LEADING ARTICLE

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Antitumour Effects of Bisphosphonates
First Evidence and Possible Mechanisms

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Abstract

Bisphosphonates have been used successfully for many years in the treatment of hypercalcaemia and to reduce skeletal complications of metastases. In the first years of bisphosphonate use the efficacy of these substances was thought to lie purely in the inhibition of osteoclasts. However, there is recent evidence to suggest that an antitumour effect may also play a role. As well as having an apoptotic and antiproliferative effect on osteoclasts, bisphosphonates may exert a similar influence on macrophages and tumour cells. Whether this effect (at low doses) also plays a role *in vivo* remains unclear and requires further investigation.

Improvements in the survival time of certain subpopulations have been found in many phase III studies with bisphosphonates to date, both in the setting of metastatic breast cancer and in multiple myeloma. However, because survival time in subgroups of patients was neither a primary nor a secondary objective in these studies, these advantages could only be seen as important pointers for future studies.

Some preclinical studies have shown that down-regulation of bone metabolism by bisphosphonates is associated with a lower incidence of bone metastases and destruction in animals, whereas activation is correlated with a higher number of metastases. However, varying results were found in animal experiments with regard to the effect of bisphosphonates on the incidence and growth pattern of non-osseous metastases.

The results of 3 randomised studies in patients with primary breast cancer who received clodronate 1600 mg/day orally have now been evaluated and presented. All 3 studies arrived at different results. In the Heidelberg study there was a reduction in both osseous and non-osseous metastases, whereas in a much larger study performed in Great Britain, Canada and Scandinavia there was a reduction only in the incidence of skeletal metastases. A third study from Finland found no effect on bone metastases, but an increase in the number of visceral metastases and a deterioration in overall survival. Because the dosage was identical in all 3 studies, the differing results can only be either random or methodological (for example inclusion criteria or sample size). Overall, the results are very promising, but there is a need for further studies.

Bisphosphonates are analogues of pyrophosphate, and, like it, are strongly bound to hydroxyapatite on the bone surface. Unlike pyrophosphate, which is rapidly split by the hydrolytic enzymes of osteoclasts, the bisphosphonates are stable and reduce the activity of osteoclasts by a variety of means. This antiresorptive effect is the pharmacological basis of treating tumour osteolysis.[1-4]

Bisphosphonates exert an osteoprotective effect on the remaining healthy skeleton and, in rare

cases, can also support the reossification of affected areas, particularly in patients receiving concomitant radiotherapy.

Numerous clinical trials, particularly in patients with metastasising breast cancer and multiple myeloma, have demonstrated the ability of bisphosphonates to reduce skeletal signs and symptoms. The number of expected complications was reduced by about 30 to 40%, irrespective of the individual agent and the route of administration (oral versus intravenous).[5-10] None of the studies were able to show better survival in the entire group of patients treated with bisphosphonates. However, initial evidence of longer survival has been reported in certain subpopulations of patients with multiple myeloma and metastasising breast cancer.

Several animal models, as well as experiments in cell cultures, suggest that bisphosphonates act prophylactically with regard to subsequent bone metastasis. Initial clinical trials in patients with primary breast cancer were able to show a reduction in the number of new skeletal metastases as a result of bisphosphonate therapy. This article provides a concentrated summary and discussion of current experience and clinical trials.

1. Pathophysiology of Bone Metastasis

In principle, all malignant tumours are capable of metastasising to bone. In practice, however, only a few regularly and frequently affect the skeleton. This group includes breast and lung cancer and prostate, thyroid and renal cell carcinomas, which together are responsible for about 80% of all bone metastases.[11,12] Apart from the fact that metastasis is generally a sign of incurable disease, the typical complications of bone metastases impair the quality of life of those affected. In breast cancer, the complications of bone metastases include bone pain, pathological fractures, spinal compression syndromes, hypercalcaemic episodes and bone marrow carcinomatosis with suppression of haemopoiesis.[13-15]

