

Invited review

Bone disease and bisphosphonates in multiple myeloma

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Bisphosphonates are potent inhibitors of myeloma – induced osteoclast – mediated bone resorption. On the basis of the published studies one may conclude that long – term oral clodronate administration (1600-2400 mg daily) as an adjunct to chemotherapy in patients with multiple myeloma with osteolysis is an efficient approach in prevention of hypercalcaemia and amelioration of skeletal morbidity. Intravenous clodronate and intravenous pamidronate in a single dose of 1500 mg and 90 mg, respectively is efficient in the treatment of hypercalcaemia and may be recommended in patients with normal renal function. Monthly intravenous pamidronate administration (60-90 mg) as an adjunct to chemotherapy in patients with multiple myeloma with osteolysis reduces bone pain and is efficient in the prevention and treatment of hypercalcaemia and reduces skeletal morbidity. Occurrence or worsening of anaemia during long – term pamidronate treatment deserves attention and further study. Caution must be exercised before concluding that clodronate and pamidronate improves survival in multiple myeloma. The role of bisphosphonates in the treatment of myeloma induced hypercalcaemia is already established but despite their increasing use in oncological practice the questions regarding the optimal selection of patients and the duration of treatment of myelomatous bone disease remain unanswered.

Choroba kości i bisfosfoniany w szpiczaku plazmocytozym

Bisfosfoniany hamują resorpcję kostną, związaną ze szpiczakową aktywacją układu osteoklastów. Opublikowane badania wskazują, że długotrwałe stosowanie kłodronianu doustnie (w dawkach 1600-2400 mg dziennie), jako leczenia wspomagającego chemioterapię u chorych na szpiczaka plazmocytozy z osteolizą, jest postępowaniem zmniejszającym niebezpieczeństwo występowania hiperkalcemii i łagodzącym chorobę kości. Podobnie, jak pamidronian w dawce dożyłnej 90 mg, dożylnie stosowanie kłodronianu w jednorazowej dawce 1500 mg jest skuteczne w leczeniu hiperkalcemii i może być zalecane u chorych z prawidłową czynnością nerek. Długotrwałe stosowanie comiesięcznych infuzji dożylnych pamidronianu (w dawkach 60-90 mg), jako leczenia wspomagającego chemioterapię u chorych na szpiczaka plazmocytozy z osteolizą, zmniejsza niebezpieczeństwo występowania hiperkalcemii i powikłań kostnych, a w pierwszych miesiącach leczenia zmniejsza także bóle kostne, ale częstsze występowanie lub pogłębianie się niedokrwistości wymaga uwagi i dalszych badań. Wpływ kłodronianu i pamidronianu na czas przeżycia chorych na szpiczaka jest wątpliwy. W chwili obecnej stosowanie bisfosfonianów w leczeniu hiperkalcemii szpiczakowej jest uzasadnione, natomiast nie ustalono optymalnych wskazań do ich stosowania w zapobieganiu i leczeniu szpiczakowych powikłań kostnych.

Key words: multiple myeloma, osteolytic lesions, bisphosphonates, clodronate, pamidronate

Słowa kluczowe: szpiczak plazmocytozy, osteoliza, bisfosfoniany, kłodronian, pamidronian

Bone biology in multiple myeloma

The maintenance of skeletal integrity requires a strict balance between the anabolic and resorptive actions of osteoblasts and osteoclasts, respectively. Osteoclasts are large multinucleated cells derived from the monocyte / macrophage lineage that are responsible for the resorption of bone. Its recruitment, activity, and apoptosis are under

the modulation of a series of hormones and cytokines and under the control of the cells of osteoblastic lineage. Osteoclast differentiation requires cell-cell contact between osteoblastic / stromal cells and cells of the hematopoietic lineage. Recently, proteins involved in the cell-cell interaction have been identified. RANKL (receptor activator of nuclear factor NF- κ B ligand) [which is identical to TRANCE (TNF – related activation – induced cytokine) and ODF (osteoclast differentiation factor) / OPGL (osteoprotegerin ligand)] was identified as the ligand [1]. RANKL is a member of the TNF ligand family and is expressed by osteoblastic / stromal cells. RANKL activates its receptor RANK, which is expressed on oste-

oclasts and osteoclast progenitor cells, to induce osteoclast differentiation and function. A decoy receptor, OPG / OCIF (osteoclastogenesis inhibitory factor), has also been identified. OPG (osteoprotegerin) is a secreted member of the TNF receptor family. OPG binds to RANKL, thus inhibiting osteoclast differentiation or function. Cells of lymphatic system also express RANK, RANKL, and OPG.

