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Review Article

The role of bisphosphonates in oncology

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CONTENTS

Abstract

Bisphosphonates are successfully used in the treatment of bone diseases characterized by enhanced osteoclastic bone resorption, such as osteoporosis and Paget's disease. More recently, they have also been shown to play an indispensable role in the therapy and management of cancer-induced skeletal complications. For years it was suggested that these effects were mediated solely by inhibition of bone resorption, but recent investigations also indicate an involvement of direct and indirect antitumor effects in the complex mechanism of action of bisphosphonates. Thus, bisphosphonates influence tumor cell proliferation, invasion, adhesion and growth factor release. Furthermore, these drugs induce tumor cell apoptosis, modulate cells of the immune system and have inhibitory effects on tumor angiogenesis. The exact antitumor mechanism remains unclear and needs further investigation. In this article we will provide an overview of the preclinical and clinical studies of bisphosphonates in oncology, and discuss future directions for these promising compounds in the treatment of cancer and potentially other diseases.

Introduction

Bisphosphonates are a class of drugs identified by German chemists in 1865 (1), but whose biological properties were not discovered until 1968 (2). In the last 30 years, bisphosphonates have been developed as potent compounds in the diagnosis and treatment of different bone diseases (3). They are potent inhibitors of osteoclastic bone resorption, which has led to their successful use in the treatment and prevention of postmenopausal and corticosteroid-induced osteoporosis, Paget's disease and hypercalcemia (4). Bisphosphonates have also become indispensable in the treatment and management of cancer-induced bone diseases, especially in patients with bone metastases from breast cancer, multiple myeloma and other types of tumors (5-7).

Recent preclinical and clinical studies suggest that bisphosphonates not only have antiosteoclastic activity, but also act on different types of cells, including tumor cells, endothelial cells and cells of the immune system. The exact mechanism of these antitumor properties has not yet been elucidated. Thus, here we review the well-established effects and new results of bisphosphonates and provide an overview of possible antitumor mechanisms of action and the status of clinical development. Finally, we will discuss future directions for bisphosphonates in the treatment of malignancy.

Chemical structure and SAR of bisphosphonates

Bisphosphonates are metabolically stable analogues of the naturally occurring pyrophosphate (P-O-P), in which the central oxygen atom is replaced by a carbon atom (P-C-P) (8) (Fig. 1). These drugs possess high affinity for bone mineral, like pyrophosphate (9). The P-C-P structure of bisphosphonates is stable against heat and most chemical agents and highly resistant to enzymatic cleavage, in contrast to the extremely unstable pyrophosphate. Replacement with the carbon atom allows linking of additional side-chains, indicated as R1 and R2 (10). Due to the P-C-P moiety, bisphosphonates are able to chelate divalent metal ions, such as calcium or magnesium ions. A hydroxyl group at R1 increases affinity for chelation by tridentate binding compared to bidentate

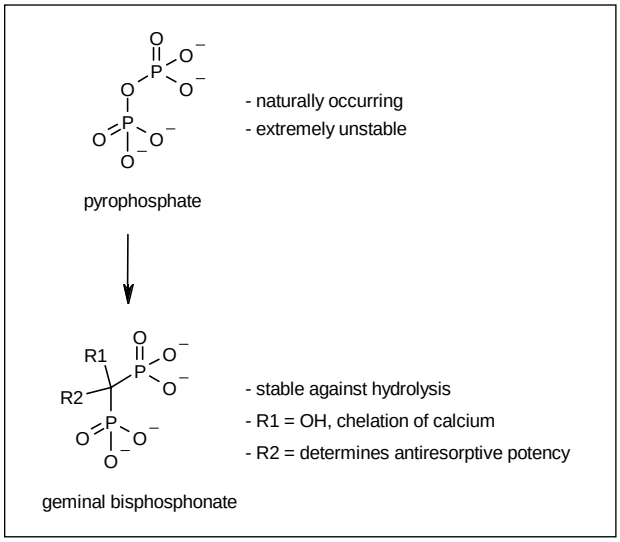

Fig. 1. Comparison of basic structures and properties of pyrophosphate and bisphosphonates

binding. The R2 substitution is key for potent inhibition of osteoclastic bone resorption.

First-generation bisphosphonates have either a single atom or a simple alkyl side-chain at R2 (*e.g.,* etidronate, clodronate). Antiresorptive activity was increased in second-generation bisphosphonates with a basic aminoalkyl group at R2, such as pamidronate, alendronate and neridronate (11). The antiresorptive activity is increased even more in bisphosphonates with a secondary amino group (*e.g.*, incadronate) or a tertiary amino group (*e.g.*, olpadronate, ibandronate). Most of the highly potent third-generation bisphosphonates contain heterocyclic rings with one or more nitrogen atoms (*e.g.*, risedronate, zoledronic acid) (12) (Fig. 2).

Pharmacokinetic properties of bisphosphonates

The oral bioavailability of all bisphosphonates is less than 2% in all species studied, due to their poor lipophilicity. Absorption from the gastrointestinal tract occurs via paracellular transport. After i.v. application, the half-life of bisphosphonates is usually between 30 min and 2 h, although a half-life up to 6 h was reported for etidronate.

