Use of Zoledronate to Treat Osteoblastic versus Osteolytic Lesions in a Severe-Combined-Immunodeficient Mouse Model

Yu-Po Lee, Edward M. Schwarz, Mark Davies, Mark Jo, Jeffrey Gates, Xuguang Zhang, Jing Wu, and Jay R. Lieberman

ABSTRACT

Prostate adenocarcinoma is associated with the formation of osteoblastic metastases in bone. It has been hypothesized that osteoclast-mediated bone resorption is a critical component before the development of these osteoblastic lesions in bone. This observation has led researchers to test agents that inhibit osteoclastic activity to prevent or halt the formation of metastatic prostate cancer lesions in bone. Bisphosphonates inhibit osteoclast activity, and previous studies showed that they have the ability to reduce the osteolytic bone resorption associated with multiple myeloma and breast cancer. The objective of this study was to evaluate the efficacy of zoledronate in limiting the formation and/or progression of osteoblastic lesions produced by the injection of known prostate cancer cells (LAPC-9 and PC-3 cells) into the tibia of SCID mice. The mice were treated with either 30-μg or 150-μg doses of zoledronate before tumor implantation (pretreatment group), or at weekly intervals after tumor implantation (weekly treatment group), or weekly starting one month after tumor implantation (delayed-treatment group). The zoledronate was very effective in limiting the formation of osteoblastic lesions in PC-3 implanted tibias by inhibiting osteoclast activity. Radiographic and histological analysis at weekly intervals revealed that osteoblastic lesions developed in the control tibias by 2 weeks, and there was complete destruction of the cortical bone in much of the proximal tibias by 4 weeks. In the treatment groups, there was minimal cortical destruction noted in the weekly treatment groups at both doses, whereas mild cortical erosion was evident in the pretreatment groups, with more cortical destruction noted in the 30-μg group compared with the 150-μg group. Tartrate-resistant acid phosphatase (TRAP) staining showed that zoledronate decreased osteoclastic numbers and that there was a dose-dependent response. In tibias implanted with the LAPC-9 cells, the zoledronate was not effective in halting the formation of the osteoblastic lesions. Radiographic and histological analysis revealed that osteoblastic lesions either had formed or were developing in 18 of 18 of the control tibias and 36 of 36 of the treated tibias at 8 weeks regardless of dose or treatment schedule. Furthermore, TRAP staining demonstrated that osteoblastic lesions had formed in the LAPC-9 tibias under conditions in which osteoclast numbers were significantly reduced. These results suggest that osteoclast activity may not be critical for the development of osteoblastic lesions associated with prostate tumor cells. Hence, bisphosphonates may not be ideal agents to prevent the formation of osteoblastic lesions associated with prostate cancer metastases to bone.

INTRODUCTION

Prostate adenocarcinoma is the second leading cause of cancer in North American men and is associated with significant morbidity and mortality (1–4). Skeletal metastases are a common occurrence in advanced disease, affecting ~70% of patients (1–4). In one series, the incidence of pure osteoblastic metastases was 95%, and the remaining 5% of prostate metastases formed mixed osteoblastic/osteolytic lesions late in the disease process (3). This hypothesis has been supported by clinical studies that suggest that prostate cancer metastases to bone have an initial bone resorptive component that is mediated by osteoclasts (5–8). This observation has led researchers to test agents that inhibit osteoclastic activity to prevent or halt the formation of metastatic prostate lesions in bone. Finding an agent that could prevent the formation of prostate cancer metastases in bone or to treat these metastases once they have become established would be a major therapeutic advance in the treatment of patients with prostate cancer. Previous studies revealed that bisphosphonates have the ability to reduce the osteolytic bone resorption associated with multiple myeloma and breast cancer (9–11). Bisphosphonates act by inhibiting the recruitment, proliferation, and differentiation of preosteoclasts, by either adhering to the mineralized matrix of bone or by impeding the resorptive activity of mature osteoclasts (12–15). They also shorten the life span of osteoclasts by inducing apoptosis (12–15). If osteoclastic activity is a necessary step in the development of metastatic prostate lesions in bone, bisphosphonates like zoledronate should be effective in halting the formation of these lesions.

