided by the pyrophosphates which have a central P-O-P binding. Pyrophosphate was widely employed in industry due to its ability to dissolve calcium carbonate. Consequently pyrophosphates were used in washing powders and other soapy solutions to inhibit scale formation. Today they are also used worldwide in toothpaste to prevent and to reduce plaque formation. Due to its strong affinity for calcium phosphate and therefore for bone, pyrophosphate can be bound to 99mTc and utilised for scintigraphy of the skeleton (bone scans).

Moreover, in vivo studies demonstrated an inhibitory effect of pyrophosphates on calcification. Various forms of ectopic calcification could be effectively avoided by parenteral, but not by oral administration. However, there was no influence on osteoclastic resorption due to enzymatic splitting of pyrophosphate when taken orally (half-life of only 16 min).

The bisphosphonates were then discovered during the search for analogues of pyrophosphate. They have similar physical and chemical effects but are resistant to enzymatic splitting and to metabolic breakdown. *This is because, in contrast to the P-O-P binding of pyrophosphate, the P-C-P binding of the bisphosphonates is stable and above all cannot be broken down enzymatically so that their activity is retained. This switch of the binding from P-O-P to P-C-P represented a genuine breakthrough which enabled the development of the potent bisphosphonates which are now in use for therapy of disorders of bone all over the world.*

The first medical application of a bisphosphonate was published in the Lancet in 1969. A 16 month old baby, diagnosed as having progressive myositis ossificans, was successfully treated with oral etidronate to inhibit the extra-osseous calcification.

Subsequently H. Fleisch and coworkers demonstrated, by means of animal experiments, that bisphosphonates inhibit osteoclastic bone resorption and thereby achieve a positive calcium balance. The rapid advances in the diagnosis and therapy of the osteopathies is thus closely bound up with the history of the bisphosphonates – a story of genuine and lasting success in the treatment of disorders of bone.

During the past 30 years new, more potent bisphosphonates have been developed. These have now been extensively applied in medicine, particularly in the fields of osteology, orthopedics, surgery (as a consequence of accidents and other emergencies), as well as in hematology and particularly in oncology. *All osteopathies characterised by excess (absolute or relative) of osteoclastic activity are now treated with bisphosphonates, and it should be noted that this comprises about 90% of all disorders of bone*. Bisphosphonates are now the major drugs used in the treatment of postmenopausal osteoporosis and represent the first-line therapy in the majority of patients. The latest applications of bisphosphonates include their administration for prevention of osseous metastases (administered during adjuvant chemotherapy), for alleviation of bone pain, and for their modulation of the immune and stromal systems in the bone marrow and the bone. Their anti-proliferative activity is under close investigation and some results have already been published for example in multiple myeloma and in metastatic bone disease, and experimentally in sarcomas.

An additional novel application is inhibition of proliferation of the causative organisms in some parasitic infections.

Chemistry

Bisphosphonates are analogues of pyrophosphates which occur physiologically, and in which the oxygen atom of the central P-O-P structure has been replaced by carbon, resulting in a P-C-P group (Fig. 3.1), and this exchange has made them resistant to heat and enzymatic hydrolysis. These bisphosphonates exert strong effects on bone; they also have a high affinity for metal ions, forming soluble or insoluble complexes and aggregates, depending mainly on the pH of the solution.

Further substitutions have enabled synthesis of a series of biologically active bisphosphonates, each of which has its own characteristic potential activity and effect on bone (Table 3.1. and Fig. 3.2). Therefore every bisphosphonate has to be evaluated individually. This is of particular importance because of the rare occurrence of side effects such as renal damage and necrosis of the jaw bones (see below).

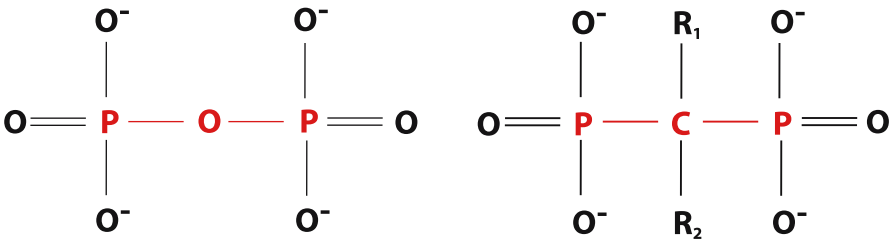
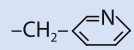
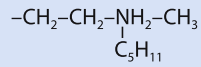
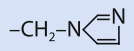


Fig. 3.1 Chemical structure of pyrophosphate and of bisphosphonates

Table 3.1. List of available bisphosphonates according to side chains and relative potency

Substance	Trade Name	R1	R2	Relative Potency
Etidronate	Didronel®	–OH	$-CH_3$	1×
Clodronate	Ostac®	–CL	–CL	10×
Pamidronate	Aredia®	–OH	$-CH_2-CH_2-NH_2$	100×
Alendronate	Fosamax®	–OH	$-CH_2-CH_2-CH_2-NH_2$	1000×
Risedronate	Actonel®	–OH		5000×
Ibandronate	Bondronat® Bon(v)iva®	–OH		10 000×
Zoledronate	Zometa® Aclasta®	–OH		20 000×

For practical purposes, the bisphosphonates are sub-divided into chemical groups according to the alphabetic order of the side chains (Table 3.1):

- Bisphosphonates without nitrogen substitution: etidronate, clodronate, tiludronate
- Aminobisphosphonates: pamidronate, alendronate, neridronate
- Aminobisphosphonates with substitution of the nitrogen atom: olpadronate, ibandronate
- Bisphosphonates with basic heterocycles containing nitrogen: risedronate – pyridine-ring, zoledronate – imidazol-ring

The bisphosphonates used to be given in grams, now only milligrams are given because of their greatly increased potency.

Pharmacodynamics

The bisphosphonates are poorly absorbed when taken orally, but this is compensated for by their greatly increased potency – even 1% of a given dose is effective! They are distributed in the body via the blood stream, stored in the bones, and excreted unchanged by the kidneys. Interactions with other pharmaceutical agents have not been observed. Four compartments of bisphosphonate distribution are distinguished; these determine their pharmacodynamics (Fig. 3.3):

- Gastro-intestinal tract
- Blood
- Bone
- Kidneys

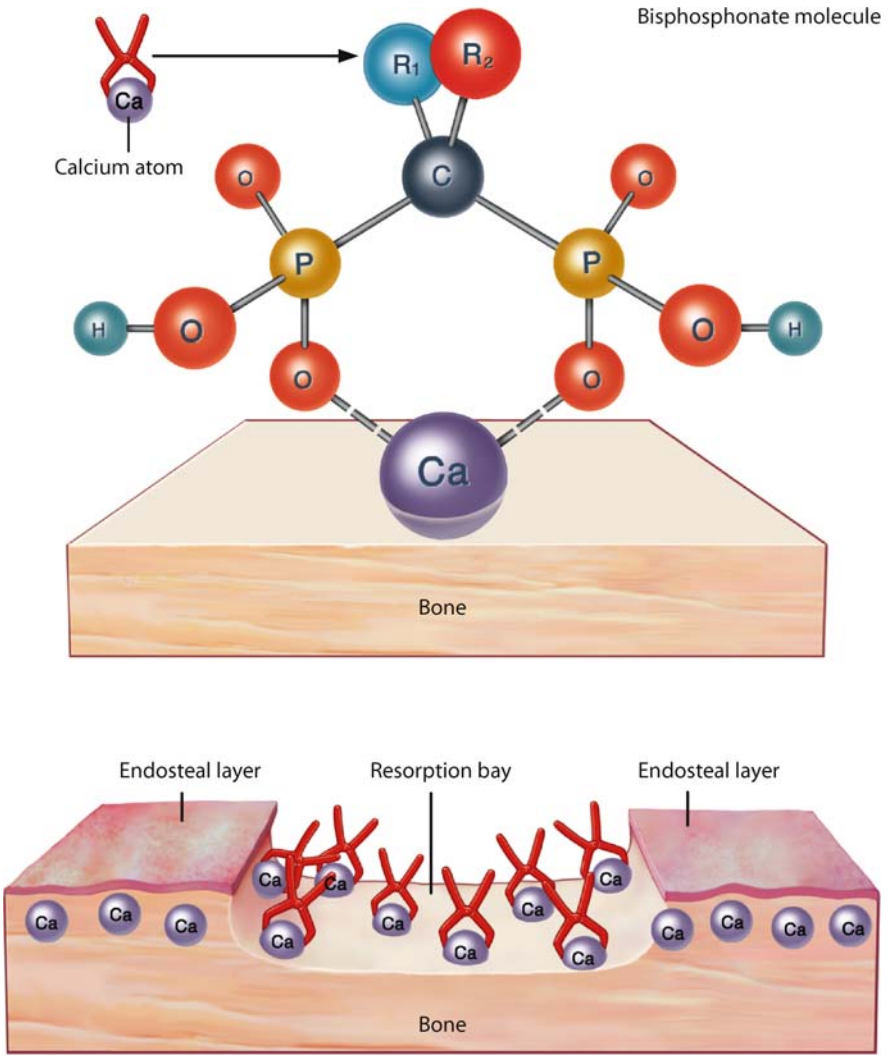


Fig.3.2 Molecular structure of bisphosphonates: they are stable analogues of pyrophosphate with a central P-C-P binding instead of the P-O-P. The various bisphosphonates are distinguished one from another by the ligands R1 and R2. The bisphosphonates depicted here as small tongs, are deposited on the surface of the bone in the resorption lacunae. Here they are taken up by osteoclasts or incorporated into bone by osteoblasts

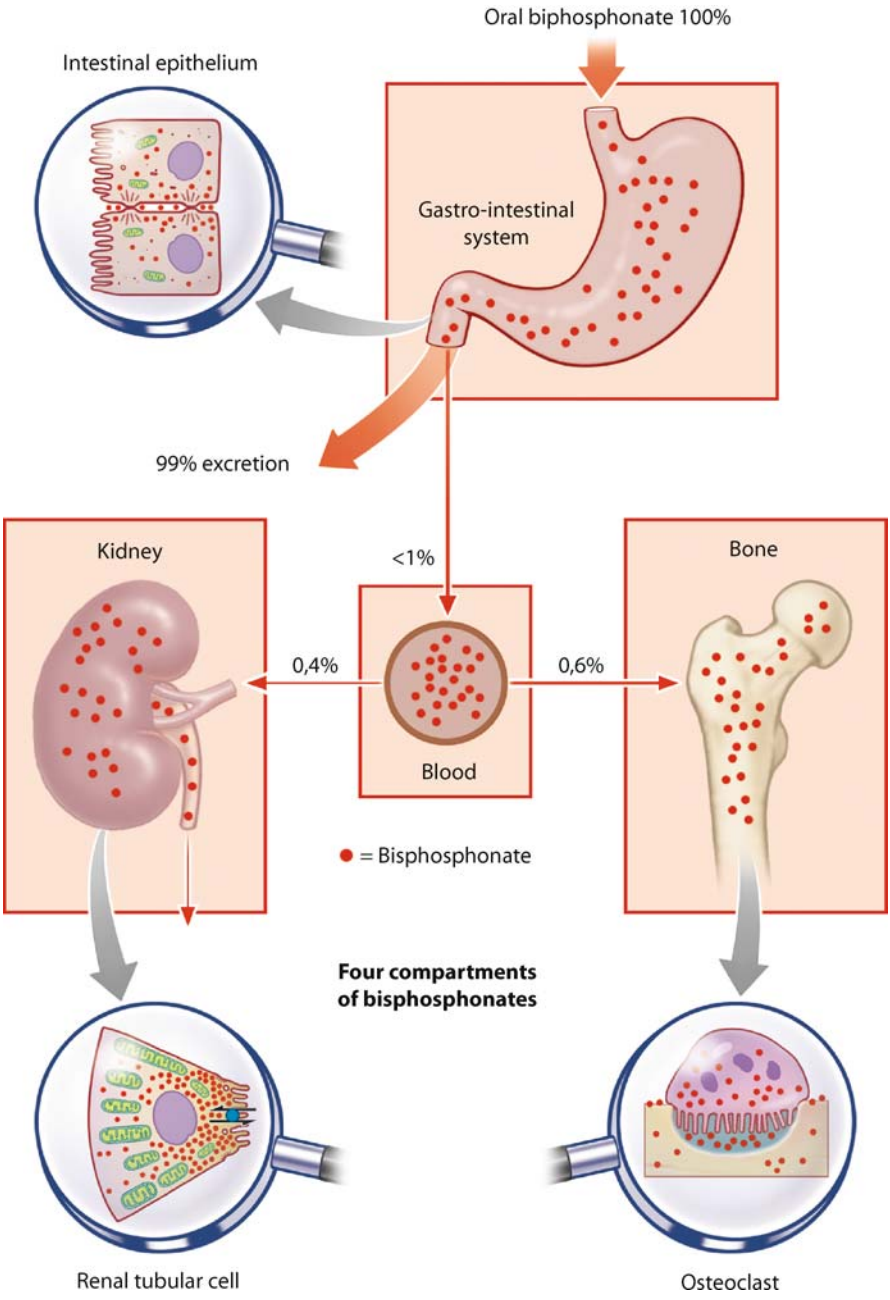


Fig.3.3 Diagrammatic representation of the four compartments of bisphosphonate absorption and excretion: Gastro-intestinal tract, blood, bone and kidney