Bisphosphonates are either taken up by bone tissues (about 30-60% of the reabsorbed dose) or excreted by the kidneys, the only route of elimination (13). Studies in rats with alendronate suggested that this compound is actively secreted by a transport mechanism on the brush border membrane of kidney epithelial cells (14). The elimination of bisphosphonates after bone uptake is very slow and is dependent on bone resorption activity. The half-life of bisphosphonates in bone ranges from 200 days in rats to 3 years in dogs and to 12 years in humans.

A recent study investigated the tisssue distribution of different bisphosphonates (zoledronic acid, clodronate and ibandronate) in rats. As expected, substantial concentrations were observed in bone, reaching a plateau 1 h after administration, and in the kidneys. Interestingly, all bisphosphonates also accumulated in the prostate, with a maximum concentration after 30-60 min. In comparison, the concentrations of the compounds in other tissues, such as the lung, liver or spleen, were very low (15).

Effects of bisphosphonates on bone cells

In the treatment of osteoporosis and Paget's disease, the efficacy of bisphosphonates is mostly attributed to reduced bone destruction. Bone undergoes a permanent turnover, which is regulated by osteoclasts and osteoblasts through a complex network of different factors (16, 17). The main target of bisphosphonates is the bone-resorbing osteoclast, although different mechanisms of action appear to be involved. First, these drugs have inhibitory effects on osteoclastogenesis (18, 19). Recent studies showed direct effects of bisphosphonates on early osteoclast precursors (20).

A potential new mechanism of nitrogen-containing bisphosphonates is an increase in osteoprotegrin release by primary human osteoblasts. Osteoprotegrin antagonizes the osteoclastogenic activity of RANKL (receptor activator of nuclear factor-κB ligand). RANKL interacts with the receptor activator of NF-κB on the surface of osteoclast precursors. This receptor activation leads to differentiation of mature osteoclasts. Thus, osteoprotegrin can block this interaction, thereby inhibiting osteoclastogenesis (21).

Furthermore bisphosphonates prevent the adhesion of osteoclasts to the bone surface. The compounds mostly accumulate within osteoclasts and are apparently incorporated by endocytosis (22). This intracellular accumulation leads to critical changes in cell morphology, especially on the ruffled border and the cytoskeleton (23). Bisphosphonates also influence enzyme activity and acid production and secretion.

Another mechanism of action involves the induction of apoptosis in osteoclasts (24, 25). The effects of bisphosphonates on the proliferation and activity of osteoclasts involve interference with important intracellular pathways, and two different intracellular mechanisms have been identified (26). Non-nitrogen-containing bisphosphonates are metabolized to nonhydrolyzable, cytotoxic ATP analogues that influence cell function and induce apoptosis (27). They also have an impact on important metabolic processes, such as glycolysis, lactate production and fatty acid oxidation (Fig. 3). In contrast, second- and third-generation bisphosphonates with either an aliphatic or a heterocyclic nitrogen-containing side-chain inhibit a key enzyme in the mevalonate pathway, farnesyl diphosphate synthetase (geranyltransferase) (28) (Fig. 4). This enzyme inhibition prevents the formation of isoprenoids such as farnesyl diphosphate (FPP) or geranylgeranyl diphosphate (GGPP), which are required for the regular prenylation of small GTP-binding proteins such as Rho

