Impact of Bisphosphonates on Bone Quality in a Rat Model of Mixed Femoral Metastases

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INTRODUCTION: The skeleton is one of the most common sites of metastatic disease, especially in trabecular regions (i.e., vertebrae and metaphases of long bones). Metastases impact the bone remodelling process, compromising the mechanical integrity of the bone, and increasing the risk of pathological fractures. Systemic administration of zoledronic acid (ZA), a bisphosphonate, is considered standard of care to reduce skeletal-related events in patients with bone metastases. Despite clinical efficacy, the impact of ZA on bone quality and biomechanics in the presence of mixed osteolytic/osteoblastic metastasis is not well understood. This study aimed to quantify the effects of ZA on bone quality in a preclinical model of mixed metastases.

METHODS: Twelve 6-week-old athymic male rats (Hsd: $^{RH\text{-}Foxn1rnu}$, Envigo) were randomly assigned to the following groups: healthy (Healthy untreated, n=3), tumor cell injected control (ACE-1 untreated, n=3), healthy zoledronic acid (ZA) treated (Healthy ZA, n=2) and tumor-cell injected ZA treated (ACE-1-ZA, n=4). Institutional approval was obtained, and the ARRIVE guidelines were followed. Seven animals were inoculated with luciferase-transfected ACE-1 canine prostate cancer cells via an intracardiac injection after a week of acclimation (day 0). ZA treatment (Zometa®, Norvartis; 60µg/kg) was administered subcutaneous on day 10. In vivo bioluminescence imaging (BLI, day 15, day 21) was used to assess tumor burden. All animals were euthanized on day 21. For stereological analysis, both excised femora underwent µCT scanning (µCT100, Scanco, 20µm isotropic voxels), and trabecular bone within the distal femora were segmented using an in-house stereology algorithm. For histological analysis, the right femur (n=12) was fixed in 10% buffered formalin and decalcified in 10% EDTA. Thin sections (5-7 µm) were stained with hematoxylin and eosin (H&E) to evaluate tissue morphology and bone histoarchitecture. Staining with an anti-wide cytokeratin antibody was used to identify tumor burden. The left distal femur (n=12) was cut to a 1cm length using a diamond wafering blade on a low-speed saw (Isomet 1000, Buehler Corporation). The samples were stained with $BaSO_4$ and µCT imaged (90kVp, 44µA, 4.9µm) to visualize microdamage location and volume. Damage volume fraction (SV/BV) was calculated as the ratio of $BaSO_4$ stain volume (SV) to bone volume (BV). Finally, the samples were loaded to failure under compression, and force-displacement data was recorded. Two-factor ANOVAs were used to evaluate the effects of cell line (healthy vs. ACE-1 cell-injected) and treatment (untreated vs. ZA treated) on bone quality.

RESULTS: Of the seven rats injected, bioluminescence imaging confirmed femoral metastases in 4 rats. Only rats that had developed femoral metastases were included in the subsequent analyses. Specimen with metastatic bone changes had significantly higher bone mineral density (BMD, p=0.016) and bone volume fraction (BV/TV, p=0.024) compared to healthy bone. Treatment had a significant effect, showing an increase in BMD (p=0.032), BV/TV (p=0.0022), and trabecular thickness (TbTh, p=0.0031) in ZA-treated animals compared to untreated animals (Table 1). Animals treated with ZA showed a lower SV/BV (p=0.144) and higher load to failure (p=0.238) compared to untreated animals; however, additional samples (currently underway) are needed to demonstrate significance.

DISCUSSION: The ACE-1 cell line produced a mixture of osteolytic destruction and areas of new osteoblastic bone (Figure 1), contributing to an overall increased bone mineral density and bone volume fraction compared to healthy animals.[1] ZA effectively diminished tumor-induced osteolysis, consistent with its known capacity to inhibit osteoclast activity. The subsequent bone formation without proportional bone resorption and the newly formed osteoblastic bone resulted in increased BMD, BV/TV, and TbTh.[2,3] As observed with a purely osteolytic model, there was a general trend (non-significant) suggesting that ZA strengthens the bone and reduces SV/BV compared to untreated animals (Figure 2), implying an advantageous impact of the treatment on bone quality.[4] Future work includes increasing the sample size per group to achieve significance in SV/BV as well as load to failure. Histological analysis confirmed tumor cell location within the newly formed bone (Figure1).

SIGNIFICANCE/CLINICAL RELEVANCE: Skeletal related events post cancer treatment can significantly impact the quality of life. Assessing how treatment affects the quality of both healthy and metastatically affected bone is crucial for comprehending the consequences of cancer therapies on fracture risk.

REFERENCES: 1. Burke MV et al. JOR, 34 (12) 2126–36, 2016. 2. Won E et al. Breast Cancer Res Treat. 124 (1) 111-9, 2010. 3. Akens MK et al. Photodiagnosis and photodynamic therapy. 11(3), 426-33, 2014. 4. Tolgyesi, A et al. Orthop Procs. *105-B*(SUPP_7) 73, 2023

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Table 1: Summary of stereological parameters

Treatment group	BMD [mgHA/cm³]	TMD [mgHA/cm³]	TBV [%]	TbTh [µm]
Healthy untreated	283.62±40.7	752.23±92.08	29.04±1.96	138.67±16.02
ACE-1 untreated	346.58±34.77	779.23±84.15	34.38±3.98	144.30±11.46
Healthy ZA	360.63±38.92	772.56±70.24	36.90±2.25	166.97±20.73
ACE-1 ZA	387.38±70.86	815.57±75.57	37.98±4.81	188.01±33.93

All data represented as mean ± standard deviation

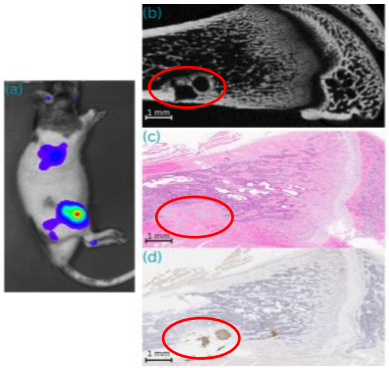

Figure 1: (a) BLI, (b) *ex-vivo* µCT, (c) H&E stain, and (d) Cytokeratin stain of an ACE-1 untreated rat, red circle indicates mixed lesions in the distal metaphysis of the right femur

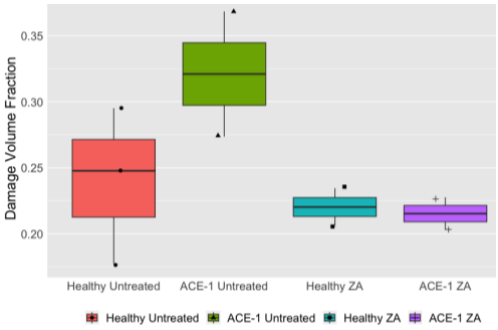

Figure 2: Damage volume fraction in left femora of healthy and ACE-1 cell injected rats following ZA treatment