It is difficult to study the pathophysiology of prostate cancer metastases to bone because most prostate tumors harvested from human patients do not survive in tissue culture and most prostate cancers that induce osteoblastic lesions in humans produce osteolytic lesions in animal models (7, 16–19). In our laboratory, we have developed a model in which both osteoblastic and osteolytic prostate cancer lesions can be reliably produced. In a previous study, human prostate cancer cells were implanted into the tibia of SCIDmice (20). PC-3 prostate tumor cells formed osteolytic lesions, whereas LAPC-9 prostate tumor cells formed osteoblastic lesions. PCR and immunohistochemical analysis of these tumors revealed that PC-3 cells produced TNF-α and RANKL. Conversely, LAPC-9 cells did not secrete RANKL and secreted only a limited amount of TNF-α. Instead, the LAPC-9 cells produced IL-6, OPG, and bone morphogenetic proteins. It was hypothesized that the PC-3 cells formed osteolytic lesions via the RANK:RANKL and TNF-α pathway. In contrast, the OPG secreted by the LAPC-9 cells may block the formation of osteolytic lesions in the LAPC-9 implanted tibias by RANK blockade. It was also hypothesized that the secretion of IL-6, bone morphogenetic proteins, and other osteoinductive proteins by the LAPC-9 cells contributed to the formation of osteoblastic lesions. TRAP staining showed that whereas there was abundant osteoclastic activity present during the formation of the osteolytic lesions in the PC-3 implanted tibias, there was no discernable osteoclastic activity present during the formation of osteoblastic lesions in the LAPC-9-implanted tibias. Osteoclasts were not noted until the osteoblastic lesions had formed and were actively involved in remodeling. These data suggest that bisphosphonates may be more effective in treating osteolytic lesions instead of osteoblastic lesions. Hence, the purpose of this study was to evaluate the efficacy of zoledronate in preventing and treating osteolytic versus osteoblastic lesions formed by prostate tumor cells in a SCID mouse model.
MATERIALS AND METHODS

Cell Culture

All of the reagents were purchased from Life Technologies, Inc. (Rockville, MD) unless otherwise mentioned. Human prostate cancer cell lines LAPC-9 and PC-3 were cultured in Iscove’s medium (Irvine Scientific, Irvine, CA) supplemented with 15% fetal bovine serum and 1% glutamine and maintained at 37°C in a humidified atmosphere with 5% CO2.

Cell Lines

The human prostate cancer cell lines PC-3 and LAPC-9 were used in this study. PC-3 (American Type Culture Collection) cells were chosen for this study because our prior experience with this cell line demonstrated that it produces an osteolytic lesion when implanted into bone (18). LAPC-9 was derived from a metastatic lesion to bone in a human patient (17). In prior studies, LAPC-9 produced pure osteoblastic lesions when implanted into bone (18, 20).