Bone metastases develop in the same way as other metastases: the tumour releases cells that pass through the extracellular matrix, penetrate the basal membrane and are then transported to distant organs by the circulation. In the target organ, the process runs in reverse: the metastatic cells enter the perivascular space and are deposited there. This process is facilitated by adhesion molecules and chemotaxis.[16] The majority of the disseminated cells die, but a few are capable of micrometastatic proliferation or remain dormant, only to grow later. Disseminated tumour cells are found in the bone marrow of 30 to 45% of patients with breast cancer, but it is still not possible to differentiate between cells that die and those that survive and are capable of proliferation.[17]

Although the processes of tumour seeding, cell dormancy and the early division phase are only poorly understood, we now have detailed insights into the interaction between micrometastases in the bone marrow and the bone and its cell systems. In the early phase of bone metastasis the bone is not destroyed by the tumour itself, but by the osteoclasts, which are activated by paracrine secretion of substances. A typical substance produced by metastatic tumour cells is parathyroid hormone-related peptide (PTHrP). The paracrine activation of osteoclasts leads to degradation of mineralised bone matrix. Growth factors and cytokines (for example insulin-like growth factor, platelet-derived growth factor, transforming growth factor β) that were stored in the bone are possibly able to increase the proliferation rate of the micrometastases. This self-sustaining cycle of interactions characterises the process of tumour osteolysis (fig. 1). The therapeutic use of bisphosphonates considerably disrupts or interrupts the dialogue between the tumour cells and bone cells.[18,19]

2. *In Vitro* Studies of the Cytotoxicity of Bisphosphonates

Surprisingly, there are only a few investigations from the first years of bisphosphonate research that confirm or refute a direct or indirect cytotoxic effect of these drugs. However, as long ago as 1982, Reitsma et al.[20] demonstrated a cytotoxic effect in macrophages. With clodronate this effect was seen with doses in the therapeutic range, but only at

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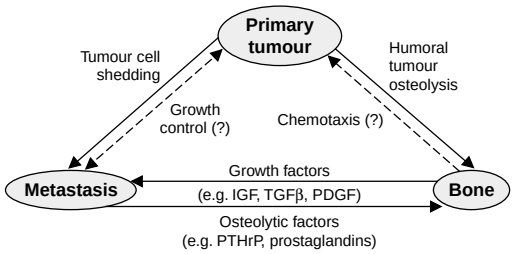

Fig. 1. Tumour osteolysis is characterised by a self-sustaining cycle of interactions between metastases and the cell systems of the bone. Metastatic tumour cells produce osteolytic factors such as parathyroid hormone-related peptide (PTHrP) and prostaglandins. The paracrine activation of osteoclasts leads to degradation of mineralised bone matrix. Growth factors and cytokines [for example insulin-like growth factor (IGF), platelet-derived growth factor (PDGF) and transforming growth factor β (TGFβ)] stored in the bone may increase the proliferation rate of the micrometastases.

much higher doses with pamidronate. Mönkkönen's group in Finland confirmed this effect of clodronate in numerous studies, and discovered that liposomal clodronate is much more potent than the free substance. They were also able to demonstrate a cytotoxic effect for liposome-encapsulated etidronate and pamidronate.[21,22] Several studies in recent years have confirmed that the cytotoxic effect in both macrophage-like cells and osteoclasts is achieved by the induction of apoptotic processes.[23-25] Interestingly, there appear to be differences between the various bisphosphonates. Clodronate induces apoptotic cell death following metabolism to the nonhydrolysable ATP analogue adenosine 5′-[β,γ-dichloromethylene]triphosphate.[26,27] In the case of aminobisphosphonates, apoptosis seems to be caused by inhibition of the mevalonate pathway with subsequent prevention of prenylation.[28-30] There are now several studies in cell lines that underline this apoptotic effect for both clodronate and the aminobisphosphonates.[31-35] It remains uncertain whether this effect is only an additional explanation of the direct action of bisphosphonates on tumour cells, or whether apoptosis of macrophages and osteoclasts can alter the microenvironment of the tumour cells. However, whether an identical effect (at low doses) can also be achieved *in vivo* remains unclear and requires further investigation.

