Treatment with ibandronate preserves bone in experimental tumour-induced bone loss


From Harvard Medical School, Boston, USA and Roche Diagnostics Boehringer, Mannheim, Germany

Cancer-induced bone diseases are often associated with increased bone resorption and pathological fractures. In recent years, osteoprotective agents such as bisphosphonates have been studied extensively and have been shown to inhibit cancer-related bone resorption in experimental and clinical studies. The third-generation bisphosphonate, ibandronate (BM 21.0955), is a potent compound for controlling tumour osteolysis and hypercalcaemia in rats bearing Walker 256 carcinosarcoma.

We have studied the effect of ibandronate given as an interventional treatment on bone strength and bone loss after the onset of tumour growth in bone. Our results suggest that it is capable of preserving bone quality in rats bearing Walker 256 carcinosarcoma cells. Since other bisphosphonates have produced comparable results in man after their success in the Walker 256 animal models our findings suggest that ibandronate may be a powerful treatment for maintaining skeletal integrity in patients with metastatic bone disease.

Received 14 September 1998; Accepted after revision 19 March 1999

The prevention and treatment of metastases of solid bone tumours in patients with cancer are a major problem to oncologists, radiotherapists and orthopaedic surgeons. Two-thirds of women with breast cancer who develop skeletal metastases will sustain fractures of the spine or long bones within their remaining lifetime.1,2 Carcinoma of the breast, lung and prostate probably account for more than 80% of cases of metastatic bone disease.3 By the time that bone metastases are detected the underlying cancer is considered to be incurable. Patients may survive for many years4,5 and the demand for palliative treatment is high6,7 Despite the major incidence of skeletal complications in malignancies, there are still notable gaps in the regimes for their prevention and treatment with drugs.

Cancer-induced bone diseases are often associated with increased bone resorption and hypercalcaemia because of neoplastic activation of osteoclasts and renal impairment. In the case of breast carcinoma, the final step in bone destruction by metastasis is mediated by osteoclasts which are stimulated by local production of parathyroid hormone-related tumour peptide.8 Ultimately, tumour osteolysis is the cause of pathological fractures, and once they occur immediate surgical intervention is required. Despite increasing numbers of such fractures, the current approaches for the prevention and palliative treatment of tumour-induced bone resorption are insufficient. Non-surgical treatment aims to control neoplastic cell growth and pain.

The osteoclast is a suitable site for therapeutic intervention and in recent years interest has grown in the inhibition of osteoclast-mediated bone resorption. Osteoprotective agents such as bisphosphonates have been studied extensively and some have also been shown to inhibit cancer-related bone resorption in experimental and clinical studies.2,9-12 The proper indication for these agents, however, remains undefined. The third-generation bisphosphonate, ibandronate (BM 21.0955), is a potent compound for controlling tumour osteolysis and hypercalcaemia in rats bearing Walker 256 carcinosarcoma,13 and has been shown to be an effective and well-tolerated treatment for hypercalcaemia in patients.11,14,15 There have been only a few studies on the beneficial effect of bisphosphonates on the prevention of pathological fractures due to bone metastases.16-19

We have attempted to determine the effect of ibandronate on bone strength and bone loss when given as an interventional treatment after the onset of tumour growth in bone as measured by densitometry and mechanical testing.
Materials and Methods

We used a previously described animal model for the direct evaluation of the densitometric and structural consequences of a tumour-induced osteolysis. A series of 30 four-month-old virgin Sprague-Dawley rats (Taconic Co Laboratories, Germantown, New York) was randomly divided into two experimental groups. All the animals were maintained in accordance with the US federal regulations and the study was carried out with approval of the Institutional Animal Care and Use Committee.

