Bisphosphonates and Cancer-Induced Bone Disease: Beyond Their Antiresorptive Activity

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Abstract

Bisphosphonates are primarily known for their ability to inhibit osteoclast-mediated bone resorption. They are an indispensable part of therapy for patients with cancers that cause osteolysis. However, there is now a growing body of evidence from preclinical research showing that bisphosphonates also exhibit antitumor activity, both *in vitro* and *in vivo*. They can affect molecular mechanisms of tumor cell adhesion, invasion, and proliferation; reinforce the effects of cytotoxic agents in a synergistic manner; and exhibit antiangiogenic and immunomodulatory effects. These preclinical findings reveal exciting ways of optimizing bisphosphonate therapy in oncology to fully exploit their antitumor potential. (Cancer Res 2005; 65(12): 4971-4)

Introduction

Bisphosphonates are analogues of the naturally occurring compound PP_i (P-O-P) in which the oxygen in P-O-P has been replaced by a carbon, forming a P-C-P structure. Two chains (called R_1 and R_2) are covalently bound to the carbon atom. The P-C-P backbone and the R_1 chain (preferably a hydroxyl group) allow the binding of bisphosphonates to bone mineral, whereas the R_2 chain determines the potency of bisphosphonates to inhibit osteoclast-mediated bone resorption (Fig. 1; ref. 1). Bisphosphonates that lack a nitrogen functional group in the chemical structure of the R_2 chain (such as clodronate) condense with an aminoacyladenylate to form nonhydrolyzable analogues of ATP that inhibit ATP-dependent intracellular enzymes (1). Nitrogen-containing bisphosphonates (NBP; such as pamidronate, ibandronate, risedronate, zoledronate, and minodronate; Fig. 1) inhibit the activity of farnesyl diphosphate synthase, a key enzyme in the mevalonate pathway (1). This leads to a reduction in the levels of geranylgeranyl diphosphate, which is required for the prenylation of small GTPases (such as Rho, Rab, and Rac) that are essential for osteoclast activity and survival (1).

Because of their potent antiresorptive activity, some bisphosphonates (clodronate, pamidronate, ibandronate, zoledronate) have shown clinical utility in the treatment of complications associated with cancers that cause osteolysis. However, there is now a growing body of evidence from preclinical research showing that bisphosphonates also exhibit antitumor activity. Here, we review the preclinical evidence and discuss the possible antitumor mechanisms of action of bisphosphonates.

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In vitro Antitumor Effects of Bisphosphonates

Tumor cell adhesion to bone. Pretreatment of cortical bone slices or tumor cells with different NBPs leads to the inhibition of breast and prostate cancer cell adhesion to bone (2, 3). Maximal inhibitory activity of NBPs upon treatment of tumor cells is achieved at concentrations (10^{-8}-10^{-6} mol/L) that are much lower than those required when cortical bone slices (instead of tumor cells) are pretreated with these compounds (10^{-5}-10^{-4} mol/L; refs. 2–4). This suggests that soluble NBPs have a higher potency than matrix-bound NBPs in inhibiting tumor cell adhesion to bone. Although integrins mediate cell adhesion, NBPs do not modulate integrin cell surface expression (2, 5). However, these findings (2, 5) do not preclude the possibility that NBPs are inhibiting integrin function. Small GTPases play a key role during integrin activation and zoledronate has been shown to inhibit geranylgeranylation, which is required for the prenylation of small GTPases and the subsequent tumor cell adhesion to bone (4), suggesting that NBPs could inhibit integrin activation.

Tumor cell invasion. Tumor cell invasion is intrinsically linked to a localized cell surface proteolytic activity driven by matrix metalloproteinases (MMP), which favors cell detachment from matrix proteins, thereby promoting cell migration. NBPs inhibit breast and prostate cancer cell invasion (2, 3, 6–8). Bisphosphonates also inhibit the zinc-dependent proteolytic activity of MMPs (2). However, the inhibition of MMP activity only occurs when high concentrations (10^{-4} mol/L) of bisphosphonates are used, whereas submicromolar concentrations of NBPs are sufficient to inhibit tumor cell invasion. Thus, NBPs might rather inhibit cell migration. Indeed, alendronate and zoledronate inhibit ovarian and breast cancer cell migration, respectively, by attenuating the geranylgeranylation of RhoA (6, 8), a key player in cell adhesion dynamics that drive cell motility. Zoledronate also inhibits the chemokine CXCL12–induced breast cancer cell migration by decreasing the cell surface expression of CXCR4, the receptor for CXCL-12 (6). Therefore, the anti-invasive properties of NBPs may be the result of the inhibition of distinct molecular pathways (the mevalonate and chemokine signaling pathways) that mediate in a coordinated fashion cancer cell invasion. These compounds may eventually also inhibit MMP activity if high local bisphosphonate concentrations are achieved in the tumor microenvironment.