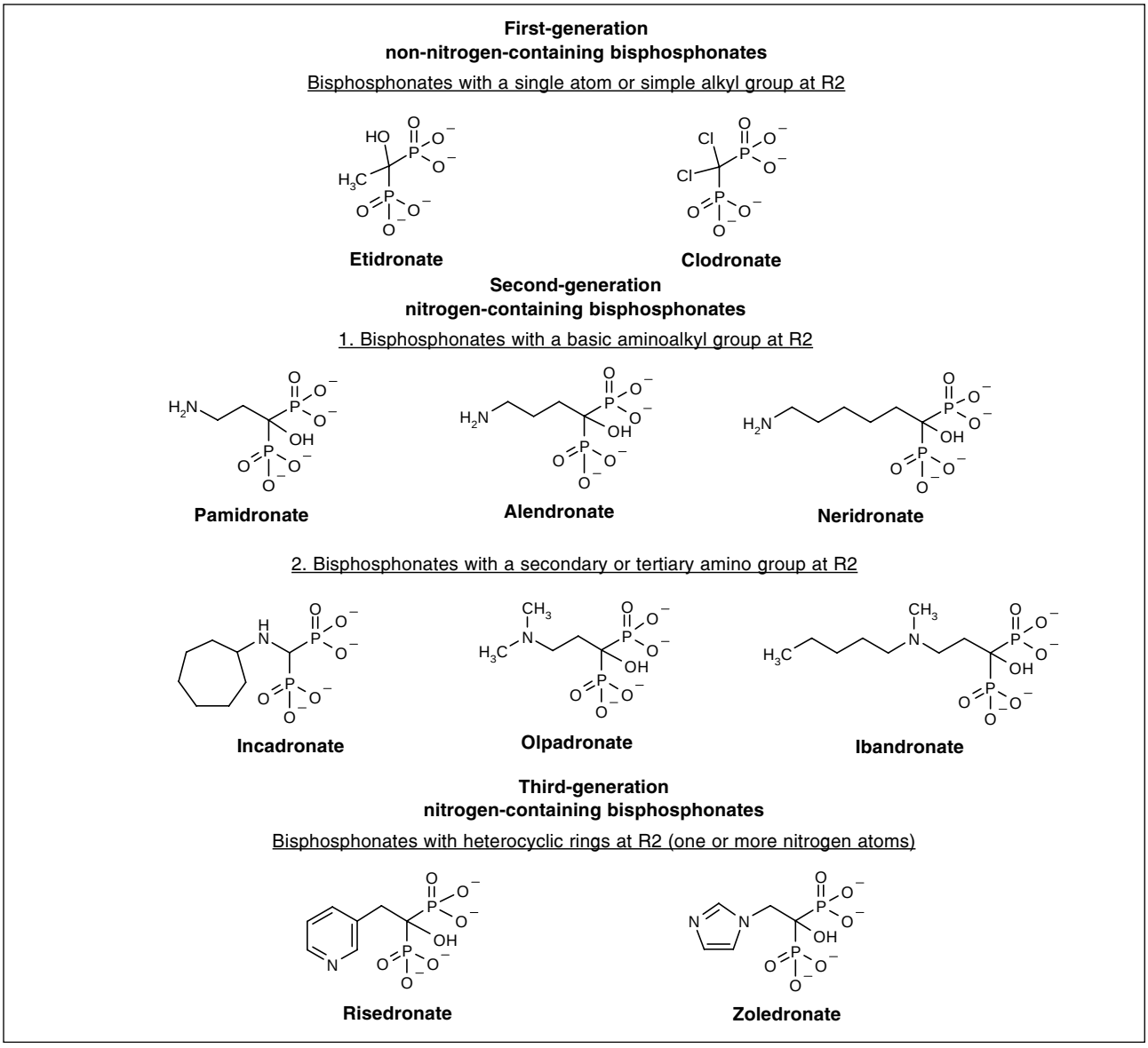

Fig. 2. Classification of bisphosphonates according to different side-chains.

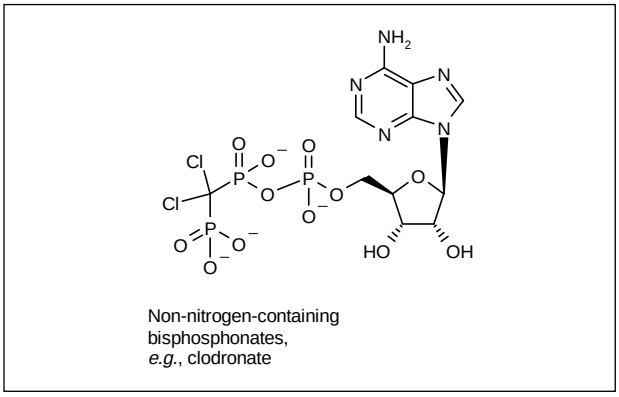

Fig. 3. Formation of toxic ATP analogues from non-nitrogen-containing bisphosphonates.

and Ras. Because these proteins are essential for correct osteoclast cell function, this dysfunction leads to decreased activity and an increased rate of apoptosis in osteoclasts (26) (Fig. 5). *In vitro* experiments indicated that the effects of nitrogen-containing bisphosphonates can be prevented by adding FPP and GGPP (29).

Antitumor activity of bisphosphonates

Bisphosphonates have become important agents in the treatment of cancer-induced bone diseases, such as bone metastases, hypercalcemia, spinal cord compression, pathological fractures or chronic bone pain. For a long time it was assumed that the inhibitory effects on