A number of studies have demonstrated that bone resorption is increased in patients with multiple myeloma [2, 3, 4, 5]. Increased resorption has also been detected in patients defined as having early myeloma [2], or with a low tumour infiltration of the bone marrow [4], suggesting that increased resorption is an early event in the disease process. The increased bone resorption observed in patients with multiple myeloma is associated with tumour infiltration and correlates with tumour burden [4, 6]. Myeloma cells are found closely associated with bone surfaces actively undergoing bone resorption, suggesting that tumour cells stimulate the resorption process directly. Apart from that, myeloma cells can secrete, or stimulate other local cells to secrete, a series of bone – resorbing cytokines such as IL-6, IL-1 β , TNF β , MIP1a, PTH-related protein which induce osteoclasts to resorb bone. RANKL / TRANCE, a cytokine normally responsible for the generation and survival of osteoclasts, mediates myeloma-associated bone destruction. RANKL / TRANCE expression is stimulated by ligands which signal through G – proteins, STATs, TNF and TGF- β receptors. As mentioned previously malignant plasma cells produce a number of osteoclast activating factors that stimulate stromal cell expression of RANKL / TRANCE by triggering these signaling pathways. RANKL / TRANCE, in turn, binds to its receptor on myeloid precursor to foster the development of osteoclasts. Pearse et al. [7] identified TRANCE as the common mediator of myeloma – induced osteolysis. They demonstrated that bone marrow biopsies from patients with myeloma – induced osteolysis display overexpression of TRANCE mRNA by bone stroma and they also found that inhibitors of TRANCE signaling inhibit myeloma – induced osteoclastogenesis, whereas inhibitors of any single osteoclast activating factor give variable results.

Bone destruction caused by aberrant production and activation of osteoclasts, results in significant morbidity for over 80% of patients with multiple myeloma [8]. Localized osteolysis can lead to fractures, pain and hypercalcaemia.

Mechanisms of action of bisphosphonates

Bisphosphonates are analogs of endogenous pyrophosphate in which a carbon atom replaces the central atom of oxygen (Fig 1). This carbon substitution makes these compounds resistant to hydrolysis (to endogenous phosphatases). Binding to the mineral appears to be due to the P-C-P structure, while the antiresorptive activity is influenced both by the P-C-P part and by the structure of the side chains. Bisphosphonates have a high affinity for bone mineral and will selectively target to bone surfa-

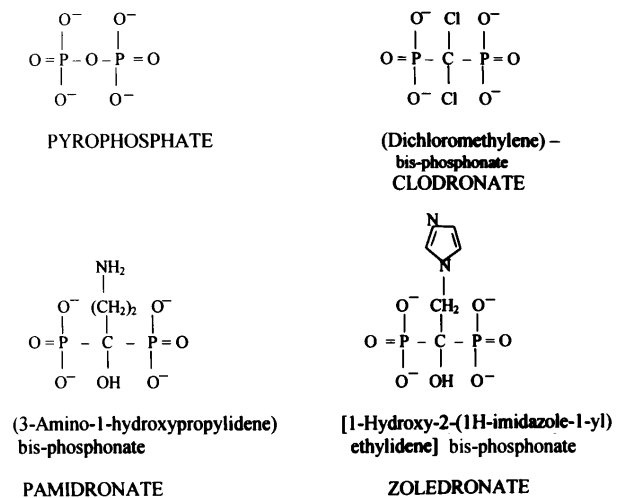


Fig.1. Chemical structure of bisphosphonates

ces. They preferentially bind to bones that have high rates of bone turnover (ie, undergoing increased bone resorption or formation). Thus these agents are concentrated at the exposed bone surface that undergoes active remodeling. The binding of these substances is usually reversible at sites where the bone surface is accessible to the extracellular fluid. However, it is irreversible at sites which become buried by new bone formation, until the bone with the bisphosphonate is destroyed again during modeling or remodeling.

The following bisphosphonates have been investigated in animals and humans with respect to their effect on bone: alendronate, clodronate, EB-1053, etidronate, ibandronate, incadronate, minodronate, neridronate, olpadronate, pamidronate, risedronate, tiludronate, zoledronate. The potency of different bisphosphonates on bone resorption varies from 1 for etidronate to approximately 10,000 for zoledronate in the rat, about 10 or more times less in humans (Fig. 2).

