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Low intensity vibration mitigates tumor progression and protects bone quantity and quality in a murine model of myeloma

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ABSTRACT

Myeloma facilitates destruction of bone and marrow. Since physical activity encourages musculoskeletal preservation we evaluated whether low-intensity vibration (LIV), a means to deliver mechanical signals, could protect bone and marrow during myeloma progression. Immunocompromised-mice (n = 25) were injected with human-myeloma cells, while 8 (AC) were saline-injected. Myeloma-injected mice (LIV; n = 13) were subjected to daily-mechanical loading (15 min/d; 0.3 g @ 90 Hz) while 12 (MM) were sham-handled. At 8w, femurs had 86% less trabecular bone volume fraction (BV/TV) in MM than in AC, yet only a 21% decrease in LIV was observed in comparison to AC, reflecting a 76% increase versus MM. Cortical BV was 21% and 15% lower in MM and LIV, respectively, than in AC; LIV showing 30% improvement over MM. Similar outcomes were observed in the axial skeleton, showing a 35% loss in MM with a 27% improved retention of bone in the L5 of LIV-treated mice as compared to MM. Transcortical-perforations in the femur from myeloma-induced osteolysis were 9× higher in MM versus AC, reduced by 57% in LIV. Serum-TRACP5b, 61% greater in MM versus AC, rose by 33% in LIV compared to AC, a 45% reduction in activity when compared to MM. Histomorphometric analyses of femoral trabecular bone demonstrated a 70% elevation in eroded surfaces of MM versus AC, while measures in LIV were 58% below those in MM. 72% of marrow in the femur of MM mice contained tumor, contrasted by a 31% lower burden in LIV. MM mice (42%) presented advanced-stage necrosis of tibial marrow while present in just 8% of LIV. Myeloma infiltration inversely correlated to measures of bone quality, while LIV slowed the systemic, myeloma-associated decline in bone quality and inhibited tumor progression through the hindlimbs.

1. Introduction

In the United States, there is a 0.7% lifetime risk of acquiring multiple myeloma, a cancer that forms from plasma cells and accumulates within the bone marrow, thus crowding out healthy blood cells [1,2]. Myeloma is the second most prevalent hematologic malignancy [3,4], representing approximately 15% of all hematologic cancers [5], with 27,000 new U.S. cases projected to be diagnosed this year [1]. Recently developed therapeutics have extended 5-year survival to 44%, yet over 11,000 patients still die of this disease each year in the U.S. Bone resorption is one of the defining comorbidities of myeloma, compromising skeletal quality and increasing fracture susceptibility in those with the disease [6,7]. The spread of malignant plasma cells through the bone marrow (BM) space also disrupts resident hematopoietic progenitors and weakens the immune response [8–10], and, in aggregate, consequences of BM crowding by myeloma contributes to decreased quality-of-life despite advances in treatment.

Pathologically, myeloma is characterized by a marked increase in plasma cell density within the BM [11–13], with tumor cell invasion disrupting the tightly orchestrated mechanisms that control bone remodeling while simultaneously creating an environment conducive to osteolytic lesions [4,14]. Further, the transformation of the BM into a tumor supportive niche leaves a less viable milieu for the mesenchymal stem cell (MSC) populations that are critical for tissue regeneration and hematopoietic stem cells (HSC) central to hematopoiesis and myelopoiesis [11,15].

Cancer progression and the array of treatment strategies employed to manage the disease often result in a significant challenge to the skeleton [16–19]. The long-term, catabolic impact of chemotherapy, irradiation, and immunosuppressive therapies on bone endpoints contribute heavily to osteopenia [20], especially for the very young [18,21], frail elderly [22], and immunosuppressed [23]. Routine treatment strategies, from high-dose chemotherapy to fractionated radiotherapies [24,25],

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are aimed at slowing tumor expansion, but each approach is limited by adverse effects. For example, while irradiation is effective in combating tumor burden [24], it degrades the bone matrix [20] and damages the spectra of cellular constituents which govern bone remodeling [26]. Chemotherapy diminishes tumor burden but, amongst other side effects, can be associated with renal toxicity and pancytopenia [25,27]. Extensive radiation and/or chemotherapy may necessitate BM transplantation, increasing the likelihood of secondary side effects (i.e., graft-vs.-host disease, acute myelodysplastic leukemia, or myelodysplastic syndrome) [28,29]. Glucocorticoids (e.g. Dexamethasone) are efficacious in diminishing tumor burden, primarily as a function of dosing, yet, high-dose and/or chronic use are heavily associated with toxicity and result in osteoporotic bone [30,31] by inducing apoptosis in osteoblasts and osteocytes [32]. Immunosuppressive agents (e.g., corticosteroids) are administered as primary therapy or to permit graft tolerance (e.g. BM transplantation) but, in doing so, predispose the patient to infection. Anti-resorptives (e.g., bisphosphonates) have been shown to mitigate bone loss but are limited by inconsistent outcomes and negative side effects of short and long-term use, including osteonecrosis of the jaw and atypical fractures [33,34].