Walker carcinosarcoma 256 malignant breast cancer cells (WCS 256) were obtained from the Pathology and Oncology Research Group of McMaster University in Hamilton, Ontario, Canada. This cell line has been developed by Orr, Varani and Ward and has been widely used for invasive and metastatic models in research on bone cancer. The animals were anaesthetised by an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). After dislocation of the patella of the left femur of both groups an agarose gel cap containing the WCS 256 tumour cells, at a concentration of $5 \times 10^5$ cells/ml, was implanted into the medullary canal through a hole drilled through the intercondylar notch. The gel cap was obtained by stabbing the prepared WCS agarose gel with a 20-gauge blunt needle. The drill hole was sealed with standard bone wax, the extensor mechanism was reconstructed and the skin closed. Based on the dose-finding studies of Sedar-Obermeier and Baus, one group received a daily dose of 0.003 mg P/kg (phosphorus per kilogram body-weight) of ibandronate subcutaneously for a period of 28 days starting at the day of implantation of the tumour cells (Tu-Iban). The control group (Tu-Saline) had a placebo with saline for the same period. The animals were allowed free activity in their cages and food and water were supplied ad libitum. Twenty-eight days after implantation of the tumour all the rats were killed in a CO chamber. The time chosen for killing was based on the experience gained from multiple pilot studies, in which we had determined the 28th day to be the time when all the animals were still alive and sufficient and reproducible bone loss was detectable. Both femora of each rat were removed, cleaned of soft tissue and stored in isotonic-saline-soaked gauze at -20°C for further analysis.

After thawing, anteroposterior radiographs were taken and dual-energy x-ray absorptiometry (DEXA) (QDR 2000plus; Hologic Inc, Waltham, Massachusetts) and peripheral CT (pQCT) (XCT 960A; Norland Stratec, Pforzheim, Germany) were carried out on all femora. To determine the bone mineral density (BMD) (g/cm$^3$) and bone mineral content (BMC) (g) we performed a subregion analysis of the distal 25% of the length of the bone using DEXA. The average bone density (BD) (g/cm$^3$) was determined by pQCT from one 1 mm slice scanned in the distal metaphysis of the femur at the boundary of the condyles.

For mechanical testing the thawed femora were embedded distally and proximally in Fuji Rock dental stone (GC America Inc, Chicago, Illinois), using aluminium-squared endcaps to ensure proper concentric and axial alignment. All specimens were torqued to failure in external rotation with 6°/s. The loading parameters of the torsional test apparatus were based on standard methods. Structural properties such as ultimate failure torque (N-m) and torsional stiffness (N-m/°) were determined graphically directly from the load-deformation curve using XMGR-software (developed by P. J. Turner at the Oregon Institute of Science and Technology).

Statistical analysis. An analysis of variance between left and right femora within the group (Tu-Iban and Tu-Saline) as a factor revealed that the difference depends on the group. A paired $t$-test was therefore used to test the null hypothesis that the tumour-bearing bones are weaker than the contralateral control bones. An unpaired $t$-test was used to test the differences in stiffness and failure torque between tumour-bearing bones and the sham-operated control group. The same statistical methods were applied to determine the significance in mean differences for the densitometric values. All statistical analyses were performed using RS1 and BMDP statistical software.

Results

There were no deaths related to anaesthesia but three rats (two Tu-Saline and one Tu-Iban) died or had to be killed between the 24th and the 28th days after surgery because of either tumour-related lung problems or paralysis and limping. In all tumour-bearing animals, macroscopic evidence of soft-tissue tumours was found which indicated the presence of tumour cells after implantation. There were no signs of any adverse effects of ibandronate during the 28-day period of administration. Radiological examination showed that all untreated tumour-bearing bones had intra-medullary lesions and severe compromise of the metaphyseal bone, but no gross pathological fractures. In the ibandronate-treated group, only one bone showed tumour-related resorption similar to that found in the untreated group, but it was not as severe and did not have cortical compromise. Intramedullary changes in the treatment group.