The inhibition of bone resorption occurs as a result of the effect of these drugs on osteoclast, both directly and indirectly. Bisphosphonates were first shown to reduce oste-

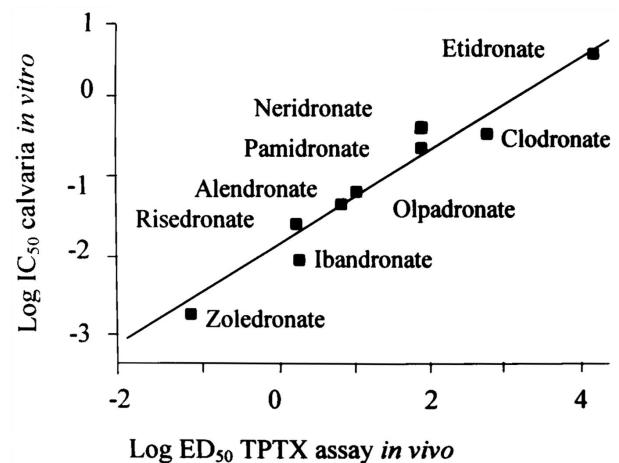


Fig.2. Inhibitory activity of bone resorption of various bisphosphonates *in vitro* in mouse calvariae and *in vivo* in the thyroparathyroidectomized rat

oclast development from their precursors, as well as inhibiting movement of osteoclasts to the bone surface, where they would normally resorb bone. Recent studies have shown that these compounds may be internalised by osteoclasts and interfere with specific biochemical pathways that ultimately lead to osteoclast apoptosis. The nature of the specific molecular targets is only now becoming clear. It was found that nitrogen containing bisphosphonates (risedronate, zoledronate, ibandronate, alendronate, pamidronate) can inhibit the mevalonate pathway, by inhibiting farnesyl pyrophosphate synthase. This leads to a decrease of the formation of isoprenoid lipids such as farnesyl- and geranyl-geranyl-pyrophosphates. These are required for the post-translational prenylation of proteins, including the GTP-binding proteins Ras, Rho, Rac, and Rab. These proteins are important for many cell functions, including cytoskeletal assembly and intracellular signaling. Therefore, disruption of their activity will induce a series of changes leading to decreased activity, probably the main effect, and to earlier apoptosis in several cell types, including osteoclasts. In osteoclasts the lack of geranylgeranylpyrophosphate is probably responsible for the effects [9, 10, 11]. It was also shown that some non-nitrogen-containing bisphosphonates such as etidronate, clodronate, and tiludronate, can be incorporated into the phosphate chain of ATP-containing compounds so that they become nonhydrolyzable. The new P-C-P containing ATP analogs inhibit cell function and may lead to apoptosis and cell death [10, 12, 13].

Several *in vivo* animal and *in vitro* human studies supported the possible role of bisphosphonates as antimyeloma agents. By inhibiting bone resorption bisphosphonates may alter the local factors that may be important in the growth and survival of the tumour cells. A reduction in the production of the cytokine IL-6 from bone marrow stromal cells exposed to bisphosphonates has been found (Savage et al. 1996 – Congress publication). The osteoclast itself is a potent producer of this same cytokine. IL-6 not only enhances bone resorption but also is an important growth factor for myeloma. As such, reducing the availability of this cytokine in the bone microenvironment by exposure to bisphosphonates may not only inhibit bone resorption but may inhibit tumour growth as well.

Recent studies have suggested that pamidronate, incadronate and zoledronate can inhibit myeloma cell proliferation and induce tumour cell apoptosis *in vitro* [14, 15]. This effect has also been observed in primary myeloma cells isolated from patients with multiple myeloma [14]. Furthermore, bisphosphonates appear to be able to cause apoptosis of human myeloma cells by inhibiting enzymes of the mevalonate pathway, suggesting a similar molecular mechanism of action to that seen in osteoclasts [16].

Yaccoby et al. [17] have shown a reduction in lytic bone lesions and tumor burden in severe combined immunodeficiency mice that were implanted with fresh human myeloma bone marrow and fetal bone and were treated with pamidronate. However, treatment with ibandronate, which is more potent than pamidronate, in a murine model of myeloma showed only a reduction in

lytic bone disease, without an impact on tumour burden [18]. Croucher et al. have investigated the effect of ibandronate in the 5T2 murine model of myeloma in which the bisphosphonate was administered once the myeloma disease had established. Treatment with ibandronate had no effect on tumour burden or tumour cell apoptosis. Taken together these murine studies suggest that bisphosphonates are able to modulate the development of the bone disease but have little effect on the tumour itself. However, clinically in a study of two patients with established myeloma, pamidronate was shown to significantly decrease the proportion of bone marrow plasma cells and concentration of serum monoclonal protein [19].