Cell Suspension Protocol

Both PC-3 and LAPC-9 tumor cells were isolated ~24 h before use. Mice with s.c. tumors were anesthetized (100 mg ketamine/kg body weight, 10 mg xylazine/kg body weight) and sacrificed. The overlying skin was then shaved and prepared with 70% ethanol and Betadine. The tumor was explanted in a sterile fashion and placed in a sterile 50-ml conical Falcon tube (Becton Dickinson Labware, Franklin Lakes, NJ). The tumor was then finely minced with a sterile razor blade in sterile PBS (Life Technologies, Inc.). This slurry was spun in a centrifuge at 1300 rpm for 5 min at room temperature. The supernatant was aspirated, and the pellet was resuspended with a sterile razor blade in sterile PBS (Life Technologies, Inc.). This slurry was spun in a centrifuge at 1300 rpm for 5 min at room temperature. The supernatant was aspirated, and the pellet was resuspended in Iscove’s medium and then centrifuged again at 1300 rpm for 5 min at room temperature. Once again, the supernatant was aspirated, and the pellet was resuspended with a 0.1% Pronase E/Iscove’s mix (which was previously filtered through a Steriflip Millipore filter with a 0.22 μm membrane (Millipore Corporation, Bedford, MA) and then gently shaken on a rotating platform for 18 min at room temperature in a sterile 50-ml conical Falcon tube. The mix was placed on ice for 2 min and then poured over a 70-μm Falcon cell strainer (Becton Dickinson Labware). Once strained, the mix was spun at 1300 rpm for 5 min at room temperature. The supernatant was aspirated and the pellet resuspended in Iscove’s medium with 15% fetal bovine serum and 1% glutamine and was plated in 10-ml culture dishes (Becton Dickinson Labware). After this, 1× fungizone (Life Technologies, Inc.) was added to each plate, and the plates were incubated at 37°C in 5% CO2 until use.

Animals

Eight-week-old male SCID mice were housed under pathogen-free conditions in accordance with the protocol approved by the Chancellor’s Animal Research Committee at the author’s institution.

Tibial Implantation

Single-cell suspensions of LAPC-9 or PC-3 cells were combined with an equal amount of Matrigel (Collaborative Biomedical Products, Bedford, MA) so that 10 μl of the mixture contained 1 × 106 cells. Ten μl of either the PC-3-Matrigel mix or the LAPC-9-Matrigel mix was injected into the left tibia of 8-week-old SCID mice. To summarize, the mice were anesthetized (100 mg ketamine/kg body weight, 10 mg xylazine/kg body weight) and the left hind limb was shaved and prepared with 70% ethanol and betadine. A 3-mm longitudinal incision was made with a no. 15 scalpel blade over the patellar ligament. Then a 2-mm incision was made along the medial border of the patellar ligament with the same scalpel blade. A 27 gauge half-inch needle was inserted through the tibial plateau with the knee flexed and 10 μl of the desired mixture was injected. The skin was closed with 5–0 Vicryl suture (Ethicon Inc., Somerville, NJ). Animals were sacrificed at 8 weeks. Before sacrifice, the animals were anesthetized and radiographs were taken with a Faxitron (Field Emission Corporation, McMinnville, Oregon). At sacrifice, all of the major organs and both hind limbs were harvested for histological analysis.

Treatment

Zoledronate solutions were prepared with sterile water and stored at 4°C in a plastic container in the dark. This was done because zoledronate interacts with glass and is photosensitive. Also, zoledronate interacts with calcium and magnesium and must be prepared in solutions free of divalent cations. These treatments were administered s.c. to SCID mice based on three treatment protocols (Fig. 1).

Protocol 1: Effects of a Short Period of Prophylactic Treatment (Pre-treatment Group). Zoledronate (0, 30 μg, or 150 μg) was administered s.c. into male SCID mice 5, 3, and 1 days before tibial implantation with either PC-3 or LAPC-9 cells. No additional treatments were administered after the tibial injections.

Protocol 2: Effects of Interventional Treatment (Weekly Treatment Group). Zoledronate (0, 30 μg, or 150 μg) was administered s.c. into male SCID mice at weekly intervals starting on the day of tibial implantation with either PC-3 or LAPC-9 cells.

Protocol 3: Effects of Treatment on Established Disease (Delayed Treatment Group). Zoledronate (0, 30 μg, or 150 μg) was administered s.c. into male SCID mice at weekly intervals starting one month after tibial implantation with either PC-3 or LAPC-9 cells.

In all of these groups, there were six animals for each prostate cell line and for each dose. PBS administered in the contralateral limb acted as a control, and the animals were sacrificed at 8 weeks.

Histology

The tibias were fixed in 10% buffered formalin, followed by decalcification in 10% EDTA solution for 2 weeks at room temperature with gentle stirring. Sections were paraffin embedded, sectioned (3 μm), and stained with H&E, orange G, and TRAP.

