Bisphosphonate Effects on Breast Cancer Colonization of Three-Dimensional Osteoblast Tissue

Genevieve N. Miller, McNair Scholar
The Pennsylvania State University

McNair Faculty Research Advisors:
Erwin A. Vogler, Ph.D.
Professor of Materials Science and Engineering
Departments of Materials Science and Engineering and Bioengineering
College of Engineering
The Pennsylvania State University
Andrea M. Mastro, Ph.D.
Professor of Microbiology and Cell Biology
Department of Biochemistry and Molecular Biology
Eberly College of Science
The Pennsylvania State University

ABSTRACT

The purpose of this study was to characterize the effect of zoledronic acid (a bisphosphonate used clinically to treat bone metastasis) on osteoblasts and a model of breast cancer metastasis to bone *in vitro*. Murine calvarial pre-osteoblasts (MC3T3-E1) were grown to various stages of maturity in tissue culture and continuously treated with zoledronic acid (ZA) at 0.05, 0.50 or 5.00 μM concentrations. Drug effects on osteoblast proliferation, differentiation and mineralization were assessed. Additionally, mineralized 3D osteoblastic tissue was grown in a specialized bioreactor based on the principle of simultaneous-growth-and-dialysis. This 3D bone model provides a unique test system in which cancer cell interactions with osteoblastic tissue at controlled phenotypic maturities can be monitored in real time. Using this system, human metastatic breast cancer cells (MDA-MB-231) were co-cultured for 6 days with osteoblastic tissue in the actively-mineralizing phase (120 days of continuous culture). Without added ZA, cancer cells were observed to attach, penetrate, and colonize osteoblastic-tissue in a continuous process that ultimately marshaled osteoblasts into linear files similar to that observed in authentic pathological tissue. A single dose of ZA (at 0.50 μM and 0.05 μM administered 3 days after co-culture was initiated) delayed cancer-cell penetration and colony formation, with osteoblasts retaining the characteristic cuboidal shape observed in controls for the first 2 days of co-culture. Thereafter, cancer-cell colonization progressed to the filing stage.

INTRODUCTION

Breast Cancer in Bone

Breast cancer is the second most commonly diagnosed cancer in women in the United States, with an estimated 192,370 new cases of invasive breast cancer in 2009 [1]. Breast cancer also ranks second as the cause of cancer deaths, with an expected 40,610 breast cancer deaths this year [1].

Breast cancer may progress to become invasive, i.e. cancer cells spread throughout the breast tissue, or metastatic, i.e. cancer cells spread to other organs in the body [2]. Breast cancer frequently metastasizes to bone [3], where it disrupts the normal balance between the function of osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells), favoring osteolysis [4]. Tumor cell production of parathyroid hormone-related protein (PTHrP) signals osteoblasts to increase expression of receptor activator of nuclear factor κB ligand (RANKL), which activates osteoclasts to begin bone resorption [5]. Transforming growth factor-β (TGF-β) is released from the bone matrix as it is degraded, which further stimulates cancer cell production of PTHrP [6], generating a "vicious cycle" between breast cancer and the bone environment.

Osteoblasts also contribute to bone loss during metastasis. Breast-cancer cells disrupt normal osteoblast function [7, 8] and suppress the production of matrix proteins. In addition they elicit an osteoblast stress response in which osteoblasts release inflammatory cytokines known to activate osteoclasts [9].

Bisphosphonate Therapy

A family of drugs called bisphosphonates has been widely used clinically for the management and prevention of skeletal complications from bone metastases [10]. Bisphosphonates are chemically stable synthetic analogues of inorganic pyrophosphate (P-O-P), a molecule that inhibits calcification [11], in which the oxygen atom has been replaced by a carbon atom (P-C-P). The third-generation, nitrogen-containing bisphosphonates, such as zoledronic acid, target the "vicious cycle" of breast cancer metastasis to bone in two ways – by reducing osteoclast activity and exhibiting direct antitumor effects on cancer cells. Bisphosphonates bind avidly to bone mineral, where they are internalized by osteoclasts during dissolution [12]. Once internalized, nitrogen-containing bisphosphonates inhibit the enzyme farnesyl diphosphate (FPP) synthase in the mevalonate pathway and interfere with functions essential for osteoclast survival [12, 13]. Bisphosphonates also directly affect tumor cells by inhibiting proliferation, inducing apoptosis, and interfering with adhesion, invasion, and angiogenesis [14].