Bisphosphonate treatment in multiple myeloma

Bisphosphonates have become useful in the treatment of diseases characterised by increased bone resorption. The following bisphosphonates are commercially available in some countries for use in human bone disease: etidronate, clodronate, pamidronate, alendronate, ibandronate, risedronate, tiludronate. A number of studies have investigated the effect of etidronate, clodronate and pamidronate on the development of osteolytic bone disease in patients with multiple myeloma [20-33].

Oral etidronate has been found to be ineffective in patients with multiple myeloma [20].

The Finnish Myeloma Trial with 350 patients was multicenter study where all patients received melphalan – prednisolone and were randomized to receive oral clodronate 2400 mg daily or placebo for 24 months. The proportion of patients with progression of osteolytic bone lesions was 24% in the placebo group and 12% in the clodronate group ($p=0.026$). Progression of vertebral fractures was lower in the clodronate group but the difference was not significant (30% vs 40%) [29].

In the Mc Closkey et al. study [31] carried out within the framework of the VI th MRC Multiple Myeloma Trial 536 patients with recently diagnosed multiple myeloma were randomized to receive either clodronate 1600 mg daily or placebo in addition to polychemotherapy (ABCM, ABCMP). The minimum follow-up for all patients was 1.3 years. Treatment with clodronate decreased the incidence of severe hypercalcaemia (5% vs 10%, $p=0.06$). The occurrence of pathological fractures at both non-vertebral (6.8% vs 13.2%) and vertebral (38% vs 55%) sites was significantly reduced with a significant prevention of height loss. The reduction in the incidence of non-vertebral and vertebral fractures was observed within the first year of treatment and persisted throughout the duration of exposure. The median overall survival from the entry into the study was 2.9 years for patients receiving clodronate and 2.8 years for patients receiving placebo.

In the Kraj et al.' trial [26] a comparative study on clodronate efficacy in prevention of new osteolytic foci occurrence, prevention and treatment of hypercalcaemia and hypercalciuria as well as bone pain has been carried out in 61 multiple myeloma patients with osteolysis. In all

patients chemotherapy according to VMBCP or VMCP/VBAP regimens was administered. Thirty one patients received clodronate (Bonafos; Leiras) *per os* 2.4 g/24hrs. Median treatment duration amounted 17 months and in 14 patients treatment duration exceeded 24 months. Assessment of patients' survival time was further conducted during the next 5 years after discontinuation of clodronate treatment. Bone pain reduction was not significantly greater and incidence of hypercalcaemic episodes was lower in the group of patients treated with clodronate compared to the control group (8 vs 14, $p < 0.05$). After 24 months a further osteolysis progression occurred in 43% of clodronate treated patients and in 60% of those in the control. The number of new vertebral fractures observed during the first year of treatment in the control group and in the clodronate group were similar – 79 versus 72/100 patient – year, respectively. Proportions of patients experiencing new vertebral fractures after the second year of the study was insignificantly lower in the clodronate group than in the control (37% versus 46%, $p = 0.1$) while the corresponding ratios of new vertebral fractures /100 patients – year were 28 and 41 ($p = 0.08$), respectively. Patients' survival time did not differ between two compared groups. Median survival time since the diagnosis of multiple myeloma in the clodronate treated patients is 60 months while in the control group – 64 months ($p = 0.9$).

The outcomes of clodronate efficacy in multiple myeloma achieved in Kraj et al.' study [26] are not so convincing as those obtained in Finnish and British trials- so far the largest investigations dealing with oral clodronate effectiveness in myelomatosis [29, 31]. This inconsistency is perhaps connected with the fact that clodronate in aforementioned studies was administered in patients with recently diagnosed multiple myeloma both with and without osteolysis and presenting also early stages of malignancy.

In one open controlled prospective study of parenterally administered clodronate, at a dose of 600-1000 mg /4-6 weeks, in addition to cytostatic therapy survival for multiple myeloma patients on clodronate prophylaxis was longer than for those who did not receive clodronate prophylaxis [32]. Another study of 1600 mg/day oral clodronate showed less progression in bone and less pain in the clodronate group [25].