Histomorphometric analysis was performed on an Olympus system (Olympus, Melville, NY). The osteoclast perimeter (osteoclast number/mm bone) was compared between the PC-3- and LAPC-9-implanted tibias by examination at ×10 from TRAP-stained slides (21). Four tibias from each treatment group and dose were analyzed in this manner. Statistical analysis was performed using the Wilcoxon rank sum test. To determine the area of bone formation, the image of a trichrome-stained slide was captured and the area quantified with the use of Image One software (Universal Imaging Corporation, Downingtown, PA). Three tibias from each treatment group and dose were analyzed in this manner.

RESULTS

Radiographic Evidence That Zoledronate Inhibits Osteoclastic but not Osteoblastic Prostate Cancer Lesions. To evaluate the effects of zoledronate on the establishment and progression of osteolytic and osteoblastic tumor lesions, PC3 and LAPC9 cells were injected into the tibia of SCID mice that were given the pretreatments.

Protocol #1: Pre-treatment protocol.

Zoledronate

Sacrifice

Protocol #2: Weekly treatment protocol.

Zoledronate

Sacrifice

Protocol #3: Delayed treatment protocol.

Zoledronate

Sacrifice

Fig. 1. Summary of treatment protocols. Prostate tumors PC-3 or LAPC-9 were implanted into the tibia of male SCID mice on day 0. Zoledronate was administered s.c. at either 150-μg or 30-μg doses. All of the mice were sacrificed at 8 weeks.
Zoledronate in Treatment of Lesions in a SCID Mouse Model

Table 1 Summary of Radiographic Data

<table>
<thead>
<tr>
<th></th>
<th>150 µg</th>
<th>30 µg</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3 implanted tibia[s]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>0/6</td>
<td>1/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Weekly treatment</td>
<td>0/6</td>
<td>2/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Delayed treatment</td>
<td>6/6</td>
<td>5/5</td>
<td>5/6</td>
</tr>
<tr>
<td>LAPC-9 implanted tibia[a]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>2/6</td>
<td>3/6</td>
<td>5/6</td>
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<tr>
<td>Weekly treatment</td>
<td>4/6</td>
<td>4/6</td>
<td>5/6</td>
</tr>
<tr>
<td>Delayed treatment</td>
<td>5/6</td>
<td>5/6</td>
<td>3/6</td>
</tr>
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</table>

[s] P = 0.001 between both pretreatment 150-µg and 30-µg groups and control. P = 0.001 between weekly treatment 150-µg group and control. Weekly treatment 30-µg and both of the delayed treatment groups were not statistically different (P > 0.05).[a] P > 0.05 between all of the treatment groups and their respective controls. Histology showed that LAPC-9 cells were present in the intramedullary canal and that bone was being formed in all of the tibias in both the control and the treatment groups even though an osteoblastic lesion was not clearly evident on radiographs.

Histological Evidence That Zoledronate Inhibits Osteoclastic but not Osteoblastic Prostate Cancer Lesions. H&E and orange G stains were performed on all of the tibias that were implanted with PC-3 cells. In the control-tibias and the delayed-treatment groups, the cortical bone in the proximal tibias was completely destroyed and PC-3 cells were noted in the space between the remaining proximal and distal ends of the tibias (Fig. 4). The pretreatment and the weekly-treatment groups were markedly different. H&E and orange G stains showed that there was minimal cortical destruction in the weekly-treatment tibias at both the 150-µg and the 30-µg doses. Mild cortical destruction was evident in the proximal tibias of the pretreatment groups with more cortical destruction noted in the 30-µg group compared with the 150-µg group.

In the tibias implanted with LAPC-9 cells, there was abundant bone formation in the control and in all of the treatment groups. In all of the groups, H&E and orange G stains showed that bone completely filled the intramedullary canal with pockets of tumor cells encased in bone (Fig. 5). There were no noticeable histological differences between the controls and the treated groups nor were there any differences evident between the different doses.