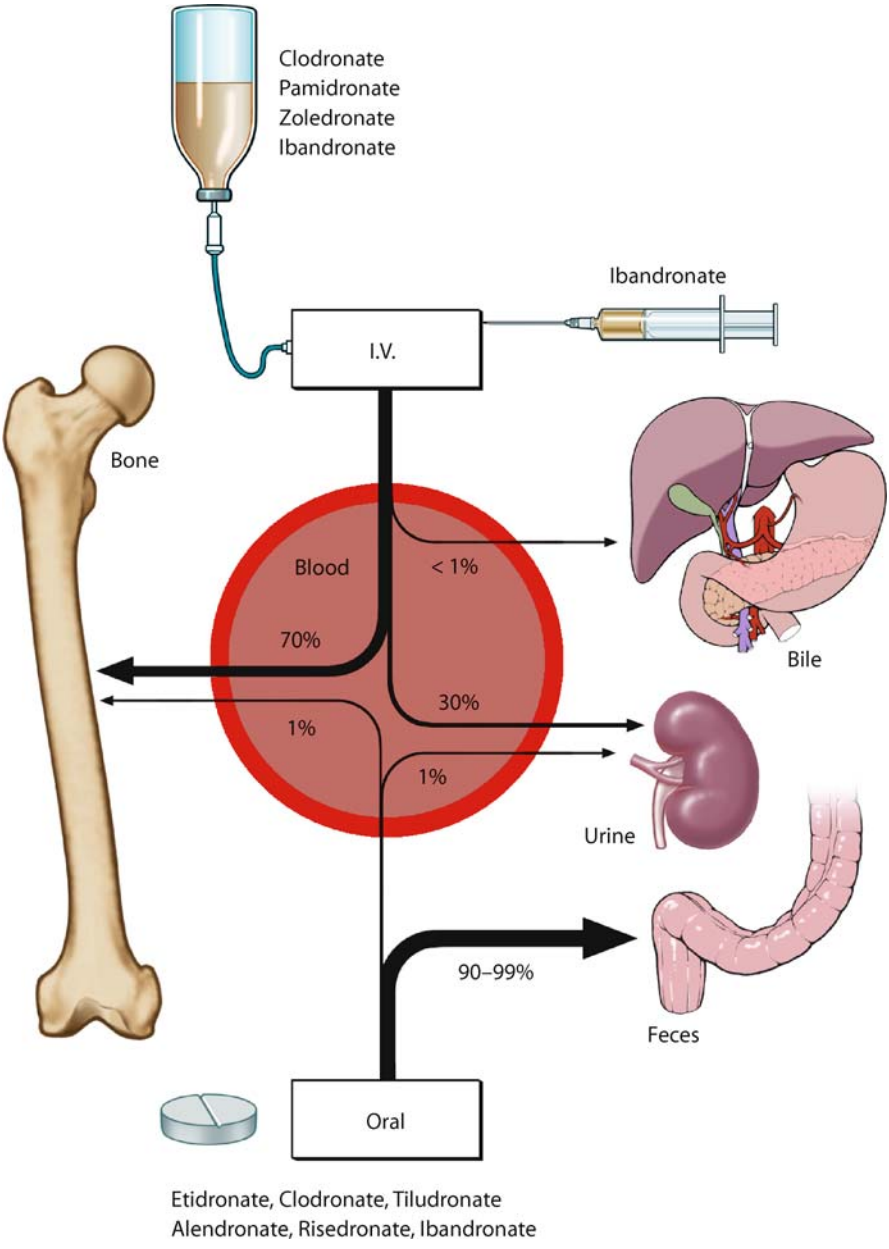


Fig. 3.4 Pharmacokinetics of bisphosphonates

Administration

Bisphosphonates may be taken orally as tablets, given intravenously as infusions, or more rarely as injections (Fig. 3.4). They are also effective when given intranasally or transdermally, and by intramuscular (multiple myeloma) and intra-articular (osteoarthritis of the knee) injections, but these forms of administration are no longer carried out.

Intestinal Absorption

The intestinal absorption of bisphosphonates is minimal. It varies from <1% to 10%, is dose-dependent, therefore increases with higher doses: alendronate 0.76%, risedronate 0.62% and ibandronate 0.63%. However, as mentioned above these doses are effective.

Two characteristics of bisphosphonates are responsible for their poor absorption: their low affinity for lipids, which hinders transport through membranes and into the cell, and their polarity, their negative charge, which prevents paracellular transport. Bisphosphonate absorption is further decreased when ingested together with food, especially food rich in calcium, such as milk and milk products because bisphosphonates form insoluble chelates with the calcium in these products. The presence of other substances in the gastro-intestinal tract such as fruit juices, iron, coffee etc. likewise decreases their absorption. Bisphosphonates are absorbed in the stomach and upper part of the intestine by passive diffusion within about an hour after ingestion. Studies are underway to increase their lipophilicity and facilitate their absorption.

Distribution Half-life

Bisphosphonates are bound to albumin in the blood. Insoluble complexes are formed by means of bi-valent cations: for example two bisphosphonate molecules are attached to magnesium, to calcium or to iron (Fig. 3.5). The variable polarity and lypophilia of the bisphosphonate side chains are responsible for considerable differences in their attachment to plasma proteins which in turn accounts for differences in their half–life values. There are also big differences in the strength of the albumin bonds (from 22% for zoledronate to 87% for ibandronate) and therefore in the time it takes for the bisphosphonates to be eliminated from the plasma (Fig. 3.6). The half-life of zoledronate in the plasma is only 1–2 hours, while that of ibandronate is 10–16 hours. The kinetics of the elimination of the bisphosphonates follows the 4 compartment model (see above). The plasma binding of the bisphosphonates determines their half-life and the kinetics of their elimination by the kidneys.

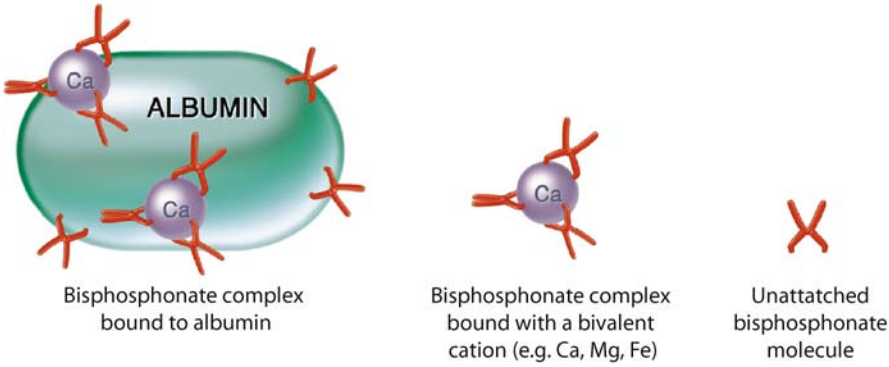


Fig. 3.5 Formation of complexes of bisphosphonates in the serum

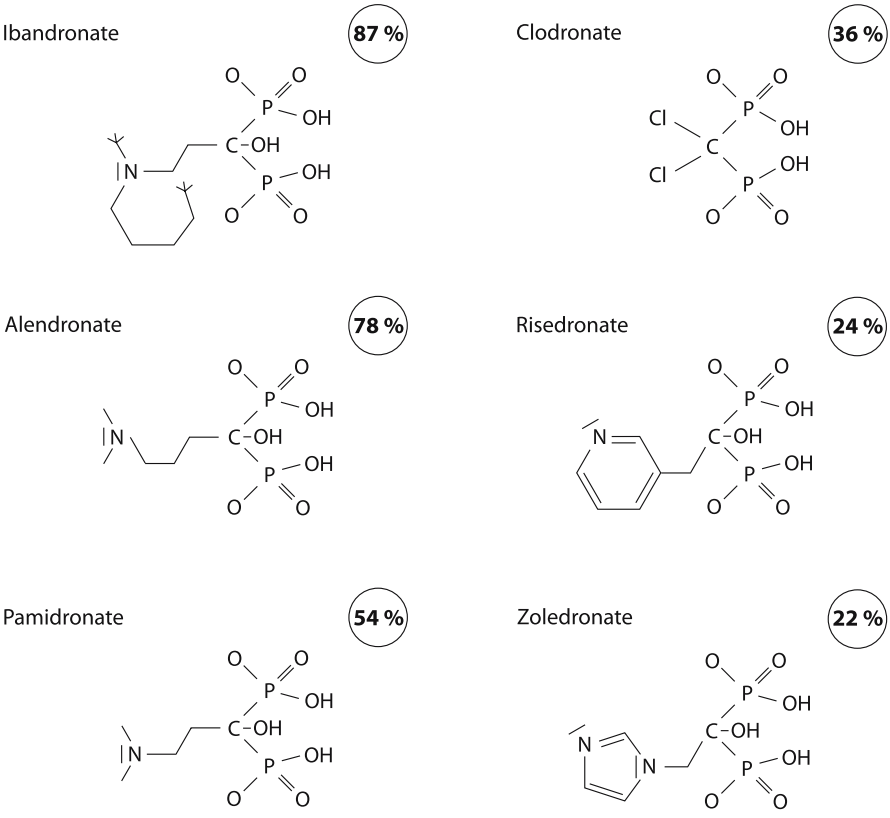


Fig. 3.6 Quantities of binding of various bisphosphonates to plasma proteins

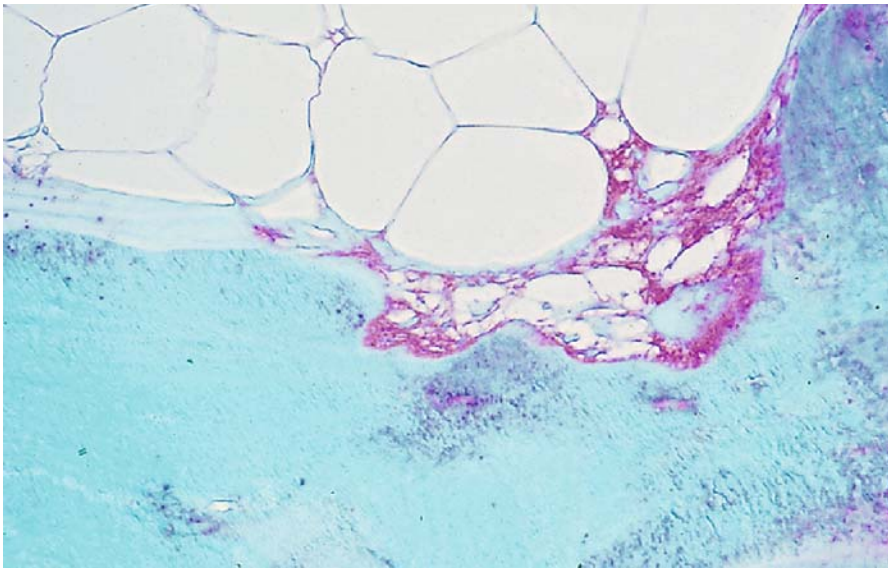


Fig. 3.7 Deposition of bisphosphonate (*red*) on bone in a resorption lacuna and in the cytoplasm of an osteoclast visualised by means of an antibody to ibandronate, in sections of a plastic embedded undecalcified iliac crest biopsy taken from a patient 2 days after 4 mg ibandronate i.v.

Bisphosphonates from the plasma are actively bound to the surface of the bones, especially in the resorption lacunae where they are attached to calcium (Fig. 3.7). *The amount of deposition depends on the extent of resorption surface of bone available.*

Affinity to Bone

By binding to hydroxyapatite, bisphosphonates accumulate at sites of bone resorption and are selectively internalised by actively resorbing osteoclasts. *The different bisphosphonates have different affinities for hydroxyapatite crystals*. The values (adsorption affinity constants, K_L l/mol × 10^6) that have been determined in vitro are:

- Clodronate 0.6
- Etidronate 1.2
- Risedronate 2.2
- Ibandronate 2.4

- Alendronate 2.9
- Zoledronate 3.5

These data extend earlier work on the potential important contribution of mineral binding to the potency and duration of action of different bisphosphonates. *These differences in binding affinities and effects on mineral surface properties are likely to be reflected in the clinical differences among these bisphosphonates: uptake and retention on the skeleton, diffusion of the drug within bone, release of absorbed drug from bone, potential recycling of the desorbed drug back onto bone surface, effects on mineral dynamics and effects on bone cellular function.* Higher affinity bisphosphonates such as alendronate and zoledronate have an avide uptake, a lower desorption, a higher re-attachment and a less diffusion in bone. Risedronate for example has lower kinetic binding affinity than alendronate for the mineral substrates hydroxyapatite and octa calcium phosphate. These differences in bone affinity may contribute to the shorter terminal bone half-life of risedronate and therefore to faster clinical on- and off-responses seen with risedronate compared with alendronate (Nancollas et al. 2006). And indeed, the results of the *FACT-Study* (direct comparison of the effects of two bisphosphonates on bone mass and parameters of bone remodelling) indicated a greater effect of alendronate than risedronate on the parameters measured (BMD and bone turnover markers).