The effects of bisphosphonates on tumor cells and osteoclasts have been well documented, but reported effects on osteoblasts vary among studies. Proliferation and differentiation of human fetal osteoblasts (hFOB 1.19) was reportedly enhanced by pamidronate coated cellulose scaffolds [15], while direct treatment of hFOB cells with pamidronate and zoledronate was found to decrease cell proliferation but enhance differentiation [16]. Treatment of human osteoblast-like cells derived from trabecular bone explants with zoledronic acid (ZA) promoted differentiation and mineralization but induced apoptosis at concentrations of 0.5 μM or greater [17]. The proliferation of primary human osteoblasts cultured on ZA-coated implants was not affected by concentrations up to 100 μM, while cells directly treated with 50 μM ZA were significantly reduced in number [18]. The proliferation and osteogenic differentiation of human bone marrow

stromal cells (BMSC) were enhanced by 10^{-8} M concentrations of alendronate, risedronate and zoledronic acid [19]. Zoledronic acid (1 μM – 1 nM) treatment of human mesenchymal stem cell (hMSC)-derived osteoblasts reportedly has little effect on differentiation but inhibits mineralization in a dose-dependent manner [20]. Studies using MG63 human osteoblast-like cells indicate that aledronate and pamidronate promote both proliferation and differentiation [21, 22], but zoledronic acid decreases proliferation in a dose-dependent manner [23].

In vitro studies using murine cell lines also produce various results. Treatment of MC3T3-E1 osteoblasts in tissue culture plates with 0.1-50 μM concentrations of zoledronic acid decreased cell proliferation [23], while the viability and mineralization of MC3T3-E1 cells cultured on calcium phosphate discs were unaffected by the same concentrations [24]. Researchers using aminobisphosphonates on primary rat osteoblasts and primary mouse osteoblasts found that nanomolar concentrations of each inhibited osteoblast mineralization while micromolar concentrations were toxic to the cells [25, 26]. Cells cultured on dentine substrates responded similarly to bisphosphonate treatment but at higher concentrations [25].

The results of these studies suggest that the effects of nitrogen-containing bisphosphonates on osteoblasts vary according to tissue complexity. To study the effects of these drugs on osteoblasts *in vitro* in a manner relevant to clinical applications, a sophisticated model of bone tissue is required.

Compartmentalized bioreactor

Modeling the bone environment to gain an understanding of the mechanisms underlying breast cancer colonization of bone is difficult. Cell systems must create an environment that provides biological relevance as well as experimental control [27]. The previous lack of a sophisticated bone model has not only hindered breast cancer metastasis research, but also has hindered the development and understanding of therapeutic treatments for bone metastases.

This research utilizes a compartmentalized cell culture device [28] based on the concept of simultaneous growth and dialysis pioneered by G.G. Rose [29] to grow and develop three-dimensional osteoblastic tissue for extended culture [30]. This compartmentalized cell culture device, hereafter referred to as the "bioreactor", consists of a cell growth chamber separated from a larger medium reservoir by a dialysis membrane. Waste from the growth compartment and nutrients from the medium reservoir are capable of dialyzing, while macromolecules synthesized by the cells as they develop are maintained in the cell growth space. Media is exchanged in the medium reservoir, thereby reducing drastic environmental changes cells experience in conventional tissue culture. The bioreactor creates an extraordinarily stable cellular environment that allows for the growth of multiple-cell layer osteoblastic tissue. Three-dimensional osteoblastic tissue has been maintained for over 10 months of continuous culture using this device [30].

The 3D osteoblastic tissue grown in the bioreactor can be challenged with metastatic breast cancer cells to model the initial stages of breast cancer colonization of bone. Human metastatic breast cancer cells (MDA-MB-231) introduced into existing cultures of murine osteoblasts (MC3T3-E1) were observed to adhere to and penetrate osteoblastic tissue and form colonies

within the cultures [31]. Furthermore, breast cancer cells were observed to align into "Indian files", chains of single cancer cells, characteristic of cancer invasion [31, 32]. This system allows for the real time monitoring of breast cancer colonization of osteoblastic tissue.

The purpose of this study was to characterize the effect of a nitrogen-containing bisphosphonate, zoledronic acid, on osteoblasts in conventional tissue culture and osteoblasts challenged with metastatic breast cancer cells in the compartmentalized bioreactor.

MATERIALS AND METHODS

Cells and tissue culture conditions

Murine calvaria pre-osteoblast (MC3T3-E1) cells were a gift from Dr. Norman Karin at the Pacific Northwest National Laboratories (ATCC CRL-2593). MC3T3-E1 were cultured in an incubator at 37^{o}C with 5% CO_2 and maintained in alpha minimum-essential medium (α–MEM) (Mediatech, Herdon, VA) supplemented with 10% heat-inactivated neonatal fetal bovine serum (FBS) (Cansera, Roxdale, Ontario) and 1% 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma, St. Louis, MO), hereafter referred to as growth medium. MC3T3-E1 were passaged every 3-4 days using 0.002% pronase in phosphate buffered saline (PBS). Cells were not used above passage 20. Growth medium further supplemented with 50 μg/ml ascorbic acid and 10mM β-glycerophosphate (Sigma, St. Louis, MO), hereafter referred to as differentiation medium, was used to develop mineralized, differentiated osteoblasts.

Human metastatic breast cancer (MDA-MB-231) cells genetically engineered to produce green fluorescent protein (GFP) were a gift from Dr. Danny Welch at the University of Alabama at Birmingham (ATCC-HTB 26). MDA-MB-231^{GFP} cells are capable of forming bone metastases [33]. MDA-MB-231^{GFP} were cultured at 37^{o}C with 5% CO_2 and maintained in Dulbecco's Modified Eagle's medium (DMEM) (Mediatech, Herdon, VA) supplemented with 5% heat-inactivated neonatal FBS and 1X non-essential amino acids.