In addition, 2 investigations have since been published that demonstrate that bisphosphonates can alter the adhesion properties of tumour cells and bone surfaces. Van der Pluijm et al.[36] showed that slices of bone were resistant to tumour cell adhesion following incubation with various bisphosphonates. The strongest effect was seen with ibandronate, the weakest with clodronate. Another study[37] investigated the adhesion properties of breast and prostate carcinoma cell lines. Cell adhesion was drastically reduced following pretreatment with clodronate. It was not reported whether the influence of bisphosphonates on adhesion molecules also had an effect on tumour cells that had already colonised, leading to their remobilisation; this should be urgently investigated in animal models.

3. Prophylaxis of Metastasis in Animal Models

3.1 Etidronate

The very first evidence of a reduction in osteolytic lesions as a result of early bisphosphonate therapy was seen with etidronate.[38,39] Nemoto et al.[40] demonstrated a reduction in tumour osteolyses and prolonged survival in a mouse tumour model with bladder carcinoma cells. However, the authors were quick to note the relatively weak effect of etidronate and the mineralisation disturbances it caused.

3.2 Clodronate

The most extensive studies of the osteoprotective effect of clodronate were performed by Krempien and Manegold[41] and by Krempien.[42,43] In numerous experiments with the PTHrP-producing Walker carcinosarcoma 256, tumour cells were inoculated intra-osseously and the effects assessed by means of histology. The extent of bone destruction was drastically reduced by pretreatment with bisphosphonates. The degree of destruction was clearly dependent on the duration and intensity of the clodronate therapy: the longer the therapy-free interval, the weaker the osteoprotective effect.

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This finding was interpreted by the authors as evidence that long term therapy with bisphosphonates is more appropriate than interval therapy in a prophylactic setting.

The demonstrated effect was primarily achieved via osteoclast inhibition, but an antitumour effect may also have played a role in these investigations. In a further series of investigations in rats in which Walker cells were injected into the aorta via a catheter, it was found that up-regulation of bone metabolism [local trauma, high doses of vitamin D (colecalciferol), uraemia or calcium-deficient diet] was associated with an increase in the incidence of bone metastases and adrenal metastases. These investigations also show that there is a mutual interaction between bone metabolism and the metastatic process and highlight the difficulties in differentiating between direct and indirect antitumour effects.[44]

3.3 Pamidronate

Krempien et al.[45] and Wingen et al.[46] were able to demonstrate the same osteoprotective effect for pamidronate, again using the hypercalcaemic Walker tumour. A difference between the individual bisphosphonates was not observed.

Kostenuik et al.[47] investigated the efficacy of pamidronate on rat bone after injection of Walker cells. Fisher rats were pretreated with pamidronate 0.5 mg/kg for 7 days before intramuscular injection of tumour cells. The animals were killed 2 weeks later and the skeleton was subjected to histological investigation. The trabecular volume was 3 times higher in animals pretreated with pamidronate than in controls. Surprisingly, the tumour mass in bone was higher in the pretreated animals, although an effect on extra-osseous metastases was seen. In a further animal model, Müller et al.[48] were able to inhibit the intraperitoneal growth of myeloma cells by continuous injections of pamidronate. In some cases, the tumour weight in treated animals was 50% lower than in the controls.

3.4 Risedronate

Contradictory results were reported by Sasaki et al.[49] The authors were able to demonstrate a reduced tumour burden in nude mice previously treated with the bisphosphonate risedronate that subsequently received an intracardiac injection of a human breast cancer cell line (MD 231). In a second experiment, risedronate was not given until the first bone metastasis was observed. In both studies administration of the bisphosphonate delayed or reduced the occurrence of further skeletal metastases; survival was also significantly prolonged.