Tibial sections were also stained for TRAP to identify osteoclasts. In the PC-3-implanted tibias, very few TRAP-positive cells could be seen in the control groups and in the delayed-treatment group because the cortical bone of the proximal tibias was completely destroyed and only tumor cells could be seen (Fig. 6). In the weekly-treatment groups, no osteoclasts were evident in the 150-µg treatment group, whereas a few osteoclasts were seen in the 30-µg treatment group. Both of the pretreatment groups were positive for osteoclasts with more osteoclasts seen in the 30-µg-treated group (Table 2).

In the LAPC-9-implanted tibias, no osteoclasts were visible in the pretreatment and the weekly-treatment groups, whereas a few osteoclasts were visible in the monthly-treatment groups. In contrast, many TRAP-positive cells were evident in all of the control groups at the time of sacrifice (Fig. 7).

To quantitate the effects of the zoledronate on osteoclast numbers in the mice, histomorphometry was performed on the TRAP-stained sections. For the PC-3-implanted tibias, there were no osteoclasts evident in the control tibias because almost all of the bone had been resorbed at the 8-week time point. There were significantly more osteoclasts in the 30-µg group compared with the 150-µg group in both the pretreatment (P = 0.020) and the weekly (P = 0.014) groups. There were no osteoclasts evident in either of the doses of the delayed-treatment groups because the tibias had already been eroded before treatment with zoledronate (Table 2).

In the LAPC-9-implanted tibias, no osteoclasts were evident at either the 30-µg or the 150-µg dose in the pretreatment and weekly

weekly treatments, or delayed treatments of zoledronate as described in “Materials and Methods.” Radiographs were taken of all of the mice before sacrifice at 8 weeks (Table 1). Osteolytic lesions similar to that shown in Fig. 2A developed in 15 of 18 of the PC-3 control tibias. Histological analysis revealed that the three control tibias that did not form osteolytic lesions did not have tumor cells successfully implanted into the intramedullary canal. In the treated groups, no osteolytic lesions were noted in any of the pretreatment or weekly treatment groups treated with the 150-µg dose (Fig. 2B). In the 30-µg pretreatment group, only 1 of 6 tibias developed an osteolytic lesion, and in the 30-µg weekly treatment group 2 of 6 tibias were noted to develop an osteolytic lesion. In the delayed treatment groups, 6 of 6 of the 30-µg delayed-treatment tibias and 5 of 5 of the 150-µg delayed-treatment tibias developed an osteolytic lesion at the time of sacrifice (Table 1).

Radiographs of the LAPC-9-injected tibias taken at the time of sacrifice showed that 13 of 18 control tibias developed osteoblastic lesions similar to that shown in Fig. 3A. However, histological analysis revealed the presence of both LAPC-9 cells and bone formation in the intramedullary canal in all 18 control tibias, even though an osteoblastic lesion was not clearly evident on the radiographs (Fig. 3B). In the 150-µg pretreatment group, two of six tibias developed an osteoblastic lesion whereas four of six tibias in the 150-µg weekly-treatment group and five of six tibias in the 150-µg monthly-treatment group formed osteoblastic lesions. Similarly, in the 30-µg pretreatment group, three of six tibias formed osteoblastic lesions, whereas four of six tibias in the 30-µg weekly treatment group and five of six tibias in the 30-µg monthly-treatment group formed osteoblastic lesions. Once again, histological analysis demonstrated that LAPC-9 cells were present in the intramedullary canal and that bone was being formed in all of the tibias in both the control and the treatment groups including the ones where an osteoblastic lesion was not clearly evident on radiographs.

Fig. 2. Radiograph of SCID mouse tibia implanted with PC-3 cells at 8 weeks. A, weekly control tibia; B, weekly 150-µg-treatment group tibia.

