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Mimicking the effects of spaceflight on bone: Combined effects of disuse and chronic low-dose rate radiation exposure on bone mass in mice

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ABSTRACT

During spaceflight, crewmembers are subjected to biomechanical and biological challenges including microgravity and radiation. In the skeleton, spaceflight leads to bone loss, increasing the risk of fracture. Studies utilizing hindlimb suspension (HLS) as a ground-based model of spaceflight often neglect the concomitant effects of radiation exposure, and even when radiation is accounted for, it is often delivered at a high-dose rate over a very short period of time, which does not faithfully mimic spaceflight conditions. This study was designed to investigate the skeletal effects of low-dose rate gamma irradiation (8.5 cGy gamma radiation per day for 20 days, amounting to a total dose of 1.7 Gy) when administered simultaneously to disuse from HLS. The goal was to determine whether continuous, low-dose rate radiation administered during disuse would exacerbate bone loss in a murine HLS model. Four groups of 16 week old female C57BL/6 mice were studied: weight bearing + no radiation (WB + NR), HLS + NR, WB + radiation exposure (WB + RAD), and HLS + RAD. Surprisingly, although HLS led to cortical and trabecular bone loss, concurrent radiation exposure did not exacerbate these effects. Our results raise the possibility that mechanical unloading has larger effects on the bone loss that occurs during spaceflight than low-dose rate radiation.

1. Introduction

During spaceflight, astronauts face many biological and biomechanical challenges to their skeleton, including microgravity and radiation. Astronauts lose 1 to 2% of bone mass per month during spaceflight (Vico et al., 2000; Lang et al., 2004a), and recovery may be incomplete even several years after returning to Earth (Lang et al., 2006). This bone loss can increase risk of fracture immediately upon return to normal gravitational loading, and may exacerbate the development of normal age-related osteoporosis (Sibonga et al., 2015). Thus, it is important to better understand the mechanism of spaceflight-related bone loss in the quest towards developing better countermeasures against disuse osteoporosis and risk of bone fracture in astronauts.

Hindlimb suspension (HLS) as a ground-based disuse model has been used widely for simulating the effects of microgravity in spaceflight, and has improved understanding of mechanisms of disuse-induced bone loss (Morey-Holton and Globus, 2002a. The loss of bone mass in a mature rodent subjected to hindlimb suspension for 2 to 3 weeks is comparable to the loss of bone mass for a human who spends 4–6 months in space (Squire et al., 2004; Judex et al., 2004). However, during spaceflight astronauts are also exposed to solar radiation in the form of particle events (SPEs) and galactic cosmic radiation (GCR), the former (consisting mainly of protons) originating from solar flares delivering high radiation doses in a short time, and the later (consisting of heavy ions and protons) providing continuous low-dose rate exposure throughout the space mission (Benton and Benton, 2001; Blakely, 2000; Townsend, 2005). Astronauts also experience secondary radiation generated by heavy ions striking the space craft shielding (Board, 1997;

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Cucinotta et al., 2001; Edwards, 2000). A proportion of the radiation dose received within a spacecraft in deep space will be from particles with high Linear Energy Transfer (LETs of 10 keV/μm or greater) (Slaba et al., 2016). While the negative effects of microgravity mimicked by hindlimb suspension have been widely studied (as reviewed in (Morey-Holton et al., 2005)), there are fewer studies that simultaneously account for the detrimental effects of space radiation, which is complex and difficult to replicate on Earth. Total body radiation doses of 1–2 Gy would be experienced during some long-duration mission scenarios (Bandstra et al., 2009). Whole body doses of 2 Gy gamma radiation or 1 Gy proton radiation are considered good ground-based models of space radiation and result in significant bone loss in murine models (Lloyd et al., 2012; Kondo et al., 1985). For logistical reasons, combined radiation and hindlimb unloading have previously been given sequentially in ground-based mouse models, with high dose rate radiation delivered in a single fraction (lasting about 2 minutes) prior to hindlimb unloading (Lloyd et al., 2012). However, low dose rate exposure better replicates the conditions of normal tissue injury, repair and repopulation, cell cycle redistribution, and normal tissue radiosensitivity as seen in spaceflight. Few animal models to date have accounted for the simultaneous effects of radiation and microgravity on skeletal tissues (Willey et al., 2016; Walb et al., 2015; Macias et al., 2016).