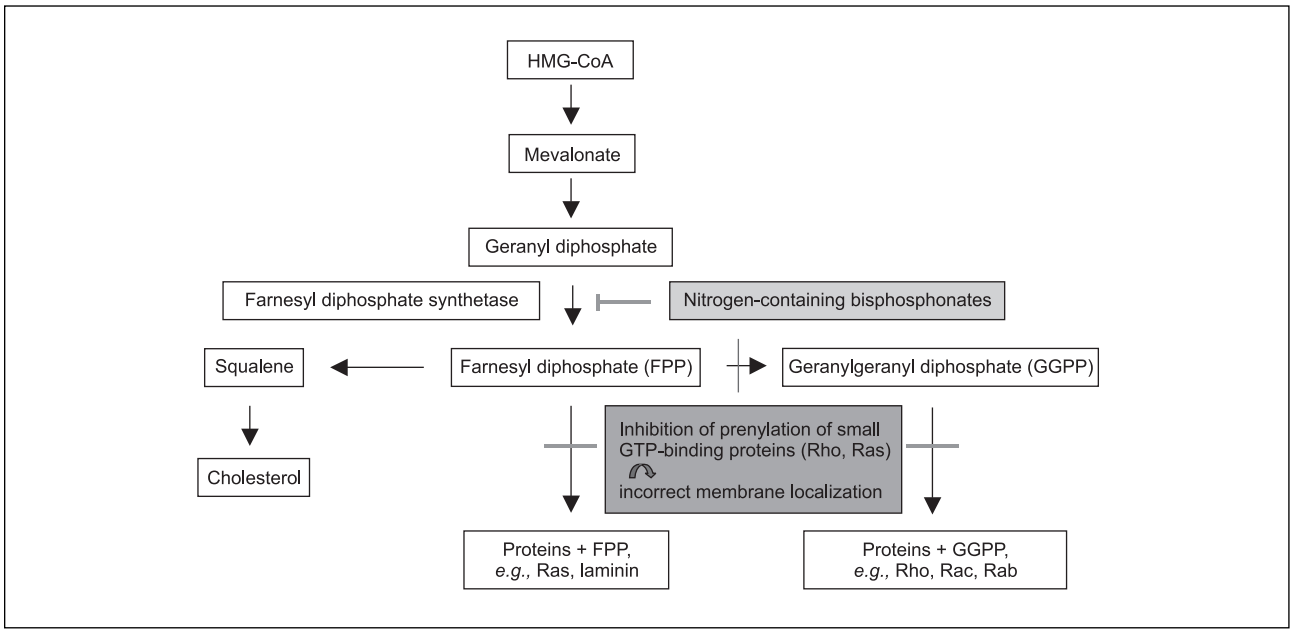

Fig. 4. Inhibition of farnesyl diphosphate synthetase (geranyltransferase) in the mevalonate pathways by nitrogen-containing bisphosphonates.

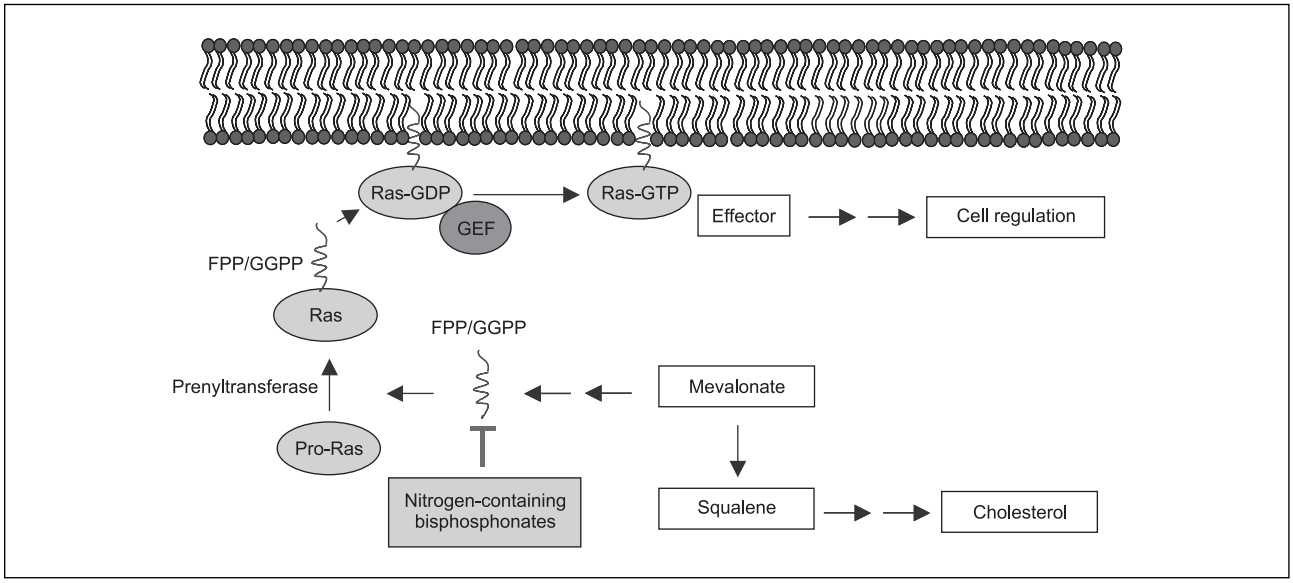

Fig. 5. Posttranslational modification, membrane localization and activation of small GTP-binding proteins (*e.g.*, Ras). Isoprenoids such as farnesyl diphosphate (FPP) or geranylgeranyl diphosphate (GGPP) are linked with an SH group of cysteine. These enzyme-catalyzed reactions enable anchoring to the cell membrane and subsequent activation. GEF = guanine nucleotide exchange factor.

bone resorption were solely responsible for the efficacy of bisphosphonates. However, a wealth of recent preclinical and clinical data suggests a direct antitumor effect for the bisphosphonates. Other indirect antitumor effects also appear to be involved in the complex mechanism of action of bisphosphonates. Here we present a comprehensive overview of the antitumor effects of bisphosphonates *in vitro* and *in vivo* (Fig. 6).