Studies with hydroxyapatite crystals and later with fetal mouse bone explants showed that the presence of a OH substitution in R_1 increases their binding to bone mineral, and that this action was independent of the structure of the R_2 substitutions (van Beck et al. 1998). In contrast, bisphosphonates lacking an R_1 substitution or compounds with other substitutions such as Cl (clodronate) or H (etidronate) had significantly lower binding affinities. The following *ranking of binding affinities of bisphosphonates* for bone according to substitutions at R_1 can be given: OH and NH_2 > H > "no R_2" > Cl, provided that the phosphonate groups remained intact (vanBeck et al. 1998). *But in spite of the specific functions of the ligands R_1 and R_2, all studies strengthen the view that the whole molecule is necessary for the full range of their action on bone.*

Cellular Uptake

Few studies have addressed the question of how bisphosphonates actually enter the cell. Since no specific transport mechanisms have yet been elucidated, the assumption has been made that bisphosphonates are taken up from the surrounding fluid by non-specific pino- and endocytosis. Bisphosphonates have been demonstrated in the cytoplasm, in mitochondria and in other organelles within the cytoplasm of the osteoclasts. Relatively speaking, macrophages, a cell line to which osteoclasts belong, are also active in their up-take. *However, the concentration of bisphosphonates in extra-osseous cells is very low, which explains the lack of toxicity.*

Elimination

20–50% of the bisphosphonate in the plasma is deposited on the bone; about 1% is excreted with the gall, the rest is eliminated by the kidneys into the urine. There are considerable differences between the various bisphosphonates with respect to their elimination. *Long-term studies (more than 10 years) have now clearly demonstrated that the (relatively) minimal amount of bisphosphonate deposited on and in bone has absolutely no influence on bone "quality"; whether it has any biological or clinical significance when subsequently released and excreted and/or "recycled" is still unknown.*

Bisphosphonates exhibit a very strong affinity for hydroxyapatite crystals which are avid bisphosphonate grabbers, and this "binding" process is strictly pH-dependent, so that when, during active resorption, the interface between osteoclasts and bone becomes strongly acidic the previously bound bisphosphosnate is released from its binding to calcium. In contrast to the blood (half-life of 1 to 15 hours) the half-life on the surface of the bone varies from 150 to 200 hours; but once inside the bone, and after the resorption cavity has been filled by the osteoblasts (see below), the bisphosphonates remain attached even for years.

Skeletal retention varies with the different bisphosphonates and a major factor in retention is the rate of bone turnover and the amount of bone surface available. This retention in bone is similar to that of substances such as tetracyclines, fluoride and strontium.

The uptake on the osseous surface appears to be the major determinant of the antiresorptive effect simply because osteoclasts cannot attach to bone covered by a layer of bisphosphonate. *The bisphosphonates are also taken up by the joints and therefore may decrease bone resorption and cartilage degradation in disorders of the joints such as rheumatoid arthritis.*

The prolonged surface attachment of bisphosphonates explains their extended duration of action. The earliest pharmacologic effect is manifest 24 hours after administration and lasts for 2 to 3 weeks after a single dose. After longer periods of administration the effect lasts for 2 to 3 months. There is no evidence that bisphosphonates within the bones retain any pharmacologic activity or exert any harmful effects on the "quality" of the bone involved. Such bone can be resorbed normally even many years later and the bisphosphonates within the bone released.

Following resumption of bone remodelling at previously exposed sites, the incorporated bisphosphonate will be liberated once from the hydroxyapatite crystals, but the fate of this locally released compound is uncertain (Papapoulos 2006). While some will enter the circulation and will appear in the urine, it is not known whether and to what extent the released biphosphonate will be active for the suppression of bone resorption. In all studies with alendronate, risedronate and pamidronate, cessation of bisphosphonate treatment given for 2 to 7 years was not associated with a rebound increase in bone turnover and rapid bone loss, as it occurs after stopping hormone therapy. *These results support the hypothesis that some of the embedded bisphosphonate that is released later is active again at the bone surface* (Landman et al. 1995).

It should be born in mind that different bisphosphonates have different affinities for bone, which influence their activities at the time of initiation, duration, and termination of administration. Moreover, the total amount of bisphosphonate retained in the body varies widely and is related to many factors including type, mode of administration, duration of treatment and others.

Soft tissues and internal organs are only briefly exposed to bisphosphonates in the blood because of their rapid uptake by bone. Occasionally, bisphosphonates may be deposited in organs such as the liver and spleen but only in very small quantities – about 2% of the absorbed dose. The bisphosphonate complexes are then taken up by macrophages of the reticuloendothelial system and excreted. This extra-osseous deposition occurs only with high doses and rapid intravenous infusions.

Renal Clearance

Renal clearance of bisphosphonates is accomplished by glomerular filtration as well as active tubular excretion. Bisphosphonates are passively bourne by the blood stream to the kidneys, the quantity depends on the concentration gradient of the bisphosphonate in the blood. Bisphosphonates released from the surface of bone (T½ 150–200 h) also reach the kidneys by way of the blood stream and are actively eliminated by the proximal tubules.

The process of elimination varies for each of the bisphosphonates as it depends on the properties of their side chains which also determine the different half-lives of the bisphosphonates. *This elimination is supported by evidence for a tri-phasic excretory pattern*:

- T ½ alpha: rapid distribution of the infused bisphosphonates onto bone (and very little elsewhere), with concurrent renal elimination
- T ½ beta: elimination from the blood stream by way of the kidneys
- T ½ gamma: release from the osseous surface, renal elimination

Consequently, excretion of bisphosphonates given by intravenous infusion is multi-phasic – a fast bi-phasic elimination from the blood stream, followed by a lengthier phase with a final elimination half-life of several days. *Even after administration of a number of doses, accumulation in the plasma does not occur. The total body plasma clearance is 7.8 l/h for ibandronate, and 5.0 l/h for zoledronate.*

About half of the amount of bisphosphonate given at any time is excreted unchanged by the kidneys within 24 hours. This renal clearance is dose-dependent. Bisphosphonates have a negative charge so they are only minimally filtered by the glomerular membranes which also have a negative charge. In experiments with rodents, most of the absorbed bisphosphonate was eliminated by active excretion through the proximal tubules, and only about 15% by glomerular filtration.

In the event of very high doses of bisphosphonates (concentrations of more than 4000 ug/min/kg) glomerular excretion can rise to about 54%. The passage from the plasma into the cytoplasma of the tubular cells is passive and depends

on the concentration of the bisphosphonates and their binding to plasma proteins, especially albumin. The transport of the bisphosphonate across the tubular membrane into the lumen is active and therefore requires energy and is limited (Fig. 3.8). The *half-life-time of the bisphosphonates in renal tissue* is very variable from 24 days (ibandronate) up to 200 days (zoledronate). *It is clear that these dif-*

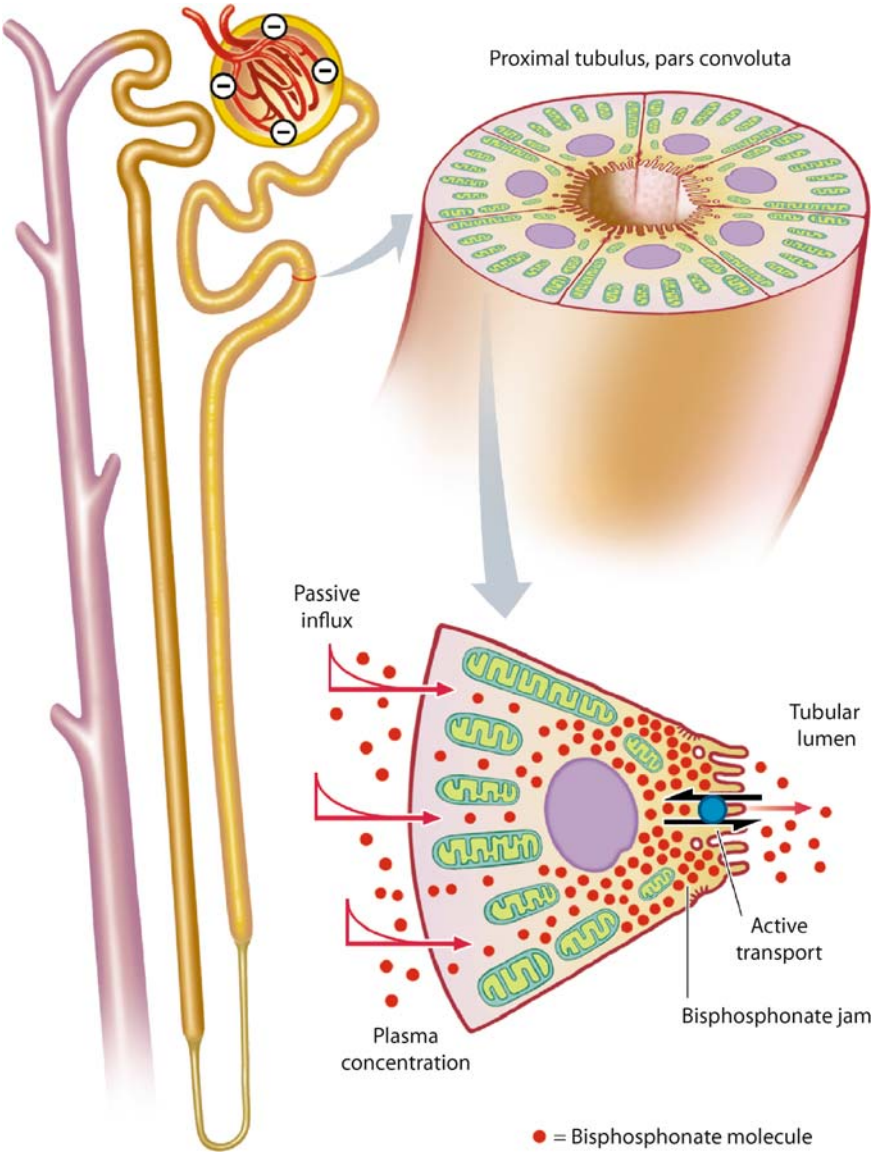


Fig. 3.8 Active elimination of bisphosphonate by renal tubular cells

ferences are responsible for differences in toxicity to the kidney, particularly if and when administration is repeated. These values should be taken into consideration when decisions are made concerning higher dosages and shorter time intervals.

The dose and half-life of the bisphosphonates given to patients with *renal insufficiency* and on hemodialysis must be carefully calculated individually for each patient. Patients whose renal function is limited to a creatinine clearance of 30 ml/min do not require a reduction in the amount of ibandronate administered. But with a lower creatinine clearance, the dose should be reduced from 6 mg to 2 mg. About a third of the dose given to patients on dialysis finds its way into the dialysate. Therefore, patients on dialysis should be given about a third to two thirds of the normal dose i.v. immediately on completion of the dialysis. Patients with renal insufficiency must be treated with special care. Recommendations of the manufacturer should always be followed. *In summary, when dealing with patients with impaired renal function the following precautionary measures apply:*

- Meticulous monitoring of renal function, including creatinine clearance.
- Increase time of the infusion to 1–2 hours.
- Increase the quantity of the infusion solution (cave overhydration).
- Reduce the dose to about 30% to 40% to attain the normal renal elimination rate.
- Check manufacturer's instructions for the particular bisphosphonate used.
- Administration of bicarbonate could be considered as required.

Actions of Bisphosphonates

Clinically bisphosphonates act almost exclusively on bone as outlined above. The mechanisms of action of the bisphosphonates include the following:

- *Inhibition of Crystallisation and Mineralisation*: The major physicochemical effects of the bisphosphonates on bone are decreased solubility of bone substance and changes in mineralisation because of their incorporation into hydroxyapatite crystals and into bone matrix. Due to their affinity for and adherence to solid-phase calcium phosphate, bisphosphonates inhibit the formation, aggregation and dissolution of crystals; but the aminobisphosphonates take up only 1/1,000 to 1/10,000 of the surface saturation capacity of the hydroxyapatite And this plays no part whatsoever in the clinical effects of the modern bisphosphonates. *Clinically significant inhibition of mineralisation with its attendant consequences of fractures and delayed healing simply does not occur. In addition, it is worth noting that the physico-chemical effects of the modern bisphosphonates are clinically insignificant.*
- *Inhibition of mineralisation* is however exploited clinically with the first generation etidronate for prevention and treatment of ectopic calcification. An additional future application of etidronate could be inhibition of calcification

of prosthetic heart valves. Etidronate decreases the experimental formation of kidney stones. It also inhibits plaque formation on teeth and therefore is incorporated in some tooth pastes. However, effective doses of etidronate inhibit normal mineralisation so that its use is strictly limited to the indications listed above.