Tumor cell proliferation and survival. Submicromolar concentrations of bisphosphonates that inhibit tumor cell adhesion and invasion do not inhibit tumor cell proliferation (2, 3, 6, 8). Yet, higher bisphosphonate concentrations do reduce proliferation and induce apoptosis of a variety of human cancer cell lines, including breast, prostate, ovarian, melanoma, colon, osteosarcoma, pancreatic, myeloma, and leukemia cells (2, 3, 6–10). The mechanisms of apoptosis seem to be through the mevalonate

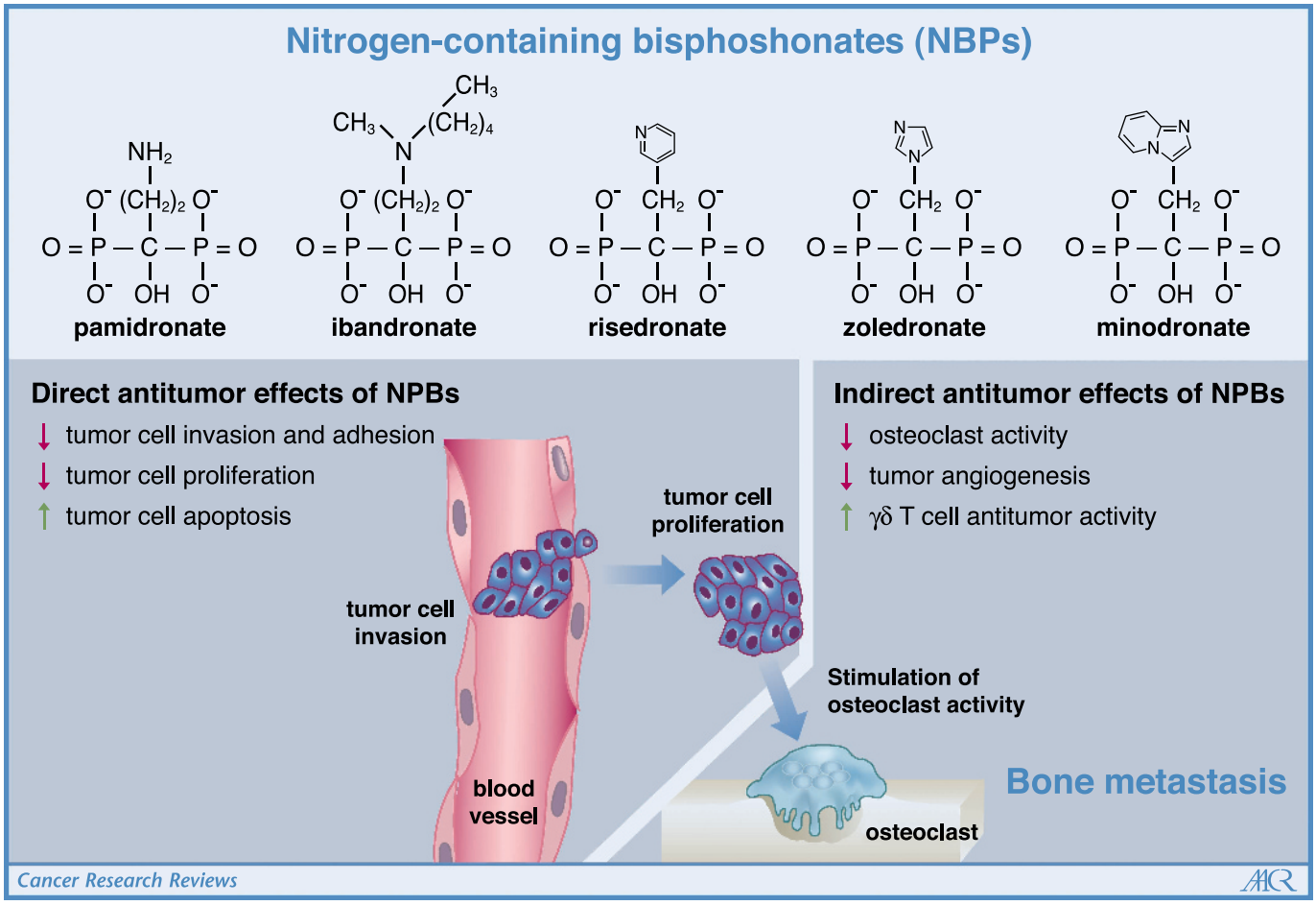

Figure 1. *Top,* chemical structure of NBPs in clinical use and development. NBPs are an indispensable part of therapy for patients with cancers that cause osteolysis. *Bottom,* NBPs affect molecular mechanisms of tumor cell adhesion, invasion, proliferation, and survival *in vitro* (*left*), suggesting they may prevent the formation of (bone) metastases *in vivo*. NBPs also inhibit osteoclast-mediated bone resorption and tumor angiogenesis and stimulate the antitumor activity of γδ T cells (*right*), which could indirectly contribute to the *in vivo* antitumor activity of these compounds.

pathway for NBPs (2, 3, 10). In addition, pamidronate- or zoledronate-mediated apoptosis in breast and prostate cancer cells is associated with the release of mitochondrial cytochrome *c* into the cytosol, leading to the activation of caspases (2, 3). How does caspase activation by NBPs relate to the mevalonate pathway? Failure of the small GTPase Ras to translocate to the plasma membrane in zoledronate-treated cancer cells has been reported (2, 3, 10). This leads to the inhibition of the downstream Ras/Raf-1/MEK/ERK1-2 mitogenic and pKB/Akt antiapoptotic pathways in these cells, and to the subsequent activation of caspases (2, 5). The antiproliferative effects of NBPs are, however, not always the result of apoptotic cell death. Cell cycle analysis of prostate cancer cells treated with NBPs shows, for example, that pamidronate induces a substantial increase of cell apoptosis, whereas zoledronate is more effective at