Effects on the release of growth factors and cytokines

Tumor cells secrete factors, such as parathyroid hormone-related peptide (PTHrP), which stimulate osteoclasts to degrade the bone matrix. This degradation leads to the release of regulatory growth factors and cytokines. These factors, in turn, are able to stimulate tumor cell proliferation (30). However, bisphosphonates break this

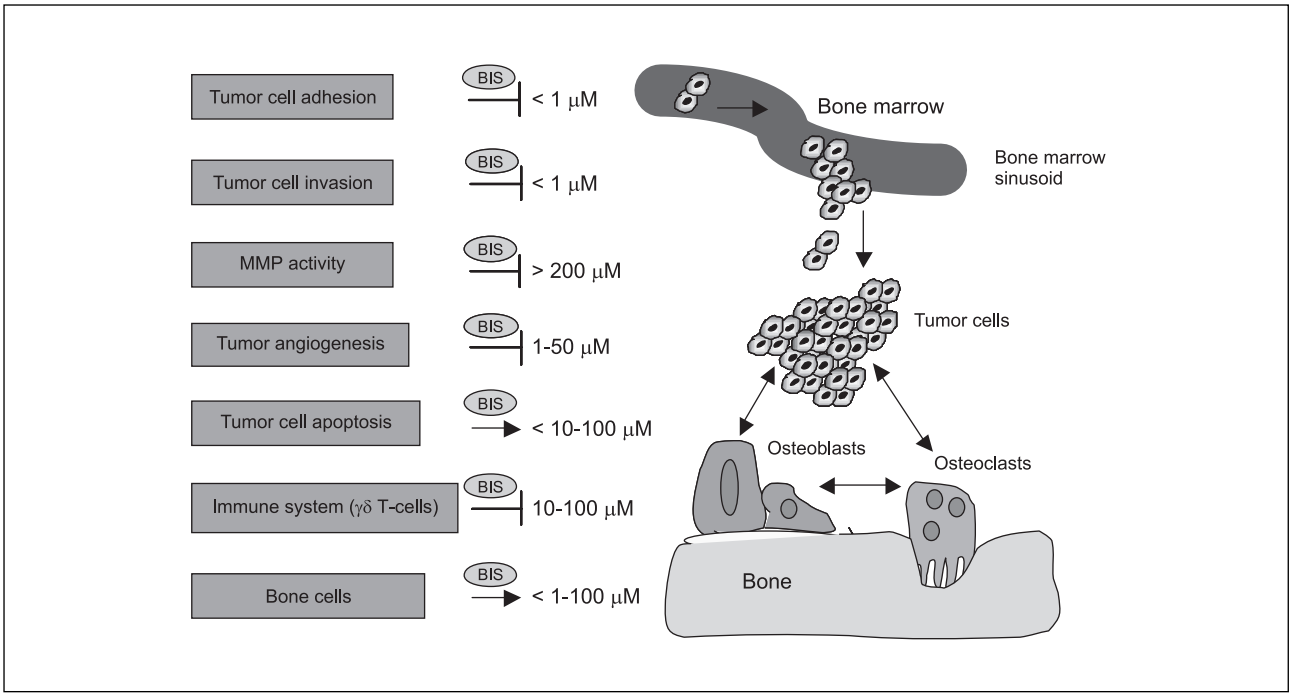

Fig. 6. Potential mechanisms of action of bisphosphonates in the treatment of cancer.

vicious circle by reducing the release of tumor cell-stimulating factors from bone via inhibition of osteoclastic bone resorption.

In addition to these effects, bisphosphonates also interact with the secretion of growth factors and cytokines by osteoblasts, macrophages, monocytes and bone marrow stromal cells. In macrophage-like RAW 264 cells, clodronate and pamidronate inhibited the lipopolysaccharide (LPS)-induced secretion of IL-1β, IL-6 and TNF-α (31). In bone marrow stromal cells, zoledronic acid and pamidronate decreased the release of IL-6 and significantly inhibited the IL-1β-stimulated production of the matrix metalloproteinase MMP-1, which is involved in the initiation of bone resorption. This study also revealed an upregulation of MMP-2 secretion in bone marrow stromal cells with zoledronic acid and, to a lesser extent, pamidronate. MMP-2 plays an important role in both bone resorption and metastasis, suggesting a potential risk for bisphosphonate therapy *in vivo* (32).

Effects on tumor cells

1. Inhibition of tumor cell proliferation and induction of apoptosis *in vitro*

Numerous *in vitro* studies have clearly demonstrated antitumor activity of different bisphosphonates against breast cancer, pancreatic cancer, myeloma, melanoma, prostate cancer and osteosarcoma cell lines (33-39), with antiproliferative and proapoptotic effects at concentrations of 5-2000 μM. Adequate concentrations can probably be achieved *in vivo* at the active resorption site in bone.

A study using four bisphosphonates found differences in growth inhibition of three breast cancer cell lines (MDA-MB-231, MCF7, T-47D) after various incubation times. Zoledronic acid was the most potent bisphosphonate in short-term cell cultures (3 h), whereas ibandronate was more potent in long-term cell cultures (1-6 days). Clodronate and pamidronate showed lower activity compared to zoledronic acid and ibandronate. Interestingly, MCF7 cells were killed primarily by apoptosis, whereas T-47D cells died due to necrosis. Both these breast cancer cell lines were also more sensitive to bisphosphonate therapy than MDA-MB-231 cells (39). Other studies with breast cancer cells confirmed that the order of potency is zoledronic acid > pamidronate > clodronate.