Zoledronic acid

Zoledronic acid (2-(imidazol-1-yl)-hydroxyehtylidene-1,1-bisphosphonic acid, disodium salt) was purchased from Toronto Research Chemicals, North York, Ontario and dissolved in 0.1 N NaOH to make a 5 mM stock solution.

Tissue culture

MC3T3-E1 were plated at a sub-confluent density (10^4 cells/cm^2) onto 24-well plates (Corning, Corning, NY). Differentiating cells were maintained with periodic media changes every 3-4 days.

Bioreactor design and implementation

Compartmentalized bioreactors based on the principal of simultaneous growth and dialysis were constructed as described previously [30]. Briefly, 316L stainless steel stock rings were tightly secured together by stainless steel screws to create the body of the device. Two compartments – a cell growth chamber (5 ml volume) and a larger (30 ml volume) medium reservoir – were formed with two outer gas-permeable and liquid-impermeable films and an inner dialysis membrane. The films forming the outer barriers were approximately 3 mm thick and made by hot pressing Surlyn 1702 resin (DuPont, Wilmington, DE) using simultaneous application of heat (220^{o}C) and pressure (245 Pa) in a laboratory hot press. The inner film was cellulosic-dialysis membrane (Spectrapor-13266; Spectrum Medical Industries, Rancho Dominguez, CA) and was hydrated in de-ionized water for 2 hours prior to assembly of the device. Assembled bioreactors had a cell-growth area of 25 cm^2. Once assembled, bioreactor chambers were filled with 0.1% sodium azide in PBS, packaged in plastic bags, and sterilized using 10 Mrad γ-ray irradiation at the Breazeale Nuclear Reactor Facility at the Pennsylvania State University. Sterile bioreactors were rinsed 3 times with PBS and incubated overnight with basal medium (aMEM, 1% penicillin-streptomycin) prior to cell inoculation.

MTT assay for cell proliferation

MC3T3-E1 were plated at 10^4 cells/cm^2 in growth medium. Following overnight incubation, zoledronic acid was added at 0.05, 0.50 and 5.00 μM concentrations for 24, 48, and 72 hours, upon which cell viability was assessed with an MTT assay. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, St. Louis, MO) at 5 mg/ml in PBS was added to the cultures equivalent to 1/10th of the culture volume (50 μl/500 μl per well). Cells were incubated at 37^{o}C in 5% CO_2 for 2 hours. Cells were then rinsed once with PBS and 1 ml solubilization solution (0.1 N HCl, 1% Triton X-100 in isopropanol) was added to dissolve the formazan crystals. Samples were read at 570 nm on a spectrophotometer with 650 nm background subtraction. Viable cell numbers were used to determine proliferation over time.

Alkaline phosphatase activity

MC3T3-E1 were plated at 10^4 cells/cm^2 in differentiation medium and maintained with periodic media changes for 17 days. After 17 days, differentiation medium was exchanged with zoledronic acid in concentrations of 0.05, 0.50 and 5.00 μM diluted from a 5 mM stock concentration with differentiation medium. Cells were cultured an additional week in the presence of zoledronic acid with two medium changes containing the drug. Following exposure to zoledronic acid for 7 days, MC3T3-E1 were stained for alkaline phosphatase activity. Culture medium was removed and the cells were rinsed twice with PBS. The cells were fixed for 10 minutes with 10% formaldehyde in PBS and then rinsed three times with PBS. Cells were

stained for alkaline phosphatase with a solution consisting of napthol, pre-warmed dH_2O, 0.2 M Tris (Sigma, St. Louis, MO) and Fast Blue RR Salt (Sigma, St. Louis, MO) and then incubated at 37^oC (no CO_2) for 30 minutes.

Von Kossa stain for mineralization

MC3T3-E1 were plated at 10^4 cells/cm^2 in differentiation medium and maintained with periodic media changes exactly as described for the alkaline phosphatase assay except the cells were grown for 28 days. After 28 days, differentiation medium was exchanged with zoledronic acid in concentrations of 0.05, 0.50 and 5.00 μM. Following exposure to zoledronic acid for 7 days, MC3T3-E1 were stained for calcium phosphate and calcium carbonate salts. Culture medium was removed and the cells were rinsed twice with PBS. The cells were fixed for 10 minutes with 10% formaldehyde in PBS and then rinsed three times for five minutes each with dH_2O. A 5% silver nitrate solution (diluted in dH_2O) was added to the cells, and they were incubated in the dark for 30 minutes at room temperature. The cells were rinsed three times with dH2O, a final volume of 0.5 mL dH2O was added to the cells, and they were incubated for 2 hours under a fluorescent lamp.

