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Tiludronate: Bone Pharmacology and Safety

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The pharmacological properties of tiludronate (4-chlorophenyl)thiomethylene bisphosphonate), a sulfured bisphosphonate, have been characterized in a series of preclinical in vivo and in vitro studies. In vivo, tiludronate exerts a dose-dépendent inhibitory activity on bone resorption. This property was demonstrated in several animal models, including rats, ewes, and dogs, when bone resorption was induced by administration of retinoid acid or parathyroid hormone, or by immobilization, ovariectomy or orchidectomy. By uncoupling bone resorption from bone formation, tiludronate can induce a positive calcium and phosphate balance. When administered either continuously or intermittently to ovariectomized osteoporotic rats, tiludronate promotes a significant increase in bone mass. This positive effect is associated with an increase in mechanical resistance. Bone tolerance studies indicate that tiludronate is a safe compound with an appreciable therapeutic margin since it can effectively inhibit bone resorption without reducing bone mineralization and strength. In vitro, tiludronate added to bone tissue culture inhibits calcium release, lysosomal enzyme secretion and collagen matrix degradation when induced by various stimulators of bone resorption. At the cellular level, tiludronate does not appear to exert its inhibitory effect on bone resorption by impairing either the recruitment, the migration or the fusion of osteoclast precursors. Tiludronate could act on mature osteoclasts by reducing their capacity to secrete proton into the resorption space and also by favoring their detachment from the bone matrix. The available preclinical data indicate that tiludronate should be an efficacious bisphosphonate in the management of clinical conditions characterized by excessive bone resorption. (*Bone 17:473S–477S; 1995*)

Key Words: Bone resorption; Bone mass; Bone formation; Mineralization; Tiludronate.

Introduction

Various experimental tools can be used to assess the activity of pharmacological agents aimed at preventing or curing human bone diseases. In vivo, several animal models have been shown to be predictive of positive and negative pharmacodynamic effects on bone and calcium metabolism observed in human clinical conditions. Ex vivo experiments allow the precise evaluation of whether any positive effect of a drug on bone mass is accompanied by a commensurate modification in the resistance to mechanical stress. Biomechanical tests are now being adapted to various skeletal sites, including vertebral bodies, proximal femur, and long-bone metaphysis, of small laboratory animals. These technical developments improve the overall capability of preclinical tools in predicting the quantitative relationship between increase in bone mass, observable in human clinical trials, and expected enhancement in mechanical resistance. In vitro, tissue and cell culture systems provide insight into the mechanism by which pharmacological compounds can affect the processes of bone remodeling and mineralization.

Among the different classes of osteotropic agents, the bisphosphonates have been extensively evaluated both in preclinical studies and in various clinical disorders.[20] Preclinical observations made with bisphosphonates have been predictive of results obtained in clinical investigations, particularly in the relative potency to inhibit bone resorption.[10,20,21,35] This remarkable predictability of preclinical pharmacology with bisphosphonates also holds true for the therapeutic margin separating the most efficacious dose inhibiting bone resorption from that impairing matrix mineralization.[10,20]

In this article we review preclinical studies with sulfured bisphosphonate tiludronate (4-chlorophenyl)thiomethylene bisphosphonate, in which the pharmacological properties have been characterized in a series of in vivo and in vitro studies.[6] The results of these preclinical investigations indicate that tiludronate will be an effective bisphosphonate in the management of human clinical conditions characterized by excessive bone resorption.

In Vivo Studies on Bone Resorption

The effects of tiludronate have been studied in models previously shown to be predictive of the activity of bisphosphonates and other antiresorbing drugs when applied to human conditions such as Paget's disease, hypercalcemia of malignancy and, more recently, osteoporosis. Tiludronate, given to rats, ewes, and dogs, inhibits dose-dependent bone resorption induced by administration of retinoid acid or parathyroid hormone (PTH), or by immobilization, ovariectomy, or orchidectomy.