Early studies documented a reduction of biochemical markers of bone resorption and bone pain in patients with myeloma which could be attributed to the effects of pamidronate treatment [23, 30, 33].

In the Berenson's et al. [21] study 392 stage III multiple myeloma patients with osteolysis were randomized to obtain either pamidronate at a dose of 90 mg or placebo both in 4 – hour intravenous infusion administered every four weeks and combined with anti – tumour chemotherapy. The outcomes of a such treatment presented in 1996 after administration of 9 cycles showed in the pamidronate arm a remarkable decrease in proportion of patients experiencing bone fractures and requiring orthopedic – surgical management, radiotherapy (24% v 42%) as well

as a significant decrease in proportion of those with episodes of hypercalcaemia during first three months of treatment (1% v 5%). Pamidronate treatment was accompanied by significant bone pain diminishing, decrease in antianalgesic drugs consumption and improvement of the patients' quality of life. In 150 patients the treatment with pamidronate was continued and 41% of them obtained 21 cycles of therapy. After administration of 21 treatment cycles in the pamidronate – treated group there was observed a decrease both in the proportion of patients experiencing various bone complications and in number of bone complications. Among all 392 patients there was no difference in overall survival between the pamidronate and placebo groups while the median survival of patients included into the study during second – line or greater chemotherapy programs was 21 months for patients receiving pamidronate and 14 months for patients receiving placebo [22].

In the Kraj et al.' study [27, 28] since October 1995 the efficacy of pamidronate, has been evaluated in multiple myeloma patients all receiving anti-myeloma chemotherapy acc. to VMCP/VBAP alternating regimen. Forty – six patients with stage III myeloma and osteolytic lesions were randomized to receive either pamidronate (Aredia; Novartis) 60 mg *i.v.* in 4- hour infusion monthly ($n = 23$) or chemotherapy alone (control group $n = 23$). During the pamidronate treatment there was observed bone pain reduction and improvement of bone turnover indices. However, only in the first 8 months of treatment the reduction of clinical symptoms related to bone destruction was greater in patients treated with pamidronate in comparison to control group receiving only chemotherapy. The pamidronate administration was associated with a not significant decrease in the proportion of patients with hypercalcaemia but the mean serum calcium concentration remained in the normal limits during the whole period of study. At skeletal X-ray examination performed after 6, 12, 18 and 21 cycles of pamidronate and by comparing each consecutive imaging with previous one the progression of osteolysis was found respectively in 67%, 39%, 27% and 33% of patients. In the control group corresponding figures were: 79%, 70%, 30% and 25%. The mean number of skeletal events (pathologic fractures, radiation or surgery to bone and spinal cord compression) per year was lower in the pamidronate group (1.82) than in the control patients (2.72), $p > 0.013$. The proportion of patients who had developed skeletal events (excluding vertebral fractures) was lower in the pamidronate group – 34% v 52% ($p > 0.023$). The proportion of patients with pathologic vertebral fractures was similar 69% v 70%, respectively but the number of vertebral fractures was lower in the pamidronate group, 45 v 64 (1.4 v 2.3 per patient per year; $p > 0.04$). Decreases of blood haemoglobin level occurred more frequently in pamidronate patients than in the controls (72% v 41%, respectively) and mostly were accompanied by progression of proliferation. Survival was not different between the pamidronate – treated group and control patients (20 v 19 months since randomisation, $p > 0.65$ and 62 v 50 months since mul-

tiple myeloma diagnosis, $p > 0.45$). Thirteen (=56%) patients in the pamidronate group died, as did 12 (=52%) in the control group [23]. Continuation of the study up to 34 cycles of pamidronate have shown that along with extension of treatment the effect of pamidronate on skeletal morbidity becomes less pronounced [34].