Bioreactor co-cultures

MC3T3-E1 were inoculated into bioreactor cell growth chambers at 10^4 cells/cm^2 and were cultured for 120 days with medium changes to the medium reservoir every 30 days. After 120 days, osteoblast tissue was stained with Cell Tracker Orange™ (Invitrogen, Carlsbad, CA) for live confocal imaging to monitor osteoblast morphology throughout the experiment. MDA-MB-231^{GFP} cancer cells were inoculated into the cell growth chambers containing osteoblast tissue at a 1:10 ratio of breast cancer cells to osteoblasts (10^5 cancer cells/bioreactor). Cancer cells were observed using confocal microscopy. Zoledronic acid was added to bioreactor cell growth chambers in concentrations of 0.05 and 0.5 μM after 48 hours, a time when the cancer cells and the osteoblasts were re-organizing into files. Cultures were monitored for an additional 72 hours using confocal microscopy. On day 5, bioreactors were disassembled and the film containing adherent osteoblast tissue was carefully cut into pieces for various assays. Medium from the cell growth space and medium reservoir was also collected for analysis.

Confocal microscopy

Cultures maintained in bioreactors were observed daily using an Olympus FV-300 laser-scanning microscope (Olympus America Inc., Center Valley, PA) at 20x and 40x magnifications. Cell Tracker OrangeTM was excited using a 543 nm helium-neon laser and collected through a 565 nm long-pass filter. GFP was excited using a 488 nm argon laser and collected through 510 nm long-pass and 530 nm short-pass filters. The emission was split through a 570 nm dichroic

long-pass filter. Adherent osteoblast tissue from disassembled bioreactors was fixed in 2.5% glutaraldehyde in PBS and stained for actin filaments with Alexa Fluor 568 phalloidin stain (Invitrogen, Carlsbad, CA) for further image analysis.

RESULTS

Zoledronic acid effects on osteoblast proliferation.

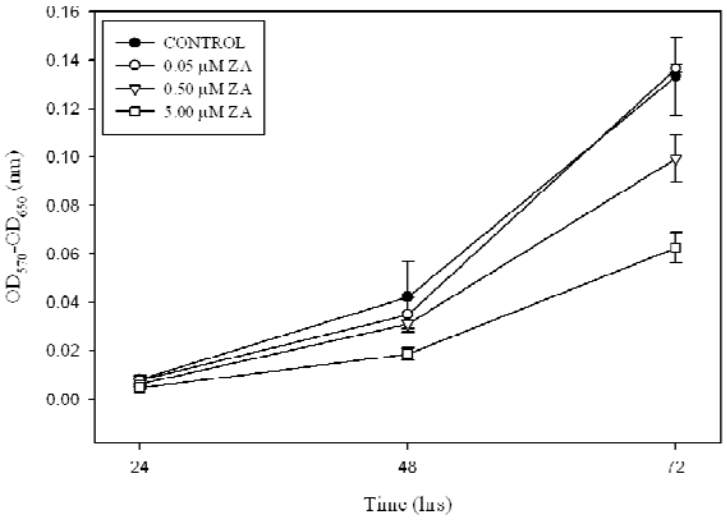


FIGURE 1. Effect of zoledronic acid (ZA) on osteoblast proliferation. MC3T3-E1 were plated at 10^4 cells/cm^2, incubated overnight, and treated with ZA at 0.05, 0.50 and 5.00 μM concentrations for 24, 48, and 72 hours (control cells were untreated). Cell proliferation was assessed with an MTT assay.

Osteoblast proliferation was measured by quantifying mitochondrial activity using an MTT assay. MC3T3-E1 were continuously treated with zoledronic acid in 0.05, 0.50 or 5.00 μM concentrations for 24, 48 or 72 hours. Results are reported as average optical density at 570 nm with a background subtraction of 650 nm, plus or minus standard deviation between triplicate samples (Figure 1). Continous exposure to zoledronic acid at 0.50 and 5.00 μM concentrations for 48 and 72 hours inhibited osteoblast proliferation. Treatment with low concentration (0.05 μM) zoledronic acid, however, did not result in the same inhibition; the optical densities at all times were similar to the untreated controls.

Zoledronic acid effects on osteoblast differentiation and mineralization.

Effects of zoledronic acid on osteoblast differentiation were measured by staining for alkaline phosphatase production. Alkaline phosphatase is an enzyme linked to osteoblast differentiation. Cells were cultured in differentiation medium for 17 days and then continously treated with zoledronic acid in 0.05, 0.50 or 5.00 μM concentrations for an additional 7 days. The culture dish was scanned to generate images of the tissue (Figure 2, A-D). Zoledronic acid at 5.00 μM (D) exhibited relatively strong inhibitory effects on alkaline phosphatase production.

Drug effects on mineralization were assessed by a Von Kossa stain for calcium phosphate and calcium carbonate salt deposits. Von Kossa's stain indirectly measures calcium in mineralized tissue. MC3T3-E1 were cultured in differentiation medium for 28 days and continuously treated for 8 days with zoledronic acid in 0.05, 0.50 or 5.00 μM concentrations. Images of the stained tissue were taken with a digital camera (Figure 2, E-H). All concentrations of zoledronic acid disrupted osteoblast mineralization (F-H) when compared to controls (E).