In thyroparathyroidectomized (TPTX) rats in which bone resorption is stimulated by retinoid derivatives,[38] tiludronate can completely inhibit retinoid-induced bone resorption at doses of 0.16–0.32 mmol/kg/day (50–100 mg/kg/day) orally, or 0.016 mmol/kg/day (5 mg/kg/day) subcutaneously.[1–4] The minimal active dosage by the oral route is 0.04 μg L/kg/day (1.25 mg/kg/day).[6] When given subcutaneously and simultaneously with the retinoid derivative, the rise in calcemia and calciuria was fully prevented at doses that did not alter food intake and renal function.[1] These findings are consistent with a selective inhibitory

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activity on calcium mobilization from bone. Other experiments suggest that, when given orally before the administration of the retinoid agent, larger doses of tiludronate would be required to inhibit bone resorption.[4] Whether this difference is due to localization of the bisphosphonate in bone[7] in relation to its mechanism of action in the process of osteoclastic resorption remains to be clarified. When tiludronate is given orally, during or after retinoid exposure, it appears to be more powerful than etidronate in inhibiting bone resorption.[4] Compared with other bisphosphonates, the inhibitory activity of tiludronate on bone resorption in the retinoid-treated TPTX rat model is close to that of clodronate, or similar to that of pamidronate, depending to the timing sequence of administration. It is less potent than alendronate and other third generation bisphosphonates.[20,35,37] With respect to bone tolerance, however, the absolute potency of bisphosphonates to inhibit resorption is of less importance than the dose margin between the therapeutic response and the occurrence of any adverse effect on mineralization.

In a model of immobilization induced by sciatic neurectomy in growing rats, tiludronate prevented bone loss[27] in agreement with the princeps study carried out with clodronate, a first generation bisphosphonate.[19] Histomorphometric analysis of the metaphyseal primary and secondary spongiosa of the proximal tibia indicates that, when given immediately after neurectomy, tiludronate can prevent reduction in bone volume at these sites. This protective effect appeared to be mainly due to an inhibition of osteoclastic mediated bone resorption.[27] At the level of the secondary spongiosa of the tibial metaphysis, tiludronate was shown to reduce the number of osteoclasts attached to the trabeculae but not the number of TRAP-positive cells localized in the marrow space. These results suggested that tiludronate exerts its inhibitory activity on bone resorption by interfering with the attachment of osteoclasts to the bone matrix rather than by blocking the osteoclastogenesis process.[27] As described below, in vitro observations support this hypothesis.[28]

Other evidence for inhibition of bone resorption was obtained by using a histomorphometric technique and by the determination of markers of bone remodeling, such as osteocalcin and pyridinolin in both ovariectomized dogs[15] and aged ewes.[16] In the ewe model, bone turnover was increased by the simultaneous administration of PTH. Tiludronate prevented the elevation in bone resorption due to either estrogen deficiency or pharmacological application of PTH.[16] In this last study it was shown that tiludronate prevented the marked rise in bone formation rate induced by PTH. This observation suggested that blockage of bone resorption by tiludronate or other bisphosphonates may blunt the anabolic effect of PTH. This possible antagonism should be further documented using various PTH-bisphosphonate dosage regimens. Likewise, whether bisphosphonates block the anabolic response to other bone forming agents is an important issue that also deserves further examination. Recent studies in adult ovariectomized rats indicated that the anabolic effect of insulin-like growth factor (IGF-1) on cortical bone mass and strength was actually enhanced by bisphosphonate therapy.[2,3]

In Vitro Studies on Bone Resorption

Tiludronate added to bone tissue culture inhibits calcium release, lysosomal enzyme secretion, and collagen matrix degradation, as induced by various molecules including PTH, prostaglandin E2, and interleukin-1.[4] In agreement with the in vivo data on bone resorption, tiludronate is more potent than etidronate in inhibiting both calcium release and the secretion of lysosomal enzymes from mouse calvaria. As observed with other bisphosphonates,[39] there is a strong parallel between the inhibitory activity of the bisphosphonates on bone calcium release and lysosomal enzyme secretion.

In other tissue culture experiments, tiludronate was also shown to block the effect of 1,25-dihydroxyvitamin D_3 $1,25(OH)_2D_3$ on bone resorption. Thus, in fetal mouse long-bone organ culture, tiludronate added at 10^{-4} mol/L greatly inhibited bone resorption induced by $1,25(OH)_2D_3$.[26] The bisphosphonate added at the same concentration, however, did not inhibit osteoclast-like cell formation induced by $1,25(OH)_2D_3$ in cocultures of mouse bone marrow cells and osteoblastic cells.[29] Thus, it appears that tiludronate does not exert its inhibitory effect on bone resorption by impairing either the recruitment, the migration or the fusion of osteoclast precursors.