Hall and Stoica[50] were also able to show a reduction in the number, extent and size of osseous metastases as a result of bisphosphonate therapy. In their experiments, rats also received an intracardiac injection of a breast cancer cell line (ENU 1564). Thereafter, the animals were treated with the bisphosphonate risedronate; control animals received saline solution. The rats were killed after 4 weeks and the pattern of metastasis was investigated. Whereas visceral metastasis was similar in the 2 groups, the animals that had received adjuvant bisphosphonate treatment exhibited considerably fewer bone metastases (33 metastases) than the controls (151 metastases). Furthermore, 30% of the bisphosphonate-treated animals were completely free from skeletal metastases compared with 16% of the controls (table I).

4. Initial Evidence of Prophylaxis in Clinical Trials

The therapy of tumour osteolysis and its associated complications is currently highly unsatisfactory and of a purely palliative nature. As a result, it would be extremely useful to provide prompt osteoprotective therapy for at least those patients at high risk of subsequent bone metastasis. Older clinical trials provided initial evidence that patients

Table I. Reduction of osseous metastases in rats as a result of bisphosphonate therapy. The animals received an intracardiac injection of a breast cancer cell line and were then treated with risedronate or placebo for 4 weeks[50]

Treatment	Total number of metastases	Rats with multiple metastases (%)	Metastasis-free rats (%)
Placebo	151	71	16
Risedronate	33	33	30

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treated with bisphosphonates developed fewer new metastases. In particular, Elomaa et al.[51,52] were able to observe positive effects after administration of clodronate to patients with breast cancer and bone metastases, albeit in a very small group. If the bisphosphonate was discontinued, the number of new metastases became the same in both groups.

Interestingly, many studies, both in metastatic breast cancer and in multiple myeloma, have shown survival advantages for subgroups of patients. However, these findings must be interpreted with caution, since survival was not a primary or secondary objective for subsets of patients in these studies. Such observations have been made in breast cancer patients on oral clodronate,[53] in premenopausal patients on intravenous pamidronate,[10] and in patients with osseous and visceral metastases on intravenous ibandronate.[54] Similar findings have been made in multiple myeloma patients without vertebral fractures on oral clodronate[9] and in patients receiving second-line chemotherapy and intravenous pamidronate.[8]

4.1 Clodronate in Secondary Prevention of Metastases

The osteoprotective effect of clodronate was first investigated in a double-blind, randomised, placebo-controlled study[55] in patients with advanced breast cancer (local recurrence or distant metastases, but not bone metastases). 66 women received clodronate 1600 mg/day orally for 3 years, and 67 women received placebo for the same period. At the end of therapy it was found that both the number of patients with bone metastases (15 *vs* 19) and the total number of metastases (32 *vs* 63; $p < 0.005$) were lower in the clodronate group than in the control group. As expected, the number of skeletal complications was also lower in the treatment group.

4.2 Clodronate in the Primary Prevention of Metastases

The first study into the adjuvant use of bisphosphonates was presented by our group at the 1997 meeting of the American Society for Clinical Oncology in Denver, and has meanwhile been published.[56] In this randomised but not placebo-controlled study, 157 patients were treated with clodronate 1600 mg/day orally for 2 years and a further 145 patients served as controls. At the time of the primary operation, all patients had immunocytologically detectable tumour cells in the bone marrow (minimal residual disease) and were therefore at high risk of subsequent metastasis.[57] The study was evaluated after a median observation period of 36 months. In the bisphosphonate group there was a significant reduction not only in the number of bone metastases, but, surprisingly, also in the number of non-osseous metastases (table II). In addition, the number of bone metastases per patient in the bisphosphonate group was half that seen in the control patients.