- *Inhibition of bone resorption: Clinically the most important therapeutic action of bisphosphonates is inhibition of bone resorption, which commences within 1 to 2 days after administration, regardless of the route and frequency of administration, the total amount given determines the overall effect.* The reduction in bone resorption is accompanied by a positive calcium balance. The mechanisms of action of bisphosphonates in the inhibition of resorption are complicated and operate at both the cellular and molecular levels (Fig. 3.9). The target cells are osteoclasts and their precursors. At the biochemical level bisphosphonates interfere with the mevalonate pathway by inhibiting formation of the lipid chains of prenylated proteins and thus also with metabolism of steroids. Bisphosphonates inhibit the formation of lipid chains of prenylated proteins. While statins effect the synthesis of mevalonic acid by inhibition of HMG-CoA-reductase, the bisphosphonates interfere with the earlier phases of prenylation and of steroid synthesis (Fig. 3.9). The following steps in the process of mevalonic acid synthesis are clinically relevant and are targets of the bisphosphonates:
- *The first generation bisphosphonates* (Fig. 3.10) – together with adenosine monophosphate they form an ATP analogue (for example APPCCL2P) which cannot be hydrolised and thereby withholds the energy required for the synthesis of isopentenyl-pyrophosphate.
- *The second generation bisphosphonates* (Fig. 3.11) – these prevent the enzymatic switch of Dimethylallylpyrophosphate (DMAPP,C5-building block) to Geranyl-Pyrophosphate (GPP,C10-building block). The linear formulae demonstrate the steric likeness of ammonium bisphosphonate to the DMAPP-Carbocation stabilised within the enzyme.
- *The third generation bisphosphonates* (Fig. 3.12) – these additionally block the next step in the enzymatic reaction, i.e. conversion of Geranylpyrophosphate to Farnesylpyrophospate (FPP,C15) or to Geranylgeranylpyrophosphate (GGPP,C20). In this instance also, the linear formulae of the ammonium bisphosphonates demonstrate the steric likeness to the GPP-Carbocation which in turn enables competitive inhibition of the enzymic activity.

Small proteins, such as the GTPases, attach themselves to the cellular membrane with the help of the Farnesyl- and the Geranylgeranyl side chains and send specific signals into the cell which regulate numerous cellular functions (Fig. 3.13). However, since these proteins do not possess lipid side chains they are not able to transfer the signals to the cell membrane. Consequently the cells become inactive, they loose their membrane-specific properties, and eventually induce programmed cell death, i.e. apoptosis (Fig. 3.14 and 3.15). Initially, this blockage takes place in the osteoclasts, due to their uptake of bisphosphonates from the osseous

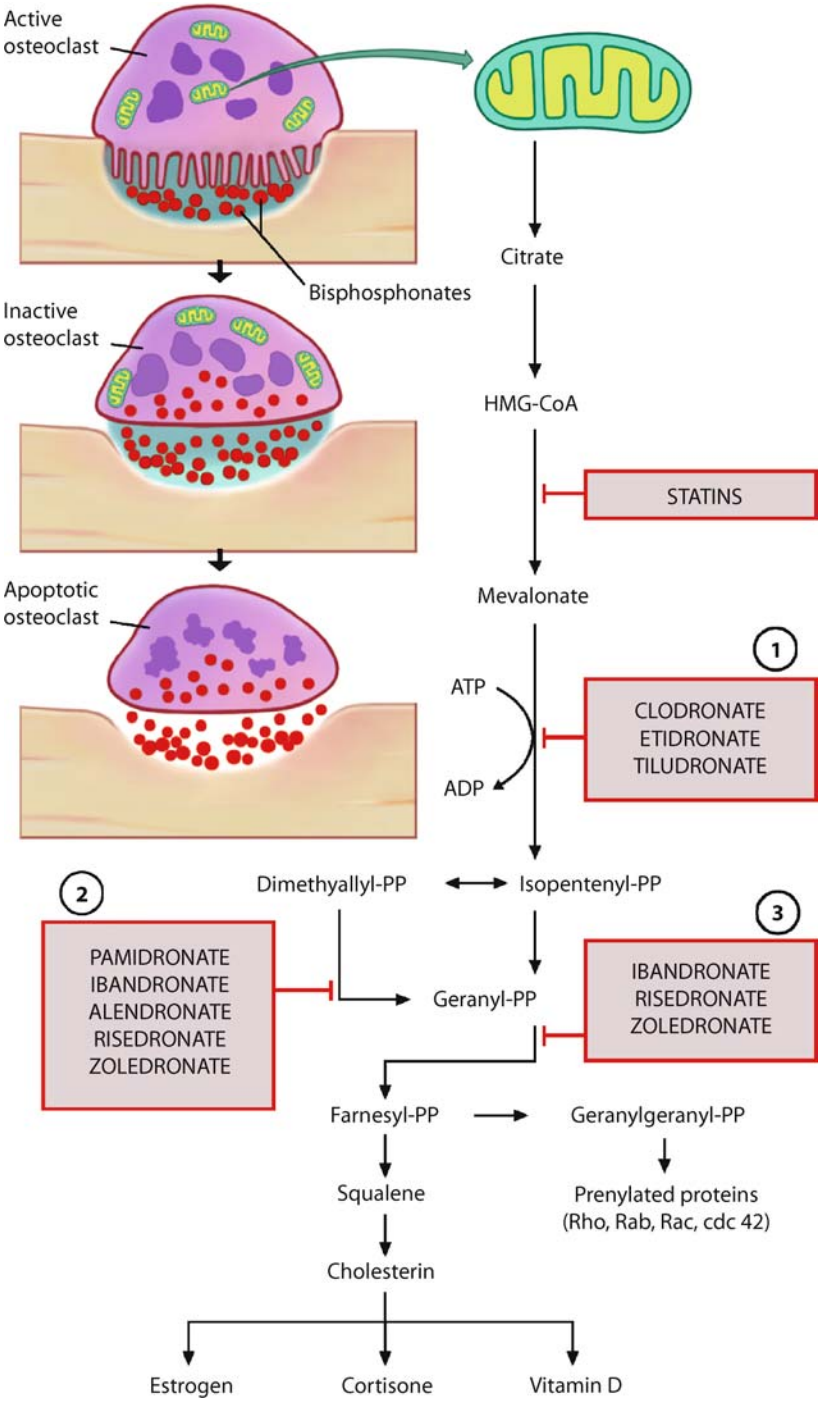

Active osteoclast
Bisphosphonates
Inactive osteoclast
Apoptotic osteoclast
Citrate
HMG-CoA
STATINS
Mevalonate
1
ATP
ADP
CLODRONATE
ETIDRONATE
TILUDRONATE
Dimethyallyl-PP
Isopentenyl-PP
2
PAMIDRONATE
IBANDRONATE
ALENDRONATE
RISEDRONATE
ZOLEDRONATE
Geranyl-PP
3
IBANDRONATE
RISEDRONATE
ZOLEDRONATE
Farnesyl-PP
Geranylgeranyl-PP
Squalene
Prenylated proteins
(Rho, Rab, Rac, cdc 42)
Cholesterin
Estrogen
Cortisone
Vitamin D

Fig. 3.9 Cellular and biochemical mechanisms of action of the nitrogen-containing bisphosphonates: Left: Layer of bisphosphonate (*red dots*) on bone beneath osteoclasts in resorption lacunae. The bisphosphonates are taken up by the osteoclasts which leads to their inactivation and retraction of the ruffled membrane. Higher doses lead to increased apoptosis of the osteoclasts. Right: Biosynthetic pathway for sterols and isoprenoids, which takes place in the cytoplasm of the osteoclasts. Steps of inhibition by statins and bisphosphonates. HMG Co-A = 3-hdroxy-3-methylglutaryl-Co-A, PP = pyrophosphate. 1, 2 and 3 shows the different generations of bisphosphonates each with its own specific targets. Effects of the 2nd and 3rd generation lead to an accumulation of isopentenyl-PP, which in turn stimulates the acute phase reaction. However, this may be reduced by simultaneous administration of clodronate

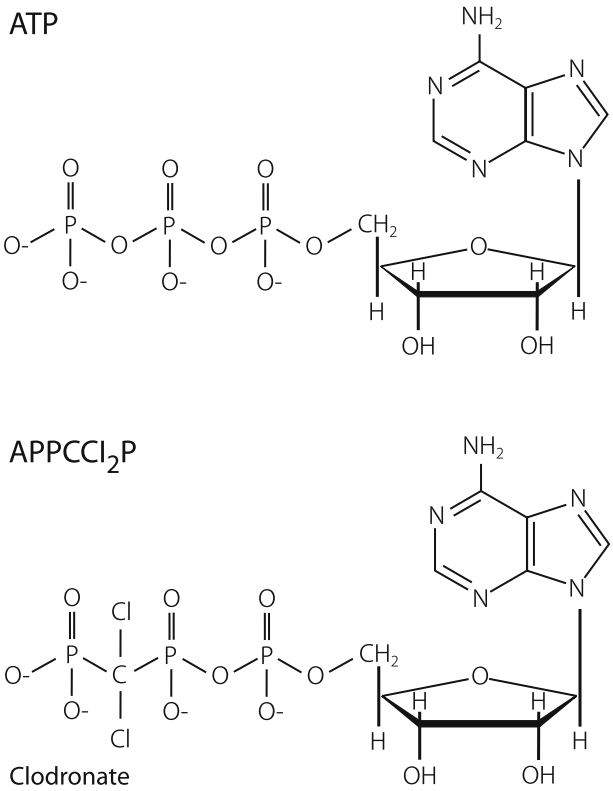


Fig. 3.10 First generation bisphosphonates: Formation of ATP-analogues

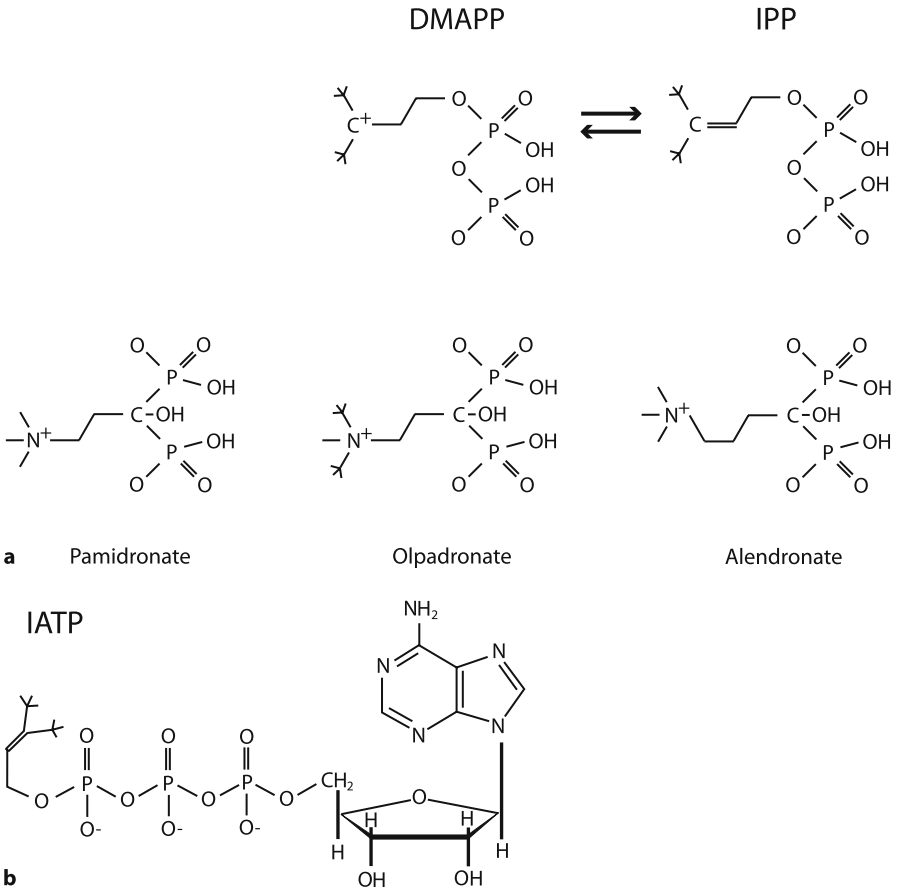


Fig. 3.11 **a** Second generation bisphosphonates: Competitive inhibition of dimethylallylpyrophosphate (DMAPP). **b** Reaction of IPP (isopentenyl-pyrophosphate) with AP (adenosin-phosphate) leads to IPPPA or IATP (isopentenyl-adenosintriphosphate). This substance triggers the release of caspases and thereby programmed cell death (i.e. apoptosis) of the osteoclasts or other macrophages

surface. Within osteoclasts, bisphosphonates cause many changes that affect their ability to resorb bone, such as loss of the ruffled border, disruption of the cytoskeleton and inability to migrate or bind to bone (Russell et al. 1999). Because of the inhibitory effect of nitrogen-containing bisphosphonates, there is an increase in the concentration of IPP, which in turn results in the formation of *isopentenyl ATP* by means of its reaction with AMP. *This combination triggers the excretion of caspases and thereby programmed cell death, i.e. apoptosis.* It should be stressed that the same process occurs in all cells in which bisphosphonates accumulate and it

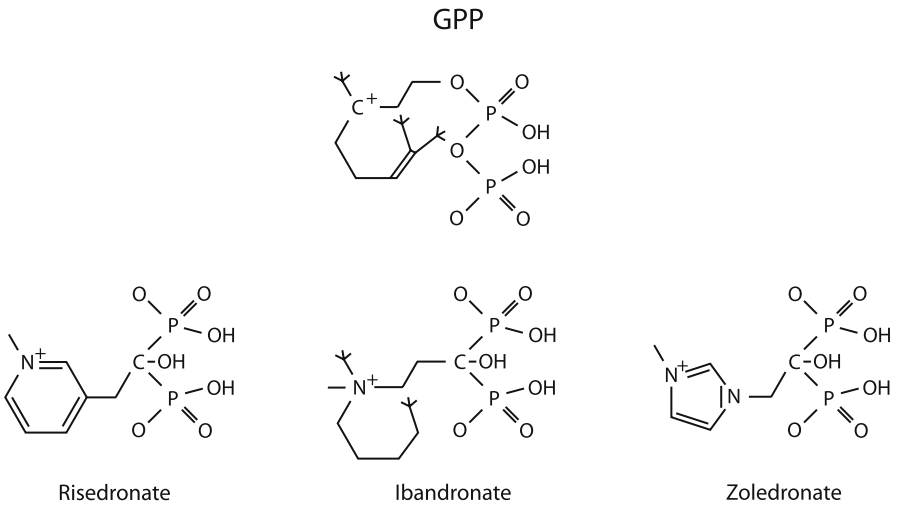


Fig. 3.12 Third generation bisphosphonates: additional competitive inhibition of geranyl-pyrophosphate (GPP)

is responsible for the (desired) effects as well as the (unwanted) side effects of the bisphosphonates. For example, excessive accumulation of bisphosphonates in the renal tubules results in apoptosis and in toxic damage to the renal tubules, leading to renal functional impairment.