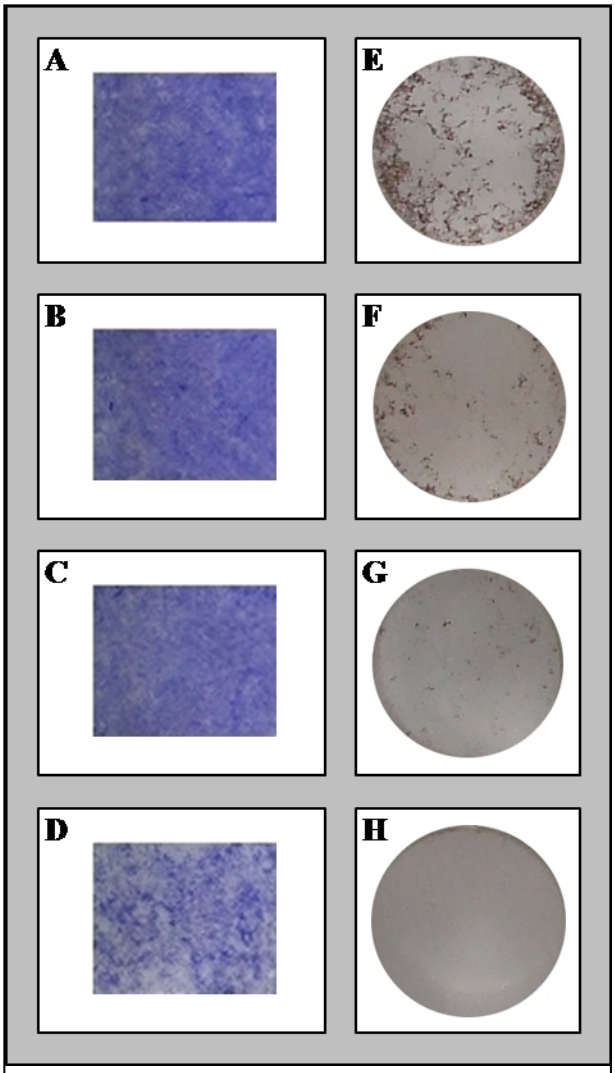
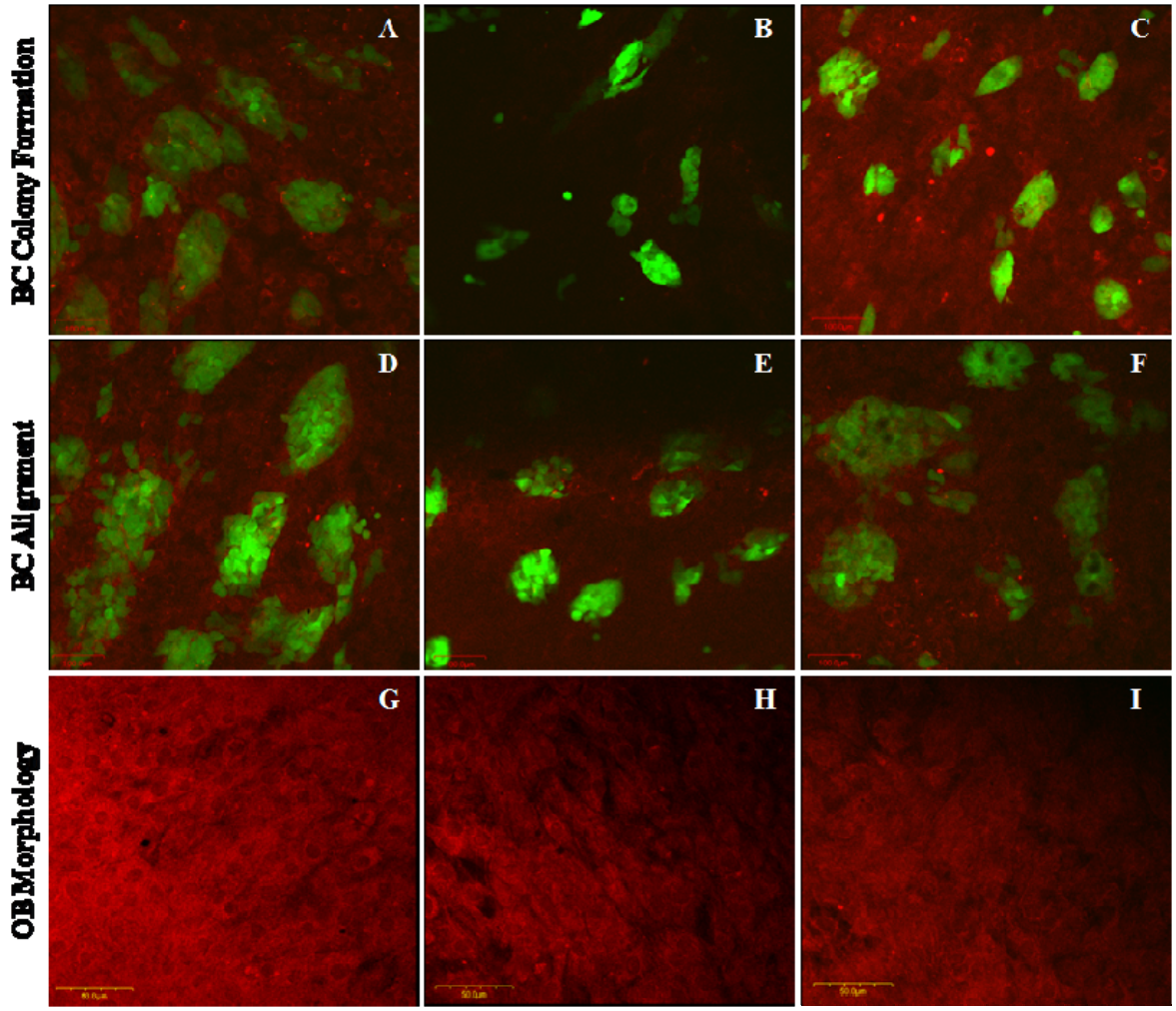