Results from experiments in prostate cancer cells indicated that bisphosphonates may act in different ways. Thus, at a concentration of 100 μM, pamidronate and zoledronic acid clearly exerted tumor growth inhibition. However, whereas pamidronate was associated with apoptosis, zoledronic acid showed marked effects on cell proliferation, characterized by an increase in cells present in the G_0-G_1 and S phases (37).

The addition of geranylgeraniol and farnesol, two intermediates in the mevalonate pathway, was reported in several studies to protect tumor cells from nitrogen-containing bisphosphonate-related growth inhibition and apoptosis, indicating that the established mechanism is also responsible for these effects. Zoledronic acid leads to an incorrect membrane localization of the Ras protein

due to inhibition of prenylation, which represents the initial step of apoptosis. However, a study with pamidronate and zoledronic acid indicated that inhibition of the mevalonate pathway is involved in their effects on osteoblasts, but not in their inhibition of MDA-MB-231 breast cancer cell proliferation (40), suggesting cell type-specific mechanisms of action. On the other hand, the formation of toxic ATP analogues by non-nitrogen-containing bisphosphonates, which accumulate intracellularly, is crucial for the induction of tumor cell apoptosis, which is associated with decreased expression of Bcl-2 (antiapoptotic protein), release of mitochondrial cytochrome *c* in the cytosol and the subsequent activation of the caspase cascade.

Combinations of bisphosphonates with common cytostatic drugs clearly exert synergistic effects. Zoledronic acid in combination with paclitaxel or tamoxifen enhanced proapoptotic activity in MCF7 and MDA-MB-231 breast carcinoma cells (41). Synergistic efficacy was also seen in myeloma cells after treatment with zoledronic acid and dexamethasone (42).

2. Effects on tumor cell adhesion and invasion *in vitro*

Tumor cell migration, adhesion and invasion are important steps in the development of metastases, especially in bone tissue. In principle, every malignant tumor is able to develop metastases, but metastatic invasion is especially common in breast, prostate, lung, thyroid and kidney cancer.

The first study in pretreated cortical bone slices demonstrated inhibition of the adhesion of MDA-MD-231 cells at very high concentrations of nitrogen-containing bisphosphonates (10-100 μM), whereas non-nitrogen-containing bisphosphonates had weak or no effects (43). In subsequent experiments, bisphosphonates (clodronate, pamidronate, risedronate, ibandronate) concentration-dependently inhibited tumor cell adhesion to bone and the potency of each compound corresponded to its activity on osteoclastic bone resorption (ibandronate > risedronate > pamidronate > clodronate). The maximal inhibitory effects were observed at 0.01-1 μM (44), concentrations likely to be achieved *in vivo*.

The mechanism of action by which nitrogen-containing bisphosphonates inhibit adhesion remains unclear, but an interaction with important cell adhesion molecules, such as laminins and integrins, may be involved. The inhibition is probably due to incorrect prenylation of small GTP-binding proteins, which are essential for important signaling pathways involved in tumor cell adhesion. However, no changes in the cell-surface expression of integrins in breast and prostate carcinoma cells could be detected after exposure to bisphosphonates (44). Recently, zoledronic acid was reported to interfere with endothelial cell adhesion and migration by affecting integrin-dependent postreceptor pathways (45).

Studies with bisphosphonates using different breast and prostate cancer cells also showed an inhibition of tumor cell invasion at concentrations of 1 pM to 1 μM (46, 47). The inhibitory potency again correlated with the antiresorptive potency. The antiinvasive effects of nitrogen-containing bisphosphonates, but not clodronate, could be reversed by addition of *trans,trans*-farnesol and geranylgeraniol.

Proteolytic enzymes, including the matrix metalloproteinases (MMPs), are essential in the invasion process of tumor cells. Interestingly, bisphosphonates are able to inhibit the activity of MMPs secreted by tumor cells, although at concentrations 1,000-fold higher than those required for inhibition of tumor cell invasion (48). Recent data have demonstrated that alendronate (50-150 μM) reduces mRNA and cellular levels of MMP-2 in osteosarcoma cell lines in a time- and concentration-dependent manner (49), an effect that could be prevented by the addition of zinc (46).

In summary, two mechanisms are likely to be involved in inhibition of tumor cell invasion: interference with the mevalonate pathway at lower concentrations and inhibition of MMPs at much higher concentrations. Furthermore, it was suggested, that the R2 side-chain of the molecule and not the P-C-P moiety is essential for the effects of lower concentrations of these compounds on invasion and adhesion (44, 46). Moreover, combination of bisphosphonates with taxoids resulted in enhanced inhibitory efficacy against tumor invasion and adhesion (50).