Osteoclasts and their precursors are the target cells of the bisphosphonates. At the molecular level, effects such as the inhibition of protein-tyrosine phosphatases, as well as of cell growth and differentiation play important parts. Once inside the cell, bisphosphonates are able to inhibit production of acids, Proton-ATPase, lysosomal enzymes and prostaglandins. It should be stressed that given in sufficient dosages, bisphosphonates also act on tumor cells by inactivation of mevalonic acid metabolism and induction of apoptosis.

In summary, inhibition of osteoclastic resorption is accomplished by means of three different mechanisms corresponding to the 3 generations of bisphosphonates. At the molecular level, inhibition of tyrosine phosphatases (which participate in regulation of cell growth and differentiation) plays a significant role. Once inside the cell, bisphosphonates can inhibit the secretion of acids, proton ATPases, lysosomal enzymes and prostaglandins.

Direct Effects on Osteoclasts

- *Reduction of osteoclastic activity:* As soon as the bisphosphonates have entered the osteoclasts, their cellular activity decreases: synthesis of prenylated pro-

teins such as RAS, Rho, Rac and Rab stops, production of acids and enzymes is halted. Structural alterations of the cytoskeleton (actin, vinculin) can be seen on electron microscopy. Microtubules are depolymerised and the "ruffled membrane" is retracted. The levels of products of bone resorption in the serum are reduced, and the serum calcium concentration is lowered. The toxic damage to the osteoclasts can also be observed morphologically in 3 phases:

- *Inhibition of osteoclast adhesion:* The layer of bisphosphonates on the surface of bone prevents attachment of osteoclasts and thereby development of the appropriate acidic environment essential for resorption. Bisphosphonates are preferentially deposited on the osseous surface beneath the osteoclasts. In addition, as soon as the bisphosphonate has entered the osteoclast cytoplasm, it retracts its ruffled membrane.
- *Decrease in number of osteoclasts:* Bisphosphonates inhibit the proliferation of macrophages that are recruited and undergo fusion to become osteoclasts. This action is possibly mediated by TGFβ. Inhibition of osteoblastic recruitment of osteoclasts may also occur.
- *Induction of osteoclast apoptosis:* Bisphosphonates trigger apoptosis, that is premature cell death, by advancing the time of genetically programmed cell death (Fig. 3.13). This leads to a reduction in osteoclast numbers. The bisphosphonates vary considerably with respect to this action. For example, clodronate induces apoptosis after it has been metabolised and converted into the non-hydrolyzable ATP analogue AppCCl$_2$p. The nitrogen-containing bisphosphonates (N-BPs) induce formation of ApppI, an ATP-analogue, which evokes mitochondria-mediated apoptosis (Mönkkönen et al. 2006). The N-BPs exert their apoptotic effect by inhibiting the metabolism of mevalonic acid with subsequent modification of the prenylation of various intracellular proteins. *To*

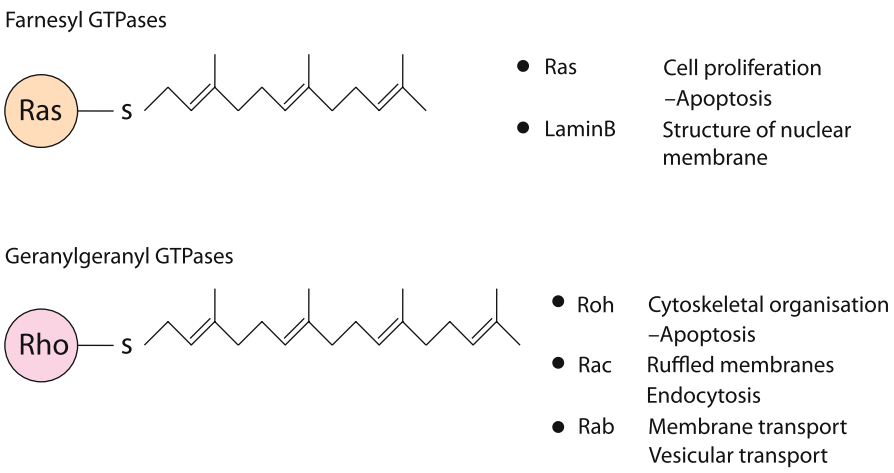


Fig. 3.13 The two most important membrane proteins inhibited by bisphosphonates and their functions

summarise: the bisphosphonates inhibit lipopolysaccharide and parathyroid hormone induced osteoclast differentiation, fusion, attachment, actin ring formation and activation, in simple terms, the whole process of resorption of bone.

Effects on Osteoblasts

It was recently shown that bisphosphonates stimulate osteoblasts to produce a factor (osteoclast resorption inhibitor, ORI), which inhibits osteoclast recruitment and activation (Fig. 3.14). *Bisphosphonates stimulate proliferation and osteogenic differentiation of bone marrow stromal cells and thus promote osteoblastic bone formation*; therefore the function of osteoblasts is influenced by bisphosphonates both directly and indirectly (Fig. 3.15). This was demonstrated in bone biopsies taken from patients with multiple myeloma under therapy with bisphosphonates. An increase in osteoblasts and osteoid seams was observed and confirmed by histomorphometry.

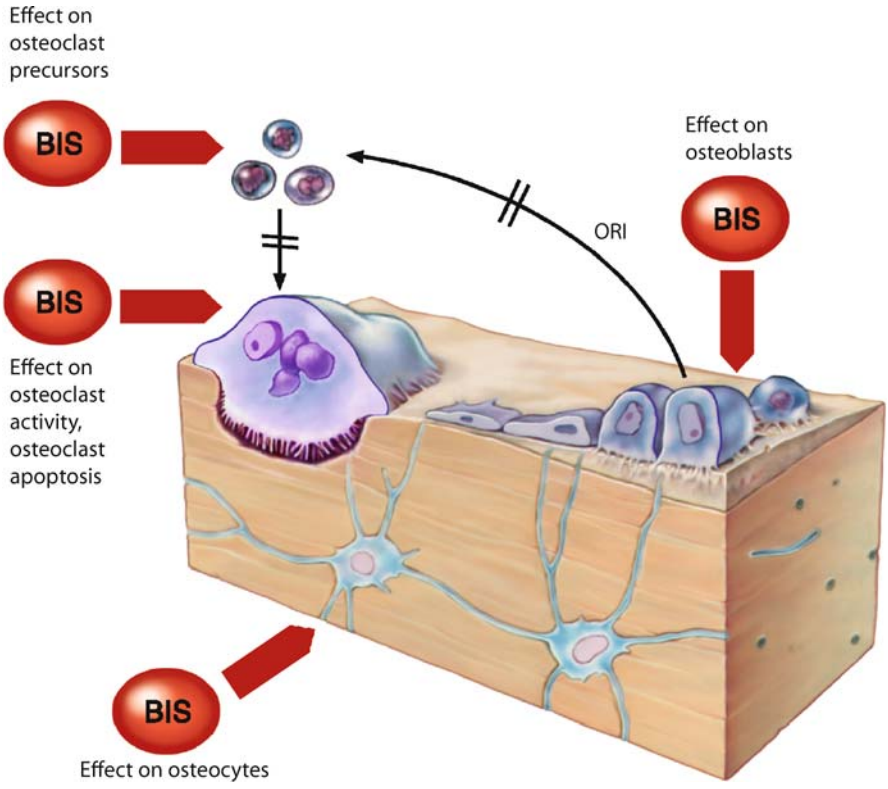


Fig. 3.14 The four most important cellular targets for bisphosphonates in the bone remodelling unit (BRU). *ORI* Osteoclast Resorption Inhibitor

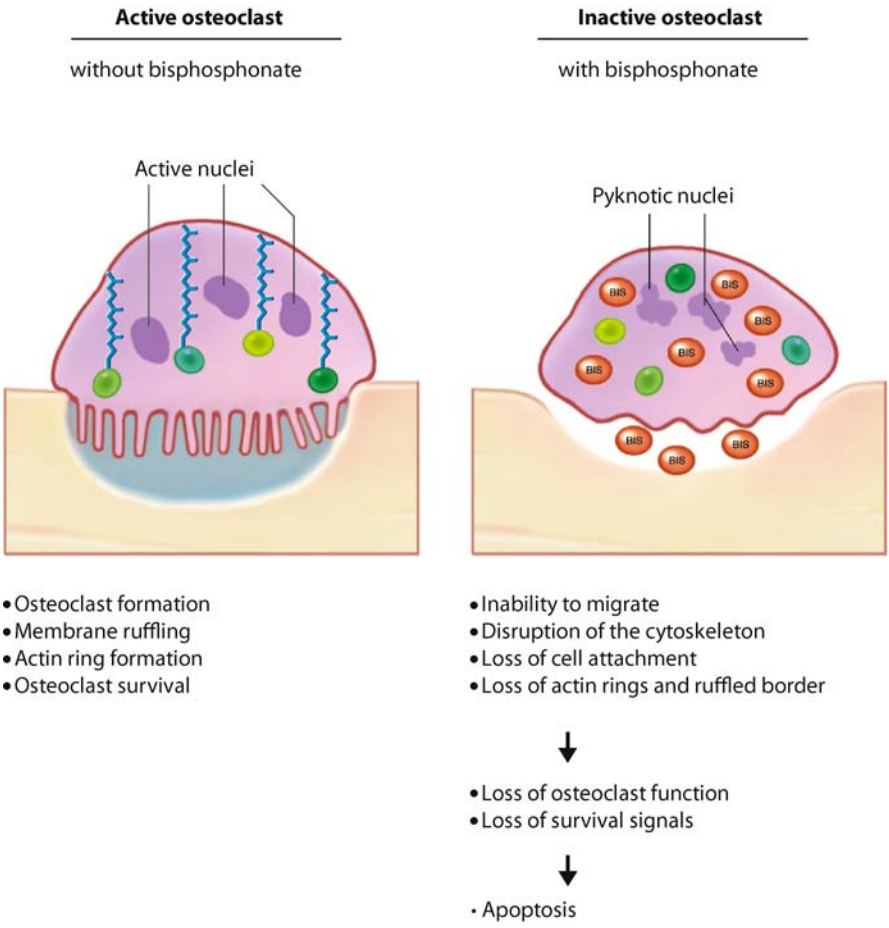


Fig. 3.15 Bisphosphonates inhibit the production of membrane proteins in the osteoclast causing its inactivation and apoptosis

Effects on Osteocytes

Few studies have dealt with the influence of bisphosphonates on osteocytes. It is well known that glucocorticoids have a negative effect on osteoblasts and osteocytes and that this may be mitigated by bisphosphonates, thereby increasing the pool of osteoblasts and osteocytes and exercising a positive influence on osteocyte function and on bone quality. The presence of bisphosphonates in osteocytes and in their canaliculi has been demonstrated by immunohistochemistry on sections of bone biopsies. Recent evidence suggests that the *inhibition of osteocyte apoptosis* by bisphosphonates is mediated through the opening of connection 43 hemichan-

nels and activation of extracellular signal-regulated kinases (Plotkin et al. 2005). More studies are urgently required to clarify the direct effects of bisphosphonates on the micro-architecture of bone, including the trabecular network, and on serologic estimates of osteocyte function.