Two further studies with oral clodronate 1600mg/day have now been presented: a multicentre, double-blind, placebo-controlled study performed in Great Britain, Canada and Scandinavia with a large sample size (n = 1076)[58] and a single-centre, randomised, non–placebo-controlled study in node-positive patients performed in Finland (n = 299).[59] In the multicentre study, there was a significant reduction in skeletal metastases of the same order as that seen in the Heidelberg study.[56] A (late) trend towards a reduction in visceral metastases was observed, but overall survival was unchanged compared with that in the placebo group.[58] The study from Helsinki showed no changes for osseous metastasis, but a more fre-

Table II. Reduction of osseous and non-osseous metastases in patients with primary breast cancer by adjuvant clodronate treatment (1600 mg/day orally for 2 years). Follow-up was for a median of 36 months. Values show actual number of events[56]

Outcome	Clodronate (n = 157)	Control (n = 145)	p value
Distant metastases	21	42	<0.001
Bone metastases	12	25	0.003
Visceral metastases	13	27	0.003
Death	6	22	0.001
Bone metastases per patient	3.1	6.3	0.004

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quent incidence of visceral metastases and a deterioration in overall survival.[59]

The differing results of the 3 studies require critical interpretation. Until now, both preclinical and clinical trials have failed to demonstrate a toxic effect of clodronate. The prevention study by Saarto et al.[59] is the first of its kind. If a toxic effect is indeed present, this would have enormous repercussions, since it might have an impact on other bisphosphonates, not just on clodronate. A toxic effect could not go unregarded in view of the use of bisphosphonates over a period of years in osteoporosis.

Even so, and this must also be remembered when considering Saarto's study, the best effect was still seen with regard to bone metastases (i.e. no significant deterioration). After careful consideration, the reason behind the conflicting results in the 3 prophylaxis studies can lie only in methodological differences. Only a small number of patients (about 300 in each case) were enrolled in the Heidelberg and Finnish studies, neither of which was double-blind. Such a small sample size can produce random results. From a methodological point of view, the multicentre study is the best: it is most trustworthy with its large sample size, and its results are closer to those of the Heidelberg study. The inclusion criteria were different in all 3 studies (table III). In the largest study, all patients with primary breast cancer were enrolled. This is perhaps the reason why effect occurred somewhat later. Patients with tumour cells in the bone marrow were enrolled in the Heidelberg study. It can be speculated that such patients have the best preconditions for prophylactic therapy because an antitumour effect of bisphosphonates accumulated on the bone surface could influence the individual tumour cells.

4.3 Pamidronate in Secondary and Primary Prevention of Metastases

In a non–placebo-controlled randomised study[60] in patients with breast cancer and bone metastases, 152 patients received chemotherapy alone and 143 patients received chemotherapy supplemented by infusion of 45mg of pamidronate every 3 weeks. The therapy was continued at least until renewed skeletal progression occurred. Evaluation of the study revealed a prolongation of the time to bone progression (249 *vs* 168 days; p = 0.02) and of the complication-free period (533 *vs* 490 days), but not a reduction in the number of new bone metastases. Another study[61] also failed to show a reduction in the number of metastases. Here, 124 patients with breast cancer (local or distant metastases, but no bone metastases) were enrolled and treated continuously with 300mg pamidronate orally, or simply followed up. There was no difference in the frequency of metastases between the 2 groups at the end of the study. Identical (negative) results were seen in 3 studies with 304 myeloma patients and 610 patients with advanced breast cancer but without skeletal metastases.[62] After randomisation, the myeloma patients received 300mg pamidronate orally or placebo, and the breast cancer patients 150mg pamidronate orally or placebo for an unlimited period. Both investigations failed to show a reduction in the frequency of bone metastases. It would, however, be wrong to consider pamidronate ineffective in prophylaxis. The problem with oral administration of pamidronate is its extremely poor absorption from

Table III. Comparison of 3 studies with adjuvant clodronate treatment (1600 mg/day orally) in patients with primary breast cancer

Study	No. of patients	Selection	Placebo control	Duration (y)	Centre	Effect on		
						bone metastases	visceral metastases	overall survival
Diel et al.[56]	302	TCD positive	No	2	Single	+	+	+
Powles et al.[58]	1079	No	Yes	2	Multi	+	↔	↔
Saarto et al.[59]	299	Node positive	No	3	Single	↔	–	–

TCD = tumour cell detection in bone marrow; + indicates improvement; ↔ indicates unchanged; – indicates deterioration.