Further studies suggest that the bisphosphonate could act on polarized mature osteoclasts by favoring their detachment from the matrix by a mechanism involving the disruption of the podosomal-ringed structure.[29] In addition, inhibition of osteoclast activity by tiludronate could involve a reduction in matrix acidification by impairing a proton ATPase pump present in the ruffled-border membrane of osteoclasts.[14] An increase in the phosphorylation of certain proteins associated with an inhibition of protein phosphatase activity has been recently detected in osteoclast-like cells treated with tiludronate.[29] The causal relationship between such an inhibition of some protein phosphatase activity and the tiludronate-induced changes in osteoclastic function remains to be established.

In Vivo Studies on Bone Formation and Mineralization

Tiludronate can reduce bone resorption and turnover without affecting the process of mineral deposition onto the organic matrix laid down by the osteoblasts. Previous studies with other bisphosphonates have shown that all such compounds, when given in appropriate doses, can lead to a decrease in bone remodeling. The reduction in bone formation follows inhibition in bone resorption after a period of delay varies according to the chemical structure and the dose of the bisphosphonate tested. This phenomenon, which secondarily reduces bone formation, is also classically observed with hormonal and "natural" inhibitors of bone resorption such as estrogen and calcitonin. It should be distinguished from possible additional and untoward inhibitory activity on bone mineralization. A dissociation between the rate of matrix and mineral deposition has been observed with some bisphosphonates, such as etidronate, when given in doses not much greater than those therapeutically used to inhibit bone resorption.[10,20] Tiludronate was tested in a model previously developed to assess the effect of drugs on hydroxyapatite crystal deposition in intact rats.[17] This experimental setting consists of the formation of calcified plaques by subcutaneous injections of a saturated potassium permanganate solution. Tiludronate, given either orally or subcutaneously at doses that maximally inhibited bone resorption, did not reduce the formation of the calcified plaques, but completely blocked their spontaneous dissolution.[4] The same results were obtained with pamidronate. In this model, etidronate, given at the same dose as tiludronate, reduced the size of the plaques by about 30%, a result that can be explained in terms of known potent inhibitory activity of this bisphosphonate on hydroxyapatite deposition.[4] These results confirm that tiludronate, in contrast to etidronate, does not interfere with hydroxyapatite deposition when given at doses that fully inhibit crystal dissolution.

In the castrated rat model, chronic administration of tilu-

dronate, at a dose that prevented bone loss, did not lead to a reduction in mineralization as indirectly assessed by determining the bone calcium/hydroxyproline ratio.[4]

In Vitro Studies on Osteoblastic Function

In vitro studies using mouse calvaria showed that incubation with tiludronate from 1–100 μmol/L did not appear to interact negatively with the incorporation of ^{45}Ca or proline into collagen, and in the activity of alkaline phosphatase.[4] These findings suggest that tiludronate does not interfere with bone matrix formation. Other experiments using rat- or human-isolated osteoblastic cells indicated that tiludronate could interfere with cell proliferation and differentiation, but only when added at a concentration higher than that required to inhibit bone resorption in vitro.[23]

Effect on Calcium and Phosphate Balance

Animal experiments sustain the notion that the inhibitory activity of tiludronate on osteoclastic-mediated bone resorption can lead to an increase in calcium balance. Thus, in intact rats, tiludronate dose-dependently increased the intestinal absorption of calcium without affecting the calciuria.[1] This observation indicates that this bisphosphonate can uncouple bone resorption from bone formation, at least transiently, and thereby promote a positive bone calcium balance. As previously documented[8] with another bisphosphonate (hydroxypentane-bisphosphonate), the increase in intestinal calcium absorption probably results from the stimulation of $1,25(OH)_2D_3$ production. Animal and human studies indicate that bisphosphonates do not interfere with the secretion of the main hormonal elements, namely PTH and $1,25(OH)_2D_3$, controlling calcium homeostasis.[10] Nevertheless, the plasma calcium level can no longer be maintained within the normal range if the calcium supply is markedly reduced in the presence of a marked inhibition of bone resorption. In other words, the risk of hypocalcemia with bisphosphonate therapy can be avoided by maintaining a normal dietary calcium supply, as shown in rats treated with tiludronate.[1]