Table I. Mean values (so) for BMC, BMD and BD in the treated and untreated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>BMC (g)</th>
<th>BMD (g/cm$^3$)</th>
<th>BD (g/cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tu-Saline</td>
<td>0.0759 (0.01076)</td>
<td>0.185 (0.0185)</td>
<td>0.588 (0.092)</td>
</tr>
<tr>
<td>Tu-Saline control</td>
<td>0.0871 (0.00806)</td>
<td>0.205 (0.0131)</td>
<td>0.699 (0.042)</td>
</tr>
<tr>
<td>Tu-Iban</td>
<td>0.102 (0.00987)</td>
<td>0.237 (0.0149)</td>
<td>0.798 (0.058)</td>
</tr>
<tr>
<td>Tu-Iban control</td>
<td>0.096 (0.00775)</td>
<td>0.225 (0.0878)</td>
<td>0.794 (0.049)</td>
</tr>
</tbody>
</table>
were due mostly to the reaming with sclerotic formations along the needle track.

The densitometric assessment revealed that the mean values for BMD, BMC and BD in the tumour-bearing bones were 10%, 13% and 15% (p < 0.001) lower, respectively, than those in the corresponding contralateral control (Table I). In the untreated tumour-bearing bones they were 21%, 26% and 26% (p < 0.001) lower, respectively, than in the ibandronate-treated tumour-bearing bones (Tu-Saline v Tu-Iban).

Table II. Mean values (sd) for load and stiffness in the treated and untreated groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Load (N-m)</th>
<th>Stiffness (N-m/°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tu-Saline</td>
<td>0.249 (0.1025)</td>
<td>0.596 (0.168)</td>
</tr>
<tr>
<td>Tu-Saline control</td>
<td>0.344 (0.0903)</td>
<td>0.759 (0.147)</td>
</tr>
<tr>
<td>Tu-Iban</td>
<td>0.429 (0.0499)</td>
<td>0.778 (0.146)</td>
</tr>
<tr>
<td>Tu-Iban control</td>
<td>0.409 (0.0662)</td>
<td>0.803 (0.147)</td>
</tr>
</tbody>
</table>

Ultimate failure torque in the untreated tumour-bearing group was 28% (p < 0.05) lower than in the corresponding contralateral control and 42% (p < 0.001) lower than that in the ibandronate-treated tumour-bearing bones (Table II; Fig. 4). In torsional stiffness there was a significant decrease of about 20% between the tumour-bearing bones and their contralateral controls (p < 0.05) and of 22% between tumour-bearing bones and ibandronate-treated tumour-bearing bones (p < 0.01). Comparison of the non-tumour-bearing contralateral control bones of both groups showed a significant increase in the ibandronate-treated group in most of the measured outcome variables; BMC by 9% (p < 0.01), BMD by 10%
(p < 0.001), BD by 12% (p < 0.001), and ultimate load by 16% (p < 0.05). Only with regard to torsional stiffness was a difference between the groups not detected.