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the small intestine (<1%). At a dose of 600mg, oral pamidronate is also extremely effective, e.g. in the treatment of tumour osteolysis, but its adverse effects (oesophagitis and gastritis with ulceration) are unacceptable. It is likely that prophylaxis studies with intravenous pamidronate will produce better results.

5. Arguments in Favour of Adjuvant Therapy with Bisphosphonates

Most drugs that are now used in the systemic adjuvant therapy of primary malignancies were first tested with regard to their efficacy and tolerability in patients receiving palliative treatment. The same is likely to be true of the bisphosphonates. There are now a number of studies that demonstrate the effectiveness of the individual substances in preventing skeletal complications.

In comparison to classical cytotoxic substances, the rate of complications and adverse effects produced by the bisphosphonates is extremely low, and is comparable to that seen with tamoxifen. Not a single study has shown any signs of long term toxicity to bone, an effect that was previously feared. Bisphosphonates are also used in non-oncological indications (Paget's disease, osteoporosis).

Animal studies and initial clinical experience suggest that further investigation of bisphosphonates in the adjuvant setting is likely to be very promising. It is not clear whether the effect arises as a result of a direct cytotoxic action, or whether micrometastatic cells are prevented from growing as a result of changes to the microenvironment. Almost all studies to date suggest that it is logical to treat the metastatic target organ as well as the primary tumour. The skeleton, with its pronounced interaction between bone cells and metastatic tumour cells, offers an excellent model for this approach.

It is now urgently necessary to check the preliminary but encouraging results of adjuvant bisphosphonate therapies. Candidates for clinical trials of such treatments are patients with tumours that preferentially metastasise to bone, particularly those with breast, lung and prostate carcinoma, as well as patients with multiple myeloma. To obtain results quickly, it would be best to start by enrolling only patients at high risk of early metastasis, i.e. patients with regional lymph node involvement, local progression or tumour cells in the bone marrow, or, alternatively, patients with elevated concentrations of specific prognostic factors for bone metastasis. A promising prognostic factor could be the detection of bone sialoprotein in serum in patients with primary breast cancer. Bone sialoprotein is a non-collagenous matrix protein that can also be formed by breast cancers themselves. In an extensive study[63] we were able to show that, with very few exceptions, patients who developed subsequent skeletal metastases had excessively high serum levels of bone sialoprotein preoperatively. Here too, another piece in the jigsaw puzzle of the pathogenesis of bone metastasis seems to fall into place, since bone sialoprotein acts as an adhesion molecule with which tumour cells are provided by the primary tumour, and which the cells need to establish contact with the bone matrix. However, because crosslaps (collagen degradation fragments in serum) were elevated in some patients, it is not completely clear whether some of the serum bone sialoprotein detected is derived from bone metabolism rather than from the primary tumour.[64] This could mean that bone sialoprotein is also an early marker for an onsetting bone metastasis. Possibly, bone sialoprotein is both a prognostic factor and an early marker, and thus identifies a risk group that would profit from preventive bisphosphonate therapy.

At present it is completely unclear whether adjuvant bisphosphonate as an experimental new treatment should be given continuously and orally, or whether intravenous interval therapy would produce the same results. Another matter of speculation is whether the dosage used in the palliative setting is sufficient, or whether lower dosages would also suffice. The duration of adjuvant therapy is also unclear. In fact, the only issue about which there is no doubt is that we need trials to confirm the initial clinical results.

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