Tiludronate positively uncouples bone formation from resorption and, as may be expected, induces an increase in both the calcium and inorganic phosphate (P_i) balance. In our own investigation we observed that oral administration of 0.32 mmol/kg/day tiludronate significantly increased the P_i balance from 10.2 ± 1.3 to 17.8 ± 2.1 mg/day ($p < 0.05$). This effect was the result of a stimulation of the net intestinal P_i absorption (+ 9%, $p < 0.001$) without any significant alteration in urinary P_i excretion. Associated with this positive effect on the P_i balance, the maximal tubular P_i transport per glomerular filtration rate (TmP_i/GFR) was significantly increased in TPTX rats treated with 0.32 mmol/kg of tiludronate (3.79 + 0.12 mmol/mL GFR, $p < 0.01$) compared with vehicle treated animals (3.19 + 0.08 mmol/mL GFR). In intact rats the renal tubular capacity to reabsorb P_i was not changed. This observation indicates that tiludronate can stimulate TmP_i/GFR by a PTH-independent mechanism. This stimulation, which is not expressed in parathyroid intact animals, probably corresponds to an adequate homeostatic response related to the enhanced P_i demand due to the increased bone mineral balance.[9]

Effect on Bone Mass

Long-term tiludronate treatment was investigated in 6-month-old Sprague-Dawley female rats made osteoporotic by ovariectomy.[1] This model of adult ovariectomized (OVX) rats is considered to resemble the human postmenopausal status. As in estrogen deprivation in women, OVX in adult rats induces an increased bone turnover and a decrease in area bone mineral density (BMD in g/cm^2) as assessed by dual X-ray absorptiometry (DXA), particularly in sites with a prevailing proportion of trabecular bone such as lumbar vertebrae and proximal tibia. In this OVX rat model, maturity of the skeleton is almost reached, linear bone growth is virtually stopped, and the growth plates start to close. Therefore, the changes in BMD can be interpreted without the need to consider possible variation in bone growth. The tiludronate caused a time- and dose-dependent increase in BMD at the levels of the lumbar spine, the caudal vertebrae, and the proximal tibia.[1] Continuous or intermittent cyclic administration of tiludronate over a 4-month period were similarly found to increase bone mass. The increment in BMD was maintained for at least 2 months after discontinuation of therapy. In the OVX rat model, the positive effect of tiludronate on bone mass is associated with an increase in bone strength.[30]

Tiludronate was also tested in other models of osteoporosis. In the castrated male rat model, tiludronate given orally at doses of 50–200 mg/kg prevented the decrease in the skeletal mass, assessed physically by measuring the bone weight and density or chemically by determining the calcium and phosphate content.[4] Similar protection was obtained against the bone loss induced by a low-calcium diet in the rat.[6] A low calcium diet results in an augmentation in bone resorption. This is induced by several mechanisms, among which the increased production and plasma levels of both PTH and $1,25(OH)_2D_3$ certainly play an important role. As discussed below and observed with other bisphosphonates, tiludronate is capable of blocking the stimulatory effect of these two calciotropic hormones on bone resorption, thus explaining, at least in part, the protective action detected in the calcium-deficient rat model of osteoporosis. More difficult to explain is the protective effect of tiludronate[4] observed in rats in which low bone mass was obtained by "inflammation" after either implantation of a cotton pellet or injection of magnesium silicate.[25] Indeed, in this inflammation-induced osteoporosis model, in which similar results were obtained with pamidronate, the reduction in bone mass appears to be due more to a reduction in bone formation rather than a stimulation in bone resorption.[24,31] In this model the bisphosphonate preventive effect might be mediated by influencing, either directly or indirectly, the osteoblastic bone formation process. Recent evidence indicates that bisphosphonates exert a pharmacodynamic effect on osteoblasts by influencing the production of factor(s) capable of modulating osteoclast activity.[26] However, to our knowledge, a direct positive effect of bisphosphonates on osteoblastic bone formation has not yet been demonstrated. Thus, it remains possible that in the inflammation-induced osteoporosis model, bisphosphonates, by reducing below normal the bone resorption rate, counteract the detrimental effect of the inflammatory process on bone formation.