inducing cell cytostasis (3). Similarly, zoledronate-treated BV173 leukemic cells are arrested in the S phase (10). Thus, antiproliferative mechanisms of action of NBPs may vary according to the cell types and/or the bisphosphonates used. Whatever the mechanisms are, the combination of zoledronate with antineoplastic agents (paclitaxel, docetaxel, doxorubicin, imanitib, dexamethasone) results in synergistic apoptotic effects on tumor cell lines (2, 3, 10, 11).

Antitumor Effects of Bisphosphonates in Animal Models

Animal models of tumorigenesis. Risedronate, alendronate, ibandronate, and zoledronate do not inhibit tumor growth when human breast or prostate cancer cells are injected s.c. or orthotopically in immunodeficient animals (2, 3, 12). Similarly, zoledronate or ibandronate does not inhibit the growth of syngeneic 4T1/luc mammary tumors in mice (7, 13). Because of the rapid accumulation of NBPs in bone, tumor xenografts may be exposed to these compounds for too brief a period of time to observe cytotoxicity. However, there are a few reports of growth reduction of melanomas and cervical carcinomas upon treatment of animals with minodronate and zoledronate, respectively (9, 14).

Animal models of bone metastases caused by carcinoma cells. NBPs reduce the development and progression of osteolytic lesions when breast (2, 3), prostate (2, 3), small-cell lung (15), or neuroblastoma (16) tumor cells are inoculated into immunodeficient animals. Zoledronate also impairs the development and progression of osteoblastic lesions caused by human LuCaP 23.1 prostate cancer cells (12). The formation of osteoblastic lesions is often preceded by a wave of bone resorption, explaining the efficacy of the treatment with zoledronate in LuCaP 23.1–bearing mice. Moreover, metastatic animals treated with NBPs experience a

decrease in skeletal tumor burden (2, 3, 12, 16). Similarly, zoledronate and ibandronate decrease the formation of spontaneous bone metastases and reduce bone tumor burden in syngeneic mice bearing 4T1/luc mammary tumors (7, 13).