Fig. 3. Radiograph of SCID mouse tibia 8 weeks after implantation with LAPC-9 cells. A, pretreatment control tibia; B, pretreatment 150-µg-treatment group tibia.
groups; therefore, there were no significant differences with respect to osteoclast number in either group (Table 2). However, there were significant differences between these groups and their respective controls \((P = 0.0006\) for all four groups). In the delayed treatment group, there were a few osteoclasts present in the 150-\(\mu\)g treatment group, whereas there were no osteoclasts seen in the 30-\(\mu\)g treatment group. Statistical analysis showed that there were significantly fewer osteoclasts at the 150-\(\mu\)g dose as compared with the control \((P = 0.025)\).

Bone area was also calculated to determine the amount of bone loss or new bone formation in the PC-3- and the LAPC-9-implanted tibias. In the PC-3-implanted tibias, significant differences were noted in the amount of cortical bone destruction between the control group and the pretreatment group \((P = 0.0219)\) for both the 150-\(\mu\)g and the 30-\(\mu\)g groups (Table 3). Significant differences were also noted between the control group and the weekly-treatment group \((P = 0.0219)\) for both the 150-\(\mu\)g and the 30-\(\mu\)g treatments. No significant differences were identified between the control group and the delayed-treatment group \((P > 0.05)\). Also, no significant differences in bone loss were seen between the 150-\(\mu\)g and the 30-\(\mu\)g groups in either the pretreatment or the weekly-treatment groups.

In the LAPC-9-injected tibias, no significant differences were noted in the amount of bone formation between the control group and any of the treatment groups at either the 150-\(\mu\)g or the 30-\(\mu\)g dose \((P > 0.05\) for both doses; Table 3). These results suggest that inhibiting osteoclastic activity does not limit the formation of osteoblastic metastases.

![Fig. 4. H&E stains of SCID mice tibias implanted with PC-3 cells. A, pretreatment 150 \(\mu\)g; B, weekly-treatment 150 \(\mu\)g; C, delayed-treatment 150 \(\mu\)g; D, control. (“T” signifies tumor cells).](image)

![Fig. 5. H&E stains of SCID mice tibias implanted with LAPC-9 cells. LAPC-9 weekly control: A, \(\times\)2x; B, \(\times\)10; C, \(\times\)40. T and arrows, tumor cells.](image)
DISCUSSION

Metastatic prostate adenocarcinoma is associated with significant morbidity and mortality (1–4). Complications include bone pain, disability, and spinal cord compression (1–4). It has been suggested that the abnormal osteoblastic bone formation within metastatic lesions is preceded by osteoclastic activation (5–8). This provides the rationale for using bisphosphonates, which are powerful and selective inhibitors of osteoclastic bone resorption. Clinical studies using bisphosphonates to treat established prostate tumor metastases have demonstrated that they are effective in decreasing bone pain (14, 22).

Bisphosphonates are agents that selectively inhibit osteoclastic bone resorption in ways that currently are only partially understood (12–15). A direct cytotoxic effect on osteoclasts has been shown for

Table 2 Histomorphometric analysis of implanted tibias (osteoclasts/millimeter of bone surface)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pretreatment</th>
<th>Weekly</th>
<th>Delayed</th>
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</thead>
<tbody>
<tr>
<td>PC-3 150 µg</td>
<td>148</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PC-3 30 µg</td>
<td>425</td>
<td>82</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LAPC-9 150 µg</td>
<td>299</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>LAPC-9 30 µg</td>
<td>299</td>
<td>0</td>
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PC-3 implanted tibias: there were significantly more osteoclasts in the 30-µg group compared with the 150-µg group in both the pretreatment (P = 0.020) and the weekly (P = 0.014) groups. Comparisons between these groups and their respective controls were not possible to assess because the proximal tibias of the controls had been destroyed.

LAPC-9 implanted tibias: P = 0.025 for the 150-µg delayed-treatment group. P = 0.0006 for all of the other treatment groups and the controls.