Effects on Immune System

Some bisphosphonates (e.g. pamidronate) stimulate cytokine production by macrophages and other immunocompetent cells. There is also a significant decrease in the number of circulating lymphocytes, especially natural killer cells and T lymphocytes both CD4 and CD8 positive. This decrease is probably caused by an increase in acute-phase reactants such as C-reactive protein, IL-6 and TNFα. In contrast, ibandronate stimulates a moderate increase in lymphocytes within 10 hours, whereas clodronate has no apparent effect. *Afferent nerve fibres in bone may also be influenced by inhibition of release of neuropeptides and neuromodulators, which would explain the rapid analgesic effect of bisphosphonates on bone pain.*

Anti-angiogenic Effects

Both in vivo and in vitro studies have demonstrated the qualitative and quantitative anti-angiogenic actions of bisphosphonates, illustrated in bone biopsies of patients with multiple myeloma on therapy with bisphosphonates. The mechanism of endothelial cell inhibition presumably includes down-regulation of integrins and laminin receptors. Possibly negative actions on vascular endothelial growth factors (VEGFs) are also involved. This is indicated by the rapid decrease in the levels of these factors in the serum of patients shortly after administration of bisphosphonates. *Combinations with chemotherapeutic agents such as the taxanes increase the anti-angiogenic action of bisphosphonates.*

Effects on Tumor Cells

Interactions of tumor cells with the vascular system, the immune system and the bone marrow stroma are outlined in Fig. 3.16.

Bisphosphonates appear to slow down the rate of tumor growth by inhibiting intracellular signal transduction, which stimulates apoptosis, i.e. an antiproliferative effect. This apoptotic effect of pamidronate has been demonstrated in human myeloma cells. The inhibition of osteoclasts results in decreased IL-6 production and thereby release of growth factors from the bone matrix is also decreased. There are indications that bisphosphonates interfere with the establishment of osseous and probably also visceral metastases. Recent in vitro studies have highlighted the

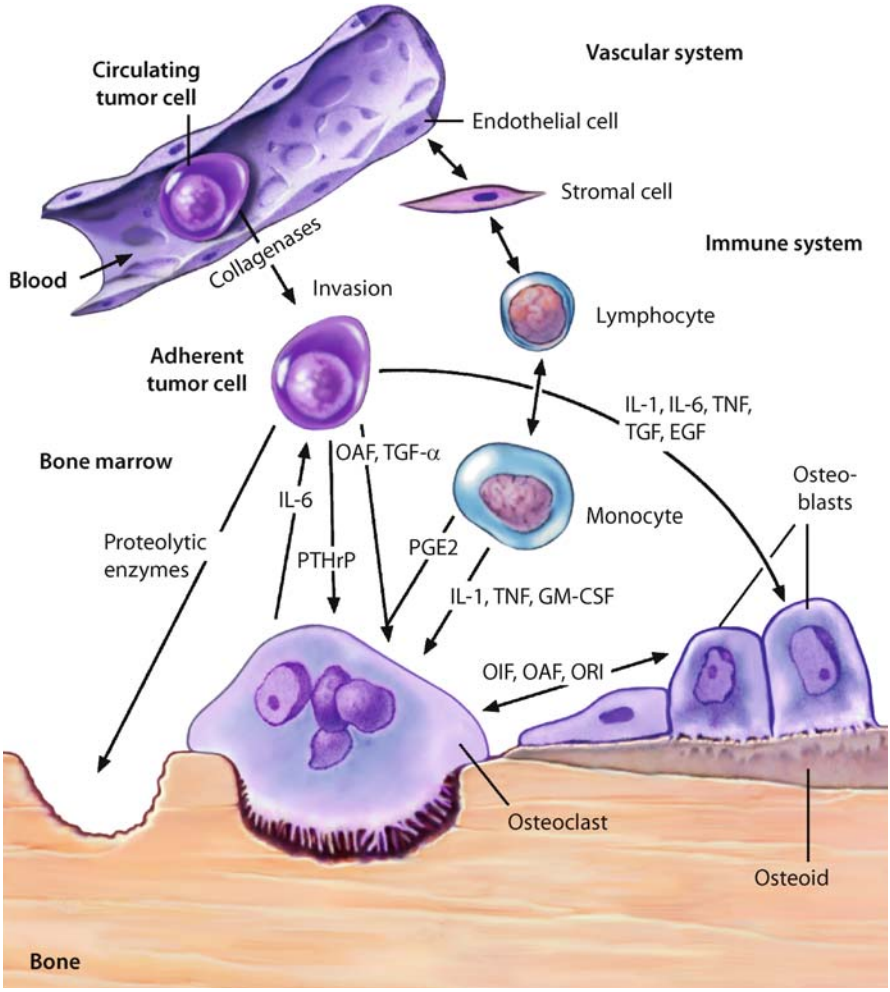


Fig. 3.16 Demonstration of interactions between tumor cells, blood vessels, bone, bone marrow, stroma and immune system

direct toxic effect of the modern bisphosphonates on tumor cells leading to their apoptosis. *Recently it was shown that nitrogen-containing bisphosphonates induce formation of a novel ATP analogue (ApppI), which evokes mitochondria-mediated apoptosis (Mönkkönen et al. 2006). This action may account for the direct antitumor effects of the nitrogen-containing bisphosphonates. They also prevent cancer adhesion to bone by their inhibitory effect on protein prenylation (Roelofs et al. 2006). In addition, as shown in these experiments, bisphosphonates together with standard chemotherapeutic agents induced a greater degree of toxicity and apoptosis of tumor cells than that achieved by chemotherapy alone.*

Effects on Protozoa

Bisphosphonates inhibit proliferation of trypanosoma cruzi, leishmania donovani, toxoplasma gondii and plasmodium falciparum. This has been demonstrated by studies in vitro and in animals, indicating the potential of bisphosphonates for treating parasitic protozoan infections responsible for major social hardships and economic losses in countries in which these parasites are endemic. One of the actions of bisphosphonates in these organisms is inhibition of sterol synthesis at the pre-squalene level, thereby inhibiting their proliferation.

Effects on Arterial Calcification

The bisphosphonates alendronate and ibandronate inhibit calcification of arteries and heart valves at doses comparable to those that inhibit bone resorption, as shown in experiments with rats. These results support the hypothesis that arterial calcification is linked to bone resorption. The mechanism of this linkage remains to be established; results of clinical trials have not yet been reported. New data have shown that the RANK/RANKL/OPG-system plays an important role in the linkage of osteoporosis and arteriosclerosis.

Effects on Fracture Healing

Animal experiments had previously shown that high doses of etidronate interfered with the healing and mineralisation of fractures. This does not apply to the modern aminobisphosphonates, which can be taken without risk by patients with fractures. In addition, animal experiments have shown that under therapy with these bisphosphonates

- the formation of callus as well as its calcium content were increased,
- the disruption of the healing process did not occur, and
- the final weight-bearing capacity of the healed bone was not reduced.

Effects on fractured bone in special conditions, e.g. osteogenesis imperfecta are dealt with in the appropriate chapters. *To summarise: bisphosphonates can safely be given to patients with osteoporosis who have sustained a fracture. In cases with severe osteoporosis and/or multiple fractures anabolic agents are preferable as first-line therapy followed by bisphosphonates, (see appropriate chapter).*

Effects on Resorption of Cartilage

Some of the modern bisphosphonates are able to suppress local resorption of cartilage. Moreover, it has been demonstrated that the inflammatory reactions ac-

companying artificially induced arthritis could be suppressed by bisphosphonates; thereby preserving the architecture of the joint involved. These encouraging experimental results have lead to clinical studies of bisphosphonates in patients with osteochondrosis and osteoarthritis; results of which are awaited.

Structure-Related Actions

Initial studies in the 1960s to explain the action of bisphosphonates on bone resorption focused on their physicochemical effects. However, it became apparent that these could not explain the antiresorptive action of bisphosphonates which was rather due to their cellular effects. It was demonstrated in the 1990s that bisphosphonates act mainly on osteoclasts and induce their apoptosis. This is achieved either by intracellular formation of a toxic ATP analogue (*first generation, bisphosphonates without a nitrogen functionality*) or by inhibiting the enzyme farnesylpyrophosphate synthase of the mevalonic acid metabolic pathway and subsequently the prenylation of small GTPase signalling proteins that leads to inactivation of the osteoclasts (*second and third generations, nitrogen-containing bisphosphonates*). Numerous bisphosphonates have been developed over the past 30 years. Their antiresorptive activity is 20,000 times greater than that of etidronate. They differ from each other with respect to the ligands bound to their carbon atoms.

Substitutions at R_2 determine the antiresorptive and antiproliferative activity – the *bioactive moiety;* while substitutions at R_1 determine the binding site for attachment to the bone: the *bone hook* (Fig. 3.17). The R_1 moiety confers additional

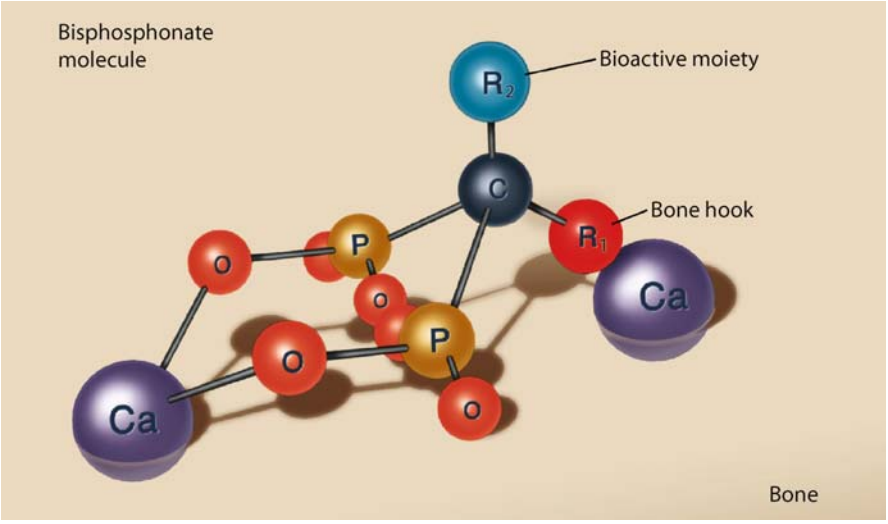


Fig. 3.17 Spatial structure of bisphosphonate binding to the surface of bone

binding activity, for example, replacing a hydrogen atom by a hydroxyl group at R_1 increases the affinity for hydroxyapatite by about two-fold. However, differences in bisphosphonate binding affinities suggest that the nature of the R_1 moiety may not be the sole determinant of binding ability. Derivatives with one amino group at the end of the side chain are particularly active. The introduction of nitrogen components such as primary and tertiary nitrogens or heterocyclic rings at the R2 position increased the antiresorptive potency of biphosphonates by up to three orders of magnitude compared to that of non-nitrogen containing bisphosphonates (e.g. etidronate or clodronate). However, it is not only the presence of the nitrogen atoms that is important but also their position in the molecule since potency can differ by >700-fold between isomers of the same bisphosphonate. According to the *molecular mechanism of action* two groups of bisphosphonates can be distinguished:

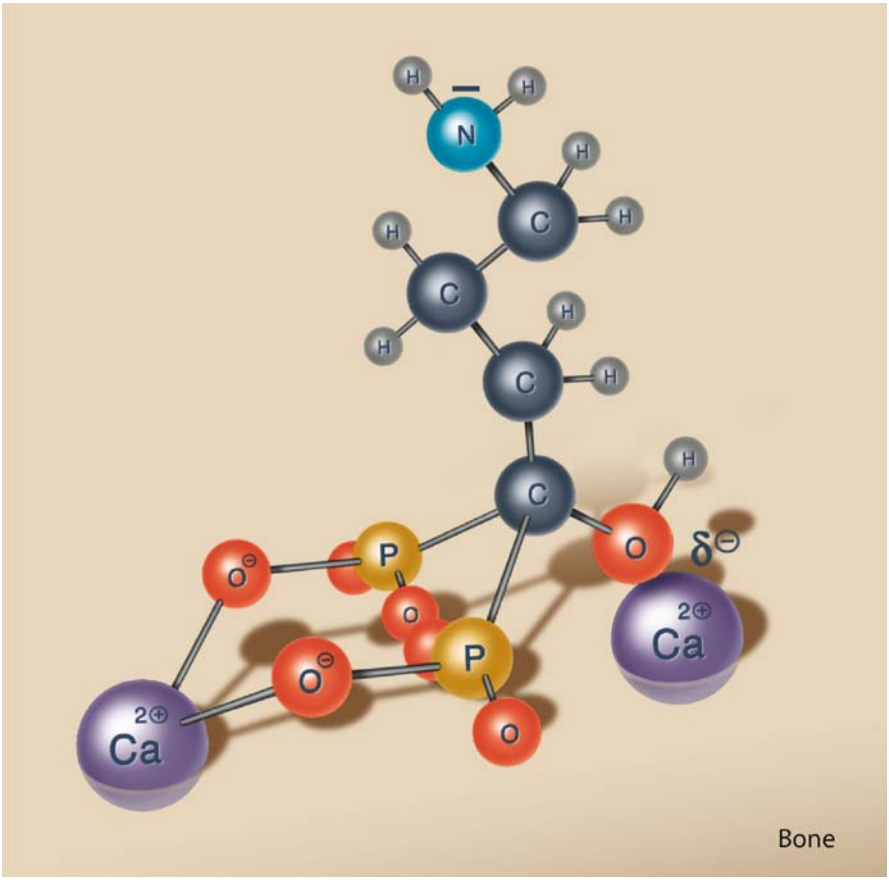


Fig. 3.18 Spatial structure of alendronate (second generation bisphosphonate) on the surface of bone