Bone Tolerance

Bone tolerance studies[6] indicate that tiludronate is a safe compound with an appreciable therapeutic margin, since it can effectively inhibit bone resorption without reducing bone mineralization. Thus, in a study carried out in old castrated male rats, oral administration of tiludronate (0.16–0.64 mmol/kg/day, i.e., 50–200 mg/kg/day for 2–3 months) prevented bone loss without affecting the calcium/hydroxyproline ratio of the tibiae.[5] These results are consistent with the notion that tiludronate, given at doses that effectively prevent the increase in bone resorption

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resulting from sex hormone deprivation, does not impair mineralization of the bone matrix. The same conclusion regarding the absence of mineralization impairment can be drawn from the results of another study in which chronic treatment with oral tiludronate was shown to prevent bone loss induced by inflammation without affecting the calcium/hydroxyproline ratio of the tibiae.[5] In growing monkeys, the continuous administration of tiludronate for 6 months at doses of up to 16 times the dose pharmacologically active on bone resorption did not induce the histomorphometric expression of osteomalacia as assessed from examination of iliac crest biopsies.[12] Furthermore, after 1 year of continuous treatment, bone strength was conserved.[22] Increase in mechanical resistance at the femoral level was observed in OVX dogs treated for 1 year, at doses normalizing the elevated bone remodeling.[5] Moreover, in the same toxicity study, biomechanical properties of the radius were also evaluated and indicated that chronic oral administration of tiludronate at doses of up to 40 mg/kg/day did not decrease bone strength. On the contrary, tiludronate dose-dependently increased the mechanical resistance of long bones to fracture induced by torsional stress. This increased strength correlated with an increased bone mineral content. Thus, no evidence was found that the quality of bone would be negatively altered by long-term administration of tiludronate.[5]

Finally, tiludronate was also tested on the fracture-healing process in beagle dogs. No impairment was observed in the animals treated with tiludronate.[13]

Conclusions

In several animal models previously shown to be predictive of the activity of bisphosphonates in human pathological conditions, administration of tiludronate promotes a dose-dependent inhibition of bone resorption. Compared with other bisphosphonates, the bone resorption inhibitory activity of tiludronate is greater than that of etidronate, close to that of clodronate, and to pamidronate in some experimental conditions, but less than that of alendronate and other third generation bisphosphonates. In vitro tiludronate inhibits increased bone resorption induced by various mediators. The mechanism of action probably involves an inhibition of osteoclastic activity with a probable reduction in the enzymatic and proton transport processes. In addition, tiludronate appears to favor the detachment of mature osteoclasts from the matrix in disrupting the podosomal-ringed structure. As with other bisphosphonates such as clodronate, pamidronate or alendronate, tiludronate can markedly inhibit bone resorption without significantly depressing bone mineral deposition. In this respect, the therapeutic margin of tiludronate is larger than that of etidronate. Besides the maintenance of a normal mineralization rate, bone tolerance is good with no impairment in the mechanical strength of the skeleton. Furthermore, the transient maintenance of a normal bone formation rate in the presence of marked inhibition of the resorptive process leads to a positive body calcium balance, and thereby to an increase in bone mass. The suggestion that tiludronate may attenuate the stimulatory activity of bone forming agents, such as PTH, remains to be thoroughly investigated.

Considering this analysis of preclinical data, it is not surprising that tiludronate has already been shown to be an efficacious bisphosphonate in the treatment of human bone diseases that can be improved by reducing the rate of bone resorption. The inhibitory activity on bone resorption has already been demonstrated in Paget's disease,[32,34] postmenopausal osteoporosis,[33] immobilization secondary to spinal cord injury,[11] and hypercalcemia of malignancy.[18] Finally, from the results of preclinical studies on the relation between changes in bone mass and in mechanical resistance, one may predict that, when administered to osteoporotic patients, tiludronate will not only prevent further bone loss but will also confer protection against the occurrence of fragility fractures.

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