FIGURE 2. Effect of zoledronic acid (ZA) on osteoblast mineralization and differentiation. MC3T3-E1 were plated at 10^4 cells/cm^2 in differentiation medium and allowed to grow for 14 days (A-D) or 28 days (E-H) with periodic medium changes. Cells were then continuously treated with ZA at 0.05 (B,F), 0.50 (C,G) and 5.00 (D,H) μM concentrations for 8 days. Control cells (A,E) were untreated. Cell differentiation was assessed by alkaline phosphatase activity (A-D). Mineralization was determined with a Von Kossa stain (E-H).

Effects of zoledronic acid on breast cancer colonization of osteoblast tissue.

MC3T3-E1 were cultured in the bioreactor for 120 days with medium changes to the medium reservoir every 30 days. The osteoblast tissue was then challenged with metastatic breast cancer cells. Cancer cells were observed to colonize the tissue and organize into linear files after 48 hours, at which time zoledronic acid was added to the cultures in 0.05 and 0.5 μM concentrations. Cultures were maintained and monitored using confocal microscopy for an additional 72 hours.

Confocal images show breast cancer cells expressing green fluorescent protein (green) and osteoblasts stained with Cell Tracker Orange or Alexa Fluor 568 phalloiding (red) (Figure 3). Treatment with zoledronic acid reduced the formation of breast cancer cell colonies (Figure 3, compare A with B,C) and disrupted the alignment of breast cancer cells within the osteoblast tissue (Figure 3, compare D with E,F). Zoledronic acid also inhibited the penetration of breast cancer cells through the multiple-layer osteoblast tissue (Figure 3, table). Osteoblasts in cultures treated with zoledronic acid retained the characteristic cuboidal shape observed in the control (untreated) culture.



Experimental Parameter	Culture Conditions		
	OB + BC	OB + BC + 0.05 μM ZA	OB + BC + 0.50 μM ZA
BC Colony Formation	+++	+	++
BC Alignment	+++	+	++
Spindle-shaped OB Morphology	+++	+	++
Tissue Penetration	+++	+	++

FIGURE 3. Qualitative analysis of the effects of zoledronic acid (ZA) on MDA-MB-231 metastatic breast cancer cell (BC) colonization of osteoblast (OB) tissue. Cancer cells (green) were observed to penetrate and colonize osteoblast tissue (red) in the bioreactor (A,D,H). Addition of ZA to co-cultures in the bioreactor resulted in reduced breast cancer colony formation (B – 0.05 μM ZA, C – 0.50 μM ZA, 24 hr exposure) and disruption of cancer cell alignment with osteoblast tissue (E – 0.05 μM ZA, F – 0.50 μM ZA, 48 hr exposure). ZA delayed breast cancer cell penetration of osteoblast tissue (table) and osteoblasts retained characteristic cuboidal shape (I – 0.05 μM ZA, 72 hr exposure) consistent with controls (G). Scale bar = A-F: 100μM, G-I: 50 μM.

DISCUSSION AND FUTURE WORK

In this study, zoledronic acid was observed to have a notable effect on osteoblast proliferation, differentiation and mineralization. Treatment of sub-confluent MC3T3-E1 osteoblasts with zoledronic acid yielded a dose-dependent effect on proliferation (Figure 1), with higher concentrations of zoledronic acid inhibiting cell proliferation. Treatment with 0.50 μM zoledronic acid for 72 hours resulted in an approximate 25 percent reduction in osteoblast number, while treatment with 5.00 μM zoledronic acid for the same time inhibited proliferation by approximately 50 percent of the control. Exposure to a lower dosage (0.05 μM) of zoledronic acid had no statistically significant effect on osteoblast proliferation. However, these studies were carried out with cells grown in conventional tissue culture conditions.

Continuous treatment of 17- and 28-day old differentiated osteoblasts with zoledronic acid for one week resulted in decreased alkaline phosphatase production and calcium deposition, respectively (Figure 2). A continuous dose of zoledronic acid at 5.00 μM led to a reduction in alkaline phosphatase (Figure 2, D), while lower concentrations had little effect (Figure 2, B-C). All tested concentrations of zoledronic acid inhibited mineralization (Figure 2, F-H). Again, these tests were carried out with cells grown in tissue culture.