Animal models of osteolytic lesions caused by myeloma cells. When myeloma cells isolated from patients with medullary disease are injected in human bones implanted s.c. into severe combined immunodeficient mice, zoledronate or pamidronate reduces the progression of osteolysis and tumor burden (2, 3). In contrast, these NBPs do not inhibit tumor burden when myeloma cells are derived from patients with extramedullary disease. Similarly, ibandronate inhibits osteolysis in 5TGM1 and ARH-77 murine models of myeloma; however, myeloma cell growth is not confined to bone, thereby masking the inhibitory effect of ibandronate on skeletal tumor burden (2, 3). Conversely, when the growth is restricted to bone, as it is observed in the 5T2MM murine myeloma model, zoledronate reduces the progression of osteolysis and decreases skeletal tumor burden (17).

Animal models of visceral metastases. The effects of NBPs on visceral metastases are difficult to interpret. Minodronate treatment of animals bearing small-cell lung cancer cells inhibits bone metastasis formation, but has no effect on lymph node, lung, and liver metastases (15). Similarly, the administration of ibandronate in animals bearing 4T1/luc mammary tumors inhibits the spontaneous development of osteolytic lesions, whereas lung metastasis formation remains unaffected (13). In sharp contrast, the administration of zoledronate to 4T1/luc-tumor-bearing animals decreases tumor burden in bone, as expected, but also in the liver and lungs (7). Alendronate also inhibits the i.p. dissemination of Caov-3 ovarian cancer cells *in vivo* (18).

Indirect Antitumor Effects of Bisphosphonates

The peak plasma concentration of bisphosphonates in humans is in the micromolar range (1), suggesting that *in vitro* anti-adhesive and anti-invasive effects of NBPs observed at sub-micromolar concentrations could also take place *in vivo*, especially when combined with standard neoplastic agents (Fig. 1). In bone, local concentrations in the range 0.1 to 1 mmol/L have been calculated for alendronate (1), suggesting that such concentrations in bone could be also achieved with other NBPs. NBPs bound to bone are released during the malignant bone destruction process and could therefore locally promote tumor cell apoptosis (Fig. 1). However, the experimental conditions used *in vitro* to treat tumor cells are far removed from those pertaining to the treatment of osteolytic lesions in animals. Bone is a rich source of growth factors that are released during bone resorption (2). Bisphosphonates, by inhibiting bone resorption, may cause not only a reduction in the extent of osteolytic lesions, but also deprive tumor cells of bone-derived growth factors that are required for tumor-cell proliferation (Fig. 1). In addition, it is most likely that NBPs have direct inhibitory effects on the stroma that supports skeletal tumor growth *in vivo*. Indeed, NBPs can act directly on endothelial cells that are part of the stroma surrounding the tumor. They reduce endothelial cell adhesion and proliferation, and decrease capillary-like tube formation (2, 3, 5, 9). They also inhibit the formation of blood vessels in different animal models of angiogenesis (2, 3). Moreover, minodronate (9) and zoledronate (14) impair the growth of melanomas and cervical carcinomas in animals, respectively, by suppressing tumor-associated angiogenesis. These observations (2, 3, 5, 9, 14), taken together with the fact that NBPs decrease circulating levels of vascular endothelial growth factor in metastatic cancer patients (2), raise the exciting possibility that NBPs could be potent antiangiogenic agents.

NBPs also have immunomodulatory effects; they stimulate the expansion of the most abundant population of human γδ T cells (Vγ9Vδ2 T cells; refs. 3, 19). In addition, the accumulation of mevalonate metabolites in NBP-treated tumor cells renders these cells sensitive to lysis by human Vγ9Vδ2 T cells (19). Thus, NBPs could have a pronounced effect on the immune system, which might contribute to their *in vivo* antitumor activity (Fig. 1). Indeed, a pilot study using pamidronate in lymphoma or multiple myeloma patients has recently shown a significant *in vivo* expansion of Vγ9Vδ2 T cells and an objective tumor response in some of these patients (20).

Conclusion and Future Directions

In conclusion, NBPs have antitumor effects via direct (tumor cell adhesion and invasion, apoptosis) and indirect mechanisms (angiogenesis, γδ T cells) in preclinical research (Fig. 1). However, doses of NBPs currently used in clinical trials do not show any convincing antitumor effect. Higher doses or more frequent dosing may be required to achieve clinically meaningful antitumor effects. Therefore, it will be an important task in the future to determine the most effective doses and schedules of NBPs to maximize their *in vivo* antitumor potential and to take advantage of the observed synergy between NBPs and standard neoplastic agents.

Acknowledgments

Due to space limitations, we have endeavored to describe recent, representative studies, and to direct the reader to previous reviews in which other relevant literature has been cited.

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