In this study, we investigated the effects of low-dose gamma irradiation when administered simultaneously with disuse from HLS on the skeleton. We hypothesized that concomitant radiation would exacerbate bone loss in mice exposed to HLS. We exposed mice to 8.5cGy gamma radiation per day for 20 days, amounting to a total dose of 1.7 Gy. These studies build on previous work by using an irradiation protocol that more closely mimics space low-dose rate radiation (and its effects on bone cells) by delivering the radiation slowly over a 20 day period as compared to previous studies where delivery occurred as a single radiation dose lasting about 2 minutes (Lloyd et al., 2012; Kondo et al., 1985). The goal of our study was to mimic the effects of microgravity and radiation on the skeleton to compare the negative effects between HLS and long term radiation on the bone, with the ultimate hope of developing an effective, novel model to study potential countermeasures against spaceflight-induced bone loss in astronauts.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice were obtained from the National Institutes of Health at 14 weeks of age. Body masses were measured and used to randomize animals between study groups, which included: weight bearing + radiation (WB + RAD), weight bearing with no radiation (WB + NR), hindlimb suspension + radiation (HLS + RAD), and hindlimb suspension with no radiation (HLS + NR) (n = 5/group). Mice were allowed to acclimate to the vivarium and cages of interest (standard or HLS) for 7 days under standard conditions on a 12 h light: 12 h dark schedule prior to onset of experiments. All mice were provided with standard laboratory rodent chow and water ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee at Colorado State University (protocol 13–4463A).

2.2. Hindlimb suspension (HLS)

Hindlimb suspension was performed using a modification of the Morey-Holton method, as described previously (Turner et al., 2006; Morey-Holton and Globus, 2002b). Briefly, mice were suspended at a 30° angle (torso relative to floor of cage) using a custom-built tail harness consisting of strips of Skin Trac adhesive traction strips (Zimmer), extending from 1 cm past the hairline to 2 cm beyond the end of the tail. Plastic shields, made from conical tubes, were placed over the tail to protect the harness attachment. Swivel clips were attached to the tail harness and secured to an adjustable-height pulley system that allowed mobility about the cage in the x- and y-directions. Mice were checked daily to ensure harness integrity, adequate food and water consumption by each animal, and to monitor for signs of animal distress. Animals remained suspended for 20 days, and body mass data were collected at the conclusion of the experiment. Weight bearing controls were also monitored daily and weighed to collect body mass data at the conclusion of the experiment.

2.3. Irradiation

Two groups of animals were irradiated (WB + RAD and HLS + RAD), and for the HLS + RAD group, irradiation and hindlimb suspension were conducted simultaneously throughout the study. Whole-body irradiation was performed using a ^{137}Cs gamma-ray irradiator in a vivarium facility. The dose rate to the mice was 8.5 cGy/day over a 20 day period (20 hours per day irradiation, 4 hours per day for animal husbandry procedures), a total dose for each mouse of 1.7 Gy. Exact dosimetry was calculated for each cage through thermoluminescent dosimeters (TLDs) affixed to each cage. Measurements from individual TLDs affixed to the mouse cages throughout the 20 day experiment averaged 5.89 μC, corresponding to a dose of gamma radiation of 182.59 cGy or approximately 1.83 Gy. Background measurements of 0.947 ± 0.031 nC were determined for unexposed TLDs (n = 10) preselected from the same lot as the exposed TLDs and kept throughout the experiment in an adjacent room.

2.4. Tissue collection

At the end of the 20 day experimental period, all mice were anesthetized with inhalation isoflurane (2%) and sacrificed by cervical dislocation and exsanguination. Hindlimbs were removed and femurs and tibias were cleaned of soft tissue. The right femur from each mouse was stored in micro-centrifuge tubes at −20 deg C for mechanical testing, and the left femur from each mouse was fixed in 10% neutral buffered formalin for 48 hours and stored at 4 deg C in 70% ethanol. The left tibia from each mouse was flash frozen in liquid nitrogen and stored at −80 deg C, but was later thawed for analysis of bone architecture.

2.5. Micro-computed tomography (trabecular and cortical bone microarchitecture)

Bone architecture of the left femur and tibia was analyzed using micro-computed tomography (microCT). The central portion of the femoral diaphysis and secondary spongiosa in the distal femoral metaphysis and proximal tibial metaphysis of each bone were scanned in 70% ethanol on a μCT80 scanner (Scanco Medical AG, Basserdorf, Switzerland). Cortical and trabecular bone scans were performed with 10 μm voxel size using an energy setting of 70 kVp and an integration time of either 500 ms (femur) or 800 ms (tibia). For the distal femoral metaphysis scans, a region of interest that was 1.0 mm long (100 slices) was analyzed beginning 1.5 mm proximal to the growth plate. For the proximal tibia metaphysis scans, a region of interest that was 1.0 mm long (100 slices) was analyzed beginning 1.25 mm distal to the growth plate. Trabecular bone volume fraction (Tb. BV/TV, %), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), and trabecular separation (Tb.Sp, mm) were computed using the manufacturer's software (Bouxsein et al., 2010). For the femoral diaphysis scans, a region of interest that was 1.0 mm long and centered about the exact midshaft of the bone was analyzed in each sample. Cortical bone volume (Ct.BV, mm^3), cortical bone area (Ct.B.Ar, mm^2), and cortical bone tissue mineral density (Ct.Tiss.Mn.Dn, mg hydroxyapatite (HA)/cm^3) were computed with the manufacturer's software (Bouxsein et al., 2010). Maximum section modulus, quantified as the maximum moment of inertia divided by the distance from the centroid to the farthest bone

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