Exposure of three-month old osteoblasts grown in the bioreactor and challenged with metastatic breast cancer cells delayed the progression of cancer cells within the osteoblast tissue and temporarily maintained osteoblast tissue integrity (Figure 3). ZA treatment reduced the formation of breast cancer colonies (Figure 3, A-C) and inhibited breast cancer cell penetration of osteoblast tissue (Figure 3, table). Exposure of cancer cells to zoledronic acid also disrupted cancer cell alignment within the osteoblast tissue (Figure 3, D-F).

These results indicated that concentrations of zoledronic acid that minimally affect osteoblast function are capable of delaying breast cancer progression to bone. These data should be interpreted with caution, however. Other studies indicate that low concentrations (10 nM) of ZA do not significantly inhibit osteoblast proliferation until after 7 days of continuous culture [25]. Additionally, little is known about the adsorption kinetics of bisphosphonates. It is unclear whether ZA treatment of osteoblasts challenged with cancer cells in the bioreactor resulted in a true delay of breast cancer progression or occurred due to diffusion of ZA from the growth chamber to the medium reservoir. To address these concerns, future work will ensure that osteoblast proliferation is measured over an extended growth period and zoledronic acid is added to both bioreactor chambers.

Further analysis of the effects of zoledronic acid on breast cancer colonization of osteoblast tissue in the bioreactor is also required. RNA isolated from the bioreactor cultures will be analyzed for expression of osteocalcin, Type I collagen, and other bone proteins. In addition,

increases or decreases in cytokine production will be measured using medium collected from the bioreactor compartments.

Further studies will introduce the chemotherapeutic drug docetaxel into the bioreactor system, as literature suggests that zoledronic acid enhances the effects of docetaxel [34-39]. This study has shown that the bioreactor is a useful device for the study of drug effects on the early stages of breast cancer cell interactions with bone tissue.

ACKNOWLEDGEMENTS

This work was supported by the U.S. Army Medical Research and Material Command Breast Cancer Research Program WX81XWH-06-1-0432, the Susan G. Komen Breast Cancer Foundation BCTR 0601044, the Pennsylvania State University Office of Undergraduate Education, and the Penn State Ronald E. McNair Postbaccalaureate Achievement Program. I would like to thank Dr. Mastro, Dr. Vogler, and Donna Sosnoski for their guidance and assistance on this project. I would also like to thank Teresa Tassotti, Felicia Sanders and Judy Banker for their support.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2009. Atlanta, GA: American Cancer Society, Inc. 2009; 1-72.
2. American Cancer Society. Breast Cancer Facts & Figures 2007-2008. Atlanta, GA: American Cancer Society, Inc. 2007; 1-36.
3. Rubens RD. Bone Metastases - The Clinical Problem. European Journal of Cancer 1998; 34 (2): 210-3.
4. Taube T, Elomaa I, Blomqvist C, et al. Histomorphometric evidence for osteoclast-mediated bone resorption in metastatic breast cancer. Bone 1994; 15 (2): 161-6.
5. Guise TA. Molecular mechanisms of osteolytic bone metastases. Cancer 2000; 88 (12 Suppl): 2892-8.
6. Bierie B, Moses HL. Tumour microenvironment: TGFβ: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 2006; 6 (7): 506-20.
7. Mercer RR, Miyasaka C, Mastro AM. Metastatic breast cancer cells suppress osteoblast adhesion and differentiation. Clinical and Experimental Metastasis 2004; 21: 427-35.
8. Mastro AM, Gay CV, Welch DR, et al. Breast Cancer Cells Induce Osteoblast Apoptosis: A Possible Contributor to Bone Degradation. Journal of Cellular Biochemistry 2004; 91: 265-76.
9. Kinder M, Chislock E, Bussard KM, et al. Metastatic breast cancer induces an osteoblast inflammatory response. Experimental Cell Research 2008; 314: 173-83.
10. Costa L, Lipton A, Coleman R. Role of bisphosphonates for the management of skeletal complications and bone pain from skeletal metastases. Supportive cancer therapy 2006; 3 (3): 143-53.
11. Fleisch H. Bisphosphonates: mechanisms of action. Endocrine reviews 1998; 19 (1): 80-100.
12. Green J. Bisphosphonates: Preclinical Review. The Oncologist 2004; 9(suppl 4): 3-13.
13. van Beek E, Pieterman E, Cohen L, et al. Farnesyl Pyrophosphate Synthase Is the Molecular Target of Nitrogen-Containing Bisphosphonates. Biochemical and biophysical research communications 1999; 264 (1): 108-11.
14. Green J. Antitumor effects of bisphosphonates. Cancer 2003; 97 (3 Suppl): 840-7.
15. Ponader S, Brandt H, Vairaktaris E, et al. In vitro response of hFOB cells to pamidronate modified sodium silicate coated cellulose scaffolds. Colloids and Surfaces B: Biointerfaces 2008; 64 (2): 275-83.
16. Reinholz GG, Getz B, Pederson L, et al. Bisphosphonates Directly Regulate Cell Proliferation, Differentiation, and Gene Expression in Human Osteoblasts. Cancer Res 2000; 60 (21): 6001-7.