Table 3 Bone histomorphometry (area of bone/total area on slide)

<table>
<thead>
<tr>
<th></th>
<th>150 µg</th>
<th>30 µg</th>
<th>Control</th>
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<tbody>
<tr>
<td>PC-3 implanted tibias&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.8 ± 5</td>
<td>36.7 ± 17</td>
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<tr>
<td>Pretreatment</td>
<td>58.9 ± 11</td>
<td>58.9 ± 18</td>
<td>0</td>
</tr>
<tr>
<td>Weekly treatment</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Delayed treatment</td>
<td>62.0 ± 8</td>
<td>76.2 ± 3</td>
<td>62.5 ± 3</td>
</tr>
<tr>
<td>LAPC-9 implanted tibias&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.0 ± 8</td>
<td>75.0 ± 7</td>
<td>62.5 ± 3</td>
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<tr>
<td>Pretreatment</td>
<td>60.1 ± 3</td>
<td>71.0 ± 8</td>
<td>62.5 ± 3</td>
</tr>
<tr>
<td>Weekly treatment</td>
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<tr>
<td>Delayed treatment</td>
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<sup>a</sup> In the PC-3-implanted tibias, significant differences were noted in the amount of cortical bone destruction between the control group and the pretreatment group (P = 0.0219) for both the 150 µg and the 30 µg. Significant differences were also noted between the control group and the weekly treated group (P = 0.0019) for both the 150 µg and the 30 µg.

<sup>b</sup> In the LAPC-9 injected tibias, no significant differences were noted in the amount of bone formation between the control group and any of the treatment groups at either the 150-µg or the 30-µg dose (P > 0.05 for both doses).
ZOLEDRONATE IN TREATMENT OF LESIONS IN A SCID MOUSE MODEL

clostronate, whereas the aminobisphosphonates impede the attachment of the osteoclasts to the bone surface and promote their apoptosis (12–15). It also has been suggested that bisphosphonates might inhibit the attachment of tumor cells to the bone matrix (23–24). Thus, in this study, prostate tumor cells were implanted into the tibias of SCID mice and these mice were treated with three different treatment protocols, and two different doses of zoledronate, to study the efficacy of bisphosphonates in treating osteolytic and osteoblastic lesions formed by these prostate tumor cells.

The zoledronate was very effective in treating the tibias implanted with PC-3 cells. Radiographic and histological analyses demonstrated that osteolytic lesions developed in the control tibias resulting in destruction of much of the proximal tibias. In the treated groups, osteolytic lesions were rarely seen in any of the pretreatment or weekly-treatment groups on radiographs. Histological analysis revealed that there was minimal cortical destruction noted in the weekly-treatment groups at both of the doses given, whereas mild cortical erosion was evident in the pretreatment group with more cortical destruction noted in the 30-µg group compared with the 150-µg group. Bone histomorphometry showed that there were no significant differences with respect to bone loss between the weekly-treatment and the pretreatment groups at either dose. However, there was significantly less bone destruction associated with these groups than with their respective controls. TRAP staining revealed that zoledronate significantly decreased osteoclastic activity and that there was a definite dose-dependent response. These results suggest that zoledronate is effective in halting the formation of osteolytic lesions mediated by osteoclastic activity and that zoledronate would be more effective if used earlier in the course of the disease.

In the LAPC-9-implanted tibias, zoledronate was not effective in halting the formation of the osteoblastic lesions. Radiographic and histological analysis demonstrated that osteoblastic lesions had formed or were being formed in all of the tibias in both the control and the treated tibias regardless of dose or treatment protocol. Furthermore, even though tibias from both of these groups formed osteoblastic lesions, TRAP staining showed that no osteoclasts were evident in the treated tibias, whereas there were many osteoclasts present in the control tibias. Also, bone histomorphometry revealed that there were no significant differences in the amount of bone formation within the tibias in any of the groups and their respective controls.