Antitumor effects in animal models

In recent years, bisphosphonates have been shown to decrease cancer-induced osteolysis in different animal models of breast, prostate and bladder cancer and myeloma (51-57). This decrease can be explained by the induction of osteoclast apoptosis as a result of inhibition of bone resorption. Furthermore, these compounds reduce skeletal tumor burden in *in vivo* studies. Interestingly, the inhibitory effect of bisphosphonates could be visualized in a fluorescent bone metastasis model in animals bearing MDA-MB-231 cells (58). Recently, further animal studies were able to show increased apoptosis of tumor cells in osteolytic metastases of different tumors in mice treated with bisphosphonates.

Due to the pharmacokinetic properties of bisphosphonates, the effects on tumor cells appear to be restricted to bone tissue. However, there are contradictory data on the activity of bisphosphonates on extraskeletal metastases. Most investigators observed no significant effects, but several studies reported a decrease in the number of visceral metastases. Thus, the preventive administration of minodronate reduced the growth of soft tissue metastases (59). Furthermore in a murine 4T1/luc breast cancer model in mice, a single injection or four i.v. injections of zoledronic acid significantly reduced the occurrence of lung and liver metastases, in addition to bone metastases, and prolonged overall survival of the mice (60, 61). The formation of lung metastases was also reduced in rats bearing syngeneic 13762 mammary

carcinoma cells in the bone treated with alendronate (62). In contrast, there are also several reports of a small increase in visceral metastases (63-65), and one study even showed an increase in bone metastases (66). It must be noted that these results were not reproducible in other tumor models.

Effects on the immune system

Another remarkable aspect of the antitumor mechanism of bisphosphonates is their influence on the immune system. Up to 60% of patients receiving pamidronate for the first time experience an acute-phase reaction. Significant effects on circulating lymphocytes were seen in one study. Interestingly, pamidronate decreased circulating lymphocyte counts, whereas ibandronate increased the number of lymphocytes (67). The administration of alendronate results in inhibition of the antigen-presenting cell function of monocytes, an effect that was reversed by addition of exogenous IL-1 (68).

Furthermore, nitrogen-containing bisphosphonates induce a significant expansion of the γδ T-cell subset in peripheral blood mononuclear cell cultures. The effect on the proliferation of this T-cell subset was dependent on IL-2, whereas the activation of γδ T-cells occurred without exogenous cytokines. Only a slight effect of the non-nitrogen-containing bisphosphonates clodronate and etidronate was seen in this study (69). However, another study with clodronate in a γδ T-cell-restricted system showed a measurable effect on proliferation and cytotoxicity (70). The activation of γδ T-cells is associated with upregulation of CD25 and CD69 and increased secretion of cytokines, *e.g.*, interferon gamma, which exerts cytotoxic effects against neuroblastoma cells (MC-IXC), human Burkitt lymphoma Daudi cells, myeloma cells (RPMI 8226 and U266), human colon carcinoma cells (COLO 205), erythroleukemia cells (K-562) and human neuroepithelioma cells (SK-N-MC) (68, 69). Human γδ T-cells appear to be activiated via nitrogen-containing bisphosphonate antigen-presenting cells (71).

In conclusion, bisphosphonates have complex effects on the immune system that presumably play an important role in their antitumor mechanism.

Antiangiogenic activity

Angiogenesis is a prerequisite for the progressive growth of solid tumors and their metastases (72). Above a few millimeters in diameter, connection to the nutritive system of the body is essential for tumor progression. In malignant tumors, the development and spread of new vessels is directed and regulated by a complex network of endogenous proangiogenic factors, *e.g.,* vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and endogenous antiangiogenic factors like angiostatin or endostatin. Neovascularization can be further stimulated by factors secreted by the tumor. These "leaky" vessels enable the tumor to metastasize to various sites (73, 74).

The first study on the antiangiogenic effects of zoledronic acid demonstrated inhibition of the proliferation of human umbilical vein endothelial cells (HUVECs) stimulated with VEGF, bFGF or fetal calf serum (FCS). Zoledronic acid also inhibited angiogenesis induced by subcutaneous implants impregnated with bFGF in mice (75). In another study, the treatment of endothelial cells with bisphosphonates decreased capillary tube-like formation. Moreover, ibandronate and zoledronic acid inhibited the testosterone-induced revascularization of the prostate gland in castrated rats (13). The administration of zoledronic acid (120 g/kg twice weekly) in a 5T2 model of myeloma decreased osteolysis, tumor burden and angiogenesis, and even increased survival (76). A novel non-nitrogen-containing bisphosphonate, BP-7033, also exerts marked antiangiogenic effects *in vitro* and *in vivo*. Thus, BP-7033 inhibited both the angiogenesis and growth of human epidermoid carcinoma A-431 xenografts in nude mice (77).

The treatment of cancer patients with pamidronate and zoledronic acid also significantly decreased circulating angiogenic factors (78, 79).

The antiangiogenic mechanism of action is still unclear, however, although it may involve inhibition of endothelial cell adhesion. A decrease in $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin and 67-kD laminin receptor expression on endothelial cells has been observed (45). The integrin family of cell adhesion proteins promotes the attachment and migration of cells on the surrounding extracellular matrix (ECM). This family of proteins plays key roles in regulating tumor growth and metastasis, as well as tumor angiogenesis. Recently, it was reported that zoledronic acid inhibited the sustained phosphorylation of focal adhesion kinase (FAK), a regulator of endothelial cell adhesion and migration, and protein kinase B (PKB/Akt), which promotes cell survival and protects cells against death induced by cytotoxic stimuli. Clodronate had no effect on endothelial cell adhesion, migration and survival. Thus, it was suggested, that zoledronic acid sensitizes endothelial cells to TNF-induced, caspase-independent apoptosis (80). Further investigations in this interesting field of research are urgently needed.