In contrast to pharmacological-centric therapies, exercise is recognized as a non-drug deterrent of cancer, as well as a means to protect musculoskeletal health. Indeed, the attributes of physical activity have helped promote it as a foundation of any therapeutic plan [35–37]. Paradoxically, for cancer patients already at risk for developing a fracture, even a moderate exercise regimen may precipitate the fractures that the treatment is prescribed to prevent. In an effort to incorporate non-drug strategies for those with a compromised musculoskeletal system, low-intensity vibration (LIV), a mechanical signal which mimics the dynamics of muscle contraction, has been shown to protect bone quality in a murine model susceptible to ovarian cancer [38]. LIV's effects have been observed in both *in vitro* and *in vivo* systems to promote highly-ordered tissue synthesis [39,40], upregulate musculoskeletal quality [41–43], and enhance the cytoskeletal architecture of precursor bone cells [44,45], while preserving the viability of the BM niche [46–48]. Previous work by our group and others have demonstrated LIV as having an anabolic effect that encourages, at the level of the MSC, lineage differentiation towards osteogenesis and away from an adipogenic phenotype [49,47]. Contrarily, in the absence of mechanical loading, administration of LIV has been shown to reduce osteoclast activity across the endosteal surface [50]. On the order of the cell, LIV has been shown to both decrease resorptive activity while also enhancing cytoskeletal proteins [51]. To determine whether LIV is an effective agent against bone loss associated with myeloma, a xenograft mouse model was developed and, with the disease allowed to progress for 8w, quantified to what degree osteolysis and tumor progression had been influenced.

2. Materials and methods

2.1. Human myeloma cell culture and expansion

A cryogenically preserved human-myeloma cell line (U266β1; *ATCC*; Manassas, VA, USA), was thawed and cultured using aseptic techniques. Centrifugation was used to separate (4 °C, 125 g, 6 min) cell pellets from media containing dimethyl-sulfoxide (DMSO). Pellets were resuspended in a 25 cm^2 tissue culture flask with 10 mL of growth media and incubated horizontally (37 °C in 5% CO_2 atmosphere). Cell viability was quantified every 3d using an automated cytometer (Countess; *Invitrogen*; Rockville, MD, USA) until confluence (~97% viability), at which time the cell suspension was centrifuged (24 °C, 2200 rpm, 5 min), resuspended, and split 1:3 into 75 cm^2 tissue culture flasks. According to manufacturer's recommendations, subcultures were maintained at a density of 3.5×10^5–1×10^6 cells/mL until day of injection.

2.2. Murine model

All mice were individually housed. Power calculations were performed to account for effect-size (0.25) and for statistical power of at least 0.8. Age-Matched Control (AC; n = 8), Myeloma-Injected (MM; n = 12), and Myeloma-Injected treated with Low Intensity Vibration (LIV; n = 13) groups were distributed using a *Matlab* (*The MathWorks, Inc.*; Natick, MA, USA) algorithm that randomizes samples by weight matching. U266β1 cells were inoculated intravenously via tail vein into 7w-old, male immunodeficient NSG mice (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ; *The Jackson Laboratory*; Bar Harbor, ME, USA) [52]. 25 NSG mice (MM and LIV) were injected with 0.3 cm^3 of 2×10^6 U266β1 via a sterile saline vehicle, while AC mice were injected with 0.3 cm^3 of sterile saline as control. Of the 25 U266β1-injected mice, 13 were subjected to 8w of LIV, while the remaining 12 received sham-LIV (MM). Criteria were established to exclude tissue samples from analyses if the associated mouse died prematurely, else all samples were utilized. Mice were maintained in accordance with the Institutional Animal Care and Use Committee guidelines at Stony Brook University and the NIH Guide for the Care and Use of Laboratory Animals.

2.3. Daily mechanical loading protocol

Loading regimens commenced 4 h post-inoculation providing a rest period in order to mitigate stress induced from handling. Mice assigned to the mechanical loading regimen were subject to LIV ($0.3g \pm 0.025$ @ 90 Hz, where $1g$ = Earth's gravitational field or 9.8 m/s^2) [38,47,50], for 15 min/d, 5 d/w, while AC and MM groups underwent identical handling and loading protocols as LIV mice but without activation of the platform. The daily loading regimen consisted of placing mice into individual 12 cm × 12 cm containers on a fixed, vertically-oscillating platform (*Marodyne Medical*; Tampa, FL, USA) to administer the LIV signal. Displacements required to produce accelerations at 90 Hz are well below 100 μm and are barely perceptible to human touch. The lead investigator was not blinded to the experimental groups during the delivery of LIV.

2.4. Tissue harvest and preservation

At the end of the 8w protocol, each mouse was anesthetized using isoflurane inhalation and whole blood collected via cardiac puncture. Blood was then heparinized and aliquoted (100 μL) for FACS analysis after erythrocyte lysis (1× Pharmalyse; *BD Biosciences*, San Jose, CA, USA). Euthanasia was achieved by cervical dislocation. Left femora were briefly preserved on ice, and BM was extracted and isolated using supplemented Dulbecco's Modified Eagle's Medium (DMEM; *GIBCO*; Grand Island, NY, USA) containing 2% FBS, 10 mM HEPES Buffer, and 1% penicillin–streptomycin ($DMEM^+$). Tissues for histological processing, including right femora and tibia, were fixed in 10% neutral buffered formalin, replaced at 48 h with 70% ethanol, and subsequently sectioned. Bone specimens were decalcified (DECAL; *Decal*; Suffern, NY, USA) and 5 μm paraffin-embedded sagittal cross-sections were stained with hematoxylin and eosin (H&E). Prevalence of MM and evaluation of tumor burden were determined via histologic examination, performed by a histopathologist (KRS) who was blinded to the status of any mouse's experimental regimen.

2.5. Flow cytometric analyses of femoral bone marrow

Flow cytometric analyses (FACSAria Cytometer; *BD Pharmingen*; San Diego, CA, USA) utilized specific markers to isolate and quantify cells of both the lymphoid and myeloid lineages. As reported, the FACS data represents the average of all cell populations quantified separately for each mouse across each group. Since the immune deficient mice do not have viable, native plasma cells, FACS analysis was performed using an antibody for the surface marker CD138 (syndecan-1), a cell-