A special attention should be paid to one patient (Kraj et al. [35]) in whom the treatment composed of radiotherapy combined with pamidronate administration resulted in reconstruction of myelomatous vertebral lesions. Such diminishing of osteolytic lesions with simultaneous improvement in bone structure calcification is an extremely rare event in multiple myeloma even in patients in whom their tumour proliferation process was inhibited in result of successful chemotherapy. In this patient, aged 38, engineer, trade specialist, having multiple myeloma with monoclonal protein IgG κ and BBJ κ , 15% rate of bone marrow plasma cells and osteolysis there was observed the following course of malignancy, diagnosing and treatment. In 1992 a X-ray survey revealed osteolytic lesion of C-2 vertebra. In 1995, radiologic skeletal examination disclosed – in addition to osteolytic vertebral destruction of C-2 – the osteolytic foci in C-5 and C-6 vertebrae; magnetic resonance imaging showed alterations in spinal cervical region excluding however spinal cord lesion. The same year too, the patient apart from continuing chemotherapy according to VMCP/VBAP program was subjected radiation to cervical vertebral region, and beginning from 1996 he started the treatment with pamidronate (Aredia) systematically, every month, administered in dose of 60 mg in intravenous infusions. Till February 2000, he received 36 cycles of such treatment. The treatment resulted in bone reconstruction of vertebra C-2 and stabilisation of osteolysis in other bones. The patient continues to perform his full-time job and does not require analgesic treatment. This case was presented at XVII Congress of the Polish Society of Haematology and Blood Transfusion in Łódź, June 1999 [35].

The encouraging data on early progression adapted use of bisphosphonates in myeloma were presented at VII International Multiple Myeloma Workshop in Stockholm, Sweden, September 1999 by R. Bartl [36] from the University Hospital Grosshadern, Munich, Germany. One hundred sixty myeloma patients in stage I and without osteolytic lesions were treated with aminobisphosphonates from time of diagnosis. Pamidronate (60-120 mg) or ibandronate (2-6 mg) were used intravenously and the dosages and infusion intervals (from 1 to 3 months) depending on the intrinsic malignancy of the disease (spectrum from smouldering to rapidly progressive variants). In this non – randomized study he could demonstrate a marked reduction of skeletal events in the first 3 years and especially a reduction of tumour growth (shown in MRI of the skeleton, bone biopsy and PCLI) and a decline of the M – protein levels in the first year of treatment (no concomitant chemotherapy!).

Oral pamidronate (300 mg/24h) was compared with placebo in 300 newly diagnosed myeloma patients who were also receiving intermittent oral melphalan and pred-

nisone [11]; pamidronate had no effect on skeletal – related morbidity or survival.

Early clinical studies used bisphosphonates in the treatment of hypercalcaemia of malignancy [37, 38]. In the study of Kraj et al. [26] in 11 patients with hypercalcaemia clodronate was applied *i.v.* 0.3g/24hrs in 4-hour infusion for 5 days and in 3 patients-1.5g/24hrs for 1 day. Intravenous administration of clodronate in all cases led to normalisation of serum calcium concentration during 2-4 days. In this study, similar to the O`Rourke et al. [38] experience, intravenous clodronate at dose of 1500mg was efficient in the treatment of hypercalcaemia and a single-day 1500 mg was as efficient as a 5-day treatment with 300 mg daily. Another study has shown the efficacy of intravenous pamidronate in reversing hypercalcaemia in cancer patients and revealed the drug dose of 90mg as optimal [37].

A randomized trial has shown the superiority of pamidronate over clodronate in patients with tumour-induced hypercalcaemia essentially about the duration of normocalcaemia, because the median duration of action of clodronate was 14 days compared with 28 days for pamidronate [39].

Zoledronate is a heterocyclic imidazole containing third generation bisphosphonate (Fig.1), which is so far the most potent bisphosphonate evaluated in humans. It is 100 times more potent than pamidronate in inhibiting 1.25(OH) $_2$ D induced release of calcium from mouse calvaria *in vitro*. In the *in vivo* animal model of calcitriol induced hypercalcaemia in thyroparathyroidectomized rats, zoledronate is 850 times more active than pamidronate and more than 4 orders of magnitude more potent than clodronate (Fig.2) [40]. Of all bisphosphonates clinically evaluated, zoledronate has the largest therapeutic ratio between the desired inhibition of bone resorption and the unwanted inhibition of bone mineralization.

Zoledronate is under development but not yet approved for marketing in any country. Body et al. (41) conducted an open-label, dose-finding, single-dose phase I study in tumour-induced hypercalcaemia in 33 patients. The primary objective was determine, with a dose escalation schedule, two nontoxic dose levels of zoledronate able to induce normocalcaemia in at least 80% of patients with hypercalcaemia of malignancy after rehydration. The two effective dose levels were 0.02 mg/kg and 0.04 mg/kg (i.e. 1.2 mg and 2.4 mg for a 60-kg individual, respectively). At the latter dose, the first day of normocalcaemia was day 2 or 3, and normocalcaemia was often maintained throughout the trial – 32 to 39 days.

Zoledronate is, now being compared with pamidronate in a large randomized double-blind trial for patients with multiple myeloma related bone lesions.

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