Antitumor effects in clinical studies

A number of trials have been conducted to investigate the potential clinical use of bisphosphonates in the treatment of cancer. Bisphosphonates have become indispensable agents in the treatment of osteolytic metastases in cancer patients. Bone metastases are associated with skeletal complications, such as pathological fractures, bone pain, impaired mobility, spinal cord compression and hypercalcemia (81, 82). A systematic review of 30 randomized controlled trials in patients with malignant disease and bone metastases, who were treated with different bisphosphonates, revealed a significant decrease

Table I: Comparision of the results of 3 clinical studies on adjuvant application of clodronate (1600 mg/day orally) in the treatment of patients with primary breast cancer.

			Median		Bone metastases		Nonskeletal metastases		Mortality	
Study (ref.)	No. of patients	Duration (years)	follow-up (years)	Placebo -controlled	Clodronate (n)	Control (n)	Clodronate (n)	Control (n)	Clodronate (n)	Control (n)
Diel *et al.* (85)	302	2	3	No	12	25	13	27	6	22
Saarto *et al.* (86)	299	3	5	No	29	24	60	36	42	24
Powles *et al.* (87)	1079	2	5.5	Yes	63	80	112	128	98	129

in skeletal-related events (SRE), except for spinal cord compression. Moreover, bisphosphonate-treated patients had a significantly increased time to first SRE, although treatment did not influence survival (83). Clinical studies have indicated that bisphosphonates can reduce SREs in breast cancer, myeloma, hormone-refractory prostate cancer, non-small cell lung cancer and a range of other solid tumors. In large multicenter, randomized trials, a reduction in the occurrence of SREs of 20% was obtained after intravenous administration of zoledronic acid every 3-4 weeks (84).

Another interesting clinical approach is the administration of bisphosphonates to prevent the development of metastases, especially bone metastases. Three randomized trials were performed with adjuvant oral clodronate therapy in patients with breast cancer, and contradictory results emerged (Table I). One study found a significant reduction in osseous and even visceral metastases and an improvement in overall survival compared to the control group (85), whereas another non-placebo-controlled trial showed no benefit for breast cancer patients under adjuvant clodronate treatment. The results from the latter study even showed a detorioration in survival and an increase in the incidence of metastases (86). Recently, a third larger multicenter clinical trial demonstrated a decrease in bone metastases, a significant reduction in mortality, but no significant effect on visceral metastases (87).

The relevance and benefit of adjuvant bisphosphonate therapy are presently unclear and require further investigation. The results of large confirmatory studies (NSABP B34 and SWOG S9905) are expected to provide new insights. Similar clinical studies with clodronate and zoledronic acid in prostate cancer are under way and the results are awaited with interest. However, new trials of adjuvant administration should be initiated, especially with highly potent nitrogen-containing bisphosphonates, particularly since all bisphosphonates are well tolerated with a low incidence of serious side effects.

Summary and future directions

Available evidence indicates that bisphosphonates have pronounced antitumor activity in addition to inhibitory effects on bone resorption. Different direct and indirect antineoplastic effects appear to be involved in the complex mechanism of action. In clinical studies, bisphosphonates are clearly effective in reducing the skeletal complications of breast cancer, myeloma and most likely prostate cancer. Moreover, bisphosphonates are also able to reduce tumor burden in bone tissue. The efficacy of adjuvant bisphosphonate treatment remains unclear and large confirmatory studies with clodronate and similar trials with potentially more potent aminobisphosphonates, especially zoledronic acid, should clarify the conflicting results. It should be noted that, so far, no published clinical study has investigated a direct randomized comparision of oral and intravenous bisphosphonates (83).

In the future, a number of issues have to be clarified to optimize the safety and benefit of bisphosphonate therapy for cancer patients. The optimum schedule and duration of treatment, as well as the relative efficacy of third-generation bisphosphonates such as risedronate and zoledronic acid compared to clodronate and pamidronate, have not yet been elucidated. Due to their known pharmacokinetic properties, the biological activity of bisphosphonates appears to be limited to bone tissue. However, more frequent dose schedules or higher doses may increase the antitumor activity in other organs, such as the prostate or kidney. Preclinical data also clearly demonstrated synergistic effects of bisphosphonates in combination with other compounds. The greatest clinical benefit can likely be achieved using combined therapy with established antineoplastic drugs, *e.g.*, taxanes, or novel antitumor compounds, such as specific kinase inhibitors, aromatase inhibitors or inhibitors of tumor angiogenesis.

In summary, bisphosphonates possess promising antitumor potential. Further preclinical and clinical investigations must be performed to elucidate the complex biological activity of bisphosphonates and possibly to extend their indication to malignancy and other diseases.

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