- *Bisphosphonates without nitrogen:* These are metabolised within the cell to form cytotoxic ATP analogues, which inhibit the mevalonate pathway in osteoclasts. This group comprises the first generation bisphosphonates: etidronate, clodronate and tiludronate.
- *Bisphosphonates with nitrogen* (Figs. 3.18 and 19): These inhibit mevalonate metabolism and prenylation of proteins. Ibandronate (Fig. 3.19) primarily inhibits the enzyme squalene synthase. The lack of prenylated proteins within the osteoclast leads to structural changes such as dissolution of the ruffled membrane, so that the osteoclast cannot function. All the data available indicate

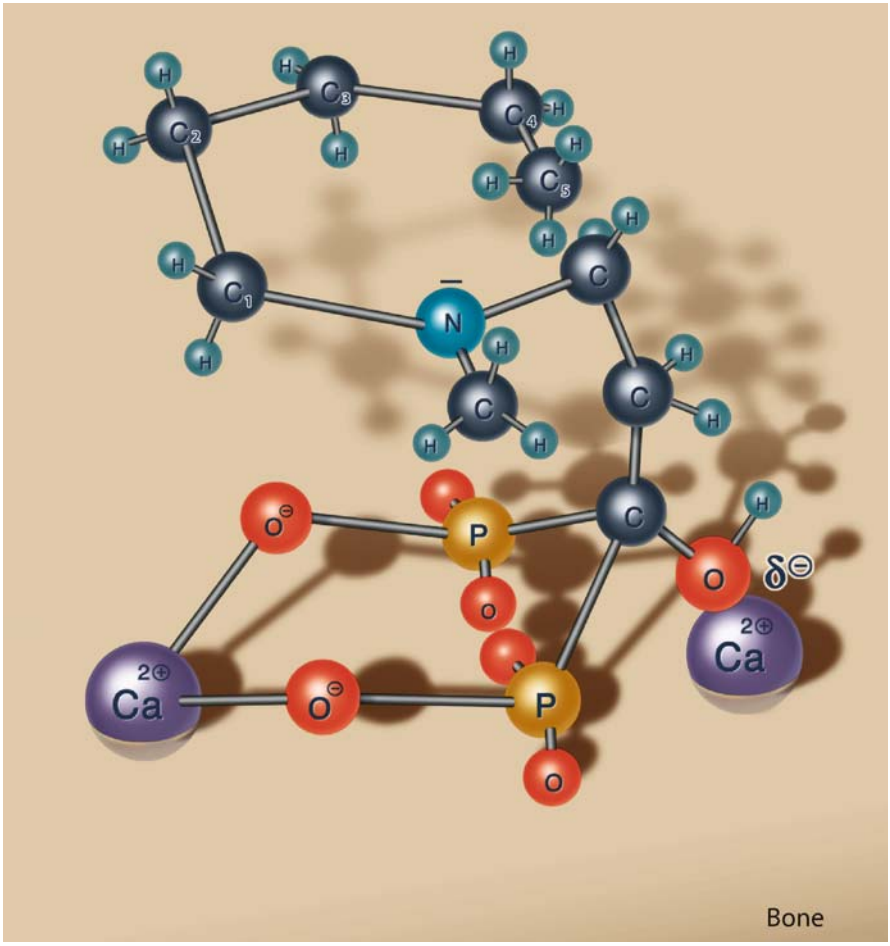


Fig. 3.19 Spatial structure of ibandronate (third generation bisphosphonate) on the surface of bone

that farnesyl diphosphate synthase is the major pharmacological target of the nitrogen-containing bisphosphonates in vivo, and that small changes to the structure of the R_2 side chain alter antiresorptive potency by affecting the ability to inhibit farnesyl diphosphate synthase (see also Fig. 3.9). The order of potency inhibiting farnesyl diphosphate synthase in vitro is closely matching the order of antiresorptive potency in vivo: zoledronate > ibandronate > risedronate > alendronate > pamidronate (Dunford et al. 2001). This group comprises both second and third generation bisphosphonates: risedronate, alendronate, pamidronate, olpadronate, ibandronate and zoledronate.

Both mechanisms, i.e. intra-cellular metabolic inhibition and structural alterations, induce apoptosis of osteoclasts and tumor cells (e.g. myeloma cells) as well as inhibiting proliferation of various microorganisms (e.g. trypanosoma cruzi).

Side Effects

The bisphosphonates are well tolerated. Their side effects are few and rarely significant (see below). Nevertheless, patients must be informed about possible complications and asked to report occurrence of any side effects and complaints during the course of treatment, particularly if the bisphosphonate prescribed has not yet been authorised for that particular indication. In such a situation, the patient's informed consent must be obtained in writing before the initiation of treatment. In addition, also before starting therapy, the patient must be examined, especially the oral cavity, and basic investigations must be carried out. These should include kidney and liver function, complete blood count, and levels of calcium, magnesium, phosphate and alkaline phosphatase in the serum.

Relatively minor and usually transient side effects which occur mostly after i.v. administration include: "flu-like" symptoms and bone pain 9%, fever 7%, fatigue 4%, occasionally arthralgia and myalgia 3%. Should significant side effects occur, these may warrant a change in the mode of administration or type of bisphosphonate. The most clinically important side effects are considered in detail below. Before treating children (for example suffering from osteogenesis imperfecta) written consent of the parents and of the responsible ethics committee must be obtained.

Hypocalcemia

This indicates acute toxicity due to formation of bisphosphonate-calcium complexes, which cause a drop in the serum calcium concentration. Usually it is transitory and does not cause symptoms. When bisphosphonates are administered intravenously, especially in high doses, it is important to monitor the speed of infusion. Clinically relevant hypocalcemia has been observed after rapid infusion of

high doses and with concomitant administration of aminoglycosides; both these substances may cause long-lasting hypocalcemia and they should not be given together. *Bisphosphonate-induced hypocalcemia has also been associated with vitamin D deficiency, especially in seriously ill and older patients.* Hypomagnesemia may also occur by similar mechanisms, i.e. due to the binding of bisphosphonate to magnesium cations.

Disturbance of Mineralisation

Etidronate given at high doses (>5 mg/kg body weight daily) for prolonged periods (>6 months) can cause osteomalacia. Mineralisation is usually normalised within 3 months after discontinuation of the bisphosphonate. However, osteomalacia can be avoided by administration of calcium and vitamin D concomitantly with the etidronate, so that even very long treatment for example with 400 mg etidronate daily for 2 weeks every 3 months does not induce any significant inhibition of mineralisation. *With the latest bisphosphonates (3rd generation) this side effect no longer occurs and is not seen in bone biopsy sections. In fact, a wider than normal osteoid seam denotes increased bone formation indicating that patients on bisphosphonates should always be given vitamin D and calcium.*

Gastro-intestinal Side Effects

Mild gastrointestinal side effects may occur when bisphosphonates are taken orally. These include diarrhea, nausea, bloating, gastric pain and other uncharacteristic abdominal complaints, which had previously been reported in 2–10% of patients. However, large placebo-controlled studies have not confirmed these reports. In one case of ulcerative esophagitis, the patient had received a nitrogen-containing bisphosphonate. *It should be emphasised that such serious side effects can occur only if:*

- the patient and doctor overlooked a reflux esophagitis or other similar pathologic conditions,
- the patient swallowed the tablets with too little water (minimum 200 ml recommended),
- the patient lay down within 30 minutes of taking the medication,
- the patient continued to take the tablets after symptoms of esophagitis had occurred.

This example highlights the importance of making all patients aware of exactly how these tablets should be taken. No cases of esophagitis have yet been reported with the latest tablets of 70 mg alendronate, taken once a week. The effects of the bisphosphonate on the bones have remained the same (that is, effects of daily or weekly inges-

tion). It is worth noting that monthly tablets have already become available and are equally effective.

Acute-Phase Reactions

The day after aminobisphosphonate infusion, 20–40% of all patients experience fever and lymphocytopenia as well as a rise in C-reactive protein, in IL-6 and in TNFα. These patients experience flu-like symptoms such as headache, bone and joint pains and fatigue. *The reactions begin 10 hours after the first infusion, last only 1 to 2 days and do not leave any long-term side effects. Symptomatic therapy can be given, but is rarely required.*

Elderly patients with cardiac insufficiency have reported occasional cardiac irregularities, but these paroxysmal disturbances in cardiac rhythm were never severe enough to require treatment. Generally speaking, an acute-phase reaction occurs only after the first infusion, rarely after the second and then is very mild. More such reactions have been observed after treatment of patients with CRPS (Sudeck's disease) and patients with asthma who are sensitive to aspirin: they occur less frequently in patients with osteoporosis and very rarely in patients with malignancies. It is prudent therefore to give lower doses to vulnerable patients when first starting treatment and to monitor the patients for several hours after completion of the infusions.

Renal Side Effects

In the past, rapid infusion (or injection) of large amounts of etidronate or clodronate led to acute renal failure, which is also a danger when hypercalcemia is accompanied by dehydration. Insoluble complexes formed in the blood most probably caused the impairment of renal function. Consequently, intravenous administration of bisphosphonates should be slow and considerably diluted. Highly potent bisphosphonates such as ibandronate are effective even at a dosage of 2 mg, which can easily be administered and which, so far, has not caused any significant renal complications. This was clearly shown in patients with breast cancer given infusions of 2–6 mg ibandronate. Minimal, short-term excretion of protein has also been observed in some patients. A low blood volume, for example in patients with multiple myeloma, must always be corrected before intravenous infusion of bisphosphonates. Moreover, it is particularly important to remember that patients with multiple myeloma are especially vulnerable to renal complications because of the possibility of the presence of light chains, paraproteins and deposition of amyloid in the kidneys, so extra care is required.

Renal insufficiency per se is not a contraindication for bisphosphonates. Administration of pamidronate is not limited by renal insufficiency. Ibandronate, on the other hand, should only be given to patients with creatinine values up to 5 mg/

dl. It is advisable to reduce the dose and prolong the infusion time in patients with renal insufficiency. In patients on hemodialysis, the dose should be reduced by 25%, and the different half-lives of the bisphosphonates must be taken into account (pamidronate 1 hour, ibandronate 10 to 16 hours).

No renal toxicity has been reported following oral ingestion of bisphosphonates, probably because of their minimal and slow absorption in the gastro-intestinal tract.

Bisphosphonates may cause a rise in plasma phosphate together with an increase in renal tubular reabsorption of phosphate, but this effect is not associated with any clinical problem.

Ocular Side Effects

Isolated cases have been reported of ocular side effects including visual disturbances in patients taking aminobisphosphonates, especially pamidronate. These effects included inflammatory reactions, such as *conjunctivitis*, *scleritis*, *episcleritis, uveitis* and even *retinitis*. *Any patient with a "red eye" or complaints of visual disturbances should immediately be sent for specialist examination.* These inflammations are generally unilateral and reversible after discontinuation of intravenous bisphosphonates, but may recur when the infusions are resumed. In one patient with unilateral uveitis, the condition slowly regressed under therapy with prednisone and atropine eye drops. In another patient with unilateral scleritis, the symptoms fully resolved under therapy with prednisone.

Central Nervous System (CNS)

Toxicity within the CNS is an extremely rare side effect, which may be expressed by the patient as "hearing voices in the head" and "as colored visual disturbances" (not connected to inflammatory manifestations in the eyes). *Visual, olfactory and auditory hallucinations* have also been reported after therapy with pamidronate.

Hematopoietic Side Effects

Effects on hematopoietic cells have rarely been observed, even after long-term bisphosphonate therapy. Occasionally, anemia or other cytopenias may be observed after zoledronate, but these are very rarely symptomatic.

Other Side Effects

Ototoxicity has been reported in a few isolated cases during pamidronate therapy, the cause is not known. Unilateral deafness was observed in one patient with osteogenesis imperfecta (OI) while on pamidronate therapy, but as this may also

occur in patients with OI who are not receiving any therapy there may not be a causal relationship.

Clodronate and etidronate have been known to trigger *asthmatic attacks* in patients with asthma who are sensitive to aspirin. Allergic *skin rashes* have also been reported, and rapid, highly concentrated infusions may cause *local phlebitis*. Infusions of bisphosphonates may cause a *transient loss or alteration of taste (metallic taste)*, observed in about 5% of the patients.