In our previous work with PC-3 and LAPC-9 cells, these prostate tumor cell lines were noted to have different cytokine profiles (20). Our hypothesis was that the different cytokines expressed by these tumors might influence osteoclasts or osteoblasts to form the lesions characterized by these tumors. PC-3 expressed RANKL, TNF-α, and other cytokines that promote the activation and formation of osteoclasts. LAPC-9 expressed BMP-2, BMP-4, BMP-6, and IL-6, whereas it did not express TNF-α or RANKL. Both of the tumors expressed OPG. The LAPC-9 cells characteristically expressed cytokines that promoted osteoblastic activity and did not express cytokines that increased osteoclastic activity. Conversely, PC-3 expressed TNF-α and RANKL, which promotes osteoclast activity. This could explain why PC-3 cells formed osteolytic lesions, whereas LAPC-9 cells formed osteoblastic lesions. However, we have not yet delineated the factor(s) that lead to the formation of osteoblastic lesions. The zoledronate inhibits osteoclasts, and, therefore, it was effective in preventing the osteolytic lesions associated with the PC-3 cells. However, LAPC-9 cells formed osteoblastic lesions even when no osteoclasts were present. This suggests that osteoclastic activity may not be necessary for the formation of an osteoblastic lesion. In addition, zoledronate did not have any apparent effect on the soft tissue lesions formed by either the LAPC-9 or the PC-3 cells.

It has been hypothesized that the osteoblastic lesions formed by metastatic prostate tumors are preceded by osteolytic lesions mediated by osteoclasts (5–8). Zhang et al. (25) recently demonstrated that osteoclastogenesis was critical in the development of some prostate cancers in bone. In the study, C4-2B prostate cancer cells produced mixed osteolytic/osteoblastic lesions 16 weeks after injection into the tibia of SCID mice. These cells secrete RANKL, which stimulate osteoclast activity. Animals treated with OPG did not develop these mixed lesions, and histomorphometric analysis demonstrated a corresponding decrease in osteoclast number. In our study, the tibias into which LAPC-9 cells were injected developed pure osteoblastic lesions in both the control and the treatment groups. TRAP staining revealed that LAPC-9 cells induced the formation of osteoblastic lesions regardless of the presence of osteoclastic activity. This suggests that OPG, zoledronate, and other agents that inhibit osteoclast activity may not be ideal agents for preventing the formation of the osteoblastic lesions formed by prostate cancer metastasizes to bone. However, they may limit the progression of established metastatic disease.

Zoledronate is a potent inhibitor of osteoclast formation and is effective in reducing the cortical destruction seen in osteolytic metastases. However, we were unable to determine whether the bisphosphonate directly inhibited tumor cell growth. In addition, we have demonstrated that zoledronate is not effective in stopping the formation of osteoblastic metastases. Osteoblastic lesions can form in the absence of osteoclastic activity. This suggests that osteocytic activity may not be necessary for the formation of osteoblastic lesions. Previous studies demonstrated an increased release of various collagen breakdown products in patients with metastatic prostate cancer (26–29). Investigators concluded from this data that osteoclastic activity was critical for the development of an osteoblastic lesion (28–29). Yet, these findings were generally noted in patients with advanced disease, in which there may have been increased remodeling of the new and host bone (26–29). Our data suggest that osteoclasts do not play a critical role in the early development of osteoblastic lesions. However, they may be important once an osteoblastic lesion is established. In our previous study, we noted the presence of osteoclasts after the osteoblastic lesions had formed (20). This would explain the presence of increased collagen breakdown products in the urine of patients with metastatic prostate cancer. Furthermore, osteoclastic activity may lead to the release of cytokines from the bone that may enhance tumor cell behavior (30–36). This suggests that bisphosphonates may have therapeutic benefits in patients with established disease. Further study is necessary, however, to determine the therapeutic benefits of zoledronate in the treatment of established prostate cancer metastasis.

REFERENCES


Use of Zoledronate to Treat Osteoblastic versus Osteolytic Lesions in a Severe-Combined-Immunodeficient Mouse Model

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