17. Pan B, To LB, Farrugia AN, et al. The nitrogen-containing bisphosphonate, zoledronic acid, increases mineralisation of human bone-derived cells in vitro. Bone 2004; 34 (1): 112-23.
18. Greiner S, Kadow-Romacker A, Lbberstedt M, et al. The effect of zoledronic acid incorporated in a poly(D,L-lactide) implant coating on osteoblasts in vitro. Journal of biomedical materials research Part A 2007; 80 (4): 769-75.
19. von Knoch F, Jaquiery C, Kowalsky M, et al. Effects of bisphosphonates on proliferation and osteoblast differentiation of human bone marrow stromal cells. Biomaterials 2005; 26 (34): 6941-9.
20. Kellinsalmi M, Mnkknen H, Mnkknen J, et al. In vitro comparison of clodronate, pamidronate and zoledronic acid effects on rat osteoclasts and human stem cell-derived osteoblasts. Basic & clinical pharmacology & toxicology 2005; 97 (6): 382-91.
21. Panzavolta S, Torricelli P, Bracci B, et al. Alendronate and Pamidronate calcium phosphate bone cements: setting properties and in vitro response of osteoblast and osteoclast cells. Journal of inorganic biochemistry 2009; 103 (1): 101-6.
22. Xiong Y, Yang HJ, Feng J, et al. Effects of alendronate on the proliferation and osteogenic differentiation of MG-63 cells. The Journal of International Medical Research 2009; 37 (2): 407-16.
23. Peter B, Zambelli PY, Guicheux J, et al. The effect of bisphosphonates and titanium particles on osteoblasts: an in vitro study. Journal of Bone and Joint Surgery; British volume 2005; 87 (8): 1157-63.
24. Schindeler A, Little D. Osteoclasts but not osteoblasts are affected by a calcified surface treated with zoledronic acid in vitro. Biochemical and biophysical research communications 2005; 338 (2): 710-6.
25. Orriss IR, Key ML, Colston KW, et al. Inhibition of osteoblast function in vitro by aminobisphosphonates. J Cell Biochem 2009; 106 (1): 109-18.
26. Idris A, Rojas J, Greig I, et al. Aminobisphosphonates Cause Osteoblast Apoptosis and Inhibit Bone Nodule Formation In Vitro. Calcified Tissue International 2008; 82 (3): 191-201.
27. Mastro AM, Vogler EA. A Three-Dimensional Osteogenic Tissue Model for the Study of Metastatic Tumor Cell Interactions with Bone. Cancer Res 2009; 69 (10): 4097-100.
28. Vogler EA. A compartmentalized device for the culture of animal cells. Biomaterials, artificial cells, and artificial organs 1989; 17 (5): 597-610.
29. Rose GG. Cytopathophysiology of tissue cultures growing under cellophane membranes. Int Rev Exp Pathol 1966; 5: 111-78.
30. Dhurjati R, Liu X, Gay CV, et al. Extended-Term Culture of Bone Cells in a Compartmentalized Bioreactor. Tissue Engineering 2006; 12 (11): 3045-54.
31. Dhurjati R, Krishnan V, Shuman LA, et al. Metastatic breast cancer cells colonize and degrade three-dimensional osteoblastic tissue in vitro. Clin Exp Metastasis 2008.
32. Friedl P, Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat Rev Cancer 2003; 3 (5): 362-74.
33. Cailleau R, Oliv M, Cruciger QV. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In vitro 1978; 14 (11): 911-5.
34. Brubaker KD, Brown LG, Vessella RL, et al. Administration of zoledronic acid enhances the effects of docetaxel on growth of prostate cancer in the bone environment. BMC Cancer 2006; 6 (15).
35. Fabbri F, Brigliadori G, Carloni S, et al. Zoledronic acid increases docetaxel cytotoxicity through pMEK and Mcl-1 inhibition in a hormone-sensitive prostate carcinoma cell line. Journal of Translational Medicine 2008; 6 (43).
36. Inoue K, Karashima T, Fukata S, et al. Effect of CombinationTherapy with a Novel Bisphosphonate, Minodronate (YM529), and Docetaxel on aModel of Bone Metastasis by HumanTransitional Cell Carcinoma. Clin Cancer Res 2005; 11 (18): 6669-77.
37. Karabulut B, Erten C, Gul MK, et al. Docetaxel/zoledronic acid combination triggers apoptosis synergistically through downregulating antiapoptotic Bcl-2 protein level in hormone-refractory prostate cancer cells. Cell Biology International 2009; 33: 239-46.
38. Kim S-J, Uehara H, Yazici S, et al. Modulation of Bone Microenvironment with Zoledronate Enhances the Therapeutic Effects of STI571 and Paclitaxel against Experimental Bone Metastasis of Human Prostate Cancer. Cancer Res 2005; 65 (9): 3707-15.
39. van Beek ER, Lowik CWGM, van Wijngaarden J, et al. Synergistic effect of bisphosphonate and docetaxel on the growth of bone metastasis in an animal model of established metastatic bone disease. Breast Cancer Res Treat 2008.