Osteomyelitis/Osteonecrosis of the Jaw Bones

These side effects were first reported in a single patient in 2003, and the literature reviewed in 2004 and 2005 (Fig. 3.20a,b). The following significant points were recognised: almost all cases reported had previously experienced a dental complication such as extraction of a tooth (or teeth), undergone a dental implant, had a buccal infection, or were receiving some other form of generally invasive dental treatment. *In one study, 56/63 of the patients were under long term therapy with bisphosphonates: pamidronate or zoledronate i.v.* The remaining 7 patients were taking oral bisphosphonates. All the patients were suffering from pain, non-healing extraction wounds, exposed bone with sequestration, and inflammatory reactions in the mouth. The lesions were refractory, i.e. did not respond to antibiotics. *All patients had malignancies: 44% multiple myeloma, 32% breast cancer, and 5% prostatic cancer (Ruggieri et al. 2004). So far, a satisfactory elucidation of the mechanism of the necrosis of the jaw bones has not been given*. The following possibilities have been put forward:

- Microfractures in heavily burdened jaw bones
- Anomalies of the vascular system in the jaws
- Infectious inflammatory processes during immuno-suppression
- Anti-angiogenic effects of bisphosphonates leading to local necrosis
- Inhibitory effect on local physiological bone remodelling
- Enhancement of inflammatory/necrotizing processes (caused by prior chemotherapy and corticosteroids) already present before administration of bisphosphonates

However, these explanations are only speculative. Histologic investigation of necrotic material obtained by bone biopsies taken from the jaw bones of 15 patients demonstrated vascular and inflammatory reactions as well as ostoclastic remodelling typical of a subacute to chronic abacterial osteomyelitis. Presence of bacteria was never documented. However, there were also clear signs of necrosis of bone, soft tissues and bone marrow, as well as the presence of newly formed bone containing osteocytes and showing cement lines. Practically all the cases reported so far, have implicated i.v. therapy with pamidronate or zoledronate. Only isolated instances have been reported of patients receiving a different bisphosphonate (i.e. ibandronate) because of a malignant condition or osteoporosis. *In the vast major-*

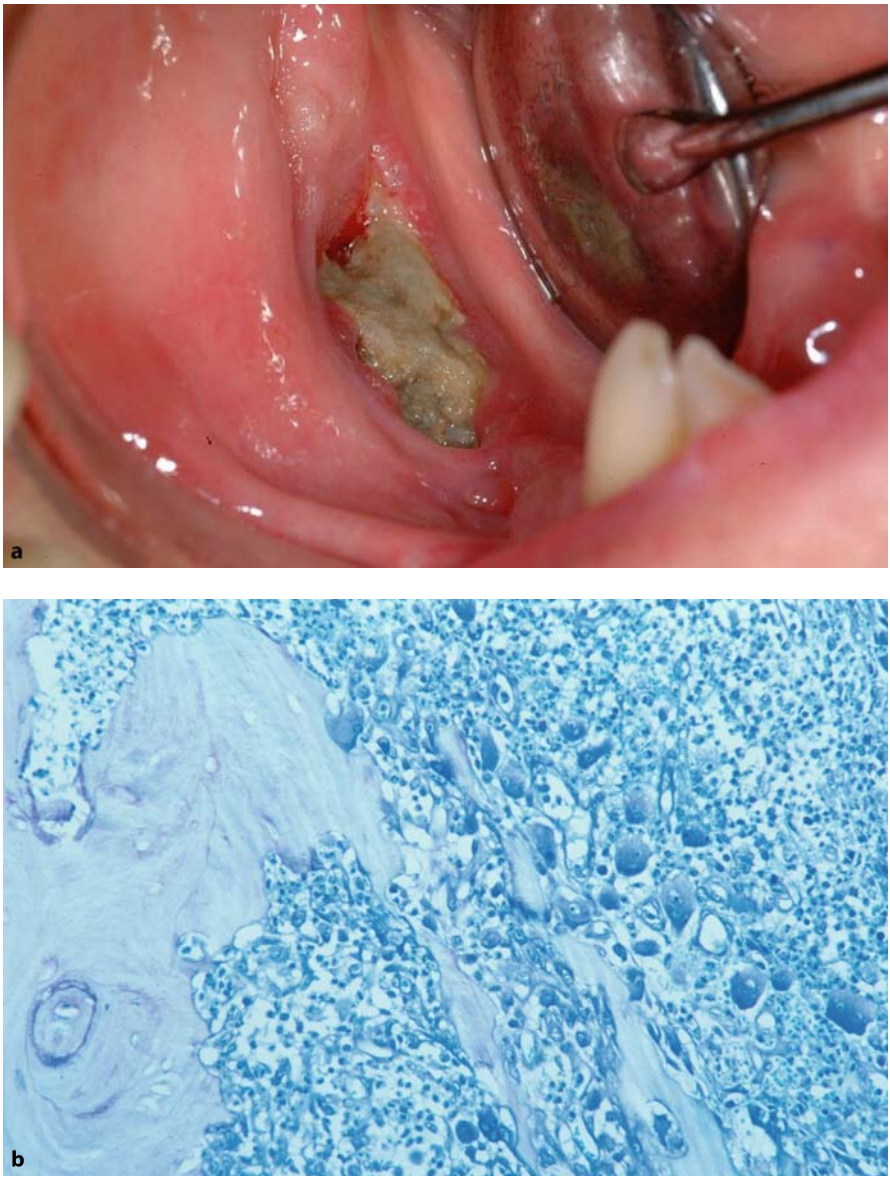


Fig. 3.20 **a** Osteonecrosis of the jaw in a patient with breast cancer, 1 year after therapy with zoledronate 4 mg monthly, **b** Histology from the necrotic jaw of the same patient showing chronic osteomyelitis with necrotic bone and osteoclastic bone resorption, and increase of large macrophages, plasma cells, lymphocytes and granulocytes in the surrounding bone marrow (Giemsa 100x, plastic embedded biopsy)

ity of the cases the occurrence of necrosis of the jaw bone was preceded by an invasive dental intervention, for example extraction of a tooth or teeth.

As outlined above, many speculative theories have been proposed, and intensive investigations are underway. But, more to the point, specific and detailed guidelines for the prevention, diagnosis and therapy have been published (2004), and one of the manufacturers (Novartis) has also sent round a circular outlining recommendations for the administration of zoledronate (Zometa®). It must be clearly stated that these cases emphasise the importance of a careful medical history and physical examination before starting treatment with bisphosphonates, especially with the new potent ones given i.v. long term and in patients with malignancies who possibly had already received, or were currently on chemotherapeutic protocols, possibly containing glucocorticoids. Only when the above precautions have been taken will it be possible to institute the preventive measures now recommended. *It is also worth while pointing out that international studies have clearly demonstrated that in more than 20 million patient years of oral therapy with bisphosphonates such problems have not been reported.*

Long Term Side Effects

Patients have now been treated with bisphosphonates for over 12 years without any recorded long term side effects. A single report has recently appeared expressing concern on the possibility of potential over-suppression of bone turnover during long term use (Odvina et al. 2004) so far this has not been confirmed. In a few patients *fracture healing* was delayed and both osteoclastic and osteoblastic activities were reduced. These observations stress the need for continued monitoring of patients on bisphosphonate therapy.

Non-traumatic Avascular Necrosis of the Femoral Head

This condition has been recognised for many years, even before the era of bisphosphonates. It occurs in children as well as in adults. A fairly frequent cause is steroid therapy, or other treatments which include steroids. Pardoxically, when compared to the situation outlined above, *patients with avascular necrosis of the femoral head treated with bisphosphonates showed reduction of pain, improvement of function, and retardation of progression, so that early surgical intervention could be avoided in many cases.*

Contraindications and Precautions

So far the only absolute contraindications are *pregnancy and lactation* because some bisphosphonates cross the placental barrier and may also be excreted in the milk. Animal studies have not demonstrated any mutagenic effects on the

fetus. Recent studies on maternal and fetal outcome after bisphosphonate treatment before conception found no evidence for adverse effects on mothers or babies.

The presence of fractures or orthopedic prostheses do not constitute contraindications to bisphosphonate therapy. On the contrary, callus formation is increased and fractures are repaired more rapidly under bisphosphonate therapy. *When rigidly defined indications are present, aminobisphosphonates can also be given to infants and children*. Disturbances of growth and mineralisation have not been reported. Bisphosphonates should be administered by the intravenous route to patients with difficulties in swallowing and inflammatory or other esophageal, gastric or intestinal disorders. Aminoglycosides should not be given together with bisphosphonates, nor should different bisphosphonates be given simultaneously. *Patients with multiple myeloma on therapy with thalidomide should not be given zoledronate.*

Practical Recommendations and Guidelines

Oral Administration of Bisphosphonates

Etidronate, clodronate, tiludronate, alendronate, risedronate, ibandronate:

The poor gastro-intestinal absorption of these modern bisphosphonates is offset by their high effectiveness. Before initiating oral bisphosphonate therapy, the following points should be considered:

- Exclusion of reflux esophagitis, difficulties in swallowing and elucidation of dental status in the patient's clinical history.
- Instructions provided by the manufacturer of the bisphosphonate to be noted and followed.
- Other medication(s) should not be taken together with bisphosphonates.
- The patient must not lie down for at least 30 minutes after taking the tablet, preferably should be active physically during this time.
- Bedridden patients should not be prescribed oral bisphosphonates.
- Possible side effects should be discussed with the patient to increase awareness and compliance.
- During trips abroad, mineral water poor in calcium and carbon dioxide can be used instead of tap water. Alternatively, the time abroad can be bridged by an intravenous infusion given beforehand.

Intravenous Administration of Bisphosphonates

Clodronate, pamidronate, ibandronate, zoledronate:

The infusions are usually administered on an ambulatory basis in the out-patients clinic. The following points should be noted:

- The doses and intervals between treatments depend on the type and severity of disease, on osteoclastic activity and urgency of achieving therapeutic success. The infusions are usually administered at intervals of 3 weeks to 3 months, in some cases 6 months or even annually.
- Calculation of the dose according to body weight is not required.
- Dehydration must be recognised and treated before bisphosphonate administration to avoid renal damage through precipitation of complexes in the renal tubules; basic biochemical values (e.g. creatinine) should also be obtained beforehand.
- The infusion should be slow, e.g. 250–500 ml physiological saline in about 1 hour (or longer as required), to avoid local and renal reactions as well symptomatic hypocalcemia. Recently an infusion time of 15 min has been approved also for ibandronate 6 mg.
- The instructions of the manufacturer must be followed when treating patients with partial renal insufficiency.
- The dose should be reduced by 25% for patients with complete renal failure, and be given immediately after completion of the haemodialysis.
- The half-life of the bisphosphonate must be taken into account when patients are on hemodialysis.
- Patients must be informed of the possibility of an acute-phase reaction on the day after the first infusion.
- The patient's mouth and jaws must be examined before i.v. therapy, and appropriate treatment given and completed beforehand. Surgical intervention in the mouth and jaws should be avoided during this period, i.e. while the patient is receiving bisphosphonates especially i.v.
- Bisphosphonates do not interact with other medications or drugs.
- Simultaneous administration of aminoglycosides may cause symptomatic hypocalcemia and should be avoided.
- Should drug resistance occur, the dose should subsequently be increased by about 50% if possible, alternatively switch to another more potent bisphosphonate.
- With the introduction of zoledronate in oncology three considerations are routinely applied: 1) Careful adherence to the manufacturer's instructions especially with respect to monitoring of renal function. 2) Attention to the possibility of a toxic reaction. 3) Reduction in dosage in patients with impaired renal function.

Effects of Cessation of Bisphosphonate Therapy

Within 2–4 months of stopping therapy with bisphosphonates, indices of bone turnover begin to increase and these should be monitored – a negative bone balance that is bone loss, becomes evident 1–2 years later. Therefore annual measurement of BMD is recommended so that appropriate preventive therapy can be re-instated. Data are not yet available on the incidence of fractures during this period.

Long Term Effects of Bisphosphonate Therapy

Investigations of the effects of bisphosphonates on bone for periods of up to 10 years have not revealed any deleterious or damaging effects on bone (Papapoulous 2005). The main 4 parameters which are responsible for bone strength (bone mineral density, bone architecture, bone remodelling and bone material) are all positively influenced by modern bisphosphonates in combination with calcium and vitamin D_3.

Combination of Bisphosphonates with Other Drugs

The combination of bisphosphonates with *other inhibitors of bone resorption* such as raloxifen has a positive additive effect on bone density and on fracture incidence. The addition of calcitonin to bisphosphonates appears to be useful in the treatment of bone pain because of the apparent stimulation of endogenous opiates in the brain. *Calcitonin* is a rapidly acting peptide hormone and therefore may control severe hypercalcemia faster than bisphosphonates, thus allowing time for their effect to take place.

Sequential administration of bisphosphonates after *stimulators of bone formation* such as *parathyroid hormone (PTH)* or with low doses of fluoride has proved to be effective and has so far induced the greatest increase in bone mass in clinical trials. It has recently been shown that statins given to reduce cholesterolemia also reduce fracture risk: they appeared to have additive effects on bone density in combination with a bisphosphonate.

As mentioned previously, it is mandatory to obtain the *patient's written consent* before administration of a bisphosphonate especially if it has not yet been officially authorised for that particular condition. The written consent of the parents must be obtained before children are treated. It is advisable to complete a special consent form which states the condition for which the bisphosphonate is recommended, the name of the bisphosphonate, the exact dose, and the duration of the oral administration and/or number of infusions.