Discussion

We used an in vivo model in which WCS 256 tumour cells were implanted in the medullary canal of the long bones, and caused significant bone resorption and subsequent increased skeletal fragility at the site of implantation. Several methods for assessing the effect of bisphosphonates in metastatic bone disease have previously been described. One common outcome variable was the survival time of the animals suffering from tumour growth in bone, while other studies used biochemical markers of bone turnover such as the urinary excretion of calcium, pyridinium crosslinks or urinary hydroxyproline. Classifying radiographs according to their severity as a variable is another approach to determining the effect of the treatment of tumour-induced bone resorption, but it has been shown to be less precise and reader-dependent. The most direct quantification of the changes in tumour-induced bone diseases has been by histomorphometry, including measurement of the mass of the tumour, the area of necrosis and counts of osteoclastic indices. None of the previously used experimental approaches to assess the effects of different bisphosphonates on tumour-induced bone loss has taken into consideration the direct changes in bone density and bone strength due to osteolytic resorption. Since pathological fractures result from the deterioration of bone quality, as defined by bone mass and bone architecture, the direct measurements of bone quality used in our study provide the most direct assessment of the effect of osteoprotective drugs such as ibandronate. The increase in bone density and bone stability in the ibandronate-treated animals, and even in the control bones, confirms the assumption that ibandronate suppresses osteoclastic action and inhibits bone resorption. This supports the results of Leal et al in a comparable study which showed preservation of bone strength and a significant decrease in the number of osteoclasts per tissue area and tumour mass after an intermittent dose of alendronate. The increase in bone densitometric parameters as well as the strength in tumour-bearing bones treated with ibandronate in our study indicates a reduced osteoclastic activity, with little or less effect on osteoblast activity as demonstrated in the same species by Ca kinetics. This is supported by healing of endosteal damage due to the intramedullary reaming as shown by the radiological appearance of a sclerotic rim in the canal after 28 days. The healing of the endosteal damage is also the explanation for the higher values for all outcome variables for the Tu-Iban than the Tu-Saline groups in which the intramedullary canal had not been reamed.

Using an intravascular WCS 256 tumour model Guaitani et al showed an inhibiting effect on the formation of new bone metastases in rats receiving etidronate, and a significant reduction in serum calcium level in the same species which received arterial injections of WCS 256 cells followed by treatment with clodronate. Similarly, Jung et al showed a significant decrease in the number of osteoclasts in affected bones. Sasaki et al showed a reduction in tumour burden in bone and a prolonged survival time of animals treated with risedronate. Similar results were found using ibandronate in a mouse model. In our study the presence of soft-tissue tumours in all tumour-bearing animals indicated that ibandronate does not have an antineoplastic effect on the WCS 256 cell line in rats and is therefore not active on the tumour itself. This agrees with recent findings with ibandronate and other bisphosphonates used in animal studies and in clinical trials.

Ibandronate, one of the most recent bisphosphonates, is about 2, 10, 50 and 500 times more effective on bone resorption in rats than risedronate, alendronate, pamidronate and clodronate, respectively. It has been found to be very effective in the treatment of Paget’s disease, tumour-related hypercalcaemia in animals and man, and in the prevention of bone loss in ovariectomised dogs and osteoporotic women. Little is known, however, about the prevention of cancer-related bone loss. A first hint that ibandronate can prevent bone loss in rats bearing Walker 256 bone metastases was revealed by the studies of Sedar-Obermeier and Bauss, who found that treatment with the drug prevented tumour osteolysis. Yoneda et al had similar results in human breast cancer in nude mice. During the last few years observations on the effect of bisphosphonates in the treatment and prevention of osteoclast-mediated bone resorption have increased because of their primary effect in blocking the osteoclastic activity in both formation and activation. No data are available, however, on the effect of ibandronate in preserving structural integrity and preventing bone alterations by a tumour ultimately causing pathological fractures. Since the aim of treating tumour-induced bone diseases is the prevention of progression and skeletal complications, the inhibition of increased osteoclastic activation is currently a promising approach.

Our results imply that an interventional treatment with ibandronate, currently the most powerful available bisphosphate, is capable of preserving bone quality in rats bearing Walker 256 carcinosarcoma cells. Since other bisphosphonates have produced comparable results in man after success in the Walker 256 animal models, our findings strongly suggest that ibandronate may be a powerful treatment for patients suffering from metastatic bone disease to help maintain their skeletal integrity. This treatment would improve the quality of life in cancer patients by reducing the incidence of pathological fractures.

We wish to thank Marie Shea, M.S., Conrad Wang, MD and Elisabeth Meyers, PhD (OBL Beth Israel Deaconess Medical Centre, Boston/MA) for their input and their help designing the study and statistical analysis. This work was supported in part by the German National Science Foundation (Deutsche Forschungsgemeinschaft) (AKK), and the G. Wallrabenstein Foundation.
One or more of the authors have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article.

References